Short Report

High frequency of \textit{OPA1} mutations causing high ADOA prevalence in south-eastern Sicily, Italy


Optic atrophy type 1 (\textit{OPA1}) gene mutation causes autosomal dominant optic atrophy (ADOA, MIM \#165500). Prevalence of ADOA ranges from 1:50,000 in most populations to 1:12,000 in Denmark. Seventy members of nine families were analysed for the presence of \textit{OPA1} gene mutations by polymerase chain reaction (PCR) and direct sequencing. We identified three \textit{OPA1} gene mutations in 48 patients with variable signs of optic atrophy. Two mutations, c.784-21_784-22insAluYb8 and c.876_878delTGT, were found in two different families. The third mutation, c.869G>A, was found in 28 patients from seven families. The haplotype analysis data suggested that the c.869G>A mutation is a founder mutation. Our main result suggests a higher ADOA prevalence in south-eastern Sicily than previously found in Denmark. This is because of not only the founder effect but also to the presence of three different mutations in the geographical area of the study. Our hypothesis is that a combination of social pressure because of blindness and migration factors is involved. In fact, in Siracusa, a provincial capital in south-eastern Sicily, St. Lucy, the patron saint of the blind was born and died.

Conflict of interest

The authors report no conflicts of interest.
The advent of multiplex ligation-dependent probe amplification (MLPA) has facilitated the definitive identification of deletions and duplications in the OPA1 gene, thereby increasing the spectrum and prevalence of known OPA1 mutations in ADOA patients (13).

In some cases mutations in the OPA1 gene lead to additional neurological symptoms, identified as the so-called ‘ADOA plus’ phenotypes. Multiple mtDNA deletions have only been observed in these ADOA plus phenotypes, explaining their atypical multi-systemic phenotype with visual failure and optic atrophy in childhood, followed by PEO, ataxia, deafness and sensory-motor neuropathy in adulthood (14–16).

**Materials and methods**

**Standard protocol approvals, registrations and patient consents**

The ethics committee approval is not necessary in Italy for this kind of research. Informed consent was obtained from all patients participating in the study.

**Family study**

Seventy individuals of nine Italian families (age range 8–77 years) were assessed clinically and genetically. All families originate in the province of Siracusa, south-eastern Sicily (Fig. 1), an area with 123,487 inhabitants (ISTAT, National Institute of Statistics, 2010). All patients harbouring pathogenic OPA1 mutations underwent a detailed neuro-ophthalmological examination including visual acuity (high contrast Snellen charts at 6 m), colour vision (Ishihara plates), slit-lamp biomicroscopy, intra-ocular pressure assessment by applanation tonometry and direct or indirect fundoscopy. Eye movements and confrontation visual fields were evaluated clinically. When possible, visual field was defined by computerized (Humphrey Field Analyzer 24.2, Carl Zeiss Meditec, Dublin, CA) or Goldmann perimetry, and optic disc area and diameters were measured by ocular coherence tomography (Stratus OCT, Carl Zeiss Meditec, Dublin, CA). Clinical neurological evaluation was also performed in all patients. The genealogical data reported were obtained through interviews.

**Mutation analysis**

**OPA1 gene analysis**

Having obtained informed consent, genomic DNA was extracted from the blood samples of family members and healthy controls using a QIAamp DNA blood kit (Qiagen, Limburg, The Netherlands). All 30 coding exons (comprising intron–exon boundaries) were amplified by PCR using primers specific for the OPA1 gene (primer sequences available upon request).

Following purification of the PCR products using QIAquick PCR Purification kits (Qiagen),
sequencing was performed using the ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA). The results were analysed using Chromas 2.33 (Technelysium Pty Ltd, Australia) software and compared with reference sequence NM_015560.2 (OPA1 transcript variant 1).

**Generation of haplotypes**

We analysed 27 patients with the c.869G>A mutation and 11 unaffected familial for a founder effect. Haplotype analysis was performed with single nucleotide polymorphisms (SNPs) surrounding the OPA1 gene using the human genome sequence as a reference (GRCh37.p2). A total of 13 SNPs in the Chr3 189,800,000–199,200,000 region were used. We selected the SNPs with minor allele frequency (MAR) >25% in European descendants (Table 1). We also studied Chr3 189,800,000–199,200,000 region SNPs, selected based on their position. SNPs were analysed by Restriction Fragment Length Polymorphism (RFLP) or direct sequencing. Measures of linkage disequilibrium (LD) between the SNPs (D’ and LOD score) and haplotype frequencies were calculated by the Gerbil algorithm, implemented in Gevalt 2.0 (GENotype Visualization and ALgorithmic Tool) (17). Gevalt is based on Haplovew’s source code (18). The allele frequencies of the SNP and haplotypes were compared between patients and controls using chi square test. For precise results, p values of the alleles were permuted 1,000,000 times.

**Results**

**Clinical findings in carriers of mutations**

At the time of examination four subjects were clinically asymptomatic. In the others, various degrees of visual impairment were observed. The median age of the first diagnosis of visual impairment was 7.5 years. Visual acuity ranged from normal to less than 1.70 LogMAR (mean 0.50). Colour vision was normal in two symptomatic subjects, whereas the others had different extents of colour sensitivity deficiency, ranging from mild (14/15 Ishihara plates) to severe (0/15). Intra-ocular pressure (IOP), as measured by applanation tonometry, was in the normal range in all cases except in one patient who had high IOP in one eye (40 mmHg). In the symptomatic patients, ophthalmoscopy revealed optic nerve head pallor, often limited to the temporal quadrant and only rarely associated with the temporal crescent. Although increased nonglaucomatous cupping was shown in a few patients, the cup to disc ratio, optic disc horizontal and vertical diameters and optic disc area were generally within the normal range. Of the four asymptomatic subjects, optic nerve head appearance was normal only in two. The most common visual field defect was central and paracentral scotoma, although cecocentral scotoma and reduced peripheral sensitivity were also detected in a few cases. Symptoms showed a high intra- and inter-familial variability. No additional neurological features were present in the carriers observed.

**Molecular studies**

We screened nine independent families with a clinical diagnosis of ADOA for the presence of mutations in the OPA1 gene. We found three mutations: c.784-21_22insAluYb8, c.869G>A and c.876_878delTGT. In total, we identified OPA1 mutations in 48 out of the 70 family members enrolled.

**Table 1. Single nucleotide polymorphisms (SNPs) surrounding the OPA1 gene used for generation of haplotypes**

<table>
<thead>
<tr>
<th>SNP #</th>
<th>SNP name</th>
<th>NCBI reference sequence</th>
<th>Position</th>
<th>Distance to mutation (bp)</th>
<th>Gene</th>
<th>Alleles</th>
<th>Allele Frequency dbSNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs67960000</td>
<td>NT_005612.15</td>
<td>189,874,213</td>
<td>4,963,546</td>
<td>LPP</td>
<td>A/T</td>
<td>A: 0.325 T: 0.675</td>
</tr>
<tr>
<td>2</td>
<td>rs4677728</td>
<td>NT_005612.15</td>
<td>192,576,004</td>
<td>2,261,755</td>
<td>CCDC50</td>
<td>A/G</td>
<td>A: 0.508 G: 0.492</td>
</tr>
<tr>
<td>3</td>
<td>rs4453795</td>
<td>NT_005612.15</td>
<td>193,576,699</td>
<td>1,261,090</td>
<td>FGF12</td>
<td>A/G</td>
<td>A: 0.725 G: 0.275</td>
</tr>
<tr>
<td>4</td>
<td>rs3905277</td>
<td>NT_005612.15</td>
<td>194,466,392</td>
<td>371,367</td>
<td>HRASLS</td>
<td>A/G</td>
<td>C: 0.617 G: 0.383</td>
</tr>
<tr>
<td>5</td>
<td>rs6444720</td>
<td>NT_005612.15</td>
<td>194,609,153</td>
<td>228,606</td>
<td>ATP13A4</td>
<td>C/G</td>
<td>C: 0.475 T: 0.525</td>
</tr>
<tr>
<td>6</td>
<td>rs6788484</td>
<td>NT_005612.15</td>
<td>194,691,872</td>
<td>145,887</td>
<td>ATP13A4</td>
<td>C/T</td>
<td>C: 0.475 T: 0.525</td>
</tr>
<tr>
<td>Our mutation</td>
<td>c.869G&gt;A</td>
<td>NT_005612.15</td>
<td>194,837,759</td>
<td>OPA1</td>
<td>G/G</td>
<td>G: 1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>rs1007408</td>
<td>NT_005612.15</td>
<td>194,957,963</td>
<td>120,204</td>
<td>Intron</td>
<td>C/G</td>
<td>C: 0.733 G: 0.267</td>
</tr>
<tr>
<td>8</td>
<td>rs1976393</td>
<td>NT_005612.15</td>
<td>195,055,317</td>
<td>217,558</td>
<td>Intracne</td>
<td>G/T</td>
<td>C: 0.617 G: 0.383</td>
</tr>
<tr>
<td>9</td>
<td>rs9866605</td>
<td>NT_005612.15</td>
<td>195,158,806</td>
<td>321,047</td>
<td>Intracne</td>
<td>C/T</td>
<td>C: 0.617 G: 0.383</td>
</tr>
<tr>
<td>10</td>
<td>rs4686674</td>
<td>NT_005612.15</td>
<td>195,381,966</td>
<td>544,207</td>
<td>Intracne</td>
<td>G/T</td>
<td>C: 0.475 T: 0.525</td>
</tr>
<tr>
<td>11</td>
<td>rs4677655</td>
<td>NT_005535.16</td>
<td>196,307,426</td>
<td>1,469,667</td>
<td>C3orf21</td>
<td>C/T</td>
<td>C: 0.475 T: 0.525</td>
</tr>
<tr>
<td>12</td>
<td>rs3772109</td>
<td>NT_009928.12</td>
<td>197,489,626</td>
<td>2,651,867</td>
<td>PCYT1A</td>
<td>C/T</td>
<td>C: 0.642 T: 0.358</td>
</tr>
<tr>
<td>13</td>
<td>rs10428227</td>
<td>NT_009928.12</td>
<td>199,050,937</td>
<td>4,213,178</td>
<td>LRCH3</td>
<td>A/G</td>
<td>A: 0.475 G: 0.525</td>
</tr>
</tbody>
</table>
The first mutation, c.876_878delTGT, was found in exon 9 and leads to the deletion of one amino acid, p.Val294del. We identified this mutation in only one family (Fig. 2a). The second mutation, c.784-21_784-22insAluYb8, was found in intron 7 and leads to the skipping of exon 8 in mRNA. This mutation was only identified in one family (Fig. 2b). The third mutation, c.869G>A, was found in exon 8 and leads to an Arginine to Glutamine substitution in the OPA1 protein (p.Arg290Gln). We identified this mutation in seven families, accounting for a total of 28 individuals, that live in the same town (Fig. 2c–i).

**Discussion**

Here we report data from our study, indicating exceptionally high ADOA prevalence rates in the province of Siracusa, south-eastern Sicily. We studied nine families, detecting 3 OPA1 gene mutations in 48 cases.

Our results for the local population suggest a prevalence of up to 1:1200, more than forty times the prevalence in the general Caucasian population (1:50,000) and ten times the prevalence in Denmark (1:12,000) (3, 4). The 48 patients with OPA1 mutations lived in three particular towns, which account for a total of 57,800 inhabitants (ISTAT 2009 and 2010). Even considering the population of the whole province of Siracusa (402,840 inhabitants, ISTAT 2010), the prevalence was 1:8375, higher than previously found in Denmark (1:12,000). In addition, data from the Siracusa branch of the Italian Union of the Blind suggest the presence of other families affected by dominant optic atrophy who have yet to be officially diagnosed. Therefore, further investigations are necessary to verify the presence of the mutations in other clinically affected family members and in the general population, in order to establish the real prevalence rate in this area.
High frequency of \textit{OPA1} mutations causing high ADOA prevalence

An important finding of this study is the identification of three different mutations, one of which was a novel mutation (previously described by us) (19), in three neighbouring towns. Only one of these mutations was defined in each town, referred to simply as town A, B or C (Fig. 1). The first mutation (c.876_878delTGT) was found in town A, the second mutation (c.784-21_784-22insAluYb8) in town B and the third (c.869G>A) in town C. The distances between the towns were A–B: 35 km, A–C: 22 km, B–C: 43 km. The distance from each town to the city of Siracusa, the provincial capital, was 39, 22 and 35 km, respectively.

The two mutations, c.784-21_784-22insAluYb8 and c.876_878delTGT, were found in two different families (Fig. 2a and b). The c.876_878delTGT mutation was described in British patients (20). Interestingly, the c.869G>A mutation, previously described in Cuban and British patients (21, 22), was found in 28 patients from seven families (Fig. 2c–i). Haplotype analysis suggests that the families were related due to the founder effect.

Previous studies have indicated that a high prevalence in isolated communities or small geographic areas is because of factors such as selective pressure, founder effect, genetic drift and others (23). Our hypothesis, taking into account the dominant inheritance of ADOA, is that a combination of social pressure because of blindness and migration factors is involved. It is interesting to note that the city of Siracusa, situated near the three towns, is home to the cult of St. Lucy (who was born and died there). St. Lucy is one of the most popular saints in Sicily, patron saint of the blind and of Siracusa. Therefore we hypothesize that some families may have come to this area due to the presence of institution for the blind, charitable societies or community-oriented organizations. A somewhat similar phenomenon was described by Gerber et al. in the Poitou-Charentes region. The authors hypothesized that several USH1 families came to the region because of the presence of the Institution for Deaf and Blind Children (24).

From a clinical standpoint, the phenotypic expression of these mutations presents characteristic features, as no additional findings identifying syndromal ADOA variants were detected. The variability in visual impairment and incomplete penetrance observed in our patients have also frequently been described in previously reported \textit{OPA1} mutations (2).

To conclude, the results of this study demonstrate a high prevalence of ADOA in the south-east of Sicily, and highlighted the presence of three \textit{OPA1} mutations in 48 patients. The final aim of this research is to extend the genetic and clinical study to as many persons as possible from the area.

\textbf{Acknowledgements}

We acknowledge Prof. Joel Zlotogora for helpful suggestions and all families for their participation. This work was supported by a grant from Regione Toscana and Monte dei Paschi Foundation to A. F.
References