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OPA1 analysis in an international series of probands with bilateral optic atrophy

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ABSTRACT.

Purpose: **To determine the molecular genetic cause in previously unreported probands with optic atrophy from the United Kingdom, Czech Republic and Canada.**

Methods: ***OPA1*** **coding regions and flanking intronic sequences were screened by direct sequencing in 82 probands referred with a diagnosis of bilateral optic atrophy. Detected rare variants were assessed for pathogenicity by *in silico* analysis. Segregation of the identified variants was performed in available first degree relatives.**

Results: **A total of 29 heterozygous mutations evaluated as pathogenic were identified in 42 probands, of these seven were novel. In two probands, only variants of unknown significance were found. 76% of pathogenic mutations observed in 30 (71%) of 42 probands were evaluated to lead to unstable transcripts resulting in haploinsufficiency. Three probands with the following disease-causing mutations c.1230+1G>A, c.1367G>A and c.2965dup were documented to suffer from hearing loss and/or neurological impairment.**

Conclusions: ***OPA1*** **gene screening in patients with bilateral optic atrophy is an important part of clinical evaluation as it may establish correct clinical diagnosis. Our study expands the spectrum of *OPA1* mutations causing dominant optic atrophy and supports the fact that haploinsufficiency is the most common disease mechanism.**

Key words: DOA *plus* syndrome – dominant optic atrophy – haploinsufficiency – novel mutations – *OPA1*

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Introduction

Autosomal dominant optic atrophy (DOA, OMIM 165500) is the most common hereditary optic neuropathy with an estimated prevalence of 1:12 000–1:50 000 (Lyle 1990; Kjer et al. 1996). The disease is genetically heterogeneous, with five mapped loci (Votruba et al. 1997; Kerrison et al. 1999; Anikster et al. 2001; Barbet et al. 2005; Carelli et al. 2011). To date however, only two genes have been identified as disease causing: optic atrophy gene 1 (*OPA1*; MIM 165500) (Alexander et al. 2000; Delettre et al. 2000) and optic atrophy gene 3 (*OPA3*; MIM 606580) (Anikster et al. 2001; Reynier et al. 2004). *OPA1* is the most frequently mutated gene causing DOA accounting for up to 60% of cases with inherited optic atrophies (Yu-Wai-Man et al. 2009).

Autosomal dominant optic atrophy (DOA) shows highly variable expression, onset and progression. In most patients, the disease manifests by the end of the first decade of life. Typically, loss of central vision, optic nerve pallor, a centrocaecal scotoma and colour vision deficit are observed (Votruba et al. 1998; Rönnbäck et al. 2015). In some individuals, DOA *plus* phenotype is found, characterized by variable presence of sensorineural hearing loss, ataxia, axonal sensory-motor polyneuropathy, multiple sclerosis-like phenotype, chronic progressive external ophthalmoplegia and mitochondrial myopathy (Amati-Bonneau et al. 2009; Yu-Wai-Man et al. 2010; Liskova et al. 2013). Most recently association of *OPA1* mutations with a multisystem disorder characterized by

age-related parkinsonian features as well as cognitive deterioration has been described (Carelli et al. 2015).

The OPA1 protein is a mitochondrially targeted dynamin-related GTPase located on the inner mitochondrial membrane functioning mainly in mitochondrial fusion and regulation of apoptosis (Cho et al. 2010). Initial studies suggested that OPA1 dysfunction leads to primary retinal ganglion cell degeneration (Olichon et al. 2007); however, recent evidence proposes that more diverse mechanisms are implicated in the disease pathogenesis (Bertholet et al. 2013).

The aim of our study was to explore the involvement of *OPA1* mutations in a large cohort of probands with bilateral optic atrophy.

Material and Methods

Patients

In this study, we have included samples of 81 probands of white British, Canadian and Czech origin and one proband of Czech Roma origin referred with the diagnosis of bilateral optic atrophy to the All Wales Genetic Testing Service; University Hospital of Wales and General University Hospital in Prague for laboratory investigation. The study followed the tenets of the Declaration of Helsinki and was authorized by local Ethical boards. All investigated individuals signed informed consent.

Clinical referral notes of the patients indicated bilateral impairment of best corrected visual acuity, pallor of the optic nerve head, evidence of colour vision deficit and no evidence of other factors causing compressive or optic neuropathy. In some probands but not all clinical data included also family history for optic atrophy and results of neurological and audiometry examinations.

Molecular genetic analysis

Prior to the start of the study, participating probands were tested negative for the three most prevalent mitochondrial mutations associated with Leber hereditary optic neuropathy (Yu-Wai-Man et al. 2011). Positive family history of the disease was not a prerequisite for the initiation of *OPA1* molecular genetic testing.

Genomic DNA was extracted from venous blood samples using conventional protocols. PCR amplification and bidirectional Sanger sequencing of the *OPA1* coding regions and intron–exon boundaries were performed in probands as previously described (Thiselton et al. 2002). Primer sequences are listed in Table S1. DNA samples from available first degree relatives were also collected and used for targeted genetic testing of variants considered as potentially pathogenic.

Sequence variants were described as per the Human Genome Variation Society recommendations (den Dunnen & Antonarakis 2000), and with reference to NG_011605.1 and *OPA1* transcript variant 8 (NCBI Reference Sequence: NM_130837.2) containing two additional exons 4b and 5b compared to transcript variant 1 (NM_015560.2) maintaining however the same reading frame and encoding a protein of 1015 amino acids.

Interpretation of mutation pathogenicity

Frequency of the changes detected in this study was searched in the following population databases: The Exome Aggregation Consortium (ExAC) (http://exac.braoadinstitute.org) showing exome sequencing data from more than 60 000 unrelated individuals and The Exome Variant Server (EVS, NHLBI Exome Sequencing Project; http://evs.gs.washington.edu/EVS/), which includes data from more than 6000 individuals (both accessed 7 May 2016). Only rare variants (i.e. minor allele frequency ≤ 0.001) were further evaluated for potential pathogenicity. Identified sequence changes were further cross-referenced with published literature and the e*OPA1* mutational database (http://mitodyn.org, accessed December 2015).

As it has been convincingly documented that reduction in OPA1 protein levels is a disease mechanism in DOA (Marchbank et al. 2002; Schimpf et al. 2008; Fuhrmann et al. 2009) variants predicted to lead to an absence of the gene product due to the mRNA nonsense-mediated decay mechanism (e.g. nonsense or frameshifting mutations located 50–55 bp upstream from the last intron–exon junction) were considered as pathogenic.

The pathogenicity of missense variants was predicted using various algorithms; Sorting Intolerant From Tolerant (SIFT) (Kumar et al. 2009), Polymorphism Phenotyping v2 (PolyPhen-2) (Adzhubei et al. 2010), MutPred (Li et al. 2009), Mutation Taster (Schwarz et al. 2010), SNPs&GO (Calabrese et al. 2009), PhD-SNP (Capriotti et al. 2006), PROVEAN (Choi et al. 2012) and Panther (Capriotti et al. 2006). Novel *OPA1* missense variants with no entry in ExAC and EVS and predicted harmful by at least three tools were regarded as pathogenic. Heterozygous missense variants previously observed in DOA patients and not present in population databases were also considered pathogenic, regardless of their scoring by *in silico* algorithms.

As cDNA was not available to experimentally evaluate whether canonical (± 1 or 2), non-canonical splice variants and coding variants close to intron–exon boundaries (i.e. +1, +2 or −1, −2) affect pre-mRNA splicing, the wild-type and mutated sequences were analysed by splice site prediction tools Human Splicing Finder (Desmet et al. 2009), NNSPLICE (Reese et al. 1997), MaxEntScan (Yeo & Burge 2004) and NetGene2 (Brunak et al. 1991). Mutations leading to the disruption of splice site predicted by at least three of the four tools used were considered pathogenic. Conservation analysis of affected amino acid residues across 16 species was performed using T-coffee (Di Tommaso et al. 2011).

Sequence variants not segregating with the disease phenotype, that is not present in all affected members of a given family were considered benign. As penetrance of DOA maybe as low as 43% (Toomes et al. 2001), the presence of a mutation in clinically unaffected family members was not considered to be excluding its pathogenic nature.

Results

Summary of the study cohort demographics and rare variants identified is provided in Table 1. In total, 32 rare sequence changes in a heterozygous state were detected in *OPA1* coding region and intron–exon boundaries in 44 probands, of 82 tested. Pathogenicity scores of missense variants and effect predictions of variants potentially affecting splicing are provided in Tables S2, S3, respectively. All novel pathogenic variants were submitted to the *OPA1* locus specific database (http://opa1.mitodyn.org/) (Ferre et al. 2015).

Table 1. Disease-causing and rare *OPA1* variants of unknown clinical pathogenicity found in British, Czech and Canadian white probands, and one Roma proband with optic atrophy. Allele count in the ExAC database is shown as number of alleles found for with a given variant of the total number of alleles tested. In compliance with the HGVS nomenclature guidelines, no brackets in description of mutation at the protein level indicate that the effect on mRNA has been experimentally verified.

Proband no/origin	Family history	Mutation description		mRNA transcript alteration	Position at chromosome 3	ExAC	Mutation classification	Reference
		DNA	Protein domain (missense mutations)					
1/B	Y	c.678+1G>T	p.?	Splicing	3:193336726	N	Pathogenic	Novel
2/B	Y	c.800_801del	p.(Lys267Argfs*4)	Frameshift	3:193349411	1/120600	Pathogenic	(Toomes et al. 2001)
3/CZ	N	c.943C>T	p.(Leu315Phe) Downstream from coiled coil 1	Missense	3:193353306	N	Unknown significance	Novel
4/B	N	c.949-1G>C	p.?	Splicing	3:193354983	N	Pathogenic	Novel
5/B	N	c.949-2A>G	p.?	Splicing	3:193354982	N	Pathogenic	Novel
6/B	N	c.980T>C	p.(Leu327Pro) Downstream from coiled coil 1	Missense	3:193355015	N	Pathogenic	(Baris et al. 2003; Almind et al. 2012)
		c.1805C>A	p.(Ala602Glu) Dynamin, GTPase domain	Missense	3:193364904	N	Pathogenic	Novel
7/CZ	Y	c.1034G>A	p.Arg345Gln	Missense	3:193355069	N	Pathogenic	(Lunkes et al. 1995; Alexander et al. 2000; Gallus et al. 2012; Russo et al. 2013)
8/CZ	Y		Dynamin, GTPase domain					
9/B	Y							
10/B	Y	c.1035+5G>A	p.Lys317_Arg345del	Splicing	3:193355075	1/121286	Pathogenic	(Toomes et al. 2001)
11/B	N							
12/B	NA	c.1148A>G	p.Val346_Phe383del	Splicing	3:193355853	N	Pathogenic	(Baris et al. 2003)
13/CZr	N	c.1230+1G>A	p.Val384_Asp410del	Splicing	3:193360635	N	Pathogenic	(Schimpf et al. 2008)
14/C	Y	c.1305+5G>C	p.Leu411_Glu435del	Splicing	3:193360843	N	Pathogenic	(Schimpf et al. 2008)
		c.2355G>C**	p.(Leu785Phe) Middle domain	Missense	3:193376699	N	Unknown significance	Novel
15/B	N	c.1367G>A	p.(Gly456Asp) Dynamin, GTPase domain	Missense	3:193361223	N	Pathogenic	(Kim et al. 2005; Ke et al. 2006; Leruez et al. 2013)
16/B	N	c.1377_1377+4del	p.?	Splicing	3:193361233	N	Pathogenic	(Puomila et al. 2005)
17/B	Y	c.1673C>A	p.(Thr558Lys)	Missense	3:193363406	N	Pathogenic	(Toomes et al. 2001; Thiselton et al. 2002)
18/B	Y		Dynamin, GTPase domain					
19/B	Y							
20/B	N							
21/B	Y	c.1681+1G>T	p.?	Splicing	3:193363415	N	Pathogenic	(Toomes et al. 2001)
22/B	NA							
23/B	Y							
24/B	Y							
25/B	N							
26/B	Y	c.1834C>T	p.(Arg612*)	Nonsense	3:193364933	1/114298	Pathogenic	(Toomes et al. 2001)
27/B	N	c.1835G>C	p.(Arg612Pro) Dynamin, GTPase domain	Missense	3:193364934	N	Pathogenic	(Kim et al. 2005)
28/B	Y	c.1935+3A>G	p.?	Splicing	3:193365926	1/120536	Unknown significance	(Toomes et al. 2001)
29/B	Y	c.1943T>C	p.(Leu648Pro)	Missense	3:193366591	N	Pathogenic	(Ferre et al. 2009; Manners et al. 2015)
30/B	N		Middle domain					
31/B	NA	c.1945_1948del	p.(Phe649Lysfs*14)	Frameshift	3:193366593	N	Pathogenic	(Toomes et al. 2001)
32/B	Y	c.1989del	p.(Ser664Alafs*24)	Frameshift	3:193366637	N	Pathogenic	Novel

Table 1. (Continued)

Proband no/origin	Family history	Mutation description		mRNA transcript alteration	Position at chromosome 3	ExAC	Mutation classification	Reference
		DNA	Protein domain (missense mutations)					
33/B	Y	c.2102del	p.(Ser701*)	Nonsense	3:193372740	N	Pathogenic	Novel
		c.2873_2876del	p.Val958Glyfs*3	Frameshift	3:193384959	N	Pathogenic	(Delettre et al. 2000, 2001; Murton et al. 2001; Pesch et al. 2001; Toomes et al. 2001; Baris et al. 2003; Schimpf et al. 2008)
34/B	N	c.2296C>T	p.(Arg766*)	Nonsense	3:193374986	N	Pathogenic	(Delettre et al. 2001)
35/B	Y	c.2734C>T	p.(Arg912*)	Nonsense	3:193382741	N	Pathogenic	(Ferre et al. 2009)
36/B	NA	c.2778+1G>A	p.?	Splicing	3:193382786	N	Pathogenic	(Toomes et al. 2001)
37/C	NA	c.2779-1G>A	p.?	Splicing	3:193384084	N	Pathogenic	(Ferre et al. 2009)
38/B	N	c.2858_2865dup	p.(Thr956Leufs*9)	Frameshift	3:193384164	N	Pathogenic	Novel
39/CZ	N	c.2873_2876del	p.Val958Glyfs*3	Frameshift	3:193384959	N	Pathogenic	(Delettre et al. 2000, 2001; Murton et al. 2001; Pesch et al. 2001; Toomes et al. 2001; Baris et al. 2003; Schimpf et al. 2008)
40/B	Y							
41/B	Y							
42/B	Y							
43/B	N							
44/CZ	Y	c.2965dup	p.(Gln989Profs*10)	Frameshift	3:193385051	N	Pathogenic	Novel

B = British, C = Canadian, CZ = Czech, CZr = Czech Roma, Y = yes, N = none, NA = not available data, ExAC = Exome Aggregation Consortium.
NM_130837.2 was used as the reference sequence. Chromosomal position corresponds to the human genome build GRCh37/hg19 and in case of a deletion or duplication of multiple nucleotides the position of the first nucleotide is indicated.

Altogether, 29 mutations observed in 42 probands were evaluated as pathogenic and three sequence variants were classified as being of unknown significance, of these one was present in a proband carrying a different *OPA1* disease-causing mutation. The predicted and/or in previous studies already functionally verified effect of disease-causing variants was missense ($n = 7$), splicing ($n = 11$), nonsense ($n = 4$) and frameshifting ($n = 7$) (Table 1).

Eight mutations identified are in bases of canonical dinucleotides at the splice-acceptor or splice-donor site. In addition, three other mutations found in the current study c.1035+5G>A, c.1305+5G>C and c.1148A>G were previously experimentally shown to cause a splicing defect (Baris et al. 2003; Schimpf et al. 2008). *In silico* analysis supported deleterious effect of nine mutations on pre-mRNA splicing (Table S3).

Six mutations were found in more than one proband, c.1034G>A in two Czech and one UK proband, c.1035+5G>A in two UK probands, c.1673C>A in four UK probands, c.1681+1G>T in five UK probands, c.1943T>C in two UK probands, c.2873_2876del in one Czech and four UK probands.

In three patients, two rare variants were detected; p.(Leu327Pro) and p.(Ala602Glu) in proband 6; p.Leu411_Glu435del and p.(Leu785Phe) in proband 14; p.(Ser701*) and p.Val958Glyfs*3 in proband 33. Unfortunately, in families of probands 6 and 33, we were not able to determine whether these mutations were present on the same allele or in a compound heterozygous state due to the unavailability of DNA samples from other family members. In family of proband 14, targeted mutational screening of similarly affected proband's mother confirmed their *cis* position. *In silico* analysis (Table S2) suggested that p.(Leu785Phe) is of unknown significance therefore less likely to be disease causing.

None of the identified 32 rare sequence *OPA1* variants was found in the EVS database. Three pathogenic mutations and one variant of unknown significance had an entry in the ExAC database (Table 1).

Altogether, 16 probands of 39 did not report a family history of visual loss, and in five probands this

information was not available. Mutational screening and clinical evaluation to assess possible *de novo* occurrence and penetrance could be performed (due to sample unavailability) only in two-first degree relatives from pedigrees of probands 8 and 14. Both had signs of bilateral optic atrophy and carried c.1034G>A and c.1305+5G>C mutations, respectively.

DOA *plus* phenotype was documented in three individuals. Proband 13 harbouring c.1230+1G>A noticed gradual visual loss since childhood and hearing loss since 23 years of age, which was confirmed by brainstem auditory evoked potential examination. Analysis of cerebrospinal fluid at the age of 22 years revealed four positive oligoclonal bands of immunoglobulin G and slightly decreased protein level which is consistent with multiple sclerosis-like phenotype. The patient denied permission to undergo brain MRI. CT scan of the brain revealed only the presence of bilateral optic atrophy. Proband 15 harbouring c.1367G>A had hearing loss, peripheral neuropathy and proband 44 with c.2965dup had hearing loss and peripheral sensitive axonal neuropathy. Interestingly, she reported that her mother also suffered from severe neurological impairment and that she was diagnosed with multiple sclerosis. Unfortunately, her mother could not be examined as she had died of cancer. In addition, proband 3 found to carry c.943C>T (classified as variant of unknown significance) was reportedly diagnosed elsewhere with peripheral neuropathy.

Discussion

Herein, we report on rare *OPA1* sequence variants identified in a large cohort of international patients (white British, Canadian, Czech and one proband of Czech Roma origin) with bilateral optic atrophy. Ten novel sequence changes were detected, of these seven were considered pathogenic and three as variants of unknown significance. Two probands carried two different pathogenic mutations. Similarly, to other studies aiming at the identification of the underlying cause of bilateral optic atrophy (Toomes et al. 2001; Ferre et al. 2009; Chen et al. 2014), all probands with no paternal family history were prescreened for common mitochondrial mutations associated with Leber hereditary optic neuropathy prior to *OPA1* screening.

The great majority 22 (76%) of the detected 29 pathogenic mutations observed in 30 (71%) of 42 probands (including one individual with two frameshifting mutations) were predicted to lead to unstable transcripts likely to be degraded by mRNA nonsense-mediated decay cell mechanism (Pesch et al. 2001; Schimpf et al. 2006; Zanna et al. 2008) confirming that lack of functional protein product underlies the great majority of DOA cases (Pesch et al. 2001; Marchbank et al. 2002).

Only seven pathogenic missense mutations in this study were found in 12 (29%) of 42 probands (including one individual with two different missense mutations). This corresponds to frequency estimated in other studies concluding that about 30% of patients with DOA carry missense *OPA1* mutations (Amati-Bonneau et al. 2008; Ferraris et al. 2008; Hudson et al. 2008; Yu-Wai-Man et al. 2010).

Two mutations c.1034G>A and c.1148A>G in near proximity to intron–exon boundaries (2nd exonic 3′ nucleotides) were predicted to lead to an amino acid substitution. While c.1034G>A was indeed experimentally verified by Schimpf et al. (2006) to cause p.Arg345Gln, c.1148A>G leads to in-frame skipping of exon 11 changing p.Val346_Phe383del at the protein level (Baris et al. 2003) highlighting the fact that interpretation of mutations needs always to be put into context of nucleotide position within the open reading frame so that variants interfering with splicing process are not wrongly indicated as substitutions.

The clear limitation of the current study is that the majority of first degree relatives were not available for our investigation; in part, information on family history was also missing. Negative family history for DOA was reported by 38% of probands with pathogenic mutation in *OPA1* which we attribute to *de novo* occurring changes and incomplete penetrance.

Three previously reported mutations in association with DOA and one variant of unknown significance are recorded in ExAC database, each with an occurrence 1 allele of ~120 000. As it has been shown that penetrance of *OPA1* mutations may be as low as 43% (Toomes et al. 2001), in addition to mild phenotypes that may remain unnoticed if subjects do not undergo comprehensive neuro-ophthalmological examination, we think that the presence of these mutations in public datasets does not exclude their pathogenic nature. Variant c.1935+3A>G previously reported as benign (Toomes et al. 2001) has been regarded as of unknown significance as functional study could not be performed due to sample unavailability.

Further limitations of this study were that results of examinations to assess extraocular neurodegenerative impairment and hearing loss were not available for all individuals tested. Autosomal dominant optic atrophy (DOA) *plus* phenotype was therefore documented in only three probands of 42 with *OPA1* disease-causing mutations and in one proband with a missense variant classified as of unknown significance. Of the four sequence variants detected in our patients with DOA *plus* syndrome only p.(Gly456Asp) in Dynamin, GTPase domain has been previously reported to cause this phenotype (Leruez et al. 2013). Although the remaining probands were not aware of any other than visual symptoms, it needs to stressed out that mild forms of hearing impairment or peripheral neuropathy might have not been noticed.

In summary, our study expands the *OPA1* mutational spectrum and shows that the proportion of pathogenic variants leading to insufficient OPA1 protein expression level may be higher than it has been anticipated to date.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primer sequences used for mutation analysis of *OPA1*.
Table S2. *In silico* analysis of *OPA1* missense variants identified in patients with optic atrophy in the current study.
Table S3. *In silico* analysis of *OPA1* variants potentially affecting splicing identified in the current study.
Figure S1. Evolutionary conservation of the OPA1 protein.