Clinical and molecular genetic findings in autosomal dominant *OPA3*-related optic neuropathy

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31   **ABSTRACT**

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33   Leber hereditary optic neuropathy and autosomal dominant optic atrophy are the two most

34   common inherited optic neuropathies. The latter has been associated with mutations in the

35   *OPA1* and *OPA3* genes. To date, only six families with *OPA3*-associated dominant optic

36   atrophy have been reported. In order to identify additional families we performed Sanger

37   sequencing of the OPA3 gene in 75 unrelated optic neuropathy patients. Affected individuals

38   from two families were found to harbour the c.313C>G, p.(Gln105Glu) change in

39   heterozygous state; this genetic defect has been previously reported in four dominant optic

40   atrophy families. Intra- and inter-familial variability in age of onset and presenting symptoms

41   was observed. Although dominant *OPA3* mutations are typically associated with optic

42   atrophy and cataracts, the former can be observed in isolation; we report a case with no lens

43   opacities at age 38. Conversely, it is important to consider *OPA3*-related disease in

44   individuals with bilateral infantile onset cataracts and to assess optic nerve health in those

45   whose vision fail to improve following lens surgery. The papillomacular bundle is primarily

46   affected and vision is typically worse than 20/40. Notably, we describe one subject who

47   retained normal acuities into the fifth decade of life. The condition can be associated with

48   extraocular clinical features: two affected individuals in the present study had sensorineural

49   hearing loss. The clinical heterogeneity observed in the individuals reported here (all having

50   the same genetic defect in *OPA3*) suggests that the molecular pathology of the disorder is

51   likely to be complex.

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55   **KEYWORDS**

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57   OPA3; optic atrophy; inherited optic neuropathy; 3-methylglutaconic aciduria type III;

58   congenital cataract; genetic ophthalmology

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INTRODUCTION

Inherited optic neuropathies are a clinically and genetically heterogenous group of disorders associated with selective loss of retinal ganglion cells. Clinically, they are characterised by colour vision deficits, visual field defects and, typically, bilateral, symmetrical and irreversible visual loss [1]. Mitochondrial dysfunction appears to play a central role in the pathophysiology of these disorders and inheritance can be mitochondrial [MIM #535000] or monogenic; autosomal dominant (for example [MIM #165500] and [MIM #165300]), autosomal recessive (for example [MIM #612989]) and X-linked [MIM %311050] subtypes have been described. Notably, certain types of inherited optic neuropathy form part of clinical syndromes that include additional ocular or non-ocular features [2].

Defects in the OPA3 gene [MIM *606580] have been previously associated with both recessive and dominant optic neuropathy. Biallelic *OPA3* mutations cause 3-methylglutaconic aciduria type III [MIM #258501], a recessive neuro-ophthalmological syndrome, most prevalent amongst individuals of Iraqi-Jewish origin and classically characterized by the following triad: (i) bilateral optic atrophy diagnosed in the first decade of life; (ii) a movement disorder (ataxia or extrapyramidal dysfunction) of variable severity beginning in the first or second decade of life; (iii) increased urinary excretion of 3-methylglutaconic acid [3-8]. Disease-causing *OPA3* variants inherited in an autosomal dominant fashion can also cause optic neuropathy. This is often associated with congenital/infantile lenticular opacities; hearing loss and neurological symptoms can also be features of the disorder. Autosomal dominant *OPA3*-related disease is less common than the recessive form and only six families have been identified to date. The following dominant disease-associated variants have been reported [NCBI Reference Sequence: NM_025136.3]: c.277G>A, p.(Gly93Ser); c.313C>G, p.(Gln105Glu) (recurrent mutation); c.10_11insCGCCCG, p.(Val3_Gly4insAlaPro) [9,10].

The OPA3 gene is composed of at least 3 exons that are alternatively spliced to produce two major transcripts: *OPA3A* (exon 1 plus exon 2a; encodes the 179 amino acid isoform b; NCBI Reference Sequence: NM_025136.3) and *OPA3B* (exon 1 plus exon2b; encodes the 180 amino acid isoform a; NCBI Reference Sequence: NM_001017989.2). Although cDNA studies indicate ubiquitous expression of both transcripts, *OPA3A* is much more strongly expressed in most tissues, including the brain [11]. Notably, the OPA3A amino acid sequence

94   appears to be more conserved in evolution and yet no human disease has been associated with

95   mutations in the *OPA3B*-specific exon 2b [7,11]. At the subcellular level, OPA3 localises

96   predominantly to the mitochondrial inner membrane and although its function remains

97   unclear previous studies have suggested involvement in the regulation of mitochondrial

98   morphology [10,12].

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100   In the present study we report clinical and genetic findings in two families with dominant

101   *OPA3*-related optic neuropathy. The phenotypic spectrum of the disorder is broadened and

102   intra- and inter-familial variability is discussed.

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105   **MATERIALS & METHODS**

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107   Initially, 74 unrelated individuals with a presumed diagnosis of inherited optic neuropathy

108   (mean age 43 years; range 24 to 66 years) were tested for *OPA3* mutations using Sanger

109   sequencing of the three exons and flanking intron-exon boundaries of the *OPA3* gene

110   (primers and conditions available on request). All these subjects were previously screened

111   and excluded for: (i) defects in the *OPA1* gene ([MIM *****605290]; the major cause of

112   dominant optic neuropathy [2,13]) and (ii) the three primary mitochondrial mutations

113   associated with Leber hereditary optic neuropathy ([MIM #535000]; m.11778G>A,

114   m.14484T>C and m.3460G>A). Subsequently, members of an additional family with

115   suspected dominant optic atrophy and cataracts were recruited and tested for *OPA3* mutations

116   in a similar fashion.

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118   Clinical assessment of individuals with *OPA3*-related disease included detailed history, best

119   corrected Logarithm of the Minimum Angle of Resolution (logMAR) visual acuity, dilated

120   fundus examination and optic disc imaging. Optical coherence tomographs of the optic nerve

121   head were obtained with the spectral-domain Cirrus platform (Carl Zeiss Meditec, Dublin,

122   CA, USA) in two cases. An audiogram was performed in three patients. Informed consent

123   was obtained from all participants and all investigations were conducted in accordance with

124   the principles of the Declaration of Helsinki. Institutional Review Board (IRB)/Ethics

125   Committee approval was obtained from the Multicentre Research Ethics Committee

126   (MREC).

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RESULTS

Three *OPA3* coding variants that have not been previously reported in publicly available databases (1000 genomes project database, National Heart, Lung, and Blood Institute Exome Sequencing Project or NHLBI ESP, dbSNP Build 139, accessed Jun 2014) were identified in the 180 amino acid isoform a (OPA3B; [NCBI Reference Sequence: NM_001017989.2]: c.227C>T, p.(Ala76Val); c.379G>A, p.(Gly127Ser) and c.389G>A, p.(Gly130Glu) (Supplementary Table S1). Each of these changes was detected in the heterozygous state in a simplex sporadic case and it was not possible to perform segregation analysis to support their pathogenicity. Also, none of these alters the amino acid sequence of isoform b (OPA3A) and wWe therefore, consider them to be variants of unknown significance.

In addition to these variants, a heterozygous c.313C>G, p.(Gln105Glu) change (only affecting *OPA3A*, the transcript encoding the 179 amino acid isoform b; [NCBI Reference Sequence: NM_025136.3]) was identified in an individual with a diagnosis of dominant optic atrophy (subject C1; Figure 1A). This sequence alteration is the most common *OPA3* mutation associated with dominant disease as it was previously identified in four families segregating optic atrophy [9,10]; therefore no further evidence was needed to confirm that this is a functional variant. The proband as well as his affected sister (subject C2) and daughter (subject C3) presented in the first years of life with nystagmus and abnormal optic disc appearance. The clinical findings are detailed in Table 1.

The same disease-associated variant, c.313C>G, p.(Gln105Glu), was detected in all three affected members of a family (Figure 1B) that were recruited and tested for *OPA3* mutations at a later time. Colour disc images and clinical findings are presented in Figure 1C and Table 1 respectively.

DISCUSSION

Dominant *OPA3*-related disease is a clinically heterogeneous disorder (Table 1); some patients present with poor visual behaviour and nystagmus from birth (for example subject

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162 C1) while others remain asymptomatic until later in life (for example subject M2). The optic
163 neuropathy is characterised by primary involvement of the papillomacular bundle and has
164 similarities with that observed in individuals with *OPA1* mutations [14]. Age of onset is in
165 the first two decades of life (Table 1) and, although comprehensive longitudinal data are
166 lacking, patients typically experience a slowly progressive, symmetrical decrease in vision
167 [9,10]. Visual acuity is usually worse than 0.3 logMAR and it is of interest that one subject in
168 the present series had preserved acuity and no visual complaints at age 41 (subject M2; 0.1
169 logMAR right, 0.2 logMAR left; Table 1 and Figure 1D). Previous reports have shown that
170 peripheral visual fields are spared and that colour vision is impaired [9,10]. Nevertheless, loss
171 of colour discrimination is variable and without a systemic axis [10]. Subject M3 reported
172 dyschromatopsia only in the right eye, in keeping with findings from colour vision testing
173 with the Ishihara plates (right eye 4 of 17 plates; left eye 16 of 17 plates).

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175 On fundus examination, temporal optic disc pallor was the most common finding in the
176 present cohort although diffuse pallor involving the whole neuroretinal rim and optic disc
177 excavation are not unusual [9,10]. Notably, subject M1 was referred to the clinic after a
178 routine eye test at age 60 revealed an enlarged cup-to-disc ratio (0.8) in the left eye (Figure
179 1C); an erroneous initial diagnosis of glaucoma was made as consideration was not given to
180 the fact that she had infantile-onset cataracts operated at age 35. Furthermore, subject M4 had
181 a healthy optic disc appearance in the right eye and only subtle pallor in the left eye at age 14.
182 In such equivocal cases measurement of retinal nerve fibre layer thickness with optical
183 coherence tomography can be helpful (for example Figure 1D). Future imaging studies in
184 patients with *OPA3*-related disease are expected to provide further insights.

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186 Crystalline lens opacities (i.e. cataracts) are observed in most affected individuals [9,10].
187 Nevertheless, a patient with a heterozygous p.(Val3_Gly4ins2) mutation and no cataracts at
188 age 52 has been reported by Grau and colleagues [10]. Only one of the seven patients
189 reported here had no clinical history of cataract: subject C3 was examined at age 38 and no
190 lens opacity was detected. It is worth highlighting that a number of patients with *OPA3*-
191 related disease presented with bilateral lens opacities at birth or in early childhood (including
192 subject M4 who was only noted to have optic atrophy after lens extraction at age 15);
193 therefore, *OPA3* mutations should be in the differential in cases of bilateral infantile-onset
194 cataract and it is important to assess optic nerve function and health before concluding about
195 the likely amblyogenicity of the lens opacities.

Ocular *OPA3*-related disease often occurs in parallel with extraocular clinical features. Reynier and colleagues reported affected members of a family with a heterozygous p.(Gly93Ser) change in *OPA3* to have mild neurological signs including spasticity and extrapyramidal dysfunction [9]. Subsequently, Grau and colleagues reported four individuals with a heterozygous p.(Val3_Gly4ins2) change to have hearing loss. Expression analysis of OPA3 in murine cochlear tissue was consistent with the notion that auditory neuropathy can be an extraocular feature of *OPA3*-related disease [10]. Notably, two affected individuals from the present cohort had sensorineural hearing loss (Table 1); hearing impairment has been previously described in a patient with the same genetic defect [10]. Clinicians should be vigilant to the development of such a complication and audiograms should be performed in all patients with *OPA3*-related disease at least once.

All *OPA3* families with dominant disease reported to date have affected individuals in two or more generations and disease-associated variants appear to be highly penetrant (Figure 1A-B, [9,10]). The p.(Gln105Glu) change identified in both families reported here has been previously shown to be a recurrent mutation [10]; it was not possible to perform haplotype analysis in subjects from the present cohort and thus, the possibility of a recent common ancestor in the two families cannot be excluded. The p.(Gln105Glu) change affects an amino acid in a predicted coiled-coil structure (Uniprot) but it is unclear how this common cause of *OPA3*-related disease affects mitochondrial function and/or structure.

Findings from this and other studies [9,10] suggest that dominant *OPA3*-related disease is associated with significant intra- and inter-familial phenotypic variability. Any attempt to draw genotype-phenotype correlations would therefore be highly speculative and secondary genetic factors are likely to be the basis for the observed clinical heterogeneity; a similar conclusion has been reached for the much commoner *OPA1*-associated optic neuropathy [14]. Expansion of the phenotypic spectrum of *OPA3*-related disease has led to greater understanding of the condition and the risks of developing extraocular complications. However, it is still unclear what disease mechanism explains multi-system tissue involvement and future studies on the pathogenesis of the disorder are expected to provide important insights.

230 **ACKNOWLEDGEMENTS**

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TABLE

Table 1. Clinical and genetic findings in subjects with dominant *OPA3*-related disease reported here and elsewhere.

Subject (family ID)	Age; sex	VA (logMAR) right/left	Presentation/ onset	Lens	Heterozygous *OPA3* change identified	Other features
C1 (GCC1)	72; M	1.30/1.30	Nystagmus in infancy	Blue-dot cataracts diagnosed at age 2	p.(Gln105Glu)	-
C2 (GCC1)	62; F	1.00/1.00	Nystagmus in infancy	Cataracts operated at age 4	p.(Gln105Glu)	-
C3 (GCC1)	38; F	0.80/0.80	Nystagmus in infancy	No significant lens opacity at age 38	p.(Gln105Glu)	-
M1 (G43755)	65; F	0.72/1.04	Cataracts at age 34; re-referred as found by optometrist to have cupped left disc at age 60	Cataracts operated at age 34; bilateral aphakia	p.(Gln105Glu)	Right mild-moderate s/n hearing loss; left moderate-severe s/n hearing loss; bilateral ocular hypertension on topical treatment
M2 (G43755)	41; M	0.10/0.20	Mild cataracts at age 9; presently asymptomatic	Nuclear cataracts operated at age 35	p.(Gln105Glu)	Right and left mild-moderate s/n hearing loss
M3 (G43755)	36; F	0.60/0.30	Decrease in VA from age 19	Blue-dot cataracts operated at age 36&37	p.(Gln105Glu)	Normal audiogram; familial hypercholesterolaemia
M4 (G43755)	13; F	0.08/0.18	Cataracts at age 13	Lamellar cataracts operated at age 15	p.(Gln105Glu)	Congenital AV malformation in frontal lobe
III.3 (#1) [9]	38; F	0.70/0.70	Poor vision from infancy	Posterior cortical cataracts operated at age 51	p.(Gly93Ser)	Tremor of hands; extrapyramidal rigidity of upper limbs; absence of deep tendon reflexes
IV.1 (#1) [9]	15; F	0.30/0.30	Decreased VA before age 10	Posterior cortical cataracts operated at age 47&48	p.(Gly93Ser)	Mild postural tremor & mild rigidity of upper extremities
IV.2 (#1) [9]	57; F	0.60/0.50	Decreased VA before age 10	Anterior cortical cataracts operated at age 45&46	p.(Gly93Ser)	Postural tremor without extrapyramidal signs
V.1 (#1) [9]	29; F	0.70/0.52	Decrease in VA from age 12	Anterior cortical cataracts operated at age 25	p.(Gly93Ser)	Normal neurological examination
VI.1 (#1) [9]	4; F	0.15/0.15	Visual impairment investigated at age 3	Anterior & posterior cortical cataracts operated at age 4	p.(Gly93Ser)	Normal neurological examination
IV.6 (#1) [9]	50, F	NA	Visual impairment from infancy	Cataracts	p.(Gly93Ser)	Normal neurological examination
V.6 (#1) [9]	5; F	NA	Visual impairment from infancy	Cataracts operated at age 5	p.(Gly93Ser)	Normal neurological examination
III.3 (#2) [9]	37; F	1.70/1.70	Decrease in VA from age 12	Posterior capsular cataract diagnosed at age 56	p.(Gln105Glu)	-
III.7 (#2) [9]	49; F	"legally blind"	Decrease in VA from age 10	Cataracts diagnosed at age 45	p.(Gln105Glu)	-
IV.1 (#2) [9]	33; F	1.30/1.30	Decrease in VA from age 6	Cataracts diagnosed at age 10; cerulean cataracts at age 33	p.(Gln105Glu)	Normal neurological examination

IV.2 (#2) [9]	12; M	0.52/0.52	Decreased VA at age 12	Posterior capsular cataracts operated at age 19	p.(Gln105Glu)	-
II.2 (OAK1) [10]	57	1.00/1.00	Diagnosed at age 18	Monocular cataract	p.(Gln105Glu)	-
II.6 (OAK1) [10]	54	0.40/0.50	Onset in infancy	Cataracts	p.(Gln105Glu)	Hearing loss
III.1 (OAK1) [10]	35	0.52/0.30	Onset in infancy	No significant lens opacity at age 19	p.(Gln105Glu)	-
III.2 (OAK1) [10]	31	0.40/0.52	Onset in infancy	Cataracts	p.(Gln105Glu)	-
III.3 (OAK1) [10]	17	0.52/0.40	Onset in infancy	Cataracts	p.(Gln105Glu)	Chiari malformation type I
I.1 (OAK61)[10]	57	1.30/1.40	Diagnosed at age 10	No information	p.(Gln105Glu)	-
II.1 (OAK61)[10]	33	0.40/0.40	Diagnosed at age 19	No significant lens opacity at age 25	p.(Gln105Glu)	-
II.4 (OAK105)[10]	84	1.15/1.30	Onset in infancy	Congenital cataracts	p.(Val3_Gly4ins 2)	Hearing loss
III.2 (OAK105)[10]	61	0.30/0.40	Onset in adolescence	No significant lens opacity at age 52	p.(Val3_Gly4ins 2)	Hearing loss
III.3 (OAK105) [10]	62	0.52/0.40	Diagnosed at age 19	Cataracts	p.(Val3_Gly4ins 2)	Hearing loss
III.5 (OAK105) [10]	57	1.15/1.15	Onset in adolescence	Congenital cataracts	p.(Val3_Gly4ins 2)	Hearing loss
IV.1 (OAK105) [10]	32	1.30/1.30	Diagnosed at age 19	Congenital cataracts	p.(Val3_Gly4ins 2)	Morbus Scheuermann
III.1 (OAK255) [10]	46	NA	Onset in infancy	Cataracts	p.(Gln105Glu)	Intestinal pseudo-obstruction
III.2 (OAK255) [10]	47	NA	Onset in infancy	Cataracts	p.(Gln105Glu)	-

F, female; M, male; VA, visual acuity; logMAR, logarithm of the minimum angle of resolution (equivalent); BE, both eyes; NA, not available; s/n, sensorineural; AV, arteriovenous. The term cataracts is used to denote bilateral crystalline lens opacities. Variants are annotated according to the NCBI Reference Sequence NM_025136.3 (OPA3 isoform b).

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Figure 1.

A. Pedigree from a family segregating optic atrophy. Subjects C1 (IV:2), C2 (IV:5) and C3 (V:3) had a heterozygous *OPA3* change, c.313C>G, p.(Gln105Glu).

B. Pedigree from a family segregating optic atrophy and cataracts. DNA from subjects M1 (II:6), M2 (III:3) and M3 (III:4) was available for testing; a heterozygous c.313C>G, p.(Gln105Glu) change in *OPA3* was identified in all there patients. See text and Table 1 for clinical findings of all tested individuals as well as subject M4 (IV:2).

C. Right and left optic disc appearance of an individual with a confirmed *OPA3* mutation (subject M1) showing pallor of the neuroretinal rim, which is more marked temporally. Left optic nerve appears more affected than the right and has optic disc excavation in addition to the temporal pallor.

D. Left optic disc appearance and pattern of retinal nerve fibre layer (RNFL) thinning in an individual with a confirmed *OPA3* mutation (subject M2). Sparing of the nasal, superior and inferior peripapillary quadrants is observed. The RNFL profile for each eye is superimposed on the normal distribution percentiles. The normal distribution indices are colour-coded: (i) red <1%, (ii) yellow <5%, (iii) green <95%, and (iv) white >95%.