Short communication

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

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1. Introduction

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.

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

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.
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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 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 extracellular 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 underlying 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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