Clinical and genetic investigation of the epsilon-sarcoglycan complex in neurologic and psychiatric disease

A thesis submitted for the Degree of Doctor of Philosophy at Cardiff University School of Medicine

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2012

Supervised by Professor Huw Morris, Professor Derek Blake and Professor Michael Owen

Acknowledgements

This work would not have been possible without the help and encouragement of many people. Firstly I would like to thank my supervisors Professor Huw Morris, Professor Derek Blake and Professor Michael Owen whose advice and support have been invaluable over the past three years.

I owe a huge amount to all those who have helped me in the lab; Kate, Lesley, Sarah, Hywel, Kiran, Rhodri, Liam, Vicky, Laura, Caz, Mark, Ria and Alys, their advice, assistance and good humour have helped me through many a difficult day. However, it is to Ade that I owe the greatest thanks, for his continued support, patience and tireless explanations, I am enormously grateful. To Katharine, Sam, Mark C, Gill, Mark W, Justin, Joe, Dee, June, Josephine, Matt and Seb, your humour, coffee making and tolerance of my endless chatter know no bounds. I will always look back upon the last three years with enormous fondness and hope that I've been able to provide a small fraction of the support you've given me. Mark Wardle, for your quiet patience and willingness to ignore my technological incompetence, an enormous thank you.

My colleagues who have collaborated with this project; Dr JP Lin, Dr Tammy Hedderly, Dr Martin Smith, Dr Hardev Pall, Professor Patrick Chinnery, Prof Tim Lynch, Dr Danny Smith, Professor Mary King, Dr Sean O'Riordan, Dr Bryan Lynch, Dr Cathy White, Dr Alan Whone, DrAndrew Lux, Dr Philip Jardine, Dr Nin Bajaj, Dr George Kirov, Dr Mike Samuel and Professor Tom Warner, thank you all for your support from the very beginning of this work, without your contribution none of this would have been possible. My mentor, Dr Manju Kurian, I would have been lost without her. From the very first MD-SIG meeting, the support, advice and encouragement has been overwhelming. I hope someday to be able to repay you this enormous debt.

To Celia, Tina, Jef, Chris and Dobrina, your kindness, humour, company on some great nights out and your ability to ensure that I retain my perspective on life knows no bounds. Thank you a million times. My family, Mum, Dad, Alistair, Neil and Edward, who despite my sometimes trying nature, offer an endless supply of love and support and without which I would never have made it this far. Finally, Adrian, a ceaseless source of support, encouragement and love – thank you from the bottom of my heart.

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Abbreviations

ABN	Association of British Neurologists
ACA-A	Acid citrate dextrose
AD	Anxiety Disorder
ADHD	Attention Deficit Hyperactivity Disorder
AS	The Anxiety Scale
ATP1A3	Na^+, K^+-ATPase alpha 3 subunit gene
AUDIT	Alcohol Use Disorders Identification Test
BAI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
BFMDRS	Burke-Fahn Marsden Dystonia Rating Scale
BNSU	British Neurological Surveillance Unit
BPNA	British Paediatric Neurology Association
BTX	Botulinum Toxin
cDNA	Complementary Deoxyribonucleic Acid
CGH	Comparative Genomic Hybridization
CIDI	Composite International Diagnostic Interview
CMA	Chromosome Microarray
CNV	Copy Number Variant
CPN	Community Psychiatric Nurse
D1R	Dopamine 1 Receptor
D2R	Dopamine 2 Receptor
DAT	Dopamine Transporter
DBS	Deep Brain Stimulation
DGC	Dystrophin-associated Glycoprotein Complex
DIGS	Diagnostic Interview for Genetic Studies
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate

DRD2	D2 Dopamine Receptor Gene
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders IV
DTI	Diffusion Tensor Imaging
EBV	Epstein Barr Virus
ECACC	European Collection of Cell Cultures
EDTA	Ethylenediaminetetraacetic acid
EEG	Electroencephalogram
EMG	Electromyogram
ER	Endoplasmic Reticulum
EtBr	Ethidium Bromide
FA	Fractional Anisotropy
FBS	Fetal Bovine Serum
FISH	Fluorescence In Situ Hybridisation
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma-Aminobutyric Acid
GAD	Generalised Anxiety Disorder
GAG	Guanine-adenine-guanine
GARS	Gilliam Autism Rating Scale
GCH1	GTP Cyclohydrolase 1 gene
GMC	General Medical Council
GP_e	Globus Pallidus Externus
GP_i	Globus Pallidus Internus
GRM3	Glutamate receptor type 3 gene
HPA	Health Protection Agency
ICF	Intracortical Facilitation
KO	Knock Out
L-SAS	Liebowitz Social Anxiety Scale
LICI	Long Term Intracortical Inhibition

MAD	Major Affective Disorder
MADRS	Montgomery-Asberg Depression Rating Scale
MC	Manifesting Carrier
MDS	Myoclonus Dystonia Syndrome
Mg Cl_2	Magnesium Chloride
M.I.N.I.	MINI International Neuorpsychiatric Interview
M.I.N.I. KID	MINI International Neuropsychiatric Interview for Children and Adolescents (Parent Version)
MLPA	Multiplex Ligation-Dependent Probe Amplification
MR-1	Myofibrillogenesis regulator gene
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
NC	Non-Carrier
NKX2-1	NK2 homeobox 1 gene
NMC	Non-Manifesting Carrier
OCD	Obsessive Compulsive Disorder
OCS	Obsessive Compulsive Symptoms
PAS	Paired Association Stimulation
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
PHQ-9	Patient Health Questionnaire 9
PNKD1	Paroxysmal Non-Kinesigenic Dyskinesia 1
PTD	Primary Torsion Dystonia
SCID-II	Structure Clinical Interview for DSM-IV: Personality Disorder Questionnaire
SF-36	Short Form Health Survey
SGCE	Epsilon-sarcoglycan gene
SGCZ	Zeta-sarcoglycan gene

SICI	Short Term Intracortical Inhibition
SMA	Supplementary Motor Area
SNP	Single Nucleotide Polymorphism
SPECT	Single Photon Emission Computed Tomography
THAP1	Thantos-associated protein domain-containing apoptosis-associated protein 1
TMS	Transcranial Magnetic Stimulation
TOR1A	Torsin A GAG deletion
TorsinA	Torsin family 1, member A
UK	United Kingdom
UMRS	Unified Myoclonus Rating Scale
UPD	Uniparental Disomy
VIM	Ventral Intermediate Nucleus of the thalamus
WGD	Whole Gene Deletion
YBOCS	Yale-Brown Obsessive Compulsive Scale

Abstract

Myoclonus Dystonia Syndrome is a childhood onset hyperkinetic movement disorder characterised by alcohol responsive upper body myoclonus and dystonia. A

proportion of cases are due to mutations in the maternally imprinted *SGCE* gene, which encodes the transmembrane epsilon-sarcoglycan protein. Previous studies suggest an increased rate of psychiatric disorders in those with *SGCE* mutations. This study aimed to establish a cohort of myoclonus dystonia syndrome patients, identify the rate and type of *SGCE* mutations, determine differences in motor characteristics between mutation positive and negative cases and whether psychiatric disorders form part of the disease phenotype.

Eighty-nine probands with clinically suspected MDS were recruited. Information regarding onset and distribution of motor symptoms was collected via systematic questionnaires and video taped examination. *SGCE* was analysed using direct sequencing and for copy number variants. Psychiatric symptoms were assessed using systematic and standardised questionnaires and compared to a disability-matched, alcohol responsive tremor control group.

Nineteen (21%) probands had an *SGCE* mutation. All had evidence of upper body predominant myoclonus and dystonia during their disease course. Five had contiguous gene deletions ranging from 0.7 to 2.3Mb in size with distinctive clinical features. Recruitment of family members increased the affected *SGCE* mutation positive group to 27 of whom 21 (77%) had psychiatric symptoms. Obsessive-Compulsive Disorder was eight times more likely ($p<0.001$) in mutation positive cases, compulsivity being the predominant feature ($p<0.001$). Generalized Anxiety Disorder ($p=0.003$) and alcohol dependence ($p=0.02$) were five times more likely in cases than tremor controls.

Overall, *SGCE* mutations are associated with a narrow clinical and specific psychiatric phenotype. The presence of myoclonus, dystonia, age at onset ≤10 years and a positive family history of the disorder are the strongest predictors of an *SGCE* mutation. *SGCE* mutations are likely to have a pleiotropic effect in causing both motor and specific psychiatric symptoms.

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CHAPTER 1

Introduction

1.1 Introduction

This thesis examines the clinical characteristics of Myoclonus Dystonia Syndrome (MDS) and the subsequent role of the epsilon-sarcoglycan (ε-sarcoglycan) protein in its pathogenesis. In this chapter I will initially outline the classification and clinical features of the different forms of dystonia, while also discussing treatment options and current models of pathogenesis. In the final part I will explore the clinical features and genetic aetiology of MDS while also discussing the current understanding of the role of the ε-sarcoglycan protein.

1.2 Dystonia

The dystonias are a group of hyperkinetic movement disorders characterised by involuntary sustained muscle contractions causing twisting, repetitive movements and abnormal postures.[1] First described by Schwalbe in a Jewish family in 1908, Oppenheim later introduced the term '*dystonia musculorum deformans*' to characterise the autosomal dominant inheritance of abnormal movements in a single family.[2]

Prevalence studies have reported varying rates of dystonia dependent upon the study population and the type of dystonia identified. However, all values are believed to be underestimates owing to lack of recognition and under diagnosis. Rates of primary dystonia vary between 152 per million in Europe[3] and 330 per million in North America, the later estimating a total of 88,000 patients with primary focal dystonia throughout the USA.[4] Lower rates have been identified elsewhere, between 0.7 and 50 per million in an Italian study of patients greater than 50 years of age[5] and 30 per million in a Chinese cohort.[6] The highest reported rates are within the Ashkenazi Jewish population, estimated at 111 per million[7] and thought to be due to a founder mutation in the *DYT1* gene.[8]

Until the mid-1970's many believed that dystonia constituted a non-organic pathology, with patients presenting with what were considered 'bizarre' and variable symptoms, frequently exacerbated in situations of stress or anxiety. This resulted in a large proportion of individuals being given a diagnosis of 'hysteria' and prescribed

treatment with psychotherapy or surgical intervention more typical in the treatment of psychiatric disorders.[9]

1.3 Diagnosis

The diagnosis of dystonia is predominantly clinical, and although electromyogram (EMG) mapping can be used as an aid in determining antagonistic muscle co-activation and various phases of movement, there is no specific diagnostic test. The clinical features of dystonia are varied; they may be slow or rapid, involve flexion and/or extension and may be regular or irregular in frequency. However, they are repetitive, affect the same body part and with time abnormal postures may be seen at rest. Some dystonias are triggered by voluntary activity and may be termed 'action dystonias' while others occur in response to specific tasks, 'task-specific dystonias' e.g. playing a musical instrument or writing.

Dystonia may also be described and diagnosed dependent upon the body parts affected and their proximity to one another (Table 1.1).

Table 1.1: Terms used to describe dystonia based upon body part affected

Descriptive term	Body parts affected
Focal	One body part or region
Segmental	Two or more adjacent regions
Multifocal	Two or more nonadjacent regions
Generalised	Trunk, one or both legs and one other body part
Hemidystonia	Ipsilateral arm and leg

Clinical features helpful in determining a diagnosis of dystonia are *gestes antagonistes*, overflow dystonia and mirror movements. Gestes antagonistes are sensory tricks where light touch of a body region can help alleviate the muscle spasm of the dystonia. Overflow dystonic movement is the spread of the involuntary, unwanted muscle activity from the initial site to an adjacent body part. Finally, mirror movements occur when the unaffected side of the body performs a particular task e.g. finger tapping or writing, these same movements can then be seen to occur involuntarily on the opposite side of the body i.e. the side affected with dystonia.

Dystonia may be difficult to determine from spasticity especially in cases of childhood hypertonia. The Task Force on Childhood Movement Disorders attempted to aid in separating these two diagnoses by defining spasticity as requiring either increased resistance to externally imposed movement which increases with speed of stretch and varies with direction of joint movement and/or a resistance of externally imposed movement that rises rapidly above a threshold speed or joint angle.[10]

1.4 Classification of dystonia

The classification of different forms of dystonia remains a controversial area, revised on multiple occasions as understanding of the disorder has improved. Much of this ambiguity is due to 'dystonia' being used to describe multiple clinical entities: symptoms, signs, syndromes and specific diagnoses. Three different means of classification are in common use: age at onset, topography (Table1.1) and underlying aetiology.

Age at onset is usually divided into early (<26 years) and late (>26 years) onset. This age definition is used as most cases of dystonia caused by *DYT1* mutations are expected to have manifested symptoms by this age. Age at onset is also closely linked to topography with specific disease patterns being more common in certain age groups. Those who develop symptoms in childhood typically have a focal limb dystonia, often involving the lower limbs, which becomes generalized over time. Focal dystonia involving the cranio-cervical musculature is more common in those whose symptoms begin >26 years with less likelihood of generalization.

Fahn and Marsden classified dystonia into: primary dystonia, dystonia-plus syndromes, paroxysmal dystonias/dyskinesias and secondary dystonias.[11] This last group also includes the heredodegenerative dystonias, although some classification systems place them as a distinct group (Figure 1.1). Primary dystonias are those with no additional neurological abnormality (with the exception of tremor of the arms, head or neck), typically normal brain imaging and no known underlying aetiology other than a recognized genetic cause, e.g. Primary Torsion Dystonia (PTD). Dystonia-plus syndromes include those with additional movement disorders, for example myoclonus, while paroxysmal dystonias/dyskinesias involve intermittent

combinations of dystonia, chorea, ballism and athetosis. Secondary dystonias refer to those with an identifiable cause for the movement disorder e.g. structural lesion or drug-induced dystonia. The heredodegenerative dystonias are a more complex group involving neurodegeneration and additional pyramidal and extra-pyramidal neurological signs.

1.5 Primary Torsion Dystonia (PTD)

PTD totals approximately 75% of all dystonia cases and can vary from the childhood-onset generalized forms, which are usually genetic in aetiology (Table 1.2), to the adult onset focal dystonias.[12] Overall the focal dystonias are ten times more common than the generalized forms[13] and usually involve the upper limbs, head or neck. Focal dystonias very rarely become generalized but in approximately 30% of cases spread to an adjacent body region forming a segmental dystonia.[14] The different forms of focal dystonia exhibit some sex bias with craniocervical involvement more common in women and limb involvement more common in men.[15, 16] There may be a family history of focal dystonia, usually with an autosomal dominant pattern of inheritance and incomplete penetrance. Several loci have been mapped (DYT7, DYT13) and in some cases the causative gene identified (*DYT1* and *DYT6*). These are discussed in further detail below.

Cervical dystonia

This is the most common form of focal dystonia, typically involving the sternocleidomastoid, trapezius and posterior cervical muscles. Onset is usually in the fifth decade of life[3] presenting with neck stiffness, a pulling sensation and restricted movement. Symptoms typically progress over the initial five years and then become static, often resulting in abnormal postures, increased muscle tone and bulk, and on occasion development of a head tremor. Spontaneous remission is seen in approximately 20% of cases, although these will frequently relapse.[17]

Cranial dystonia

Cranial dystonia can involve the muscles of the eyelids, jaw, vocal cords, face, tongue, platysma and pharynx. Involvement of the orbicularis oculi muscles in the form of blepharospasm is the most common form, usually beginning between the fifth

and seventh decades of life and affecting women more commonly than men.[5] Initial symptoms include dry eyes and irritation typically evolving to include increased blinking, forced eye closure and difficulties with eye opening. Symptoms are often worse with bright lights, reading and driving, and may cause functional blindness with sustained eye closure.[18]

Oromandibular dystonia

This can involve the muscles of the mouth, tongue or neck leading to involuntary clenching and opening of the mandible as well as jaw deviation.[12] Symptoms are often worse with particular tasks e.g. eating and talking, causing dysarthria, dysphagia, difficulty chewing and temporomandibular joint dysfunction.

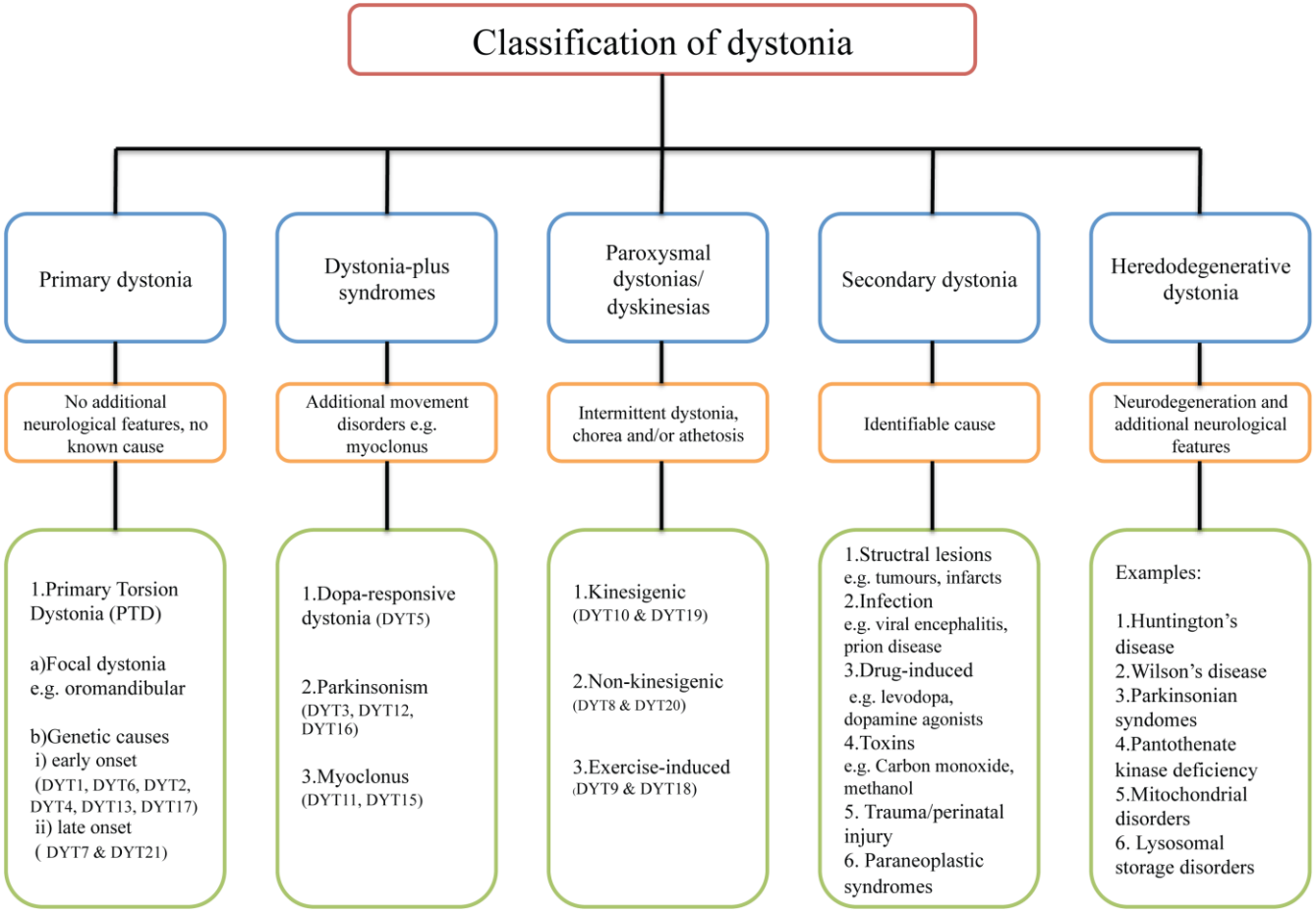
Laryngeal dystonia

Laryngeal dystonia is an action dystonia caused by abnormal abduction or adduction of the vocal cords usually triggered by speaking. Adductor dysphonia is the more common form, occurring in approximately 90% of cases and is caused by over contraction of the thyroarytenoid muscles. In this setting the voice becomes strained and strangled. Abductor dysphonia involves over activity of the posterior cricoarytenoid muscles causing separation of the vocal cords and an intermittent, breathy voice particularly exacerbated when pronouncing consonants. Tremor can also develop with both forms of dystonia.[19]

Limb dystonia

This is the least common adult-onset focal dystonia and predominantly affects the upper limbs. Onset is usually between 30 and 50 years of age and can involve abnormal flexion, extension and twisting postures. These forms of dystonia are frequently task-specific, particularly amongst people regularly performing highly practiced motor skills such as musicians and sportsmen.[20] The most common form of limb dystonia is writer's cramp, involving the hands and triggered by holding a pen and writing. Symptoms usually begin as cramp and an aching sensation in the fingers, progressing to an abnormal handgrip with pronation and ulnar deviation of the wrist. Tremor may also become a feature of this form of dystonia.[21]

Figure 1.1: Diagram of the current classification of dystonia



Adapted diagrammatic representation of Fahn and Marsden classification of dystonias[22]
Key: blue boxes: represent dystonia sub-types, orange boxes: describe typical clinical features, green boxes: examples of each subtype

1.6 Genetic forms of dystonia

Significant progress has been made over the past 25 years in identifying the genetic loci of several disparate forms of dystonia. Currently 20 monogenic dystonia loci have been identified[23-25] and the causative gene identified in ten of these (Table 1.2).

1.6.1 Primary dystonia

This group constitutes a broad spectrum of clinical disease ranging from the early-onset generalized to the adult-onset focal forms. Genetic loci have been identified in six of the early-onset forms (DYT1, DYT2, DYT4, DYT6, DYT13 and DYT17) and two late-onset forms (DYT7 and DYT21). Within this group only two genes have been identified, *DYT1*[26] and *DYT6*.[27]

DYT1

The clinical phenotype is consistent across ethnicities and is typically an early onset (mean age at onset 13 years and most cases <26 years)[26] focal limb dystonia. In the majority of cases symptoms progress to become multifocal or generalized with cranio-cervical involvement less common.[28-33]

Linkage analysis of a single large North American family identified a locus on chromosome 9 (9q34). Sequencing of genes within this region found a 3-bp deletion (guanine-adenine-guanine (GAG)) in the *TOR1A* gene (encoding torsinA), removing a single in-frame amino acid from the C-terminus of the protein. Inheritance is autosomal dominant with reduced penetrance of approximately 30%.[26] Two further missense mutations have been described in *TOR1A*, each in a single case.[34, 35]

TorsinA is a member of the AAA+ superfamily of ATPase proteins that function as molecular chaperones with a variety of cellular functions. The wild-type isoform is located within the endoplasmic reticulum (ER) but in its mutant form becomes associated with the nuclear envelope (NE).[36, 37] In addition wild-type torsinA has been shown to be involved in neurite extension,[36, 37] synaptic vesicle recycling, impaired dopamine release[38] and altered tyrosine hydroxylase activity.[39, 40]

DYT6

Typical clinical characteristics are of an early onset dystonia (mean age at onset 16 years), usually beginning in the upper limbs (50%) before progressing to involve the head or neck (25%). Lower limb involvement is rare. Symptoms later progress, becoming generalised or multifocal in the majority of cases. Laryngeal involvement is seen in more than two-thirds of all cases.[41]

Linkage analysis in three Amish-Mennonite families mapped to a locus on chromosome 8.[42-45] Subsequent studies identified mutations in the thanatos-associated protein domain-containing apoptosis-associated protein 1 (*THAP1*) gene, pathogenic mutations of which have a penetrance of approximately 60% independent of sex.[27, 46] The majority of mutations have been identified in European populations[27] but case reports have also identified a number in Chinese and Brazilian patients.[47]

THAP1 is a transcription factor with a conserved DNA binding domain at its N-terminus, a coiled-coil domain and a nuclear localization signal at its C-terminus. Its role within the brain is not known, however wild-type Thap1 protein has been shown to bind to the *TOR1A* promoter, suppressing its expression, this function being impaired with *THAP1* mutations.[48]

DYT2 and DYT4

Clinical presentation of DYT2 patients is of an early onset, focal limb dystonia with rapid generalization and autosomal recessive inheritance.[49-51] DYT4 was described in a single large Australian family with an autosomal dominant pattern of inheritance and characterized by whispering dysphonia.[52, 53] In neither case has a chromosomal locus or gene been identified.

DYT13 and DYT17

An Italian family with autosomal dominant PTD was used to map the DYT13 gene to chromosome 1p36.[54] Clinically the patients had segmental dystonia with prominent cranio-cervical involvement.[55] DYT17, located on chromosome 20, is of autosomal recessive inheritance and was first described in a consanguineous Lebanese family.

Initial symptoms were cervical, together with dysphonia and dysarthria, these later progressing to a segmental or generalized dystonia.[56]

DYT7 and DYT21

Both of these loci are characterized by later-onset symptoms and have each been described in single families. DYT7, located on the short arm of chromosome 18, was described in a German family with predominantly cervical dystonia and mean age at onset of 43 years (range: 28-70 years).[57] DYT21 was identified by linkage to chromosome 2q14.3-q21.3 in a Swedish family with predominantly cranial/cervical dystonia at onset, which then became multifocal or generalized.[58]

1.6.2 Dystonia-plus syndromes

These are a group of disorders with other neurological features in addition to dystonia, specifically Parkinsonism or myoclonus.

DYT3

Onset is typically in the mid-30s (range: 12-52 years) with a focal dystonia, which within the subsequent five years becomes multifocal or generalized. Approximately 50% of individuals develop features of Parkinsonism which can then become the predominant symptom.[59, 60] This disorder was initially identified on Panay Island in the Philippines,[61] linkage analysis identifying a locus on the X chromosome (Xq13.1).[62-64] A number of disease specific changes were later identified including a retrotransposon insertion in an intron of the TATA-binding protein-associated factor (*TAF1*) gene. This appears to reduce neuron-specific expression of *TAF1* as well as that of the dopamine receptor D2 (*DRD2*) gene in the caudate nucleus, suggesting a likely role in pathogenicity.[62-64]

DYT5

A childhood onset limb dystonia, with symptoms that progressively worsen throughout the day, improve with sleep and show significant improvement with levodopa therapy. Additional clinical features may include: oromandibular dystonia,[65] spasticity,[66] scoliosis,[67] psychiatric abnormalities,[68] generalized hypotonia and proximal weakness.[69] In the majority of cases symptoms are caused by autosomal

dominant inheritance of mutations in the GTP cyclohydrolase 1 (*GCH1*) gene located on chromosome 14 (14q13), referred to as DYT5a or Segawa's Disease.[70] This enzyme is the rate-limiting step in the synthesis of tetrahydrobiopterin, an essential cofactor for tyrosine hydroxylase, needed to synthesise dopamine. Less common are autosomal recessively inherited mutations in the other enzymes involved in the dopamine synthesis pathway: tyrosine hydroxylase (DYT5b),[71] 6-pyruvoyltetrahydropterin synthase[72] and sepiapterin reductase.[73]

DYT12

Also known as rapid-onset-dystonia-parkinsonism, symptoms usually begin during teenage years or early 20s. Onset of the dystonia is sudden (hours to weeks) followed by the development of parkinsonian features. The dystonia develops in a rostro-caudal pattern (face-arms-legs), typically with significant bulbar involvement. An emotional stressor e.g. fever, childbirth, alcohol binge, usually precedes symptom onset.[74] Inheritance is autosomal dominant with reduced penetrance, mutations of the Na^+, K^+-ATPase alpha 3 subunit gene (*ATP1A3*) on chromosome 19 are thought to be responsible.[75, 76] The function of the Na^+, K^+-ATPase is to maintain an electrochemical gradient across the plasma membrane, *ATP1A3* mutations are believed to reduce it's catalytic activity by several mechanisms including reduced Na^+ affinity.[77, 78]

DYT16

An autosomal recessive form of dystonia-parkinsonism identified in two consanguineous Brazilian families. Symptoms are characterized by early onset (2-8 years) limb dystonia progressing to a more generalized form. There is typically pronounced bulbar involvement, including dysarthria, spasmodic dysphonia and dysphagia. A missense mutation in the protein kinase, interferon-inducible double-stranded RNA-dependent activator (*PRKRA*) gene on chromosome 2 was identified in all affected individuals. The exact role of this protein is unknown but it is believed to be involved in the cell stress response.[79]

DYT11 & DYT15

These are discussed in detail in Section 1.8

1.6.3 Paroxysmal Dystonias/Dyskinesias

This is a group of disorders characterized by sudden, brief attacks of involuntary movements. They may be subdivided into kinesigenic (DYT10 & DYT19), non-kinesigenic (DYT8 and DYT20) and exercise-induced (DYT9 and DYT18) paroxysmal dyskinesias.

DYT8

Known as Paroxysmal Non-Kinesigenic Dyskinesia 1 (PNKD1), this disorder is characterized by intermittent episodes of dystonia, chorea, ballismus or athetosis often triggered by alcohol or caffeine that first become evident during childhood or adolescence. Episodes can last minutes to hours and at a frequency ranging from daily to annually.[80] Inheritance is autosomal dominant with linkage identifying a locus on chromosome 2 (2q33-36). Missense mutations have been identified in the myofibrillogenesis regulator gene (*MR-1*), the product of which is believed to be involved in the breakdown of oxidative stress by-products caused by alcohol and caffeine consumption.[81, 82] More recent studies have shown that a mitochondrial targeting sequence, found in the N-terminus of the protein, is removed in the mature protein. All missense mutations have been found in this region, suggesting that a toxic gain-of-function role may be responsible for the pathogenesis.[83]

DYT9

Identified in a single large German family linked to chromosome 1p21-p13.3, symptoms include episodes of limb dystonia, dysarthria, diplopia, paroxysmal choreoathetosis, ataxia and dyskinesia. These events may be precipitated by alcohol, emotional stress and physical exercise, lasting up to 20 minutes at a time.[84]

DYT10

Inheritance is autosomal dominant and with onset of symptoms in childhood or adolescence. Attacks are short and frequent (up to 100 per day), characterized by dystonic or choreiform movements triggered by sudden movements.[85] Linkage in 13 families has identified a locus on chromosome 16p11.2-q12.1.[86] More recent whole exome sequencing identified mutations in the proline-rich transmembrane protein 2 (*PRRT2*) that co-segregated with the disorder in affected families. Little is known of

the function of this protein although it is highly expressed in the developing nervous system, localises to axons, interacts with synaptic protein SNAP25 and may play a role in synaptic regulations.[87] Truncating mutations are thought to alter its subcellular localization.[87]

DYT18

A childhood onset exercise-induced dyskinesia involving dystonia, choreoathetosis and ballism, lasting minutes to hours.[88] Mutations have been identified in the *SLC2A1* gene (chromosome 1p35-p31) encoding the glucose transporter 1 protein (GLUT1), the predominant glucose transporter in the brain.[89, 90] Other clinical characteristics include epilepsy, haemolytic anaemia, migraine and developmental delay.

DYT19

Mapped in a single large Indian family, this locus on chromosome 16 (16q13-q22.1) overlaps with that of DYT10 and is similar in phenotype: short, frequent attacks of dystonia or chorea in response to sudden movement.[85, 91]

DYT20

The second of the paroxysmal non-kinesigenic dyskinesias, this locus was mapped to chromosome 2q31 in a single large Canadian family, just proximal to the DYT8 locus. No mutations were identified in the *MR-1* gene and hence a second locus has been assigned.[92]

Table 1.2: The monogenetic dystonias

Dystonia subgroup	Other names	MIM Number	Locus	Chromosome	Inheritance	Gene	Mutation	Penetrance
Primary dystonia								
Dystonia 1	Primary torsion dystonia, idiopathic torsion dystonia, Oppenheim dystonia, TOR1A	128100	DYT1	9q34	Autosomal dominant	*TOR1A*	GAG deletion	30-40%
Dystonia 2	Autosomal recessive primary torsion dystonia	224500	DYT2	Unknown	Autosomal recessive	Unknown	Unknown	Unknown
Dystonia 4	Non-DYT1 primary torsion dystonia	128101	DYT4	Unknown	Autosomal dominant	Unknown	Unknown	40% of patient's offspiring over 40 years affected
Dystonia 6	Adult onset primary torsion dystonia of mixed type	602629	DYT6	8p21-8p22	Autosomal dominant	*THAP1*	Nonsense, missense and frameshift mutations identified. Concentrated in the THAP domain	30%
Dystonia 7	Adult-onset focal primary torsion dystonia	602124	DYT7	8p11.3	Autosomal dominant	Unknown	Unknown	Incomplete (<40%)
Dystonia 13	Focal dystonia with cranio-cervical features	607671	DYT13	1p36.13-1p36.32	Autosomal dominant	Unknown	Unknown	58%
Dystonia 17	Autosomal recessive torsion dystonia (may overlap with dystonia 2)	612406	DYT17	20p11.22-20q13.12	Autosomal recessive	Unknown	Unknown	Unknown
Dystonia-plus syndromes								
Dystonia 3	X-linked dystonia parkinsonism, 'lubag'	314250	DYT3	Xq13.1	X-linked	*TAF1*	Retrotransposon insertion in intron of *TAF1*	100% by 5th decade
Dystonia 5a	Dopa-responsive dystonia, Segawa syndrome, hereditary progressive dystonia with marked diurnal fluctuation	128230	GCH1	14q22.1-14q22.2	Autosomal dominant	*GCH1*	Multiple types, >60 different mutations reported to date	30%

Dystonia 5b	Tyrosine Hydroxylase Deficiency, DYT14	191290	TH	11p15.5	Autosomal recessive	*TH*	Predominantly homozygous and compound heterozygous missense and frameshift mutations. >20 different mutations identified	Unknwon
Dystonia 11	Myoclonus dystonia; alcohol-responsive dystonia	159900	DYT11	7q21-7q31	Autosomal dominant	*SGCE*	Multiple types (>50 different mutations identified to date)	Incomplete due to maternal imprinting
Dystonia 12	Rapid-onset dystonia-parkinsonism	128235	DYT12	19q13	Autosomal dominant	*ATP1A3*	11 different mutations in 19 families, mostly heterozygous missense mutations	Incomplete
Dystonia 15	Myoclonus dystonia	607488	DYT15	18p11	Autosomal dominant	Unknown	Unknown	Incomplete
Dystonia 16	Autosomal recessive dystonia parkinsonism	612067	DYT16	2q31.3	Autosomal recessive	*PRKRA*	Homozygous 665C to T transition in exon 7 causing pro222-to-leu (P222L) substitution	
Paroxysmal dystonia/dyskinesia								
Dystonia 8	Paroxysmal dystonic choreoathetosis, paroxysmal nonkinesigenic dyskinesia, Mount-Reback syndrome	118800	DYT8	2q33-2q36	Autosomal dominant	*MR-1*	Missense mutations in mitochondrial targeting sequence	Incomplete
Dystonia 9	Paroxysmal choreoathetosis with episodic ataxia and spasticity; choreoathetosis, spasticity and episodic ataxia	601042	DYT9 (also CSE)	1p13.3-1p21	Autosomal dominant	Unknown	Unknown	Unknown

Dystonia 10	Primary kinesigenic choreoathetosis; paroxysmal kinesigenic dyskinesias; periodic dystonia	128200	DYT10	16p11.2-16p12.1	Autosomal dominant	*PRRT2*	Four truncating mutations identified to date	Incomplete
Dystonia 18	Paroxysmal exercise-induced dyskinesia (PED); paroxysmal exertion-induced dyskinesia	612126	DYT18	1p31.3-p35	Autosomal dominant	*SLC2A1*	Missense, nonsense, frameshift and splice-site mutations identified	Reduced
Dystonia 19	Paroxysmal kinesigenic dyskinesia 2 (PKD2)	611031	DYT19	16q13-q22.1	Unknown	Unknown	Unknown	Unknown
Dystonia 20	Paroxysmal non-kinesigenic dyskinesia 2 (PNKD2)	611147	DYT20	2q31	Unknown	Unknown	Unknown	Unknown

1.7 Pathophysiology of dystonia

The pathophysiological mechanisms that give rise to dystonia are thought to centre upon disruption of the direct putamen-pallidal pathway in the basal ganglia (Figure 1.2). Three main models have been proposed, the first described by Berardelli *et al.* suggests that reduced activity of the inhibitory systems in the motor cortex, brainstem and spinal cord is the predominant cause of recruitment of unwanted motor units and muscle co-contraction. This model also supports hyperactivity of the direct striato-pallidal pathway causing disruption to the thalamo-cortical loops.

The second model is based upon the hypothesis that in normal basal ganglia circuitry the striatum provides context dependent inhibition of the globus pallidus internus (GP_i) with excitation from the subthalamic nucleus (STN). In dystonia it is proposed that there is over activity of the GABAergic pathway from the striatum to the GP_i, causing excessive GP_i inhibition with subsequent reduced inhibition to larger cortical areas resulting in co-contraction and overflow to adjacent muscle groups.

The final model involves decreased GABAergic transmission of the indirect pathway from the striatum to the globus pallidus externus (GP_e), causing GP_e overactivity and excessive inhibition of the STN. This causes a deficit in surround activation of GP_i causing loss of inhibition of the competing motor pattern in the thalamo-cortical pathway.

A number of research techniques have been employed to try and improve our understanding of these models and how disruption of their normal activity can contribute to pathogenesis. These will be discussed in further detail below.

1.7.1 Neurophysiology

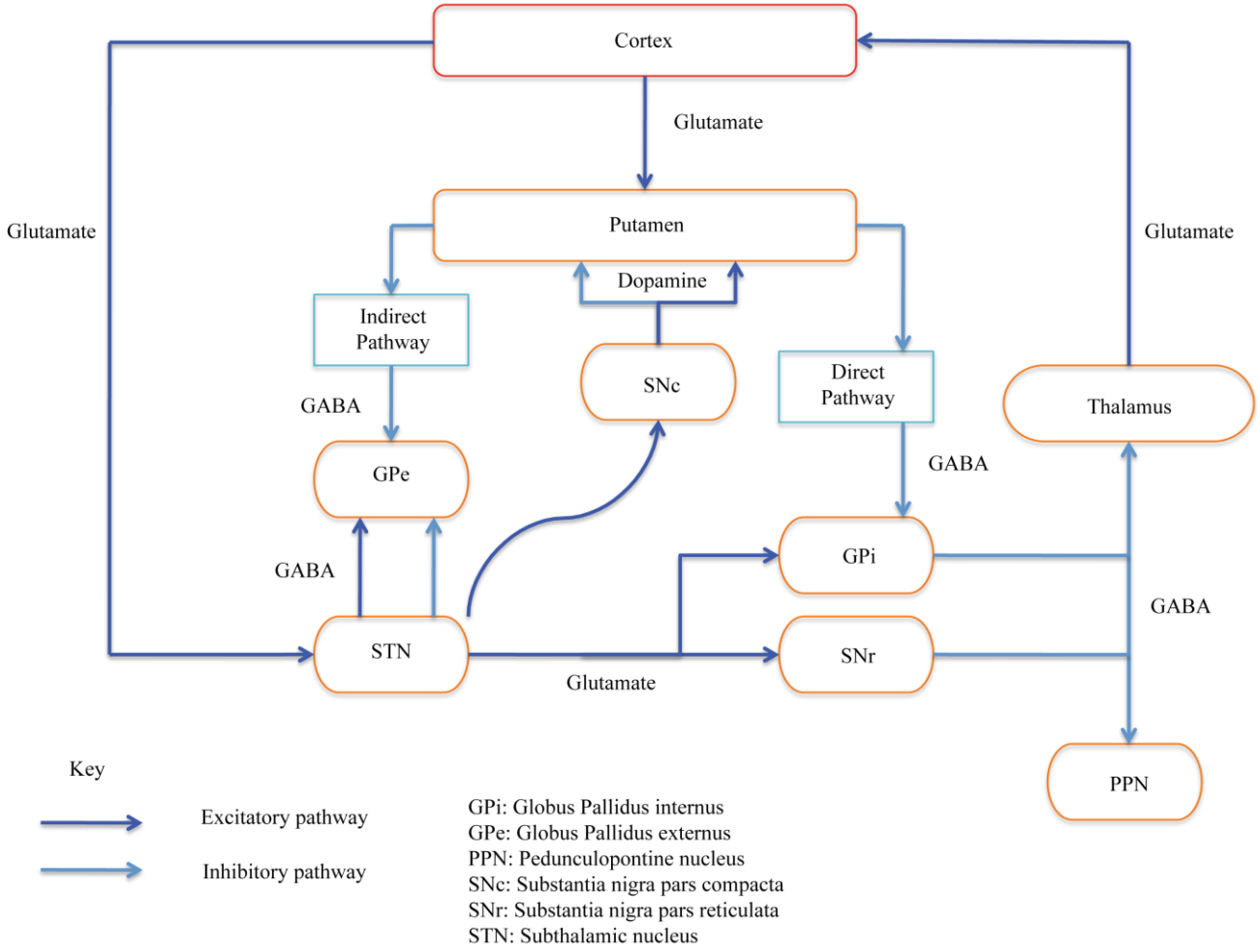
Neurophysiological studies have identified three main areas of abnormality that are believed to contribute to the pathogenesis of dystonia: impaired inhibition and impaired surround inhibition, increased or maladaptive neural plasticity and sensory processing dysfunction.

Impaired inhibition

During normal movement, neuronal activity stimulates the wanted movement while inhibiting unwanted movement. With dystonia it is believed that there is loss of inhibition at multiple levels within the nervous system (spinal cord, brain stem and cortex) causing stimulation of this unwanted, additional movement. This reduction in reciprocal inhibition between antagonistic muscle groups has been identified at the level of the spinal cord during arm movements in patients with writer's cramp, generalized dystonia and cervical dystonia compared to controls.[93] Similar abnormalities have been identified in the blink reflex recovery cycle in blepharospasm.[94]

Transcranial magnetic stimulation (TMS) is a non-invasive technique used to stimulate a restricted part of the cortex and has been used to measure cortical inhibition. This process works by generating a current perpendicular to the stimulating coil, which in turn generates an electric field, affecting the membrane potential of nearby neurons. This has allowed measurement of both short and long intracortical inhibition as well as the length of the cortical silent period. Assessment of both affected and unaffected hands of those with writer's cramp and blepharospasm has shown loss of both short and long intracortical inhibition.[95] In comparison with healthy controls, a decreased silent period was observed in the affected muscles of those with cervical and hand dystonia.[96] Loss of surround inhibition has also been identified in patients with writer's cramp by measuring the amplitude of motor evoked potentials of surrounding antagonistic muscles.[97]

Figure 1.2: Diagram representing proposed basal ganglia circuitry under normal conditions



Maladaptive neural plasticity

Neuronal plasticity is an essential component of the nervous system's ability to adapt and learn from its surrounding environment. This process may become deranged either due to intrinsic abnormalities of this process or destruction of these processes over time due to excess stimulation. Some of the first evidence for altered neural plasticity in dystonia came from the Byl monkey model of task-specific hand dystonia.[98] This showed dedifferentiated hand representation in the somatosensory cortex and overlapping representation of individual digits. Breakdown of the normal homuncular pattern is also seen in people with focal hand dystonia[99] as well as musicians. In this latter group there appears to be a larger represented proprioceptive region in the hand motor cortex, which is larger again in musicians with dystonia compared to unaffected musicians.[100] One explanation is that with excessive use there is repeated afferent input, which together with maladaptive cortical plasticity result in the generation of dystonic movements. Impaired plasticity can also be measured using paired association stimulation (PAS) and TMS, these tools being used as indirect measures of long-term potentiation and depression. In cases of focal dystonia, PAS showed an enhanced effect consistent with increased plasticity and loss of spatial specificity.[101, 102]

Sensory processing dysfunction

Despite no overt clinical sensory pathology in dystonia patients, the sensory system does appear to be involved e.g. gestes antagonistes, dystonias seen with chronic regional pain syndromes and disturbances to the sensory homunculus. Abnormalities have also been reported in spatial and temporal sensory discrimination patterns of those with dystonia compared to controls[103] and that the degree of these abnormalities is related to the severity of the dystonia.[104] These abnormalities are not however exclusively seen in those affected with dystonia but also in non-manifesting DYT1 mutations carriers[105] suggesting that this may form a dystonia endophenotype.

1.7.2 Neuroimaging

Several imaging modalities have been used in an attempt to determine structural and functional abnormalities in patients with dystonia, these include magnetic resonance imaging (MRI), positron emission tomography (PET), functional MRI (fMRI) and diffusion tensor imaging (DTI). Examples of these modalities can be seen in Figure 1.3.

Lesion studies

Lesions of the spinal cord, basal ganglia, brainstem, cerebellum, thalamus and parietal cortex have been associated with varying forms of dystonia, earlier studies suggesting that the type of dystonia may be related to the location of the lesion.[106] Spinal cord lesions, predominantly those involving the cervical cord, are usually associated with cervical dystonia. Suggested mechanisms include disruption of either sensory feedback from the cervical musculature to the brain or of motor impulses from the brain to the neck.[107] Interpretation of the impact of specific lesions within the brainstem is difficult as they often span large areas impacting upon a number of different pathways and potentially reducing neural input to the basal ganglia.[108, 109] Cerebellar lesions are associated with a number of different forms of dystonia; hemidystonia and craniofacial dystonia, the dystonia frequently resolving after surgical resection of the lesion (Section 1.6.4).[110] Location of thalamic lesions appear to have an impact upon the type of dystonia, twisting/writhing dystonias being seen with disruption to the striatopallidal circuits and involvement of the cerebellar circuitry causing more tremulous, jerky dystonias.[111, 112]

Quantitative volumetric studies

These forms of investigation look for apparent size differential in various brain regions comparing those with dystonia to controls. Imaging of the basal ganglia found significantly larger putamen amongst those with cranial or focal hand dystonia compared to unaffected controls.[113] Voxel based morphometry, a method looking for structural differences in local tissues rather than global abnormalities, has found consistent differences in the basal ganglia, cortex, thalamus and cerebellum of dystonia patients.[114-116]

Positron emission tomography (PET)

PET studies are used to look for changes, either in local metabolism using a [^{18}F]-fluorodeoxyglucose tracer (believed to be a marker of regional neural activity) or regional blood flow with [^{15}O] H_2O. *DYT1* and sporadic dystonias were found to have increased metabolic activity in the basal ganglia, cerebellum and supplementary motor area (SMA).[117, 118] Carbon and Eidelberg[119] compared cohorts with *DYT1* and *DYT6* mutations, the former with increased activity in the globus pallidus, cerebellum and SMA while the *DYT6* cohort showed decreased metabolism in the putamen, cerebellum and upper brainstem and increased activity in the temporal cortex. These results suggest that although different dystonia subtypes may be associated with different metabolic patterns both affected *DYT1* and *DYT6* mutation carriers had relative increases in activity in the pre-SMA and parietal association regions, suggesting some area of overlap.[120] Variation in regional blood flow involving primary motor and sensory cortices, motor planning regions of the frontal cortex, posterior parietal and temporal lobes have also been reported in several forms of dystonia.[121]

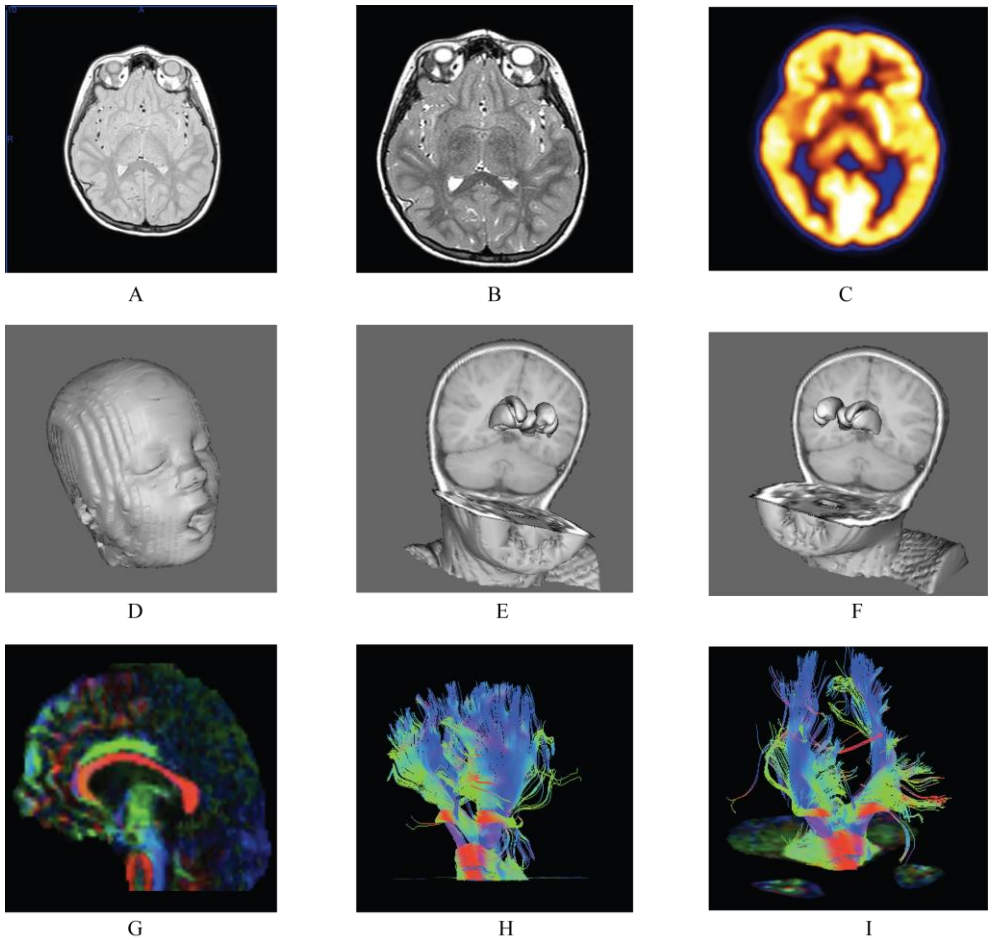
Functional magnetic resonance imaging (fMRI)

Using a contrast that is dependent upon blood oxygen levels, fMRI is also able to look at regional metabolic activity. Regions highlighted with this scanning technique include the basal ganglia, cerebral cortex and cerebellum. However these differences in metabolic activity were found to be dependent upon the type of dystonia and the task being performed.[122, 123]

Diffusion tensor imaging (DTI) and tractography

Diffusion tensor imaging (DTI) is used to measure directionality of water molecule movement in the brain. This is also referred to as anisotropy, is measured as fractional anisotropy (FA) and thought to reflect microstructural white matter changes e.g. number of axons or myelination. A study of both manifesting and non-manifesting *DYT1* and *DYT6* mutation carriers found abnormalities in the cerebellothalamic tracts. However, non-manifesting mutation carriers had an additional more distal thalamocortical tract disruption suggesting that clinical penetrance of a mutation may relate to the number and location of pathway disruptions.[124, 125]

Figure 1.3: Examples of imaging studies in dystonia



Examples taken from a single patient with cervical dystonia. Image A: MRI Proton Density weighted image (useful for grey/white differentiation and demarcating pallidal boundaries), B: T2 weighted MRI, C: resting state PET scan (no evidence of abnormal uptake), D: three dimensional reconstruction from T1 weighted MRI, E & F: cut-away images showing segmented thalami, pallidi, putamen and caudates, G: colour coding of diffusion tensor image (DTI), H & I: DTI tractography results showing radiations through the cerebral peduncles.
Images courtesy of Dr Daniel Lumsden, Evelina Chidren's Hospital, London

1.7.3 Neuropathology

A number of case reports and case series have reported the co-morbidity of posterior fossa masses and cervical dystonia, implying a functional link between disruption to the cerebellum and the development of dystonia. Support for this theory is seen in a small number of cases where resection of the mass has led to resolution of the dystonia. The mechanism by which this process is likely to occur is not clearly understood however, it has been postulated that the masses may have a direct impact upon the cerebellum and this in turn is a generator of dystonic symptoms. Alternatively dystonic symptoms may arise due to disruption of one or more of the tracts communicating with the cerebellum.[110, 126]

1.7.4 Neurosurgery

Significant developments have been made in the use of neurosurgical techniques in the treatment of movement disorders (Section 1.7.2). Several brain regions have been used as targets including the basal ganglia, thalamus, cerebellum and interstitial nucleus of Cajal (INC). Deep brain stimulation (DBS) is widely used in the treatment of movement disorders, the GPi of the basal ganglia being an effective target for several forms of dystonia. Other forms of dystonia respond better to stimulation of the thalamus and thalamotomies, now less common, are still used in certain clinical settings.[127, 128] The post-operative improvement seen with these interventions is not usually immediate and may not be seen until months or years later, suggesting that some degree of neural reorganization is required to bring about this improvement. Imaging studies have suggested that this reorganization may not be limited to the immediate area around the operative site, with changes seen in both cortex and cerebellum following pallidal DBS.[129, 130]

1.7.5 Animal models

Animal models have been used to investigate both *in vivo* and pathological elements of the dystonic model. Focal techniques such as ablation or microinjection of

stimulants or inhibitors to the cortex, basal ganglia, cerebellum and thalamus have all been able to produce movements akin to dystonia in the animals.

Several specific models have been generated, firstly the dystonic (dt) rat, exhibits movements that are both clinically and electrophysiologically consistent with dystonia. The mutation is in the *Atcay* gene, which encodes caytaxin, expressed at high levels throughout the cerebellum. Surgical removal of the cerebellum in these rats results in resolution of the dystonia, but its replacement with ataxia.[131, 132] The second animal model, the tottering mutant mouse has symptoms of paroxysmal dystonia.[133, 134] Here the mutation is in the *Cacna1a* gene, encoding a subunit of the P/Q-type calcium channel that is widely expressed throughout the brain. Mapping the time course of brain activation during a dystonic episode showed early activation of the cerebellar Purkinje neurons, followed by the red nucleus, thalamus and cortex.[135] Removal of the cerebellum stopped these attacks as did selective removal of the Purkinje neurons by cross breading with another mouse strain in which these neurons degrade.[136, 137] These data suggest that, in this model at least, the dystonic movements are generated by the cerebellum and are caused by increased rather than decreased neuronal activity.

Lesional and pharmacological manipulation studies in primate models have also suggested a role for several brain regions in the generation of dystonia. In the Rhesus monkey, lesions to the Red nucleus caused symptomatic torticollis,[138] while ventromedial tegmentum,[139] midbrain[140] and dorsomedial mesencephalic tegmentum disruption produced similar results in the Macaque model.[141] Interestingly, vestibular lesions produce a peculiar pattern of decreased severity upon ascending the phylogenetic scale with chimpanzees exhibiting only transient symptoms of torticollis.[142]

1.8 Treatment

Treatment of dystonia is broadly divided into medical and surgical therapies. Medical treatment can be further divided into oral therapies, botulinum toxin (BT) and intrathecal baclofen, while surgical interventions are either peripheral or central. Overall efficacy of some treatments is poor either due to minimal response or

development of side effects. Response may also vary between different forms of dystonia. Current management is to begin treatment with medical therapies and only in those who fail to respond or show a poor response is surgical intervention considered.

1.8.1 Medical therapies

Oral therapies

Current mainline treatments include anticholinergics (e.g. trihexyphenidyl), levodopa, benzodiazepines (e.g. clonazepam) and baclofen. Levodopa is used predominantly in the treatment of dopa-responsive dystonia where a dose of up to 300mg per day of carbidopa/levodopa should be tried before determining a response.[143, 144] Whether other forms of dystonia are responsive to levodopa remains uncertain with responses reported in a few *DYT1* cases and several forms of secondary dystonia.[145, 146] Trihexyphenidyl was the first drug to be found to be effective in the treatment of primary generalized dystonia and is generally used as a first line therapy in non-dopamine responsive forms, with approximately 40-50% of patients showing a moderate response.[147]

Botulinum Toxin

BT serotypes A and B may be injected locally into dystonic muscles, inhibiting local release of acetylcholine into the neuromuscular junction and allowing a decrease in localized muscle spasm with no systemic effects. BT may be used to treat most forms of focal dystonia; cervical, blepharospasm, laryngeal, oromandibular and limb dystonias. It may also be used as a focal treatment in more generalized or task-specific dystonias.[148] Principal side effects are associated with localized muscle weakness, not always predicted by site of injection or volume of toxin used.

Baclofen

Baclofen may be used intra-thecally in those unresponsive to oral therapy being most effective in those with secondary dystonia associated with pain or spasticity.[149] Intraventricular treatment into the third ventricle has been trialed recently in a small cohort of secondary dystonia patients, reporting an improvement in 80%.[150]

1.8.2 Surgical interventions

Peripheral denervation

Selective peripheral denervation of dystonic musculature is occasionally used to treat forms of focal dystonia such as cervical dystonia and blepharospasm.[151, 152] Although overall benefit from this form of intervention may be variable, some studies have suggested that this is an effective form of treatment in secondary, not primary, dystonias resistant to BTX therapy.[153] The major limitation with this form of surgery is the risk of re-innervation post-procedure, accompanied by pain and in the case of cervical dystonia, dysphagia.[154]

Lesional surgery

Until the development of DBS in the mid-1990s, stereotactic lesioning was the most common form of surgical intervention in patients with dystonia. Bilateral thalamic lesions (Section 1.6.4) were the most common form of intervention, providing significant symptomatic improvement, although associated with not infrequent complications, usually in the forms of further neurological disability, most typically dysarthria. The finding that pallidotomies were an effective target in the treatment of dystonia in Parkinson's disease resulted in more recent surgical intervention being targeted towards this region, and in particular the GP_i.[155]

Deep Brain Stimulation

DBS, initially used in the treatment of Parkinson's disease and tremor, has been used increasingly over the last 10 years as a treatment for dystonia (Section 1.6.4). Several studies have shown stimulation of the GPi to provide significant improvement such that it is now the preferred target in this form of therapy.[156, 157] The greatest improvement is seen amongst those with *DYT1* mutations[158] and is also not restricted to the immediate post-operative period but may continue up to a decade later.[159] Response to this form of treatment varies significantly between different forms of primary and secondary dystonia.[160-162] Those that are younger in age, have a shorter disease duration, lower pre-operative severity scores, *DYT1* mutations and lack fixed skeletal deformities tend towards a better outcome.[163-165]

1.9 Common themes in dystonia

The discovery of genes responsible for different dystonic syndromes has not only allowed a refinement of specific disease phenotypes but also enabled observation of common features across different forms of dystonia.

1.9.1 Inheritance

The majority of the DYT genes follow a Mendelian autosomal dominant pattern of inheritance, although reduced penetrance is observed in a number of cases suggesting additional genetic or environmental factors may contribute towards the expression of the dystonic phenotype. *DYT1* (GAG deletion) has an approximately 30% penetrance in familial studies which is thought to be influenced by coding variant D216H. Haplotype analysis found the H allele in *trans* with the GAG deletion to be highly protective against the development of dystonia with the suggestion that *cis* D216 is required for disease penetrance.[166] Similarly in those inheriting *DYT6* mutations only approximately 60% of those manifest symptoms,[27] although the mechanism here is less clearly understood. *DYT11*, discussed below, shows reduced penetrance owing to maternal imprinting (Section 1.9.2.2).

1.9.2 Environmental factors

Epidemiological studies have identified a small number of associations between exogenous factors and the genetically defined dystonias. In carriers of the *DYT1* mutation onset of motor symptoms has been associated with recent or concurrent injury, hypoxia and viral infections.[167, 168] Rapid onset dystonia-parkinsonism (DYT12) is often triggered by an emotional or physical stressor,[169] and alcohol or caffeine ingestion is frequently linked to the paroxysmal dystonia and chorea of DYT8.[82]

1.9.3 Altered neurochemical transmission

Dopamine and cholinergic neurotransmission have been implicated repeatedly in dystonia, not least due to the varying response to medical therapies (Section 1.7.1). The protein product of *TOR1A* (*DYT1*), torsinA is expressed at high levels in

dopaminergic neurons[170] although affected patients with this mutation show little improvement with L-dopa therapy, responding instead to treatment with anti-cholinergic agents. Treatment of dopa-responsive dystonia (DYT5) with L-dopa results in dramatic improvement to the motor symptoms although some change is also observed with anti-cholinergic agents. DYT3 patients have clinical evidence of Parkinsonism and postmortem histopathology shows evidence of degeneration of striatal neurons that receive dopaminergic input.[171] In a similar context treatment of adult-onset idiopathic Parkinson's disease with L-dopa therapy can result in significant dystonic posturing both at onset and weaning of treatment.[172] Collectively these findings suggest that dopamine levels within the basal ganglia play a critical role with either too little of too much dopamine in susceptible individuals resulting in dystonic symptoms.

1.9.4 Psychiatric disorders

Longstanding anecdotal evidence has suggested an increased rate of psychiatric symptoms across the broad spectrum of dystonias, not least with the long held assumption that dystonia was a non-organic disorder. Prior to the advent of genetic testing, large studies of mixed groups of dystonias found on overall increased rate of psychiatric symptoms when compared to both healthy and hemifacial spasm control groups.[173] Some found anxiety-related symptoms to be most common while others noted an increased rate of depression amongst those with cervical dystonia and blepharospasm.[174]

Using standardised diagnostic methods, Wenzel et al noted an increased psychiatric burden amongst patients with spasmodic torticollis, noting agoraphobia and panic disorder to be the most common and with over half reporting onset of their psychiatric symptoms prior to their movement disorder.[175] Other studies have suggested an overall increased rate of social phobia[176] and elevated levels of depression and anxiety when compared to a matched chronically disabled control group.[177]

Discovery of the *DYT1* mutation allowed studies of genetically defined cohorts. Dividing participants according to genetic and motor disorder status found an increased rate of major depressive disorder amongst manifesting carriers that was

independent of motor symptom severity and tended to occur at a younger age than in unaffected individuals.[178] Others have also reported elevated rates of anxiety disorders with almost a fifth of the cohort reporting onset of psychiatric symptoms prior to any motor manifestations.[179]

Despite not being recognized as a secondary feature of chronic disease, elevated rates of Obsessive Compulsive disorder (OCD) have also been observed amongst a number of dystonia cohorts. A family study of idiopathic focal dystonias found almost 20% to meet the DSM-IV diagnostic criteria for OCD and a 13.8% increased morbidity risk in unaffected family members.[180] Similar findings were also observed when comparing patients with blepharospasm to those with hemifacial spasm.[181] However, use of a genetically defined *DYT1* cohort found similar rates to controls[182] suggesting that unlike depressive and anxiety related symptoms, OCD may relate to specific forms of dystonia.

1.10 Myoclonus Dystonia Syndrome

1.10.1 Prevalence and clinical description

Myoclonus Dystonia Syndrome (MDS) is a childhood onset hyperkinetic movement disorder typically characterized by myoclonus of the trunk and upper limbs together with dystonia of the neck and/or hands (writer's cramp).[1, 183] Age at onset of motor symptoms is usually under 20 years of age, with girls typically affected at a younger age than boys (median age at onset 5 years vs. 8 years).[184] Clinical features appear to be consistent across ethnicities.[185-190] Several case reports and case series have also suggested the presence of co-morbid psychiatric pathology, including OCD, alcohol excess, depression, anxiety, panic attacks.[191, 192]

The myoclonic jerks are usually the predominant and most disabling feature, typically seen in the upper body[193] and are stimulus-insensitive. They are often precipitated with posture or action and exacerbated by an external stressor. Other patterns of involvement have been reported, including the lower limbs,[185, 186, 194, 195] face and larynx.[186, 196] The myoclonic jerks are alcohol-responsive in the majority of cases, allowing patients to 'self-medicate'. Dystonia is typically observed in the neck and

hands causing pain and impairing endurance and performance of fine motor tasks. Case reports have also reported evidence of dystonia in the lower limbs, spine and larynx.[185, 197]

The true prevalence of MDS is still unknown. It is often referred to as a rare disorder and therefore considered to occur at a rate of less than 1 in 200,000 within the population. Others have suggested that a rate of 1 in 100,000 population for essential myoclonus may be more accurate.[198] Overall it is believed that estimates for MDS, as with most dystonic disorders, are lower than actual population values owing to under reporting, poor recognition, misdiagnosis and failure to seek medical attention. Difficulties also exist in the nomenclature and phenomenology used by adult and paediatric movement disorder communities.[10, 199] In defining a hyperkinetic movement disorder decisions are often made as to whether they are discrete, rhythmic, random or repetitive.[200] However unlike adult forms individual, discrete types of movement disorder are both rare and difficult to discern in children.[201]

1.10.2 Genetic aetiology

Multiple attempts during the late 20th century were made to identify the gene or genes responsible for MDS. Initial exhaustive genomic screens, each in single large families, led to exclusion of major parts of the genome.[202, 203] A candidate gene approach including sequencing of *DYT1* (chromosome 9), various subunits of the $GABA_A$ receptor (on chromosomes 4, 5, 6 and 15) and the alpha-subunit of the glycine receptor (chromosome 5) was also unsuccessful.[204, 205]

1.10.2.1 Linkage studies

The first breakthrough came in 1999 when Klein et al identified linkage to a 23cM region on chromosome 11q23. The D2 dopamine receptor (*DRD2*) gene was one of the genes within this region and sequencing of this found a heterozygous Val154Ile polymorphism in a highly conserved region of exon 3 that co-segregated with the disorder.[206] This polymorphism was not identified in the remaining unaffected family members or in 250 control DNA samples. However, attempts to replicate these findings in larger cohorts of both familial and sporadic cases were unsuccessful.[207,208]

At a similar time two-point and multipoint linkage analysis using an autosomal dominant model of inheritance in a single family found linkage to the 7q21 region with a peak pairwise LOD score of 3.91 and a flat plateau at 3.9 multipoint analysis. The glutamate receptor type 3 gene (*GRM3*) was considered a candidate gene within this region, but no mutations were identified upon direct sequencing.[209] Linkage analysis in eight families enabled refinement of this region to a 14cM interval,[210] while a second study using microsatellite markers in four families reduced this to 7.2cM.[211] A bacterial artificial chromosome clone reduced the critical region to 3.2Mb, within which there were 15 genes (14 known, 1 unknown) and 2 pseudogenes. Ten genes from the critical region were sequenced, identifying five different heterozygous loss-of-function mutations in the ε-sarcoglycan gene (*SGCE*). These mutations co-segregated with the disease in all families and showed evidence of reduced penetrance if the mutated allele were maternally inherited.[212]

The original family, in whom the *DRD2* polymorphism was identified, was later also found to carry a novel 5bp deletion of *SGCE* exon 7 (c.835-839delACAAA) resulting in a frameshift and premature stop codon (p.K278fs>295X).[213] To date in excess of 40 different *SGCE* mutations have been identified (Table 5.1).

1.10.2.2 Imprinting

Initial linkage studies suggested that the pathogenic *SGCE* mutations followed an autosomal dominant pattern of inheritance but with evidence of reduced penetrance. The majority of affected individuals inherited the mutant allele from their father while those who inherited the mutation from their mother were clinically unaffected, leading to the suggestion of maternal imprinting (Figure 1.4). *Sgce* is also maternally imprinted in mouse models, although it is also weakly expressed in the brain when maternally inherited.[214]

Grabowski et al used bisulphite genomic sequencing of Human DNA to investigate differential methylation, a hallmark of genomic imprinting. They found sequences upstream of *SGCE* showed extensive methylation of the maternal alleles in both leukocytes and brain tissue. Within *SGCE* itself, the potential promoter region, exon 1

and the beginning of intron 1 were embedded within a CpG island. Sequencing of DNA derived from maternal and paternal uniparental disomy 7 (UPD7) lymphoblastoid cell lines showed a corresponding parent-of-origin specific methylation pattern. The effect of which found only weak *SGCE* expression in matUPD7 cell lines while strong expression was observed in patUPD7 lines.[215, 216] Four other genes on chromosome 7 are imprinted in humans: *MEST (PEG1)*, *COPG2*, *GRB10* and *PEG10*, the latter being adjacent to *SGCE* and simultaneously maternally imprinted.[215]

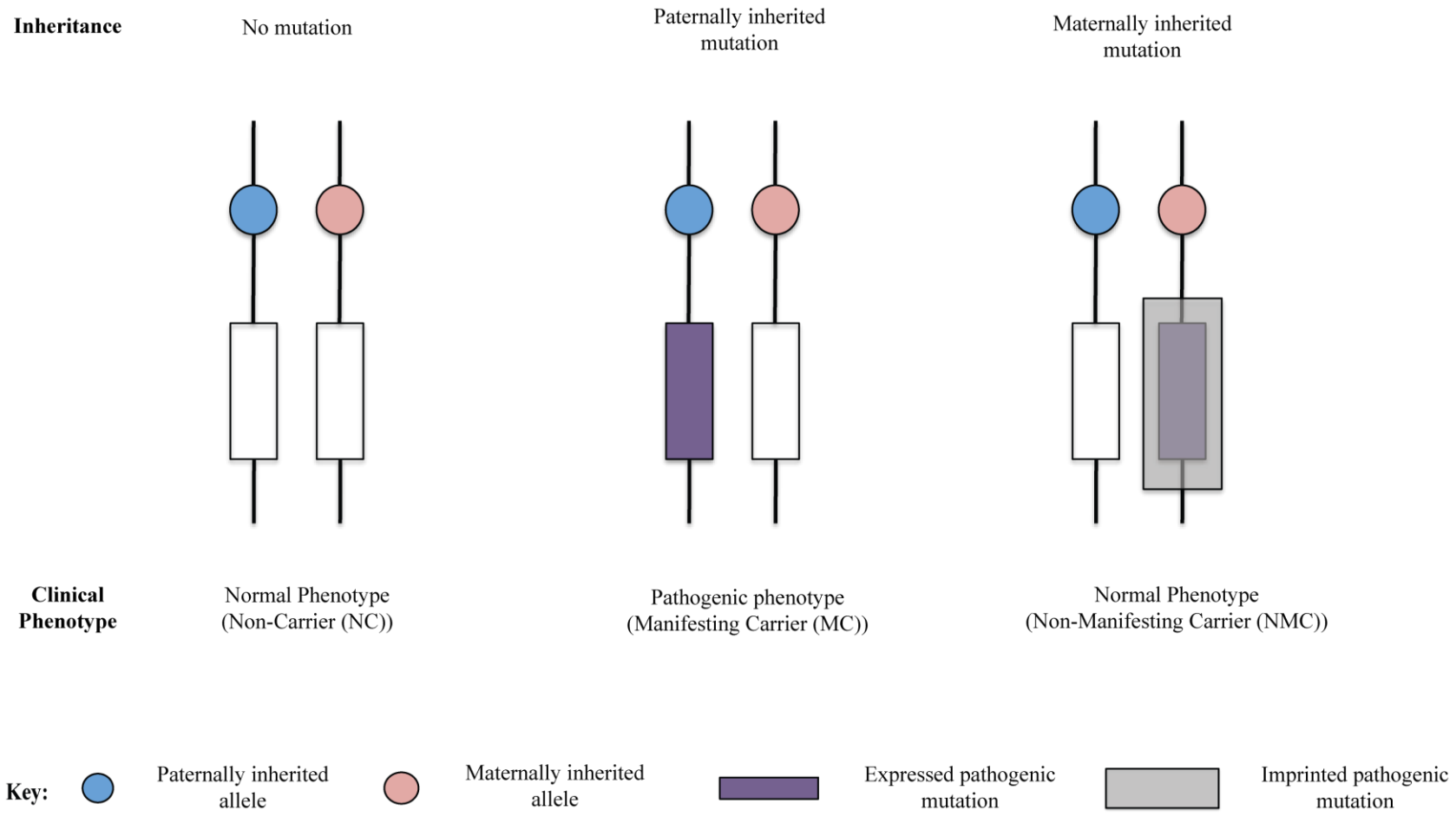
1.10.2.3 Genetic Heterogeneity

Rates of reported *SGCE* mutations have varied significantly between published cohorts, ranging from 0%[217] to 82%[186] (Table 5.2), leading to the suggestion of genetic heterogeneity amongst MDS patients.

Chromosome 18 (DYT15)

Linkage studies in a single large Canadian family have found a locus on chromosome 18p11 with LOD score >3.0 which has been narrowed to a 3.2Mb candidate region. This region was given the DYT15 locus although no pathogenic genetic mutations have as yet been identified.[218, 219] Analysis of 10 further MDS families found linkage to this region in only two, however within each of these families linkage did not co-segregate with motor symptoms.[220] Involvement of chromosome 18 has also been implicated in the 18p deletion syndrome, deletion of the chromosome's short arm being observed in more than ten patients with dystonic symptoms[221-225] and a single patient with features of myoclonus dystonia, although onset of symptoms was in late twenties and had been preceded by growth and developmental delay.[226] Fluorescence in situ hybridization (FISH) analysis of this case found the deletion to involve the entire short arm of chromosome 18 including DYT7 and DYT15 loci.[227]

Figure 1.4: Diagrammatic representation of maternal imprinting



Copy Number Variants (CNVs)

Deletions and duplications of the *SGCE* gene provide another possible explanation for genetic heterogeneity. Several studies have reported single exon or multiple exon deletions within *SGCE.* Despite large deletions, these cases have been described as having a typical MDS motor phenotype with no additional clinical characteristics.[228, 229] Others have described large contiguous gene deletions involving *SGCE,* ranging between 0.17Mb and 16.5Mb in size and involving a varying number of surrounding genes (Figure 5.1).[230-233] Additional clinical characteristics have also been reported in these patients e.g. microcephaly and short stature, these will be discussed in further detail in Chapter 5.

1.10.3 The ε-sarcoglycan protein

The *SGCE* gene encodes the ε-sarcoglycan protein, a member of the sarcoglycan family of proteins, of which there are five other members: α, β, γ, δ and ζ. The sarcoglycans are predominantly expressed in striated and smooth muscle and the Schwann cells of peripheral nerves. In these tissues they form a heterotetrameric complex typically with a βδ core which is then associated with α/ε and γ/ζ.[234, 235]

1.10.3.1 Dystrophin-associated glycoprotein complex

Within muscle and peripheral nerve the sarcoglycan complex forms part of the dystrophin-associated glycoprotein complex (DGC), a membrane spanning protein complex, which in muscle protects the sarcolemma from mechanical damage. The other components of the DGC include the dystroglycan complex and a cytoplasmic sub-complex. Dystroglycan is a matrix receptor that spans the plasma membrane linking the cytoplasmic components of the DGC to the extra-cellular matrix. The cytoplasmic sub-complex is composed of:

a) Dystrophin: encoded by the *DMD* gene and a vital component in the building and stability of the DGC. *DMD* mutations result in Duchenne and Becker muscular dystrophy.

b) Dystrobrevins: two subtypes exist (α and β). These bind directly to dystrophin, the resultant complex then binding to the syntrophin family of proteins. Dystrobrevins may have a role in $GABA_A$ receptor clustering.[236]
c) Syntrophins: These are a family of adaptor proteins (α, β_1, β_2, γ_1 and γ_2) that interact with a number of nerve-related proteins e.g. aquaporin-4[237] and neuronal nitric oxide synthase.[238]

1.10.3.2 Sarcoglycan complex in the brain

The exact function of the sarcoglycan complex is still not clearly understood however, mutations of the α, β, γ or δ subunits result in different forms of autosomal recessive limb-girdle muscular dystrophy.[239] However patients with MDS due to *SGCE* mutations do not have any associated muscle pathology,[240] suggesting that the complex may play a different functional role in the brain. Unlike the other forms of sarcoglycans that are predominantly expressed in muscle and nerve, ε is more widely distributed, being expressed at its highest levels in heart and lung tissue.[241] In the brain it is found predominantly in midbrain monoaminergic neurons, cerebellar Purkinje cells, the hippocampus and cortex.[241-243] Little is known of the composition and structure of the brain sarcoglycan complex. However, in addition to ε-sarcoglycan, β appears to be only weakly expressed while ζ is present at much higher levels.[244, 245] Therefore, if a sarcoglycan complex does exist in the brain it is likely to differ in composition to that seen in muscle.[246]

1.10.3.3 Cellular handling of mutant ε-sarcoglycan proteins

The majority of *SGCE* mutations are nonsense mutations that would abolish the synthesis of the full length protein. Missense mutations in the extracellular domain of the protein impair trafficking to the plasma membrane, the proteins instead being retained intracellularly, misfolded and degraded by the ubiquitin proteasome system. There also appears to be some interaction with torsinA as co-expression of the two proteins reduces the steady-state levels of the mutant ε-sarcoglycan but has little effect on overall levels of the wild-type protein.[247]

This process does not appear to be consistent across all missense mutations. A missense mutation in the Ig-like domain of ε-sarcoglycan resulted in a gain of glycosylation mutation, producing a protein that continued to be targeted to the plasma membrane albeit at lower levels than the wild-type protein.[248] Therefore intracellular handling of mutant ε-sarcoglycan proteins may vary dependent upon the nature of the genetic mutation, resulting in varying quantities of the protein being expressed at the cell surface.

1.11 Thesis Objectives

The principal aims of this thesis are:

- To establish a cohort of patients with clinically suspected MDS and determine the rate of *SGCE* mutations within this cohort (Chapter 3).
- To determine differences in motor phenotype between those with an *SGCE* mutation and those without (Chapter 3).
- To assess the rate and type of psychiatric symptoms amongst those with *SGCE* mutations and to determine whether psychiatric disorders are significantly increased amongst this cohort compared to familial controls, an external control group of alcohol responsive tremor and estimated population levels (Chapter 4).
- To identify the different types of *SGCE* mutations within this cohort and whether any genotype-phenotype correlation exists (Chapter 5).
- To characterise the additional clinical features of those with contiguous gene deletions or duplications involving *SGCE* and whether, these can be predicted by the genes involved (Chapter 5).

CHAPTER 2

Materials and Methods

2.1 Introduction

This chapter outlines the materials and methods used for participant recruitment, assessment and genetic analysis. All work detailed below was performed by myself unless otherwise stated.

2.2 Case ascertainment

2.2.1 Clinically probable Myoclonus Dystonia Syndrome cases

Patient recruitment took place throughout the United Kingdom (UK) and Ireland via both adult and paediatric movement disorder specialists. A number of methods were employed to establish a large cohort of patients with clinically suspected Myoclonus Dystonia Syndrome (MDS).

1) Initially adult movement disorder specialists who had previously collaborated with the group were contacted and asked to identify any patients either with a clinical syndrome consistent with MDS or who had previously undergone genetic testing confirming an *SGCE* mutation.

2) The British Paediatric Neurology Association (BPNA) movement disorders special interest group (MD-SIG) meet quarterly to discuss clinical cases and ongoing research. Through attendance of this meeting I was able to establish awareness of our research and establish collaborations with a number of paediatric groups throughout the UK and Ireland.

3) This research project was registered with the British Neurological Surveillance Unit (BNSU), an epidemiological and research branch of the Association of British Neurologists (ABN). This allows adult Neurologists to alert the research group of patients who may be interested in participating in research. The ABN issues a monthly electronic newsletter listing all current projects and asks their members to complete a form documenting the number of relevant clinical cases they have seen in routine clinical practice over the preceding month. This information is then emailed to the research project lead as a

monthly update. A research pack including a brief summary of the project and a form allowing contact from the research group is then sent to the primary caring consultant. The consultant sends this information to the patient, asking them to complete the contact form and return it to the research group. Having received this form I was then able to contact the patient and arrange a face-to-face assessment. Patients recruited via this method included both those with known *SGCE* mutations and those with clinically suspected MDS but yet to undergo genetic testing.

4) All cases identified as having an *SGCE* mutation were asked to contact both affected and unaffected family members. The proband participant was asked to give a copy of the patient information sheet, participant contact form and a stamped addressed envelope to each family member who may be interested in partaking. This ensured that the research team was only able to contact those individuals interested in receiving further information regarding the study. Having received a completed contact form indicating that the family member was willing to participate, they were contacted by the research team as detailed in Section 2.2.1 and data collected as outlined in Section 2.3.

All *SGCE* mutation positive patients and family members were classified according to their motor and genetic status into 3 groups: 1) manifesting carriers (MC): *SGCE* mutation and movement disorder; 2) non-manifesting carriers (NMC): *SGCE* mutation and no movement disorder; 3) non-carriers (NC): neither *SGCE* mutation nor movement disorder.

2.2.2 Alcohol responsive tremor cases

Patients with tremor who reported an improvement with alcohol were recruited from general neurology and movement disorder clinics, forming the control group for assessment of psychiatric co-morbidity. These patients were examined using the same protocol (Sections 2.2.1 and 2.3)

A diagrammatic description of the recruitment and assessment process can be seen in Figure 2.1.

2.2.3 Patient information and informed consent

We explained the nature of the study, its purpose and associated procedures, the expected duration and the potential risks and benefits of participation to each patient prior to clinical evaluation. Patients would be provided with a patient information sheet and given an opportunity to ask questions (Appendix B.1.1 and B.1.3). During this process emphasis was placed upon the voluntary nature of participation, the right to withdraw from the study at any time without any disadvantage to his or her ongoing care and without the need to provide a reason for his or her decision. Following this discussion patients were asked to sign a statement of informed consent for participation into the study and for medical illustration. This signed statement of informed consent was subsequently filed and stored in the patient's research records (Appendix B.2.1 and B.2.3).

For participants under the age of 16 years and unable to consent for themselves, assent for participation in the study was asked for from their parent or legal guardian. Again we discussed the nature of the study it's aims, risks, benefits and what it entailed. The parent/guardian would then be provided with a third party information sheet detailing specific aspects of the study and both participant and parent/guardian given the opportunity to ask questions (Appendix B.1.2 and B.1.4). The parent/guardian would then be asked to sign an assent form allowing their child to participate in the study and also for medical illustration, stating clearly their relationship to the child (Appendix B.2.2 and B.2.4). This form was then filed in the participant's research folder and stored in a secured cabinet, inside a locked room, within the research department.

2.3 Data collection

2.3.1 Self-completed questionnaires

For adult only participants, prior to the face-to-face evaluation and with their consent, a small pack of standardised questionnaires were sent in the post for self-completion. These questionnaires were sent only after telephone discussion with the participant and with plenty of time prior to the examination. Participant's were also given

multiple means of contacting the research team (telephone, email and postal address) and encouraged to do this should they encounter any problems or require any further assistance. These questionnaires included the Patient Health Questionnaire (PHQ-9)[249] (Appendix B.5), Structured Clinical Interview for DSM-IV Axis II: Personality Disorder questionnaire (SCID-II)[250] (Appendix B.6) and Short Form Health Survey (SF-36)[251] (Appendix B.7).

2.3.2 Clinical evaluation

Clinical information was collected by two methods either direct face-to-face clinical evaluation or where this was not possible by retrospective data collection from the clinical records.

During direct assessment a detailed standardised data collection booklet was created to collect structured data including patient demography, past medical history, previous investigations, movement disorder symptomatology at onset and examination, current medication, response to medication used to treat the movement disorder and family history (Appendix B.3). In addition several standardised data collection tools were used including the MINI International Neuropsychiatric Interview (M.I.N.I.) (Appendix B.7) or where appropriate the MINI International Neuropsychiatric Interview for children and adolescents (Parent Version) (M.I.N.I. KID)[252] (Appendix B.8), The Alcohol Use Disorders Identification Test (AUDIT)[253] (Appendix B.9), Yale-Brown Obsessive Compulsive Scale (YBOCS)[254, 255] (Appendix B.10) and Montgomery-Asberg Depression Rating Scale (MADRS)[256] (Appendix B.11). Only adult participants were asked to complete the later three questionnaires.

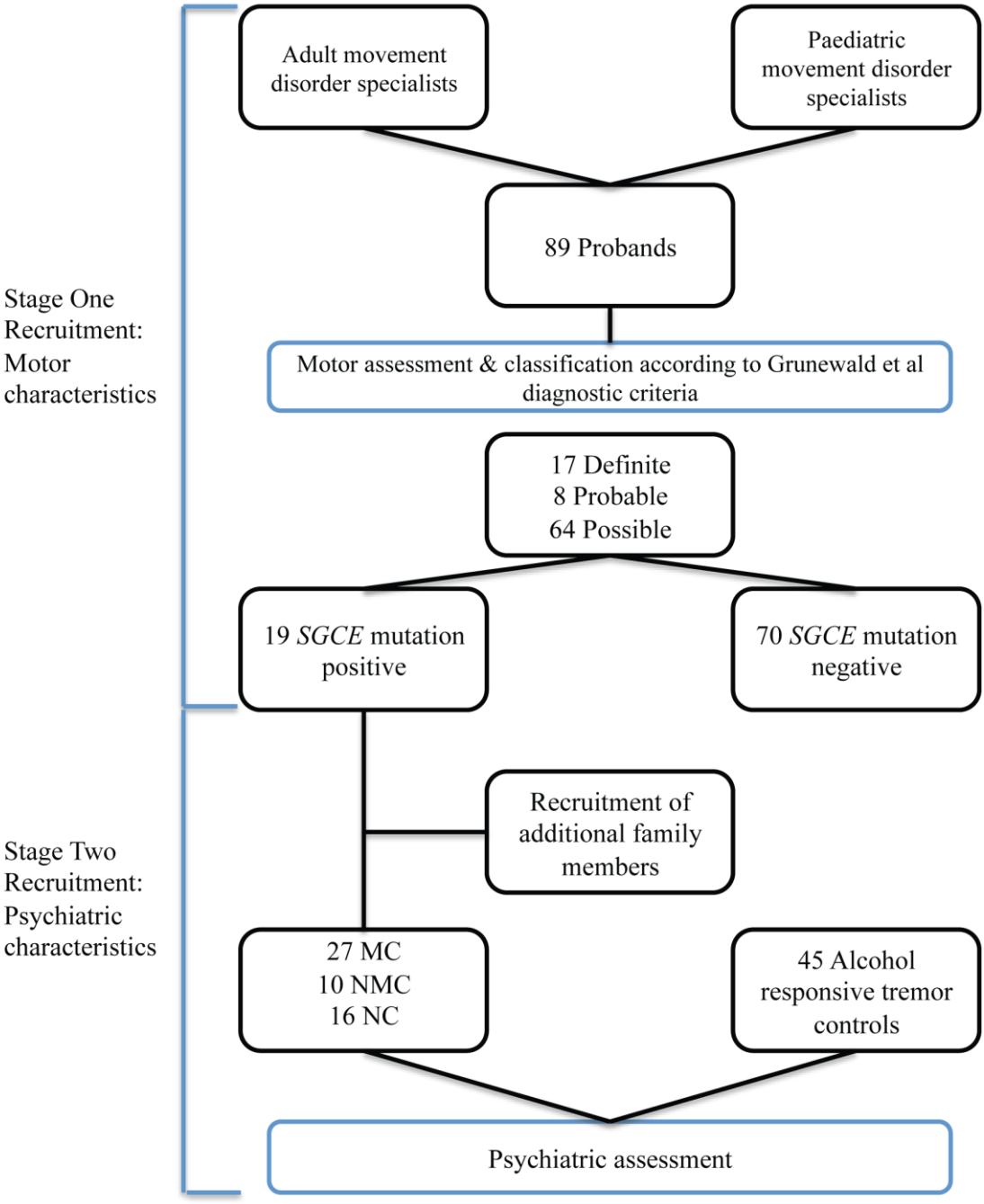
When face-to-face clinical evaluation was not possible, data collection was completed using a systematic *pro forma*. Information collected included patient demography, past medical history, previous investigations, movement disorder symptomatology at onset and at the time or their most recent clinic appointment, current medication, response to medication used to treat the movement disorder and any family history.

2.3.3 Videotaped clinical examination

All participants undergoing face-to-face examination were asked to consent to a standardised videotaped clinical examination, which would then be stored upon a

secure computer database (Appendix B.4). Examination followed a modified version of that suggested by the Unified Myoclonus Rating Scale (UMRS)[257] (Appendix B.12) and was subsequently rated by a single assessor using both the UMRS and Burke-Fahn Mardsen Dystonia Rating Scale (BFMDRS)[258] (Appendix B.13).

Figure 2.1: Diagrammatic representation of recruitment



Key: Grunewald et al[259] clinical diagnostic criteria used for motor classification.

MC = manifesting carrier, NMC = non-manifesting carrier, NC = non-carrier

2.3.4 DNA and serum collection

When possible, 20-30ml of blood was taken in ethylenediaminetetraacetic acid (EDTA) and acid citrate dextrose (ACA-A) tubes, the former to allow extraction and storage of DNA and the later to be sent to the European Collection of Cell Cultures (ECACC) part of the Health Protection Agency Culture Collections (HPA) located at Porton Down, Salisbury, UK for cell preservation. A DNA bank was established in conjunction with the Institute of Medical Genetics, University Hospital of Wales, Cardiff who undertook DNA extraction and subsequent storage.

2.3.5 Project database

Two databases were utilized for the purposes of this research study. The first a pre-existing Access database used for storing the contact details and basic clinical information of all those participants with DNA samples stored within the research group's bio-bank. This was stored on a secure NHS computer, locked in a secure room and password protected. The second database was specifically designed for this study using File-maker software. Here patients were anonymised using their study codes and initials. Further information stored included:

- Patient recruitment information
    - Current demographic information
    - Referring Consultant and NHS centre
    - Proband or related family member
- Patient clinical information
    - Age and clinical symptoms reported at onset
    - Diagnostic and medication histories
    - Family history
- Standardised questionnaires
    - Results and total scores of those standardised questionnaires outlined in Section 2.3.2 (see also Appendix B)

2.3.6 Study Compliance and confidentiality

This study was performed in compliance with the guidelines of the Declaration of Helsinki in the Tokyo version of 2004[260], the European ICH Guideline for Good Clinical Practice (1997), the Medical Research Council (MRC, UK) Operational and

Ethical Guidelines of human tissue and biological samples for use in research[261] and the General Medical Council (GMC, UK) Guidelines in Research: roles and responsibilities of doctors (2000). Project protocols and documentation were reviewed and agreed by the Research Ethics Committee for Wales (MREC 09/MRE09/56 & 09/MRE09/35) and local Research and Development Committees (Cardiff and Vale NHS Trust & Aneurin Bevan Health Board). To ensure confidentiality was maintained at all times several steps were put in place to ensure good practice. Where possible automated safeguards were introduced at the planning and design stages avoiding the need for manual checking and possibility of investigator error. At all times during case ascertainment the nominated primary carer for the patient was asked to inform the patient that there was an ongoing research opportunity and seek their consent for involvement. These methods prevented contact with patients for research purposes without their specific consent.

2.4 Materials and Equipment

2.4.1 Materials

DNA extraction and quantification chemicals

DNA was extracted from peripheral blood leucocytes using the Nucleon Genomic DNA extraction kit obtained from Tepnel Life Sciences (Manchester, UK). Absolute ethanol, chloroform and TE buffer (made from 10mM trishydroxymethyl-aminomethane-hydrochloride (Tris-HCl) and 1mM EDTA to pH 8) were used to complete the extraction process. Quantification was performed using PicoGreen (Invitrogen) and TE buffer.

PCR chemicals and biochemical

Those used included 10X polymerase chain reaction (PCR) buffer containing 15mM magnesium chloride ($MgCl_2$) and HotStar Taq DNA polymerase at 5 units/µl concentration (Qiagen, Crawley, UK). Deoxynucleoside triphosphates (dNTPs) in 100mM aqueous solutions were sourced from Invitrogen (Paisley, UK), oligonucleotide primers and Dimethyl sulphoxide (DMSO) from Sigma, and sterile water from Hameln Pharmaceuticals (Gloucester, UK).

Electrophoresis Reagents

These included Hi-Res Agarose powder (Fisher Scientific), 10X TBE buffer (0.89M Tris-boric acid, 20mM disodium EDTA at pH 8.3) from National Diagnostics (Hessle, UK) and 10mg/ml Ethidium Bromide (EtBr) (Amersham Biosciences UK Ltd). A 1kb plus DNA reference ladder at 1.0µg/µl was sourced from Invitrogen and 10X loading dye consisting of 1.5g Ficoll, 0.02g bromophenol blue, 0.02g xylene cyanole FF and water was prepared within the department by the laboratory technicians.

PCR and sequencing Purification products

The two principle products used were Agencourt Ampure and ClenSEQ magnetic bead solutions (Beckman Coulter, UK) and 70% ethanol, diluted from absolute ethanol (Fisher Scientific) with purified water.

Sequencing reaction reagents

These included The BigDye Terminator v.3.1 Cycle sequencing kit consisting of BigDye Ready Reaction premix and 5X BigDye Terminator sequencing buffer (Applied Biosciences, UK) and forward and reverse primers (Sigma)

Multiplex Ligation-Dependent Probe Amplification Kits

Multiplex ligation-dependent probe amplification (MLPA) probe set P099B (MRC Holland, Amsterdam, Holland) was used to detect deletions or duplications of *SGCE*, *GCH1* and *TH* genes.

Additional consumables

Additional items used in the laboratory included micro-centrifuge tubes (100µl & 200µl) (ELKAY, UK), thermo-fast 96-well skirted and non-skirted microtitre plates, adhesive PCR cover film (Thermo Scientific), single and multi-channel pipettes (Jencons, UK), general purpose pipette tips (alpha laboratories, UK) and Biomek robot pipette tips (Beckman Coulter).

2.4.2 Equipment

DNA extraction and quantification equipment

Three thousand, five hundred standard gravity centrifuge and a 15,000g micro-centrifuge were used for DNA extraction. DNA quantification was achieved through

use of NanoDrop 1000 UV-visible spectrophotometer (Thermo Scientific) initially and then subsequent Fluororskan Ascent fluorometer (Thermo Scientific).

Thermal cyclers

Those used for PCR and BigDye sequencing reactions were the '1000 Series' Thermal Cyclers (Bio-Rad, UK)

Gel tanks, power supplies and photography

Thermo Hybaid Electro 4 gel tanks (Fisher Scientific) connected to a Bio-Rad Model 200/2.0 power supply were used for agarose gel electrophoresis. A dual intensity transilluminator box (Ultra-Violet Products, UK) was used for DNA visualization and photographed using a Kodak digital camera (Kodak Digital Science Electrophoresis Documentation System 120 v.2.0.3. USA).

Biomek robots

The Biomek NX MC "Laboratory Automation Workstation" robot (Beckman Coulter) was used for PCR product and sequencing product clean-up

Sequencers

ABI Prism 3100 "Genetic Analyser" sequencing machines (Applied Biosystems) were used for sequencing prior to mid-2011, after this "cleaned-up" PCR products were outsourced to Source BioScience (UK).

2.4.3 Computer Software and online databases

General Software

Word® 2007/2010 (MS Office, Microsoft Corporation) was used as a text processor throughout. Powerpoint® 2007/2010 (MS Office, Microsoft Corporation) was used to generate electronic genealogies, presentations and conference posters and Adobe Illunstrator CS4 for manipulation of pictorial and graphical data. Data was stored in a specifically designed Filemaker database and extracted in the form of .csv files, the data being analysed using statistical software R v.2.10.1[262] freely available for download at http://www.R-project.org

Specific software programs

Lasergene SeqMan Pro

SeqMan Pro forms part of the Lasergene package (DNASTAR Inc, USA) and was used for the analysis of Sanger sequencing data. Data was entered as raw fluorescent trace material in a .abi format which was then converted to readable sequences and aligned to a reference sequence. Typically the sequences were assembled into contigs with base calls deviating from the reference sequence being called based upon a quality score assigned to each variant by the program, which could then be assessed by visual inspection of the raw sequence trace.

Online databases

UCSC Genome Browser

This website (http://genome.ucsc.edu) was used to obtain information regarding the genes of interest and their genomic regions. During primer design the 'In-Silico PCR' function was used to check whether primers designed using the Primer 3 program contained any Single Nucleotide Polymorphisms (SNPs) and aligned to the correct genomic sequence. The GRCh37/hg19 genome build from February 2009 was used throughout in this study.

Primer 3

Available online at http://frodo.wi.mit.edu/primer3 this program was used for the design of both PCR oligonucleotide and sequencing primers. During primer design melting temperature, maximum GC base content allowed and the length of the primer had to be entered into the program as required.

2.5 Laboratory methods used for genetic analysis

All laboratory work was performed by myself, unless otherwise stated.

2.5.1 DNA extraction and purification

Genomic DNA was extracted from peripheral blood leucocytes at the Institute of Medical Genetics, Cardiff University. The standard procedure involved the use of Nucleon kits (VWR International, UK) in which each sample was mixed with four times its volume of working concentration 'Reagent A'. The combined solution was

then centrifuged at 1300g for 10 minutes and the supernatant discarded, 2.0ml of 'Reagent B' was then added to the pellet lysing the cells. Five hundred microlitres of sodium perchlorate was mixed with this solution, 2ml chloroform was then added and mixed and finally 300µl of Nucleon Resin added, this time without mixing, achieving de-proteinisation. The solution was then centrifuged at 1300g for 3 minutes and the phase above the resin transferred to a fresh tube. Absolute ethanol (~2.5ml) was added to this phase to precipitate the DNA and centrifuged at 4000g for 5 minutes. The supernatant was then discarded. The DNA was washed with 2ml cold 70% ethanol and re-centrifuged. The pellet was air dried for 10 minutes and re-suspended in 680µl of TE buffer, the DNA then being stored at -20 degrees Celsius (°C)

2.5.2 DNA quantification

Samples were first quantified using the NanoPore spectrophotometer, the machine first equilibrated using a water or TE blank and 1µl of each sample subsequently added. The spectrophotometer is able to measure A_{260} and A_{280} of the sample, using these to calculate the DNA concentration. However, spectrophotometers are unable to distinguish double-stranded DNA from degraded DNA in the sample and therefore the later may be counted towards the total concentration using this method.
Each sample was then quantified using PicoGreen, a fluorescent dye that intercalates with double-stranded DNA only and therefore is more accurate. DNA concentration is then calculated from the strength of the PicoGreen fluorescence, this being more intense when bound to DNA.[263] PicoGreen was diluted to a working dilution of 1:200 with TE buffer and a standard curve calculated. PicoGreen was then added to each DNA sample and concentration measured with a UV excitation wavelength of 485nm and emission wavelength of 520nm

2.5.3 Oligonucleotide Primer Design

The online program Primer3 was used for the purposes of designing PCR and sequencing primers. Each primer was designed to be 18-25 nucleotides long. DNA regions that could result in non-specific binding (e.g. Alu elements) were excluded using the Primer3 mis-priming library. Guanine/Cytosine (G/C) content of each primer was limited to 60% to avoid GC-rich regions. In the majority of cases the forward and/or reverse primer designed for PCR was also used in sequencing. Primers used can be seen in Tables 2.1 - 2.5.

Table 2.1: *SGCE* primer sequences

Exon	Primer sequence
Exon 1	
Forward	GGGATGCTGATGCTGAACTGGCCA
Reverse	AGAGAGGCTGGTGCCCAA
Exon 1a	
Forward	TTAGCTGGGCTGGAAGGAAT
Reverse	GCAGACATTTAATTGGCTCCCC
Exon 2	
Forward	CTGAATTATCAAGGGCGTATC
Reverse	CCATTTGAAATAATGTTAATG
Exon 3	
Forward	AGACAGAATGTTTTGATTGAAAC
Reverse	AGAAGAATGGCACATTTCCAAA
Exon 4	
Forward	GTTCTCATTGCCCAGAGAAGG
Reverse	TCAGTTATATTAGGTATGTGGC
Exon 5	
Forward	CTACTTCATTAAAGATATGCATGC
Reverse	ATAAGTTTGATAAGATCACCG
Exon 6	
Forward	AAGGCTAAATCCTGCTTTTAAGGTGG
Reverse	TTATTCCTAAAAGCAGTTCAG
Exon 7	
Forward	AAGAATGCTTTAGTGTAT
Reverse	TTGTTATCTTAGCAGGATCTC
Exon 8	
Forward	GACAATGTCAGCATTTCCACAT
Reverse	GTTTTAGTTTGTACCCTCCA
Exon 9	
Forward	CAAATTGATGACCCATCAGGC
Reverse	CATGCATATTAATAATTATGGCTC
Exon 10	
Forward	TAATGTAGCCTAGTGGCCACA
Reverse	AGCCAACTTCATGACTTGTAG
Exon 11	
Forward	GACTGGGGTCATAGTTTACCCG
Reverse	ATTTGGTGAAGATAAAGCTTCAT
Exon 11b	
Forward	GGCATTGTGGTAGGGAAACT
Reverse	GCTTACAAAGTAGCACCAACAC
Exon 12	
Forward	GATGGAAACTTTCTCCTTGCC
Reverse	CAACATGCATAACATATGCCAG

Table 2.2: *TOR1A* (GAG deletion) and *GCH1* primer sequences

Exon	Primer sequence
TOR1A	
Forward	CCTGGAATACAAACACCTA
Reverse	GGTGGAAGGACTGAGTGTTG
GCH1	
Exon 1	
Forward	CGGCTCGGAGTGTGATCTA
Reverse	GTTCTCGCCCAGAAAGTGAG
Exon 2	
Forward	TTCCATTGGATTAACGTTCG
Reverse	TTGCTGGGAAACAACAAAGA
Exon 3	
Forward	TTGTCACAAAGAAGGCACTG
Reverse	CAGCAGATGAGGGCAGGT
Exon 4	
Forward	ATTTCCTCTTGCAGCCCACT
Reverse	CTCATCAGCCTGGGTGACA
Exon 5	
Forward	CTGCATCTGCAGAAGTCTGATT
Reverse	GCATCACCTGGTGCTACAAA
Exon 6a	
Forward	CTCGGGAATGGTAACTGTGA
Reverse	AGCACTTTCGGCACTACACC
Exon 6b	
Forward	TTGGGAATGAGAGGGAACTG
Reverse	TGCAGACCTGAAAATGATGG
Exon 6c	
Forward	GACATTTAACTCTCTGTGCCTTGA
Reverse	CATCTTGCCCCATCATAACC
Exon 6d	
Forward	CATCTCTGCCACTTTGATGC
Reverse	TGGGAGAAGCCCTTATGATG

Table 2.3: *THAP1* primer sequences

Exon	Primer sequence
Exon 1a	
Forward	ACCTGGCCTCAGCCAATAGT
Reverse	AGGGTCCTCACTTGTGGAAA
Exon 1b	
Forward	AAACGGGCACACTAGTCACC
Reverse	AAAACACCTGGCTGCTCTGT
Exon 2	
Forward	GGAAAGTTTGGGTGCCTTTA
Reverse	TGCATTTTGTGTTTTCAGAAGTG
Exon 3a	
Forward	CCCACCTCTTCCTCACAAAA
Reverse	GTGCGGTCTTGAGCTTCTTT

Exon 3b	
Forward	CCCTGTTAATCTCTCAGTTTTC
Reverse	ATCCTCCTCTAGCCTGTAAAGGA
Exon 3c	
Forward	GGAGGTTGTTCACTTCCAGAAA
Reverse	TCCCATGATCTGACCCATACT
Exon 3d	
Forward	GAACAGTGTGGTAAAAGGGTGA
Reverse	ACTACAGCTGGGGAACTGGA
Exon 3e	
Forward	TTTCCCCTACTGTCTTGCATT
Reverse	TCACAGTTTGAACAGAAACCTCA
Exon 3f	
Forward	CTGAGTTGGGACAAGGCTTC
Reverse	GCATGAATCACAGTGCTATCC

Table 2.4: *NKX2-1* primer sequences

Exon	**Primer sequence**
Exon 1	
Forward	CTCGGATTCTCTCCGGTAGG
Reverse	GCACGGACAGGTCTTTAGGA
Exon 2a	
Forward	GTGGGCATGAAGGTAACACC
Reverse	CAGGTTGCCGTTGCAGTAG
Exon 2b	
Forward	ACAAGAAAGTGGGCATGGAG
Reverse	GGCTCCCCGAGGTCTTCTGA
Exon 3a	
Forward	GCTAGGCTGCCTGGGTCA
Reverse	CCTGGCGCTTCATTTTGTAG
Exon 3b	
Forward	CCAGCATGATCCACCTGAC
Reverse	ACTGCTGCTGAGCCTGTTG
Exon 3c	
Forward	GAACCACCGCTACAAAATGAA
Reverse	GAGGAGTTCAGGTGGGACAG
Exon 3d	
Forward	CCAGGTATCCAGCCTGTCC
Reverse	CAGAGTGTGCCCAGAGTGAA
Exon 3e	
Forward	AGAGGGCTCTGTGCTGACAT
Reverse	CCCTCAAAGCCATTTAAAGC

Table 2.5: *SGCZ* primer sequences

Exon	Primer sequence
Exon 1a	
Forward	GGTGGTGAAGGCCAGTAAAA
Reverse	TCCCAGGCATTAGCAATGAT
Exon 1b	
Forward	GGAGAACGTTCCCTTCTGACT
Reverse	CAACACAGCTGAGTCGATTG
Exon 1c	
Forward	CTTGAAATTTGCCGCATGAT
Reverse	CGGGACCACCAACTACTCCG
Exon 2	
Forward	TGAACAAAATGATTCGAAGTTTTC
Reverse	CCCACACTTAAATGGCAGGT
Exon 3	
Forward	TTGAGCTCACTGTTTTCTCAATTT
Reverse	TGCTGAAGAGATAAGGGGATTC
Exon 4	
Forward	TGAGGCTCTCAGTTTTGTACATTG
Reverse	CAAGCACAGTAGGCCAGATG
Exon 5	
Forward	TCCCAAATTAGCCTCCTGAA
Reverse	GGCATAGGAATCATCCATCTT
Exon 6	
Forward	TGTTTTGGAAGAATATTTGATGC
Reverse	GCCTCAGGATCCCTGTTTTT
Exon 7	
Forward	GCCTGTCTGTCTGGTTGTTG
Reverse	ACAACTCCATTTATTTTCTACTGAAAG
Exon 8a	
Forward	GACCATGTTGAGGAGGGATG
Reverse	AAGGGAAACCGAGCAGAACT
Exon 8b	
Forward	ATCTGCCTGTGGAGCTGAAG
Reverse	TGCCATTGGATACTGGGAAT
Exon 8c	
Forward	GGGTGTGATTGACACAGCAG
Reverse	TTGCTGAGTGCTTTCAAAATTA

2.5.4 Polymerase Chain Reaction

PCR is used to amplify specific fragments of DNA nested between a forward and reverse primer.[264] The main components of a PCR reaction are a double stranded DNA template, Taq DNA polymerase (a heat stable enzyme able to synthesize new strands of DNA), dNTPs, primers, $MgCl_2$ containing buffered solution and DMSO

added if required. Volumes and concentrations of these reagents are detailed in Table 2.6, *NKX2-1* exon 3b was the only exon that required the addition of DMSO.

Table 2.6: PCR reagents (volumes and concentrations)

Reagent	Volume per 96 plate well (µl)	Volume per 96 plate well (µl)
H_2O	4.5	3.87
PCR Buffer	1.2	1.2
dNTPs (2.5mM)	1.2	1.2
Forward/Reverse Primers (5µM)	2.0	2.0
Taq polymerase	0.1	0.1
DMSO (7%)	-	0.63
DNA	3	3
Total	12.0	12.0

The overall PCR reaction is subdivided into three steps, which are then repeated 12-40 times as required. Initially the template DNA is denatured to a single-strand using high temperatures (~95°C). Secondly the temperature is reduced to the annealing temperature specific to the set of primers (usually 55-60°C) allowing binding/annealing of the primers to the DNA template. Finally, at a higher temperature (~72°C) the Taq DNA polymerase extends the DNA fragment from the primers' 5' end. Once a new double-stranded DNA sequence has been synthesized the cycle is repeated, each new fragment serving as a template for the next and therefore increasing the number exponentially until 1µg of DNA has been synthesized.[265] (see Figure 2.2)

For those fragments where amplification was more difficult, touchdown PCR was used.[266] During touchdown PCR the annealing temperature is lowered at 0.5°C intervals during the first 10 cycles and then held at a constant temperature for the remaining 28 cycles. At higher annealing temperatures primers will anneal with higher specificity but a lower yield. During subsequent cycles, at a lower temperature, the DNA target yield will increase at the expense of specificity. However, due to the first cycles and the exponential nature of PCR amplification the sequence of interest, correctly amplified during the first few cycles, will be at a much higher concentration at the end of the reaction than the non-specific by-products.
PCR conditions for all exons of *SGCE*, *SGCZ*, *GCH1*, *TOR1A*, *THAP1* and *NKX2-1* genes can be seen in Tables 2.7 and 2.8.

Figure 2.2: Diagrammatic description of PCR conditions

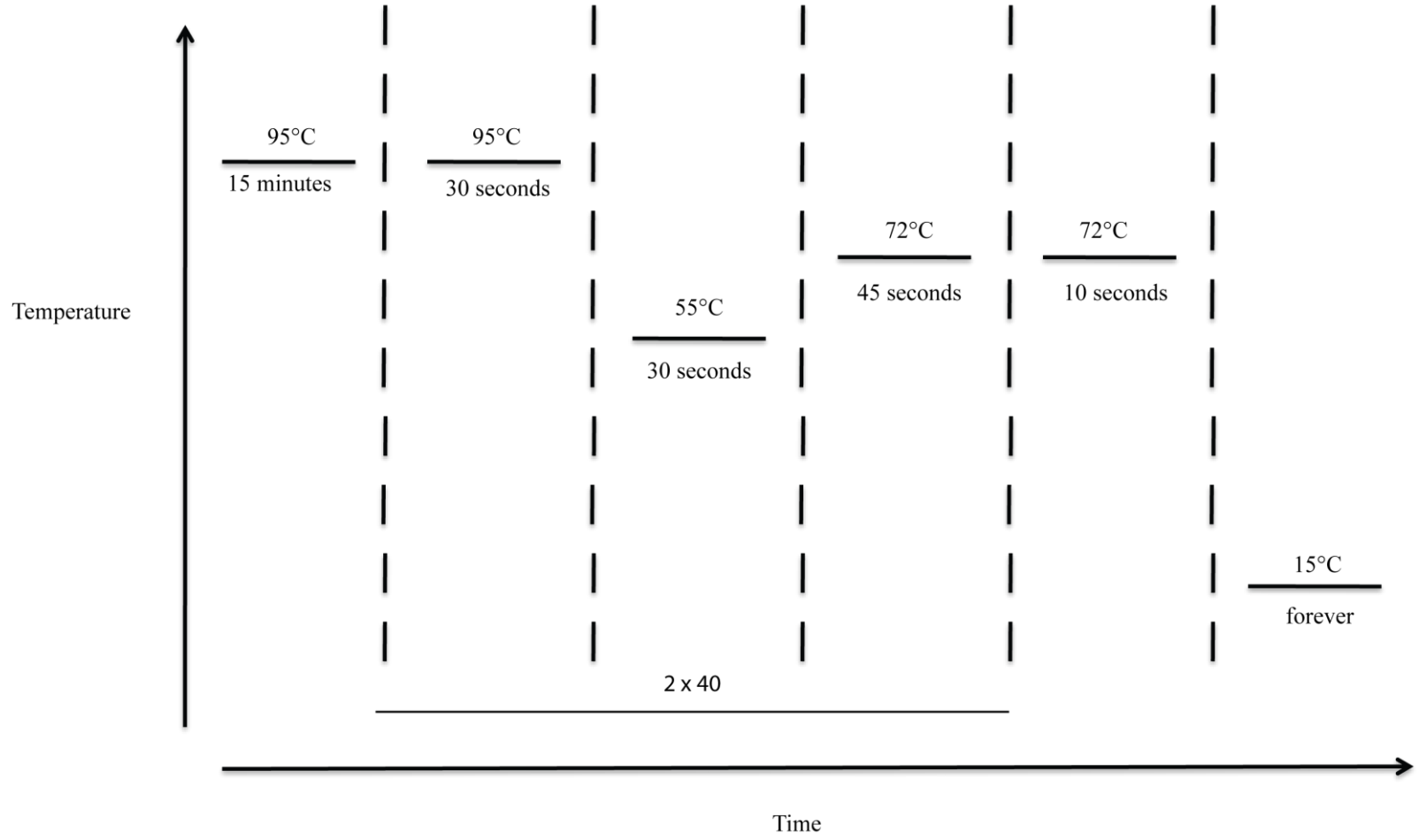


Figure 2.2 represents the changes in temperature and time spent at each stage during the PCR reaction cycle

Table 2.7: PCR conditions for *SGCE*, *SGCZ* and *GCH1*

Gene	Start temperature		Annealing temperature	Extension time	Cycles		
SGCE (exons 1-12 except exon 2)	95°C (15 minutes)	95°C (30 seconds)	55°C (30 seconds)	72°C (45 seconds)	2 x 40	72°C (10 seconds)	15°C (forever)
SGCE exon 2	95°C (15 minutes)	95°C (30 seconds)	53°C (30 seconds)	72°C (45 seconds)	2 x 34	72°C (5 minutes)	12°C (forever)
SGCZ	95°C (15 minutes)	95°C (30 seconds)	55°C (30 seconds)	72°C (45 seconds)	2 x 40	72°C (10 seconds)	15°C (forever)
GCH1	95°C (15 minutes)	95°C (30 seconds)	55°C (30 seconds)	72°C (45 seconds)	2 x 40	72°C (10 seconds)	15°C (forever)

Table 2.8: Touchdown PCR conditions for *TOR1A*, *THAP1* and *NKX2-1* genes

Gene	Start temperature		Step 1		Cycles	Step 2			Cycles		
TOR1A	95°C (15 minutes)	95°C (30 seconds)	59°C (45 seconds)	72°C (2 minutes)	2 x 15	95°C (30 seconds)	52°C (45 seconds)	72°C (2 minutes)	2 x 20	72°C (2 minutes)	12°C (forever)
THAP1 (exons 1a-3f)	95°C (15 minutes)	95°C (30 seconds)	60°C (45 seconds)	72°C (1 minute)	2 x 5	95°C (30 seconds)	55°C (45 seconds)	72°C (1 minute)	6 x 30	72°C (10 minutes)	15°C (forever)
THAP1 (exon 3b)	95°C (15 minutes)	95°C (1 minute)	65°C (1 minute)	72°C (2:30 minutes)	2 x 5	95°C (1 minute)	60°C (1 minute)	72°C (2:50 minutes)	6 x 30	72°C (10 minutes)	15°C (forever)
NKX2-1 (exon 2)	95°C (15 minutes)	95°C (30 seconds)	60°C (45 seconds)	72°C (2 minutes)	2 x 15	95°C (30 seconds)	52°C (45 seconds)	72°C (2 minutes)	6 x 20	72°C (7 minutes)	12°C (forever)
NKX2-1 (exon 3a)	95°C (15 minutes)	95°C (30 seconds)	62°C (45 seconds)	72°C (2 minutes)	2 x 15	95°C (30 seconds)	52°C (45 seconds)	72°C (2 minutes)	6 x 20	72°C (7 minutes)	12°C (forever)
NKX2-1 (exon 3b)	95°C (15 minutes)	95°C (30 seconds)	57°C (45 seconds)	72°C (2 minutes)	2 x 15	95°C (30 seconds)	52°C (45 seconds)	72°C (2 minutes)	6 x 20	72°C (7 minutes)	12°C (forever)

2.5.5 Agarose Gel Electrophoresis

PCR products were run on ethidium bromide containing agarose gels in order to confirm successful amplification of the appropriate DNA target sequence. Ethidium bromide intercalates DNA molecules, allowing for visualization using UV light. To make a 2% agarose gel, 2g of agarose powder was dissolved in 100ml 10X TBE buffer and heated in a 950 Watts microwave at maximum heat for 90 seconds. The solution was briefly cooled, 1µl of EtBr added to the liquid agar and then the combined solution left to set in a gel mould for 20 minutes. One microlitre of each PCR product was then mixed with 1µl of bromophenol blue loading dye, allowing visualization of the speed of electrophoresis. Two microlitres of a 1kb Plus DNA ladder was also added to the first well of each row. The gel was then run in a gel tank filled with 10X TBE buffer at 120 Volts for 30 minutes or until the loading dye had reached the bottom of each column. The gel was then transferred to the UV light box and photographed using the Kodak camera. The image was viewed and saved for later use with the Kodak digital science ID software. The PCR amplification process was considered successful if only one well-defined band was visible and the same size as the DNA sequence of interest as compared to the DNA ladder.

2.5.6 PCR/Sequencing Product Purification

PCR products were purified using Agencourt Ampure reagent on a Biomek robot using an automated program. This technique is based on solid phase reversible immobilization. Eighteen microlitres of Ampure reagent containing para-magnetic carboxyl-coated beads was added to 10µl of PCR product and incubated for 5 minutes. This allows binding of the PCR amplicons to the magnetic beads. The robot then moves the plate to a central magnet, magnetizing the beads to the bottom of each well and allowing separation of the amplicons from potential contaminants e.g. primers, primer dimers, unused dNTPs and salts. While on the magnet, the amplicons are washed with 150µl of 70% ethanol twice over for a total of 10 minutes. The amplicons are then eluted from the beads using 100µl of sterile water for 5 minutes and 90µl of the clean aqueous PCR product transferred to a new plate.

The purification of the sequencing products of the Big Dye sequencing reaction is similar to that described above. This requires use of a different automated program on the Biomek robot, during which 10µl CleanSEQ reagent is added to 10µl of the sequencing product and incubated for 3 minutes. The products from this reaction are

then washed twice with 70% ethanol and then eluted with sterile water. Eighty microlitres of the clean aqueous product is then transferred to a new plate.

2.5.7 Cycle Sequencing

This is used for elucidation of DNA sequence reads and the detection of point or frameshift mutations.[267] Amplified single stranded target DNA is used as a template and extended by a DNA polymerase using chain termination agents that mimic the four dNTPs but lack the hydroxyl group at the 3' position of the deoxyribose sugar. Incorporation of the termination agent prevents the polymerase continuing DNA extension, terminating the reaction. This allows single DNA strands to be formed that differ from each other by one nucleotide at the end. The terminator agents are fluorescently labeled allowing the last base of all fragments to be visualized using the ABI sequencing machine. Sequencing conditions used in these experiments are detailed in Table 2.9.

Table 2.9: Thermacycler conditions for sequencing reaction

Start temperature				Cycles	
95°C (2 minutes)	95°C (10 seconds)	50°C (5 seconds)	60°C (4 minutes)	2 x 25	15°C (forever)
	Stages repeated during each cycle				

2.5.8 Sequencing Reaction

The sequencing reaction mixture was made using the BigDye Terminator v3.1 Cycle sequencing kit in a reaction volume of 10µl. The mix contained 5µl of Ampure-cleaned PCR product, 0.25µl BigDye Ready Reaction premix, 0.125µl of either forward or reverse primers at a concentration of 3.2pmol/µl, 1µl of sequencing buffer and 3.5µl of sterile water. The standard cycling parameters of the automated thermal cyclers are detailed in Table 2.9.

2.5.9 Automated ABI sequencing

Following sequencing product clean up, the samples were loaded into the ABI3100 Prism Genetic Analyser capillary sequencer, analyzing the samples in sets of 16. Once in the sequencer, each sample was electrophoresed and the last base of each DNA fragment being detected using laser fluorescence. Each of the four bases emit a

different wavelength of fluorescence and the fragments are then sorted by size and the sequence of bases deduced by inbuilt software.

2.5.10 Sequence Analysis

Sequences obtained from the ABI sequencing machine were analysed using the Lasergene SeqMan Pro software. Using a reference sequence obtained from the UCSC genome browser, the sequences were then assembled into 'contigs'. Variants were identified by a quality score, calculated by the program, and visual inspection and comparison of the DNA chromatograms. All putative sequence variants were compared against known mutations and SNPs found on dbSNP and The 1000 Genomes Project.

All PCR and sequencing was performed by myself with the exception of case 20 (Family XIV). DNA from this case had been analysed previously by Dr Adrian Waite.

2.5.11 Multiples Ligation-dependent Probe Amplification

MLPA reactions were performed using commercially available kits from MRC Holland. This technique uses multiplex PCR and capillary electrophoresis to detect small CNVs in specific genes. Target DNA is denatured and a pair of specifically designed MLPA probes in the SALSA MLPA kits are hybridized to the target sequence. Successful ligation of both hybridization probes is necessary for PCR amplification using universal primers. Using capillary electrophoresis the PCR products are separated and the peak patterns compared to a reference sample. If the hybridization probes fail to successfully ligate, this is seen in a reduced sample peak to reference peak ratio, suggesting the presence of a deletion. A normal sample will have a peak ratio of 1 with a recommended range of 0.7 to 1.3. A heterozygous deletion will have a peak ratio of 0.5 (range: 0.3-0.7) while a homozygous deletion will have a peak ratio of 0. Heterozygous and homozygous duplications will have peak ratios of 1.5 (range: 1.3-1.7) and 2 (range: 1.7-2.3) respectively. The size of any deletion or duplication detected was then analysed using a custom oligonucleotide CGH array platform (Roche Nimblegen) with 5900 probes covering chr7:88,000,000-98,000,000 (NCBI36/hg18 genome build). Data was analyzed using the segment tool and visualized using SignalMap (Roche Nimblegen).

CHAPTER 3

Motor characteristics of MDS cohort

3.1 Introduction

As discussed in Chapter 1 Myoclonus Dystonia Syndrome (MDS) is a rare hyperkinetic movement disorder usually with onset in the first two decades of life. The typical clinical pattern is of alcohol responsive myoclonus in the trunk and upper limbs with cervical dystonia and/or writer's cramp.[195, 268] This disorder affects males and females equally[184] and is clinically consistent across ethnicities.[47, 186, 189]

Following a number of linkage studies, positional cloning techniques identified that *SGCE* mutations were causative in a number of MDS cases.[212] The rate at which these mutations occur in MDS populations varies significantly between published reports, ranging from 0[217] to 21-80%[186, 188, 269, 270] Explanations for the apparent variation in mutation rates include occult copy number variants (CNVs) in cases studied with Sanger sequencing and genetic heterogeneity. Linkage analysis in a single family identified a region on chromosome 18 but despite sequencing all known genes in this region, a causative gene has not been identified.[218, 219] A number of contiguous gene deletion cases involving *SGCE* have been documented and described, this will be discussed in further detail in Chapter 5. Genetic analysis of *SGCE* does however allow comparison between *SGCE* positive and negative cases.

This section of the study examines the nature and distribution of movement disorder symptoms in a population with clinically suspected MDS. This has included combined cross-sectional and longitudinal data to allow assessment of presenting symptomatology, disease progression, efficacy of therapeutic interventions and impact upon quality of life.

3.2 Diagnostic criteria

Multiple attempts have been made at establishing MDS diagnostic criteria as understanding of this complex disorder has improved. The first of these more recent updates was by Asmus & Gasser[193] which attempted to take into account a number of preceding proposals.[271-273]

Table 3.1 Diagnostic criteria proposed by Asmus & Gasser[193]

Brief, "lightning-like" myoclonus as a primary feature; focal or segmental dystonia of subtle to marked severity may also be seen but is rarely sole feature
Autosomal-dominant inheritance with incomplete penetrance and variable expressivity; in *SGCE* mutation cases suppression of phenotype upon maternal transmission or "pseudo-sporadic" inheritance
Onset usually in the first or second decade
Exclusion of additional neurologic features e.g. cerebellar ataxia, spasticity, dementia and seizures
No structural abnormalities in cranial imaging, normal EEG and somatosensory evoked potentials
Usually benign clinical course with no progression of symptoms, normal life expectancy but great social stigmatization

Following the identification of *SGCE* mutations in a proportion of MDS cases, Grunewald et al attempted to refine the diagnostic criteria by allowing sub-grouping of patients according to their clinical probability of having a mutation into 'definite', 'probable' and 'possible' groups. This was not only a useful research tool but also provided indirect guidelines for those most likely to harbour a *SGCE* mutation and therefore, in whom genetic testing would be most clinically pertinent.[259]

Table 3.2 Classification criteria of MDS phenotype by Grunewald et al[259]

Description	Phenotype
Definite	Early-onset myoclonus and dystonia OR Isolated myoclonus predominantly in upper body AND Positive family history
Probable	Early onset myoclonus and dystonia OR Isolated myoclonus predominantly in upper body
Possible	"jerky dystonia" of neck OR Isolated jerky movements of variable distribution OR Signs of dystonia and/or myoclonus in lower body half OR No response to alcohol

Following the development of the initial criteria two cases of co-morbid MDS and epilepsy with associated EEG abnormalities were reported.[274, 275] This together with a number of electrophysiological findings[276, 277] led to revised diagnostic criteria in 2009.[194] These attempted to include the broader clinical requirements, in particular any features that required immediate exclusion, while retaining the elements introduced in Grunewald's 'definite' category.

Table 3.3 Diagnostic criteria proposed by Kinugawa et al[194]

Diagnostic criteria for definite MDS
Early onset (<20 years)
Myoclonus predominantly in the upper body, either isolated or associated with dystonia
Positive family history with paternal transmission
Exclusion of additional neurologic features e.g. cerebellar ataxia, spasticity and dementia
Normal brain MRI
Additional suggestive features
Short myoclonic bursts (25-250ms) without cortical pre-myoclonic potential: negative C-reflex response and lack of giant somatosensory evoked potentials
Spontaneous remission of limb dystonia during childhood or adolescence
Alcohol responsiveness

3.3 Patients and methods

3.3.1 Patients

Patients were recruited via adult and paediatric movement disorder specialists throughout the United Kingdom (UK) and Ireland from 2009 to 2012 (Figure 3.1). Criteria for referral included:

- known cases of MDS with confirmed *SGCE* mutations
- suspected MDS cases with no *SGCE* mutation following direct DNA sequencing
- suspected MDS cases not yet tested for *SGCE* mutations
- any prospective case of clinically suspected MDS

Figure 3.1: Patient recruitment sites throughout UK and Ireland

Patients were also recruited via the British Neurological Surveillance Unit (BNSU), an electronic system by which Neurology consultants throughout the UK are able to notify a central registry of known MDS cases. This registry is run by the Association of British Neurologists (ABN) and subject to ethical approval (Section 2.2)

Patients were evaluated by two methods (Figure 3.2). In the first specialists were asked to identify those patients meeting the study inclusion criteria and inform the patients, or in the case of children, their parents of the ongoing study. Contact details of those interested in participating or those requiring further information were given to the research team in Cardiff, provided consent/assent had been given by the patient/parent/guardian. After obtaining informed consent or consultee assent, patients underwent a face-to-face clinical evaluation either in hospital or their home. In this setting the patient/parent/guardian were asked to invite any other family members,

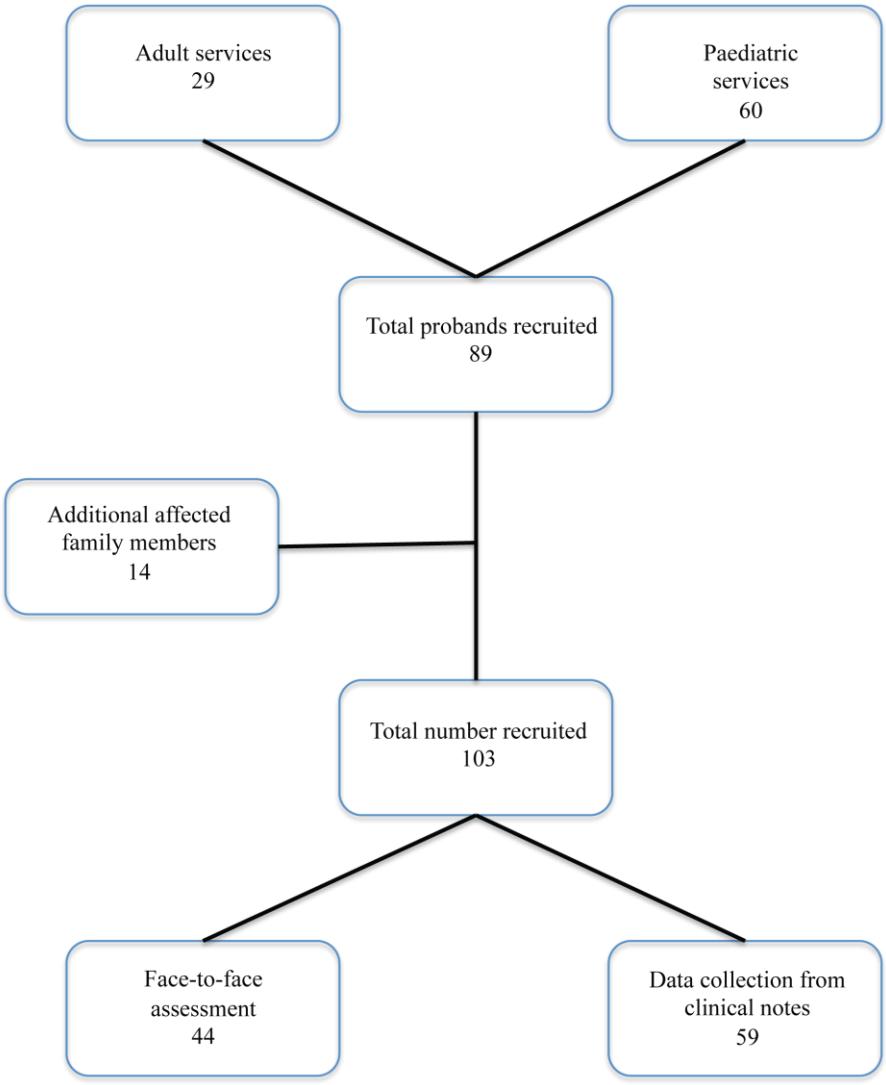
affected or unaffected by a movement disorder, who were willing to participate and have a face-to-face evaluation (Section 2.1.1).

The second means of evaluation again involved identification of suspected MDS cases by the movement disorder specialist. The patient/parent/guardian were then informed of the ongoing study and consent/assent obtained for a blood sample to be taken, DNA extracted and the sample to be sent to the research laboratory in Cardiff for *SGCE* sequencing. Genetic sequencing reports were issued to the referring clinician for all samples received. In those where an *SGCE* mutation was identified, patients were asked if they were willing to undergo face-to-face evaluation via their referring consultant. Individuals in whom consent/assent was obtained underwent assessment either in hospital or their home in the same manner as the first group. For those not undergoing face-to-face assessment, clinical details were collated by retrospective data collection from the clinical notes using a systematic protocol (Section 2.3.2) (Appendix B.3).

DNA samples from all participants underwent *SGCE* sequencing, in those where no mutation was identified Multiplex Ligation-dependent Probe Amplification (MLPA) analysis was performed using the commercially available probe set P099B (MRC Holland, Amsterdam, The Netherlands) according to manufacturer's instructions to identify CNVs involving *SGCE*. In those samples where no *SGCE* mutations were identified further sequencing of *TOR1A* (GAG deletion), *GCH1, THAP1* and *NKX2-1,* was performed.

A standardized data collection protocol was used to document clinical and investigative results, and data was stored in a dedicated database in accordance with the Data Protection Act, 1998. The project was approved by the Multicentre Research Ethics Committee (MREC) for Wales and NHS Research and Development office (section 2.3.5).

Figure 3.2 Summary of recruitment and assessment methods



3.3.2 Neurological evaluation

Neurological history included questions relating to age, type of movement disorder (myoclonus, dystonia, tremor, chorea, tics) and body parts affected at onset as described by a movement disorder specialist. These features were revisited at time of evaluation to determine any change in the type of movement disorder, progression in severity or number of body parts involved.

Information regarding medical co-morbidities including hypertension, hypercholesterolaemia, hyperthyroidism, hypothyroidism, insulin dependent diabetes mellitus (IDDM), non-insulin dependent diabetes mellitus (NIDDM) and epilepsy was

documented on a two-point scale (0 = absent, 1 = present). Past medical history questions were directed towards determining risk factors that may relate to a secondary dystonia, these included; complications during pregnancy or delivery, cerebrovascular accident (CVA), prior central nervous system (CNS) infections e.g. meningitis or encephalitis, CNS tumour, mitochondrial disorder, heredo-degenerative or metabolic disorder, previous head or peripheral trauma, neuropathy, prior general anaesthetic or previous neuroleptic or dopamine agonist exposure, again documented as absent (0) or present (1).

Past and present drug treatments and their efficacy in the management of the movement disorder were documented. Response to alcohol consumption was also recorded, if it provided symptomatic relief the extent (% improvement) and quantity (units) required to produce this effect was also documented. Information collected regarding other potential stimulants or depressants included caffeine consumption, smoking and effect of social settings. In addition, information was sought regarding the ability to perform specific tasks including, writing, eating and drinking, washing, dressing, pouring liquids, walking and sporting activities.

A full family history was taken where possible, including drawing of an extended family tree. The number of family members affected with the same form of movement disorder was documented including the number of these individuals who were alive or deceased. Further information regarding consanguinity, place of birth and maiden names of females was also documented where available (Appendix B.3).

For those undergoing a face-to-face evaluation a systematic videotaped clinical examination was performed following informed consent from the patient or assent from parent or guardian. This was then retrospectively rated using modified forms of the Unified Myoclonus Rating Scale (UMRS)[257] and Burke-Fahn Marsden Dystonia Rating Scale (BFMDRS)[258] (Appendix B.12 and B.13)

In the cases where no face-to-face evaluation took place the same information was collected retrospectively from the clinical notes using a systematic protocol. Following data collection all patients were rated as ‘definite’, ‘probable’ or ‘possible’

in terms of their clinical likelihood of having an *SGCE* mutation as per Grunewald's diagnostic criteria.[259]

3.4 Results

3.4.1 Demographics, co-morbidities and past medical history

One hundred and three patients were assessed and underwent DNA sequencing for *SGCE* mutations. Overall, males and females were near equally distributed (52% vs 48%) with a predominance of males in those presenting over the age of 20 years (83% vs 17%) and an excess of females amongst those with an *SGCE* mutation (63% vs 37%). In the overall cohort 27 patients (19 probands) were identified as having an *SGCE* mutation, giving a mutation rate of 21% among probands. Median age at onset was 5 years across the whole cohort, reduced in those with *SGCE* mutations (3 years) and 4 years younger than the mutation negative group (7 years). Mean disease duration was highest amongst those >20 years old at the time of data collection (33.7 years) and those with an *SGCE* mutation (25.6 years).

Hypertension (4%), hypercholesterolaemia (5%) and hypothyroidism (3%) were the most common medical co-morbidities, the majority occurring in those >20 years at time of data collection. A single case of epilepsy was reported in the mutation negative group. The most common previous medical problems included a complicated pregnancy or delivery (15%) and previous general anaesthetic (24%). Gestational complications were highest amongst those with disease onset <10 years (18%) and *SGCE* mutation negative cases (17%), compared to only 7% in the mutation positive group. Two cases of head injury and single case each of CNS infection and CVA were reported prior to onset of their movement disorder in the overall cohort. Statistical comparison found a significant difference between mutation positive and negative only in relation to general anaesthetic exposure (OR=3.88, 95% CI 1.33-11.41, p=0.008).

Full details of demographic, co-morbid and past medical features of the overall cohort can be seen in Table 3.4. Analysis of the probands reduced the cohort to 89 (Table 3.5) with no significant change to the overall characteristics of each subgroup.

Table 3.4: Demographic, co-morbid and past medical features grouped by age at onset, age at data collection and *SGCE* mutation status (Whole cohort)

Feature	All	Age at onset			Age at data collection		*SGCE* mutation	
		<10 years	10-20 years	>20 years	<20 years	>20 years	Positive	Negative
Demographics								
n	103	65	21	6	59	34	27	76
Male	54 (52 %)	34 (52%)	12 (57%)	5 (83%)	33 (56%)	18 (53%)	10 (37%)	44 (58%)
Female	49 (48 %)	31 (48%)	9 (43%)	1 (17%)	26 (44%)	16 (47%)	17 (63%)	32 (42%)
Median age at onset	5	3	13	32.5	4.5	7.8	3	7
Median age at data collection	16	13	19	46	11	41	28	14
Disease duration								
Mean	15.7	7.5	16.5	12.8	5.4	33.7	25.6	11.7
<5 years	30 (29%)	22 (34%)	7 (33%)	1 (17%)	30 (51%)	1 (3%)	5 (19%)	26 (34%)
5-10 years	23 (22%)	18 (28%)	5 (24%)	1 (17%)	22 (37%)	1 (3%)	4 (15%)	17 (22%)
>10 years	39 (38%)	25 (38%)	9 (43%)	4 (67%)	7 (12%)	32 (94%)	18 (67%)	23 (30%)
Co-morbidities								
Hypertension	4 (4%)	3 (5%)	0	1 (17%)	0	4 (12%)	2 (7%)	2 (3%)
Hypercholesterolaemia	5 (5%)	3 (5%)	1 (5%)	1 (17%)	0	5 (15%)	2 (7%)	3 (4%)
Hyperthyroidism	0	0	0	0	0	0	0	0
Hypothyroidism	3 (3%)	3 (5%)	0	0	1 (2%)	2 (6%)	0	3 (4%)
Insulin dependent diabetes	1 (1%)	1 (2%)	0	0	1 (2%)	0	0	1 (1%)
Non-insulin dependent diabetes	0	0	0	0	0	0	0	0
Epilepsy	1 (1%)	1 (2%)	0	0	1 (2%)	0	0	1 (1%)
Past Medical History								
Complicated delivery/pregnancy	15 (15%)	12 (18%)	1 (5%)	1 (17%)	10 (17%)	4 (12%)	2 (7%)	13 (17%)
Cerebrovascular accident	1 (1%)	0	0	0	0	0	0	1 (1%)
CNS Infection	1 (1%)	1 (2%)	0	0	0	1 (3%)	1 (3.7%)	0
CNS tumour	0	0	0	0	0	0	0	0
Mitochondrial disorder	0	0	0	0	0	0	0	0
Heredodegenerative/metabolic disorder	0	0	0	0	0	0	0	0
Head trauma	2 (2%)	0	2 (10%)	0	1 (2%)	1 (3%)	1 (3.7%)	1 (1%)
Peripheral trauma	0	0	0	0	0	0	1 (3.7%)	0
Neuropathy	0	0	0	0	0	0	1 (3.7%)	0
General anaesthetic	25 (24%)	18 (28%)	5 (24%)	2 (33%)	8 (14%)	17 (50%)	12 (44%)**	13 (17%)**
Neuroleptic/dopamine antagonist exposure	0	0	0	0	0	0	0	0

Key: Statistical comparison between *SGCE* mutation positive and negative groups using Chi-square analysis. *=p<0.05, **p<0.01, ***p<0.001

Table 3.5: Demographic, co-morbid and past medical features grouped by age at onset, age at data collection and *SGCE* mutation status (Proband only cohort)

Feature	All	Age at onset			Age at data collection		*SGCE* mutation	
		<10 years	10-20 years	>20 years	<20 years	>20 years	Positive	Negative
Demographics								
n	89	56	20	4	56	24	19	70
Male	50 (56%)	33 (59%)	10 (50%)	4 (100%)	33 (59%)	14 (58%)	8 (42%)	42 (60%)
Female	39 (44%)	23 (41%)	10 (50%)	0	23 (41%)	10 (42%)	11 (58%)	28 (40%)
Median age at onset	5	3	13	32.5	4.5	9.3	3	7
Median age at data collection	14	11.5	17.5	45.5	11.5	40	28	14
Disease duration								
Mean	13.6	14.1	12.2	15.5	5.4	32.6	22.7	10.7
<5 years	50 (56%)	37 (66%)	12 (60%)	1 (25%)	49 (88%)	1 (4%)	7 (37%)	43 (61%)
5-10 years	13 (15%)	7 (13%)	3 (15%)	2 (50%)	7 (12%)	6 (25%)	2 (11%)	11 (16%)
>10 years	17 (19%)	12 (21%)	5 (25%)	1 (25%)	0	17 (71%)	10 (53%)	7 (10%)

3.4.2 Motor characteristics

Clinical features at onset

Within the overall cohort dystonia was the most common presenting symptom (50 cases) and chorea the least common (7 cases). The upper limbs were the most commonly affected body part across all symptom groups. However, the neck and trunk were affected in almost half of all cases with myoclonus, and more than a third of those with dystonia had lower limb involvement. Median age at onset was lowest for those presenting with dystonia (4 years) and highest in those with tremor as their initial symptom (10 years).

When groups were divided according to *SGCE* mutation status, dystonia was the most common presenting symptom and the upper limbs the most commonly affected body part in both groups. Median age at onset of dystonia was 1.5 years younger in the mutation positive compared to the mutation negative group (3 years vs. 4.5 years). Median age at onset of myoclonic symptoms was similar between the two groups (4 years vs. 4.75 years) although upper limb involvement was more common in those with an *SGCE* mutation (94% vs. 75%). No cases of tremor, chorea or tics were observed in those later found to have an *SGCE* mutation.

A summary of the reported clinical features at onset can be seen in Table 3.6.

Clinical features upon examination

Of the 103 patients in the overall cohort, only 44 were examined as part of this study (Table 3.7). Dystonia was the most common clinical finding (43 cases) although a greater proportion had evidence of myoclonus than reported at presentation. There were no patients with chorea and smaller numbers with tremor (4 cases) and tics (5 cases). The upper limbs were the most commonly affected body part across all symptom groups.

All *SGCE* mutation positive cases had evidence of myoclonus and dystonia at examination with the exception of one case, in whom, early childhood myoclonus had resolved. No chorea, tremor or tics were observed. The upper limbs were the most commonly affected body part, while truncal (65%) and cervical myoclonus and dystonia (73% and 74% respectively) were seen in over two thirds of cases. In the mutation negative group myoclonus and dystonia remained the most common symptoms, although tremor and tics were also observed in 9 cases. Upper limbs were again the most frequently affected body part, however, lower limb dystonia (38%) and cervical myoclonus (38%) were substantially lower than in the mutation positive group.

Overall myoclonus ($p<0.0001$) and dystonia ($p<0.0001$) were strongly associated with positive *SGCE* mutation status, while tics ($p=0.0007$) and tremor ($p=0.002$) were more common in mutation negative cases. Stepwise multiple logistic regression saw a significant association of both myoclonus ($p<0.001$) and dystonia ($p=0.006$) with *SGCE* mutation status.

Table 3.6: Clinical symptoms and distribution at onset

Feature	All	*SGCE* mutation	
		Positive	Negative
Clinical features at onset			
Myoclonus			
n	34	18	16
Median age at onset (range)	4.5 (0-50)	4 (1.5-18)	4.75(0-15)
Neck	17 (50%)	9 (50%)	8 (50%)
Jaw	0	0	0
Voice	0	0	0
Trunk	15 (44%)	5 (28%)	8 (50%)
Upper limbs	31 (91%)	17 (94%)	12 (75%)
Lower limbs	7 (21%)	1 (6%)	5 (31%)
Dystonia			
n	50	21	29
Median age at onset (range)	4 (0-48)	3 (1.5-11)	4.5 (0-48)
Neck	14 (28%)	5 (24%)	9 (31%)
Jaw	2 (4%)	0	2 (7%)
Voice	5 (10%)	1 (5%)	4 (14%)
Trunk	1 (2%)	1 (5%)	0
Upper limbs	26 (52%)	11 (52%)	15 (52%)
Lower limbs	19 (38%)	7 (33%)	12 (41%)
Tremor			
n	15	0	15
Median age at onset (range)	10 (0.25-48)	0	10 (0.25-48)
Neck	4 (27%)	0	4 (27%)
Jaw	0	0	0
Voice	0	0	0
Trunk	0	0	0
Upper limbs	13 (87%)	0	13 (87%)
Lower limbs	1 (7%)	0	1 (6%)
Chorea			
n	7	0	7
Median age at onset (range)	4.5 (0-21)	0	4.5 (0-21)
Neck	3 (43%)	0	3 (43%)
Jaw	0	0	0
Voice	1 (14%)	0	1 (14%)
Trunk	0	0	0
Upper limbs	6 (86%)	0	6 (86%)
Lower limbs	3 (43%)	0	3 (43%)
Tics			
n	16	0	16
Median age at onset (range)	7 (0.5-14)	0	7 (0.5-14)
Face	6 (38%)	0	6 (38%)
Neck	7 (44%)		7 (44%)
Jaw	0	0	0
Voice	8 (50%)	0	8 (50%)
Trunk	1 (6%)	0	1 (6%)
Upper limbs	11 (69%)	0	11 (69%)
Lower limbs	2 (13%)	0	2 (13%)

Table 3.7: Clinical symptoms and distribution at examination

Feature	All	*SGCE* mutation	
		Positive	Negative
Clinical features at examination			
Myoclonus			
n	39	26	13
Neck	24 (62%)	19 (73%)	5 (38%)
Jaw	0	0	0
Voice	2 (5%)	0	2 (15%)
Trunk	25 (64%)	17 (65%)	8 (62%)
Upper limbs	35 (90%)	25 (96%)	11 (85%)
Lower limbs	9 (23%)	5 (19%)	3 (23%)
Dystonia			
n	43	27	16
Neck	34 (79%)	20 (74%)	13 (81%)
Jaw	1 (2%)	0	1 (6%)
Voice	10 (23%)	5 (19%)	5 (31%)
Trunk	3 (7%)	3 (11%)	0
Upper limbs	37 (86%)	22 (81%)	14 (88%)
Lower limbs	18 (42%)	12 (44%)	6 (38%)
Tremor			
n	4	0	4
Neck	4 (100%)	0	4 (100%)
Jaw	0	0	0
Voice	1 (25%)	0	1 (25%)
Trunk	0	0	0
Upper limbs	4 (100%)	0	4 (100%)
Lower limbs	1 (25%)	0	1 (25%)
Chorea			
n	0	0	0
Head	0	0	0
Jaw	0	0	0
Voice	0	0	0
Neck	0	0	0
Trunk	0	0	0
Upper limbs	0	0	0
Lower limbs	0	0	0
Tics			
n	5	0	5
Face	1 (20%)	0	1 (20%)
Jaw	0	0	0
Voice	0	0	0
Neck	0	0	0
Trunk	1 (20%)	0	1 (20%)
Upper limbs	5 (100%)	0	5 (100%)
Lower limbs	2 (40%)	0	2 (40%)

3.4.3 Therapeutics

Information regarding past and present treatments was collected from 51 patients. In the remaining 52 cases, either no medication had been tried or this data was unavailable (Table 3.8).

Overall, benzodiazepines were the most frequently prescribed form of oral medication, while primidone and tetrabenazine were the least commonly used. Trihexyphenidyl (78%) and gabapentin (67%) improved symptoms in the greatest number of cases while haloperidol most frequently worsened motor symptoms (75%). Intramuscular botulinum toxin (BT) injection was used in eight patients with symptomatic improvement being reported in six. Only a single case underwent GP_i DBS surgery resulting in substantial reduction of both myoclonic and dystonic symptoms.

Response to levodopa, benzodiazepines, carbamazepine and haloperidol treatment differed between mutation positive and negative groups. Benzodiazepines were the most frequently prescribed in both subgroups with reported improvement in the mutation negative group twice that of those with a mutation. Levodopa was prescribed four times more frequently in the mutation negative group and provided no symptomatic improvement in all mutation positive cases in which it was used. Carbamazepine improved symptoms in all mutation positive patients but caused noticeable deterioration in more than a third of those without an *SGCE* mutation. Haloperidol resulted in worsening of motor symptoms in all mutation positive cases while producing symptomatic improvement in the single *SGCE* negative case in which it was used.

3.4.4 Effects of alcohol, caffeine, smoking and social environments

Overall, alcohol consumption data was collected from 99 individuals, of which 30 (30%) were consumers of alcohol at the time of data collection (Table 3.9). The highest level of response was seen amongst those with *SGCE* mutations (100%) of which 36% exceeded the recommended weekly levels of consumption and more than half (56%) reported complete resolution of their symptoms. The amount of alcohol required to produce this effect was reported to be between 1 and 12 units. The number of individuals who

regularly consumed alcohol (OR=3.77, 95% CI 1.34 - 10.74, p=0.004), exceeded the weekly recommended intake (OR=7.96, 95% CI 1.23 - 64.37, p=0.02), found symptomatic improvement (OR=inf, 95% CI 9.59 – inf, p<0.001) and no effect (OR= 0, 95% CI 0 - 0.10, p<0.001) of alcohol consumption were significantly different between mutation positive and negative groups.

Across the whole cohort, the majority of participants reported no effect of smoking or caffeine consumption on their symptoms. Data of the impact of participating in social gatherings was collected in 40 patients. The majority (58%) noted their symptoms to deteriorate under these circumstances and was near equally distributed between mutation positive and negative groups. However, a higher number of those with an *SGCE* mutation avoided these settings as a result of their movement disorder than those without (5 vs. 1). No statistical difference between *SGCE* mutation positive and negative groups was found when comparing effects of smoking, caffeine or social settings.

3.4.5 Impact of movement disorder on day-to-day living

Data relating to the impact of the movement disorder upon tasks of day-to-day living was collected from 36 patients. Overall the most frequently affected tasks included writing, pouring liquids and eating/drinking. Writing was the most affected amongst the mutation positive group and eating/drinking and pouring liquids caused most difficulty in those without a mutation.

3.5 Utility of current diagnostic criteria

As discussed in section 3.2 a number of diagnostic criteria have been proposed and used as both clinical and research tools. Although those proposed by Kinugawa et al are the most recent, the criteria of Grunewald et al are frequently used to stratify large cohorts as to their likelihood of an *SGCE* mutation.[187] The efficacy of these criteria were calculated for this cohort, and I attempted to identify any addition clinical characteristics that could be used to increase the yield of *SGCE* mutations.

Within the ‘definite’, ‘probable’ and ‘possible’ categories of the criteria, the factor separating ‘definite’ and ‘probable’ groups is a positive family history of a similar movement disorder. In order to accurately assess this, only probands were included in the analysis. This reduced the size of the overall cohort to 89, divided into 17 definite, 8 probable and 64 possible. Following genetic analysis, 15 definite, 4 probable and 0 possible were found to have an *SGCE* mutation and 2 definite, 4 probable and 72 possible were within the mutation negative group. The mutation rates were 88% and 50% in the definite and probable categories respectively.

Applying the definite diagnostic criteria, these had a 79% sensitivity, 97% specificity and 88% positive predictive value (PPV) in anticipating an *SGCE* mutation. Combining ‘definite’ and ‘probable’ criteria increased sensitivity to 100% while reducing specificity and PPV to 91% and 76% respectively. As discussed above, age at onset was noted to be significantly different between *SGCE* positive and negative groups. As this was not a factor included in the Grunewald probabilistic stratification, I propose several modifications to the ‘definite’ criteria to reflect this (Table 3.11). Replacing a positive family history with an age at onset of symptoms ≤10 years increased sensitivity (89%) but saw reductions in specificity (94%) and PPV (81%). Option 2 allowed for the greatest flexibility, including those with either a positive family history or age at onset ≤10 years, increasing sensitivity (100%) with little change to specificity (94%) and PPV (83%). Imposing the most restrictive criteria (option 3) resulted in a marked fall in sensitivity (68%), while specificity (97%) and PPV (87%) were similar to the original criteria.

Table 3.8: Use of oral medication, Botulinum toxin and Deep Brain Stimulation

Medication	All	*SGCE* mutation	
		Positive	Negative
Beta-blockers	9	5	4
Improvement	1 (11%)	0	1 (25%)
Deterioration	1 (11%)	1 (20%)	0
No effect/Uncertain	7 (78%)	4 (80%)	3 (75%)
Levodopa	15	3	12
Improvement	1 (7%)	0	1 (8%)
Deterioration	2 (13%)	0	2 (17%)
No effect/Uncertain	12 (80%)	3 (100%)	9 (75%)
Primidone	1	1	0
Improvement	0	0	0
Deterioration	0	0	0
No effect/Uncertain	1 (100%)	1 (100%)	0
Gabapentin	6	3	3
Improvement	4 (67%)	2 (67%)	2 (67%)
Deterioration	0	0	0
No effect/Uncertain	2 (33%)	1 (33%)	1 (33%)
Benzodiazepines	26	13	13
Improvement	14 (54%)	5 (38%)	9 (69%)
Deterioration	3 (12%)	3 (23%)	0
No effect/Uncertain	9 (35%)	5 (38%)	4 (31%)
Haloperidol	4	3	1
Improvement	1 (25%)	0	1 (100%)
Deterioration	3 (75%)	3 (100%)	0
No effect/Uncertain	0	0	0
Tetrabenazine	1	1	0
Improvement	0	0	0
Deterioration	0	0	0
No effect/Uncertain	1 (100%)	1 (100%)	0
Carbamazepine	11	3	8
Improvement	4 (36%)	3 (100%)	1 (13%)
Deterioration	3 (27%)	0	3 (38%)
No effect/Uncertain	4 (36%)	0	4 (50%)
Sodium Valproate	15	6	9
Improvement	1 (7%)	0	1 (11%)
Deterioration	2 (13%)	1 (17%)	1 (11%)
No effect/Uncertain	11 (73%)	5 (83%)	7 (78%)
Leviteracetam	7	4	3
Improvement	2 (29%)	1 (25%)	1 (33%)
Deterioration	2 (29%)	1 (25%)	1 (33%)
No effect/Uncertain	3 (43%)	2 (50%)	1 (33%)
Trihexyphenidyl	9	2	7
Improvement	7 (78%)	2 (100%)	5 (71%)
Deterioration	0	0	0
No effect/Uncertain	2 (22%)	0	2 (29%)
Botulinum toxin	8	5	3
Improvement	6 (75%)	3 (60%)	3 (100%)
Deterioration	0	0	0
No effect/Uncertain	2 (25%)	2 (40%)	0
Deep brain stimulation	1	1	0
Improvement	1 (100%)	1 (100%)	0
Deterioration	0	0	0
No effect/Uncertain	0	0	0

Table 3.9: Effects of alcohol, smoking, caffeine consumption and social settings

Feature	All	*SGCE* mutation		p-value
		Positive	Negative	
Alcohol				
n (currently drink alcohol)	30	14	16	**0.004**▪
n (currently don't drink alcohol)	69	13	56	
mean volume per week/units	16.28	18.92	12.23	0.43◆
Exceed weekly recommended volume (n)*	7	5	2	**0.02**•
Improvement	18 (60%)	14 (100%)	2 (13%)	**<0.001**•
Deterioration	0	0	0	
No effect	12 (40%)	0	14 (88%)	**<0.001**•
% Improvement				
100%	10 (56%)	8 (57%)	2 (100%)	
75%	5 (%)	5 (36%)	0	
50%	2(%)	2 (13%)	0	
25%	0	0	0	
Uncertain	1 (6%)	1 (6%)	0	
Volume required to produce effect (Units)				
1-4	4 (22%)	4 (25%)	1 (50%)	
4-8	7 (39%)	6 (38%)	1 (50%)	
8-12	4 (22%)	4 (25%)	0	
12-16	2 (11%)	2 (13%)	0	
16-20	0	0	0	
>20	0	0	0	
Smoking				
n	8	6	2	
Improvement	1 (13%)	1 (17%)	0	1.00•
Deterioration	1 (13%)	1 (17%)	0	1.00•
No effect	6 (75%)	4 (67%)	2 (100%)	1.00•
Caffeine				
n	31	20	11	
Improvement	0	0	0	
Deterioration	4 (13%)	4 (20%)	0	0.27•
No effect	27 (87%)	16 (80%)	11 (100%)	0.27•
Social settings				
n	40	25	15	
Improvement	0	0	0	
Deterioration	23 (58%)	15 (60%)	8 (53%)	0.75•
No effect	10 (25%)	5 (20%)	5 (33%)	0.46•
Avoids these settings (movement disorder)	6 (15%)	5 (20%)	1 (7%)	0.38•
Avoids these settings (other cause)	1 (3%)	0	1 (7%)	0.38•

Key: *recommended maximal weekly alcohol consumption from www.drinkaware.co.uk[278]
Bold denotes statistically significant result.
p-values calculated using ▪Pearson uncorrected chi-square, ◆paired t-test, •Fisher's exact test

Table 3.10: Number of participants reporting an impact of their movement disorder upon tasks of day-to-day living

Feature	All	*SGCE* mutation	
		Positive	Negative
n	36	21	14
Tasks			
Writing	27 (75%)	18 (86%)	8 (57%)
Eating & Drinking	24 (67%)	14 (67%)	9 (64%)
Washing	13 (36%)	11 (52%)	1 (7%)
Dressing	16 (44%)	11 (52%)	4 (29%)
Pouring liquids	26 (72%)	16 (76%)	9 (64%)
Walking	15 (42%)	9 (43%)	5 (36%)
Sporting activities	15 (42%)	9 (43%)	5 (36%)

Table 3.11: Modified 'definite' diagnostic criteria for MDS

Description	Phenotype
Definite	
Option 1	Early-onset myoclonus and dystonia OR Isolated myoclonus predominantly in upper body AND Age at onset ≤10 years
Option 2	Early-onset myoclonus and dystonia OR Isolated myoclonus predominantly in upper body AND Age at onset ≤10 years OR Positive family history
Option 3	Early-onset myoclonus and dystonia OR Isolated myoclonus predominantly in upper body AND Age at onset ≤10 years AND Positive family history

3.6 Discussion

In this study patients with a suspected diagnosis of MDS have been recruited from a number of different centres, covering a large geographical area and both adult and paediatric specialties. This pragmatic approach has allowed collection of data from a large cohort, representing typical clinical practice and the types of cases in whom genetic testing is frequently considered.

Despite this there are a number of limitations to this work. Firstly, data was collected in multiple formats, either face-to-face or by retrospective data collection from the clinical notes. In addition the assessment of age at onset was retrospective, based upon patient recall. Recruitment from adult and paediatric specialists also raises potential differences in use of nomenclature, partly attributed to differing clinical experiences and exposure to demographically distinct groups.[10] Finally, a number of patients were identified as having *SGCE* mutations prior to recruitment to the study and therefore the number of patients with *SGCE* mutations within this cohort is likely to be over represented.

3.6.1 Demographic characteristics

Amongst those with an *SGCE* mutation almost two thirds were female. This finding may be related to chance, as there is no evidence at present to suggest that the disorder is sex-related.[184] Age at onset significantly differed between those with and without *SGCE* mutations, being 4 years younger amongst the mutation positive group. This supports the notion that onset of MDS is usually in early childhood and that symptom onset during later adolescence and early adulthood makes this diagnosis unlikely.

Despite recent modification to the MDS diagnostic criteria allowing inclusion of those with epilepsy and EEG changes,[194] none of those with an *SGCE* mutation within this cohort were known to have a history of seizures or associated EEG changes. This may reflect that the burden of epilepsy amongst the MDS population is relatively low, or alternatively those patients with epilepsy may initially present to epileptologists, their myoclonus attributed to their convulsions, and not reviewed by a movement disorder specialist until a later stage.

A statistical difference between mutation positive and negative cohorts was seen only with prior general anaesthetic exposure. However, during data collection it wasn't clarified as to whether the anaesthetic pre- or post-dated onset of the movement disorder and therefore should be interpreted with caution. A higher proportion of those within the mutation negative group reported gestational complications. This together with the high level of dystonia suggests that in a proportion of this group their symptoms may be secondary rather than primary.

3.6.2 Motor Characteristics

Analyzing symptoms at onset and then at a subsequent time point enables assessment of disease progression. However, no fixed time point was stipulated in this study, thus providing only limited insight into disease patterns. In addition while only signs of myoclonus and dystonia were both observed and reported in the *SGCE* mutation positive group, chorea, tremor and tics were reported in the mutation negative group. Although early pre-genetic descriptions of these patients frequently reflected clinical difficulty in segregating these movement disorders,[183] improved diagnostic criteria[194, 259] suggest that these additional movements likely indicate that a proportion of the *SGCE* mutation negative group do not meet diagnostic criteria for MDS.

Previous studies have suggested that ~20% of those with *SGCE* mutations present initially with dystonia,[184, 188, 195] substantially lower than the 78% observed in this study. However, 53% of these cases had simultaneous onset of myoclonus such that isolated dystonia was seen in only 33% of the total *SGCE* positive population. Median age at onset of dystonic symptoms was one year younger than myoclonus in the mutation positive cohort and 1.5 years younger than onset of dystonia in the mutation negative cohort. As has been described previously, this likely reflects a subgroup of *SGCE* mutation positive patients presenting at a younger age with predominantly lower limb dystonic symptoms.[279, 280]

Consistent with the recognized upper body predominant pattern of MDS,[193, 194] upper limbs were the most commonly affected body part both at onset and follow-up examination and in both *SGCE* mutation positive and negative groups. Although less frequently described, 19% of the mutation positive groups were noted to have lower

limb myoclonus at examination, similar to the estimated 25% described in other cohorts.[185, 186, 195, 197] While in the majority of cases this took the form of spontaneous myoclonus at rest, negative myoclonus, was observed while mobilizing in a single case, causing significant impairment to mobility.

Only 43% (44/103) of the overall cohort were examined at a later time point, this smaller cohort being biased towards those with an *SGCE* mutation. Within the *SGCE* mutation positive group a greater number were observed to have myoclonus and to a lesser extent, dystonia than at onset. With the exception of a few cases in which symptom progression is described,[162, 281] MDS symptoms are usually stable and compatible with a normal life span.[209, 282] The changes observed therefore likely reflect an evolution of symptoms during childhood and adolescence with a more stable clinical picture emerging in early-adult life, changes that may reflect a functional maturation of the basal ganglia pathways.[283] There are also reports of spontaneous improvement of dystonic symptoms,[195, 271] similar to that seen with primary focal dystonias, and to a lesser degree myoclonus. Despite some reports of subjective improvement no spontaneous resolution of dystonic symptoms were reported in this cohort. A single case had no evidence of myoclonus upon examination despite this being a prominent childhood feature. This is similar to the rates reported in other cohorts.[186, 284]

3.6.3 Treatment strategies

No randomized clinical trials of treatment in genetically defined MDS cohorts have been performed to date and symptomatic response to currently available medication is general poor.[183, 193] Observational studies in large cohorts and single case reports have reported treatment with benzodiazepines,[1, 39, 183, 186, 220, 272, 282, 285, 286] trihexyphenidyl,[1, 183, 287, 288] levodopa,[1, 184, 289, 290] dopamine agonists,[1, 272] serotonergic agents,[1, 272, 285] anticonvulsants (sodium valproate, leviteracetam,, primidone, piracetam, carbamazepine, gabapentin),[1, 186, 220, 284, 285, 287, 291] neuroleptics (tetrabenazine, haloperidol)[1, 272] and beta-blockers.[1, 284]

Segregating treatment responses according to *SGCE* mutation status in this study found notable differences. Trihexyphenidyl and carbamazepine were the most

beneficial in the mutation positive group, although the numbers within each group were small and should be treated with caution. A generic overview of trihexyphenidyl use in movement disorders found 80% of cases with tonic torticollis and 90% with rhythmic oscillatory movements inclusive of myoclonus, were considered to have improved clinically upon examination.[292] More specifically a single case report describes an *SGCE* mutation positive patient whose symptoms resolved with trihexyphenidyl (2mg three times daily) returning upon cessation of treatment.[293]

Haloperidol caused worsening of symptoms in all mutation positive cases in this cohort, although improved symptoms in the single mutation negative case. This suggests that use of this drug and possibly neuroleptics as a whole, should be avoided in MDS, and may aid in differentiating from other similar disorders. In our cohort benzodiazepines were prescribed equally in both mutation positive and negative groups, although a larger proportion of those without a mutation reported symptomatic improvement. Previous studies suggesting benzodiazepine responsivity were conducted pre-discovery of *SGCE* involvement and may have included a mixed cohort of those with and without mutations.[1, 183, 282, 284] Further work is required before excluding this group of drugs in the treatment paradigm, although results from this cohort suggest they could be used as an adjunctive therapy rather than a first line agent.

Non-oral treatments included intramuscular BT injections and surgical DBS treatment. BT is known to be beneficial in treatment of focal dystonias and in particular cervical dystonia,[294-297] although it often has a variable response and exact injection sites are difficult to replicate.[298] Unfortunately detailed information regarding injection sites was not collected in this cohort, although a higher proportion of the mutation negative group reported symptomatic improvement. Future studies should include more detailed information regarding injection sites, degree and duration of response. Only a single mutation positive case underwent GP_i DBS with improvement to both myoclonic and dystonic symptoms. Previous studies have suggested GP_i stimulation to be superior[299] to thalamic VIM for improvement of motor symptoms,[300] although a case series of five *SGCE* mutation positive patients found worsening of their psychiatric symptoms following GP_i surgery.[301]

3.6.4 Impact of alcohol, caffeine, smoking and social stressors

Data collection of behaviour and response to social stressors, in particular alcohol intake, was important due to the observed alcohol sensitivity amongst *SGCE* mutation positive cohorts,[191] and its inclusion in the most recent diagnostic criteria.[194] The sizeable paediatric population within this cohort limited the size of the cohort in which this data was available and its collection should be repeated in a larger adult-only cohort before drawing substantive conclusions.

All *SGCE* mutation positive consumers of alcohol reported an improvement to their motor symptoms, significantly higher than that reported in the mutation negative group and exceeding that of any other intervention. There were also a significantly greater number of those with mutations who regularly consumed alcohol and exceed the recommended weekly intake. Despite this there was no statistical difference in the mean amount of alcohol consumed per week between the two groups, suggesting that MDS is particularly alcohol sensitive and reinforces the importance of this response in clinical diagnosis. Despite this the amount of alcohol required to produce an effect was, on average, relatively small (4-8 units) although some reported that the amount required had increased over time. Anecdotally a number of mutation positive patients reported a rebound worsening of their symptoms the day following a period of heavy alcohol intake. Our recent systematic review also showed a higher rate of alcohol excess/dependence amongst NMC than NC individuals within MDS families, suggesting that excess alcohol consumption may not purely be related to therapeutic response and may, in part, be a gene independent effect.[302]

Socially stressful situations were found to have a greater impact upon the *SGCE* mutation positive rather than negative cohort, although no statistical difference was observed. This again may reflect underlying psychiatric co-morbidity and a tendency towards anxiety-related disorders. This will be reviewed in greater detail in chapter 4. It does however highlight the importance of clinician awareness of these symptoms, their details elicited during the clinical history and appropriate treatment sought when required.

3.6.5 Diagnostic criteria

Stratification of this cohort according to Grunewald's[259] probability of *SGCE* mutation positive MDS found an *SGCE* mutation rate of 88%, amongst the highest reported cohort mutation rates but consistent with those in which more stringent diagnostic criteria have been applied.[186] As *SGCE* is subjected to maternal imprinting, mutations may be 'silenced' for several generations resulting in a false negative family history. Consequently the absolute requirement of a positive family history within the diagnostic criteria will inevitably result in *SGCE* mutation cases being missed in clinical practice and accounts for the 50% mutation rate within our 'probable' cohort. Modification of the 'definite' criteria to allow age at onset ≤10 years or a positive family history allows for the flexibility required with imprinting and retains the important age at onset characteristics observed in this cohort. These changes increased sensitivity of the criteria in detecting *SGCE* mutations, suggesting that this amendment may aid identification of mutation positive cases, important when considering patients for DBS surgery and also should any disorder specific treatments become available in the future.

3.7 Conclusion

Overall *SGCE* mutations appear associated with a younger age at onset than other *SGCE* mutation negative hyperkinetic disorders. Three patterns of motor involvement were seen a) younger onset, lower limb predominant dystonia that gradually evolves to a more upper body distribution of both myoclonic and dystonic symptoms; b) upper body, myoclonus predominant symptoms of onset in later childhood and c) improvement of myoclonus in adult life with only subtle residual dystonic features. Treatment with trihexyphenidyl, carbamazepine and DBS resulted in greatest improvement to symptoms in those with a mutation while the effects of haloperidol were deleterious in all cases. Alcohol response remains an important diagnostic clue for *SGCE* mutation positive cases and a proportion of MDS patients may also consume to excess. Modification of diagnostic criteria to include a lower age at onset (≤10 years) than currently suggested (<25 years) is likely to increase the sensitivity, specificity and PPV of anticipating an *SGCE* mutation.

CHAPTER 4

Psychiatric characteristics of MDS cohort

4.1 Introduction

In addition to the motor characteristics discussed in Chapter 3, it has been suggested that psychiatric symptoms also form part of the MDS phenotype.[209, 303] A number of single family, multi-family and large cohorts have reported a range of psychiatric disorders,[186, 304] while others have described a complete absence of psychiatric symptomatology within their cohorts.[188] A broad spectrum of psychiatric disturbances have been described with depression and anxiety, including phobic and agoraphobic symptoms, being most common.[185, 303] Obsessive-Compulsive disorder (OCD) and alcohol excess have also been reported in a large number of cases.[191, 192] The former is an interesting finding as OCD is not a psychiatric sequela normally attributed as a secondary consequence in a chronic disabling disorder.[305] Given the alcohol sensitive nature of the MDS motor symptoms, alcohol excess and dependence have been thought to be secondary to its therapeutic benefits, patients 'self-medicating' to control their symptomatology.[191] Single case reports have also reported Attention Deficit Hyperactivity Disorder (ADHD),[303] anorexia nervosa,[186] schizoaffective disorder[306] and suicide[304] in conjunction with *SGCE* mutations. These findings raise the possibility that *SGCE* may have a pleiotropic function, which in its mutated form can give rise to both motor and psychiatric symptomatology.

4.2 Systematic review of previously published literature

Prior to embarking upon the standardised psychiatric assessment of our *SGCE* mutation positive cohort, I systematically reviewed previously published reports and case studies in this area.

I performed a systematic literature search in Pubmed and OVID databases. The search strategy included key words "psychiatric disorders", "depression", "major affective disorder", "unipolar depression", obsessive-compulsive disorder", "anxiety", "mania", "schizophrenia" in combination with "Myoclonus Dystonia Syndrome", "*SGCE*" and "epsilon-sarcoglycan". I also checked the reference lists of each relevant study that resulted from this search for further appropriate articles. There was no restriction on year of publication but only those published in English and in peer-reviewed journals until April 2010 were included. Those reports published can be broadly divided into single family, multiple family and larger scale studies. In order to

distinguish between competing causal explanations I divided cases into the following categories; manifesting carriers (MC), those with an *SGCE* mutation and motor symptoms, non-manifesting carriers (NMC), those with an *SGCE* mutation but no motor symptoms and non-carriers (NC), those with no *SGCE* mutation and no motor signs or symptoms.

Studies were categorized into one of two levels. Level 1 studies required the use of recognized diagnostic criteria for MDS, comparison of cases with controls (in most studies this was unaffected family members), inclusion of only those individuals/families with *SGCE* mutations and the use of standardized tools for the diagnosis of psychiatric disorders according to DSM-IV criteria. Any families/individuals reported in multiple papers were included only once, where the information was most complete. For studies to be included in the level 2 grouping the same basic set of criteria were required although use of clinical history, patient reporting or case notes were also allowed in the recognition of past or present psychiatric disorders.

DSM-IV diagnoses were divided into four principal diagnostic groups:

1) Major Affective Disorder (MAD) including depression, bipolar disorder, unipolar depression and suicide.
2) Anxiety Disorder (AD) including generalized anxiety disorder, panic attacks, social phobia, specific phobia and agoraphobia
3) Obsessive-Compulsive Disorder (OCD) (Diagnostic criteria are seen in Table 4.1)
4) Alcohol excess or alcohol dependence

There were single reports of psychosis[306] and anorexia[186] that were not included in further analyses. Both level 1 studies and combined level 1 and 2 studies were analyzed using Chi-square testing with Yates' corrected p-value.

Table 4.1: DSM-IV diagnostic criteria for obsessive-compulsive disorder[307]*

Obsessions	
	1) recurrent and persistent thoughts, impulses, or images that are experienced as intrusive and inappropriate and that cause marked anxiety or distress
	2) the thoughts, impulses or images are not simply excessive worries about real-life problems
	3) the person attempts to ignore or suppress such thoughts, impulses or images, or to neutralize them with some other thought or action
	4) the person recognizes that the obsessional thoughts, impulses or images are a product of his/her own mind (not imposed from without as in thought insertion)
Compulsions	
	1) repetitive behaviour (e.g. hand washing, ordering, checking) or metal acts (e.g. praying, counting, repeating words silently) that the person feels driven to perform in response to an obsession, or according to rules that must be applied rigidly
	2) the behaviours or mental acts are aimed at preventing or reducing distress or preventing some dreaded event or situation; however, these behaviours or mental acts either are not connected in a realistic way with what they are designed to neutralize or prevent or are clearly excessive.

* Adapted from the DSM-IV diagnostic criteria for OCD[307]

The literature review identified 22 non-duplicated publications (Table 4.1), these were classified as single family,[197, 209, 213, 275, 303, 306, 308, 309] multiple family[185, 186, 191, 192, 212, 228, 268, 310] and larger scale studies with sequential probands or multiple unrelated cases.[187, 188, 195, 311, 312] Of these, eight studies met level 1 criteria[185, 187, 192, 228, 303, 308, 311, 312] with a further three meeting level 2 criteria,[186, 212, 275] resulting in eleven being included in the final analysis (Table 4.2)

Of those meeting level 1 criteria, almost two thirds of the MC patients were found to have psychiatric symptoms (65.5%), anxiety disorders (AD) being the most common (60%) and OCD the least (26%). More than two thirds of the NMC group had no features of psychiatric disease with AD again being the most common diagnosis (6/9). Psychiatric disease was seen in a quarter of the NC cohort, more than half of whom suffered from symptoms of MAD. Combining level 1 and 2 studies found little overall change in the NMC and NC, save for a relative increase in alcohol excess/dependence amongst the NMC group (55%). Within the MC group the overall rate of psychiatric

disease fell (65.5% vs 53.1%) while AD remained the most common subtype (57%). (Table 4.3)

Psychiatric disease was three times as likely amongst the MC cohort compared to both NMC (OR=3.09, 95% CI 1.44 - 6.62, p=0.006) and NC (OR= 3.5, 95% CI 2.18 - 5.60, p<0.001) groups. When the MC cohort was compared to the motor unaffected, non-mutation carriers (NC) the greatest differences were seen with OCD and AD. OCD was ten times more likely to occur amongst MC individuals (OR=10.72, 95% CI 3.25 - 35.18, p<0.001) and AD five times more likely than in the NC group (OR=5.63, 95% CI 3.01 - 10.51, p<0.001). Statistically significant differences were also seen with both MAD (OR=2.19, 95% CI 1.22 - 3.91, p=0.012) and alcohol excess (OR=3.36, 95% CI 1.40 - 8.08, p=0.011). MAD was almost seven times more likely in MC than NMC patients (OR=6.87, 95% CI 1.71 - 26.93, p=0.008), while AD was three times more likely in the former group (OR=3.12, 95% CI 1.23 - 7.83, p=0.026). There was no difference in OCD rates between the two groups (p=0.19). No significant differences were seen between NMC and NC cohorts with the exception of excess alcohol consumption, four times more likely to occur amongst the unaffected *SGCE* mutation carriers (OR=4.03, 95% CI 1.37 - 11.87, p=0.025). (Table 4.4)

Table 4.2: Twenty-two non-duplicated publications identified by systematic literature search

Author	Year	Study type	Number of individuals				Psychiatric disease			Diagnostic tools
			Total	MC	NMC	NC	MC	NMC	NC	
Nygaard et al	1999	SF	49	10	0	39	9 Dep, Anx, OCD	-	No psych symptoms	Reported History
Zimprich et al	2001	MF	36	22	6	8	4 PhD, 1 OCD, 1 Anx, 1 Alc dep	No psych symptoms	No psych symptoms	Reported History
Klein et al	2002	SF	32	12	0	0	10 non-specified psych symptoms	No psych symptoms	No psych symptoms	Reported History
Doheny et al	2002	SF	6	3	0	3	2 Dep, 2 Anx, 1 PhD, ADHD, Alc dep, 1 No psych symptoms	-	No psych symptoms	DIGS, YBOCS
Doheny et al	2002	MF	27	12	4	12	11 Dep, 2 SA, 4 Anx/PhD, 1 PhD, 3 OCD	1 Dep, 3 SA	7 Dep, 4 SA, 1 Anx/PhD, 1 psychosis	DIGS, YBOCS
Asmus et al	2002	MF	24	24	0	0	5 PA, Dep, Ag, alc dep	-	-	Reported History
Marechal et al	2003	SF	14	6	2	6	1 Dep, 3 Anx, 2 OCD	No psych symptoms	1 Anx	MADRS, YBOCS, GARS
O'Riordan et al	2004	SF	6	4	0	2	3 alc dep	-	No psych symptoms	Reported History
Hedrich et al	2004	MF	19	6	0	13	3 Dep	-	No psych symptoms	Reported History
Asmus et al	2005	MF	11	8	1	2	1 Alc dep	1 Alc dep	No psych symptoms	CIDI
Tezenas du Montcel et al	2006	LS	76	16	0	60	No psych symptoms	-	No psych symptoms	Reported History
Misbahuddin et al	2007	SF	2	2	0	0	2 Dep, 1 Alc dep, 1 suicide	-	-	Diagnosis by Psychiatrist
Saunders-Pullman et al & Hess et al	2002 & 2007	MF	64	20	10	34	5 OCD, 4 GAD, 1 MAD, 3 Alc dep	1 OCD, 1 GAD, 1 MAD	3 GAD, 4 MAD, 4 Alc dep	CIDI
Koukouni et al	2008	SF	6	4	0	2	No psych symptoms	-	No psych symptoms	Reported History
Nardocci et al	2008	MF	20	16	0	4	4 OCD, Anx, Dep	-	No psych symptoms	Reported History

Ritz et al	2008	LS	86	13	0	73	9 Dep, 8 GAD, 2 OCD, 3 Alc dep, 1 PD	-	No psych symptoms	MINI, SCID-I
Roze et al	2008	LS	41	41	0	0	4 OCD, 2 Anx & Dep, 1 ADHD, 1 PhD	-	-	Reported History & diagnosis by Psychiatrist
Thummler et al	2009	SF	17	6	0	11	No psych symptoms	-	No psych symptoms	Reported History
Foncke et al	2009	LS	68	14	12	42	2 OCD, 2 Dep, 1 PhD, 5 SocP, 3 SP, 3 Alc dep, 2 GAD	1 OCD	1 OCD, 2 Dep, 2 SocP, 2 SP	SCID-I, BAI, AS, L-SAS, YBOCS, MADRS, BDI
Beukers et al	2009	LS	30	11	4	15	3 Dep, 2 Anx, 1 OCD	No psych symptoms	No psych symptoms	BDI, YBOCS
Wong et al	2010	SF	14	5	0	8	4 Dep or Anx, 1OCD, Anx, PhD, SA, Dep, SchAD	-	No psych symptoms	Reported History

Key: LS: Larger Study**, MF:** Multiple Family Study, **SF:** Single Family Study, **MC**: Manifesting Carriers, **NMC**: Non-manifesting carriers, **NC**: Non-carriers, **ADHD**: Attention Deficit Hyperactivity Disorder, **Ag**: Agoraphobia, **Alc dep**: Alcohol Dependence, **Anx**: Anxiety Disorder, **Dep**: Depression, **GAD**: Generalized Anxiety Disorder, **MAD**: Major Affective Disorder, **OCD**: Obsessive-Compulsive Disorder, **PD:** Personality Disorder, **PhD**: Phobic Disorder, **PTSD**: Post-Traumatic Stress Disorder, **SA**: Social Anxiety, **SocP**: Social Phobia, **SP**: Specific Phobia, **AS:** The Anxiety Scale, **BAI**: Beck Anxiety Inventory, **BDI**: Beck Depression Inventory, **CIDI**: Composite International Diagnostic Interview, **DIGS:** Diagnostic interview for Genetic Studies, **GARS**: Gilliam Autism Rating Scale, **L-SAS:** Liebowitz Social Anxiety Scale, **MADRS**: Montgomery-Asberg Depression Rating Scale, **MINI**: The Mini-International Neuropsychiatric Interview, **SCID-I**: Structured Clinical Interview for DSM disorders, **YBOCS:** Yale-Brown Obsessive Compulsive scale.

Table 4.3: Studies meeting Level 1 and Level 2 inclusion criteria

Author	Year	Type of Study	Number of cases		Diagnostic Tools
			SGCE mutation	No *SGCE* mutation	
Level 1 criteria					
Doheny et al	2002	Single family	3	3	DIGS, YBOCS
Doheny et al	2002	Multiple family	16	11	DIGS, YBOCS
Marechal et al	2003	Single family	6	6	MADRS, YBOCS, GARS
Asmus et al	2005	Multiple family	5	2	CIDI
Hess et al	2007	Multiple family	30	34	CIDI, Diagnosis made by Psychiatrist, SCID-I, BAI
Beukers et al	2009	Large scale	15	15	BDI, YBOCS
Foncke et al	2009	Large scale	27	42	MADRS
Ritz et al	2009	Large scale	13	73	SCID-I, MINI
Level 2 criteria					
Zimprich et al	2001	Multiple family	11	0	Reported History
O'Riordan et al	2004	Single family	4	2	Reported History
Nardocci et al	2008	Multiple family	16	4	Reported History

Key: BAI: Becks Anxiety Inventory, **BDI:** Becks Depression Inventory, **CIDI:** Composite International Diagnostic Interview, **DIGS:** Diagnostic Interview for Genetic Studies, **GARS:** Gilliam Autism Rating Scale, **MADRS:** Montgomery-Asberg Dperession Rating Scale, **MINI:** MINI International Neuropsychiatric Interview, **SCID-I:** Structured Clinical Interview for DSM Disorders Part I, **YBOCS:** Yale-Brown Obsessive-Compulsive Scale.

Table 4.4: Distribution of psychiatric disorders in clinical studies of *SGCE* cases and families

Clinical categories	Total number of cases						
	All psychiatric disorders	MAD	AD	OCD	Alcohol dep/excess	No psychiatric disorders	Total numbers
Level 1							
MC	57 (66%)	29 (51%)	34 (60%)	15 (26%)	14 (25%)	30 (34%)	87
NMC	9 (27%)	2 (22%)	6 (67%)	2 (22%)	4 (44%)	24 (73%)	33
NC	48 (26%)	27 (56%)	17 (35%)	3 (6%)	8 (17%)	138 (74%)	186
Level 1 + 2							
MC	68 (53%)	29 (43%)	39 (57%)	16 (24%)	14 (21%)	60 (47%)	128
NMC	11 (27%)	2 (18%)	6 (55%)	2 (11%)	6 (55%)	30 (73%)	41
NC	49 (25%)	27 (55%)	17 (35%)	3 (6%)	8 (16%)	151 (75%)	200

Key: MC: Manifesting Carriers, **NMC:** Non-manifesting Carriers, **NC:** Non-Carriers, **MAD:** Major Affective Disorder, **AD:** Anxiety Disorder, **Alcohol dep/excess:** Alcohol dependence/excess, **OCD:** Obsessive-Compulsive Disorder. Note that affected individuals may have more than one psychiatric diagnosis.

Table 4.5: Comparison of psychiatric disorders in manifesting carriers, non-manifesting carriers and non-carriers of *SGCE* mutations

Subgroup analysis	Yates corrected p-value	Odds Ratio	95% Confidence interval
MC vs NC			
All psychiatric disorders	**<0.001⬥**	3.5	(2.18, 5.60)
MAD	**0.012⬥**	2.19	(1.22, 3.91)
AD	**<0.001⬥**	5.63	(3.01, 10.51)
OCD	**<0.001⬥**	10.72	(3.25, 35.18)
ETOH dependence/excess	**0.011⬥**	3.36	(1.40, 8.08)
MC vs NMC			
All psychiatric disorders	**0.006⬥**	3.09	(1.44, 6.62)
MAD	**0.008⬥**	6.81	(1.71, 26.93)
AD	**0.026⬥**	3.12	(1.23, 7.83)
OCD	0.19	3.25	(0.79, 13.21)
ETOH dependence/excess	0.94	0.83	(0.31, 2.26)
NMC Vs NC			
All psychiatric disorders	0.91	1.13	(0.53, 2.40)
MAD	1	0.96	(0.31, 2.70)
AD	1	0.95	(0.28, 3.25)
OCD	0.45	3.3	(0.64, 17.15)
ETOH dependence/excess	**0.025⬥**	4.03	(1.37, 11.87)

Key: MC = Manifesting Carrier, **NMC** = Non-manifesting Carrier, **NC**=Non-carrier, **ETOH** = alcohol, ⬥= statistical significance

Overall review of previously published data suggests an increased rate of psychiatric disease amongst *SGCE* mutation positive MDS patients compared to both NMC and NC groups. OCD was the disorder that showed the greatest difference between MC and unaffected NMC and NC groups. Interestingly, despite its therapeutic benefits amongst MDS patients no statistical difference in excess alcohol consumption was seen between MC and NMC groups, although it was four times more likely amongst the NMC cohort than their unaffected, mutation negative relatives (NC). This finding suggests that alcohol consumption may not be purely therapeutic and that mutations in the *SGCE* gene increase the rate of addiction.

In summary, previously published data supports a psychiatric component to the MDS phenotype. However, previous studies have generally involved small numbers of patients, either individual case reports or small case series. They have used differing methods of assessment and none have compared to a control group, indicating the need for further systematic evaluation.

4.3 Patients and methods

4.3.1 Patients

Original recruitment of patients was as discussed in chapter 2 (Section 2.2 and Figure 2.1). Those MDS patients identified as having an *SGCE* mutation then underwent further face-to-face psychiatric and alcohol use evaluation. Where this was not possible details were collected from case notes including psychiatric review. Systematic questionnaires were used to determine if there had been any previous and current contact with psychiatric services (e.g. Psychiatrists, Psychologists, General Practitioners, Community Psychiatric Nurse (CPN), Support groups and others (inclusive of counsellors and therapists)). Further details were sought in relation to alcohol consumption including whether the individual was a current consumer of alcohol, how much they drank on a weekly basis (units), and whether there was a previous history of excessive alcohol consumption (Appendix B.3).

Further psychiatric assessment included the use of standardized and systematic questionnaires designed to assess both axis-I (clinical disorders, including major

mental disorders) and axis-II (personality disorders) disorders. Those used for axis-I disorders included a modified version of the MINI International Neuropsychiatric Interview (MINI) (Appendix B.8), which allowed symptoms to be classified according to DSM-IV criteria for major depressive episode, manic and hypomanic episode, panic disorder, agoraphobia, social phobia, OCD, alcohol dependence, alcohol abuse, psychotic and mood disorders, and generalized anxiety disorder (GAD). For those under 18yrs of age the M.I.N.I. Kids for Children and Adolescence (Parent version) was used (Appendix B.9). Further assessment of these symptoms was made using the Patient Health Questionnaire-9 (PHQ-9)[249] (Appendix B.5), Montgomery-Asberg Depression Rating Scale (MADRS)[256] (Appendix B.12), Yale-Brown Obsessive-Compulsive Scale (YBOCS)[254, 255] (Appendix B.11) and the Alcohol Use Disorders Identification Test (AUDIT) (Appendix B.10).[253] Axis-II disorders were assessed using the Structured Clinical Interview for DSM-IV Axis-II Personality Disorders (SCID-II) (Appendix B.6).[250] None of the latter questionnaires were completed by individuals under 18 years of age. Quality of life was assessed using the Short Form Health Survey (SF-36)[251] (Appendix B.7) and the severity of the movement disorder retrospectively rated following videotaped clinical examination using modified forms of the Unified Myoclonus Rating Scale (UMRS)[257] (Appendix B.13) and Burke-Fahn Marsden Dystonia Rating Scale (BFMDRS) (Appendix B.14).[258] Population estimates for DSM-IV diagnoses were taken from the North American National Comorbidity Survey [313] and the Dunedin Longitudinal Birth Cohort Study, [314] both large comprehensive epidemiological studies.

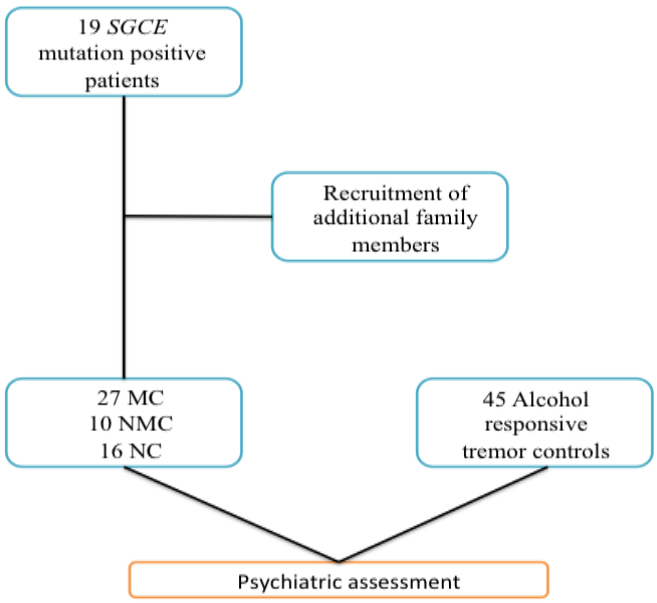
All cases with *SGCE* mutations were asked to contact additional family members to invite them to take part. These cases were assessed face-to-face using the same protocol and a blood sample taken for genetic analysis. As in other studies and the systematic review detailed above (section 4.2), all *SGCE* mutation positive patients and family members were classified according to their motor and genetic status into 3 groups: 1) manifesting carriers (MC): *SGCE* mutation and movement disorder (n=27); 2) non-manifesting carriers (NMC): *SGCE* mutation and no movement disorder (n=10); 3) non-carriers (NC): neither *SGCE* mutation nor movement disorder (n=16). Patients with tremor who reported an improvement with alcohol were recruited from general neurology and movement disorders clinics (n=45). This group of patients formed the control group for assessment of psychiatric co-morbidity (Figure 4.1), and

were examined using the same protocol described above. MC and the tremor control groups were matched for symptom severity based upon quality of life scores, assessed with the SF-36.

4.3.2 Statistical analysis

Results were analyzed using the 'R' statistical software package. Chi squared testing, Pearson correlation coefficient and binomial stepwise multiple logistic regression methods of analysis were used as appropriate. No correction for multiple testing was performed.

Figure 4.1 Methods of recruitment for psychiatric assessment



4.4 Results

Data were collected from 27 MDS (MC) patients from 11 families and 45 control cases of alcohol responsive tremor. A further 26 clinically unaffected family members were recruited from the MDS families, 10 NMC and 16 NC, the latter group including 10 married-in spouses. MC and tremor groups were matched for disability based upon median SF-36 scores, sex, disease duration and alcohol use, although they differed significantly in age at onset and age at examination ($p<0.001$) (Table 4.5).

Table 4.6: Demographics and analysis of variables

	MC	MC (>18yrs)	NMC	NC	Tremor	MC vs. Tremor (Total population)	MC vs. Tremor (Adult only population)
Total (M:F)	27 (10:7)	18 (6:12)	10 (6:4)	16 (5:11)	45 (14:31)	0.62[a]	0.59[a]
Median age at examination (range)	28 (3-74)	42.5 (18-74)	38 (29-73)	40 (16-71)	62 (19-88)	<0.001[b]	<0.001[b]
Median age at onset of movement disorder (range)	3 (1.5-18)	4.5 (1.5-18)	-	-	26.5 (3-76)	<0.001[b]	<0.001[b]
Mean duration of movement disorder	25.57	31.93	-	-	30.78	0.27[b]	0.79[b]
Alcohol consumption (%)[*]	78	78	90	59	71	0.78[a]	0.78[a]
Median SF-36 scores (range)	92 (38-111)	92 (38-111)	104 (97-106)	101 (95-118)	99 (64-125)	0.09[a]	0.09[a]

Key: MC: Manifesting Carriers, NMC: Non-manifesting carriers, NC: Non-carriers

[a] Calculated using chi-squared analysis
[b] Calculated using Student's t-test
[*] Alcohol consumption refers to whether the participant drinks any alcohol at all

4.4.1 MINI International Neuropsychiatric Interview (MINI)

M.I.N.I. questionnaires were completed by all participants with the exception of one patient <18 years where assent was declined and two adult cases; one who declined to complete the questionnaire and the second who was considered to be too unwell. Both adult cases had recently been seen by consultant psychiatrists confirming depression and anxiety in one and schizophrenia in the other.

Overall rates of psychiatric disorders were higher in MC and tremor cohorts compared to population estimates (77.8% and 62.2% vs 48%), this pattern being seen across all subgroups of psychiatric morbidity (Table 4.6). The largest differences were seen in rates of OCD and GAD. More than half of both the adult and paediatric MC cases had symptoms of OCD, occurring three times more frequently than in controls. Within the MC group, compulsive symptoms were greater than three times more common than obsessions and four times higher than observed in the tremor cohort. Rates of GAD were almost ten times higher in the MC population than population based estimates and seven times that of the tremor controls (48.1% vs 20%). Interestingly nearly a third of all NC cases were also observed to have features of generalized anxiety.

No cases of alcohol excess/dependence were seen in NMC and NC groups, and despite both groups experiencing therapeutic benefit, excessive use was more than twice as common in MC than tremor cohorts, increasing still further once cases under 18 years of age at time of data collection were excluded from analysis. Other anxiety related disorders (panic disorder, agoraphobia and social phobia) were seen at higher rates amongst MC cases than both familial and tremor control groups. Of interest, all three disorders were higher in tremor than NC populations, with panic disorder being the most common (37.8%). Although more than twice as common as population values, rates of major depressive disorder were similar across all cohorts, ranging in prevalence between 40% and 56.3%.

There was no overall excess of psychiatric disorders amongst MC patients compared to tremor controls (OR=2.13, 95% CI 0.64 - 7.31, p=0.20). However there were large differences in the rates of OCD (OR=6.7, 95% CI 2.02 - 23.27, p=0.001), GAD (OR=3.71, 95% CI 1.16 - 12.22, p=0.02) and social phobia (OR=3.7, 95% CI 1.12 -

12.56, p=0.03). Alcohol excess/dependence was also more common amongst adult MC patients (OR=4.3, 95% CI 1.08 - 18.06, p=0.02). As with the tremor control group, no overall difference in rates of psychiatric disease was seen between the MC group and unaffected familial controls (NC) (OR=1.59, 95% CI 0.32, 7.92, p=0.72). However, statistically significant differences between the two groups were again seen with OCD and alcohol excess. The largest difference was seen with OCD, almost eight times more likely amongst the MC population (OR=7.7, 95% CI 1.4, 46.52, p=0.009).

Attempts to discern a gene effect were made by comparing NMC cases to both tremor and MC cohorts. No overall or disorder specific difference was seen between NMC and tremor groups. The same pattern of increased psychiatric morbidity was seen when the MC group was compared to the NMC group as had been seen when the MC group had been compared with the tremor cohort (Table 4.7), although when the MC and NMC groups were compared the overall rate of psychiatric disorders was higher in the former (OR=5.25, 95% CI 0.88 - 34.12, p=0.05).

Stepwise multivariable logistic regression analysis found duration of motor symptoms (p=0.04), but not age at onset (p=0.20) increased the risk of psychiatric disorders. However, neither age at onset (p=0.23) nor motor disease duration (p=0.05) were found to increase the risk of OCD.

Table 4.7: Rates of psychiatric disorders determined by the MINI questionnaire

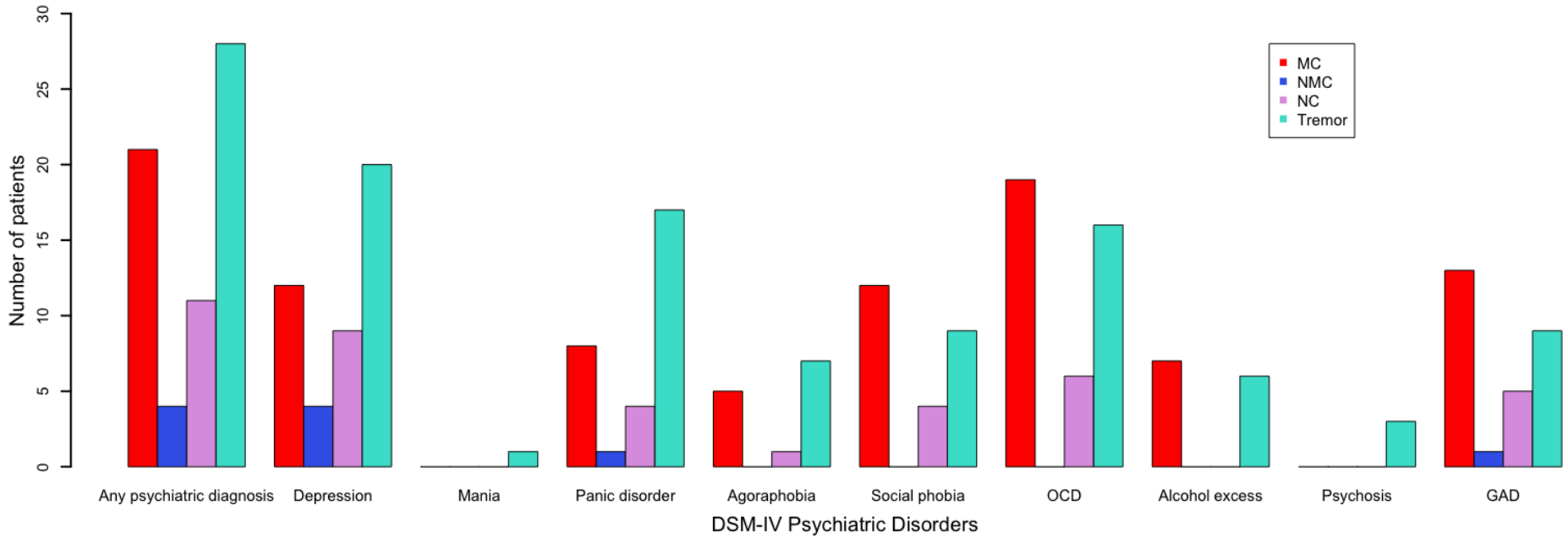
Lifetime disorder DSM-IV	MC (Total = 27)	MC >18yrs (Total = 20)	NMC (Total = 10)	NC (Total=16)	Tremor (Total = 45)	Population estimates
Any DSM-IV disorder	21 (77.8%)	16 (80%)	4 (40%)	11 (68.8%)	28 (62.2%)	48%*
Major Depressive Disorder	12 (44.4%)	9 (45%)	4 (40%)	9 (56.3%)	20 (44.4%)	17.1%*
Mania & Hypomania	0	0	0	1 (6.3%)	3 (6.7%)	1.6%*
Panic Disorder	9 (33.3%)	9 (45%)	1 (10%)	4 (25%)	17 (37.8%)	3.5%*
Agoraphobia	6 (22.2%)	5 (25%)	0	1 (6.3%)	7 (15.6%)	5.3%*
Social Phobia	12 (44.4%)	9 (45%)	0	4 (25%)	8 (17.8%)	13.3%*
OCD-overall	16 (59.2%)	11 (55%)	0	3 (18.8%)	8 (17.8%)	4%†
Obsessions	4 (14.8%)	3 (15%)	0	2 (12.5%)	8 (17.8%)	
Compulsions	14 (51.9%)	10 (50%)	0	3 (18.8%)	6 (13.3%)	
Alcohol dependence/abuse	8 (29.6%)	8 (40%)	0	0	6 (13.3%)	14.1%*
Psychotic & Mood disorders	1 (3.7%)	1 (5%)	0	0	3 (6.7%)	0.7%*
Generalized Anxiety Disorder	13 (48.1%)	10 (50%)	1 (10%)	5 (31.3%)	9 (20%)	5.1%*

Key: MC: Manifesting carriers, **NMC:** Non-manifesting carriers, **NC:** Non-carriers, **MINI:** MINI International Neuropsychiatric Interview,

* Values taken from National Comorbidity Survey

† Values from Dunedin Longitudinal Birth Cohort Study

Figure 4.2: Graphical representation of the rates of psychiatric disorders amongst MC, NMC, NC and Tremor cohorts



Key: MC: Manifesting Carrier, **NMC:** Non-Manifesting Carrier, **NC:** Non-Carrier, **GAD:** Generalised Anxiety Disorder, **OCD:** Obsessive-Compulsive Disorder

Table 4.8: Comparison or rates of DSM-IV disorders between diagnostic groups (all cases)

Psychiatric diagnosis (DSM-IV)	MC vs Tremor	NMC vs Tremor	MC vs NMC	MC vs NC	NMC vs NC
Any	0.20 (2.13; 0.64, 7.31)	0.29 (0.41; 0.08, 1.96)	**0.05 (5.25; 0.88, 34.12)**	0.72 (1.59; 0.32, 7.92)	0.23 (0.30; 0.04, 2.06)
Major Depressive Disorder	1.0 (1.0; 0.34, 2.91)	1.0 (0.83; 0.17, 4.02)	1.0 (1.2; 0.22, 6.70)	0.54 (0.62; 0.15, 2.57)	0.69 (0.52; 0.08, 3.37)
Mania & Hypomania	0.29 (0; 0, 3.8)	1.0 (0; 0, 11.37)	1.0 (unable to calculate)	0.37 (0; 0, 10.54)	1 (0; 0, 30.24)
Panic Disorder	0.8 (0.82; 0.27, 2.51)	0.14 (0.18; 0.01, 1.67)	0.23 (4.5; 0.44, 109.94)	0.74 (1.5; 0.31, 7.52)	0.62 (0.33; 0.01, 4.37)
Agoraphobia	0.54 (1.55; 0.39, 6.08)	0.33 (0; 0, 3.63)	0.16 (∞; 0.40, ∞)	0.23 (4.27; 0.42, 104.69)	1 (0; 0, 30.25)
Social phobia	**0.03 (3.7; 1.12, 12.56)**	0.33 (0; 0, 3.03)	**0.02 (∞; 1.23,** ∞**)**	0.33 (2.4; 0.52, 11.78)	0.14 (0; 0, 2.44)
OCD	**0.001 (6.7; 2.02, 23.27)**	0.33 (0; 0, 3.03)	**0.002 (∞; 2.20,** ∞**)**	**0.009 (7.7; 1.4, 46.52)**	0.26 (0; 0, 3.78)
Alcohol dependence/abuse	0.13 (2.7; 0.72, 10.61)	0.58 (0; 0, 4.46)	0.08 (∞; 0.62, ∞)	**0.018 (∞; 1.05,** ∞**)**	1 (unable to calculate)
Psychotic & Mood Disorders	1.00 (0.54; 0.02, 6.36)	1.00 (0; 0, 11.37)	1 (∞; 0.02, ∞)	1 (∞; 0.03, ∞)	1 (unable to calculate)
Generalised Anxiety Disorder	**0.02 (3.71; 1.16, 12.22)**	0.67 (0.44; 0.02, 4.36)	0.06 (8.36; 0.84, 201.48)	0.35 (2.04; 0.47, 9.20)	0.35 (0.24; 0.01, 3.03)

Table 4.9: Comparison or rates of DSM-IV disorders between diagnostic groups (cases <18 years of age at time of investigation excluded)

Psychiatric diagnosis (DSM-IV)	MC vs Tremor	MC vs NMC	MC vs NC
Any	0.25 (2.43; 0.61, 10.33)	**0.05 (6.00; 0.87, 47.22)**	0.47 (1.82; 0.32, 10.80)
Major Depressive Disorder	1.00 (1.02; 0.31, 3.35)	1.00 (1.23; 0.21, 7.52)	0.74 (0.64; 0.14, 2.91)
Mania & Hypomania	0.55 (0; 0, 5.24)	1.00 (unable to calculate)	0.44 (0; 0, 14.41)
Panic Disorder	0.60 (1.35; 0.41, 4.47)	0.10 (7.36; 0.68, 186.16)	0.30 (2.46; 0.48, 13.26)
Agoraphobia	0.49 (1.81; 0.41, 7.82)	0.14 (∞; 0.44, ∞)	0.20 (5.00; 0.45, 127.64)
Social phobia	**0.03 (3.78; 1.02, 14.37)**	**0.01 (∞; 1.16,** ∞**)**	0.30 (2.46; 0.48, 13.26)
OCD	**0.006 (5.65; 1.53, 21.69)**	**0.004 (∞; 1.73,** ∞**)**	**0.04 (5.30; 0.94, 33.37)**
Alcohol dependence/abuse	**0.02 (4.33; 1.08, 18.06)**	**0.03 (∞; 0.94,** ∞**)**	**0.005 (∞; 1.59,** ∞**)**
Psychotic & Mood Disorders	1.0 (0.74; 0.03, 8.92)	1.00 (∞; 0.03, ∞)	1.00 (∞; 0.04, ∞)
Generalised Anxiety Disorder	**0.02 (4.0; 1.11, 14.76)**	**0.05 (9.00; 0.83, 227.49)**	0.32 (2.20; 0.46, 10.98)

Key: MC: Manifesting carriers, **NMC:** Non-manifesting carriers, **NC:** Non-carriers. Figures represented as p-value (Odds Ratio; 95% Confidence Interval), **Bold** denotes statistically significant result

4.4.2 YBOCS, AUDIT, MADRS and PHQ-9 questionnaires

YBOCS, AUDIT, MADRS and PHQ-9 questionnaires were used to examine symptoms of OCD, alcohol misuse and depression in further detail and to determine whether these results corroborated the findings of the MINI questionnaire (Table 4.9). The MC group had a significantly higher total YBOCS score ($p>0.001$) and a median value nine times greater than that of all other diagnostic groups. This largely reflected a significantly increased compulsivity score ($p<0.001$) with no difference in obsessionality ($p=0.16$).

Alcohol use over the preceding year, measured with the AUDIT questionnaire, was higher amongst the MC group compared to all other groups as well as having the largest range of values (0-34). Despite alcohol's therapeutic benefit in the tremor patients, median NMC AUDIT scores were more than twice that of the tremor group. Depression self-assessment in the form of PHQ-9 found no statistical difference between the groups ($p=0.08$). However, clinician scoring of these symptoms using the MADRS questionnaire found a statistically significant difference between the MC group and all other groups ($p=0.01$).

Table 4.10: Analysis of YBOCS, AUDIT, PHQ-9 and MADRS questionnaires

	Median score (range)				
	MC	NMC	NC	Tremor	p-value
YBOCS					
Total score	9 (0-26)	0 (0)	0 (0-8)	0 (0-33)	<0.001
Obsessions	0 (0-15)	0 (0)	0 (0-8)	0 (0-17)	0.16
Compulsions	7 (0-17)	0 (0)	0 (0)	0 (0-16)	<0.001
AUDIT					
Total score	4 (0-34)	4.5 (1-9)	1 (0-4)	2 (0-18)	<0.001
PHQ-9					
Total score	6 (0-18)	2 (0-12)	2 (0-12)	4 (0-25)	0.08
MADRS					
Total score	15 (0-32)	1 (0-22)	1 (0-22)	6 (0-30)	0.01

Key: MC=Manifesting carrier, NMC=Non-manifesting carrier, NC=Non-carrier
Statistical analysis using binomial stepwise multiple logistic regression

4.4.3 Structured Clinical Interview for DSM-IV Axis-II Personality Disorders (SCID-II)

Fewer numbers of participants completed the SCID-II personality disorders questionnaire (MC=14, NMC=7, NC=12, Tremor=8) (Table 4.9). Amongst the MC patients Cluster B and Cluster C disorders were the most prevalent (85.7%), with borderline disorder (50%) and symptoms of avoidance (85.7%) being the most common in each category respectively. Cluster C symptoms were also present in over half the patients in each of the other diagnostic categories: NMC (71.4%), NC (58.3%), Tremor (62.5%). Of these, symptoms of avoidance were the most common subgroup in both NC (58.3%) and tremor (62.5%) cohorts, while obsessive-compulsive type symptoms were predominant amongst the NMC group. Overall, the proportion of patients with elevated obsessive-compulsive scores showed considerable variation between *SGCE* mutation carriers (MC: 57.1%, NMC: 71.4%) and non-mutation carriers (NC: 16.7%, Tremor: 12.5%).

Chi-squared statistical analysis found an excess of Cluster B symptoms amongst MC patients compared to all other groups, the greatest difference seen when compared to the NMC cohort (OR=15.0, 95% CI 1.16 - 316.79, p=0.02). As with the results of the MINI questionnaire the NMC group was compared to both MC and tremor groups in an attempt to determine a gene effect. In addition to Cluster B symptoms there was a statistical difference in avoidance traits between MC and NMC cohorts (OR=15.0, 95% CI 1.16 – 316.79, p=0.02). When compared with the tremor group, a statistically significant excess of obsessive-compulsive symptoms was observed within the NMC cohort (OR=17.5, 95% CI 0.84 – 813.06, p=0.04).

A full summary of statistical analysis can be seen in Table 4.10.

Table 4.11: Rates of personality disorder diagnoses determined by the SCID-II questionnaire

Personality disorder (SCID-II diagnoses)	MC (>18yrs) (Total = 14)	NMC (Total = 7)	NC (Total = 12)	Tremor (Total=8)
Cluster A	6 (42.9%)	2 (28.6%)	4 (33.3%)	2 (25%)
Paranoid	1 (7.1%)	0	2 (16.7%)	0
Schizoid	2 (14.3%)	0	1 (8.3%)	1 (12.5%)
Schizotypal	5 (35.7%)	2 (28.6%)	4 (33.3%)	2 (25%)
Cluster B	12 (85.7%)	2 (28.6%)	6 (50%)	3 (37.5%)
Antisocial	4 (28.6%)	0	1 (8.3%)	0
Borderline	7 (50%)	2 (28.6%)	4 (33.3%)	0
Histrionic	4 (28.6%)	1 (14.3%)	3 (25%)	2 (25%)
Narcissistic	3 (21.4%)	2 (28.6%)	1 (8.3%)	3 (37.5%)
Cluster C	12 (85.7%)	5 (71.4%)	7 (58.3%)	5 (62.5%)
Avoidant	12 (85.7%)	2 (28.6%)	7 (58.3%)	5 (62.5%)
Dependent	6 (42.9%)	1 (14.3%)	2 (16.7%)	2 (25%)
Obsessive-Compulsive	8 (57.1%)	5 (71.4%)	2 (16.7%)	1 (12.5%)

Key: MC: Manifesting carrier, **NMC:** Non-manifesting carrier, **NC:** Non-carrier.

Table 4.12: Comparison of rates of personality disorder diagnoses between diagnostic groups

Personality disorder (SCID-II diagnosis)	MC vs Tremor	NMC vs Tremor	MC vs NMC	MC vs NC	NMC vs NC
Cluster A	0.65 (2.25; 0.24, 24.10)	1.00 (1.2; 0.07, 19.96)	0.66 (1.88; 0.19, 20.77)	0.70 (1.5; 0.23, 10.06)	1.00 (8.00; 0.07, 8.97)
Paranoid	1.00 (∞; 0.03, ∞)	1.00 (unable to calculate)	1.00 (∞; 0.03, ∞)	1.00 (0.46; 0.01, 8.02)	0.58 (0.39; 0.01, 6.82)
Schizoid	1.00 (1.17; 0.06, 39.63)	1.00 (0; 0, 22.41)	0.53 (∞; 0.11, ∞)	1.00 (1.83; 0.10, 59.69)	1.00 (0; 0, 33.75)
Schizotypal	1.00 (1.67; 0.17, 18.05)	1.00 (1.2; 0.07, 19.96)	1.00 (1.39; 0.14, 15.60)	1.00 (1.11; 0.17, 7.57)	1.00 (0.8; 0.07, 8.97)
Cluster B	**0.05 (10.00; 0.92, 149.12)**	1.00 (0.67; 0.05, 9.24)	**0.02 (15.0; 1.16, 316.79)**	**0.005 (12; 1.62, 112.97)**	0.63 (0.4; 0.03, 4.13)
Antisocial	0.25 (∞; 0.37, ∞)	1.00 (unable to calculate)	0.26 (∞; 0.32, ∞)	0.33 (4.4; 0.34, 122.97)	1.00 (0; 0, 33.75)
Borderline	**0.02 (∞; 0.99,** ∞)	0.20 (∞; 0.26, ∞)	0.64 (2.5; 0.26, 27.76)	0.45 (2.0; 0.31, 13.46)	1.00 (0.8; 0.07, 8.97)
Histrionic	1.00 (1.2; 0.12, 13.46)	1.00 (0.50; 0.01, 11.02)	0.62 (2.4; 0.16, 71.32)	1.00 (1.2; 0.16, 9.56)	1.00 (0.5; 0.02, 8.39)
Narcissistic	0.62 (0.46; 0.04, 4.39)	1.00 (0.67; 0.05, 9.24)	1.00 (0.68; 0.06, 8.49)	0.60 (3.0; 0.21, 88.20)	0.52 (4.4; 0.22, 161.65)
Cluster C	0.31 (3.60; 0.32, 47.07)	1.00 (1.5; 0.11, 22.39)	0.57 (2.4; 0.17, 36.28)	0.19 (4.29; 0.5, 44.33)	0.66 (1.79; 0.17, 21.13)
Avoidant	0.31 (3.60; 0.32, 47.07)	0.32 (0.24; 0.01, 11.02)	**0.02 (15.0; 1.16, 316.79)**	0.19 (4.29; 0.5, 44.33)	0.35 (0.29; 0.02, 2.95)
Dependent	0.65 (2.25; 0.24, 24.02)	1.00 (0.50; 0.01, 11.02)	0.34 (4.5; 0.33, 128.70)	0.22 (3.75; 0.46, 37.03)	1.00 (0.83; 0.02, 17.03)
Obsessive-Compulsive	0.07 (9.33; 0.72, 264.60)	**0.04 (17.5; 0.84, 813.06)**	0.66 (0.53; 0.05, 5.21)	**0.05 (6.67; 0.82, 67.37)**	**0.05 (12.5; 0.94, 268.86)**

Key: MC: Manifesting carrier, **NMC:** Non-manifesting carrier, **NC:** Non-carrier. Figures represented as p-value (Odds Ratio; 95% Confidence Interval). **Bold** denotes statistically significant result

4.4.4 Contact with psychiatric services

Contact with psychiatric services was similar amongst MC (37%), NC (38%) and Tremor (32%) groups. Of those who had received psychiatric input, management by psychiatrists was the most common (MC (70%), NC (50%) and Tremor (50%)). Of the remaining patients in the MC group, a single case each was seen by a psychologist, GP and other therapists. More than a quarter (6/22) of the tremor cases were seen by a GP, while a smaller proportion were seen by either psychologists (2/22) or other therapists (3/22). Of the remaining NC patients a third of cases were seen by a psychologist and one by a GP. Twenty percent (2/10) of NMC patients had contact with psychiatric services, one in general practice and the other with a counselor. Finally hospital admissions for psychiatric symptomatology were seen only in the MC (1/27) and Tremor (6/69) subgroups (Table: 4.11).

Table 4.13 Contact with psychiatric services

Contact with psychiatric services	MC (n=27)	NMC (n=10)	NC (n=16)	Tremor (n=69)
Previous contact with psychiatric services	10 (37%)	2 (20%)	6 (38%)	22 (32%)
Type of psychiatric support				
Psychiatrist	7 (70%)	-	3 (50%)	11 (50%)
Psychologist	1 (10%)	-	2 (33%)	2 (9%)
General Practitioner	1 (10%)	1 (50%)	1 (17%)	6 (27%)
Community Psychiatric Nurse (CPN)	-	-	-	-
Support groups	-	-	-	-
Other	1 (10%)	1 (50%)		3 (14%)
Inpatient hospital stay due to psychiatric symptoms	1 (4%)	-	-	6 (9%)

Key: MC: Manifesting carrier, **NMC:** Non-manifesting carrier, **NC:** Non-carrier

4.4.5 Alcohol consumption

More than half of all patients in each diagnostic group consumed alcohol at the time of interview, the rate being highest amongst the NMC cohort (90%) and lowest in the NC (63%) controls (Table 4.12). Analysis of current consumption found mean weekly units to be highest amongst MC patients (18.5 units/week), almost twice that of the tremor group (11.5 units/week) Interestingly mean use amongst the NMC patients (13.3 units/week) was also higher than that of the tremor cohort. When asked whether

their alcohol consumption had been higher in the past the MC subgroup recorded the greatest proportion of positive responses (39%), with lower rates in the tremor (13%) and NC (10%) groups. Despite this, mean alcohol use at peak consumption was greatest amongst the tremor group (69 units/week), one and a half times greater than that of the MC group (46.3 units/week).

Table 4.14 Past and present alcohol consumption

Alcohol use	MC (n=18)	NMC (n=10)	NC (n=16)	Tremor (n=69)
Alcohol use (current)	14 (78%)	9 (90%)	10 (63%)	52 (75%)
Mean number of units/week (current)	18.5	13.3	1.6	11.5
Higher level of alcohol consumption in the past	7 (39%)	0	1 (10%)	9 (13%)
Mean number of units/week at peak consumption	46.3	0	10	69

Key: MC: Manifesting carrier, **NMC:** Non-manifesting carrier, **NC:** Non-carrier

4.4.6 Relation of psychiatric symptoms to motor severity

The severity of the hyperkinetic movements in *SGCE* mutation positive cases (MC) was retrospectively rated following videotaped clinical examination using modified forms of the Unified Myoclonus Rating Scale (UMRS)[257] and Burke-Fahn Marsden Dystonia Rating Scale (BFMDRS).[258] Total scores from both scales were then analysed alongside outcomes from MADRS, YBOCS and AUDIT questionnaires. No association between presence of overall psychiatric disorders and motor severity scores was observed (p=0.08). There was an association between motor severity and MADRS scores (p=0.05, r=0.58) but no link with overall YBOCS scores (p=0.83, r=0.06), obsessions (p=0.73, r=0.095), compulsions (p=0.98, r=0.008) and AUDIT scores (p=0.60, r=0.14).

4.5 Discussion

This study represents the largest single, multi-family cohort of MDS patients systematically assessed using validated scales for rate and type of psychiatric disorders, and the first to compare psychopathology to a disability matched control

group. We have confirmed the hypothesis that patients with manifesting *SGCE* mutations have significantly higher rates of psychiatric illness as compared to controls with a significant movement disorder. Our findings point to a specific preponderance of OCD, GAD and alcohol dependence and reveal that the association with OCD is specific to the compulsivity rather than the obsessional component of the disorder.

Although MC and tremor groups are matched for disability, movement disorder duration, sex and alcohol use, a significant difference in age at onset and age at examination ($p<0.001$) exists between the two groups (Table 4.5). However, multivariate analysis found neither the incidence of overall psychiatric pathology nor, more specifically, OCD to be influenced by age at onset of the motor disorder, and OCD alone appeared independent of the duration of movement disorder symptoms. In addition our psychiatric interview involved lifetime assessment for each of these disorders rather than relating to a restricted time frame. These analyses suggest that the specific pattern of psychiatric morbidity seen in manifesting carriers as compared to tremor controls was not due to differences in age at onset or age at examination between the two groups. In addition alcohol-responsive tremor was considered the best disease match for a chronic disabling disorder that benefits therapeutically from alcohol, in an attempt to separate therapeutic and addictive traits. Finally, a dystonia control group has not been included therefore the possibility that the specific pattern of psychiatric morbidity seen in MDS is also associated more widely with dystonia cannot be excluded.

Psychiatric assessment using the M.I.N.I. questionnaire found overall higher rates of psychiatric disorders amongst the MC group compared to the tremor controls (77.8% vs. 62.2%) and significantly higher than estimated within the general population (48%). Population estimates were taken from large cohort, epidemiological studies both of Western populations similar to the one studied in this cohort. [313, 314] In addition, no association was seen between psychiatric disorders and motor severity suggesting that the psychiatric phenotype may be independent of the motor disorder.

4.5.1 Obsessive-Compulsive Disorder

OCD was the most common disorder in MC patients (59%), almost seven times more likely to occur compared to controls. OCD is generally not recognized as a secondary psychiatric response to chronic disease, [305] and therefore is an interesting finding in a chronically disabling and disfiguring disorder. Similar results were seen with the adult only MC cohort (OR=5.65, 95% CI 1.53 - 21.69, p=0.006) suggesting that despite population estimates that rates of OCD are higher amongst children, [314] a significant difference remains between these groups. There were no differences in OCD rates between NMC and tremor groups and a significant difference between MC and NMC cohorts. These results argue against a direct gene effect, although this is based on a small number of patients. YBOCS questionnaire scores further strengthened this association with the MC median score being nine times higher and significantly different to all other groups. This effect was overwhelmingly due to compulsivity scores, seven times greater than obsessive traits and did not relate to severity of the motor phenotype.

Rates of OCD have been assessed in other forms of dystonia with conflicting results. Mixed groups of focal dystonias have been compared with groups of other disfiguring disorders [181, 315] and healthy individuals,[180] finding increased rates of OCD or Obsessive-Compulsive Symptoms (OCS) amongst the dystonic group, although unable to relate this to a specific form of dystonia. Similarly elevated rates of OCD have been noted amongst 1st degree relatives of those with dystonia/OCD compared to those with dystonia alone. In contrast, others have found no association either amongst mixed dystonia types [316] or genetically defined *DYT1* cohorts.[182]

4.5.2 Implications for the pathogenesis of dystonia and compulsivity

Comorbid OCD, dystonia and *SGCE* mutations reaffirm the likely role of the basal ganglia in the underlying pathogenesis of these disorders. PET and fMRI studies of dystonias [317] and OCD [318] have shown abnormal activation of the basal ganglia, thalamus, frontal and cingulate cortices, while an association has also been identified between dystonia severity and putaminal grey matter volume.[319] More recent fMRI studies have also shown altered patterns of activation in the sensorimotor cortex and

cerebellum, suggesting that additional brain structures may also contribute.[320] Neurophysiological studies showing impaired saccadic adaptation in *SGCE* mutation positive cases also support this, suggesting involvement of the posterior cerebellum potentially in the generation of subcortical myoclonus.[321] Use of DBS in the treatment of MDS has shown improvement of both forms of motor symptoms when stimulating the $GP_{i,}$[299] in excess of those stimulated in the region of the ventral intermediate nucleus (VIM) of the thalamus,[300] while lesions within the striatal-pallidal pathways are associated with OCD.[322]

4.5.3 Alcohol use disorders

Alcohol excess has also been frequently observed amongst DYT11 cohorts. [188, 191, 192, 268, 304] Amongst the adult MC cohort, alcohol excess was more than four times more likely than the tremor control group together with a significant difference in total AUDIT scores between the MC and all other groups ($p<0.001$). Median NMC score was also higher than that of the tremor participants, suggesting that alcohol use is higher amongst *SGCE* mutation carriers, irrespective of motor symptoms, than those without. Previous literature has suggested a link between those traits that result in excess alcohol consumption and ritualistic OCD behaviour.[323] Assessment using the YBOCS questionnaire of a population diagnosed with alcohol dependence/abuse noted a positive correlation between alcohol craving and both individual obsession and compulsion scores.[324] Similarly techniques traditionally used in the treatment of OCD have been found to reduce desire to drink and difficulty in resisting alcohol.

Further alcohol use data was collected in a systematic, although non-standardized manner, focusing upon quantity consumed rather than effect. Highest volumes of consumption were seen amongst *SGCE* mutation positive patients, with NMC individuals consuming more than the alcohol-responsive tremor controls. Data collection of previous alcohol consumption found more than a third of MC cases had drunk more heavily in the past but none within the NMC group. Anecdotally, all MC patients cited concerns regarding addiction as their predominant influence in reducing their alcohol intake coupled with a rebound worsening of their motor symptoms the day after a period of heavy consumption.

Collectively this suggests that excess alcohol consumption in *SGCE* positive individuals may not simply be a secondary therapeutic response as hitherto assumed, but rather that it is related to the compulsivity that I have demonstrated, and forms part of the phenotype of the disorder.

4.5.4 Anxiety disorders and depressive symptoms

Other psychiatric disturbances that appeared to be influenced by genetic and motor status were social phobia and GAD. Anxiety related co-morbidity has been noted during intra-familial comparisons of MDS cohorts [312] as well as other forms of dystonia. Standardised testing of patients with cervical dystonia showed an excess of anxiety disorder, specifically panic disorder and social phobia, compared to the general population.[175, 176] These features were also consistent when a similar cohort was compared to a control group of alopecia areata patients, highlighting a potential dystonia-specific feature.[177] Psychiatric features have also been known to pre-date the onset of dystonic symptoms, again suggesting that this psychopathology is a primary rather than a secondary, reactive response.[325]

Mood disorders differed between the groups when analysed using the self-completed PHQ-9 and assessor completed MADRS, although under reporting of symptoms with self-rated questionnaires is a well recognized feature.[326] Despite this excess of affective symptoms likely being a secondary effect due to a chronic, disabling disorder other genetically defined groups of dystonias have found an excess of depression,[179] again suggesting a general increase of psychiatric co-morbidity amongst this group of disorders.

4.5.5 Personality disorders

Results from the SCID-II questionnaire were limited by the small number of patients assessed, this due to failure to fully complete the questionnaire and an unwillingness to partake in this portion of the assessment. Few previous studies have systematically assessed personality disorders, only a single case of schizoaffective disorder[306] and personality disorder[185] having been reported. The same is true of other forms of

dystonia, with a single study suggesting an excess of Cluster C symptoms in a mixed group of dystonias.[325]

Within this cohort assessment of personality disorders provided further support of an excess of OCD-type symptoms among *SGCE* mutation carriers. Overall rates of OCD amongst MC and NMC patients were higher than familial and tremor controls, with a statistical difference being seen between NMC and both control groups. Of the other disorders cluster B type borderline personality disorder and avoidant personality type (Cluster C disorder) were also prominent. These results need to be viewed with caution given the small size of the cohort. However, there is certainly a suggestion that personality disorders may form part of the MDS phenotype and therefore should be investigated more thoroughly in future studies.

4.5.6 Other psychiatric disorders

Subtle unique findings were also observed when comparing psychiatric illness in the Whole Gene Deletion (WGD) cases to those with point mutations. The four cases with larger deletions (1.9-2.3Mb) all had symptoms of OCD, depression and anxiety related disorders similar to the population identified by Sanger sequencing. In contrast the fifth WGD case (0.7Mb deletion) initially presented to adult medical services with symptoms of schizophrenia requiring multiple inpatient admissions. Psychiatric features have not been observed in the majority of previous WGD case reports, the attention instead being focused upon global cognitive impairment and learning difficulties.[231, 232] An Australian family was also reported to have symptoms of psychosis and, as in the case in this study, a much smaller deletion than those described above.[233] With the exception of a single member of an MDS family being reported to have schizoaffective disorder,[327] psychosis has not been reported previously in those with *SGCE* mutations and none of the genes involved in these deletions (*PEG10, SGCE, CASD1, COL1A2*) are believed to contribute to the pathogenesis of psychosis.

4.5.7 Contact with Psychiatric Services

Many patients had received psychiatric care. The largest numbers of patients receiving one or more forms of assistance were in MC and tremor cohorts and were the only two groups in which individuals had been admitted to a psychiatric facility. This may reflect a greater severity of the psychiatric symptoms amongst these individuals or alternatively a higher rate of diagnosis due to contact with medical services brought about by their movement disorder. Interestingly the highest rate of involvement with psychiatric services was seen amongst the NC group with 50% having been seen by a psychiatrist. This may be an incidental finding amongst a small cohort or may represent environmental factors such as the stressors of co-habiting and caring for family members with chronic disease. Irrespective of the cause, these results suggest the need for increased clinician awareness of the impact upon unaffected family members.

4.6 Conclusion

This section of the study has demonstrated that an excess of specific psychiatric disorders exists amongst affected *SGCE* mutation carriers when compared both to an external control group and to unaffected family members. OCD is the most strongly associated psychopathology and this reflects compulsive rather than obsessive symptoms. Affected *SGCE* mutation carriers showed evidence of excess alcohol consumption even when compared to a control group with alcohol responsive tremor. Excess consumption in MDS is often attributed to the therapeutic effects of alcohol, but my findings suggest that this might have a more direct relationship to pathogenesis and may arise as a consequence of a primary disturbance of compulsive behaviour.

In conclusion, this study shows that psychiatric co-morbidity forms a significant part of the clinical phenotype of MDS due to *SGCE* mutations. Clinicians need to be aware of this and of the need for psychiatric symptoms to be treated effectively and early. Further work is required to define and delineate the relationship between motor and psychiatric symptoms, which will enhance our understanding of the aetiology and pathophysiology of both motor and psychiatric disorders.

CHAPTER 5

Genetic description of the MDS cohort

5.1 Introduction

As discussed in Chapter 1, mutations in the *SGCE* gene are responsible for a proportion of MDS cases.[212] Inheritance is autosomal dominant with reduced penetrance owing to maternal imprinting (Figure1.4).[215, 216] More than fifty different mutations have been identified including missense, splice-site and nonsense mutations. The majority of these have been loss of function mutations i.e. intra-genic deletions or nonsense mutations leading to premature truncation of the transcript (Table 5.1). Despite the variety of mutations identified, no true genotype-phenotype relationship has been determined.[186, 328]

A number of studies have screened large populations with clinically probable MDS, demonstrating large variation in mutation rates and leading to the suggestion of genetic heterogeneity (Table 5.2). As discussed in Chapter 1, CNVs provide a possible explanation for this heterogeneity with intra-genic deletions and duplications resulting in a clinical phenotype indistinguishable from that caused by point mutations. However, a number of contiguous gene deletions involving *SGCE* have been identified, ranging from 0.17Mb to 16.5Mb in size and involving a variable number of surrounding genes (Figure 5.1). The function of the majority of these genes in humans is unknown although *COL1A2* encodes the alpha-2 chain of type I collagen and is involved in the pathogenesis of osteogenesis imperfecta, *KRIT1* mutations are responsible for cerebral cavernous malformations and *SHFM1* is believed to contribute to split hand/split foot malformations. Additional clinical characteristics have been reported in these patients e.g. microcephaly, short stature, dysmorphic facies, joint laxity and cognitive impairment (Table 5.3). It is believed that the clinical phenotype may be related to the deletion size and genes involved, thus providing some form of genotype/phenotype correlation.[231]

This portion of the study investigates the rate and type of *SGCE* mutations identified by direct sequencing and CNV analysis. The clinical phenotype of these cases is also examined while performing extensive additional genetic analysis of the *SGCE* mutation negative group.

Table 5.1: Previously reported *SGCE* mutations

Type of mutation	Gene location	Nucleotide change*	Predicted protein	Country of origin	References
Nonsense mutations	Exon 2	c.208G>T	p.Glu70X	F	[188]
	Exon 3	c.289C>T	p.Arg97X	F/G	[192, 212, 217, 269, 275]
	Exon 3	c.300G>A	p.Trp100X	F	[188, 212]
	Exon 3	c.304C>T	p.Arg102X	F/G/C/D/I	[186, 212, 268, 270, 310, 328, 329]
	Exon 4	c.402C>A	p.Tyr134X	I	[186]
	Exon 5	c.481C>T	p.Gln161X	B	[281]
	Exon 6	nk	p.Gly227Val	nk	[280]
	Exon 6	c.709C>T	p.Arg237X	H	[185, 197, 259]
	Exon 6	c.810G>A	p.Trp270X	I	[186]
	Exon 7	c.856C>T	p.Gln286X	F/D	[212, 268, 270]
	Exon 7	c.942C>A	p.Tyr314X	F	[309]
	Exon 7	nk	p.Q281X	Ir	[279]
	Exon 9	c.1114C>T	p.Arg372X	F/I	[188, 259, 269]
Missense mutations	Exon 1	c.107C>G	p.Thr36Arg	nk	[184]
	Exon 2	c.179A>C	p.His60Pro	G/D/S	[270, 310]
	Exon 2	c.179A>G	p.His60Arg	S	[220]
	Exon 3	c.275T>C	p.Met 92Thr	F	[188]
	Exon 3	c.298T>G	p.Trp100Gly	F	[195]
	Exon 3	c.334G>A	p.Gly112Arg	I	[186]
	Exon 3	c.344A>G	p.Tyr115Cys	F	[188]
	Exon 3	c.386T>C	p.I129T	Sw	[330]
	Exon 5	c.551T>C	p.Leu184Pro	nk/D	[184, 196]
	Exon 5	c.587T>G	p.Leu196Arg	G/W/UK	[331, 332]
	Exon 5	c.662G>A	p.Gly221Asp	UK	[304]
	Exon 6	c.808T>C	p.Trp270Arg	D	[270]
	Exon 6	c.812G>A	p.Cys271Tyr	F	[188]
Deletions	Exon 2	c.110-?_232+?del	unknown	nk	[259]
	Exon 2 to 3	c.110-?_390+?del	unknown	C	[229]
	Exon 2 to 5	c.110-?_662+?del	unknown	C	[229]
	Exon 2 to 11	c.exon2_11del	unknown	T	[333]
	Exon 2	c.164delG	p.Gly55ValfsX31	W/CZ	[310]
	Exon 2	c.221delA	p.Tyr74SerfsX12	F	[188]
	Exon 3	c.256delA	p.T861tfsX91	D	[187]
	Exon 3	c.276delG	p.Gly93ValfsX39	G	[268]
	Exon 4	c.391_405del	p.Ile131_Asn135del	G	[212]
	Exon 4	c.444_447del	p.Asn149X	F	[188, 308]
	Exon 5	c.464-?_662+?del	unknown	G	[228]
	Exon 5	c.483delA	p.Ala162GlnfsX8	G	[212]
	Exon 5	c.488_497del	p.Glu163ValfsX4	G	[212]
	Exon 5	c.524_531del	unknown	T	[333]
	Exon 5	c.539_593del	p.Leu180ProfsX2	UK	[327]
	Exon 5	c.564_576del	p.Lys188AsnfsX5	nk	[269]
	Exon 5	c.566delA	p.Asn189MetfsX8	G	[212]
	Exon 5	c.619_620del	p.Arg207GlyfsX9	D	[270, 329]
	Exon 6	c.663-?_825+?del	unknown	G	[228]
	Exon 6	c.734_737del	p.Gln245ArgfsX10	F	[268]
	Exon 6	c.771_772del	p.Cys258X	?	[184, 186, 192, 259]
	Exon 6	c.783delA	unknown	Ir	[279]
	Exon 6	c.795delA	p.Gln265HisfsX24	nk	[184, 192]
	Exon 7	c.832_836del	p.Thr279AlafsX17	?	[328]
	Exon 7	c.835_839del	p.Thr279Alafsx17	F/C/W/G	[185, 188, 192, 328, 332]
	Exon 7	c.842delA	unknown	T	[333]
	Exon 7	c.946delG	p.D3161fsX318	D	[187]
	Exon 7	c.966delT	p.Val323CysfsX11	G/S	[216, 220]
	Exon 7	c.974delC	p.Ser325TrpfsX9	D	[196]
	Exon 9	c.1151delT	p.Leu384ArgfsX10		[184]
	exon 6 to 9	nk	unknown	D	[187]
	Exon 10	nk	p.fs421X	Ir	[279]
Whole gene deletions	Exons 1-12	Absence of transcript	Absence of protein	Caucasian	[230, 232, 233, 259]
Insertion	Exon 7	c.885_886insT	p.Pro296SerfsX2	G/D	[270, 274]
Duplications	Exon 5	c.625insG	p.Arg210GlnfsX7	G	[216]
	Exon 5	c.662_1insG	Unknown	Ch	[189]
	Exon 6	c.745-746insTGTA	p.Ser249MetfsX2	F	[188]
Splicing mutations	Intron 1	c.109+1G>T	Possible skipping exon 1	K	[190]
	Intron 1	c.109+1G>A	Possible skipping exon 1	nk	[184, 192]
	Intron 2	c.232+1G>A	Possible skipping exon 2	B/F	[188, 281]
	Intron 2	c.232+1G>T	Possible skipping exon 2	nk	[184]
	Intron 2	c.232+2T>C	Possible skipping exon 2	F	[188]
	Intron 2	c.233-1G>T	Skipping exon 3	F/I	[186, 188]
	Intron 2	c.233-1G>A	Skipping exon 3	F	[268, 334]
	Intron 3	c.391-3T>C	Unknown	I/F	[195, 269]
	Intron 3	c.391-43A>C	Unknown	nk	[335]
	Exon 4	c.463G>A	Skipping exon 5	G	[336]
	Intron 4	c.463+6T>C	Unknown	UK	[268]
	Intron 5	c.663-1G>A	Possible skipping exon 6	I	[186]
	Intron 6	c.825+1G>A	Possible skipping exon 6	G	[212]
	Intron 6	c.826-1G>A	Possible skipping exon 7	I	[186]
	Intron 7	c.1037+2T>C	Possible skipping exon 7	D	[270]
	Intron 7	c.1037+5G>A	Unknown	G	[268]

Key: * Numbered according to *SGCE* transcript NM_001099401.1, B: Brazilian, C: Canadian, Ch: China, CZ: Czech, D: Dutch, F: French, G: German, H: Hungarian, I: Italian, K: Korean, S: Serbia, UK: United Kingdom, W: Welsh, nk: not known
This table is adapted from a review by Kinugawa et al [194] with more recently identified mutations added

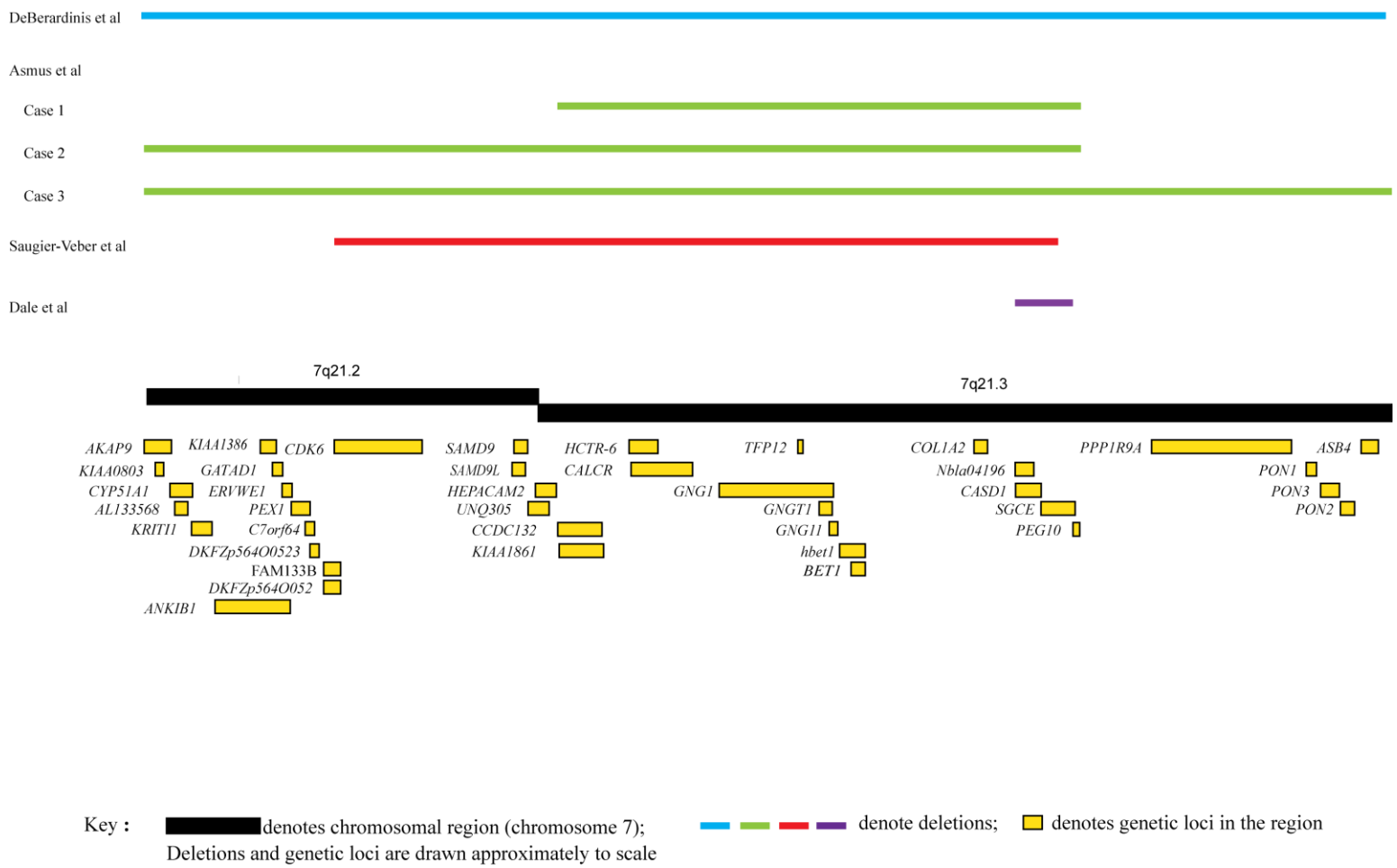
Table 5.2: Published reports of rates of *SGCE* mutations within probands cohorts

Study	Year of publication	Rate of SGCE mutations	Methods of genetic analysis		Proportion of *SGCE* mutations detected by direct sequencing	Proportion of *SGCE* mutations identified by CNV analysis
			Direct sequencing	CNV analysis		
Han et al[328]	2003	7/21 (33%)	Yes	Yes	100%	-
Valente et al[217]	2003	0/16 (0%)	Yes	No	0%	-
Schule et al[220]	2004	2/10 (10%)	Yes	Yes	100%	-
Valente et al[269]	2005	6/58 (10%)	Yes	No	100%	-
Tezenas du Montcel et al[188]	2006	16/76 (21%)	Yes	No	100%	-
Gerritis et al[270]	2006	7/31 (23%)	Yes	No	100%	-
Nardocci et al[186]	2008	9/11 (82%)	Yes	No	100%	-
Ritz et al[187]	2009	13/86 (15%)	Yes	Yes	92%	8%
Asmus et al[279]	2009	7/23 (30%)	Yes	No	100%	-

Table 5.3: Additional clinical characteristics described in previously reported *SGCE* contiguous gene deletion cases

	Deletion size	Genes involved	Intrauterine growth retardation	Microcephaly	Short stature	Dysmorphic facies	Joint laxity	Dental caries	Blue sclerae	Language delay	Cavernous cerebral malformations	Cognitive impairment	Split-hand/split-foot	Psychosis
DeBererdinis et al														
Case 1	9-16.5Mb	*SGCE*	X	X	X	X				X				
Asmus et al														
Case 1	1.63Mb	*PEG10, SGCE, COL1A2*			X		X	X						
Case 2	4.99Mb	*PEG10, SGCE, COL1A2, PEX1, KRITI*					X		X		X			
Case 3	8.78Mb	*PEG10, SGCE, COL1A2, PEX1, KRITI, DLX5*				X		X				X	X	
Saugier-Veber et al														
Case 1	1.88Mb	*SGCE, CASD1, COL1A2, BET1, GNG11, TFP12, GNG1, CALCR, HCTR-6, KIAA 1861, CCDC132, HEPACAM2 SAMD9, SAMD9L, CDK6*	X	X	X		X					X		
Dale et al														
Case 1	0.17Mb	*SGCE, CASD1*								X		X		
Case 2	0.17Mb	*SGCE, CASD1*												
Case 3	0.17Mb	*SGCE, CASD1*												X

Figure 5.1: Diagrammatic representation of contiguous gene deletions involving *SGCE*



5.2 Patients and methods

Patients were recruited as discussed in Chapter 2 (Section 2.2 and Figure 2.1). A blood sample was taken from each patient, and DNA was isolated from peripheral lymphocytes using standard protocols. All samples underwent direct sequencing of *SGCE* exons 1-12, including alternatively spliced exons 1a and 11b. All probands in whom an *SGCE* mutation was not detected by Sanger sequencing underwent gene dosage analysis using a commercially available probe set (P099B MRC Holland, Amsterdam, The Netherlands) (Section 2.5.11). The size of any deletion or duplication detected was then analysed using a custom oligonucleotide CGH array platform (Roche Nimblegen). Data was analyzed using the segment tool and visualized using SignalMap (Roche Nimblegen).

In order to thoroughly investigate the genetic aetiology of the movement disorder in all 89 proband cases, all remaining *SGCE* mutation negative samples underwent direct sequencing for the *TOR1A* (GAG deletion*)* as well as mutations in the *GCH1*, *THAP1* and *NKX2-1* genes. In view of the possible interaction of ε- and ζ-sarcoglycan proteins in the brain, *SGCZ* was sequenced in both *SGCE* mutation positive and negative cases for evidence of exonic or splice-site mutations.

All cases in whom an *SGCE* mutation was identified were asked to contact additional family members and invite them to participate in the study. In all those in whom consent or assent was obtained, a blood sample was taken for DNA extraction.

5.3 Results

Eighty-nine proband cases underwent analysis. Fifteen (16.9%) were identified as having an *SGCE* exonic or splice-site mutation using direct sequencing. MLPA analysis identified a further four (4.5%) to have deletions involving *SGCE*. Sanger sequencing also identified two (2.2%) of the proband cohort to carry an *SGCE* intronic polymorphism, of uncertain pathogenicity, ranging between 12 and 93 base pairs from their respective intron/exon boundaries. Of the remaining 67 cases, two were identified as having putative *NKX2-1* mutations, while no mutations were identified in the *TOR1A* (GAG deletion), *GCH1*, *THAP1* and *SGCZ* genes.

5.3.1 *SGCE* mutation positive cases identified by direct sequencing

Fifteen clinically affected probands were identified to have an *SGCE* mutation by direct sequencing of which 5 were novel mutations. These included 1 missense, 3 splice-site, 8 nonsense and 3 intra-exonic deletions (Table 5.4). Six additional motor affected individuals with *SGCE* mutations were recruited from the additional family members increasing the number of manifesting carriers (MC) to a total of twenty-one. The mutations in conjunction with the motor and psychiatric characteristics of these cases can be seen in Table 5.4.

Figure 5.2: Sequencing traces of mutations identified by direct sequencing of *SGCE* (1)

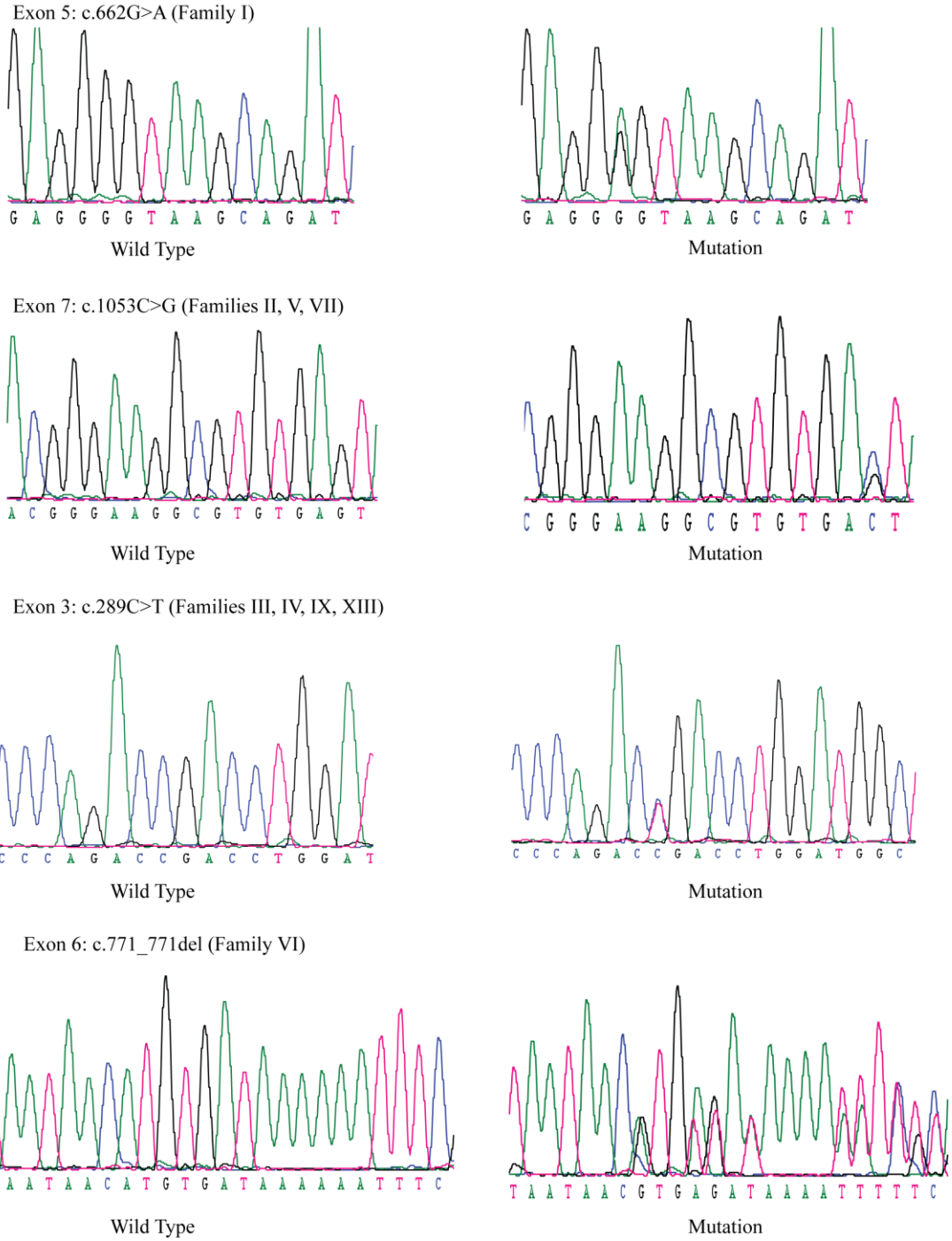
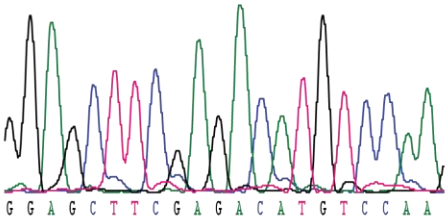
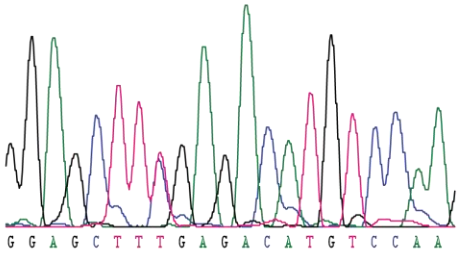


Figure 5.3: Sequencing traces of mutations identified by direct sequencing of *SGCE* (2)

Exon 9: c.114C>T \(Family VIII)

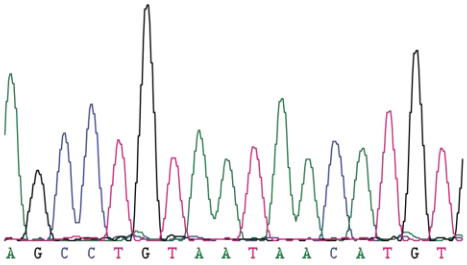


Wild Type



Mutation

Exon 6: c.765_773del (Family X)



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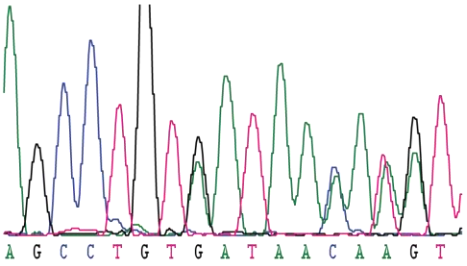
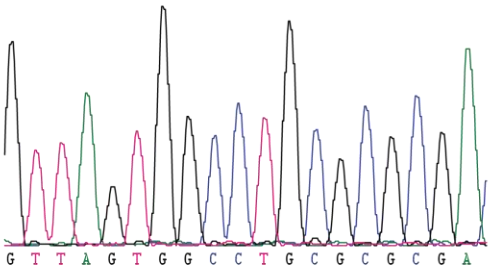


!

Mutation

Exon 1: C.109+5C>C (Family XV)



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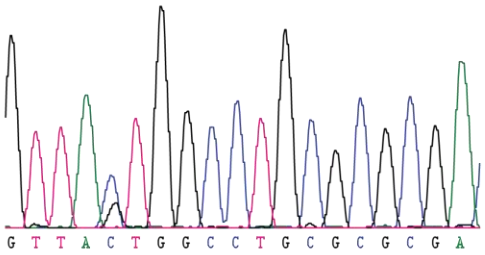


Mutation

Mutation exon 7 c.835_83del (Family XIV) was sequenced by Dr Adrian Waite and is not shown in these figures.

Table 5.4: Description of *SGCE* positive cases identified by direct sequencing

Family	Case No.	Age at onset		Age at examination	Body parts involved at onset		Body parts involved on examination		Psychiatric symptoms	Nucleotide change	Predicted protein	Mutation type	Family History	Inheritance
		Myoclonus	Dystonia		Myoclonus	Dystonia	Myoclonus	Dystonia						
I	1	4	3	6	H, UL, T	UL, LL	UL, T	UL, LL	No data	c.662G>A	p.Gly221Asp	missense	Yes	paternal
	2	7	7	68	UL, T	UL, LL	UL, T	N, UL	Nil	c.662G>A	p.Gly221Asp	missense	Yes	uncertain
II	3	14	11	19	UL, T	UL	H, UL, T	N, UL	D, OCD, Alc dep	c.1037+5G>A	unknown	splice site	Yes	paternal
	4	12	4.5	18	LL	V	H, UL, LL	N, UL, V	D, PanD, Ag, SP, OCD, GAD	c.1037+5G>A	unknown	splice site	Yes	paternal
	5	10	?	63	UL	?	UL, T	N, UL	OCD, Alc dep	c.1037+5G>A	unknown	splice site	Yes	uncertain
III	6	8.5	8.5	30	H, UL, T	N	H, UL, T	N, UL, V	PanD, Ag, SP, OCD, Alc dep, GAD	c.289C>T	p.Arg97X	nonsense	Yes	paternal
	7	11	11	62	H, UL	N	nil seen	N, UL	OCD	c.289C>T	p.Arg97X	nonsense	Yes	uncertain
IV	8	3	28	31	H	N	H, UL, T	N, UL	Ag, SP, OCD	c.289C>T	p.Arg97X	nonsense	Yes	paternal
	9	?	1.5	22	?	LL	UL, T	N, UL, LL	Nil	c.289C>T	p.Arg97X	nonsense	Yes	uncertain
V	10	3	2	15	LL	UL, LL	H, UL, T	UL, LL	D, OCD, GAD	c.1053C>G*	unknown	nonsense	Yes	paternal
	11	4.5	4.5	61	H, UL	N, UL	H, UL	N, UL	D, PanD, Ag, SP, Alc dep, GAD	c.1053C>G*	unknown	nonsense	Yes	paternal
VI	12	2.5	2.5	50	UL, T	UL	H, UL, T	N, UL, LL	D, PanD, SP, GAD	c.771_772del	p.Cys258X	deletion	Yes	paternal
VII	13	18	1.5	44	H, UL, T	UL	H, UL, T	N, UL, LL, V	D, PanD, OCD, Alc dep, GAD	c.1053C>G*	unknown	nonsense	No	uncertain
VIII	14	18	20	41	H, UL	N, V	H, UL, LL, T	N, LL, V	D, PanD	c.1114C>T	p.Arg372X	nonsense	Yes	paternal
IX	15	2	4	28	UL	N, UL	H, UL, T	N, UL	D, PanD, Ag, SP, GAD	C.289C>T	p.Arg97X	nonsense	Yes	paternal
X	16	2	1.5	4	H, UL, T	LL	H, UL, T	UL, LL	No data	c.765_773del	p.(Ile256Cys258del)	deletion	No	paternal
XI	17	10	10.5	54	H, UL	N	H, UL	N	PanD, SP, OCD, Alc dep, GAD	c.630_658del*	p.(Val211GlyfsX20)	deletion	No	uncertain
XII	18	7	7	74	UL, LL	LL	H, UL, LL	N, UL, LL, V	Nil	c.463+1G>A*	unknown	splice site	Yes	uncertain
XIII	19	1.5	1.5	47	UL	T	H, UL, T	N, UL, T	D, PanD, Ag, SP, OCD, Alc dep, GAD	c.289C>T	p.Arg97X	nonsense	Yes	paternal
XIV	20	10	19	21	UL	N, T	H, UL, T	N, T	D, PanD, SP, OCD, Alc dep, GAD	c.835_839del*	p.(Thr279AlafsX17)	deletion	Yes	paternal
XV	21	2	2	10	UL	UL	H, UL, T	UL, LL	No data	c.109+5G>C*	unknown	splice site	Yes	paternal

Key: H=head, LL=lower limbs, T-trunk, UL=upper limbs, V=voice, Ag=agoraphobia, Alc dep=alcohol dependence, D=depression, GAD=generalized anxiety disorder, OCD=obsessive-compulsive disorder, PanD=panic disorder, SP=social phobia, *=novel mutati

5.3.1.1 Case Reports

The pedigrees for these families are shown in Figures 5.4, 5.5, 5.6 and 5.7.

Family I

Patient IV: 1 (Female, aged 6 years) presented at aged 5 with 'clumsiness' at school. Symptoms began at 4 years of age with limb cramps and abnormal posturing impairing her gait and her ability to feed herself. Myoclonus, predominantly of the upper limbs, developed within the subsequent twelve months becoming the predominant symptom. On examination there was marked upper limb and truncal myoclonus, particularly prominent while feeding and writing together with cervical, hand and lower limb dystonia.

Patient II: 2 (Female, aged 68 years) reported lifelong upper body 'jerks', impairing function during adolescence but becoming less prominent with age. On examination truncal and upper limb myoclonus was visible with outstretched arms in conjunction with a mild cervical dystonia. She experienced little day-to-day functional impairment, was no longer under the care of a neurologist and not receiving any ongoing treatment. No additional neurological characteristics were noted.

Patient III: 2 (Male, aged 37 years) reported no clinical symptoms and neurological examination was unremarkable.

Family II

Patient III: 5 (Female, aged 19 years) Caucasian female presented in adolescence with writer's cramp, developing upper body action predominant myoclonus three years later. The movement disorder had little impact upon day-to-day living and was very alcohol sensitive. Upon examination there was myoclonus of the head, trunk and upper limbs together with less prominent cervical and hand dystonia.

Patient III: 6 (Female, aged 18 years) Symptoms began in childhood with reported impaired phonation followed by brief lower limb 'jerks' in early adolescence. Examination revealed characteristic upper body distribution of both myoclonus and dystonia along with occasional lower limb 'jerks' in the seated position. There was also evidence of laryngeal dystonia with sustained phonation.

Patient II: 5 (Male, aged 63 years*)* was largely unaware of his symptoms although did recall difficulty writing while at school due to upper limb 'jerks'. Examination revealed an upper body predominant movement disorder with marked restriction of neck movements.
Patient III: 3 (Male*)* Not examined as part of this study, but under the care of the regional neurologist, this patient was reported to have a very similar distribution and semiology of movement disorder to that of the other family members.

Family III

Patient III: 8 (Male, aged 30) was observed to have symptom onset in childhood with a predominant myoclonic component. Upon examination he was severely disabled with marked retrocollis and prominent upper limb myoclonus, struggling to perform basic tasks of daily living.
Patient II: 8 (Male, aged 62 years) had a much milder phenotype. Reporting occasional upper limb myoclonus in childhood, none was evident upon examination. However, when performing tasks, especially writing marked laterocollis was noted.
Patients II: 2 (Male) *& III: I* (Female) were not examined as part of this study but were both reported by other family members to have upper body jerks beginning in adolescence.

Family IV

Patient III: 6 (Female, aged 31 years) had a reported history of childhood onset myoclonus with dystonic symptoms not becoming apparent until her late twenties. Upon examination she had a typical upper body pattern of involvement with additional laryngeal dystonia, exacerbated during periods of stress and impacting upon her ability to maintain employment
Patient III: 8 (Female, aged 22 years) had a much milder phenotype, initially presenting to the regional neurologist with unilateral lower limb dystonia, particularly symptomatic in her Right foot. Examination revealed subtle upper limb myoclonus only apparent with posture.
Patient I: 2 (Male) now deceased, was reported to have had 'jerks' involving the upper limbs and trunk. He was also described to suffer from significant symptoms of anxiety.

Family V

Patient VI: 1 (Male, aged 15 years) was reported to have onset of predominantly dystonic symptoms at 2 years of age. Motor symptoms were treated successfully with GP_i DBS in early adolescence. Psychiatric symptoms persist in the form of depression, anxiety and thoughts of self-harm.

Patient V: 5 (Male, age at death 28 years) died from cardiac causes, no reported signs of a movement disorder.
Patient: III: 12 (Female, age 61 years) developed motor symptoms at 4.5 years of age, intermittently treated with oral medication. She has frequent episodes of severe depression coupled with excess alcohol intake.
Patient III: 9 (Male, age ~68 years) not examined as part of this study, currently receives treatment from local neurology services for 'twitching'.
Patients II: 8, IV: 6 & IV: 7 (Females) no evidence of movement disorder
Patient II: 4 (Female, age at death unknown) no reports of movement disorder but frequent psychiatric inpatient stays.
Patient III: 4 Not examined as part of this study due to OCD symptoms preventing visitors to the house.

Family VI

Patient III: 1 (Female, age 50 years). Motor symptoms began in childhood and have evolved into a predominant myoclonic picture with little response to oral medication.
Patient III: 4 (Male, age at death ~mid 40s). Died due to head trauma. Reported to have upper body 'jerks'.
Patient II: 4 (Male, age at death uncertain). Reported to have had episodes of significant alcohol excess but no movement disorder recalled.

Family VII

Patient III: 2 (Female, age 44 years). Symptoms began as a predominant lower limb dystonic syndrome in childhood evolving to more pronounced upper body myoclonus. Associated socially disabling OCD. No family history of movement disorders, psychiatric disorders or alcohol excess.

Family VIII

Patient II: 3 (Female, aged 41 years). Onset of symptoms in late adolescence, prominent lower limb involvement in adulthood. Symptoms of depression and panic disorder.
Patients II: 1, 4, 5, 6 & 7. Not examined as part of this study but known and genetically tested by the regional neurology service. Significant disability owing to alcohol excess.

Family IX

Patient III: 2 (Male, aged 28 years) onset of myoclonus at two years of age, with moderate current impairment upon activities of daily living. More significant impact upon working life owing to anxiety related symptoms.

Patient III: 1 (Male, aged 16 years) not examined as part of this study but reported to be having difficulties at school, partly precipitated by upper body 'jerks'.

Patient II: 4 (Male, aged ~55 years). Reported to have significant social difficulties, generalized anxiety and alcohol excess. No reported movement disorder.

Patient I: 2 (Female, age at death unknown). No reported movement disorder but several inpatient psychiatric admissions. Adopted at birth and therefore no further family history.

Family X

Patient IV: 2 (Male, aged 4 years). Welsh, Caucasian onset of lower limb dystonic symptoms at aged 18 months.

Patient III: 4 (Male, aged 28 years) No evidence of movement disorder upon examination.

Patient II: 3 (Female, aged ~60 years). Little ongoing contact with her son and not examined as part of this study. No reported movement disorder but significant anxiety-type symptoms.

Family XI

Patient III: 1 (Male, aged 54 years) Caucasian from Ireland. Onset of symptoms aged 10 years, predominant myoclonic picture with previous excess alcohol consumption. Both parents were Irish with no history of movement disorders on either side.

Family XII

Patient II: 2 (Female, aged 79 years) Caucasian from Ireland, non-consanguineous family. Not examined as part of this study but reported to have onset of lower limb dystonia aged 5 years which has progressed to involve the neck and upper limbs.

Patient II: 3 (Female, aged 76 years) Simultaneous onset asymmetric (right > left) myoclonus and dystonia aged 7 years. Dystonia beginning in the lower limbs, then progressing to involve the upper body, neck and larynx. Myoclonus noted in head, upper limbs and lower limbs at onset, distribution has remained largely unchanged.

No symptoms reported in the offspring of either patient II: 2 or II: 3

Family XIII

Patient III: 1 (Female, aged 47 years) Caucasian, originally from the Midlands. Onset of symptoms at 18 months of age with truncal dystonia, mistaken for spinal scoliosis and upper limb myoclonus.

Patient III: 3 (Male, aged ~40 years) Not examined as part of this study but reported to have upper body myoclonic and dystonic symptoms.

Patient II: 5 (Male, age at death uncertain). Family unable to recall evidence of a movement disorder however, strong history of alcohol excess and OCD

Patient II: 4 (Male) OCD and depressive symptoms. No reported movement disorder.

Patient I: 1 (Male, age at death unknown). Significant history of excess alcohol consumption, no movement disorder recalled.

Family XIV

Patient III: 2 (Female, aged 21 years). Lithuanian origin. Onset of upper body myoclonus at aged 10 years with development of subtle dystonia at nineteen. Most pronounced disability occurring with fine motor tasks. Symptoms are highly alcohol responsive and exacerbated by social stressors.

Patient III: 1 (Female, aged 25 years). Not examined as part of this study, under care of and genetically tested by local neurologist. Reported to have myoclonic and dystonic symptoms most pronounced with tasks e.g. writing.

Patient II: 1 (Female). Not examined as part of this study but reported to have motor symptoms.

Patient II: 2 (Male). Not examined as part of this study but reported to have motor symptoms.

Patient I: 1 (Male). Reported to have had upper body ‘jerks’, no recollection of psychiatric symptoms.

Family XV

Patient IV: 5 (Male, aged 10 years). Onset of predominantly upper limb symptoms at aged 2 years, upon examination additional lower limb dystonia and truncal myoclonus observed.

Patient III: 8 (Male, aged 39 years). No evidence of motor symptoms nor psychiatric disorders upon examination.

Patient IV: 1 (Male, ~6 years). Not examined as part of this study. Known to local paediatric neurologist and reported to have clinical signs consistent with a diagnosis of MDS and the same genetic mutation as patient IV: 5.

Figure 5.4: Pedigrees of families I, II and III

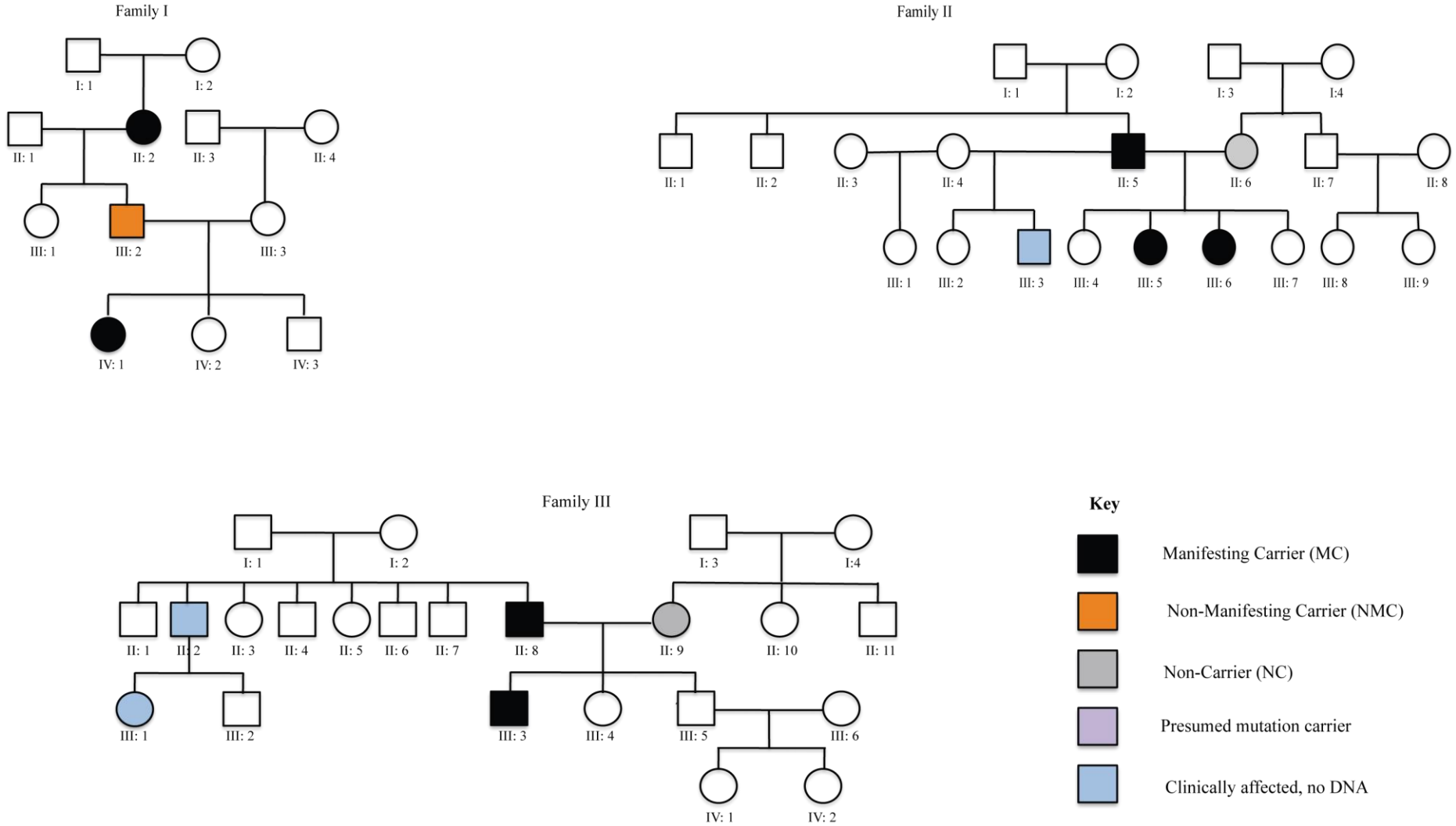


Figure 5.5: Pedigrees of families IV, V, and VI

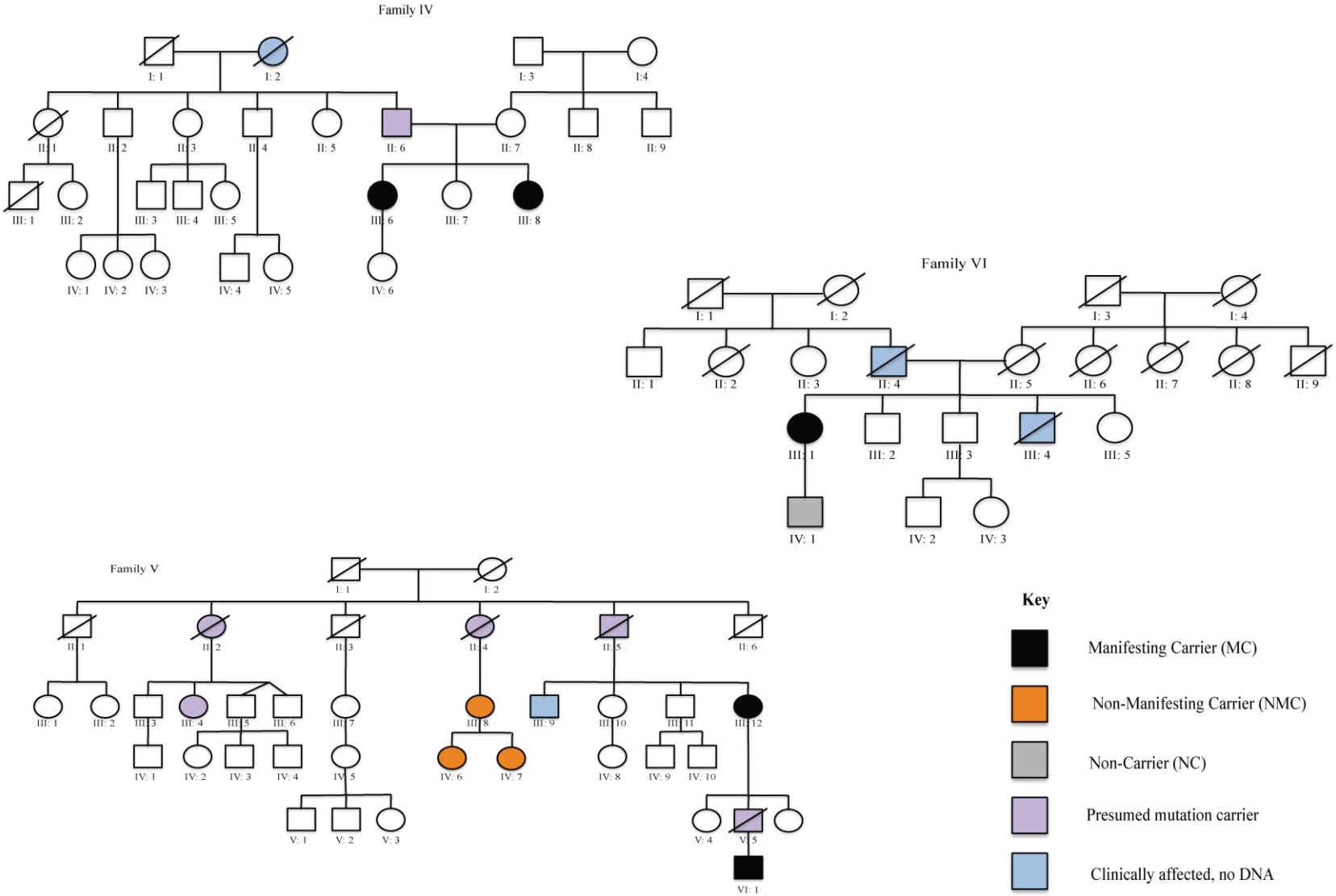


Figure 5.6: Pedigrees of families VII, VIII, IX, X and XI

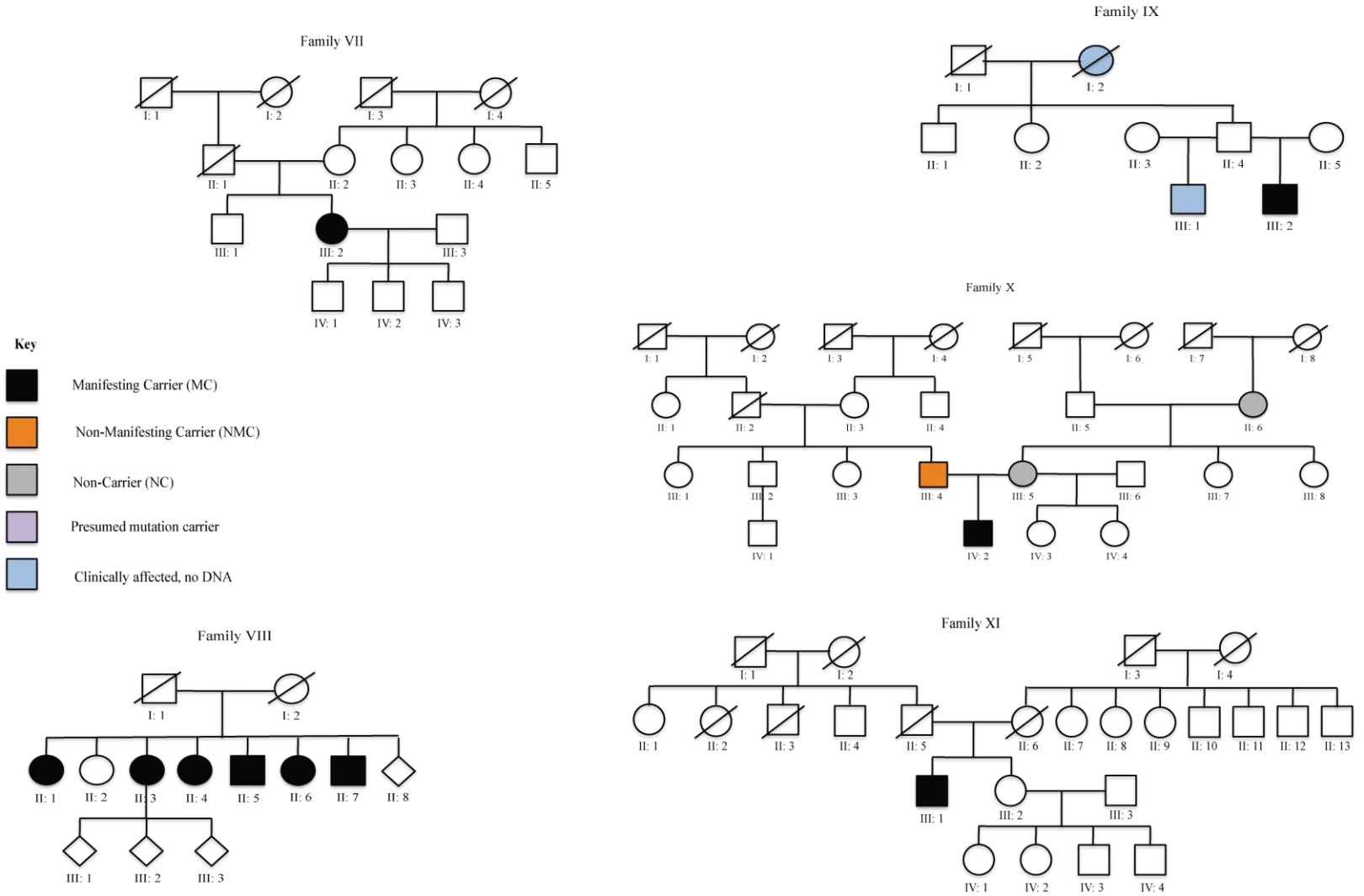
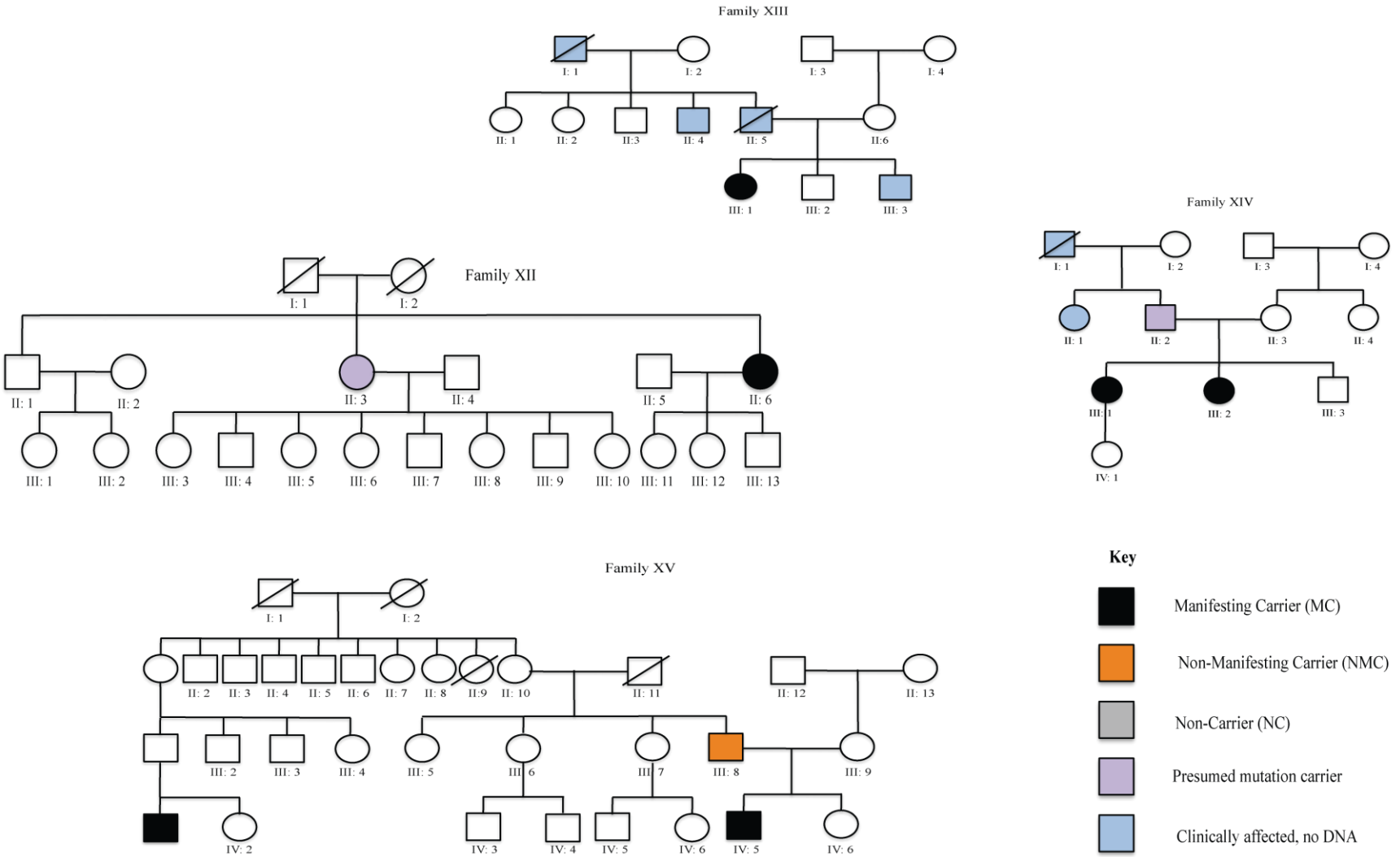


Figure 5.7: Pedigrees of families XII, XIII, XIV and XV



5.3.2 Cases identified by MLPA analysis

Four (4.5%) of the 89 probands were detected to have mutations involving the *SGCE* gene by MLPA analysis. All four were deletions, one a single exon deletion (exon 5) and the other three contiguous gene deletions involving *SGCE*. A single additional clinically affected individual was identified in two of the families with large deletions. Repeat MLPA confirmed a similar mutation in each of these cases increasing the total number identified to six. In order to determine the size of the contiguous gene deletions, these cases were analyzed on a custom oligonucleotide CGH array platform, with results analysed using the segment tool and visualized using the SignalMap software (Figure 5.8). Deletions varied in size between 0.7Mb and 2.3Mb with evidence of subtle intra-familial variation in family XVII. A comparison with previously published contiguous gene deletions is shown in Figure 5.9.

5.3.2.1 Case Reports

Pedigrees are shown in Figure 5.10

Family XVI

Patient IV: 2 (Female, aged 5 years) Simultaneous onset of myoclonus and dystonia at aged 3 years, predominantly affecting the upper limbs. On examination there was additional evidence of truncal myoclonus and lower limb dystonia as well as features of OCD with psychiatric evaluation.

Patient III: 1 (Male, aged 29 years) No evidence of motor signs nor psychiatric pathology on examination.

Patient II: 3 (Female, aged 55 years) No evidence of motor signs nor psychiatric pathology on examination.

Family XVII

Patient III: 4 (Female, aged 8 years) Symptoms began with lower limb dystonia at aged 2.5 years with evidence of upper body myoclonus by aged three. Upon examination there was evidence of dystonia affecting both upper and lower limbs. Myoclonus, although predominant in the upper body, was also evident in the lower limbs. This took the form of negative myoclonus that impaired stability during movement. Psychiatric testing revealed

evidence of a number of disorders principally anxiety and OCD related. This patient had little response to oral medication and has gone on to receive GP_i DBS with some improvement to her motor symptoms. Additional clinical characteristics included short stature, microcephaly and cognitive impairment.

Patient III: 7 (Female, aged 4 years) Initial symptoms were again of lower limb dystonia, followed by head and neck myoclonus. Paediatric assessment had also reported microcephaly, short stature and joint laxity, such that specialist rheumatological advice had been sought. Upon examination there was also evidence of dystonia involving the neck and arms. OCD was the predominant psychiatric symptom.

Patient II: 3 (Male, aged 32 years) No evidence of motor or psychiatric symptoms upon evaluation.

Patient I: 2 (Female, aged ~60 years) This lady was not examined as part of the study as her family no longer wished to be in contact with her. Reported history included epsiodes of unexplained 'leg collapse', severe recurrent depression requiring multiple inpatient psychiatric admissions and alcohol excess.

Family XVIII

Patient III: 2 (Male, aged 9 years) Onset of symptoms were at aged 4 years with both myoclonus and dystonia affecting the upper body. At time of examination symptoms had increased in severity according to observational reports but were limited to the same distribution. Psychiatric evaluation revealed predominant symptoms of anxiety and OCD related disorders, significantly worse than his other siblings. Additional clinical characteristics included short stature and language delay.

Patient III: 4 (Female, aged 3 years) Symptoms had begun a year prior to examination again with both myoclonus and dystonia limited to the upper body. This had remained unchanged at the time of examination and was less severe than that of her older brother's. She was also noted to be of short stature compared to children of the same age. OCD and anxiety related disorders were again present upon psychiatric evaluation

Patient II: 7 (Male, aged 36 years) There was no evidence of myoclonus, dystonia or psychiatric symptomatology at the time of evaluation. Despite an extended paternal family there were no other reported cases and no reported motor or psychiatric symptoms in either of the paternal grandparents. However, neither clinical assessment nor genetic analysis of the grandparents was possible therefore it is impossible to exclude a *de novo* mutation.

Family XIX

Patient IV: 4 (Male, aged 33 years) Caucasian, born in Scotland to Scottish father and Brazilian mother. Patient recalls upper limb 'jerks' as a child. Hospital records show several out-patient appointments with paediatric neurologists where upper limb myoclonus was noted and normal EEGs documented. Upon examination he was of short stature with very infrequent upper limb jerks and subtle cervical dystonia. This patient's predominant difficulty was severe schizophrenia, resulting in a number of inpatient psychiatric admissions, difficulties with education and maintaining employment.

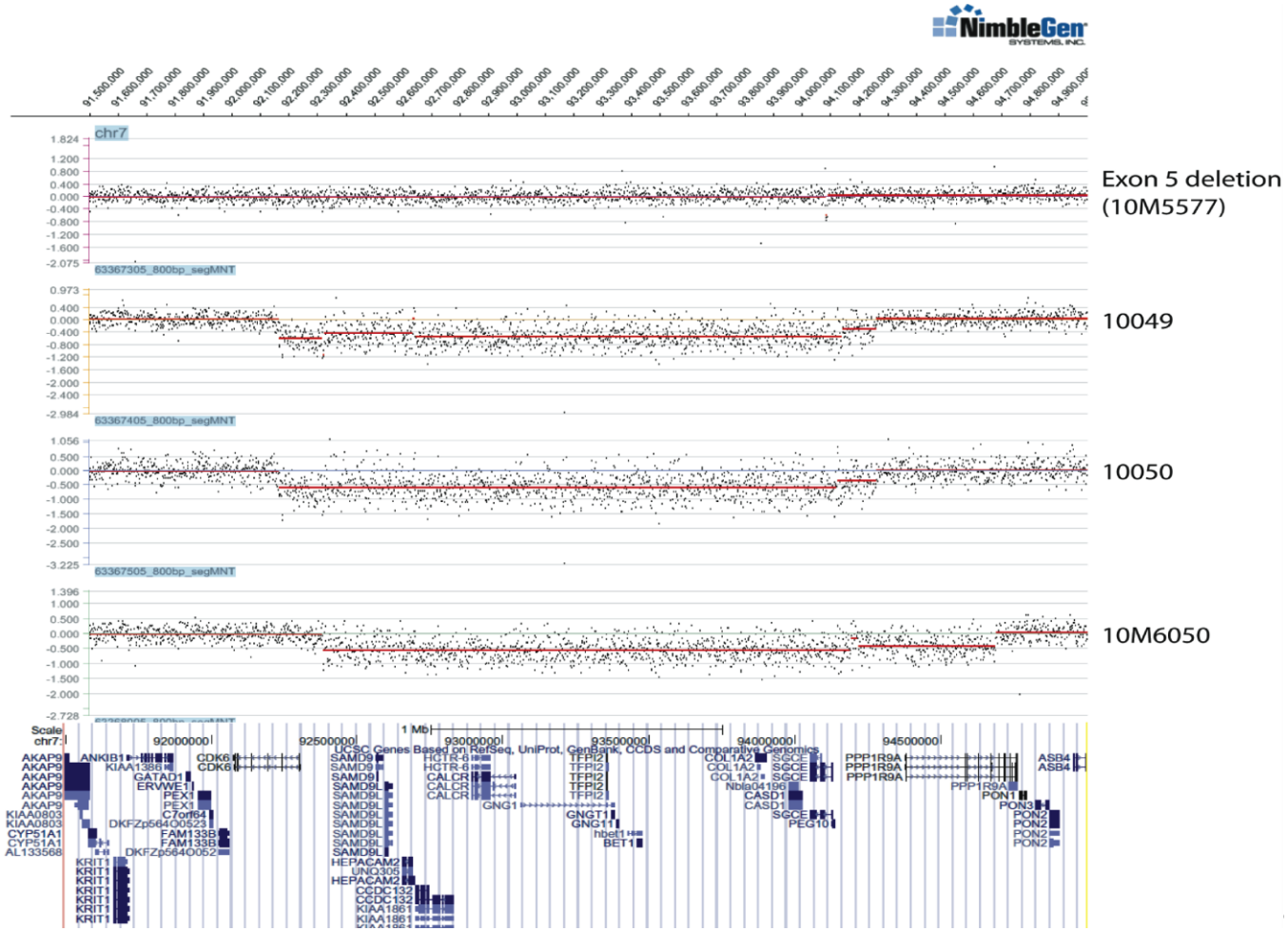
Patient III: 4 (Male, deceased) Reported to have suffered from severe depression and alcohol excess with possibly some motor symptoms. Death was thought to be related to chronic alcohol consumption.

Patients II: 1, II: 2 & III: 3 (Male) Not examined as part of this study but all were reported to have difficulties with significant excess alcohol consumption.

Patient IV: 1 (Male, aged ~30 years) This gentleman was not examined as part of the study as he was considered too unwell. He was reported to have ongoing psychiatric symptoms with a diagnosis of schizophrenia. He had not been examined for evidence of motor symptoms although there was a history of alcohol excess.

A summary of the motor and psychiatric symptoms along with the additional clinical characteristics observed and their comparison to previously published reports can be seen in Tables 5.5 and 5.6 respectively.

Figure 5.8: Results of CGH analysis used to determine size of the contiguous deletions identified by MLPA analysis



Size and location of contiguous gene deletions compared to gene locations identified using UCSC Genome Browser

Figure 5.9: Diagrammatic representation of contiguous gene deletions identified in this study and those previously reported

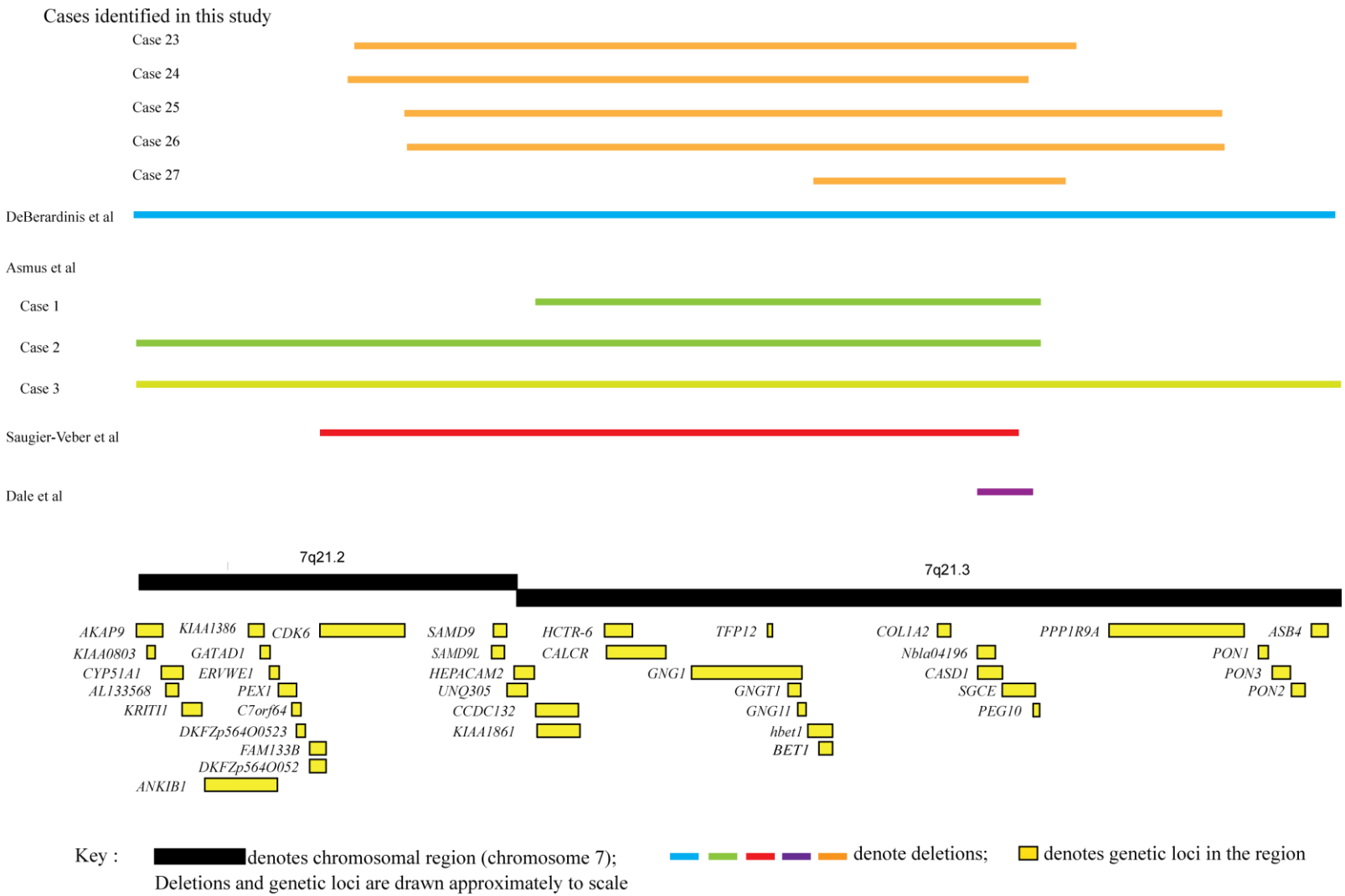


Figure 5.10: Pedigrees of families XVI, XVII, XVIII and XIX

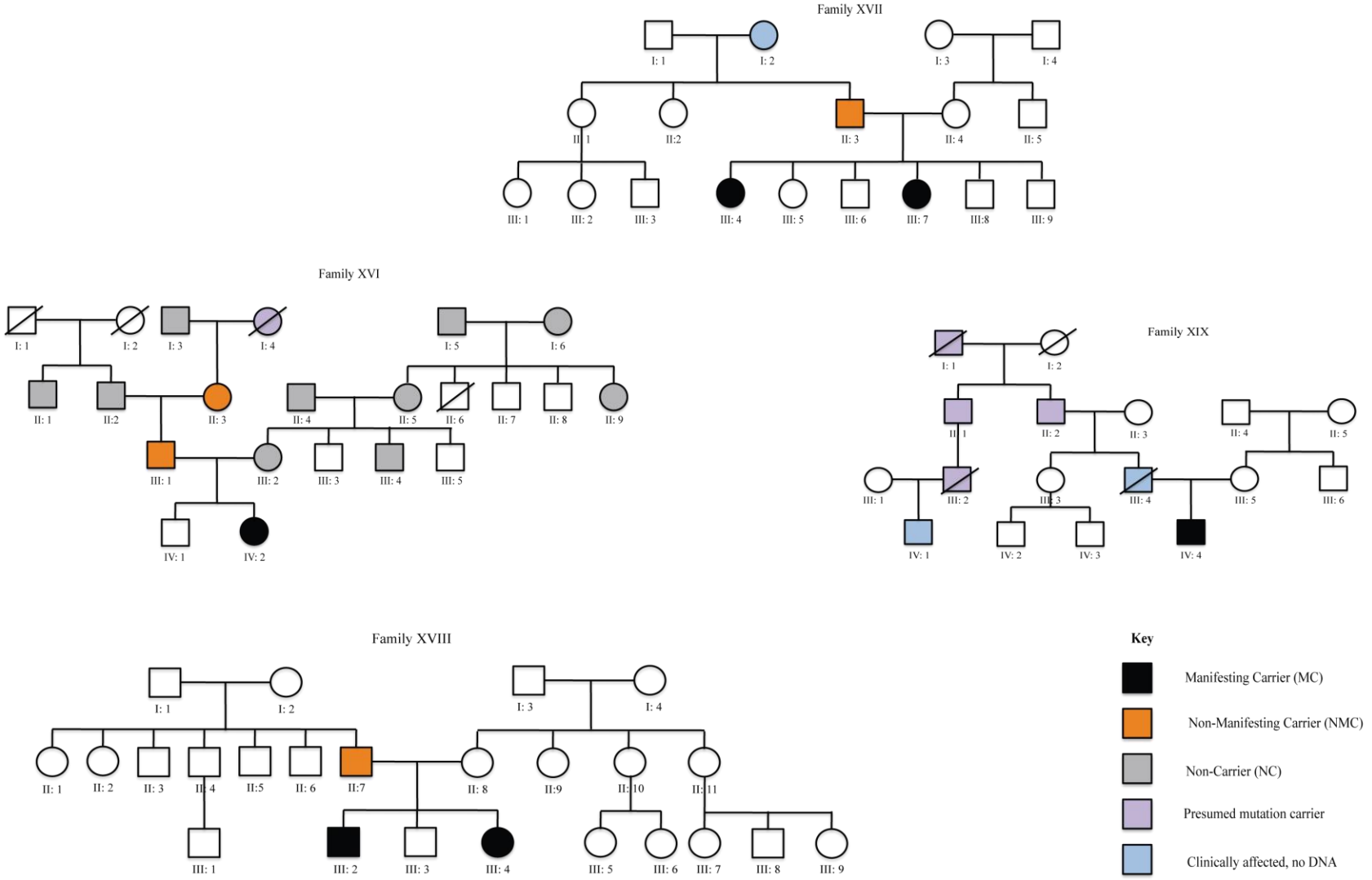


Table 5.5: Motor and Psychiatric characteristics of patients with *SGCE* mutations identified by MLPA analysis

Family	Case No.	Age at onset		Age at examination	Body parts involved at onset		Body parts involved on examination		Psychiatric symptoms	Nucleotide change	Mutation type	Family History	Inheritance
		Myoclonus	Dystonia		Myoclonus	Dystonia	Myoclonus	Dystonia					
XVI	22	3	3	5	UL	UL	UL, T	UL, LL	OCD	Exon 5 deletion	deletion	No	paternal
XVII	23	3	2.5	8	H, UL	LL	H, UL, LL	UL, LL	D, Ag, SP, OCD, GAD	WGD	WGD	Yes	paternal
	24	3.75	2	4	H	LL	H, UL	UL, LL	SP, OCD	WGD	WGD	Yes	paternal
XVIII	\												
	25	4	4	9	UL, T	N, UL	UL, T	N, UL	D, SP, OCD, GAD	WGD	WGD	Yes	paternal
	26	2	2	3	H, UL	N, UL	H, UL	N, UL	D, OCD, GAD	WGD	WGD	Yes	paternal
XIX	27	4	?	33	UL	?	H, UL	N	Schizophrenia	WGD	WGD	Yes	uncertain

Key: H=head, LL=lower limbs, T-trunk, UL=upper limbs, V=voice, Ag=agoraphobia, Alc dep=alcohol dependence, D=depression, GAD=generalized anxiety disorder, OCD=obsessive-compulsive disorder, PanD=panic disorder, SP=social phobia, WGD=whole gene deletion, *=novel mutation

Table 5.6: Clinical and genetic descriptions of contiguous gene deletion syndrome case

	Deletion size	Genes involved	Intrauterine growth retardation	Microcephaly	Short stature	Dysmorphic facies	Joint laxity	Dental caries	Blue sclerae	Language delay	Cavernous cerebral malformations	Cognitive impairment	Split-hand/split-foot	Psychosis
Case 23	1.9Mb	*SGCE, CASD1, COL1A2, BET1, GNG11, TFP12, GNG1, CALCR, HCTR-6, KIAA 1861, CCDC132, HEPACAM2 SAMD9, SAMD9L, CDK6*		X	X							X		
Case 24	2MB	*PEG10, SGCE, CASD1, COL1A2, BET1, GNG11, TFP12, GNG1, CALCR, HCTR-6, KIAA 1861, CCDC132, HEPACAM2 SAMD9, SAMD9L, CDK6*		X	X		X							
Case 25	2.3Mb	*PPP1R9A, PEG10, SGCE, CASD1, COL1A2, BET1, GNG11, TFP12, GNG1, CALCR, HCTR-6, KIAA 1861, CCDC132, HEPACAM2 SAMD9, SAMD9L*			X					X				
Case 26	2.3Mb	*PPP1R9A, PEG10, SGCE, CASD1, COL1A2, BET1, GNG11, TFP12, GNG1, CALCR, HCTR-6, KIAA 1861, CCDC132, HEPACAM2 SAMD9, SAMD9L*			X									
Case 27	0.7Mb	*PEG10, SGCE, CASD1, COL1A2*			X									X
DeBererdinis et al														
Case 1	9-16.5Mb	*SGCE*	X	X	X	X				X				
Asmus et al														
Case 1	1.63Mb	*PEG10, SGCE, COL1A2*			X		X	X						
Case 2	4.99Mb	*PEG10, SGCE, COL1A2, PEX1, KRITI*					X		X		X			
Case 3	8.78Mb	*PEG10, SGCE, COL1A2, PEX1, KRITI, DLX5*				X		X				X	X	
Saugier-Veber et al														
Case 1	1.88Mb	*SGCE, CASD1, COL1A2, BET1, GNG11, TFP12, GNG1, CALCR, HCTR-6, KIAA 1861, CCDC132, HEPACAM2 SAMD9, SAMD9L, CDK6*	X	X	X		X					X		
Dale et al														
Case 1	0.17Mb	*SGCE, CASD1*								X		X		
Case 2	0.17Mb	*SGCE, CASD1*												
Case 3	0.17Mb	*SGCE, CASD1*												X

Key: X = presence of clinical feature

5.3.3 *SGCE* deep intronic variants

Of the remaining 70 probands, two (2.9%) were identified as having an *SGCE* intronic polymorphism located between 12 and 93 base pairs from their respective splice-site boundaries. Each of these cases underwent face-to-face clinical evaluation together with any family members (with and without motor symptoms) willing to participate. A summary of their clinical findings along with details of each polymorphism can be seen in Table 5.7. These cases were also underwent *TOR1A* (GAG deletion), *GCH1*, *THAP1*, *NKX2-1* and *SGCZ* sequencing to ensure an alternative diagnosis had not been missed.

5.3.3.1 Case Reports

Pedigrees of families XX and XXI are seen in Figure 5.11.

Family XX

Patient IV: I (Male, aged 17 years) Initially presented to the paediatric service in his early teens having developed upper limb jerks. These worsened during times of stress, unresponsive to alcohol and improved with sodium valproate. There was no history of blackouts or seizures and interictal EEG was reported as normal. There was no evidence of either myoclonus or dystonia upon examination.

Patient III: 1 (Female, aged 39 years) Symptoms began at aged 12 years with 'jerks' of the head and neck followed by cervical dystonia. Motor symptoms were not alcohol responsive although treatment with clonazepam provided some symptomatic relief. The motor symptoms were later superseded by prominent psychiatric symptoms, principally alcohol excess and OCD. Upon examination there was no evidence of either spontaneous or stimulus sensitive myoclonus and only subtle signs of upper body dystonia.

II: 2 and II: 4 little is known of this part of the family and DNA was not available. Patient II: 2 is reported to have an addiction to recreational drugs and II: 4 difficulties with alcohol dependence and abuse

Table 5.7: Clinical and genetic description of cases with intronic variants

Family	Case No.	Age at onset		Age at examination	Body parts involved at onset		Body parts involved on examination		Psychiatric symptoms	Nucleotide change	Predicted protein	Polymorphism type	Family History	Inheritance
		Myoclonus	Dystonia		Myoclonus	Dystonia	Myoclonus	Dystonia						
XX	28	11	-	17	UL	-	-	-	Nil	c.109+12G>A	unknown	splice-site		maternal
	29	12	13.5	39	H	N	nil	N, UL	D, PanD, OCD, Alc dep	c.109+12G>A	unknown	splice-site	Yes	unknown
XX1	30	-	0.5	34	-	LL	nil	LL	D, PanD, OCD, GAD	c.1065-93del	unknown	intron	Yes	paternal
	31	27*	32	37	UL*	N	UL*	N, UL	D, PanD, GAD	c.1065-93del	unknown	intron	Yes	paternal
	32	50*	58.5	64	H, UL, LL*	N, UL	H, UL, LL*	N, UL	PanD, GAD	c.1065-93del	unknown	intron	Yes	unknwon

Key: H=head, LL=lower limbs, T-trunk, UL=upper limbs, V=voice, Alc dep=alcohol dependence, D=depression, GAD=generalized anxiety disorder, OCD=obsessive-compulsive disorder, PanD=panic disorder, *=not true myoclonus, more consistent with a jerky dystonia

Family XXI

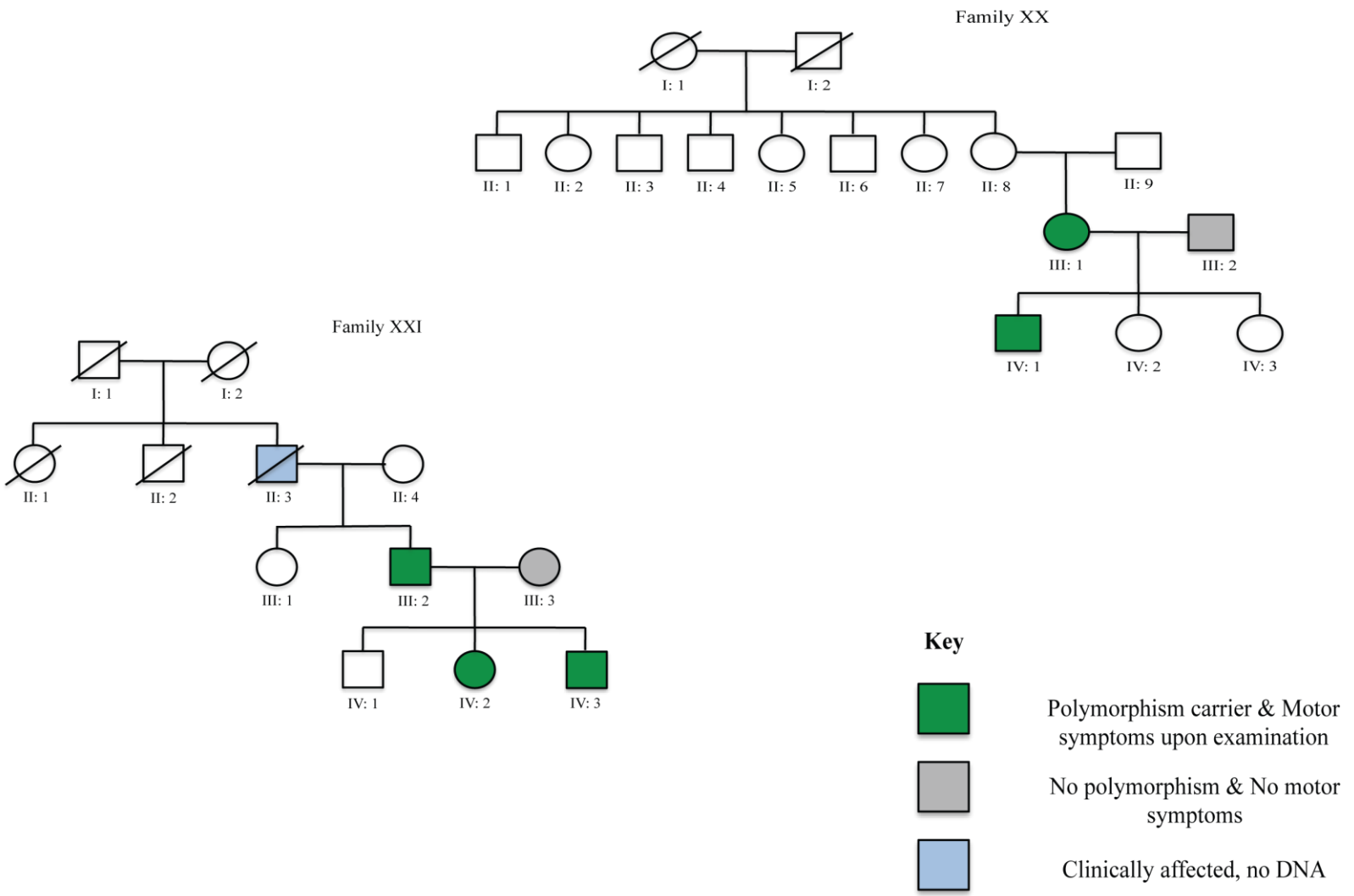
Patient III: 1 (Male, aged 64 years) Developed head and upper limb 'jerks' at aged 50 years and upper body dystonia almost a decade later. Upon examination there was clear evidence of cervical dystonia and writer's cramp with an additional 'jerky' movement disorder, less rhythmic than essential tremor and coarser in nature than myoclonus. In appearance it differed considerably from the myoclonus seen in cases described in Section 5.3.1.1. Generalised anxiety and related disorders were the predominant psychiatric phenotype.

Patient IV: 2 (Female, aged 37 years) Symptoms developed at aged 27 years with upper limb jerks similar in form to that of her father, followed by dystonic posturing of her neck and hands five years later. Both elements of the movement disorder were evident upon examination but to a milder degree than Patient III: 1. Psychiatric symptoms included depression, anxiety disorder and panic attacks, rendering her unable to maintain employment for the preceding eight years.

Patient IV: 3 (Male, aged 34 years) Noted to have an inverted Left foot in infancy, persisting into adulthood and is fully reversible. There was no reported history or evidence at examination of the more 'jerky' movement disorder seen in other family members. Symptoms of OCD and panic attacks developed at aged 18 years and have persisted into adult life having a significant impact upon ability to live independently and carry out activities of daily living.

Patient II: 3 (Male) Died in his 50s due to lung malignancy. He was reported to have developed head 'jerks', similar to those of his son towards the end of his life.

Figure 5.11: Pedigrees of families XX and XXI



5.3.4 *SGCE* mutation negative cases

The remaining 68 cases underwent direct sequencing for the *TOR1A* GAG deletion and mutations in *GCH1*, *THAP1*, *NKX2-1* and *SGCZ* genes. No mutations were detected in *GCH1*, *THAP1* or *SGCZ* genes and there was no evidence of the GAG deletion in *TOR1A*. Two cases were found to have novel *NKX2-1* variants that were considered to be potentially pathogenic. Both cases had been referred to the study by paediatric neurologists, a DNA sample being sent for analysis and clinical data collected retrospectively from the clinical notes. Further assessment of immediate family members is planned to determine if this variant co-segregates with the movement disorder. However, neither variant is recognized as a SNP in either dbSNP or The 1000 Genomes Project databases and were also not identified in any of the other remaining samples screened. In addition both variants lie within highly conserved regions of the genome.

***NKX2-1* variants**

Case 1 (c.1022C>T, p.Ala341Val)

Aged 14 years at the time of referral to the study, this Caucasian Irish female was reported to have onset of symptoms at aged 5 years. Clinical notes described her motor symptoms as a fine upper limb tremor. Symptoms were not severe enough to warrant treatment and there was no reported family history.

Case 2 (c.51C>G)

Caucasian female, 18 years of age at time of referral to the study, symptom onset was reported to have been 10 years old, again with an upper limb tremor. There were also reports that she had evidence of dystonia involving the neck, Right hand and larynx at the time of the clinical consultation. A trial of low dose trihexyphenidyl was initiated and there was no family history reported.

5.4 Discussion

A population of 89 probands, considered during clinical evaluation to have a potential diagnosis of MDS, underwent comprehensive genetic analysis of *SGCE* including direct sequencing and MLPA analysis. This identified 19 cases with pathogenic mutations involving the *SGCE* gene. This constitutes 21% of the total initial population, in keeping with the majority of previously published cohorts that have reported rates from 10%-85% dependent upon the stringency of their diagnostic criteria.[186-188, 217, 220, 269, 270, 279, 328] Of the nineteen probands, fifteen (79%) were identified by direct sequencing and four (21%) through investigation of CNVs. *SGCE* variants of unknown significance and two potentially pathogenic *NKX2-1* mutations were also identified.

5.4.1 Mutations identified by direct sequencing

Of the fifteen mutations identified by this method the majority were nonsense mutations (53%), intra-exonic deletions and splice-site mutations constituted a fifth of all cases each and there was a single missense mutation (7%). These findings are broadly in keeping with previous studies reporting loss of function mutations to be the most common, with pathogenic missense and splice-site mutations typically being half as frequent.[194]

This predominance of loss of function mutations involving *SGCE* is likely to relate to their impact upon resultant protein function. Nonsense mutations are thought to cause frame shift, resulting in premature truncation of the protein and its failure of expression at the cell surface membrane. Previous studies have also shown that the majority of missense mutations result in protein misfolding and proteosomal degradation, again resulting in failure of expression of the protein.[247] A previous report of the c.662G>A. p.Gly221Asp mutation, identified in our cohort, found evidence of exon skipping resulting in frameshift and a premature stop codon.[304] There are however, a small number of missense mutations that result in a gain-of glycosylation, causing partial expression at the cell surface membrane.[248] However, further work is required in larger cohorts to determine if these individuals differ in their clinical phenotype to those with no membrane protein expression

The most common mutation in this study was nonsense mutation c.289C>T, p.Arg97X in exon 3, occurring in four apparently unrelated Caucasian families. Internationally the most

common mutation is also a nonsense mutation within exon 3 (c.304C>T) reported in a large number of Caucasian European and North American families. This raises the possibility that exon 3, a region encoding the extracellular domain of the epsilon-sarcoglycan protein, may represent a hotspot mutation region although, if this is the case, the mechanism for this remains unclear.

All of our mutations have been identified in Caucasian individuals, the majority of Celtic or Anglo-Saxon descent and a single family from Eastern Europe. Wider publications also report the majority of mutations to be within North American or European Caucasian families. This may represent a founder effect or could be a consequence of research and resource bias. More recently there have been several case reports of mutations in Asian families including a splice site mutation in a Korean family,[190] several deletions in a Taiwanese case series[333] and a single base insertion in a Chinese family.[189] Notably no nonsense mutations have been reported amongst Asian cohorts, possibly representing regional differences.

5.4.2 Mutations identified by MLPA analysis

Of the four probands identified with a CNV involving *SGCE*, all were deletions. No duplications were identified within this cohort again consistent with previous literature.[231] The deletions identified included one case of a single exon deletion (case 22) and three contiguous gene deletions ranging in size from 0.7Mb to 2.3Mb. Inclusion of additional affected family members increased the number of these later cases to five, also demonstrating some subtle intra-familial deletion size variation, 1.9Mb vs. 2Mb (cases 23 and 24).

All six cases had features of both myoclonus and dystonia, the more subtle motor signs being seen in the smallest contiguous gene deletion case (case 27). There was also evidence of intra-familial variation in motor symptom severity, the elder sibling manifesting more pronounced and disabling symptoms in both sets of sibling pairs. This may represent a pattern of motor symptom evolution however, it is conceivable that, as is observed with *SGCE* point mutations, intra-familial phenotypic variation also exists in contiguous gene deletion cases.

As has been seen in previous case reports,[228] no additional clinical characteristics were observed in the patient with the single exon 5 deletion (case 22). The five contiguous gene deletion cases had a variety of additional clinical characteristics (Table 5.6), all of which have been previously reported. [230-233] There has been previous speculation that the size of the deletion may determine the additional characteristics.[231] Within this cohort, while all five cases were of short stature the number, type and nature of the additional characteristics appeared unrelated to deletion size, or genes involved. There was also evidence of intra-familial variation despite having the same or similar size deletion and non-motor phenotypes did not appear to relate to age - case 24 had marked joint laxity although her older sibling had no evidence of joint or connective tissue disease. Therefore the same intra-familial variability observed with motor symptom severity may also pertain to these additional clinical characteristics.

As shown in figures 5.1 and 5.7 these deletions span a large area of chromosome 7 and involve a number of genes. The genes in the region, the proteins they produce and the functions of those that are known are summarized in Table 5.7. *COL1A2* is one of the best understood, encoding the pro-alpha2 chain of type I collagen, mutations of which are associated with osteogenesis imperfect. Hence patients with CNVs involving this gene might be anticipated to have bone fractures, hypodontia and joint laxity. However, despite being involved in the deletion in all five cases in this study only a single patient (case 24) was observed to have joint laxity but had no history of fractures or problems with dentition.

KRITI1 (CCM1) mutations are thought to contribute to the development of cavernous cerebral malformation (CCM). None of the deletions in this study involved this gene however, two cases reported by Asmus et al[231] had deletions spanning this region but only one had evidence of CCMs upon cerebral imaging. These combined results suggest that not only does deletion size not necessarily determine the clinical phenotype but, deletions involving genes of known function may not necessarily result in the typical clinical picture associated with these genes. This implies that there may be additional epigenetic factors that influence phenotypic outcome. In addition a number of genes in this region are involved in cell-signaling pathways hence there may be variation in intra- and extra-cellular signaling mechanisms, which in turn result in phenotypic variation.

5.4.3 Genotype/Phenotype correlation

A few studies have attempted to identify genotype/phenotype relationships within *SGCE* mutation positive cohorts, the majority finding no clear relationship.[186, 328] Using the cases from this cohort, an attempt was made to relate the type and location of mutation within the gene to the patterns of motor symptom progression observed (Section 3.7). This is summarised in Figure 5.12. No clear relationship was identified with either type or location of mutation. However, the numbers within this cohort are relatively small and the phenotypes of motor progression may be inaccurate or in fact contain further subgroups. Similarly, if a genotype/phenotype relationship does exist this may not relate to motor phenotype alone and may also include the type and severity of psychiatric symptoms. Further work, with more detailed statistical modeling analyses is required in larger cohorts prior to determining the lack of relationship between these two aspects.

5.4.4 Intronic polymorphisms

Two probands were found to have intronic variants during direct sequencing, each being between 12 and 93 base pairs from their respective intron/exon boundaries. A number of intronic variants have been reported previously, the deepest being 43 base pairs from the intron/exon boundary.[335] Although clinical histories have been consistent with a diagnosis of MDS no functional cellular work has been performed to conclusively determine pathogenicity in any of the previously reported cases.

I examined the likelihood of pathogenicity in each of these families by addressing clinical phenotype and heritability. No true myoclonus was identified in any of the individuals examined as part of this study, either being absent at the time of examination or as seen in Family XXI, more typical of a 'jerky' dystonia rather than the 'lightening jerks' of myoclonus. In all previously published diagnostic criteria[194, 259] myoclonus forms an integral and essential component of the MDS diagnosis and therefore its absence suggests a likely alternative clinical diagnosis. Similarly, although age at onset in family XX is in keeping with current diagnostic criteria, two individuals in family XXI had onset of symptoms at ages 27 and 50 years. A previous study does report symptom onset at 60 and 75 years of two individuals within a single Dutch family.[337] However, this has not been reported in any subsequent cohorts and would generally be considered to be highly unusual in the context of MDS. Finally, only in family XXI is the pattern of inheritance via paternal transmission and therefore in keeping with maternal imprinting. In family XX there is evidence of maternal

transmission, which would suggest loss of imprinting if the *SGCE* variant were responsible for the clinical symptoms. This again has only been seen in a very small proportion of cases[320] and remains an unusual characteristic.

In combination, the atypical components of motor features, age at onset and patterns of inheritance make a clinical diagnosis of MDS and pathogenicity of each of the *SGCE* polymorphisms unlikely in both families. However, further investigation involving cDNA analysis and function protein expression is required to provide a conclusive answer.

5.4.5 *SGCE* negative cases

Sixty-eight proband cases remained following comprehensive *SGCE* analysis. Two were found to have potentially pathogenic *NKX2-1* variants. Further information is now being gathered to determine if the variant co-segregates with the movement disorder within each family. If co-segregation is observed, together with their absence in controls and presence in a conserved region of the genome, this would suggest that these variants are likely novel pathogenic variants and contribute towards the disease phenotype. Both patients are also being further evaluated for evidence of lung and thyroid disease.

As discussed in Chapter 1 (Section 1.8.3) although the brain sarcoglycan complex remains to be fully understood, unlike peripheral organs ζ-sarcoglycan is expressed at much higher levels alongside the ε-isoform.[244, 245] In view of this, and the possibility that the two isoforms may either interact or have a similar function in the brain, we sequenced *SGCZ* in both *SGCE* mutation negative and positive cases for evidence of exonic or splice-site variants. It was considered possible that *SGCZ* mutations could account for some of the genetic heterogeneity observed in previous cohorts or may influence genotype/phenotype relationships in those with an *SGCE* mutation. No *SGCZ* mutations were identified in either cohort suggesting that this gene is unlikely to play a role in this group of disorders. It remains a possibility that it does however contribute to an entirely disparate group of disorders, identification of which may aid our understanding of the function and interaction of both proteins.

The remaining 65 samples were not found to have mutations of the more common dystonia genes (*TOR1A* (GAG deletion) *GCH1, THAP1*) despite the majority having a dystonia predominant clinical phenotype. This suggests that there are additional genes, not yet

identified, that may be responsible for these syndromes. Further detailed phenotypic classification and sub-grouping of clinical phenotypes would aid in this process, particularly with the advent of next generation sequence analysis.

5.5 Conclusion

Loss of function mutations constitute the majority of *SGCE* point mutations causing failure of expression of the ε-sarcoglycan protein at the cell surface membrane. The majority of these mutations worldwide are located in exon 3, possibly highlighting a region of mutational susceptibility. All CNVs identified were deletions, demonstrating intra-familial variation and additional clinical characteristics not predicted by the genes involved. Although CNVs provide some basis of genotype/phenotype relationship, analysis of types and location of mutations failed to identify any meaningful genotype/phenotype relationship. Based upon patterns of clinical characteristics and heritability it seems unlikely that the intronic variants identified in this cohort represent pathogenic mutations. However, a causative genetic mutation has not been identified in the majority of the cases in this cohort, suggesting that further work is required with clinical phenotyping and use of whole exome and genome analysis.

Figure 5.12: Diagrammatic summary of *SGCE* genotypes identified in this study in relation to patterns of motor symptom progression

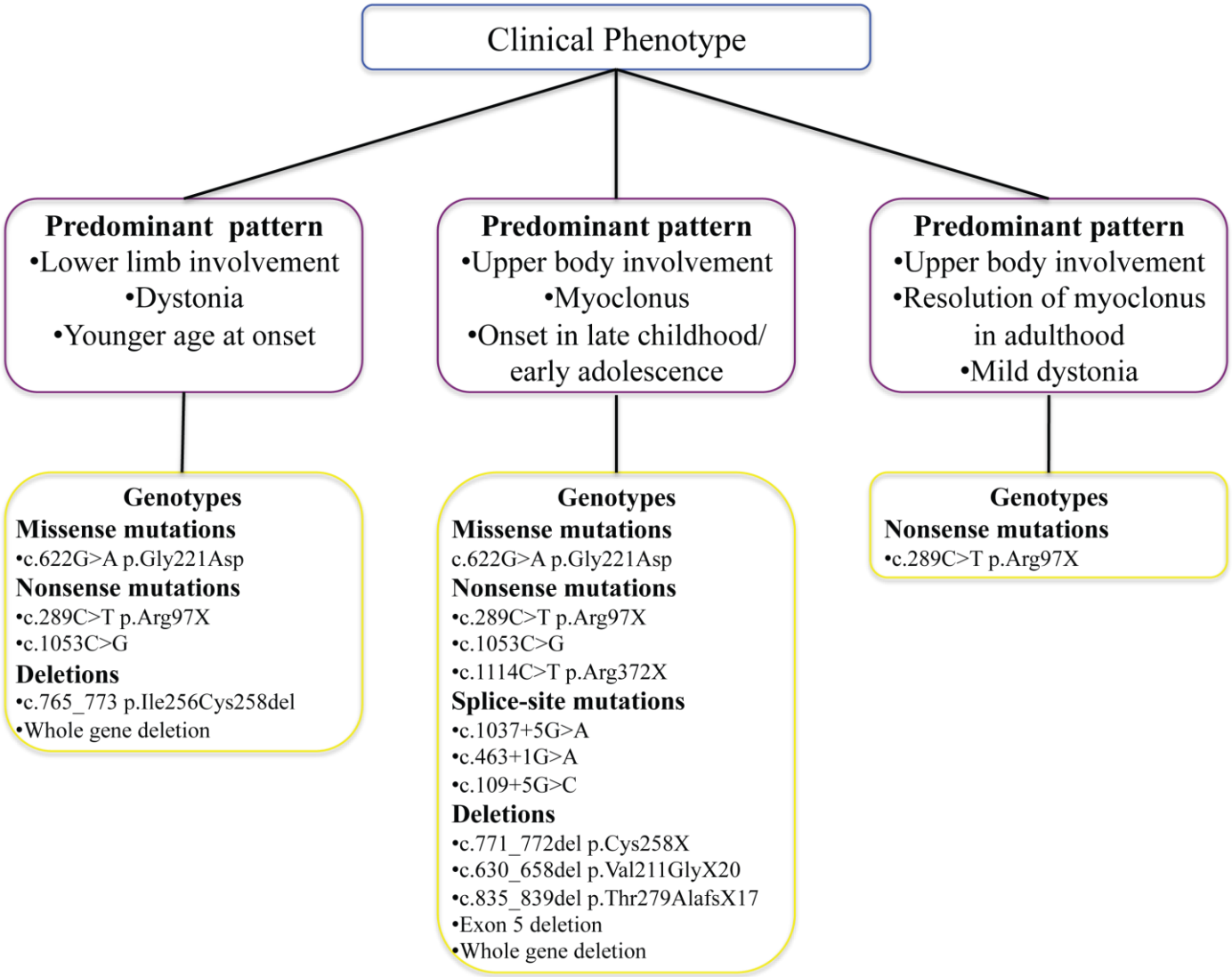


Table 5.8: Summary of chromosome 7 genes, proteins and known functions

Gene	Size	Protein	Function
AKAP9	169, 807 bases	A-kinase anchor protein	Scaffolding protein that binds to type II regulatory subunits of protein kinase A
KIAA0803	169, 797 bases	A-kinase anchor protein 9	Scaffolding protein that binds to type II regulatory subunits of protein kinase A
CYP5IA1	30, 804 bases	Member of cytochrome P450 superfamily of enzymes	Monoxygenases involved in drug metabolism and synthesis of cholesterol, steroids and other lipids
AL133508	137, 945 bases	Unknown	Unknown
KRITI1 (CCM1)	47, 198 bases	Krev interaction trapped protein 1	Mutations cause cavernous cerebral malformations
ANKIB1	155, 151 bases	Ankyrin repeat and IBR domain containing protein	Unknown
KIAA1386	29, 908 bases	Ankyrin repeat and IBR domain containing protein 1	Unknown
GATAD1	11, 978 bases	GATA zinc finger domain containing 1	Component of some chromatin complex recruited to chromatin sites
ERVWEI	9, 536 bases	Enverin/syncytin	expressed in the placental syncytiotrophoblast and involved in fusion of the cytotrophoblast cells to form syncytial layer of the placenta. HERV-W has been associated with Multiple Sclerosis & Schizophrenia in humans
PEX1	41,512 bases	Peroxisomal biogenesis factor 1	Required for stability of *PEX5* and protein import into the peroxisome matrix
C7orf64	9,233 bases	RNA binding motif protein 48	Unknown
DKFZp564O0523 (rbm48)	9,233 bases	RNA binding motif protein 48	Unknown
FAM133B	29,637 bases	family with sequence similarity 133, member B	Unknown
DKFZp564O052	29, 635 bases	Unknown	Unknown
CDK6	231,707 bases	Cyclin-dependent kinase 6	member of the cyclin-dependent protein kinase (CDK) family and may be an important regulator of cell cycle progression
SAMD9	18,511	sterile alpha motif domain containing 9	Unknown
SAMD9L	18,315 bases	sterile alpha motif domain containing 9-like	Unknown
HEPACAM2	37,939 bases	HEPACAM family member 2	Unknown
UNQ305	30, 972 bases	Unknown	Unknown
CCDC132	126,686 bases	coiled-coil domain containing 132	Unknown
KIAA1861	126686 bases	coiled-coil domain containing 132 isoform a	Unknown

HCTR-6	62521 bases	Unknown	Unknown
CALCR	150,244 bases	calcitonin receptor	high affinity receptor for the peptide hormone calcitonin and belongs to a subfamily of seven transmembrane spanning G protein-coupled receptors. The encoded protein is involved in maintaining calcium homeostasis and in regulating osteoclast-mediated bone reabsorption.
GNG1	319,500 bases	guanine nucleotide binding protein gamma 1	transduce extracellular signals received by transmembrane receptors to effector proteins
TFPI2	5,595 bases	tissue factor pathway inhibitor 2	Unknown
GNGT1	319,693 bases	guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 1	*GNGT1* encodes the gamma subunit of transducing. Transducin is a guanine nucleotide-binding protein found specifically in rod outer segments
GNGT11	4,821 bases	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-11	member of the guanine nucleotide-binding protein (G protein) gamma family and encodes a lipid-anchored, cell membrane protein.
Hbet1	41,608 bases	Homo sapiens Bet1p homolog	golgi-associated membrane protein that participates in vesicular transport from the endoplasmic reticulum (ER) to the Golgi complex
BET1	41,621 bases	BET1 homolog	Required for vesicular transport from the ER to the Golgi complex
COL1A2	36,672 bases	collagen, type I, alpha 2	encodes the pro-alpha2 chain of type I collagen. Type I is a fibril-forming collagen found in most connective tissues and is abundant in bone, cornea, dermis and tendon. Mutations in this gene are associated with osteogenesis imperfecta types I-IV, Ehlers-Danlos syndrome type VIIB, recessive Ehlers-Danlos syndrome Classical type, idiopathic osteoporosis, and atypical Marfan syndrome. Symptoms associated with mutations in this gene, however, tend to be less severe than mutations in the gene for the alpha1 chain of type I collagen (*COL1A1*)
Nbla04196	28,037 bases	Homo sapiens primary neuroblastoma cDNA	Unknown
CASD1	47,801 bases	CAS1 domain containing 1	Unknown
SGCE	70,986 bases	Epsilon-sarcoglycan protein	Component of the sarcoglycan complex, a subcomplex of the dystrophin-glycoprotein complex which forms a link between the F-actin cytoskeleton and the extracellular matrix
PEG10	13,371 bases	Retrotransposon-derived protein PEG10	Prevents apoptosis in hepatocellular carcinoma (HCC) cells through interaction with SIAH1, a mediator of apoptosis. May also have a role in cell growth promotion and hepatoma formation. Inhibits the TGF-beta signaling by interacting with the TGF-beta receptor ALK1.

			May bind to the 5'-GCCTGTCTTT-3' DNA sequence of the MB1 domain in the myelin basic protein (MBP) promoter (By similarity)
PPPIR9A		Unknown	Unknown
PON1	98,686 bases	Serum paraoxonase/arylesterase 1	*PON1* is responsible for hydrolyising organophosphate pesticides and nerve gasses. is also a major anti-atherosclerotic component of high-density lipoprotein
PON3	36,504 bases	Paraoxonase 3	secreted into the bloodstream and associates with high-density lipoprotein (HDL). The protein also rapidly hydrolyzes lactones and can inhibit the oxidation of low-density lipoprotein (LDL)
PON2	30,337 bases	Serum paraoxonase/arylesterase 2	encoded protein is ubiquitously expressed in human tissues, membrane-bound, and may act as a cellular antioxidant
ASB4	61,789 bases	ankyrin repeat and SOCS box containing 4	Probable substrate-recognition component of a SCF-like ECS (Elongin-Cullin-SOCS-box protein) E3 ubiquitin-protein ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins

CHAPTER 6

Concluding Remarks

6.1 Introduction

In this final chapter I will review the key findings of this thesis and highlight areas for future work. I will discuss how results from this study could impact upon our understanding of the aetiology, phenotype and pathogenesis of MDS as well as exploring avenues for future research.

6.2 Clinical spectrum and diagnostic criteria for *SGCE* positive Myoclonus Dystonia

Myoclonus dystonia caused by *SGCE* mutations has a narrow clinical spectrum. The predominant stable motor phenotype established in late adolescence and persisting through adult life is of upper body myoclonus and dystonia. This work has highlighted three distinct patterns of clinical presentation and evolution:

1) Younger onset (typically under 5 years of age) lower limb, dystonia predominant presentation that evolves during childhood to the more typical upper body, myoclonus predominant pattern (7/27 (25.9%) in this cohort).
2) Late childhood/early adolescent presentation of the typical upper body, myoclonus-predominant pattern that persists into adult life (19/27 (70.4%) in this cohort).
3) Childhood presentation of action/posture myoclonus that diminishes during adult life to a dystonia-predominant clinical syndrome. This represents the minority of cases, seen in only a single case in this cohort and another by personal communication (Chris Roxborough, Auckland, New Zealand) (1/27 (3.7%) in this cohort).

At present there appears to be no genotype correlation with these clinical patterns and there is substantial intra-familial phenotypic variability. This suggests that end clinical phenotype may be influenced by additional epigenetic and/or environmental factors.

Our current understanding of the natural history of MDS, in this study and in others, is based on interview and case notes review, gathering retrospective information. A prospective, longitudinal clinical study is needed to fully explore and understand the

natural history of this disorder. This could include an annual review with standardised videotaped assessment ideally collated via a centralized, multi-centre, collaborative database.

Using the earliest diagnostic criteria, rates of *SGCE* mutations within MDS cohorts were found to be between 20% and 30%. This resulted in the suggestion of genetic heterogeneity and a number of familial studies attempting to identify alternative genetic loci. This rate improved (50-91%) with the introduction of the Grunewald 'definite' diagnostic criteria (Table 3.2). However, these criteria also have their limitations with some mutation positive cases being classified as 'probable' rather than 'definite' owing to the lack of a positive family history, likely to be caused by maternal imprinting 'silencing' the clinical phenotype for several generations.[259]

During analysis of the clinical data from this study I attempted to improve the diagnostic criteria, increasing the yield of positive mutations while not decreasing the sensitivity of the testing. In addition, the aim was to provide clinically useful guidelines for the practicing clinician so that costly genetic testing could be targeted more effectively. A striking feature from the cohort was the very small number of mutation positive cases presenting with motor symptoms over the age of 10 years. It seemed pertinent that refinement of the diagnostic criteria should reflect this characteristic. In addition, although stratification of cases into 'definite', 'probable' and 'possible' is useful for research purposes, clearer genetic testing guidelines are required for the general movement disorder specialist in adult and paediatric fields. I therefore propose the following guidelines (Table 6.1), which when applied to this cohort had 100% sensitivity, 94% specificity and a PPV of 83%, improved from the 79% sensitivity, 97% specificity and 88% PPV of the Grunewald 'definite' criteria when applied to the same cohort.

Table 6.1 Proposed diagnostic criteria for identifying cases of Myoclonus Dystonia caused by *SGCE* mutations

Clinical Features
Early onset myoclonus and dystonia **OR** Isolated myoclonus predominantly in the upper body **AND** Positive family history **OR** Onset of symptoms ≤10 years

6.3 *SGCE* negative cases and nomenclature of hyperkinetic disorders

Within the overall cohort, 68 probands were found to not to have *SGCE*, *NKX2-1*, *TOR1A*, (GAG deletion), *THAP1* nor *GCH1* mutations. Of these 25% (17/68) had a definite or probably family history. In addition a proportion were observed to have additional motor characteristics including tremor, tics and chorea. This suggests that the majority of those in the mutation negative group are likely to have an alternative diagnosis and possibly an alternative genetic aetiology.

It would seem important that rather than collectively ascribing a diagnosis of 'Myoclonus Dystonia' to all childhood onset, partially alcohol responsive, 'jerky' movement disorders we should, instead, aim to provide detailed clinical descriptions of these syndromes and look for evidence of specific patterns of inheritance. This would allow the sub-grouping of clinically similar disorders, which with the advent of whole exome and genome methods of analysis would increase the likelihood of identifying an underlying genetic aetiology. This approach is likely to generate a large number of smaller sub-groups of clinical syndromes, which would require a collaborative multi-centre means of assessment and analysis in order to generate meaningful results.

6.4 Psychiatric disorders

This work demonstrates that psychiatric morbidity forms part of the clinical phenotype of *SGCE* mutation positive MDS with an excess of psychiatric symptoms being observed compared to population estimates, a disability matched control group and unaffected family members. The compulsivity component of OCD provided the most striking difference, an interesting finding given that OCD is generally not considered a secondary consequence of a chronic disabling disorder.[305]

Similar to previous studies, there was also a significant excess of alcohol consumption in the *SGCE* mutation cohort. Previous reports have suggested that this is likely due its secondary therapeutic benefits in suppressing the movement disorder. However, several elements of this work suggest that this response could be primary rather than secondary phenomenon. During systematic review of previously published literature a significant difference in excess alcohol consumption was observed between NMC and NC groups ($p=0.025$) with no difference between MC and NMC groups ($p=0.94$). Although these results were not replicated in the overall cohort it does suggest that *SGCE* may have a gene independent effect in contributing to alcohol excess. In addition a small number of studies have found a link between alcohol consumption and the more ritualistic components of OCD[323], including benefits of employing techniques traditionally used in the treatment of OCD to reduce craving for alcohol.[324]

To continue this work, future studies would include a more detailed assessment and characterisation of compulsivity symptoms. This would include systematic assessment of larger dystonia cohorts, including both genetically defined and sporadic forms, in order to determine whether this is a feature unique to those with *SGCE* mutations or a wider characteristic of dystonia. If unique to *SGCE* mutation positive cases then this would provide a further step in our understanding of the mechanism and pathogenesis of this disorder.

Finally, an interesting area of work would be to perform full neurological examinations and *SGCE* sequencing of patients attending specialized OCD outpatient clinics. Results from this work would be helpful in identifying whether *SGCE* mutations contribute more generally to OCD or whether there are specific elements,

which when combined with the movement disorder give rise to the observed psychiatric morbidity.

6.5 Whole gene deletion syndromes

No direct relationship between CNVs involving *SGCE* and neighbouring genes and the resultant clinical phenotype were observed in this cohort despite this being suggested in previous studies.[231] In addition I found considerable intra-familial phenotypic variability despite near equal deletion sizes. This again suggests that additional genetic or environmental differences are likely to contribute to the clinical phenotype.

All previous work relating to *SGCE* contiguous deletion syndromes has involved either single case reports or small case series. This group of five cases (3 probands) represents the largest combined cohort that I am aware of to date. Once again further understanding of these cases would require longitudinal assessment of a much larger cohort, systematically documenting the presence or absence of previously described features (Table 5.3) in conjunction with any novel characteristics.

This work also highlights the importance of CNV analysis in patients with neurological syndromes. A recent study of a paediatric Australian movement disorder cohort with suspected genetic aetiology found nearly thirty percent to have a micro-deletion. This included recognized movement disorder genes as well as regions not known to contribute to movement disorder pathogenesis.[338] A more systematic approach to genetic analysis, using either commercial MPLA kits or chromosome microarray (CMA), would result in a much larger group of patients with CNVs being identified. Larger cohorts would again aid in our understanding of the impact of these genetic anomalies and ultimately the function of neighbouring genes.

6.6 Treatment

Despite guidelines on the treatment of generalized and sporadic forms of dystonia[12] no previous work has focused upon the treatment of those with MDS and more

specifically *SGCE* mutation positive groups. This study highlighted some unusual findings; the reported low efficacy of benzodiazepines and sodium valproate, while the majority of participants reported benefit from carbamazepine, gabapentin, and trihexyphenidyl. Several case reports have noted complete remission of motor symptoms with trihexyphenidyl treatment, the motor features spontaneously returning upon cessation of the oral therapy.[293] Interestingly, although the overall effect of these latter medications is believed to be inhibitory each produces its effects via functionally distinct mechanisms. Carbamazepine is thought to stabilize the inactive state of voltage-gated sodium channels and potentiate inhibitory GABA receptors[339], while gabapentin reduces the calcium currents of voltage-gated calcium channels.[340] In contrast the central anticholinergic effects of Trihexyphenidyl are produced by direct antagonism of acetylcholine receptors and striatal dopamine reuptake.[341]

More recent use of DBS has provided further insight into the potential pathogenesis of MDS with stimulation to both GP_i [299, 334 342] and VIM[300] proving beneficial. A study of GP_i stimulation in an *SGCE* mutation positive cohort suggested there may be an additional effect of increasing D2R binding stability.[343] However, a case series of five patients found worsening of their psychiatric symptoms following use of the same treatment.[301] Although these studies aid in the mechanistic insights of pathogenesis, multi-centre randomised control trials are required for both medical and surgical therapies. This would allow greater understanding of which treatments are most beneficial in *SGCE* mutation positive patients and highlight areas of potential commercial therapeutic development.

6.7 Improving our understanding of the effects of *SGCE* mutations

A number of studies involving imaging, neurophysiology and animal models have been used in an attempt to gain greater understand of the pathogenesis of MDS. Early fMRI imaging studies using specific motor paradigms suggested involvement of the parietal, premotor and primary somatosensory cortices, cerebellum and more specifically thalamus and dentate nuclei,[123, 344] indicating some disorganization of sensorimotor integration, consistent with other forms of dystonia. Volumetric measurements of brain regions have added to this, finding dystonia severity in a

SGCE mutation positive cohort to be correlated with increased putaminal volume.[319] Specific cerebellar Purkinje cell (*Sgce* pKO) and striatal (*Sgce* sKO) *Sgce* KO mice have shown that ε-sarcoglycan deficits in these regions contribute to impaired motor learning and motor deficits respectively,[345, 346] suggesting defects of these individual cell types may contribute to pathogenesis. However, unlike the *Sgce* Knock Out (KO) mice which have features of myoclonus, incoordination, anxiety, depression and learning difficulties,[347] the *Sgce* pKO and *Sgce* sKO models individually do not result in myoclonus or difficulties with locomotion.

Neurophysiological studies support a subcortical origin for this movement disorder, these include normal somatosensory evoked potentials, cortical silent period, TMS measured short term intracortical inhibition (SICI), intracortical facilitation (ICF) and long term intracortical inhibition (LICI) while jerk-locked back-averaged EEG failed to show any preceding cortical correlates.[276, 348, 349] This would suggest that the GABAergic circuits within the motor cortex are generally intact and that the mechanisms underlying these symptoms are different from those seen in cortical myoclonus or other forms of dystonia. Other studies have shown some evidence of cortical dysfunction suggesting that it may have a role in modulating myoclonic presentation.[277] Investigations focusing upon changes in peripheral muscular activity in dystonia patients have found little difference between *SGCE* mutation positive MDS patients and other forms of dystonia. This suggests that the central generator mechanism may differ between subtypes of dystonia but that these effects may act by a common path producing similar findings in peripheral musculature.[285]

Neurochemical models of MDS pathogenesis have focused upon dopaminergic dysfunction. SPECT imaging studies have shown reduced D2R availability in *SGCE* mutation positive patients compared to controls.[311] These levels remained unchanged when measured pre- and post- GP_i DBS compared to a population not receiving surgical intervention, suggesting that symptomatic improvement may be through stabilization of D2R binding.[343] Animal models have also proposed a hyper-dopaminergic model for MDS, with elevated dopamine and reduced serotonin levels observed in KO mouse models.[347] Similar functional changes of dopaminergic and serotonergic neurons have also been implicated in OCD.[350, 351] Western blot analysis of protein levels from these mice found reduced DR2 levels while Dopamine 1

receptor (D1R) and dopamine transporter (DAT) levels remained normal. This suggests that ε-sarcoglycan may contribute to the regulation of D2R expression, such that pathogenic mutations may reduce striatal D2R levels, increasing dopamine release and possibly contributing to the behavioural impairment observed in both humans and mice.[352]

SGCE has four alternatively spliced exons (2, 8, 10, 11b). Exon 11b is a brain-specific exon along with 11c, an elongated form of 11b, identified in mice.[241, 353] Transcripts containing exon 11b or 11c encode proteins with a different C-terminal sequence containing a PDZ-binding motif. This motif is a protein interaction domain and therefore may contribute to the unique function of *SGCE* in the brain. Further work has also identified that the ubiquitous form of *SGCE* is localised in the post-synaptic membrane while the brain-specific form containing the 11b exon is located pre-synaptically.[241] Thus both forms may produce their effects through modulation of synaptic function with the 11b isoform contributing to the neurological phenotype. It is also suggested that the brain sarcoglycan complex differs considerably from that of peripheral tissue, where replacement of one sarcoglycan subtype with another leads to normal formation and function of the sarcoglycan complex. However, failure of ε-sarcoglycan expression may not be easily replaced by other members of the sarcoglycan family in the brain and therefore result in the disorder phenotype.[246] Deep sequencing of *SGCE* isoforms has also found differential expression in the human brain with particularly high levels in cerebellar Purkinje cells and neurons of the dentate nucleus.[354] Overall further work needs to be done to fully characterise the structure of the sarcoglycan complex, if one exists at all, in the brain, how ε and possibly ζ-sarcoglycan interact with each other and other proteins and why failure of expression of these proteins gives rise to this neuro-psychiatric phenotype.

6.8 Future studies and investigations

Collectively these studies indicate the need for ongoing multi-dimensional collaborative work. As discussed above, a centralized database with multi-centre reporting would allow efficient identification of a large number of *SGCE* mutation positive cases. This would enable longitudinal epidemiological studies of clinical

patterns and the potential for instigation and monitoring of clinical trials, using established and novel therapeutic agents. Further work is also required to establish further information of the precise nature of the psychiatric pathology in MDS patients, to compare this to other forms of dystonia and also whether *SGCE* mutations are likely to play a role in psychiatric disorders independent of the motor symptoms.

The structure of the sarcoglycan complex in brain or if it even exists remains to be determined. Protein isolation studies in cellular, animal and human tissue may aid in identifying likely protein-protein interactions and subsequent hypothetical models of the likely make-up of these complexes. Cellular models, particularly induced pluripotent stem cells (IPS) may allow the generation of more brain specific models of pathogenesis in the absence of post-mortem tissue. These types of studies have the potential to allow us further understanding of the brain-specific features of this disorder, how the pathogenic state impacts upon known neurochemical pathways and whether *SGCE* mutations can influence neuro-architectural models and impact upon brain development.

APPENDIX A

Papers and presentations

A.1 Papers

A.1.1 Systematic review of psychiatric disorders in *SGCE* mutation positive Myoclonus Dystonia patients

Peall KJ, Waite AJ, Blake DJ, Owen MJ, Morris HR. Psychiatric disorders, myoclonus dystonia, and the epsilon-sarcoglycan gene: a systematic review. Mov Disord 2011;26:1939-1942.[302]

A.1.2 Rate and type of psychiatric disorders in *SGCE* mutation poistive cohort

Peall KJ, Smith DJ, Kurian MA, Wardle M, Waite AJ, Hedderly T, Lin JP, Smith M, Whone A, Pall H, White C, Lux A, Jardine P, Bajaj N, Lynch B, Kirov G, O'Riordan S, Samuel M, Lynch T, King MD, Chinnery PF, Warner TT, Blake DJ, Owen MJ, Morris HR. *SGCE* mutations cause psychiatric disorders: clinical and genetic characterisation. Brain. 2013 Jan;136(Pt 1):294-303.

A.2 Presentations and published abstracts

A.2.1 Do Psychiatric disorders form part of the Myoclonus-Dystonia Syndrome Phenotype? A systematic review of published literature.

KJ Peall, D Perera, DJ Blake, MJ Owen, HR Morris. Presentation at SWENA, 2010 (prize winning)

A.2.2 Myoclonus Dystonia: A clinical and genetic description

Peall KJ, Smith DJ, Kurian MA, Wardle M, Waite AJ, Hedderly T, Lin JP, Smith M, Whone A, Pall H, White C, Lux A, Jardine P, Bajaj N, Lynch B, Kirov G, O'Riordan S, Samuel M, Lynch T, King MD, Chinnery PF, Warner TT, Blake DJ, Owen MJ, Morris HR.

Winner of the "*Sir Charles Symmonds Best Platform Presentation*" at the ABN Annual Meeting, Sage Gateshead, Newcastle, UK, 4-7th October 2011

A.2.3 Contiguous gene deletions involving *SGCE* gene: A clinical description

Peall KJ, Waite AJ, Kurian MA, Smith M, Pall H, Nestor T, King M, Blake DJ, Owen MJ, Morris HR. Platform presentation at The *Movement* Disorder Society's 16th International Congress of Parkinson's Disease and Movement Disorders, Dublin, Ireland, 17th-21st June 2012

A.2.4 Is psychiatric disease a core phenotype of Myoclonus Dystonia Syndrome caused by *SGCE* mutations?

Peall KJ, Smith DJ, Kurian MA, Wardle M, Waite AJ, Hedderly T, Lin JP, Smith M, Whone A, Pall H, White C, Lux A, Jardine P, Bajaj N, Lynch B, Kirov G, O'Riordan S, Samuel M, Lynch T, King MD, Chinnery PF, Warner TT, Blake DJ, Owen MJ, Morris HR. Poster presentation at combined ANA/ABN Meeting, Boston, USA, 5-11th October 2012

APPENDIX B

Investigator booklet

B.1 Patient Information Sheets

B.1.1 Cardiff Neurological Disease Bio-bank and Neurogenetic Research Study (CANDAS) participant information sheet

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Cardiff Neurological Disease Bio-bank and Neurogenetic Research Study (CANDAS)

Research participant Information Sheet
We would like to invite you to participate in this research project which is studying the causes of a wide variety of neurologic diseases. We are inviting people diagnosed to have these diseases, and unaffected volunteers.

What is the aim of this study ?
Its purpose is to identify variation in inherited material (DNA, Genes) that may cause or increase the risk of disease. Sometimes this happens when many people in a family are affected by a disease, sometimes this happens when only one person is affected but they have an increased disease risk. We will also study blood markers that could help in diagnosis or in monitoring disease progression. This may lead to better diagnosis and the development of new treatments.

Why have I been asked to participate?
You have been asked to participate because you have one of the diseases that we are including in our studies or you are an unaffected individual.

How can I participate?
We will ask for your consent to review your clinical notes and investigations. We may carry out a clinical assessment (taking details of your symptoms and carrying out an examination). We may ask if you are happy for a video to be made of part of your examination, so that this can be reviewed by another specialist. We will seek your consent to take, store and analyse a blood sample for genetic and chemical analysis.

How will the blood sample be taken?
We will take about 30ml of blood from a vein in your arm. This is a standard procedure which takes place in hospitals every day. There may be some minor discomfort, and there are very small risks of bruising or a local infection which can be treated.

What will happen to my blood sample after that?
The inherited material (DNA, Genes) will be extracted from the blood sample at a local or national facility and/or may be used anonymously to set an ongoing source of inherited material (DNA, genes in a cell line) at a European centre in Salisbury, Wiltshire (European Collection of Cell Cultures – ECACC). The sample will be treated as a gift for research, stored and used in ongoing and future projects with the same study aims. The chemical parts of the blood (plasma/serum) will also be stored if possible. Plasma/serum refers to the parts of the blood that do not contain red or white blood cells but contains chemicals that might be important for disease, for example sugar in diabetes and cholesterol in heart disease.

What is ECACC?
The ECACC Cell and DNA Bank collects, store, and distribute cell lines and DNA samples from people with many kinds of disorders, from unaffected family members, and from other healthy people. The purpose of this is to make specimens available for use in research, teaching, therapeutics and diagnostic purposes to responsible investigators in the UK and around the world. ECACC will take measures to protect my privacy, my blood or tissue specimen will be given a code number, and my name will not be submitted to the ECACC. Some patient identification, such as age, sex, diagnosis, and race, will be made available.

How does the analysis work?
Analysis of inherited material (DNA, Genes) will be used to determine if common variants increase the risk of the disease in the population and/or change the characteristics of that disease. In some samples it will be possible to identify new gene changes, which may directly cause disease. Studies will be performed by the main research team and samples may be shared on an anonymous collaborative basis with other investigators working on these diseases at different sites in the UK and abroad, and this may include commercial companies.

Can I receive the direct results of my genetic analysis?
No. The tests in this study are performed on a research basis and cannot be used for clinical purposes. In some circumstances the research tests may indicate that future NHS based genetic or chemical testing may be useful in accurately diagnosing the disease you have and in determining the risk of disease to other members of your family. You can chose whether you wish to be informed about this, in advance. If you do chose to be informed of future test development we will arrange for you to be given appropriate genetic advice. This will be discussed with you by your doctor, a member of the clinical team who arranged the original research blood sample, or by Dr. Huw Morris. Currently these types of tests do not lead to any new treatments or change in your current treatment, although this may change in the coming years.

Can outside bodies like insurance companies access the research tests?
No. We will code your sample, so that a number rather than a name is used in further analysis. The link between the code and your name will be kept confidential. Coded samples (i.e. without your name) for these tests may be shared with other research groups for analyses. Any information collected during the study will be kept confidential, aside from enabling us to inform you about the development of new tests as described above. We will store the assessment and test results on a secure, confidential database. This will enable us to analyse the information gathered for this research. When this study is completed we will continue to hold the data on our computer. You may ask for your personal information to be removed from this database at any time, in accordance with the Data Protection Act 1998

What will happen to the results of the study?
We plan to publish any results in scientific journals. Your name would not be mentioned in any publication. We will make regular reports to funding bodies and to patient groups.

What will happen to the information?
The clinical information which concerns your illness and contains your personal details will be kept on a clinical database on an NHS computer system. This computer network routinely holds personal details and test results for hospital networks. An anonymized, coded database holding clinical and genetic data, without personal details will be held on research computers, may be held by collaborators at other sites and may be made publicly available to enable the combined analysis of samples from different, large patient series around the world.

What happens if I chose not to participate?
Participation in the study is voluntary and you can chose to withdraw your participation at any time. You are free to withdraw from the study at any time and if you do decline to take part, this will not affect your current or future treatment in any way.

What are the benefits of participating?
We may learn more about your condition. We cannot promise that the study will help you but the information we get might help to treat people with similar conditions better in the future.

Who can I contact about this study?
Senior Study Doctor - Dr Huw Morris on 02920 743798 or write to Dr Huw Morris, Neurology (C4), University Hospital of Wales, Cardiff CF14 4XN
Research Nurse- Mrs Rachel Salmon 02920 745821
Research Administrator – Ms Dee Perera 02920 745821

B.1.2 CANDAS Third party information sheet

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A E Rosser BA PhD FRCP
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Mr J E Morgan MA DPhil FRCOphth
Dr N P Robertson MD FRCP
Dr H R Morris BSc PhD MRCP

Cardiff Neurological Disease Bio-bank and Neurogenetic Research Study (CANDAS)

Research participant Information Sheet – Third Party
We would like to invite you to participate in this research project which is studying the causes of a wide variety of neurologic diseases. We are inviting people diagnosed to have these diseases, and unaffected volunteers. When the person is unable to give consent, we ask someone to consider the project on their behalf and this may be a parent, carer, next of kin, or "consultee".

What is the aim of this study ?
Its purpose is to identify variation in inherited material (DNA, Genes) that may cause or increase the risk of disease. Sometimes this happens when many people in a family are affected by a disease, sometimes this happens when only one person is affected but they have an increased disease risk. We will also study blood markers that could help in diagnosis or in monitoring disease progression. This may lead to better diagnosis and the development of new treatments.

Why has the patient been asked to participate?
The patient has been asked to participate because s/he has one of the diseases that we are including in our studies.

What is my role?
We will ask for your assent to review the patient's clinical notes and investigations. We will carry out a clinical assessment (taking details of your symptoms and carrying out an examination). We will also ask if you are happy for a video to be made of part of the patient's examination, so that this can be reviewed by another specialist. We will seek your consent to take, store and analyse a blood sample for genetic and chemical analysis.

How will the blood sample be taken?
We will take about 30ml of blood from a vein in the patient's arm. This is a standard procedure which takes place in hospitals every day. There may be some minor discomfort, and there are very small risks of bruising or a local infection which can be treated.

What will happen to the patient's blood sample after that?
The inherited material (DNA, Genes) will be extracted from the blood sample at a local or national facility and/or may be used anonymously to set an ongoing source of inherited material (DNA, genes in a cell line) at a European centre in Salisbury, Wiltshire (European Collection of Cell Cultures – ECACC). The sample will be treated as a gift for research, stored and used in ongoing and future projects with the same study aims. The chemical parts of the blood (plasma/serum) will also be stored if possible. Plasma/serum refers to the parts of the blood that do not contain red or white blood cells but contains chemicals that might be important for disease, for example sugar in diabetes and cholesterol in heart disease.

What is ECACC?
The ECACC Cell and DNA Bank collects, store, and distribute cell lines and DNA samples from people with many kinds of disorders, from unaffected family members, and from other healthy people. The purpose of this is to make specimens available for use in research, teaching, therapeutics and diagnostic purposes to responsible investigators in the UK and around the world. ECACC will take measures to protect the patient's privacy, the blood or tissue specimen will be given a code number,

and the patient's name will not be submitted to ECACC. Some patient identification, such as age, sex, diagnosis, and race, will be made available.

How does the analysis work?
Analysis of inherited material (DNA, Genes) will be used to determine if common variants increase the risk of the disease in the population and/or change the characteristics of that disease. In some samples it will be possible to identify new gene changes, which may directly cause disease. Studies will be performed by the main research team and samples may be shared on an anonymous collaborative basis with other investigators working on these diseases at different sites in the UK and abroad, and this may include commercial companies.

Can we receive the direct results of the genetic analysis?
No. The tests in this study are performed on a research basis and cannot be used for clinical purposes. In some circumstances the research tests may indicate that future NHS based genetic or chemical testing may be useful in accurately diagnosing the disease and in determining the risk of disease to other members of the patient's family. You can chose whether you wish to be informed about this, in advance. If you do chose to be informed of future test development we will arrange for the patient's/your family to be given appropriate genetic advice. This will be discussed with you by your doctor, a member of the clinical team who arranged the original research blood sample, or by Dr. Huw Morris. Currently these types of tests do not lead to any new treatments or change in your current treatment, although this may change in the coming years.

Can outside bodies like insurance companies access the research tests?
No. We will code the patient's sample, so that a number rather than a name is used in further analysis. The link between the code and the patient's name will be kept confidential. Coded samples (i.e. without your name) for these tests may be shared with other research groups for analyses. Any information collected during the study will be kept confidential, aside from enabling us to inform you about the development of new tests as described above. We will store the assessment and test results on a secure, confidential database. This will enable us to analyse the information gathered for this research. When this study is completed we will continue to hold the data on our computer. You may ask for the personal information to be removed from this database at any time, in accordance with the Data Protection Act 1998

What will happen to the results of the study?
We plan to publish any results in scientific journals. The patient's would not be mentioned in any publication. We will make regular reports to funding bodies and to patient groups.

What will happen to the information?
The clinical information which concerns the illness and contains personal details will be kept on a clinical database on an NHS computer system. This computer network routinely holds personal details and test results for hospital networks. An anonymized, coded database holding clinical and genetic data, without personal details will be held on research computers, may be held by collaborators at other sites and may be made publicly available to enable the combined analysis of samples from different, large patient series around the world.

What happens if I chose not to participate?
Participation in the study is voluntary and you can chose to withdraw your participation at any time. You are free to withdraw the patient from the study at any time and if you do decline to take part, this will not affect the patient's current or future treatment in any way.

What are the benefits of participating?
We may learn more about the condition. We cannot promise that the study will help the patient but the information we get might help to treat people with similar conditions better in the future.

Who can I contact about this study?
Senior Study Doctor - Dr Huw Morris on 02920 743798 or write to Dr Huw Morris, Neurology (C4), University Hospital of Wales, Cardiff CF14 4XN
Research Nurse- Mrs Rachel Salmon 02920 745821
Research Administrator – Ms Dee Perera 02920 745821

B.1.3 Cardiff Neurological and Psychiatric Phenotype Study (CANOPY) Participant Information Sheet

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Cardiff Neurological and Psychiatric Phenotype Study (CANOPY)

Research Participant Information Sheet

We would like to invite you to participate in this research project, which is studying the impact of a variety of neurological conditions on day-to-day living, and whether there is a link with psychiatric difficulties e.g. depression, anxiety, obsessions. We are inviting people diagnosed with these conditions and unaffected volunteers.

What is the aim of this study?

The purpose of the study is to attempt to improve our understanding of these conditions and how they impact upon your daily life. Up until now, although experts are aware of these conditions, our understanding of whether psychiatric problems form part of the condition and how these affect people with these conditions has been very poor.

Why have I been asked to participate?

You have been asked to participate, because you have one of the diseases that we are including in our study (for example myoclonus dystonia, essential tremor, dystonic tremor, dystonia)

How can I participate?

We will send you a pack containing this information sheet and a set of questionnaires. We will ask you to complete the questionnaires and then bring these with you to the clinical assessment, which will be arranged for your convenience.

The clinical assessment will involve asking a series of questions related to your symptoms, an examination and a further few questionnaires.

What will the questions and examination involve?

This will begin with a series of question related to your symptoms, any other medical problems, medications and whether any other family members are affected in a similar way to your self. This will be followed by a very brief examination, similar to those you may have had when seen by a specialist before. We will also ask if you are happy for a video to be made of a more specialized examination that can be reviewed by another expert, ensuring that we both agree on your diagnosis. This video will be stored on a computer in a locked room and only able to be accessed by clinicians within the research team.

What will the questionnaires involve?

This will involve a series of questionnaires that have been tried and tested by other research groups. They will cover a broad range of topics including testing different parts of memory, how the condition affects you on a day-to-day basis and in life generally. As many of these conditions are improved by alcohol, we will also ask about the average amount of alcohol you drink, if at all. As a large part of this study

focuses on psychiatric problems, there will also be a questionnaire dedicated to asking about past and present psychiatric problems or symptoms.

What will happen if I find the process too tiring or upsetting?
You can ask for the assessment to be stopped at any point, following which you may wish to not partake in the study any further, come back at a later date or take a break and continue the same day. Doing this will not affect your treatment or care in anyway. Should you become very upset by the questions asked, we would like to be able to contact your GP, CPN or psychiatrist, before the end of the assessment, so that we can arrange an appointment for you to discuss any of the issues that these questions may have raised.

Can I receive direct results of the study?
No. The assessments in this study are performed on a research basis and cannot be used for clinical purposes. Any results generated from the study will be communicated to patient support groups and newsletters such that this information is circulated to a wider group.

Can outside bodies like insurance companies access the research results?
No. We will code your information, so that a number rather than a name is used when we analyze the data. The link between the code and your name will be kept confidential. Coded data (i.e. without your name) may be shared with other research groups for analyses. Any information collected during the study will be kept confidential. We will store the assessment results on a secure, confidential database. This will enable us to analyse the information gathered for this research. When this study is completed we will continue to hold data on our computer. You may ask for your personal information to be removed from this database at any time, in accordance with the Data Protection Act 1998.

What will happen to the results of the study?
We plan to publish any results in scientific journals. Your name would not be mentioned in any publication. We will make regular reports to funding bodies and to patient groups.

What will happen to the information?
The clinical information concerning your illness and contains your personal details will be kept on a clinical database on an NHS computer system. This computer network routinely holds personal details and test results for hospital networks. An anonymized, coded database holding clinical data without personal details will be held on research computers, may be held by collaborators at other sites and may be publicly available to enable the combined analysis of samples from different, large patient series around the world.

What happens if I chose not to participate?
Participation in the study is voluntary and you can chose to withdraw your participation at any time and this will not affect your current or future treatment in any way.

What are the benefits of participating?
We may learn more about your condition. We cannot promise that the study will help you but the information we get might help to improve treatment for people with similar conditions in the future.

Who can I contact about this study?
Study Doctor – Dr Kathryn Peall on 02920 743454 or write to Dr Kathryn Peall, Neurology Research Office, B2-C2 link corridor, University Hospital of Wales, Cardiff, CF14 4XN
Senior Study Doctor – Dr Huw Morris on 02920 743798 or write to Dr Huw Morris, neurology (C4), University Hospital of Wales, Cardiff, CF14 4XN

B.1.4 CANOPY Third Party Information Sheet

School of Medicine
Department of Psychological Medicine & Neurology
Head of Department Professor Michael J Owen PhD FRCPsych FMedSci
Ysgol Meddygaeth
Adran Meddygaeth Seicolegol a Niwroleg
Pennaeth Adran Yr Athro Michael J Owen PhD FRCPsych FMedSci

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Ymchwil Biofeddygol yng Nghymru
Mynydd Bychan
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Cardiff Neurological and Psychiatric phenotype Study (CANOPY)

Research Participant Information Sheet – Third Party
We would like to invite you to participate in this research project, which is studying the impact of a variety of neurological conditions on day-to-day living, and whether there is a link with psychiatric difficulties e.g. depression, anxiety, obsessions. We are inviting people diagnosed with these conditions and unaffected volunteers. When a person is unable to give consent we ask someone to consider the project on their behalf, this may be a parent, carer, next of kin or "consultee".

What is the aim of the study?
Its purpose is to attempt to improve our understanding of these conditions and how they impact upon daily living. Up until now, although experts are aware of these conditions, our understanding of whether psychiatric problems form part of the condition, and how this then impacts upon people with these conditions has been very poor.

Why has the patient been asked to participate?
The patient has been asked to participate because s/he has one of the conditions that we are including in our studies.

What is my role?
We will ask for your assent to review the patient's clinical notes and investigations. We will carry out a clinical assessment (taking details of the symptoms and carrying out an examination). If you are happy this will also include a video to be reviewed by another specialist. Finally, if you are happy we would like to complete a questionnaire involving you answering questions in relation to the patient.

What will the questions and examinations involve?
This will begin with a series of general questions related to any past or present medical problems, medications taken and a history of any problems that may run in the family. This will be followed by a brief, general neurological examination. We will also ask if you are happy for a video to be made of a more specialized examination that can be reviewed by another expert. This video will be stored on a computer in a locked room and only able to be accessed by clinicians within the research team.

What will the questionnaires involve?
This will involve a series of questionnaires that have been tried and tested by other research groups. They will cover a broad range of topics including how the disorder affects day-to-day living. A large part of this study focuses on psychiatric problems and therefore there will also be a questionnaire dedicated to asking about past and present psychiatric problems or symptoms.

What will happen if the patient or myself find the process too tiring or upsetting?
You can ask for the assessment to be stopped at any point, following which you may wish to not partake in the study any further, come back at a later date or take a break and continue the same day. Should the patient or yourself become very upset by the

questions asked, we would like to be able to contact your GP before the end of the assessment, so that we can arrange an appointment for you to discuss any of the issues that these questions may have raised.

Can we receive direct results of the study?
No. The assessments in this study are performed on a research basis and cannot be used for clinical purposes. Any results generated from the study will be communicated to patient support groups and newsletters such that this information is circulated to a wider group.

Can outside bodies, like insurance companies, access the research results?
No. We will code the information, so that a number rather than a name is used when we analyze data. The link between the code and the patient's name will be kept confidential. Coded data (i.e. without the patient's name) may be shared with other research groups for analyses. Any information collected during the study will be kept confidential. We will store the assessment results on a secure, confidential database, this will enable us to analyse the information gathered for this research. When this study is completed we will continue to hold data on our computer. You may ask for your personal information to be removed from this database at any time, in accordance with the Data Protection Act 1998.

What will happen to the results of the study?
We plan to publish any results in scientific journals. Neither your name nor that of the patient will be mentioned in any publication. We will make regular reports to funding bodies and to patient groups.

What will happen to the information?
The clinical information which concerns the illness and contains personal details will be kept on a clinical database on an NHS computer system. This computer network routinely holds personal details and clinical information for hospital networks. An anonymized, coded database holding clinical data without personal details will be held on research computers, may be held by collaborators at other sites and may be publicly available to enable the combined analysis of samples from different, large patient series around the world.

What happens if I chose not to participate?
Participation in the study is voluntary and you can chose to withdraw your participation at any time. You are free to withdraw the patient from the study at any time and if you do decline to take part this will not affect the patient's current or future treatment in any way.

What are the benefits of participating?
We may learn more about the conditions being studied, which may help to treat people with similar condition better in the future.

Who can I contact about this study?
Study Doctor – Dr Kathryn Peall on 02920 743454 or write to Dr Kathryn Peall, Neurology Research Office, B2-C2 link corridor, University Hospital of Wales, Cardiff, CF14 4XN.
Senior Study Doctor – Dr Huw Morris on 02920 743798 or write to Dr Huw Morris, Neurology (C4), University Hospital of Wales, Cardiff, CF14 4XN.

B.2 Consent Forms

B.2.1 CANDAS Participant Consent Form

School of Medicine
Dean Professor D Wynford-Thomas MBBCh(hons) FRCPath DSc FMedSci
Department of Neurology, Ophthalmology and Audiological Medicine
Head of Department Professor C M Wiles BSc PhD FRCP

Ysgol Meddygaeth
Deon Yr Athro D Wynford-Thomas MBBCh(hons) FRCPath DSc FMedSci
Uned Niwroleg ac Offthalmoleg Awdiolegol Meddygaeth
Pennaeth Uned Yr Athro C M Wiles BSc PhD FRCP

1883-2008 125 YEARS BLYNEDD

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Fax *Ffacs* +44(0)29 2074 4394
Email *E-bost*
Neurologyadministration@cf.ac.uk

Prifysgol Caerdydd
(C2-B2 link)
Mynydd Bychan
Caerdydd CF14 4XN

Professor/*Yr Athro*
C M Wiles BSc PhD FRCP
Professor/*Yr Athro*
A E Rosser BA PhD FRCP
Professor/*Yr Athro*
Mr J E Morgan MA DPhil FRCOphth
Dr N P Robertson MD FRCP
Dr H R Morris BSc PhD MRCP

Cardiff Neurological Disease Bio-bank and Neurogenetic Research Study (CANDAS)

Participant: **Code number**

Date of birth:

Date: **Name of researcher:**

Initial boxes to agree

1. **I have read and understood Version 2 of the CANDAS patient information sheet project** and been given a copy to keep. I have had the opportunity to ask questions about the project and I understand why the research is being done and any foreseeable risks involved. ☐

2. **I agree to give a sample of blood for research in the above project.** This sample will be used to study inherited material (DNA) and the blood chemistry (serum). ☐

3. **I give permission for my medical records, including investigations and X-Rays and scans to be looked at confidentially** by members of the medical research team who would not normally be involved with my clinical care. ☐

4. **If possible, I would like to be informed of research results** that might indicate that a test could be developed which might be of use to me or my family. YES/ NO

5. **I understand that I will not benefit financially** if this research leads to the development of new treatments or tests. ☐

6. **I agree that the DNA and blood samples that I have given can be looked after and stored** for use in current and future projects, as described in the information sheet. ☐

7. **I agree that my own doctor (GP and/or hospital)** can be informed of my involvement in this study if they have not already been involved. ☐

8. **I agree that my clinical details can be stored in a clinical research database** on the NHS hospital computer network and understand that a separate anonymized research database will be used to store research results. The anonymized DNA information may be made publicly available to enable large scale analysis. ☐

9. **I am happy to be contacted** by telephone or letter to obtain more information or about future research projects. ☐

10. **I understand that information held by the NHS** and records maintained by the General Registry Office may be used to keep in touch with me and follow up my health status. ☐

11. I consent to the **collection of blood for submission to the European Collection of Cell Cultures (ECACC)**, a British based research resource, to establish an anonymous cell line. ☐

12. **I give permission for a videotape examination, in which I am personally identifiable, to be stored** as part of my clinical assessment and to be used for teaching and research purposes. ☐

Thank you for your participation in this study.

_____ /____/_____ _____

Name of subject Date Signature

_____ /____/_____ _____

Name of researcher Date Signature

B.2.2 CANDAS Third Party Assent Form

School of Medicine
Dean Professor D Wynford-Thomas MBBCh(hons) FRCPath DSc FMedSci
Department of Neurology, Ophthalmology and Audiological Medicine
Head of Department Professor C M Wiles BSc PhD FRCP

Ysgol Meddygaeth
Deon Yr Athro D Wynford-Thomas MBBCh(hons) FRCPath DSc FMedSci
Uned Niwroleg ac Offthalmoleg Awdiolegol Meddygaeth
Pennaeth Uned Yr Athro C M Wiles BSc PhD FRCP

1883-2008 125 YEARS BLYNEDD

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Neurologyadministration@cf.ac.uk

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C M Wiles BSc PhD FRCP
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A E Rosser BA PhD FRCP
Professor/*Yr Athro*
Mr J E Morgan MA DPhil FRCOphth
Dr N P Robertson MD FRCP
Dr H R Morris BSc PhD MRCP

Cardiff Neurological Disease Bio-bank and Neurogenetic Research Study (CANDAS)

Assent Third Party

Participant: **Code number**

Date of birth:

Date: **Name of researcher:**

Initial boxes to agree

1. **I have read and understood Version 2 of the CANDAS patient information sheet project** and been given a copy to keep. I have had the opportunity to ask questions about the project and I understand why the research is being done and any foreseeable risks involved. ☐

2. **I agree that .....should give a sample of blood for research in the above project.** This sample will be used to study inherited material (DNA) and the blood chemistry (serum). ☐

3. **I give permission for .....'s medical records, including investigations and X-Rays and scans to be looked at confidentially** by members of the medical research team who would not normally be involved with their clinical care. ☐

4. **If possible, I would like to be informed of research results** that might indicate that a test could be developed which might be of use to the patient or family. YES/ NO

5. **I understand that we will not benefit financially** if this research leads to the development of new treatments or tests. ☐

6. **I agree that the DNA and blood samples that have been given can be looked after and stored** for use in current and future projects as described in the information sheet. ☐

7. **I agree that the patient's own doctor (GP and/or hospital)** can be informed of their involvement in this study if they have not already been involved. ☐

8. **I agree that .....'s clinical details can be stored in a clinical research database**on the NHS hospital computer network and understand that a separate anonymized ☐

research database will be used to store research results. The anonymized DNA information may be made publicly available to enable large scale analysis.

9. **I am happy to be contacted** by telephone or letter to obtain more information or about future research projects. ☐

10. **I understand that information held by the NHS** and records maintained by the General Registry Office may be used to keep in touch with the patient and to follow their future health status. ☐

11. I consent to the **collection of blood for submission to the European Collection of Cell Cultures (ECACC)**, a British based research resource, to establish an anonymous cell line. ☐

12. **I give permission for a videotape examination, in which .....is personally identifiable, to be stored** as part of my clinical assessment and to be used for teaching and research purposes. ☐

Thank you for your participation in this study.

_____/____/_____ _____

Name of Third Party providing assent Date Signature

_____

Relationship to Patient

_____/____/_____ _____

Name of researcher Date Signature

B.2.3 CANOPY Participant Consent Form

School of Medicine
Department of Psychological Medicine & Neurology
Head of Department Professor Michael J Owen PhD FRCPsych FMedSci
Ysgol Meddygaeth
Adran Meddygaeth Seicolegol a Niwroleg
Pennaeth Adran Yr Athro Michael J Owen PhD FRCPsych FMedSci

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Adeilad Henry Wellcome ar gyfer
Ymchwil Biofeddygol yng Nghymru
Mynydd Bychan
Caerdydd CF14 4XN

Cardiff Neurological and Psychiatric Phenotype Study (CANOPY)

Participant:

Date of Birth:

Date:

Code Number:

Name of Researcher:

Please initial boxes to agree

1. I have read and understood the CANOPY project patient information sheet and have been given a copy to keep. I have had the opportunity to ask questions about the project, I understand why the research is being done and any foreseeable risks that may be involved. ☐

2. I am happy to discuss details of my past and present medical conditions, Under go a routine neurological examination and complete a series of questionnaires discussing past and present neurological and psychiatric symptoms, as well as some assessments of quality of life. ☐

3. I consent to video recording being made of myself, which will be viewed by other health professionals. ☐

4. I am happy for my primary care giver (GP/hospital consultant) to be contacted following this assessment should the research team feel that an appointment or further assessment by them be appropriate. ☐

5. I am happy for my primary care giver (GP/Hospital Consultant) to be informed of my involvement in this study. ☐

6. I give permission for my medical records, including investigations and X-Rays and scans to be looked at confidentially by members of the medical research team who would not normally be involved with my clinical care. ☐

7. I understand that I will not benefit financially if this research leads to the

development of new treatments or tests. ☐

8. I agree that my clinical details can be stored in a clinical research database on the NHS hospital computer network and understand that a separate anonymised research database will be used to store research results. ☐

9. I am happy to be contacted by telephone, letter or email to obtain more information in relation to this research project. ☐

10. I am happy to be contacted by telephone, letter or email about future research projects. ☐

11. I understand that information held by the NHS and records maintained by the General Registry Office may be used to keep in touch with me and follow up my health status. ☐

Thank you for your participation in this study.

_____ /___/_____ _____

Name of subject Date Signature

_____ /___/_____ _____

Name of researcher Date Signature

B.2.4 CANOPY Third Party Assent Form

School of Medicine
Department of Psychological Medicine & Neurology
Head of Department Professor Michael J Owen PhD FRCPsych FMedSci
Ysgol Meddygaeth
Adran Meddygaeth Seicolegol a Niwroleg
Pennaeth Adran Yr Athro Michael J Owen PhD FRCPsych FMedSci

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Ymchwil Biofeddygol yng Nghymru
Mynydd Bychan
Caerdydd CF14 4XN

Cardiff Neurological and Psychiatric Phenotype Study (CANOPY)

Assent Third Party

Participant: **Code Number:**

Date of Birth: **Name of Researcher:**

Date:

	Please initial boxes to agree
1. I have read and understood the CANOPY project consultee information sheet and have been given a copy to keep. I have had the opportunity to ask questions about the project, I understand why the research is being done and any foreseeable risks that may be involved.	☐
2. I am happy to discuss details of past and present medical conditions and complete a series of questionnaires discussing past and present neurological and psychiatric symptoms in relation to	☐
3. I give permission for .....to undergo a neurological examination.	☐
4. I consent to video recording being made of .....which will be viewed by other health professionals.	☐
4. I am happy for my primary care giver (GP/hospital consultant) to be contacted following this assessment should the research team feel that an appointment or further assessment by them be appropriate.	☐
5. I agree to .....'s primary carer (GP/hospital consultant) to be contacted following this assessment should the research team feel that an appointment or further assessment by them be appropriate	☐
6. I agree that the patient's own doctor (GP/Hospital Consultant) to be informed of my involvement in this study.	☐
7. I give permission for my medical records, including investigations and X-Rays and scans	☐

to be looked at confidentially by members of the medical research team who would not normally be involved with my clinical care.

7. I understand that we will not benefit financially if this research leads to the development of new treatments or tests. ☐

8. I agree that ……'s clinical details can be stored in a clinical research database on the NHS hospital computer network and understand that a separate anonymised research database will be used to store research results. ☐

9. I am happy to be contacted by telephone, letter or email to obtain more information in relation to this research project. ☐

10. I am happy to be contacted by telephone, letter or email about future research projects. ☐

11. I understand that information held by the NHS and records maintained by The NHS Information Centre and the NHS Central Register may be used to help contact the participant and provide information about their health status ☐

Thank you for your participation in this study.

Name of Third Party providing assent	Date	Signature

Relationship to patient

Name of Researcher	Date	Signature

B.3 History Questionnaire Pack

Date of Assessment: Assessor's initials: Study ID:

Cardiff University School of Medicine: Department of Neurology & Psychological Medicine

Cardiff Neurological and Psychiatric Phenotype Study (CANOPY)

Participant ID:

Details of Assessment
Date of Assessment:

Name of Assessor:

Location of Assessment:

Referral Source
[1] Primary Care
[2] Secondary Care (Neurology)
[3] Secondary Care (Medicine for Older People)
[4] Secondary Care (Other)
[5] Self-referral
[6] Other

Name of referring GP/Consultant :
Address 1:
Address 2:
Address 3:

Demography

Surname: **Forename(s):**

Sex: [1] Male [2] Female **Handedness:** [1] Right [2] Left

Date of Birth: **Place of Birth:**

Address 1:

Address 2:

Address 3:

Town/City

County: **Postcode:**

Contact Number: **Email Address:**

Date of Assessment: Assessor's initials: Study ID:

Demography (Continued)

Marital Status: [1 2 3 4 5 6 7]

1. Single
2. Married
3. Co-habit
4. Divorced
5. Widowed
6. Remarried
7. Separated

Employment Status: [1 2 3 4 5]

1. Working
2. Retired
3. Unemployed
4. Disabled
5. Housewife

Occupation:
If you are working or have worked in the past, what is/was you job? Please describe:

Which of the following best describes your position in your current or last job?
[1 2 3 4 5 6 7]

1. Self employed (>25 employees)
2. Self employed (<25 employees)
3. Self employed (no employees)
4. Manager (>25 employees)
5. Manager (<25 employees)
6. Supervisor
7. Employee

Education:
Age at leaving formal education:

Qualifications Attained: [1 2 3 4 5]

1. None
2. GCSE (or equivalent)
3. A-levels (or equivalent)
4. Diploma/City & Guilds/NVQ
5. Degree/Higher Education

Daily Living: [1 2 3 4 5 6]

1. Alone, independent
2. Alone, with help
3. With carer, independent
4. With carer, dependent
5. With carer plus outside help
6. Other

Date of Assessment: Assessor's initials: Study ID:

Caregiver: [1 2 3 4 5 6]

1. No caregiver necessary
2. Spouse/Partner
3. Own child
4. Close relative (cousin, grandparent, sibling etc)
5. Friend
6. Other

National Identity: [1 2 3 4 5 6]

1. English
2. Scottish
3. Welsh
4. Irish
5. British
6. Other (please document)

Ethnicity: [1a 1b 2a 2b 2c 2d 3a 3b 3c 3d 4a 4b 4c 5a 5b]

1a. White (British)
1b. White (any other white background)
2a. Mixed (White and Black Caribbean)
2b. Mixed (White and Black African)
2c. Mixed (White and Asian)
2d. Mixed (Any other mixed background – please document)
3a. Asian or Asian British (Indian)
3b. Asian or Asian British (Pakistani)
3c. Asian or Asian British (Bangladeshi)
3d. Asian or Asian British (Any other Asian background – please document)
4a. Black or Black British (Caribbean)
4b. Black or Black British (African)
4c. Black or Black British (Any other Black background – please document)
5a. Chinese
5b. Other (please document)

Date of Assessment: Assessor's initials: Study ID:

Summary of Symptoms

Please tick box corresponding to each symptom present:

Tremor	❒
Dystonia	❒
Myoclonus	❒

Complete the following questions pertaining to each symptom present.
E.g. Essential tremor = Tremor questions
Dystonic tremor = Tremor & Dystonia
Myoclonus Dystonia = Myoclonus & Dystonia

Date of Assessment: Assessor's initials: Study ID:

Tremor

1. At what age did the symptoms start?

2. How many years ago was this?

3. Which parts of your body were affected when the tremor started?

[1 2 3 4 5 6 7 8 9 10 11 12 13]

1. Head
2. Jaw
3. Neck
4. Right arm
5. Left arm
6. Right hand
7. Left hand
8. Trunk
9. Right leg
10. Left leg
11. Right foot
12. Left foot
13. Voice

4. At onset, was it symmetrical or asymmetrical? **[1 2 3]**
   1. Symmetrical
   2. Asymmetrical
   3. Not applicable (central body part)

5. If symptoms were asymmetrical at onset, which side was more affected?

[1 2 3]

1. Right
2. Left
3. Not applicable

6. Have the symptoms progressed since onset? **[1 2]**
   1. Yes
   2. No

If, answer 'yes' to question 6, in what way have symptoms progressed?

1. Yes
2. No

a) Increased number of body parts involved. **[1 2]**
b) Increased amplitude of tremor. **[1 2]**
c) Affected by symptoms for more hours per day. **[1 2]**
d) Increased frequency of tremor. **[1 2]**

7. Are symptoms worse when resting or doing an activity or task? **[1 2]**
   1. Rest
   2. Action

8. Do the symptoms improve with alcohol? **[1 2 3 4]**
   1. Yes
   2. No
   3. Uncertain
   4. Not applicable – doesn't drink alcohol

9. If symptoms improve with alcohol, what percentage of improvement is seen (gross estimate).

[1 2 3 4 5 6 7]

1. 100%
2. 75%
3. 50%
4. 25%
5. No effect
6. Uncertain
7. Not applicable

10. If symptoms improve with alcohol, how much alcohol is required to produce the maximum effect?

[1 2 3 4 5 6 7 8]

1. 1-4 units
2. 4-8 units
3. 8-12 units
4. 12-16 units
5. 16-20 units
6. >20 units
7. Uncertain
8. Not applicable

11. If you smoke, does this have any affect on the tremor?

[1 2 3 4 5]

1. Better
2. Worse
3. No effect
4. Uncertain
5. Not applicable – doesn't smoke

12. If you drink caffeinated drinks e.g. tea, coffee, coke, does this have any affect on the tremor?

[1 2 3 4 5]

1. Better
2. Worse
3. No effect
4. Uncertain
5. Not applicable – doesn't drink caffeinated drinks

13. Does being in a public or social setting e.g. restaurant/meeting/function have an effect on your tremor? **[1 2 3 4 5 6]**

1. Yes – makes it worse
2. Yes – makes it better
3. No – it has no effect
4. Uncertain
5. Completely avoids these settings – due to movement disorder
6. Completely avoids these settings – due to other factors (please document)

Date of Assessment: Assessor's initials: Study ID:

14. Do these symptoms interfere with the following tasks?

1. Yes
2. No

a) Writing	[1	2]
b) Eating	[1	2]
c) Washing	[1	2]
d) Dressing	[1	2]
e) Pouring of liquids	[1	2]
f) Walking	[1	2]
g) Sports/Energetic activities	[1	2]

Date of Assessment: Assessor's initials: Study ID:

Dystonia Symptoms

If participant uncertain what symptoms these questions pertain to describe as: an abnormal muscle contraction or position that may cause discomfort, cramp-like sensation or pain.

1. At what age did the symptoms of dystonia begin?

2. How many years ago was this?

3. At onset, how many body parts were affected?

[1 2 3 4 5 6 7 8 9 10 11 12 13 14 15]

1. Head	6. Right Arm	11. Right Leg
2. Jaw	7. Left Arm	12. Left Leg
3. Neck	8. Right Hand	13. Right Foot
4. Right Shoulder	9. Left Hand	14. Left Foot
5. Left Shoulder	10. Trunk	15. Voice

3. Did you experience any pain in the same region when the symptoms started?

[1 2 3]

1.Yes
2.No
3.Uncertain

4. At onset, were the symptoms symmetrical or asymmetrical? **[1 2 3]**

1.Symmetrical
2.Asymmetrical
3.Not applicable (central body part)

5. If symptoms were asymmetrical at onset, which side was more affected?

[1 2 3]

1. Right
2. Left
3. Not applicable

6. When the symptoms began, did they develop rapidly (i.e. within a few days)

[1 2 3]

1.Yes
2.No
3.Uncertain

7. Have the symptoms progressed since the onset? **[1 2 3]**

1.Yes
2.No

3.Uncertain

If the answer to question 7 is 'yes', in what way have the symptoms progressed?

1. Yes
2. No

a) Increased number of body parts now affected?	**[1 2]**
b) Affected by symptoms for more hours per day?	**[1 2]**
c) More affected by symptoms of pain?	**[1 2]**
d) Angle of muscle contraction (e.g. neck tilt) is greater?	**[1 2]**

8. If there are an increased number of body parts involved, which parts are now involved?

[1 2 3 4 5 6 7 8 9 10 11 12 13 14 15]

1. Head	6. Right Arm	11. Right Leg
2. Jaw	7. Left Arm	12. Left Leg
3. Neck	8. Right Hand	13. Right Foot
4. Right Shoulder	9. Left Hand	14. Left Foot
5. Left Shoulder	10. Trunk	15. Voice

9. Do these symptoms interfere with the following tasks?

1. Yes
2. No

a) Writing	**[1 2]**
b) Eating	**[1 2]**
c) Washing	**[1 2]**
d) Dressing	**[1 2]**
e) Pouring of liquids	**[1 2]**
f) Walking	**[1 2]**
g) Sports/Energetic activities	**[1 2]**

Date of Assessment: Assessor's initials: Study ID:

Myoclonus Symptoms

1.At what age did the myoclonus begin?

2. How many years ago was this?

3. At onset, how many body parts were affected?
[1 2 3 4 5 6 7 8 9 10 11 12 13 14 15]

1. Head	6. Right Arm	11. Right Leg
2. Jaw	7. Left Arm	12. Left Leg
3. Neck	8. Right Hand	13. Right Foot
4. Right Shoulder	9. Left Hand	14. Left Foot
5. Left Shoulder	10. Trunk	15. Voice

4. At onset, were the symptoms symmetrical or asymmetrical? **[1 2 3]**
1.Symmetrical
2.Asymmetrical
3.Not applicable (central body part)

5. If symptoms were asymmetrical at onset, which side was more affected?
[1 2 3]
1. Right
2. Left
3. Not applicable

6. Are these symptoms brought on by sudden loud noises? **[1 2 3]**
1. Yes
2. No
3.Uncertain

7. Are these symptoms brought on by touch? **[1 2 3]**
1. Yes
2. No
3.Uncertain

8.Are the symptoms worse while resting or while doing tasks/activities?
[1 2 3]
1. Rest
2. Tasks/Activities
3.Uncertain

Date of Assessment: Assessor's initials: Study ID:

9. Have the symptoms progressed since the onset? **[1 2 3]**
   1.Yes
   2.No
   3.Uncertain

If the answer to question 9 is 'yes', in what way have the symptoms progressed?
   1. Yes
   2. No

a) Increased number of body parts now affected? **[1 2]**
b) Amplitude of the myoclonus has increased **[1 2]**
c) Affected by the myoclonus more often during the day? **[1 2]**
d) Myoclonus is now present during increasing number of actions/activities **[1 2]**

10. If there are an increased number of body parts involved, which parts are now involved?

[1 2 3 4 5 6 7 8 9 10 11 12 13 14 15]

1. Head
2. Jaw
3. Neck
4. Right Shoulder
5. Left Shoulder
6. Right Arm
7. Left Arm
8. Right Hand
9. Left Hand
10. Trunk
11. Right Leg
12. Left Leg
13. Right Foot
14. Left Foot
15. Voice

6. Do these symptoms interfere with the following tasks?
   1. Yes
   2. No

a) Writing **[1 2]**
b) Eating **[1 2]**
c) Washing **[1 2]**
d) Dressing **[1 2]**
e) Pouring of liquids **[1 2]**
f) Walking **[1 2]**
g) Sports/Energetic activities **[1 2]**

Date of Assessment: Assessor's initials: Study ID:

General Notes History (or any addition features mentioned by participant)

Date of Assessment: Assessor's initials: Study ID:

Past Medical History

1.Is there any previous history of the following conditions or events?

1. Yes
2. No

Anoxia/complicated pregnancy or delivery	[1	2]
Cerebrovascular accident	[1	2]
CNS infection	[1	2]
CNS tumour	[1	2]
Mitochondrial Disorder	[1	2]
Heredodegenerative/metabolic disorder	[1	2]
Head Trauma	[1	2]
Peripheral Trauma	[1	2]
Neuropathy in region of dystonia/tremor	[1	2]
General Anaesthesia	[1	2]
Neuroleptic/Dopamine-atagonist exposure	[1	2]

2.Does past or current medical history include?

1. Yes
2. No

Epilepsy	[1	2]
Diabetes (insulin independent)	[1	2]
Diabetes (insulin dependent)	[1	2]
Hypertension	[1	2]
Hypercholesterolaemia	[1	2]
Hypothyroidism	[1	2]
Hyperthyroidism	[1	2]

If, answer 'yes' to any of the above please give further details:

3. Has there been any previous contact with Psychiatric services? [1 2]

1. Yes
2. No

4. If 'yes' how long ago was this?

5. If 'yes', what form did this take? [1 2 3 4 5 6]

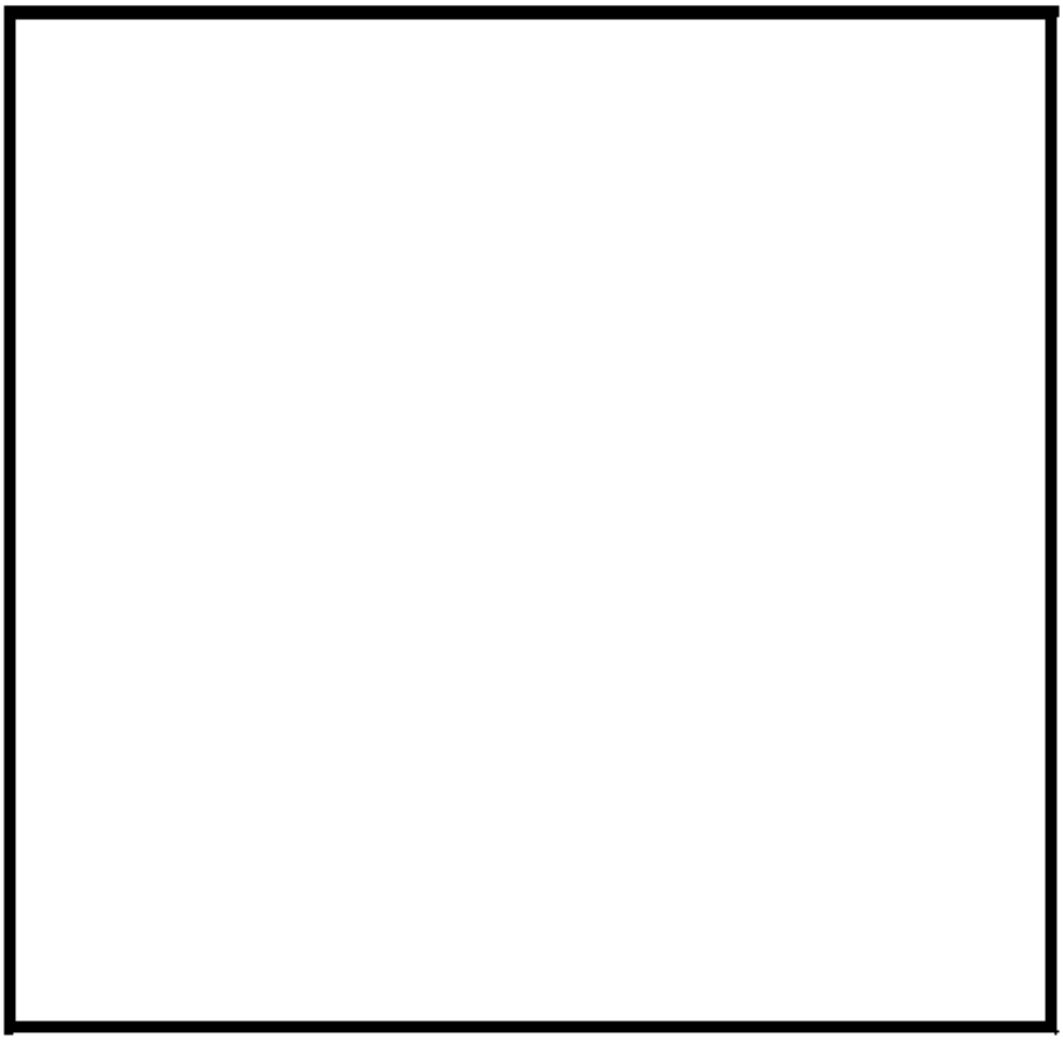
1. Psychiatrist

2. GP
3. CPN
4. Support groups
5. Other
6. Not applicable

6. Have you required, at any point in the past, an inpatient stay for treatment of these symptoms? [1 2 3]

1. Yes
2. No
3. Not applicable

Any additional Past Medical History (freehand)



Date of Assessment: Assessor's initials: Study ID:

Medication

Have you previously been prescribed any of the following medication for the treatment of your symptoms? If 'yes', was there any improvement/deterioration?

1. Yes
2. No
3. Uncertain
4. Not applicable

Response:

1. Better
2. Worse
3. No change
4. Not applicable

a) Beta-blockers e.g. propanolol	[1	2	3	4]
Response	[1	2	3	4]
b) Levodopa	[1	2	3	4]
Response	[1	2	3	4]
c) Primidone	[1	2	3	4]
Response	[1	2	3	4]
b) Gabapentin	[1	2	3	4]
Response	[1	2	3	4]
b) Benzodiazepines	[1	2	3	4]
Response	[1	2	3	4]
b) Haloperidol	[1	2	3	4]
Response	[1	2	3	4]
b) Tetrabenazine	[1	2	3	4]
Response	[1	2	3	4]
b) Sodium Valproate	[1	2	3	4]
Response	[1	2	3	4]
b) Botulinum Toxin	[1	2	3	4]
Response	[1	2	3	4]

List Current Medication & doses

Date of Assessment: Assessor's initials: Study ID:

Social History

Alcohol Intake

1. Do you drink? [1 2]
    1. Yes
    2. No

2. If 'yes', on average how many units would you drink per week?
[Guide: 1 Unit is equivalent to ½ pint of beer, lager or cider; 1 small glass of wine or sherry, 1 single measure of spirits]

3. If participant used to be a heavy drinker, what was the usual number of units per week during that time?

4. If participant used to be a heavy drinker, from and to what age did the heavy drinking occur?

Smoking History

1. Do you smoke? [1 2 3]
    1. Yes
    2. No
    3. Ex-smoke

2. How many cigarettes do you smoke per day?

Date of Assessment: Assessor's initials: Study ID:

Family History

Draw family tree on next page
(include names and ages of as many family members as is possible)

Additional Information:

Maiden name of participant if female:

Maiden name of participant's mother:

Participant's parents: **[1 2 3]**

1. Non-consanguinous
2. Consanguinous
3. Uncertain

Maiden name of maternal grandmother:

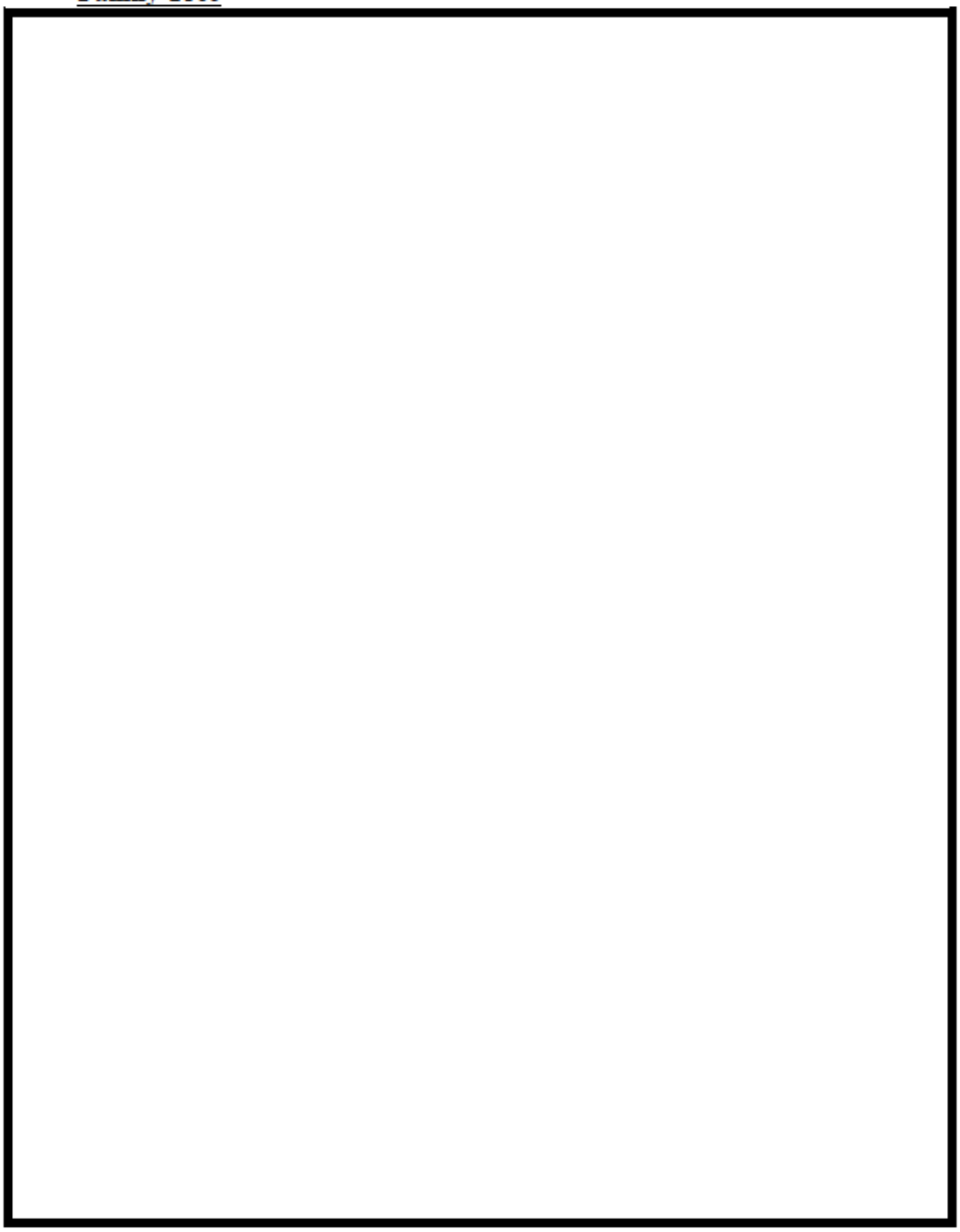
Maiden name of paternal grandmother:

List all similarly affected family members

Name	Relationship to Proband	Symptoms	Distribution	Previous diagnoses given

Date of Assessment: Assessor's initials: Study ID:

Family Tree



B.4 Examination Protocol

Video Examination Protocol

Eyes and upper face
Sit unsupported in chair, close view of head and shoulders

- Rest: 10 seconds
- Eyes open: 10 seconds
- Eyes closed: 10 seconds
- Forced eye blinks: 10 times

Lower face, jaw, tongue, larynx
Patient seated

- Close view of face at rest: 10 seconds
- Reading standardized passage aloud
- Repeat consonants: 5 times each : Tee, Mee, La, Ca
- Hold 'eeee' for 5 seconds
- Tongue protrusion: 5 seconds
- Opening and closing mouth: 5 reps

Neck
Seated in chair, close head and shoulders

- Frontal view at rest (tell patient to allow their head to move) : 10sec
- Eyes closed (allow head to move): 10 second
- Eyes closed: clap loudly 3 times
- Eyes closed: tap patient's nose
- Elicit jaw jerk
- Turn head all the way to the right: turn head all the way to the left
- Tilt ear on shoulder each side
- Look up & look down
- Lateral view (5 seconds)

Shoulders and upper arms, distal arm and hand

- Arms extended supinated: 5 seconds
- Arms extended pronated: 5 seconds
- Arms flexed in front of chest: 5 seconds
- Finger to nose: 10 repetitions
- Finger tapping, right then left: 10 repetitions
- Hand pronate/supinate separately & together: 10 repetitions
- Finger flick: right then left index finger
- Pin prick: flexor surface of wrist: right then left
- Reflexes: biceps: right and left

Upper leg, distal leg, foot and trunk

- Toe taps: shoes and socks off : 10 repetitions
- Foot stamps: 10 repetitions each side
- Knee jerks: right and left
- Toe flick: right then left (great toe)
- Pinprick: bottom of foot

Standing & Walking

- Sit to stand with arms crossed
- Standing: frontal view: 10seconds
- Standing: lateral view: 5 seconds
- Standing: back view: 5 seconds
- Walking away and towards examiner: repeat twice

Tasks

- Pouring water from one cup to another (8 transfers)
- Drinking water from a full glass (7 times each side)
- Bringing spoon of water from bowl to mouth and back (7 times each side)
- Drawing as per Tremor Rating Scale questionnaire: video hand to pen, pick pen up and writing.

B.5 Patient Health Questionnaire 9 (PHQ-9)

PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

NAME:_____ DATE:_____

Over the last *2 weeks*, how often have you been bothered by any of the following problems? *(use "✓" to indicate your answer)*	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself—or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed. Or the opposite—being so figety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead, or of hurting yourself	0	1	2	3
		add columns	+	+

(Healthcare professional: For interpretation of TOTAL, please refer to accompanying scoring card). TOTAL:

10. If you checked off *any problems*, how *difficult* have these problems made it for you to do your work, take care of things at home, or get along with other people?	Not difficult at all _____ Somewhat difficult _____ Very difficult _____ Extremely difficult _____

B.6 Structured Clinical Interview for DSM-IV Axis II (SCID-II) Personality Assessment

Please answer True (T) or False (F) to the following statements as they might apply to you over the last 5 years. If you are unsure of an answer please select the one that is more likely to be correct.

1.	I usually get fun and enjoyment out of life	T F
2.	I trust people I know	T F
3.	I'm not fussy about little details	T F
4.	I can't decide what kind of person I want to be	T F
5.	I show my feelings for everyone to see	T F
6.	I let others make my decisions for me	T F
7.	I get upset when I hear bad news about someone I know	T F
8.	Giving in to some of my urges gets me into trouble	T F
9.	Many people I know envy me	T F
10.	I give my general impression of things and don't bother with details	T F
11.	I've never been arrested	T F
12.	People think I'm cold and detached	T F
13.	I get into very intense relationships that don't last	T F
14.	Most people are fair and honest with me	T F
15.	People have a high opinion of me	T F
16.	I feel awkward or out of place in social situations	T F
17.	I'm too easily influenced by what goes on around me	T F
18.	I usually feel bad when I hurt or upset someone	T F
19.	I feel it very difficult to throw things out	T F
20.	At times I've refused to hold a job, even when I was expected to	T F
21.	When I'm praised or criticized I let others know how I feel	T F
22.	I use people to get what I want	T F
23.	I spend too much time trying to do things perfectly	T F
24.	People often make fun of me behind my back	T F
25.	I've never threatened suicide or injured myself on purpose	T F
26.	My feelings are like the weather, they're always changing	T F
27.	To avoid being criticized, I prefer to work alone	T F
28.	I like to dress so I stand out in a crowd	T F
29.	I will lie or con someone it if serves my purpose	T F
30.	I am more superstitious than most people	T F
31.	I have little or no desire to have sex with anyone	T F
32.	People think I'm to strict about rules and regulations	T F
33.	I usually feel uncomfortable or helpless when I'm alive	T F
34.	I wont get involved with people until I know that they like me	T F
35.	I would rather not be the centre of attention	T F
36.	I think my spouse (or lover) may be unfaithful to me	T F
37.	People think I have too high an opinion of myself	T F
38.	I am careful about what I tell others about myself	T F
39.	I worry a lot that people may not like me	T F
40.	I often feel "empty" inside	T F
41.	I work so hard I don't have time for anything else	T F
42.	I worry about being left alone and having to care for myself	T F
43.	I have tantrums or angry outbursts	T F
44.	I have a reputation for being a flirt	T F
45.	I feel very close to people I've just met	T F
46.	I prefer activities that I can do by myself	T F

47. I lose my temper and get into physical fights T F
48. Some people think I'm tight or stingy with my money T F
49. I often seek advice or reassurance about everyday decisions T F
50. I get people to like me. I help them with unpleasant jobs T F
51. I'm afraid of making a fool of myself with people I'm close to T F
52. I'm often mistake objects or shadows for people T F
53. I'm very moody T F
54. It's hard for me to get used to a new way of doing things T F
55. I daydream about being famous T F
56. I take chances and do reckless things T F
57. Everyone needs a friend or two to be happy T F
58. I discover hidden threats in what people tell me T F
59. I usually try to get people to do things my way T F
60. When I'm under stress, things around me don't seem real T F
61. I get annoyed when people wont do what I ask T F
62. When a close relationship ends, I can hardly wait to start a new one T F
63. I avoid unfamiliar activities so I wont be embarrassed trying to do them T F
64. People find it hard to get the point of what I'm saying T F
65. I prefer to associate with talented people T F
66. I've been the victim of unfair attacks on my character or reputation T F
67. I don't show much emotion T F
68. I do things to get people to admire me T F
69. I'm usually able to start projects on my own T F
70. People think I'm odd or eccentric T F
71. I feel at ease in social situations T F
72. I've held grudges against people for years T F
73. I find it hard to disagree with people I depend on a lot T F
74. It's hard for me to stay out of trouble T F
75. I go to extremes to try to keep people from leaving me T F
76. When I first meet someone. I don't say much T F
77. I have close friends T F

B.7 The Short Form (36) Health Survey (SF-36)

Name: **Study number:**

Date when completing form:

SF-36 Health Survey

INSTRUCTIONS: This survey asks your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Please answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

When complete, please return the questionnaire in the envelope provided.

---

1. In general, would you say your health is:

	(circle one)
Excellent	1
Very good	2
Good	3
Fair	4
Poor	5

2. Compared to one year ago, how would you rate your health in general now?

	(circle one)
Much better now than one year ago	1
Somewhat better than one year ago	2
About the same as one year ago	3
Somewhat worse than one year ago	4
Much worse now than one year ago	5

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

(circle one number on each line)

Activities	Yes, limited a lot	Yes, limited a little	No, not limited at all
Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.	1	2	3
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf	1	2	3
Lifting or carrying groceries	1	2	3
Climbing several flights of stairs	1	2	3
Climbing one flight of stairs	1	2	3
Bending, kneeling or stooping	1	2	3
Walking more than a mile	1	2	3
Walking half a mile	1	2	3
Walking one hundred yards	1	2	3
Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

(circle one number on each line)

	Yes	No
Cut down on the amount of time you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Were limited in the kind of work or other activities	1	2
Had difficulty performing the work or other activities (for example, it took extra effort	1	2

5.During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

(circle one number on each line)

	Yes	No
Cut down on the amount of time you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Didn't do work or other activities as carefully as usual	1	2

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups?

(circle one)

Not at all	1
Slightly	2
Moderately	3
Quite a bit	4
Extremely	5

7. How much bodily pain have you had during the past 4 weeks?

(circle one)

None	1
Very mild	2
Mild	3
Moderate	4
Severe	5
Very severe	6

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

(circle one)

Not at all	1
A little bit	2
Moderately	3
Quite a bit	4
Extremely	5

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks…

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
Did you feel full of life?	1	2	3	4	5	6
Have you been a very nervous person?	1	2	3	4	5	6
Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
Have you felt calm and peaceful?	1	2	3	4	5	6
Did you have a lot of energy?	1	2	3	4	5	6
Have you felt downhearted and low?	1	2	3	4	5	6
Did you feel worn out?	1	2	3	4	5	6
Have you been a happy person?	1	2	3	4	5	6
Did you feel tired?	1	2	3	4	5	6

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

(circle one)

All of the time	1
Most of the time	2
Some of the time	3
A little of the time	4
None of the time	5

11. How TRUE or FALSE is each of the following statements to you?

(circle one number on each line)

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
I seem to get ill more easily than other people	1	2	3	4	5
I am as healthy as anybody I know	1	2	3	4	5
I expect my health to get worse	1	2	3	4	5
My health is excellent	1	2	3	4	5

B.8 MINI International Neuropsychiatric Interview (M.I.N.I.)

M.I.N.I.

MINI INTERNATIONAL NEUROPSYCHIATRIC INTERVIEW

English Version 6.0.0

DSM-IV

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DISCLAIMER

Our aim is to assist in the assessment and tracking of patients with greater efficiency and accuracy. Before action is taken on any data collected and processed by this program, it should be reviewed and interpreted by a licensed clinician.

This program is not designed or intended to be used in the place of a full medical and psychiatric evaluation by a qualified licensed physician – psychiatrist. It is intended only as a tool to facilitate accurate data collection and processing of symptoms elicited by trained personnel.

A. MAJOR DEPRESSIVE EPISODE

(➡ MEANS : GO TO THE DIAGNOSTIC BOXES, CIRCLE NO IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

A1	a	Were you ever depressed or down, most of the day, nearly every day, for two weeks?	NO	YES
		IF NO, CODE NO TO **A1b**: IF YES ASK:		
	b	For the past two weeks, were you depressed or down, most of the day, nearly every day?	NO	YES
A2	a	Were you ever much less interested in most things or much less able to enjoy the things you used to enjoy most of the time, for two weeks?	NO	YES
		IF NO, CODE NO TO **A2b**: IF YES ASK:		
	b	In the past two weeks, were you much less interested in most things or much less able to enjoy the things you used to enjoy, most of the time?	NO	YES
			➡	
		IS **A1a** OR **A2a** CODED YES?	NO	YES

A3 IF **A1b** OR **A2b** = YES: EXPLORE THE **CURRENT** AND THE MOST SYMPTOMATIC **PAST** EPISODE, OTHERWISE
IF **A1b** AND **A2b** = NO: EXPLORE ONLY THE MOST SYMPTOMATIC **PAST** EPISODE

Over that two week period, when you felt depressed or uninterested:

		Past 2 Weeks		Past Episode	
a	Was your appetite decreased or increased nearly every day? Did your weight decrease or increase without trying intentionally (i.e., by ±5% of body weight or ±8 lbs. or ±3.5 kgs., for a 160 lb./70 kg. person in a month)? IF YES TO EITHER, CODE YES.	NO	YES	NO	YES
b	Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)?	NO	YES	NO	YES
c	Did you talk or move more slowly than normal or were you fidgety, restless or having trouble sitting still almost every day?	NO	YES	NO	YES
d	Did you feel tired or without energy almost every day?	NO	YES	NO	YES
e	Did you feel worthless or guilty almost every day?	NO	YES	NO	YES
	IF YES, ASK FOR EXAMPLES. THE EXAMPLES ARE CONSISTENT WITH A DELUSIONAL IDEA. Current Episode ❒ No ❒ Yes; Past Episode ❒ No ❒ Yes				
f	Did you have difficulty concentrating or making decisions almost every day?	NO	YES	NO	YES
g	Did you repeatedly consider hurting yourself, feel suicidal, or wish that you were dead? Did you attempt suicide or plan a suicide? IF YES TO EITHER, CODE YES.	NO	YES	NO	YES
A4	Did these symptoms cause significant problems at home, at work, socially, at school or in some other important way?	NO	YES	NO	YES

A5 In between 2 episodes of depression, did you ever have an interval of at least 2 months, without any significant depression or any significant loss of interest? NO YES

ARE **5** OR MORE ANSWERS (**A1-A3**) CODED **YES** AND IS **A4** CODED YES FOR THAT TIME FRAME?

SPECIFY IF THE EPISODE IS CURRENT AND / OR PAST.

IF **A5** IS CODED **YES**, CODE **YES** FOR RECURRENT.

NO	**YES**
MAJOR DEPRESSIVE EPISODE	
CURRENT	❒
PAST	❒
RECURRENT	❒

A6 a How many episodes of depression did you have in your lifetime? _____

Between each episode there must be at least 2 months without any significant depression.

C. MANIC AND HYPOMANIC EPISODES

(➡ MEANS : GO TO THE DIAGNOSTIC BOXES, CIRCLE NO IN MANIC AND HYPOMANIC DIAGNOSTIC BOXES, AND MOVE TO NEXT MODULE)

Do you have any family history of manic depressive illness or bipolar disorder, or any family member who had mood swings treated with a medication like lithium, sodium valproate (Depakote) or lamotrigine (Lamictal)? NO YES

THIS QUESTION IS NOT A CRITERION FOR BIPOLAR DISORDER, BUT IS ASKED TO INCREASE THE CLINICIAN'S VIGILANCE ABOUT THE RISK FOR BIPOLAR DISORDER .

IF YES, PLEASE SPECIFY WHO:_____

C1	a	Have you **ever** had a period of time when you were feeling 'up' or 'high' or 'hyper' or so full of energy or full of yourself that you got into trouble, - or that other people thought you were not your usual self? (Do not consider times when you were intoxicated on drugs or alcohol.) IF PATIENT IS PUZZLED OR UNCLEAR ABOUT WHAT YOU MEAN BY 'UP' OR 'HIGH' OR 'HYPER', CLARIFY AS FOLLOWS: By 'up' or 'high' or 'hyper' I mean: having elated mood; increased energy; needing less sleep; having rapid thoughts; being full of ideas; having an increase in productivity, motivation, creativity, or impulsive behavior; phoning or working excessively or spending more money. IF NO, CODE NO TO **C1b**: IF YES ASK:	NO	YES
	b	Are you currently feeling 'up' or 'high' or 'hyper' or full of energy?	NO	YES
C2	a	Have you **ever** been persistently irritable, for several days, so that you had arguments or verbal or physical fights, or shouted at people outside your family? Have you or others noticed that you have been more irritable or over reacted, compared to other people, even in situations that you felt were justified? IF NO, CODE NO TO **C2b**: IF YES ASK:	NO	YES
	b	Are you currently feeling persistently irritable?	NO ➡	YES
		IS **C1a** OR **C2a** CODED YES?	NO	YES

C3 IF **C1b** OR **C2b** = YES: EXPLORE THE CURRENT AND THE MOST SYMPTOMATIC **PAST** EPISODE, OTHERWISE
IF **C1b** AND **C2b** = NO: EXPLORE ONLY THE MOST SYMPTOMATIC **PAST** EPISODE

During the times when you felt high, full of energy, or irritable did you:

		Current Episode		Past Episode	
a	Feel that you could do things others couldn't do, or that you were an especially important person? IF YES, ASK FOR EXAMPLES. THE EXAMPLES ARE CONSISTENT WITH A DELUSIONAL IDEA. Current Episode ☐ No ☐ Yes Past Episode ☐ No ☐ Yes	NO	YES	NO	YES
b	Need less sleep (for example, feel rested after only a few hours sleep)?	NO	YES	NO	YES
c	Talk too much without stopping, or so fast that people had difficulty understanding?	NO	YES	NO	YES
d	Have racing thoughts?	NO	YES	NO	YES

		Current Episode		Past Episode	
e	Become easily distracted so that any little interruption could distract you?	NO	YES	NO	YES
f	Have a significant increase in your activity or drive, at work, at school, socially or sexually or did you become physically or mentally restless?	NO	YES	NO	YES
g	Want so much to engage in pleasurable activities that you ignored the risks or consequences (for example, spending sprees, reckless driving, or sexual indiscretions)?	NO	YES	NO	YES
C3	SUMMARY: WHEN RATING CURRENT EPISODE: IF C1b IS NO, ARE 4 OR MORE C3 ANSWERS CODED YES? IF C1b IS YES, ARE 3 OR MORE C3 ANSWERS CODED YES? WHEN RATING PAST EPISODE: IF C1a IS NO, ARE 4 OR MORE C3 ANSWERS CODED YES? IF C1a IS YES, ARE 3 OR MORE C3 ANSWERS CODED YES? CODE YES ONLY IF THE ABOVE 3 OR 4 SYMPTOMS OCCURRED DURING THE SAME TIME PERIOD. RULE: ELATION/EXPANSIVENESS REQUIRES ONLY THREE C3 SYMPTOMS, WHILE IRRITABLE MOOD ALONE REQUIRES 4 OF THE C3 SYMPTOMS.	NO	YES	NO	YES
C4	What is the longest time these symptoms lasted?				
	a) 3 days or less		❑		❑
	b) 4 to 6 days		❑		❑
	c) 7 days or more		❑		❑
C5	Were you hospitalized for these problems? IF YES, STOP HERE AND CIRCLE YES IN MANIC EPISODE FOR THAT TIME FRAME.	NO	YES	NO	YES
C6	Did these symptoms cause significant problems at home, at work, socially in your relationships with others, at school or in some other important way?	NO	YES	NO	YES

ARE C3 SUMMARY AND C5 AND C6 CODED YES AND EITHER C4a or b or c CODED YES?

OR

ARE C3 SUMMARY AND C4c AND C6 CODED YES AND IS C5 CODED NO?

SPECIFY IF THE EPISODE IS CURRENT AND / OR PAST.

NO **YES**

MANIC EPISODE

CURRENT ❑
PAST ❑

ARE C3 SUMMARY AND C5 AND C6 CODED NO AND EITHER C4b OR C4c CODED YES?

OR

ARE C3 SUMMARY AND C4b AND C6 CODED YES AND IS C5 CODED NO?

SPECIFY IF THE EPISODE IS CURRENT AND / OR PAST.

NO **YES**

HYPOMANIC EPISODE

CURRENT ❑
PAST ❑

ARE **C3** SUMMARY AND **C4a** CODED **YES** AND IS **C5** CODED **NO**? **NO** **YES**

HYPOMANIC SYMPTOMS

SPECIFY IF THE EPISODE IS CURRENT AND / OR PAST.

CURRENT ❒
PAST ❒

C7 a) IF MANIC EPISODE IS POSITIVE FOR EITHER CURRENT OR PAST ASK:
Did you have 2 or more manic episodes (**C4c**) in your lifetime (including the current episode if present)? NO YES

b) IF HYPOMANIC EPISODE IS POSITIVE FOR EITHER CURRENT OR PAST ASK:
Did you have 2 or more hypomanic EPISODES (**C4b**) in your lifetime (including the current episode)? NO YES

c) IF PAST "HYPOMANIC SYMPTOMS" IS CODED POSITIVE ASK:
Did you have 2 or more episodes of hypomanic SYMPTOMS (**C4a**) in your lifetime
(including the current episode if present)? NO YES

D. PANIC DISORDER

(➡ MEANS : CIRCLE NO IN D5, D6 AND D7 AND SKIP TO E1)

D1	a	Have you, on more than one occasion, had spells or attacks when you **suddenly** felt anxious, frightened, uncomfortable or uneasy, even in situations where most people would not feel that way?	➡ NO	YES
	b	Did the spells surge to a peak within 10 minutes of starting?	➡ NO	YES
D2		At any time in the past, did any of those spells or attacks come on unexpectedly or occur in an unpredictable or unprovoked manner?	➡ NO	YES
D3		Have you ever had one such attack followed by a month or more of persistent concern about having another attack, or worries about the consequences of the attack - or did you make a significant change in your behavior because of the attacks (e.g., shopping only with a companion, not wanting to leave your house, visiting the emergency room repeatedly, or seeing your doctor more frequently because of the symptoms)?	NO	YES
D4		**During the worst attack that you can remember:**		
	a	Did you have skipping, racing or pounding of your heart?	NO	YES
	b	Did you have sweating or clammy hands?	NO	YES
	c	Were you trembling or shaking?	NO	YES
	d	Did you have shortness of breath or difficulty breathing?	NO	YES
	e	Did you have a choking sensation or a lump in your throat?	NO	YES
	f	Did you have chest pain, pressure or discomfort?	NO	YES
	g	Did you have nausea, stomach problems or sudden diarrhea?	NO	YES
	h	Did you feel dizzy, unsteady, lightheaded or faint?	NO	YES
	i	Did things around you feel strange, unreal, detached or unfamiliar, or did you feel outside of or detached from part or all of your body?	NO	YES
	j	Did you fear that you were losing control or going crazy?	NO	YES
	k	Did you fear that you were dying?	NO	YES
	l	Did you have tingling or numbness in parts of your body?	NO	YES
	m	Did you have hot flushes or chills?	NO	YES
D5		ARE BOTH **D3,** AND **4** OR MORE **D4** ANSWERS, CODED YES? IF YES TO D5, SKIP TO D7.	NO	YES *PANIC DISORDER LIFETIME*
D6		IF **D5** = NO, ARE ANY D4 ANSWERS CODED YES? THEN SKIP TO **E1.**	NO	YES *LIMITED SYMPTOM*

D7	In the past month, did you have such attacks repeatedly (2 or more), and did you have persistent concern about having another attack, or worry about the consequences of the attacks, or did you change your behavior in any way because of the attacks?	NO	YES *PANIC DISORDER CURRENT*

E. AGORAPHOBIA

E1	Do you feel anxious or uneasy in places or situations where help might not be available or escape might be difficult, like being in a crowd, standing in a line (queue), when you are alone away from home or alone at home, or when crossing a bridge, or traveling in a bus, train or car or where you might have a panic attack or the panic-like symptoms we just spoke about?	NO	YES

IF **E1** = NO, CIRCLE **NO** IN **E2**.

E2	Do you fear these situations so much that you avoid them, or suffer through them, or need a companion to face them?	NO	YES *AGORAPHOBIA CURRENT*

IS **E2** (CURRENT AGORAPHOBIA) CODED **YES**

and

IS **D7** (CURRENT PANIC DISORDER) CODED **YES**?

NO **YES**

PANIC DISORDER with Agoraphobia CURRENT

IS **E2** (CURRENT AGORAPHOBIA) CODED **NO**

and

IS **D7** (CURRENT PANIC DISORDER) CODED **YES**?

NO **YES**

PANIC DISORDER without Agoraphobia CURRENT

IS **E2** (CURRENT AGORAPHOBIA) CODED **YES**

and

IS **D5** (PANIC DISORDER LIFETIME) CODED **NO**?

NO **YES**

AGORAPHOBIA, CURRENT without history of Panic Disorder

F. SOCIAL PHOBIA (Social Anxiety Disorder)

(➡ MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE NO AND MOVE TO THE NEXT MODULE)

F1	In the past month, did you have persistent fear and significant anxiety at being watched, being the focus of attention, or of being humiliated or embarrassed? This includes things like speaking in public, eating in public or with others, writing while someone watches, or being in social situations.	➡ NO	YES
F2	Is this social fear excessive or unreasonable and does it almost always make you anxious?	➡ NO	YES
F3	Do you fear these social situations so much that you avoid them or suffer through them most of the time?	➡ NO	YES
F4	Do these social fears disrupt your normal work, school or social functioning or cause you significant distress?	NO	YES

SUBTYPES

Do you fear and avoid 4 or more social situations?

If YES Generalized social phobia (social anxiety disorder)

If NO Non-generalized social phobia (social anxiety disorder)

EXAMPLES OF SUCH SOCIAL SITUATIONS TYPICALLY INCLUDE

- INITIATING OR MAINTAINING A CONVERSATION,
- PARTICIPATING IN SMALL GROUPS,
- DATING,
- SPEAKING TO AUTHORITY FIGURES,
- ATTENDING PARTIES,
- PUBLIC SPEAKING,
- EATING IN FRONT OF OTHERS,
- URINATING IN A PUBLIC WASHROOM, ETC.

NOTE TO INTERVIEWER: PLEASE ASSESS WHETHER THE SUBJECT'S FEARS ARE RESTRICTED TO NON-GENERALIZED ("ONLY 1 OR SEVERAL") SOCIAL SITUATIONS OR EXTEND TO GENERALIZED ("MOST") SOCIAL SITUATIONS. "MOST" SOCIAL SITUATIONS IS USUALLY OPERATIONALIZED TO MEAN 4 OR MORE SOCIAL SITUATIONS, ALTHOUGH THE DSM-IV DOES NOT EXPLICITLY STATE THIS.

SOCIAL PHOBIA
(Social Anxiety Disorder)
CURRENT

GENERALIZED ❒

NON-GENERALIZED ❒

G. OBSESSIVE-COMPULSIVE DISORDER

(➡ MEANS: GO TO THE DIAGNOSTIC BOX, CIRCLE NO AND MOVE TO THE NEXT MODULE)

G1	In the past month, have you been bothered by recurrent thoughts, impulses, or images that were unwanted, distasteful, inappropriate, intrusive, or distressing? - (For example, the idea that you were dirty, contaminated or had germs, or fear of contaminating others, or fear of harming someone even though it disturbs or distresses you, or fear you would act on some impulse, or fear or superstitions that you would be responsible for things going wrong, or obsessions with sexual thoughts, images or impulses, or hoarding, collecting, or religious obsessions.) (DO NOT INCLUDE SIMPLY EXCESSIVE WORRIES ABOUT REAL LIFE PROBLEMS. DO NOT INCLUDE OBSESSIONS DIRECTLY RELATED TO EATING DISORDERS, SEXUAL DEVIATIONS, PATHOLOGICAL GAMBLING, OR ALCOHOL OR DRUG ABUSE BECAUSE THE PATIENT MAY DERIVE PLEASURE FROM THE ACTIVITY AND MAY WANT TO RESIST IT ONLY BECAUSE OF ITS NEGATIVE CONSEQUENCES.)	NO YES ↓ SKIP TO G4
G2	Did they keep coming back into your mind even when you tried to ignore or get rid of them?	NO YES ↓ SKIP TO G4
G3	Do you think that these obsessions are the product of your own mind and that they are not imposed from the outside?	NO YES obsessions
G4	In the past month, did you do something repeatedly without being able to resist doing it, like washing or cleaning excessively, counting or checking things over and over, or repeating, collecting, arranging things, or other superstitious rituals?	NO YES compulsions
	IS G3 OR G4 CODED YES?	➡ NO YES ➡
G5	At any point, did you recognize that either these obsessive thoughts or these compulsive behaviors were excessive or unreasonable?	NO YES
G6	In the past month, did these obsessive thoughts and/or compulsive behaviors significantly interfere with your normal routine, your work or school, your usual social activities, or relationships, or did they take more than one hour a day?	NO YES *O.C.D. CURRENT*

I. ALCOHOL DEPENDENCE / ABUSE

(➡ MEANS: GO TO DIAGNOSTIC BOXES, CIRCLE NO IN BOTH AND MOVE TO THE NEXT MODULE)

		➡ NO	YES
I1	**In the past 12 months**, have you had 3 or more alcoholic drinks, - within a 3 hour period, - on 3 or more occasions?		
I2	**In the past 12 months**:		
a	Did you need to drink a lot more in order to get the same effect that you got when you first started drinking or did you get much less effect with continued use of the same amount?	NO	YES
b	When you cut down on drinking did your hands shake, did you sweat or feel agitated? Did you drink to avoid these symptoms (for example, "the shakes", sweating or agitation) or to avoid being hungover? IF YES TO ANY, CODE YES.	NO	YES
c	During the times when you drank alcohol, did you end up drinking more than you planned when you started?	NO	YES
d	Have you tried to reduce or stop drinking alcohol but failed?	NO	YES
e	On the days that you drank, did you spend substantial time in obtaining alcohol, drinking, or in recovering from the effects of alcohol?	NO	YES
f	Did you spend less time working, enjoying hobbies, or being with others because of your drinking?	NO	YES
g	If your drinking caused you health or mental problems, did you still keep on drinking?	NO	YES

ARE 3 OR MORE I2 ANSWERS CODED YES?

* IF YES, SKIP I3 QUESTIONS AND GO TO NEXT MODULE. "DEPENDENCE PREEMPTS ABUSE" IN DSM IV TR.

NO	YES*
	ALCOHOL DEPENDENCE CURRENT

I3	**In the past 12 months:**		
a	Have you been intoxicated, high, or hungover more than once when you had other responsibilities at school, at work, or at home? Did this cause any problems? (CODE YES ONLY IF THIS CAUSED PROBLEMS.)	NO	YES
b	Were you intoxicated more than once in any situation where you were physically at risk, for example, driving a car, riding a motorbike, using machinery, boating, etc.?	NO	YES
c	Did you have legal problems more than once because of your drinking, for example, an arrest or disorderly conduct?	NO	YES
d	If your drinking caused problems with your family or other people, did you still keep on drinking?	NO	YES

ARE 1 OR MORE I3 ANSWERS CODED YES?

NO	YES
	ALCOHOL ABUSE CURRENT

K. PSYCHOTIC DISORDERS AND MOOD DISORDER WITH PSYCHOTIC FEATURES

ASK FOR AN EXAMPLE OF EACH QUESTION ANSWERED POSITIVELY. CODE **YES** ONLY IF THE EXAMPLES CLEARLY SHOW A DISTORTION OF THOUGHT OR OF PERCEPTION OR IF THEY ARE NOT CULTURALLY APPROPRIATE. BEFORE CODING, INVESTIGATE WHETHER DELUSIONS QUALIFY AS "BIZARRE".

DELUSIONS ARE "BIZARRE" IF: CLEARLY IMPLAUSIBLE, ABSURD, NOT UNDERSTANDABLE, AND CANNOT DERIVE FROM ORDINARY LIFE EXPERIENCE.
HALLUCINATIONS ARE SCORED "BIZARRE" IF: A VOICE COMMENTS ON THE PERSON'S THOUGHTS OR BEHAVIOR, OR WHEN TWO OR MORE VOICES ARE CONVERSING WITH EACH OTHER.
THE PURPOSE OF THIS MODULE IS TO EXCLUDE PATIENTS WITH PSYCHOTIC DISORDERS. THIS MODULE NEEDS EXPERIENCE.

					BIZARRE
		Now I am going to ask you about unusual experiences that some people have.			
K1	a	Have you ever believed that people were spying on you, or that someone was plotting against you, or trying to hurt you? NOTE: ASK FOR EXAMPLES TO RULE OUT ACTUAL STALKING.	NO	YES	YES
	b	**IF YES OR YES BIZARRE:** do you currently believe these things?	NO	YES	YES →K6
K2	a	Have you ever believed that someone was reading your mind or could hear your thoughts, or that you could actually read someone's mind or hear what another person was thinking?	NO	YES	YES
	b	**IF YES OR YES BIZARRE:** do you currently believe these things?	NO	YES	YES →K6
K3	a	Have you ever believed that someone or some force outside of yourself put thoughts in your mind that were not your own, or made you act in a way that was not your usual self? Have you ever felt that you were possessed? CLINICIAN: ASK FOR EXAMPLES AND DISCOUNT ANY THAT ARE NOT PSYCHOTIC.	NO	YES	YES
	b	**IF YES OR YES BIZARRE:** do you currently believe these things?	NO	YES	YES →K6
K4	a	Have you ever believed that you were being sent special messages through the TV, radio, newspapers, books or magazines or that a person you did not personally know was particularly interested in you?	NO	YES	YES
	b	**IF YES OR YES BIZARRE:** do you currently believe these things?	NO	YES	YES →K6
K5	a	Have your relatives or friends ever considered any of your beliefs odd or unusual? INTERVIEWER: ASK FOR EXAMPLES. ONLY CODE **YES** IF THE EXAMPLES ARE **CLEARLY** DELUSIONAL IDEAS NOT EXPLORED IN QUESTIONS K1 TO K4, FOR EXAMPLE, SOMATIC OR RELIGIOUS DELUSIONS OR DELUSIONS OF GRANDIOSITY, JEALOUSY, GUILT, RUIN OR DESTITUTION, ETC.	NO	YES	YES
	b	**IF YES OR YES BIZARRE:** do they currently consider your beliefs strange?	NO	YES	YES
K6	a	Have you ever heard things other people couldn't hear, such as voices?	NO	YES	
		IF YES TO VOICE HALLUCINATION: Was the voice commenting on your thoughts or behavior or did you hear two or more voices talking to each other?	NO		YES
	b	**IF YES OR YES BIZARRE TO K6a:** have you heard sounds / voices in the past month?	NO	YES	
		IF YES TO VOICE HALLUCINATION: Was the voice commenting on your thoughts or behavior or did you hear two or more voices talking to each other?	NO		YES →K8b

K7 a Have you ever had visions when you were awake or have you ever seen things other people couldn't see? NO YES

CLINICIAN: CHECK TO SEE IF THESE ARE CULTURALLY INAPPROPRIATE.

b IF YES: have you seen these things in the past month? NO YES

CLINICIAN'S JUDGMENT

K8 b IS THE PATIENT CURRENTLY EXHIBITING INCOHERENCE, DISORGANIZED SPEECH, OR MARKED LOOSENING OF ASSOCIATIONS? NO YES

K9 b IS THE PATIENT CURRENTLY EXHIBITING DISORGANIZED OR CATATONIC BEHAVIOR? NO YES

K10 b ARE NEGATIVE SYMPTOMS OF SCHIZOPHRENIA, E.G. SIGNIFICANT AFFECTIVE FLATTENING, POVERTY OF SPEECH (ALOGIA) OR AN INABILITY TO INITIATE OR PERSIST IN GOAL-DIRECTED ACTIVITIES (AVOLITION), PROMINENT DURING THE INTERVIEW? NO YES

K11 a ARE 1 OR MORE « a » QUESTIONS FROM K1a TO K7a CODED **YES OR YES BIZARRE** AND IS EITHER:

MAJOR DEPRESSIVE EPISODE, (CURRENT, RECURRENT OR PAST)
OR
MANIC OR HYPOMANIC EPISODE, (CURRENT OR PAST) CODED **YES**? NO YES
↳ **K13**

IF NO TO K11 a, CIRCLE NO IN BOTH 'MOOD DISORDER WITH PSYCHOTIC FEATURES' DIAGNOSTIC BOXES AND MOVE TO K13.

b You told me earlier that you had period(s) when you felt (depressed/high/persistently irritable).

Were the beliefs and experiences you just described (SYMPTOMS CODED YES FROM **K1a** TO **K7a**) restricted exclusively to times when you were feeling depressed/high/irritable?

IF THE PATIENT EVER HAD A PERIOD OF AT LEAST 2 WEEKS OF HAVING THESE BELIEFS OR EXPERIENCES (PSYCHOTIC SYMPTOMS) WHEN THEY WERE NOT DEPRESSED/HIGH/IRRITABLE, CODE NO TO THIS DISORDER.

IF THE ANSWER IS NO TO THIS DISORDER, ALSO CIRCLE NO TO K12 AND MOVE TO K13

NO **YES**

MOOD DISORDER WITH PSYCHOTIC FEATURES

LIFETIME

K12 a ARE 1 OR MORE « b » QUESTIONS FROM K1b TO K7b CODED **YES OR YES BIZARRE** AND IS EITHER:

MAJOR DEPRESSIVE EPISODE, (CURRENT)
OR
MANIC OR HYPOMANIC EPISODE, (CURRENT) CODED **YES**?

IF THE ANSWER IS YES TO THIS DISORDER (LIFETIME OR CURRENT), CIRCLE NO TO K13 AND K14 AND MOVE TO THE NEXT MODULE.

NO **YES**

MOOD DISORDER WITH PSYCHOTIC FEATURES

CURRENT

K13 ARE 1 OR MORE « b » QUESTIONS FROM K1b TO K6b, CODED YES BIZARRE?

OR

ARE 2 OR MORE « b » QUESTIONS FROM K1b TO K10b, CODED YES (RATHER THAN YES BIZARRE)?

AND DID AT LEAST TWO OF THE PSYCHOTIC SYMPTOMS OCCUR DURING THE SAME 1 MONTH PERIOD?

NO	YES
	PSYCHOTIC DISORDER CURRENT

K14 IS **K13** CODED YES

OR

ARE 1 OR MORE « a » QUESTIONS FROM K1a TO K6a, CODED YES BIZARRE?

OR

ARE 2 OR MORE « a » QUESTIONS FROM K1a TO K7a, CODED YES (RATHER THAN YES BIZARRE)

AND DID AT LEAST TWO OF THE PSYCHOTIC SYMPTOMS OCCUR DURING THE SAME 1 MONTH PERIOD?

NO	YES
	PSYCHOTIC DISORDER LIFETIME

N. GENERALIZED ANXIETY DISORDER

(➔ MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE)

N1	a	Were you excessively anxious or worried about several routine things, over the past 6 months? IN ENGLISH, IF THE PATIENT IS UNCLEAR ABOUT WHAT YOU MEAN, PROBE BY ASKING (Do others think that you are a "worry wart") AND GET EXAMPLES.	➔ NO	YES
	b	Are these anxieties and worries present most days?	➔ NO	YES
		ARE THE PATIENT'S ANXIETY AND WORRIES RESTRICTED EXCLUSIVELY TO, OR BETTER EXPLAINED BY, ANY DISORDER PRIOR TO THIS POINT?	NO	➔ YES
N2		Do you find it difficult to control the worries?	➔ NO	YES
N3		FOR THE FOLLOWING, CODE NO IF THE SYMPTOMS ARE CONFINED TO FEATURES OF ANY DISORDER EXPLORED PRIOR TO THIS POINT.		
		When you were anxious over the past 6 months, did you, most of the time:		
	a	Feel restless, keyed up or on edge?	NO	YES
	b	Have muscle tension?	NO	YES
	c	Feel tired, weak or exhausted easily?	NO	YES
	d	Have difficulty concentrating or find your mind going blank?	NO	YES
	e	Feel irritable?	NO	YES
	f	Have difficulty sleeping (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)?	NO	YES
		ARE 3 OR MORE N3 ANSWERS CODED YES?	➔ NO	YES
N4		Do these anxieties and worries disrupt your normal work, school or social functioning or cause you significant distress?	NO	YES

GENERALIZED ANXIETY DISORDER
CURRENT

O. RULE OUT MEDICAL, ORGANIC OR DRUG CAUSES FOR ALL DISORDERS

IF THE PATIENT CODES POSITIVE FOR ANY CURRENT DISORDER ASK:

Just before these symptoms began:

O1a	Were you taking any drugs or medicines?	❒ No	❒ Yes	❒ Uncertain
O1b	Did you have any medical illness?	❒ No	❒ Yes	❒ Uncertain

IN THE CLINICIAN'S JUDGMENT: ARE EITHER OF THESE LIKELY TO BE DIRECT CAUSES OF THE PATIENT'S DISORDER?
IF NECESSARY ASK ADDITIONAL OPEN-ENDED QUESTIONS.

B.9 B.8 MINI International Neuropsychiatric Interview for children and adolescents (Parent Version) (M.I.N.I. KID)

M.I.N.I. KID

MINI INTERNATIONAL NEUROPSYCHIATRIC INTERVIEW
For Children and Adolescents
(Parent Version)

English Version 6.0

USA: **D. Sheehan, D. Shytle, K. Milo, J. Janavs**
University of South Florida - Tampa

FRANCE: **Y. Lecrubier**
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A. MAJOR DEPRESSIVE EPISODE

(➡ MEANS : GO TO THE DIAGNOSTIC BOXES, CIRCLE NO IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

At any time in his/her life:

A1 a Did (s)he feel sad or depressed? Felt down or empty? Felt grouchy or annoyed?
IF YES TO ANY, CONTINUE. IF NO TO ALL, CODE NO TO A1a AND A1b.

Did (s)he feel this way most of the time, for at least 2 weeks? NO YES

b For the past 2 weeks, did (s)he feel this way, most of the day, nearly every day? NO YES

At any time in his/her life:

A2 a Was (s)he bored a lot or much less interested in things (Like playing his/her favorite games)?
Did (s)he feel that (s)he couldn't enjoy things?
IF YES TO ANY, CONTINUE. IF NO TO ALL, CODE NO TO A2a AND A2b.

Did (s)he feel this way most of the time, for at least 2 weeks? NO YES

b For the past 2 weeks, did (s)he feel this way, most of the day, nearly every day? NO YES

➡

IS **A1** OR **A2** CODED YES? NO YES

A3 IF **A1b** OR **A2b** = YES: EXPLORE THE CURRENT AND THE MOST SYMPTOMATIC **PAST** EPISODE, OTHERWISE
IF **A1b** AND **A2b** = NO: EXPLORE ONLY THE MOST SYMPTOMATIC PAST EPISODE

In the past two weeks, when (s)he felt depressed / grouchy / uninterested:	Past 2 Weeks		Past Episode	
a Was (s)he less hungry or more hungry most days? Did (s)he lose or gain weight without trying? [i.e., by ± 5% of body weight in the past month]? IF YES TO EITHER, CODE YES	NO	YES	NO	YES
b Did (s)he have trouble sleeping almost every night ("trouble sleeping" means trouble falling asleep, waking up in the middle of the night, waking up too early or sleeping too much)?	NO	YES	NO	YES
c Did (s)he talk or move slower than usual? Was (s)he fidgety, restless or couldn't sit still almost every day? IF YES TO EITHER, CODE YES	NO	YES	NO	YES
d Did (s)he feel tired most of the time?	NO	YES	NO	YES
e Did (s)he feel bad about him/herself most of the time? Did (s)he feel guilty most of the time? IF YES TO EITHER, CODE YES IF YES, ASK FOR EXAMPLES. THE EXAMPLES ARE CONSISTENT WITH A DELUSIONAL IDEA. Current Episode ❒ No ❒ Yes Past Episode ❒ No ❒ Yes	NO	YES	NO	YES
f Did (s)he have trouble concentrating or did (s)he have trouble making up his/her mind? IF YES TO EITHER, CODE YES	NO	YES	NO	YES

	g	Did (s)he feel so bad that (s)he wished that (s)he was dead? Did (s)he think about hurting him/herself? Did (s)he have thoughts of death? Did (s)he think about killing him/herself? IF **YES** TO ANY, CODE **YES**	NO	YES	NO	YES
A4		Did these sad, depressed feelings cause a lot of problems at home? At school? With friends? With other people? Or in some other important way?	NO	YES	NO	YES
A5		In between the times of depression, was (s)he free of depression for of at least 2 months?			NO	YES

ARE **5** OR MORE ANSWERS **(A1-A3)** CODED **YES** AND IS **A4** CODED YES FOR THAT TIME FRAME?

SPECIFY IF THE EPISODE IS CURRENT AND / OR PAST.

IF **A5** IS CODED **YES**, CODE **YES** FOR RECURRENT.

NO	**YES**
MAJOR DEPRESSIVE EPISODE	
CURRENT	☐
PAST	☐
RECURRENT	☐

A6 a How many episodes of depression did (s)he have in his/her lifetime? _____

Between each episode there must be at least 2 months without any significant depression.

D. (HYPO) MANIC EPISODE

(➡ MEANS : GO TO THE DIAGNOSTIC BOXES, CIRCLE NO TO THE RELEVANT TIME FRAME IN THE DIAGNOSTIC BOXES AND THEN MOVE TO THE NEXT MODULE)

Does (s)he have anyone in his/her family who had manic depressive illness or bipolar disorder or a family member who had mood swings treated with a medication like lithium, sodium valproate (Depakote or Valproate), lamotrigine (Lamictal)? NO YES

THIS QUESTION IS NOT A CRITERION FOR BIPOLAR DISORDER BUT IS ASKED TO INCREASE THE CLINICIAN'S VIGILANCE ABOUT RISK FOR BIPOLAR DISORDER.

IF YES, PLEASE SPECIFY WHO:_____

D1 a Has there **ever** been a time when (s)he was so happy that (s)he felt 'up' or 'high' or 'hyper'? NO YES
By 'up' or 'high' or 'hyper' I mean feeling really good; full of energy; needing less sleep; having racing thoughts or being full of ideas.

DO NOT CONSIDER TIMES WHEN THE PATIENT WAS INTOXICATED ON DRUGS OR ALCOHOL OR DURING SITUATIONS THAT NORMALLY OVER STIMULATE AND MAKE CHILDREN VERY EXCITED LIKE CHRISTMAS, BIRTHDAYS, ETC.

IF PATIENT IS PUZZLED OR UNCLEAR ABOUT WHAT HIM/HER MEAN BY 'UP' OR 'HIGH' OR 'HYPER' CLARIFY AS FOLLOWS: **By 'up' or 'high' or 'hyper' I mean: having elated mood; increased energy; needing less sleep; having rapid thoughts; being full of ideas; having an increase in productivity, motivation, creativity or impulsive behavior; phoning or working or working excessively or spending more money.**

IF NO TO ALL, CODE NO TO **D1b**. IF YES TO **ANY**, ASK:

b Is (s)he currently feeling 'up' or 'high' or 'hyper' or full of energy? NO YES

D2 a Has there **ever** been a time when (s)he was so grouchy or annoyed, that (s)he yelled or started fights with people outside his/her family? Has (s)he or others noticed that (s)he have been more grouchy than other kids, even when (s)he thought (s)he was right to act this way? NO YES

DO NOT CONSIDER TIMES WHEN THE PATIENT WAS INTOXICATED ON DRUGS OR ALCOHOL.

IF NO TO ALL, CODE NO TO **D2b**: IF **YES** TO ANY, ASK:

b Is (s)he currently feeling grouchy or annoyed? NO YES

➡

IS **D1a** or **D2a** CODED **YES?** NO YES

D3 IF **D1b** OR **D2b** = **YES:** EXPLORE **THE CURRENT** AND THE MOST SYMPTOMATIC **PAST** EPISODE, OTHERWISE
IF **D1b** AND **D2b** = **NO:** EXPLORE ONLY THE MOST SYMPTOMATIC **PAST** EPISODE

During the times when him/her felt high, full of energy, or irritable did him/her:

	Current Episode		Past Episode	
a Feel that (s)he could do things others couldn't do? Feel that (s)he is a very important person?	NO	YES	NO	YES

IF **YES** TO EITHER, CODE **YES**. IF YES, ASK FOR EXAMPLES.
THE EXAMPLES ARE CONSISTENT WITH A DELUSIONAL IDEA

Current Episode ☐ No ☐ Yes
Past Episode ☐ No ☐ Yes

		Current Episode		Past Episode	
b	Need less sleep (Did (s)he feel rested after only a few hours of sleep)?	NO	YES	NO	YES
c	Talk too much without stopping? Talk so fast that people couldn't understand or follow what (s)he was saying?	NO	YES	NO	YES
d	Have racing thoughts or too many thoughts switching quickly?	NO	YES	NO	YES
e	Get distracted very easily by little things?	NO	YES	NO	YES
f	Get much more involved in things than others or much more fidgety or restless?	NO	YES	NO	YES
g	Want to do fun things even if (s)he could get hurt doing them? Want to do things even though it could get him/her into trouble? (Like staying out late, skipping school, driving dangerously or spending too much money)? IF YES TO ANY, CODE YES	NO	YES	NO	YES
D3 SUMMARY:	WHEN RATING CURRENT EPISODE: IF D1b IS NO, ARE 4 OR MORE D3 ANSWERS CODED YES? IF D 1b IS YES, ARE 3 OR MORE D3 ANSWERS CODED YES? WHEN RATING PAST EPISODE: IF D1a IS NO, ARE 4 OR MORE D3 ANSWERS CODED YES? IF D1a IS YES, ARE 3 OR MORE D3 ANSWERS CODED YES? CODE YES ONLY IF THE ABOVE 3 OR 4 SYMPTOMS OCCURRED DURING THE SAME TIME PERIOD. RULE: ELATION/EXPANSIVENESS REQUIRES ONLY THREE D3 SYMPTOMS, WHILE IRRITABLE MOOD ALONE REQUIRES 4 OF THE D3 SYMPTOMS.	NO	YES	NO	YES
D4	What is the longest time these symptoms lasted?				
a)	3 days or less		❐		❐
b)	4 to 6 days		❐		❐
c)	7 days or more		❐		❐
D5	Was (s)he put in the hospital for these problems? IF YES, STOP HERE AND CIRCLE YES IN MANIC EPISODE FOR THAT TIME FRAME.	NO	YES	NO	YES
D6	Did these symptoms cause a lot of problems at home? At school? With friends? With other people? Or in some other important way? IF YES TO ANY, CODE YES	NO	YES	NO	YES

ARE **D3** SUMMARY AND **D5** AND **D6** CODED **YES**?

OR

ARE **D3** SUMMARY AND **D4c** AND **D6** CODED YES AND IS **D5** CODED **NO**?

SPECIFY IF THE EPISODE IS CURRENT AND / OR PAST.

NO	YES
MANIC EPISODE	
CURRENT	❒
PAST	❒

Is D3 SUMMARY CODED **YES** AND ARE **D5** AND **D6** CODED **NO** AND IS EITHER **D4b** OR **D4c** CODED **YES**?

OR

ARE **D3** SUMMARY AND **D4b** AND **D6** CODED **YES** AND IS **D5** CODED **NO**?

SPECIFY IF THE EPISODE IS CURRENT AND / OR PAST.

IF **YES** TO CURRENT MANIC EPISODE, THEN CODE CURRENT HYPOMANIC EPISODE AS **NO.**

IF **YES** TO PAST MANIC EPISODE, THEN CODE PAST HYPOMANIC EPISODE AS **NOT EXPLORED.**

HYPOMANIC EPISODE

CURRENT ❒ **NO**
❒ **YES**

PAST ❒ **NO**
❒ **YES**
❒ **NOT EXPLORED**

ARE **D3** SUMMARY AND **D4a** CODED **YES** AND IS **D5** CODED **NO**?

SPECIFY IF THE EPISODE IS CURRENT AND / OR PAST.

IF **YES** TO CURRENT MANIC EPISODE OR HYPOMANIC EPISODE,
THEN CODE CURRENT HYPOMANIC SYMPTOMS AS **NO.**

IF **YES** TO PAST MANIC EPISODE OR YES TO PAST HYPOMANIC EPISODE,
THEN CODE PAST HYPOMANIC SYMPTOMS AS **NOT EXPLORED.**

HYPOMANIC SYMPTOMS

CURRENT ❒ **NO**
❒ **YES**

PAST ❒ **NO**
❒ **YES**
❒ **NOT EXPLORED**

D7 **a)** IF MANIC EPISODE IS POSITIVE FOR EITHER CURRENT OR PAST ASK:
Did (s)he have 2 or more of these (manic) episodes lasting 7 days or more (**D4c**) in his/her lifetime (including the current episode if present)? NO YES

b) IF MANIC OR HYPOMANIC EPISODE IS POSITIVE FOR EITHER CURRENT OR PAST ASK:
Did (s)he have 2 or more of these (hypomanic) episodes lasting just 4 to 6 days (**D4b**) in his/her lifetime (including the current episode)? NO YES

c) IF THE PAST "HYPOMANIC SYMPTOMS" CATEGORY IS CODED POSITIVE ASK:
Did (s)he have (hypomanic) symptoms like these lasting only 1 to 3 days (**D4a**), 2 or more times in his/her lifetime, (including the current episode if present)? NO YES

E. PANIC DISORDER

(➡ MEANS : CIRCLE NO IN E5, E6 AND E7 SUMMARY AND SKIP TO F1)

E1	a	Has (s)he ever been really frightened or nervous for no reason; or has (s)he ever been really frightened or nervous in a situation where most kids would not feel that way? IF YES TO EITHER, CODE YES. IF NO TO ALL CODE NO.	➡ NO	YES
	b	Did this happen more than one time?	➡ NO	YES
	c	Did this nervous feeling increase quickly over the first few minutes?	➡ NO	YES
E2		Has this ever happened when (s)he didn't expect it?	➡ NO	YES
E3	a	After this happened, was (s)he afraid it would happen again or that something bad would happen as a result of these attacks? Did (s)he change what (s)he did because of these attacks? (e.g., going out only with someone, not wanting to leave his/her house, going to the doctor more frequently)?	NO	YES
	b	Did (s)he have these worries for a month or more?	NO	YES
		E3 SUMMARY: IF YES TO BOTH E3a AND E3b QUESTIONS, CODE YES	NO	YES
E4		**Think about the time (s)he was most frightened or nervous for no good reason:**		
	a	Did his/her heart beat fast or loud?	NO	YES
	b	Did (s)he sweat? Did his/her hands sweat a lot? IF YES TO EITHER, CODE YES	NO	YES
	c	Did his/her hands or body shake?	NO	YES
	d	Did (s)he have trouble breathing?	NO	YES
	e	Did (s)he feel like (s)he was choking? Did (s)he feel (s)he couldn't swallow? IF YES TO EITHER, CODE YES	NO	YES
	f	Did (s)he have pain or pressure in his/her chest?	NO	YES
	g	Did (s)he feel like throwing up? Did (s)he have an upset stomach? Did (s)he have diarrhea? IF YES TO ANY, CODE YES	NO	YES
	h	Did (s)he feel dizzy or faint?	NO	YES
	i	Did things around him/her feel strange or like they weren't real? Did (s)he feel or see things as if they were far away? Did (s)he feel outside of or cut off from his/her body? IF YES TO ANY, CODE YES	NO	YES
	j	Was (s)he afraid that (s)he was losing control?	NO	YES

Were (s)he afraid that (s)he were going crazy?
IF **YES** TO EITHER, CODE **YES**

k Was (s)he afraid that (s)he was dying? NO YES

l Did parts of his/her body tingle or go numb? NO YES

m Did (s)he feel hot or cold? NO YES

E5 ARE **BOTH E3 SUMMARY,** AND **4** OR MORE **E4** ANSWERS, CODED YES? NO YES
PANIC DISORDER LIFETIME

IF YES TO E5, SKIP TO E7

E6 IF **E5=NO,** ARE ANY E4 QUESTIONS CODED YES? NO YES
LIMITED SYMPTOM ATTACKS LIFETIME

THEN SKIP TO **F1.**

E7 a. **In the past month,** did (s)he have these problems more than one time? NO YES

IF NO, CIRCLE NO TO **E7 SUMMARY** AND MOVE TO F1.

For the past month:

b. Did (s)he worry that it would happen again? NO YES

c. Did (s)he worry that something bad would happen because of the attack? NO YES

d. Did anything change for him/her because of the attack? NO YES
(e.g., going out only with someone, not wanting to leave his/her house, going to the doctor more frequently)?

E7 SUMMARY: IF **YES** TO E7b or E7c or E7d, CODE **YES** NO YES
PANIC DISORDER CURRENT

F. AGORAPHOBIA

F1 Does (s)he feel anxious, scared, or uneasy in places or situations where (s)he might become really frightened; like being in a crowd, standing in a line (queue), when (s)he is all alone, or when crossing a bridge, or traveling in a bus, train or car?
IF YES TO ANY, CODE YES NO YES

IF **F1** = NO, CIRCLE NO IN **F2**.

F2 Is (s)he so afraid of these things that (s)he tries to stay away from them? Or (s)he can only do them if someone is with him/her? Or (s)he does them, but it's really hard for him/her?
IF YES TO ANY, CODE **YES**

NO YES

AGORAPHOBIA CURRENT

IS **F2** (CURRENT AGORAPHOBIA) CODED **NO**

AND

IS **E7** (CURRENT PANIC DISORDER) CODED **YES**?

NO YES

PANIC DISORDER without Agoraphobia CURRENT

IS **F2** (CURRENT AGORAPHOBIA) CODED **YES**

AND

IS **E7** (CURRENT PANIC DISORDER) CODED **YES**?

NO YES

PANIC DISORDER with Agoraphobia CURRENT

IS **F2** (CURRENT AGORAPHOBIA) CODED **YES**

AND

IS **E5** (PANIC DISORDER LIFETIME) CODED **NO**?

NO YES

AGORAPHOBIA, CURRENT without history of Panic Disorder

H. SOCIAL PHOBIA (Social Anxiety Disorder)

(➡ MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE NO AND MOVE TO THE NEXT MODULE)

H1	**In the past month**, was (s)he afraid or embarrassed when others his/her age were watching him/her? Was (s)he afraid of being teased? Like talking in front of the class? Or eating or writing in front of others? IF YES TO ANY, CODE YES	➡ NO	YES
H2	Is (s)he more afraid of these things than other kids his/her age?	➡ NO	YES
H3	Is (s)he so afraid of these things that (s)he tries to stay away from them? Or (s)he can only do them if someone is with him/her? Or (s)he does them but it's really hard for him/her?	➡ NO	YES
H4	Do these social fears have a big effect on his/her life? Do they cause problems when (s)he interacts with others or cause problems in his/her relationships? Do they cause a lot of problems at school or at work? Do they cause him/her to feel upset and want to be alone? IF YES TO ANY, CODE YES	➡ NO	YES
H5	Did this social fear / social anxiety last at least 6 months?		

SUBTYPES

Does (s)he fear and avoid 4 or more social situations?

If YES — Generalized social phobia (social anxiety disorder)

If NO — Non-generalized social phobia (social anxiety disorder)

NOTE TO INTERVIEWER: PLEASE ASSESS WHETHER THE SUBJECT'S FEARS ARE RESTRICTED TO NON-GENERALIZED ("ONLY 1 OR SEVERAL") SOCIAL SITUATIONS OR EXTEND TO GENERALIZED ("MOST") SOCIAL SITUATIONS. "MOST" SOCIAL SITUATIONS IS USUALLY OPERATIONALIZED TO MEAN 4 OR MORE SOCIAL SITUATIONS, ALTHOUGH THE DSM-IV DOES NOT EXPLICITLY STATE THIS.

EXAMPLES OF SUCH SOCIAL SITUATIONS TYPICALLY INCLUDE INITIATING OR MAINTAINING A CONVERSATION, PARTICIPATING IN SMALL GROUPS, DATING, SPEAKING TO AUTHORITY FIGURES, ATTENDING PARTIES, PUBLIC SPEAKING, EATING IN FRONT OF OTHERS, URINATING IN A PUBLIC WASHROOM, ETC.

NO **YES**

SOCIAL PHOBIA
(Social Anxiety Disorder)
CURRENT

GENERALIZED ❐
NON-GENERALIZED ❐

J. OBSESSIVE COMPULSIVE DISORDER

(➡ MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE **NO** AND MOVE TO THE NEXT MODULE)

J1	**In the past month,** has (s)he been bothered by bad things that come into his/her mind that (s)he couldn't get rid of? Like bad thoughts or urges? Or nasty pictures? For example, did (s)he think about hurting somebody even though it disturbs or distresses him/her? Was (s)he afraid (s)he or someone would get hurt because of some little thing (s)he did or didn't do? Did (s)he worry a lot about having dirt or germs on him/her? Did (s)he worry a lot that (s)he would give someone else germs or make them sick somehow? Or was (s)he afraid that (s)he would do something really shocking? IF **YES** TO ANY, CODE **YES** DO NOT INCLUDE SIMPLY EXCESSIVE WORRIES ABOUT REAL LIFE PROBLEMS. DO NOT INCLUDE OBSESSIONS DIRECTLY RELATED TO EATING DISORDERS, SEXUAL BEHAVIOR, OR ALCOHOL OR DRUG ABUSE BECAUSE THE PATIENT MAY DERIVE PLEASURE FROM THE ACTIVITY AND MAY WANT TO RESIST IT ONLY BECAUSE OF ITS NEGATIVE CONSEQUENCES	NO YES ➡ SKIP TO J4
J2	Did they keep coming back into his/her mind even when (s)he tried to ignore or get rid of them?	NO YES ➡ SKIP TO J4
J3	Does (s)he think that these things come from his/her own mind and that they are not from outside of his/her head?	NO YES obsessions
J4	**In the past month,** did (s)he do something over and over without being able to stop doing it, like washing over and over? Straightening things up over and over? Counting something or checking on something over and over? Saying or doing something over and over? compulsions IF **YES** TO ANY, CODE **YES**	NO YES
	IS **J3** OR **J4** CODED **YES**?	➡ NO YES
J5	Did (s)he have these thoughts or rituals we just spoke about, more than other kids his/her age?	➡ NO YES
J6	Did these thoughts or actions cause him/her to miss out on things at home? At school? With friends? Did they cause a lot of problems with other people? Did these things take more than one hour a day? IF YES TO ANY, CODE YES	**NO** **YES** ***O.C.D. CURRENT***

R. PSYCHOTIC DISORDERS AND MOOD DISORDERS WITH PSYCHOTIC FEATURES

(➡ MEANS : GO TO THE DIAGNOSTIC BOXES, CIRCLE **NO** IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

ASK FOR AN EXAMPLE OF EACH QUESTION ANSWERED POSITIVELY. CODE YES ONLY IF THE EXAMPLES CLEARLY SHOW A DISTORTION OF THOUGHT OR OF PERCEPTION OR IF THEY ARE NOT CULTURALLY APPROPRIATE. BEFORE CODING, INVESTIGATE WHETHER DELUSIONS QUALIFY AS "BIZARRE".

DELUSIONS ARE "BIZARRE" IF: CLEARLY IMPLAUSIBLE, ABSURD, NOT UNDERSTANDABLE, AND CANNOT DERIVE FROM ORDINARY LIFE EXPERIENCE.

HALLUCINATIONS ARE SCORED "BIZARRE" IF: A VOICE COMMENTS ON THE PERSON'S THOUGHTS OR BEHAVIOR, OR WHEN TWO OR MORE VOICES ARE CONVERSING WITH EACH OTHER.

				BIZARRE
		Now I am going to ask you about unusual experiences that some people have.		
R1	a	Has (s)he ever believed that people were secretly watching him/her? Has (s)he believed that someone was trying to get him/her, or to hurt him/her? IF YES TO ANY, CODE **YES** **NOTE:** ASK FOR EXAMPLES TO RULE OUT ACTUAL STALKING	NO YES	YES
	b	**IF YES OR YES BIZARRE:** Does (s)he believe this now?	NO YES	YES └R6
R2	a	Has (s)he ever believed that someone was reading his/her mind or that someone could hear his/her thoughts? Or that (s)he could actually read someone else's mind or hear what they were thinking? IF YES TO ANY, CODE **YES**	NO YES	YES
	b	**IF YES OR YES BIZARRE:** Does (s)he believe this now?	NO YES	YES └R6
R3	a	Has (s)he ever believed that someone or something put thoughts in his/her mind that were not his/her own? Has (s)he believed that someone or something made him/her act in a way that was not his/her usual self? Has (s)he ever felt that (s)he was possessed? IF YES TO ANY, CODE **YES** **NOTE:** ASK FOR EXAMPLES AND DISCOUNT ANY THAT ARE NOT PSYCHOTIC	NO YES	YES
	b	**IF YES OR YES BIZARRE:** Does (s)he believe this now?	NO YES	YES └R6
R4	a	Has (s)he ever believed that (s)he was being sent special messages through the TV, radio, internet, newspapers, books, magazines or through his/her games or toys? Has (s)he ever believed that a person (s)he did not personally know was especially interested in him/her? IF YES TO ANY, CODE **YES**	NO YES	YES
	b	**IF YES OR YES BIZARRE:** Does (s)he believe this now?	NO YES	YES └R6
R5	a	Have his/her family or friends ever thought that any of his/her beliefs were strange or weird? Please give me an example. INTERVIEWER:. ONLY CODE **YES** IF THE EXAMPLES ARE **CLEARLY** DELUSIONAL AND ARE NOT EXPLORED IN QUESTIONS R1 TO R4, FOR EXAMPLE, SOMATIC OR RELIGIOUS DELUSIONS OR DELUSIONS OF GRANDIOSITY, JEALOUSY GUILT, RUIN OR DESTITUTION, ETC.	NO YES	YES
	b	**IF YES OR YES BIZARRE:** Do they think that his/her beliefs are still strange?	NO YES	YES

R6 a Has (s)he ever heard things other people couldn't hear, such as voices? NO YES
[HALLUCINATIONS ARE SCORED "BIZARRE" ONLY IF PATIENT ANSWERS YES TO THE FOLLOWING]:

IF YES: Did (s)he hear a voice talking about him/her? Did (s)he hear more than one voice talking back and forth? NO YES

b **IF YES OR YES BIZARRE TO R6:** Has (s)he heard these things in the past month? NO YES
HALLUCINATIONS ARE SCORED "BIZARRE" ONLY IF PATIENT ANSWERS YES TO THE FOLLOWING:
Did (s)he hear a voice talking about him/her? Did (s)he hear more than one voice talking? YES →R8b

R7 a Has (s)he ever had visions or has (s)he ever seen things other people couldn't see? NO YES
NOTE: CHECK TO SEE IF THESE ARE CULTURALLY INAPPROPRIATE.

b **IF YES:** Has (s)he seen these things in the past month? NO YES

CLINICIAN'S JUDGMENT

R8 b IS THE PATIENT CURRENTLY EXHIBITING INCOHERENCE, DISORGANIZED SPEECH, OR MARKED LOOSENING OF ASSOCIATIONS? NO YES

R9 b IS THE PATIENT CURRENTLY EXHIBITING DISORGANIZED OR CATATONIC BEHAVIOR? NO YES

R10 b ARE NEGATIVE SYMPTOMS OF SCHIZOPHRENIA, E.G. SIGNIFICANT AFFECTIVE FLATTENING, POVERTY OF SPEECH (ALOGIA) OR AN INABILITY TO INITIATE OR PERSIST IN GOAL DIRECTED ACTIVITIES (AVOLITION), PROMINENT DURING THE INTERVIEW? NO YES

R11 a ARE 1 OR MORE « a » QUESTIONS FROM R1a TO R7a CODED **YES OR YES BIZARRE** AND IS EITHER:

MAJOR DEPRESSIVE EPISODE, (CURRENT OR RECURRENT)
OR
MANIC OR HYPOMANIC EPISODE, (CURRENT OR PAST) CODED **YES?** NO →R13 YES

IF NO TO R11 a, CIRCLE NO IN BOTH 'MOOD DISORDER WITH PSYCHOTIC FEATURES' DIAGNOSTIC BOXES AND MOVE TO R13.

b You told me earlier that (s)he had period(s) when (s)he felt (depressed/high/persistently irritable).

Did (s)he have the beliefs and experiences (s)he just described [GIVE EXAMPLES FROM SYMPTOMS CODED YES FROM R1a TO R7a] only when (s)he was feeling depressed? high? very moody? very irritable? TO

IF THE PATIENT EVER HAD A PERIOD OF AT LEAST 2 WEEKS OF HAVING THESE BELIEFS OR EXPERIENCES (PSYCHOTIC SYMPTOMS) WHEN THEY WERE NOT DEPRESSED/HIGH/IRRITABLE, CODE NO TO THIS DISORDER.

IF THE ANSWER IS NO TO THIS DISORDER, ALSO CIRCLE NO TO R12 AND MOVE TO R13

NO	YES
MOOD DISORDER WITH PSYCHOTIC FEATURES	
LIFETIME	

R12a ARE 1 OR MORE « b » QUESTIONS FROM R1b TO R7b CODED **YES OR YES BIZARRE** AND IS EITHER:

MAJOR DEPRESSIVE EPISODE, (CURRENT)
OR
MANIC OR HYPOMANIC EPISODE, (CURRENT) CODED **YES**?

IF THE ANSWER IS YES TO THIS DISORDER (LIFETIME OR CURRENT), CIRCLE NO TO R13 AND R14 AND MOVE TO THE NEXT MODULE.

NO **YES**

MOOD DISORDER WITH PSYCHOTIC FEATURES

CURRENT

R13 ARE 1 OR MORE « b » QUESTIONS FROM R1b TO R6b, CODED **YES BIZARRE**?

OR

ARE 2 OR MORE « b » QUESTIONS FROM R1b TO R10b, CODED **YES** (RATHER THAN **YES BIZARRE**)?

AND DID AT LEAST TWO OF THE PSYCHOTIC SYMPTOMS OCCUR DURING THE SAME 1 MONTH PERIOD?

NO **YES**

PSYCHOTIC DISORDER
CURRENT

R14 IS **R13** CODED **YES**

OR

ARE 1 OR MORE « a » QUESTIONS FROM R1a TO R6a, CODED **YES BIZARRE**?

OR

ARE 2 OR MORE « a » QUESTIONS FROM R1a TO R7a, CODED **YES** (RATHER THAN **YES BIZARRE**)?

AND DID AT LEAST TWO OF THE PSYCHOTIC SYMPTOMS OCCUR DURING THE SAME 1 MONTH PERIOD?

NO **YES**

PSYCHOTIC DISORDER

LIFETIME

U. GENERALIZED ANXIETY DISORDER

(➡ MEANS : GO TO END OF DISORDER, CIRCLE NO AND MOVE TO NEXT DISORDER)

U1	a	**For the past six months**, Has (s)he worried a lot or been nervous? Has (s)he been worried or nervous about several things, (like school, his/her health, or something bad happening)? Has (s)he been more worried than other kids his/her age? IF YES TO ANY, CODE YES	➡ NO	YES
	b	Does (s)he worry most days?	➡ NO	YES
		IS THE PATIENT'S ANXIETY RESTRICTED EXCLUSIVELY TO, OR BETTER EXPLAINED BY, ANY DISORDER PRIOR TO THIS POINT?	NO	➡ YES
U2		Does (s)he find it hard to stop worrying? Do the worries make it hard for him/her to pay attention to what (s)he is doing? IF YES TO EITHER, CODE YES	➡ NO	YES
U3		FOR THE FOLLOWING, CODE **NO** IF THE SYMPTOMS ARE CONFINED TO FEATURES OF ANY DISORDER EXPLORED PRIOR TO THIS POINT. **When (s)he is worried, Does (s)he , most of the time:**		
	a	Feel like (s)he can't sit still?	NO	YES
	b	Feel tense in his/her muscles?	NO	YES
	c	Feel tired, weak or exhausted easily?	NO	YES
	d	Have a hard time paying attention to what (s)he is doing? Does his/her mind go blank?	NO	YES
	e	Feel grouchy or annoyed?	NO	YES
	f	Have trouble sleeping ("trouble sleeping" means trouble falling asleep, waking up in the middle of the night, wakening up too early or sleeping too much)?	NO	YES
		ARE **1** OR MORE **U3** ANSWERS CODED **YES**?	➡ NO	YES
U4		Do these worries or anxieties cause a lot of problems at school or with his/her friends or at home or at work or with other people?		

NO	YES
GENERALIZED ANXIETY DISORDER	
CURRENT	

IF MARKED:

- A only, then code as Adjustment disorder with depressed mood. 309.0
- B only, then code as Adjustment disorder with anxious mood. 309.24
- C only, then code as Adjustment disorder of conduct. 309.3
- A and B only, then code as Adjustment disorder with mixed anxiety and depressed mood. 309.28
- C and (A or B), then code as Adjustment disorder of emotions and of conduct. 309.4
- D only, then code as Adjustment Disorder unspecified. 309.9
- C and D, then code as Adjustment disorder of conduct. 309.3
- B and D, then code as Adjustment disorder with anxious mood. 309.24
- B, C and D, then code as Adjustment disorder with anxious mood and of conduct. 309.24 / 309.3
- A and D, then code as Adjustment disorder with depressed mood. 309.0
- A, C and D, then code as Adjustment disorder with depressed mood and of conduct. 309.0 / 309.3
- A, B and D, then code as Adjustment disorder with mixed anxiety and depressed mood. 309.28
- A, B and C, then code as Adjustment disorder with mixed anxiety and depressed mood, and of conduct. 309.28 / 309.3
- A, B, C and D, then code as Adjustment disorder with mixed anxiety and depressed mood, and of conduct. 309.28 / 309.3

IF **V1** AND **V2** AND **(V3a or V3b)** ARE CODED **YES,** AND **V5** IS CODED **NO,** THEN CODE THE DISORDER **YES** WITH **SUBTYPES.**

IF **NO,** CODE **NO** TO ADJUSTMENT DISORDER.

NO	**N/A**	**YES**
Adjustment Disorder with_____ (see above for subtypes)		

W. RULE OUT MEDICAL, ORGANIC OR DRUG CAUSES FOR ALL DISORDERS

IF THE PATIENT CODES POSITIVE FOR ANY CURRENT DISORDER ASK:

Just before these symptoms began:

W1a Was (s)he taking any drugs or medicines? ❒ No ❒ Yes ❒ Uncertain

W1b Did (s)he have any medical illness? ❒ No ❒ Yes ❒ Uncertain

IN THE CLINICIAN'S JUDGMENT: ARE EITHER OF THESE LIKELY TO BE DIRECT CAUSES OF THE PATIENT'S DISORDER?
IF NECESSARY ASK ADDITIONAL OPEN-ENDED QUESTIONS.

W2 **SUMMARY:** HAS AN ORGANIC (MEDICAL/DRUG) CAUSE BEEN RULED OUT? ❒ No ❒ Yes ❒ Uncertain

B.10 The Alcohol Use Disorders Identification Test (AUDIT)

Box 4

The Alcohol Use Disorders Identification Test: Interview Version

Read questions as written. Record answers carefully. Begin the AUDIT by saying "Now I am going to ask you some questions about your use of alcoholic beverages during this past year." Explain what is meant by "alcoholic beverages" by using local examples of beer, wine, vodka, etc. Code answers in terms of "standard drinks". Place the correct answer number in the box at the right.

1. How often do you have a drink containing alcohol?
   (0) Never [Skip to Qs 9-10]
   (1) Monthly or less
   (2) 2 to 4 times a month
   (3) 2 to 3 times a week
   (4) 4 or more times a week

2. How many drinks containing alcohol do you have on a typical day when you are drinking?
   (0) 1 or 2
   (1) 3 or 4
   (2) 5 or 6
   (3) 7, 8, or 9
   (4) 10 or more

3. How often do you have six or more drinks on one occasion?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily
   Skip to Questions 9 and 10 if Total Score for Questions 2 and 3 = 0

4. How often during the last year have you found that you were not able to stop drinking once you had started?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

5. How often during the last year have you failed to do what was normally expected from you because of drinking?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

7. How often during the last year have you had a feeling of guilt or remorse after drinking?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

9. Have you or someone else been injured as a result of your drinking?
   (0) No
   (2) Yes, but not in the last year
   (4) Yes, during the last year

10. Has a relative or friend or a doctor or another health worker been concerned about your drinking or suggested you cut down?
    (0) No
    (2) Yes, but not in the last year
    (4) Yes, during the last year

Record total of specific items here

If total is greater than recommended cut-off, consult User's Manual.

B.11 Yale-Brown Obsessive Compulsive Scale (YBOCS)

PATIENT NAME _____ DOCTOR'S NAME _____

DATE _____ ADDRESS _____

YALE-BROWN OBSESSIVE COMPULSIVE SCALE (Y-BOCS)*

Questions 1 to 5 are about your obsessive thoughts.

Obsessions are unwanted ideas, images or impulses that intrude on thinking against your wishes and efforts to resist them. They usually involve themes of harm, risk and danger. Common obsessions are excessive fears of contamination; recurring doubts about danger; extreme concern with order, symmetry, or exactness; fear of losing important things.

Please answer each question by writing the appropriate number in the box next to it.

1. TIME OCCUPIED BY OBSESSIVE THOUGHTS

Q. How much of your time is occupied by obsessive thoughts?

0 = None.
1 = Less than 1 hr/day or occasional occurrence.
2 = 1 to 3 hrs/day or frequent.
3 = Greater than 3 and up to 8 hrs/day or very frequent occurrence.
4 = Greater than 8 hrs/day or nearly constant occurrence.

2. INTERFERENCE DUE TO OBSESSIVE THOUGHTS

Q. How much do your obsessive thoughts interfere with your work, school, social, or other important role functioning? Is there anything that you don't do because of them?

0 = None.
1 = Slight interference with social or other activities, but overall performance not impaired.
2 = Definite interference with social or occupational performance, but still manageable.
3 = Causes substantial impairment in social or occupational performance.
4 = Incapacitating.

3. DISTRESS ASSOCIATED WITH OBSESSIVE THOUGHTS

Q. How much distress do your obsessive thoughts cause you?

0 = None.
1 = Not too disturbing.
2 = Disturbing, but still manageable.
3 = Very disturbing.
4 = Near constant and disabling distress.

4. RESISTANCE AGAINST OBSESSIONS

Q. How much of an effort do you make to resist the obsessive thoughts? How often do you try to disregard or turn your attention away from these thoughts as they enter your mind?

0 = Try to resist all the time.
1 = Try to resist most of the time.
2 = Make some effort to resist.
3 = Yield to all obsessions without attempting to control them, but with some reluctance.
4 = Completely and willingly yield to all obsessions.

5. DEGREE OF CONTROL OVER OBSESSIVE THOUGHTS

Q. How much control do you have over your obsessive thoughts? How successful are you in stopping or diverting your obsessive thinking? Can you dismiss them?

0 = Complete control.
1 = Usually able to stop or divert obsessions with some effort and concentration.
2 = Sometimes able to stop or divert obsessions.
3 = Rarely successful in stopping or dismissing obsessions, can only divert attention with difficulty.
4 = Obsessions are completely involuntary, rarely able to even momentarily alter obsessive thinking.

*This adaptation of the Y-BOCS is abridged from the original version with permission from Wayne Goodman. For additional information on the Y-BOCS, please contact Dr. Wayne Goodman at the University of Florida, College of Medicine, Gainesville, Florida 32610. The original version was published by: Goodman WK, Price LH, Rasmussen SA, et al. The Yale-Brown Obsessive Compulsive Scale I: Development, use, and reliability. *Arch Gen Psychiatry*. 1989;46:1006-1011.

The next several questions are about your compulsive behaviors.

Compulsions are urges that people have to do something to lessen feelings of anxiety or other discomfort. Often they do repetitive, purposeful, intentional behaviors called rituals. The behavior itself may seem appropriate but it becomes a ritual when done to excess. Washing, checking, repeating, straightening, hoarding and many other behaviors can be rituals. Some rituals are mental. For example thinking or saying things over and over under your breath.

6. TIME SPENT PERFORMING COMPULSIVE BEHAVIORS

Q. How much time do you spend performing compulsive behaviors? How much longer than most people does it take to complete routine activities because of your rituals? How frequently do you do rituals?

0 = None.
1 = Less than 1 hr/day, or occasional performance of compulsive behaviors.
2 = From 1 to 3 hrs/day, or frequent performance of compulsive behaviors.
3 = More than 3 and up to 8 hrs/day, or very frequent performance of compulsive behaviors.
4 = More than 8 hrs/day, or near constant performance of compulsive behaviors (too numerous to count).

7. INTERFERENCE DUE TO COMPULSIVE BEHAVIORS

Q. How much do your compulsive behaviors interfere with your work, school, social, or other important role functioning? Is there anything that you don't do because of the compulsions?

0 = None.
1 = Slight interference with social or other activities, but overall performance not impaired.
2 = Definite interference with social or occupational performance, but still manageable.
3 = Causes substantial impairment in social or occupational performance.
4 = Incapacitating.

8. DISTRESS ASSOCIATED WITH COMPULSIVE BEHAVIOR

Q. How would you feel if prevented from performing your compulsion(s)? How anxious would you become?

0 = None.
1 = Only slightly anxious if compulsions prevented.
2 = Anxiety would mount but remain manageable if compulsions prevented.
3 = Prominent and very disturbing increase in anxiety if compulsions interrupted.
4 = Incapacitating anxiety from any intervention aimed at modifying activity.

9. RESISTANCE AGAINST COMPULSIONS

Q. How much of an effort do you make to resist the compulsions?

0 = Always try to resist.
1 = Try to resist most of the time.
2 = Make some effort to resist.
3 = Yield to almost all compulsions without attempting to control them, but with some reluctance.
4 = Completely and willingly yield to all compulsions.

10. DEGREE OF CONTROL OVER COMPULSIVE BEHAVIOR

Q. How strong is the drive to perform the compulsive behavior? How much control do you have over the compulsions?

0 = Complete control.
1 = Pressure to perform the behavior but usually able to exercise voluntary control over it.
2 = Strong pressure to perform behavior, can control it only with difficulty.
3 = Very strong drive to perform behavior, must be carried to completion, can only delay with difficulty.
4 = Drive to perform behavior experienced as completely involuntary and overpowering, rarely able to even momentarily delay activity.

Total Score

B.12 Montgomery-Asberg Depression Rating Scale (MADRS)

1. Apparent sadness Representing despondency, gloom and despair (more than just ordinary transient low spirits), reflected in speech, facial expression, and posture. Rate by depth and inability to brighten up.	
0. = No sadness.	☐
2. = Looks dispirited but does brighten up without difficulty.	☐
4. = Appears sad and unhappy most of the time.	☐
6. = Looks miserable all the time. Extremely despondent.	☐

2. Reported sadness Representing reports of depressed mood, regardless of whether it is reflected in appearance or not. Includes low spirits, despondency or the feeling of being beyond help and without hope.	
0. = Occasional sadness in keeping with the circumstances.	☐
2. = Sad or low but brightens up without difficulty.	☐
4. = Pervasive feelings of sadness or gloominess. The mood is still influenced by external circumstances.	☐
6. = Continuous or unvarying sadness, misery or despondency.	☐

3. Inner tension Representing feelings or ill-defined discomfort, edginess, inner turmoil, mental tension mounting to either panic, dread or anguish. Rate according to intensity, frequency, duration and the extent of reassurance called for.	
0. = Placid. Only fleeting inner tension.	☐
2. = Occasional feelings of edginess and ill-defined discomfort.	☐
4. = Continuous feelings of inner tension or intermittent panic which the patient can only master with some difficulty.	☐
6. = Unrelenting dread or anguish. Overwhelming panic.	☐

4. Reduced sleep Representing the experience of reduced duration or depth of sleep compared to the subject's own normal pattern when well.	
0. = Sleeps as usual.	☐
2. = Slight difficulty dropping off to sleep or slightly reduced, light or fitful sleep.	☐
4. = Sleep reduced or broken by at least 2 hours.	☐
6. = Less than 2 or 3 hours sleep.	☐

5. Reduced appetite Representing the feeling of a loss of appetite compared with when well. Rate by loss of desire for food or the need to force oneself to eat.	
0. = Normal or increased appetite.	☐
2. = Slightly reduced appetite.	☐
4. = No appetite. Food is tasteless.	☐
6. = Needs persuasion to eat at all.	☐

6. Concentration difficulties Representing difficulties in collecting one's thoughts mounting to an incapacitating lack of concentration. Rate according to intensity, frequency, and degree of incapacity produced.	
0. = No difficulties in concentrating.	☐
2. = Occasional difficulties in collecting one's thoughts.	☐
4. = Difficulties in concentrating and sustaining thought which reduces ability to read or hold a conversation.	☐
6. = Unable to read or converse without great difficulty.	☐

7. Lassitude Representing difficulty in getting started or slowness in initiating and performing everyday activities.	
0. = Hardly any difficulty in getting started. No sluggishness.	☐
2. = Difficulties in starting activities.	☐
4 = Difficulties in starting simple routine activities, which are carried out with effort.	☐
6. = Complete lassitude. Unable to do anything without help.	☐

8. Inability to feel Representing the subjective experience of reduced interest in the surroundings, or activities that normally give pleasure. The ability to react with adequate emotion to circumstances or people is reduced.	
0. = Normal interest in the surroundings and in other people.	☐
2. = Reduced ability to enjoy usual interests.	☐
4. = Loss of interest in the surroundings. Loss of feelings for friends and acquaintances.	☐
6. = The experience of being emotionally paralysed, inability to feel anger, grief or pleasure and a complete or even painful failure to feel for close relatives and friends.	☐

9. Pessimistic thoughts Representing thoughts of guilt, inferiority, self-reproach, sinfulness, remorse and ruin.	
0. = No pessimistic thoughts.	☐
2. = Fluctuating ideas of failure, self-reproach or self-depreciation.	☐
4. = Persistent self-accusation, or definite but still rational ideas of guilt or sin. Increasingly pessimistic about the future.	☐
6. = Delusions of ruin, remorse or irredeemable sin. Self-accusations, which are absurd and unshakable.	☐

10. Suicidal thoughts Representing the feeling that life is not worth living, that a natural death would be welcome, suicidal thoughts, and preparations for suicide. Suicide attempts should not in themselves influence the rating.	
0. = Enjoys life or takes it as it comes.	☐
2. = Weary of life. Only fleeting suicidal thoughts.	☐
4. = Probably better off dead. Suicidal thoughts are common, and suicide is considered as a possible solution, but without specific plans or intension.	☐
6. = Explicit plans for suicide when there is an opportunity. Active preparations for suicide.	☐

B.13 Unified Myoclonus Rating Scale (UMRS)

The Unified Myoclonus Rating Scale

Videotape Instruction for the Unified Myoclonus Rating Scale

The following comments serve as general guidelines for obtaining videotapes of the performance of the Unified Myoclonus Rating Scale.

SPACE AND EQUIPMENT

Use a high-8 camcorder mounted on a tripod for recording. Avoid taping in front of a windrow or in poor lighting. It is recommended to play back a sample tape in a high-8 tape deck, as the quality of the image in the viewfinder of the camera is sometimes better than the recorded image. Use a room large enough to record the patient walking 15 feet. Alternatively, the tripod can be moved into a hallway for taping the patient's gait. Avoid including any identifying information in the tape that would reveal the patient's identity, date, or location. Always tape patients in the same room on each visit.

PROCEDURES

Ask the patient to sign a standard consent form at taping, giving permission to use the tape and the information contained in it for research, including publication in scientific journals. Patients will not be identified by name. The patient's name, diagnosis, medications, and date of taping can be filmed for several seconds to permanently record the information on the tape (this will later be edited out).
All recordings should have the whole body in the picture unless otherwise specified. Position the tripod approximately 10 feet from the patient.
Patients should be videotaped wearing a standard hospital gown, which allows visualization of their arms and legs. Patients will sit in a hard-backed chair, lacking arm supports if possible. For performance of the functional tests, a portable table of comfortable height should be used. Plastic, clear glasses with an easily visible 8-ounce mark and a soupspoon should be used.

Section 2: Myoclonus at rest

A. Upper face

Frequency at rest	Amplitude at rest
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

B. Lower face

Frequency at rest	Amplitude at rest
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

C. Neck

Frequency at rest	Amplitude at rest
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

D. Trunk

Frequency at rest	Amplitude at rest
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

E. R arm

Frequency at rest	Amplitude at rest
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

F. L arm

Frequency at rest	Amplitude at rest
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

G. R leg

Frequency at rest	Amplitude at rest
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

H. L leg

Frequency at rest	Amplitude at rest
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

Section 4: Myoclonus with action

A. Close eyelids

Frequency with action	Amplitude with action Ask the patient to close his eyes
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

B. Neck

Frequency with action	Amplitude with action Ask the patient to move his head in flexion-extension and side-to-side rotation
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

C. Trunk

Frequency with action	Amplitude with action Ask the patients to flex his trunk when sitting or lying down
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

MYOCLONUS WITH ACTION (CONTINUED)

D. R arm

Frequency with action	Amplitude with action Ask the patient to hold both arms forward with palms down for 10 seconds. Then ask the patient to extend both wrists for 10 seconds. Then perform the finger-to-nose test four times. Ask the patient to finish by leaving his finger on his nose for 10 seconds. Score the worst myoclonus seen on finger-to-nose testing.
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

E. L arm

Frequency with action	Amplitude with action Ask the patient to hold both arms forward with palms down for 10 seconds. Then ask the patient to extend both wrists for 10 seconds. Then perform the finger-to-nose test four times. Ask the patient to finish by leaving his finger on his nose for 10 seconds. Score the worst myoclonus seen on finger-to-nose testing.
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

F. R leg

Frequency with action	Amplitude with action Ask the patient to perform the heel-and-toe-to-shin test four times. Score the worst myoclonus seen.
❑ 0. No jerks	❑ 0. No jerks
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only (heel always remains on knee and shin)
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (heel leaves the shin at times but can complete the slide)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (heel is unable to complete the slide)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

G. L leg

Frequency with Action	Amplitude with action Ask the patient to perform the heel-and-toe-to-shin test four times. Score the worst myoclonus seen.
❑ 0. No jerks	❑ 0. No jerks
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only (heel always remains on knee and shin)
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (heel leaves the shin at times but can complete the slide)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (heel is unable to complete the slide)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

MYOCLONUS WITH ACTION (CONTINUED)

H. Arising

Frequency with action	Amplitude with action Ask the patient to arise from the chair without the use of his arms. If the patient cannot, ask them to arise using arm assist. If still unable, the examiner attempts to help the patient arise.
❑ 0. No jerks	❑ 0. Patient arises without difficulty
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Patient arises with slight difficulty but does not need arm assist
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Patient arises only by pushing off with his arms, or requires several trails to arise.
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Patient cannot arise without the help of the examiner.
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Patient cannot arise unless pulled to his feet by the examiner

I. Standing

Frequency with action	Amplitude with action Ask the patient to stand with his feel one foot apart. It necessary, the examiner helps the patient into a standing position. If the patient cannot stand unassisted, the examiner stands by the patient or supports the patient.
❑ 0. No jerks	❑ 0. No jerks
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement, does not interfere with standing
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, mildly interferes with standing
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks definitely interferes with ability to stand without assistance
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks prevent standing

J. Walking

Frequency with action	Amplitude with action Ask the patient to walk down a corridor for 15 seconds, turn, then walk back and sit down. Patients who are unsteady or at risk for falling will walk with the examiner at their side, holding one arm if necessary.
❑ 0. No jerks	❑ 0. Zero
❑ 1. ≤ 1 jerk per 10 seconds	❑ 1. Trace movement only
❑ 2. 2 or 3 jerks per 10 seconds	❑ 2. Small-amplitude jerks, easily visible (< 25% of possible maximum movement)
❑ 3. 4 to 9 jerks per 10 seconds	❑ 3. Moderate-amplitude jerks (25%-75% of possible maximum movement)
❑ 4. ≥ 10 jerks per 10 seconds	❑ 4. Large-amplitude jerks (near maximum movement)

Section 5: Functional Tests

A. Writing

Ask the patient to write "London, England," in script with their hand resting on the desk. Patients who do not write in script may print. Circle the hand used to write.

- ❑ 0 Normal
- ❑ 1 Mild sloppiness, but easily legible
- ❑ 2 Illegible
- ❑ 3 Cannot complete the words
- ❑ 4 Cannot hold the pen or keep the pen on the paper

B. R hand spiral

Ask the patient to complete the spiral connecting the dots with the right hand in one continuous motion. The hand remains off the desk during the task.

- ❑ 0 Normal
- ❑ 1 Completes the spiral, but crosses the lines ≤ 2 times
- ❑ 2 Completes the spiral, but crosses the lines 3-10 times
- ❑ 3 Completes the spiral, but crosses the lines > 10 times
- ❑ 4 Cannot complete the spiral, or cannot hold the pen or keep it on the paper

C. L hand spiral

Ask the patient to complete the spiral connecting the dots with the left hand in one continuous motion. The hand remains off the desk during the task.

- ❑ 0 Normal
- ❑ 1 Completes the spiral, but crosses the lines ≤ 2 times
- ❑ 2 Completes the spiral, but crosses the lines 3-10 times
- ❑ 3 Completes the spiral, but crosses the lines > 10 times
- ❑ 4 Cannot complete the spiral, or cannot hold the pen or keep it on the paper

D. Pouring water

Ask the patient to pour an eight-ounce glass of water into an empty eight-ounce glass with his dominant hand, without touching the two glasses to each other. Use clear plastic glasses.

- ❑ 0 Normal, no spill
- ❑ 1 Clumsy, but does not spill
- ❑ 2 Spills less than half of the water
- ❑ 3 Spills at least half of the water
- ❑ 4 Cannot hold the glass or refuses to try secondary to fear of spilling water

E. Soupspoon

Ask the patient to use a soupspoon to bring water from a cup to his mouth with his dominant hand.

- ❑ 0 Normal, no spill
- ❑ 1 Clumsy, but does not spill
- ❑ 2 Spoon reaches the mouth but spills at least some water
- ❑ 3 Cannot reach his mouth with the spoon
- ❑ 4 Cannot hold the spoon or refuses to try secondary to inability to hold the spoon

Section 6: Score Global Disability

- ❑ 0. Normal
- ❑ 1. Mild disability; completely independent
- ❑ 2. Moderate disability; depends on others for moderate assistance
- ❑ 3. Marked disability; many tasks impossible even with assistance
- ❑ 4. Severe disability; invalid

Section 7: Is Negative Myoclonus Present?

- ❑ 0. No (less than 50% likely)
- ❑ 1. Yes (more than 50% likely)

Section 8: Severity of Negative Myoclonus

- ❑ 0. Not present
- ❑ 1. Mild
- ❑ 2. Moderate
- ❑ 3. Severe

B.14 Burke-Fahn Marsden Dystonia Rating Scale (BFMDRS)

Dystonia Rating Scale

Fahn Marsden rating factors

Factor/area/rating	Criteria
I. Provoking factor	
General	
0	No dystonia at rest or with action
1	Dystonia only with particular action
2	Dystonia with many actions
3	Dystonia on action of distant part of body or intermittently at rest
4	Dystonia present at rest
Speech and swallowing	
1	Occasional, either or both
2	Frequent either
3	Frequent one and occasional other
4	Frequent both
II. Severity factor	
Eyes	
0	No dystonia
1	Slight: Occasional blinking
2	Mild. Frequent blinking without prolonged spasms of eye closure
3	Moderate. Prolonged spasms of eyelid closure, but eyes open most of the time
4	Severe. Prolonged spasms of eyelid closure, with eyes closed at least 30% of the time
Mouth	
0	No dystonia present
1	Slight. Occasional grimacing or other mouth movements (e.g., jaw opened or clenched; tongue movement
2	Mild. Movement present less than 50% of the time
3	Moderate dystonic movements or contractions present most of the time
4	Severe dystonic movements or contractions present most of the time

Speech and swallowing	
0	Normal
1	Slightly involved; speech easily understood or occasional choking
2	Some difficulty in understanding speech or frequent choking
3	Marked difficulty in understanding speech or inability to swallow firm foods
4	Complete or almost complete anarthria, or marked difficulty swallowing soft foods and liquids
Neck	
0	No dystonia present
1	Slight. Occasional pulling
2	Obvious torticollis, but mild
3	Moderate pulling
4	Extreme pulling
Arm	
0	No dystonia present
1	Slight dystonia. Clinically insignificant
2	Mild: Obvious dystonia, but not disabling
3	Moderate. Able to grasp, with some manual function
4	Severe. No useful grasp
Trunk	
0	No dystonia present
1	Slight bending; clinically insignificant
2	Definite bending, but not interfering with standing or walking
3	Moderate bending; interfering with standing or walking
4	Extreme bending of trunk preventing standing or walking
Leg	
0	No dystonia present
1	Slight dystonia, but not causing impairment; clinically insignificant
2	Mild dystonia. Walks briskly and unaided
3	Moderate dystonia. Severely impairs walking or requires assistance
4	Severe. Unable to stand or walk on involved leg

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