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Short communication

Optic atrophy plus phenotype due to mutations in the OPA1 gene: Two more Italian families

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ABSTRACT

Autosomal Dominant Optic Atrophy (ADOA) is characterized by the selective degeneration of retinal ganglion cells. The occurrence of mutations in the gene encoding the dynamin-like GTPase protein Optic Atrophy 1 (OPA1) has been observed in about 60–70% of ADOA cases. A subset of missense mutations, mostly within the GTPase domain, has recently been associated with a syndromic ADOA form called "OPA1 plus" phenotype presenting, at muscle level, mitochondrial DNA (mtDNA) instability.
In this study we disclosed two *OPA1* gene mutations in independent probands from two families affected by OPA1 plus phenotype: the previously reported c.985-2A>G substitution and a novel microdeletion (c.2819-1_2821del).
The correlation between genotype and phenotype and the effects of these variants at the transcript level and in the muscle tissue were investigated, confirming the broad complexity in the phenotypic spectrum associated with these *OPA1* mutations.

1. Introduction

Autosomal Dominant Optic Atrophy (ADOA) is due in about 60–70% of cases to mutations in the nuclear gene encoding for the OPA1 protein, mapping to chromosome 3q28-29. ADOA is the most common form of hereditary optic neuropathy, with a prevalence of 1/50,000 [1,2]. Classical ADOA usually begins before 10 years of age with slowly progressive bilateral visual loss, dyschromatopsia, centrocecal scotomas and temporal optic disc atrophy [3,4]. The disease has an incomplete penetrance and variable phenotypic expression, ranging from mild visual impairment to blindness [5]. Up to 20% of OPA1-mutated patients also develop, during clinical history, additional neuromuscular complications leading to the so-called "OPA1 plus" phenotype [6,7].

More than 200 pathogenic mutations in the *OPA1* gene have been so far described [8]. Half of these variants are predicted to result in a truncated protein producing haploinsufficiency and are usually associated to the classical non-syndromic form of optic neuropathy. Missense mutations within or close to the GTPase domain, preserving the expression of OPA1 transcript are responsible for both the classical and syndromic OPA1 phenotype.

The present study further extends the mutational spectrum of *OPA1* with the report of a novel heterozygous deletion within the GTPase effector domain (GED). We also confirmed a previously published mutation in an Italian family suggesting the existence of a mutational hot spot in *OPA1* sequence sited in the surroundings of exon 10 splice acceptor site.

2. Material and methods

2.1. Case reports

2.1.1. Family 1

The proband is an adult Italian male with a clinical history characterized by visual impairment since childhood.

He came to our attention at 48 years of age, complaining of generalized fatigue and progressive visual loss since childhood. Neurological examination showed a mild bilateral ptosis and ophthalmoparesis; he also presented pes cavus on the left side with a decreased/absent achilles tendon reflex bilaterally. Visual field revealed a peripheral concentric narrowing. Fundus oculi examination showed mild bilateral temporal pallor of the optic disc.

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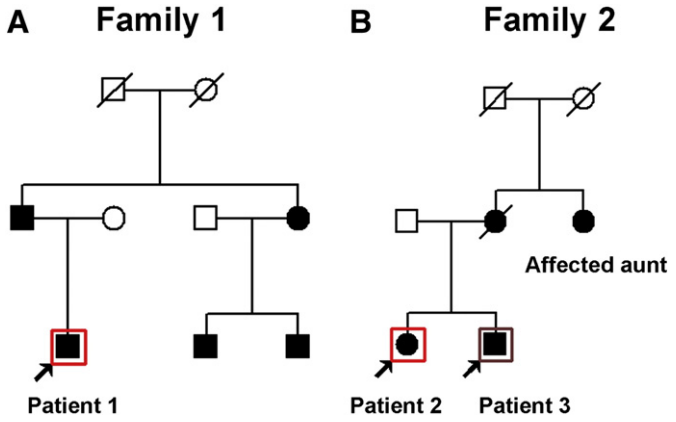

Fig. 1. Pedigrees of the described families. Black symbols indicate affected subjects. The described probands are indicated by arrows.

He underwent ocular computerized tomography, which disclosed a thinning of the peripapillar bundle nervous layers in both superior and inferior temporal quadrant bilaterally.

His father, a paternal aunt and her two sons were also affected by visual loss starting in childhood (Fig. 1A).

Brachial biceps muscle biopsy showed at the histochemical investigation three cytochrome *c* oxidase (COX) negative fibers; Succinate Dehydrogenase (SDH) activity was normal. The sequential application of these two reactions to the muscle section revealed abnormal COX deficient fibers appearing blue.

2.1.2. Family 2

The probands are two Italian siblings born from non-consanguineous parents. A 15-year-old female patient was affected by a slowly progressive visual loss with an onset at the age of 11.

Her current visual acuity is 7/10 on the right eye and 6/10 on the left one.

She underwent visual evoked potentials (VEPs), which showed a pattern characterized by increased latencies of cortical responses, with a moderate signal dispersion. Fundus examination disclosed mild temporal pallor of the optic disc bilaterally.

Her brother is an 11-year-old boy who developed a progressive visual loss at the age of 10 years with a current visual acuity of 4/10 bilaterally.

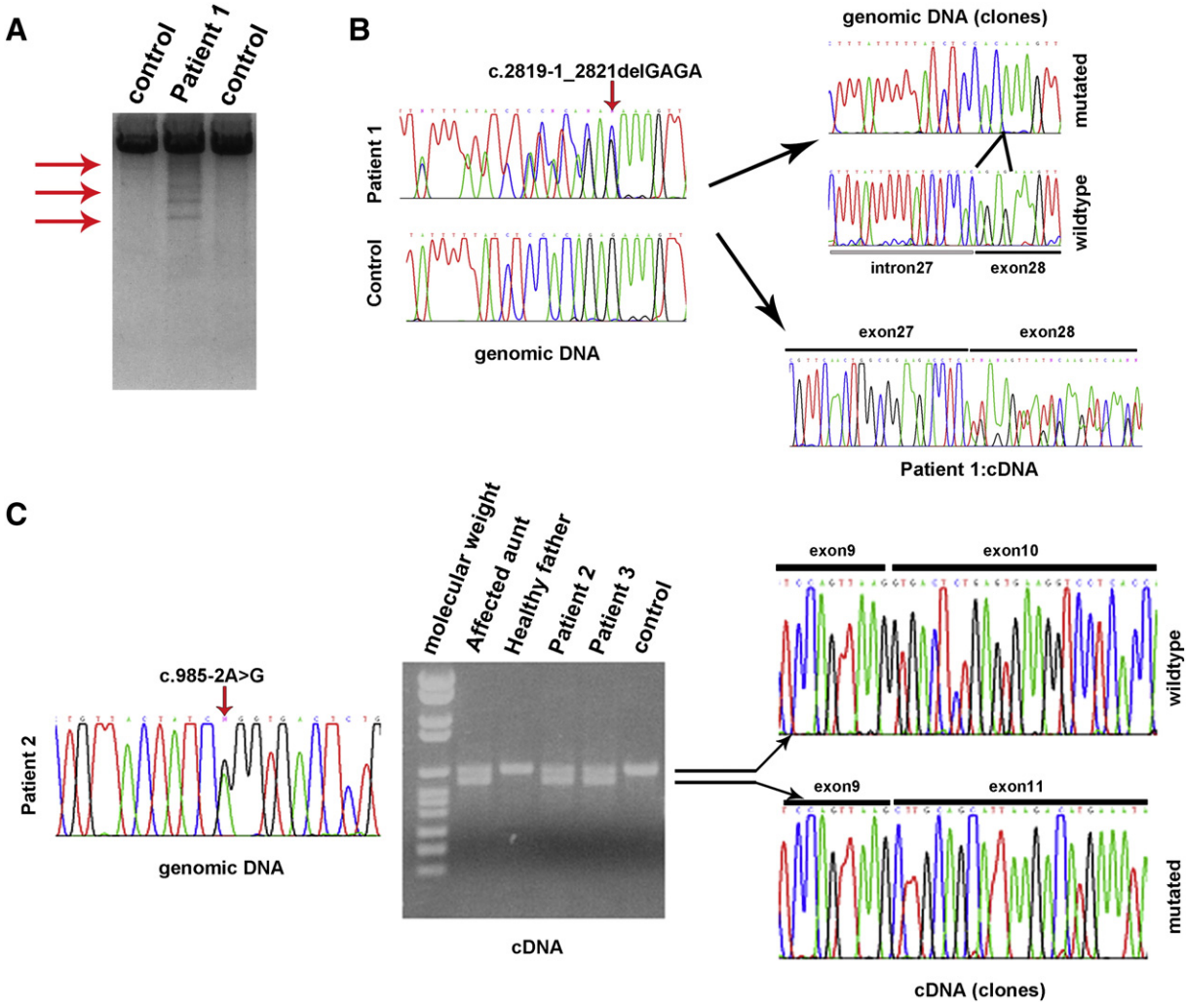

Fig. 2. (A) PCR analysis of muscle-derived mtDNA showing the presence of multiple deleted mitochondrial genomes in Patient 1. Controls are age-matched muscle biopsies. (B) Sequence analysis of OPA1 gene in Patient 1 disclosing the microdeletion c.2819-1_2921del at genomic and transcript level. PCR fragments obtained using genomic DNA as a template were subcloned to discriminate between mutated and wildtype alleles. (C) Sequence analysis of OPA1 gene in Family 2 discloses the point mutation c.985-2A>G in affected members. This substitution results in the skipping of exon 10 as showed by electrophoresis on agarose gel of RT-PCR fragments and sequence analysis of cDNA clones.

No other pathological signs were found at their neurological examination.

Their mother suffered from poor vision and died at the age of 38 for breast cancer. A 41-year old maternal aunt has presented a deterioration of visual acuity since childhood; current visual acuity is 2/10 bilaterally; a mild sensorineural hearing loss was observed in the last years (Fig. 1B).

Muscle specimens from probands of Family 2 were not available, since diagnosis was performed at the genetic level.

3. Molecular analysis

Total DNA was isolated from muscle and peripheral blood according to the standard protocols. Southern blot and long-range polymerase chain reaction analysis of muscle-derived mtDNA were performed [9,10].

The following mitochondrial DNA variants, previously associated to LHON (Leber Hereditary Optic Neuropathy) were ruled out by PCR-RFLP and sequence analysis: m.11778G>A, m.13708G>A, m.3460G>A, m.3994T>C, m.15812G>A, m.15257G>A, m.7444G>A, m.5244G>A, m.14884T>C, m.14459G>A, m.14596A>T. All OPA1 exons and at least 30 bp of flanking intronic sequences were amplified by PCR; fragments were purified and directly sequenced using BigDye Terminator protocol on an automated 3100 ABI Prism Genetic Analyzer (Applied Biosystem).

Total RNA was obtained from skeletal muscle (proband, Family1) or peripheral blood mononuclear cells (probands and healthy subjects, Family 2), according to standard procedures and reverse-transcribed. RT-PCR fragments were purified and directly sequenced. PCR products carrying the mutations were subcloned into TOPO-TA vector (Invitrogen). Nucleotide numbering of *OPA1* gene mutations reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon of the GenBank reference sequence NM_015560.2. The initiation codon is Met 1.

3. Results

Long PCR analysis of muscle-derived mtDNA of Family 1 proband disclosed several PCR products corresponding to mtDNA deletions. Southern blot analysis of muscle mtDNA did not show multiple-deleted genomes (Fig. 2A).

Sequence analysis of *OPA1* gene showed two different mutations in the affected probands.

Proband of Family 1 carried the 4 nucleotide-deletion c.2819-1_2821del, involving the last nucleotide of intron 27 and the first three nucleotides of exon 28 (Fig. 2B). This unreported rearrangement results in the activation of an exonic cryptic donor site leading to an in-frame six nucleotide-long skipping of exon 28 (r.2819_2824del) and producing a microdeletion within the GED domain (p.Lys940_Val942delinsIle). This variant was not detected in 120 ethnic-matched control subjects.

Patients from Family 2 showed the heterozygous nucleotide substitution c.985-2A>G in intron 9, previously described with incomplete penetrance, in a Chinese pedigree [11]. The same mutation was also confirmed in DNA derived from the affected maternal aunt.

This mutation is associated with the in-frame skipping of exon 10, as detected by mRNA analysis, resulting in the loss of 27 amino acids within the GTPase domain (Fig. 2C).

4. Discussion

OPA1 is a dynamin-like GTPase protein anchored to the mitochondrial inner membrane which controls the fusion of mitochondrial membranes occurring in the physiological remodeling of mitochondrial network [1,12]. Up to now more than 200 pathogenic mutations, along OPA1 coding region, have been associated to human disease. Beside optic atrophy, about 20% of patients bearing *OPA1* mutations also develop additional neuromuscular complications, mostly including deafness, progressive external ophthalmoplegia and myopathy, starting from the third decade of life onwards [13].

Our study further extends the mutational spectrum of OPA1 leading to the discovery of a novel mutation in a proband with a clinical picture of syndromic optic atrophy. This patient harbors a microdeletion located within the GED sequence confirming the importance of the integrity of this domain, responsible for the interaction between OPA1 and its partners involved in mitochondrial fusion process. The GED domain and its flanking regions in fact represent an *OPA1* mutational hotspot, since about 28% of mutations are located in this region. These findings suggest that not only missense mutation but also in frame-deletion preserving a terminal abnormal transcript, could lead to the extraneurological features observed in OPA1-plus patients [6,7].

Skeletal muscle analysis in our patient reveals mild mitochondrial defects consisting in the presence of COX-negative fibers and the occurrence of multiple deletions in mtDNA as detected by PCR analysis. The occurrence of these histological findings is frequently detected in OPA1-mutated patients with a 4-to-1 ratio in OPA1-plus patients respect to individuals with pure optic nerve involvement [14].

The mutation identified in Family 2 has been previously described in a Chinese pedigree showing an incomplete penetrance [11]. On the contrary, in our family the c.985-2A>G mutation was associated with an early-onset and a complete penetrance disease. The disclosure of the same variant in the affected members of the Italian and Chinese pedigrees supports its pathogenic nature, since it arose independently in independent genetic backgrounds. Whereas in Chinese family no mutated subjects developed any additional extraocular symptom even in late adulthood, a member of our family (the proband's aunt) showed an early onset sensorineural hearing impairment.

In our probands, transcript analysis was fundamental to characterize the effect of the genomic variants on OPA1 mRNA, but this tool is not always able to predict the resulting phenotype. In fact, in frame-deletions have been reported not only in ADOA or OPA1-plus phenotypes but even also in a multisystemic disorder in the absence of optic atrophy [15].

Recently, multiplex ligation probe amplification (MLPA) assay has allowed to detect OPA1 rearrangements in a large cohort of Danish ADOA probands, revealing that heterozygous deletions involving whole exons represent a remarkable proportion among OPA1 mutations, ranging between 10% and 19% [16]. These defects are usually missed by standard sequencing methods which are not able to detect large scale deletions as well as variants located within promoter or intronic regions.

In our opinion a combined strategy involving different techniques applied to genomic and transcript analysis could offer the most valuable option to investigate the OPA1 defects underlining the several forms of inherited optic neuropathy [17].

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Conflict of interest

The authors report no competing interests.

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