Alu-element insertion in an OPA1 intron sequence associated with autosomal dominant optic atrophy

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Purpose: Autosomal dominant optic atrophy (ADOA) is the most common form of hereditary optic neuropathy caused by mutations in the optic atrophy 1 (OPA1) gene. It is characterized by insidious onset with a selective degeneration of retinal ganglion cells, variable loss of visual acuity, temporal optic nerve pallor, tritanopia, and development of central, paracentral, or cecocentral scotomas. Here we describe the clinical and molecular findings in a large Italian family with ADOA.

Methods: Routine ophthalmologic examination and direct sequencing of all coding regions of the OPA1 gene were performed. Further characterization of a new OPA1 gene insertion was performed by reverse transcription-PCR (RT-PCR) of RNA from patients and control subjects.

Results: We identified an Alu-element insertion located in intron 7 of OPA1 causing an in-frame deletion of exon 8 in 18 family members.

Conclusions: The predicted consequence of this mutation is the loss of the guanosine triphosphatase (GTPase) activity of OPA1. Alu insertions have been reported in the literature as causing human genetic disease. However, this is the first report of a pathogenic OPA1 gene mutation resulting from an Alu insertion.

Autosomal dominant optic atrophy (ADOA; OMIM 165500) is the most common form of hereditary optic neuropathy, with an estimated prevalence between 1:10,000 and 1:50,000 in different populations [1]. It is characterized by insidious onset with a selective degeneration of retinal ganglion cells, variable loss of visual acuity, temporal optic nerve pallor, tritanopia, and development of central, paracentral, or cecocentral scotomas [2]. ADOA is inherited in an autosomal dominant manner with high interfamilial and intrafamilial phenotypic variability and incomplete penetrance.

Mutations in the optic atrophy 1 (OPA1) gene (that consists of 30 coding exons) are responsible for approximately 90% of cases. OPA1 encodes a large guanosine triphosphatase (GTPase), implicated in the formation and maintenance of the mitochondrial network [3] and in protection against apoptosis by segregating cytochrome c inside the mitochondrial cristae [4]. OPA1 comprises a highly basic N-terminus, a dynamin GTPase domain, and a C-terminus. More than 200 pathogenic mutations have been reported in the literature (see the eOPA1 database). No significant correlation has been observed between degree of visual impairment and location or type of mutation [5]. Missense mutations in OPA1, however, leading to multiple mitochondrial DNA (mtDNA) deletions in skeletal muscle and a mosaic defect of cytochrome c oxidase, were recently found. In these cases the disorder presented with visual failure and optic atrophy in childhood, followed by Progressive External Ophthalmoplegia (PEO), ataxia, deafness, and sensory-motor neuropathy in adulthood. Moreover, some cases show additional neurologic symptoms, the so-called optic atrophy plus phenotypes [6].

METHODS

Family study: Twenty-eight individuals of a single large Italian family were recruited from the Department of Neurologic, Neurosurgical and Behavioral Sciences of Siena, the Italian Union of the Blind of Siracusa and the Department of Pediatric of Catania. There were 15 females and 13 males ranging in age from 9 to 59 years. We use the term “family” to indicate descendants of a multigenerational pedigree of related individuals (Figure 1). Autosomal dominant inheritance was indicated by multiple affected individuals in each generation. All patients underwent routine ophthalmologic examination, including visual acuity, color vision, manual visual field, slit-lamp examination, intraocular pressure (IOP), and dilated fundoscopy. Clinical neurologic examination and genetic analysis was also performed in all patients.

Mutation analysis: After informed consent was obtained, 5 ml of blood from the proband, his family members, and healthy controls, was drawn into an ethylenediamine tetraacetic acid
Genomic DNA was extracted from blood samples using a QIAamp DNA blood kit (Qiagen, Limburg, Netherlands). All 30 coding exons (comprising intron–exon boundaries) were amplified by PCR using primers specific for **OPA1** (Table 1). PCR reactions were performed in 25 μl reaction volumes containing 100 ng genomic DNA, 10 pmol forward and reverse primers, 100 mM dNTPs, 1U DNA polymerase (Eurotaq DNA polymerase; Euroclone, Milan, Italy), 1.5 mM MgSO₄, 1× Reaction buffer, using a DNA thermal cycler (PTC-200; MJ Research, Waltham, MA). The PCR products of exon 8 were cloned into a PCR Cloning Vector pSC-A using the StrataClone™ PCR Cloning Kit (Agilent Technologies, Stratagene Products Division, Santa Clara, CA). After purification of PCR products with QIAquick PCR Purification kits (Qiagen), sequencing was performed using the automated sequencer ABI 3730 (Applied Biosystems, Foster City, CA).

**RESULTS**

Clinical findings in carriers of mutations: Neurologic examination was unremarkable (Table 2). Ophthalmologic examination revealed visual acuity ranging from 20/20 to less than 20/200. Color vision was normal in two subjects, the other 16 had different degrees of color sensitivity deficiency (eight of them showed generalized color sensitivity deficit). IOP with applanation was in the normal range in 11 patients (not exceeding 19–20 mmHg); four subjects had IOP over 20 mmHg. One patient had high IOP (40 mmHg). Optic nerve appearance was normal in two subjects, whereas nine showed temporal pallor and six diffuse neurorim pallor.

Molecular studies: We sequenced the 30 coding exons of **OPA1** in the propositus. We did not find any known pathogenic mutation, but amplification of exon 8 revealed a hemizygous product of about 604 bp (Figure 2, A). Cloning and sequencing of the longer band revealed an insertion of about 325 bp. Using BLAST and Repeat Masker [7] to elucidate the nature of the inserted fragment, we identified 289 bp of the insert as belonging to AluYb8 (99.65% homology with Alu Sb2). The Alu insert was composed of a 289-bp fragment and contained a 25-bp poly(A) tail at its 3′ end with perfect direct repeats of 17 bp flanking the insert (Figure 2B,C). In Figure 2C RNA polymerase III promoter (A and B boxes) and the consensus target site sequence are also indicated. The same mutation was found in 17 family members (IV-7 and IV-10) and in another family member not available for ophthalmological examination (V-15).

Reverse Transcriptase–PCR analysis: Peripheral blood, about 10 ml, was collected using vacutainer EDTA tubes (Becton-Dickinson, Franklin Lakes, NJ) from the proband (V-3, Figure 1), the proband’s mother (IV-8, Figure 1) and three healthy controls. Total RNA was extracted from peripheral blood leucocytes using the LeukoLOCK™ Total RNA Isolation System following the instruction supplied by the manufacturer (Ambion, Austin, TX). The amount of total RNA extracted from each blood sample was quantified using the NanoDrop ND-1000 photometer (NanoDrop Technologies, Wilmington, DE). For each sample, 1.0 μg of total RNA was reverse transcribed with Affinity Script (Agilent Technologies) using oligo dT primers. PCR amplifications were performed with primers AF1 (5′-GAC CAA CTT CAG GAA GAA CT-3′) and AR1 (5′-TGA GGA CCT TCA CTC AGA GT-3′). After the purification of cDNA products with QIAquick PCR Purification kits (Qiagen), sequencing was performed using the automated sequencer ABI 3730.
In the case reported here, we found an unusual molecular defect in \textit{OPA1} causing ADOA in a large Italian family. The defect is the result of an Alu element insertion.

Alu sequences are the most frequent, short, interspersed elements in the human genome, numbering 1.1 million copies and more than 10% of the mass of the genome. Alu sequences are approximated by 300 nucleotides flanked by perfect direct repeats of the host sequence, consistent with insertion into staggered nicks in the target DNA [8]. Alu and other repetitive elements have had great influence on the insertion into staggered nicks in the target DNA [8]. Alu and other repetitive elements have had great influence on the human genome and its evolution [9]. They influence mRNA splicing, RNA editing, and protein translation [10]. Not surprisingly, Alu insertions have been associated with several human genetic disorders. A total of 26 L1 trans-driven Alu insertions have so far been described as causes of diseases [11].

In our case the Alu element was inserted in the antisense direction with respect to \textit{OPA1}. Alu element insertions in introns can have strong effects on gene expression, especially when inserted in the antisense direction. Five reports indicate that Alu insertions into intronic sequences in antisense orientation and close to the affected exon (19–50 nucleotides) cause the downstream exon to shift from constitutive splicing to full exon skipping or alternative splicing. Three have been found to cause skipping of downstream exons: one impairs correct recognition of the splice acceptor site (F8 gene) [12], while the other two may affect the branch site that is usually very close to the end of the intron ([13] and [14]). The orientation and position of the Alu in the upstream intron was recently studied. Lev-Maor et al. shed light on the positive and negative effects of Alu elements inserted in antisense orientation immediately upstream of the regulated exon [15].

In our case Alu insertion probably interferes with the branch site leading to production of an alternatively spliced mRNA lacking exon 8. Loss of this exon may be similar to that reported for Alu element insertion in \textit{NF1} and \textit{TNFRSF6} genes.

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TABLE 2. PATIENTS CLINICAL FINDINGS.

| Case  | Age  | Visual acuity | Color vision Ishihara | IOP applanation (mmHg) | Ophthalmoscopy |
|-------|------|---------------|------------------------|-------------------------|----------------|
|       |      | RE LE         | RE LE                 | RE LE                   |                |
| III-3 | 59   | 20/40 20/50   | 2/15                  | 18                     | diffuse neurorim pallor C/D 0.3 |
| III-9 | 58   | <20/200       | generalized deficit   | 16                     | temporal pallor C/D 0.6–0.7 |
| III-11| 56   | <20/200       | generalized deficit   | 20                     | optic atrophy C/D 0.6–0.7 |
| III-13| 51   | <20/200       | generalized deficit   | 14                     | temporal pallor C/D 0.6–0.7 |
| IV-4  | 41   | <20/200 20/15 | 0 3/15                | 40 12                  | optic atrophy C/D 0.6 subatrophy C/D 0.4 |
| IV-7  | 20   | 20/20         | 15/15                 | 21                     | >cupping C/D 0.5–0.6 cupping C/D 0.6–0.7 |
| IV-8  | 38   | 20/20         | 12/15 15/15           | 19 22                  | slight temporal pallor C/D 0.5–0.6 |
| IV-10 | 36   | 20/20         | 15/15                 | normal                 | normal         |
| IV-22 | 33   | 20/40 20/70   | generalized deficit   | 14                     | temporal pallor C/D 0.6–0.7 |
| IV-26 | 25   | <20/200       | generalized deficit   | 16                     | temporal pallor C/D 0.3–0.4 |
| IV-27 | 27   | <20/200       | generalized deficit   | 16                     | temporal pallor C/D 0.3–0.4 |
| IV-28 | 41   | 20/70 20/100  | 4/15                  | 16 17                  | diffuse neurorim pallor C/D 0.4 normal cupping |
| IV-30 | 48   | 20/70 20/100  | generalized deficit   | 15                     | normal         |
| V-1   | 10   | 20/40 4/15    | np                    | temporal pallor C/D 0.3 |
| V-3   | 9    | 20/100        | np                    | temporal pallor C/D 0.7 |
| V-12  | 12   | 20/20 15/15   | normal                | slight temporal pallor. Normal cupping diffuse neurorim pallor |
| V-14  | 17   | 20/50 12/15   | 10/15                 | 18                     | diffuse neurorim pallor |
| V-15  | 21   | N.A.          | N.A.                  | N.A.                   | N.A.           |

The phenotypic features of seventeen family members with OPA1 mutation are shown. The numbering of patients in the first column is the same as in Figure 1. The second column indicates age at diagnosis (in years). The fourth column indicates results of the Ishihara color test (a test for color blindness). The fifth column indicates intra-ocular pressure with applanation. Abbreviations: C/D represents cup to disk ratio; IOP represents intra-ocular pressure; RE represents right eye; LE represents left eye; N.P. represents not performed; N.A. represents not available.
Phenotypic expression of this new mutation did not show particular peculiarities since ophthalmologic variability observed in our patients has also been described in other common OPA1 mutations. Moreover, the presence of the mutation in two unaffected family members suggests incomplete penetrance, as previously reported for other OPA1 gene mutations [18].

In conclusion, we describe an AluYb8 [8] retrotransposition event in an intron of the OPA1 gene leading to exon skipping. The predicted consequence of this mutation is the loss of the GTPase activity of OPA1. Alu insertions have been reported in the literature as causing human genetic disease. However, this is the first report of a pathogenic OPA1 gene mutation resulting from an Alu insertion.

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