**OPAI1 analysis in an international series of probands with bilateral optic atrophy**

Petra Liskova,¹,² Marketa Tesarova,³ Lubica Dudakova,¹ Stepanka Svecova,³ Hana Kolarova,³ Tomas Honzik,³ Sharon Seto⁴,⁵ and Marcela Votruba⁴,⁵

Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

Department of Ophthalmology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

Department of Paediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

Cardiff Eye Unit, University Hospital of Wales, Cardiff, UK

School of Optometry & Vision Sciences, Cardiff University, Cardiff, UK

**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

**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 (Thistlethwaite 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.broadinstitute.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 evaluated for potential pathogenicity. Identified sequence changes were further cross-referenced with published literature and the eOPA1 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 (Yeoh & 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.

<table>
<thead>
<tr>
<th>Family history</th>
<th>Mutation description</th>
<th>DNA alteration</th>
<th>Protein domain (missense mutations)</th>
<th>mRNA transcript alteration</th>
<th>Position at chromosome 3</th>
<th>ExAC</th>
<th>Mutation classification</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/B Y</td>
<td>c.678+1G&gt;T</td>
<td>Splicing</td>
<td>p.?</td>
<td>3:193363726</td>
<td>N</td>
<td>Pathogenic</td>
<td>Novel</td>
<td></td>
</tr>
<tr>
<td>2/B Y</td>
<td>c.800_801del</td>
<td>In frame shift</td>
<td>p.(Lys267Argfs*4)</td>
<td>3:193349411</td>
<td>1/120600</td>
<td>Pathogenic</td>
<td>(Toomes et al. 2001)</td>
<td></td>
</tr>
<tr>
<td>4/B N</td>
<td>c.949-1G&gt;C</td>
<td>Splicing</td>
<td>p.?</td>
<td>3:193354983</td>
<td>N</td>
<td>Pathogenic</td>
<td>Novel</td>
<td></td>
</tr>
<tr>
<td>5/B N</td>
<td>c.949-2A&gt;G</td>
<td>Splicing</td>
<td>p.?</td>
<td>3:193354982</td>
<td>N</td>
<td>Pathogenic</td>
<td>Novel</td>
<td></td>
</tr>
<tr>
<td>6/B N</td>
<td>c.980T&gt;C</td>
<td>Missense</td>
<td>p.(Leu327Pro)</td>
<td>3:193355015</td>
<td>N</td>
<td>Pathogenic</td>
<td>(Baris et al. 2003; Almind et al. 2012)</td>
<td></td>
</tr>
<tr>
<td>8/CZ Y</td>
<td>c.1035+5G&gt;A</td>
<td>Splicing</td>
<td>p.(Lys317_Arg345del)</td>
<td>3:193355705</td>
<td>1/121286</td>
<td>Pathogenic</td>
<td>(Toomes et al. 2001)</td>
<td></td>
</tr>
<tr>
<td>9/B Y</td>
<td>c.1035+5G&gt;A</td>
<td>Splicing</td>
<td>p.(Lys317_Arg345del)</td>
<td>3:193355705</td>
<td>1/121286</td>
<td>Pathogenic</td>
<td>(Toomes et al. 2001)</td>
<td></td>
</tr>
<tr>
<td>11/B N</td>
<td>c.1230+1G&gt;A</td>
<td>Splicing</td>
<td>p.(Val384_Asp410del)</td>
<td>3:193360635</td>
<td>N</td>
<td>Pathogenic</td>
<td>(Schimpf et al. 2008)</td>
<td></td>
</tr>
<tr>
<td>12/B NA</td>
<td>c.1305+5G&gt;C</td>
<td>Splicing</td>
<td>p.(Leu411_Glu435del)</td>
<td>3:193360843</td>
<td>N</td>
<td>Pathogenic</td>
<td>(Schimpf et al. 2008)</td>
<td></td>
</tr>
<tr>
<td>14/C Y</td>
<td>c.1377_1377+4del</td>
<td>Splicing</td>
<td>p.?</td>
<td>3:193361233</td>
<td>N</td>
<td>Pathogenic</td>
<td>(Paomila et al. 2005)</td>
<td></td>
</tr>
<tr>
<td>16/B N</td>
<td>c.1673C&gt;A</td>
<td>Missense</td>
<td>p.(Thr558Lys)</td>
<td>3:193363406</td>
<td>N</td>
<td>Pathogenic</td>
<td>(Toomes et al. 2001; Thiselton et al. 2002)</td>
<td></td>
</tr>
<tr>
<td>17/B Y</td>
<td>c.1681+1G&gt;T</td>
<td>Splicing</td>
<td>p.?</td>
<td>3:193363415</td>
<td>N</td>
<td>Pathogenic</td>
<td>(Toomes et al. 2001)</td>
<td></td>
</tr>
<tr>
<td>21/B Y</td>
<td>c.1834C&gt;T</td>
<td>Nonsense</td>
<td>p.(Arg612*)</td>
<td>3:193364933</td>
<td>1/114298</td>
<td>Pathogenic</td>
<td>(Toomes et al. 2001)</td>
<td></td>
</tr>
<tr>
<td>22/B NA</td>
<td>c.1835G&gt;C</td>
<td>Missense</td>
<td>p.(Arg612Pro)</td>
<td>3:193364934</td>
<td>N</td>
<td>Pathogenic</td>
<td>(Kim et al. 2005)</td>
<td></td>
</tr>
<tr>
<td>23/B Y</td>
<td>c.1935+3A&gt;G</td>
<td>Splicing</td>
<td>p.?</td>
<td>3:193365926</td>
<td>1/120536</td>
<td>Unknown significance</td>
<td>(Toomes et al. 2001)</td>
<td></td>
</tr>
<tr>
<td>24/B Y</td>
<td>c.1943T&gt;C</td>
<td>Missense</td>
<td>p.(Leu648Pro)</td>
<td>3:193368591</td>
<td>N</td>
<td>Pathogenic</td>
<td>(Ferre et al. 2009; Manners et al. 2015)</td>
<td></td>
</tr>
<tr>
<td>31/B NA</td>
<td>c.1945_1948del</td>
<td>Frameshift</td>
<td>p.(Phe669Lysfs*14)</td>
<td>3:193366637</td>
<td>N</td>
<td>Pathogenic</td>
<td>Novel</td>
<td></td>
</tr>
</tbody>
</table>
Altogether 29 mutations observed in 42 probands were evaluated as pathogenic and three sequence variants were classified as being of unknown significance. One mutation 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).

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, and 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 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).

<table>
<thead>
<tr>
<th>Proband no/origin</th>
<th>Family history</th>
<th>Mutation description</th>
<th>mRNA transcript alteration</th>
<th>Position at chromosome 3</th>
<th>ExAC</th>
<th>Mutation classification</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>34/B</td>
<td>N</td>
<td>c.2296C&gt;T</td>
<td>p.(Arg766*)</td>
<td>Nonsense</td>
<td>3:193374986</td>
<td>N</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>35/B</td>
<td>Y</td>
<td>c.2734C&gt;T</td>
<td>p.(Arg912*)</td>
<td>Nonsense</td>
<td>3:193382741</td>
<td>N</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>36/B</td>
<td>NA</td>
<td>c.2778+1G&gt;A</td>
<td>?</td>
<td>Splicing</td>
<td>3:193382786</td>
<td>N</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>39/CZ</td>
<td>N</td>
<td>c.2873_2876del</td>
<td>p.Va958Glyfs*3</td>
<td>Frameshift</td>
<td>3:193384859</td>
<td>N</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>40/B</td>
<td>Y</td>
<td>c.2965dup</td>
<td>p.(Gln989Profs*10)</td>
<td>Frameshift</td>
<td>3:19338051</td>
<td>N</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>41/B</td>
<td>Y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42/B</td>
<td>Y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43/B</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>44/CZ</td>
<td>Y</td>
<td>c.2965dup</td>
<td>p.(Gln989Profs*10)</td>
<td>Frameshift</td>
<td>3:19338051</td>
<td>N</td>
<td>Pathogenic</td>
</tr>
</tbody>
</table>

\(B = \text{British, C = Canadian, CZ = Czech, CZr = Czech Roma, Y = yes, N = none, NA = not available data, ExAC = Exome Aggregation Consortium.}\)

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.
information was not available. Mutation 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 neurologic 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 extracocular 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 Dynamic, 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.

**References**


Received on May 23rd, 2016. Accepted on September 2nd, 2016.

Correspondence: Marcela Votruba, BM BCh, FRCOphth, PhD School of Optometry and Vision Sciences Cardiff University Maindy Road Cathays Cardiff CF24 4HQ UK Tel: +44 (0) 29 2087 0117 Fax: +44 (0) 29 2087 4859 Email: votrubam@cardiff.ac.uk

We thank referring physicians and all family members who took part in this study. This work was supported by UNCE 204011, PRVOUK-P24/ LF1/3 and GAUK 38515/2015 programs of the Charles University, AZV 16-3241A and RVOfVN 64165 grants from the Ministry of Health of the Czech Republic and MRC Grant G0700949.

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.