

A novel OPA1 gene mutation associated with peripheral neuropathy

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Abstract

Hereditary mitochondrial fusion disorders like ADOA, due to OPA1 gene mutations, and CMT2A, due to MFN2 gene mutations, are recognized causes of optic atrophy, due to optic nerve fibers degeneration. CMT2 additional distinguishing and predominant feature includes axonal peripheral neuropathy.

We studied the clinical, laboratory, electrophysiological features, muscle biopsy and OPA1 gene DNA analysis in three sisters affected by ADOA. EMG showed a motor-sensory axonal neuropathy in the index patient, and a mild demyelinating polyneuropathy in her sister. Muscle biopsy showed neurogenic atrophy and abnormal mitochondrial granular reactions by oxidative stains within fibers in the three patients. DNA analysis showed a novel 38 base pair deletion (c1410_1443+4del38) between exon and intron 14 in the GTPase domain of OPA1 protein.

Key words: ADOA, fusion, mitochondria OPA1, optic atrophy, peripheral neuropathy.

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Autosomal dominant optic atrophy (ADOA) is the most common cause of inherited optic atrophy. Its prevalence is estimated to be between 1/10000 and 1/50000 persons. Clinically, it is typically characterized by slowly progressive bilateral visual loss with frequent onset in the first 2 decades, dyschromatopsia (often more evident in the yellow-blue spectrum), bilateral central scotoma and optic nerve atrophy, especially in the temporal side. However, recent studies demonstrated that there is high variability of the clinical severity and penetrance [21] of the disease whose spectrum can include legally blind patients as well as asymptomatic patients, chronic progressive patients as well as stabilized patients. Atypical variants with sensorineural deafness [3, 16], ptosis and ophthalmoplegia [19] have also been recognized.

ADOA can be caused by different types of mutations dispersed in the full length of OPA1 gene [8], through a controversial genetic mechanism. Haploinsufficiency is more probably implicated but a semi-dominant effect related to impaired dimerization of OPA1 protein of its coiled-coil domains has not been ruled out.

OPA1 gene is a nuclear DNA encoded gene [2, 7], composed by 30 exons, with ubiquitous expression, which is however most prominent in the retinal tissue [20], and less abundant in the cerebral, skeletal and

cardiac muscle tissue. At least eight different OPA1 isoforms have been described but their significance and regulation is still unknown. The OPA1 protein is a 960-aminoacid protein, belonging to the dynamin family, characterized by a GTPase domain, a dynamin domain, 2 coiled-coil domains, and a target sequence to mitochondria. It is localized in the inner mitochondrial membrane [18]. Its physiological function has been very recently linked to two crucial and apparently independent mitochondrial activities in experimental cellular models: mitochondrial fusion [5] regulating mitochondrial morphology [10] and dynamics, and caspase dependent apoptosis through mitochondrial cristae remodeling and cytochrome-c release [6, 9, 14, 17].

However the pathomechanism responsible of ADOA in humans is still unclear as it is why the anatomical target of the disease is apparently selective. The limited autopsic studies of patients with ADOA indicate that the principal pathological substrate of the disease is RGC degeneration [11, 13], similarly to other inherited and acquired optic neuropathies like LHON and nutritional-toxic optic neuropathies, respectively. Moreover, myelin loss in the anterior visual pathway up to the lateral geniculate bodies, and vestibulocochlear nerve degeneration had also been reported.

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In the very recent publication of the ADOA mouse model [1], carrier of an OPA1 gene splice mutation, age dependent RGC degeneration was observed corresponding to the human pathology, with associated axonal degeneration, loss of optic nerve myelin sheets, and mitochondrial cristae disorganization.

Pathological studies of tissues from living patients with ADOA are very limited. In the original study reporting the causative gene of ADOA, leukocytes ultrastructural analysis showed a mitochondrial network disruption with mitochondrial hyperfragmentation, consistent with the defect of the putative fusion activity of OPA1 [7]. Another unique study analyzed skin fibroblasts from patients affected by ADOA with sensorineural deafness [3], showed a similar hyperfragmentation of the mitochondria.

A different group found an ATP synthesis defect by muscle MR spectroscopy in a series of ADOA patients [15], suggesting a mitochondrial dysfunction also in the skeletal muscle. A muscle pathological and biochemical analysis of muscle samples from these patients was not however reported by the authors. Interestingly, similar results were obtained by the same investigators in Leber hereditary optic neuropathy, raising the possibility of a converging metabolic pathomechanism. MtDNA content was found to be decreased in ADOA patients compared to controls according to one study [12].

Methods

Three sisters from a large family affected by autosomal dominant optic atrophy were characterized by clinical history, neurological and ophthalmological examination, OPA1 gene analysis by DHPLC and subsequent DNA direct sequencing, visual evoked potentials, optic coherence tomography, audiometry, EMG, brain MRI, muscle biopsy. Respiratory chain enzyme activity of the muscle biopsy samples was also measured.

Results

The 3 sisters complained of chronic, not uniformly progressive visual loss with different clinical severity and age at onset ranging from 7 and 13 years of age. Age at clinical evaluation was between 43 and 46 years of age. The oldest, who was the most severely affected family member, complained also of mild exertional intolerance, and nocturnal distal paresthesias. There was some degree of intrafamilial phenotypic variability with different visual disability (visual acuity range: 2/10-1/50).

Fundoscopy examination showed global bilateral optic nerve pallor, more evident in the temporal sides in the 3 patients (Figure 1). Retinal nerve fiber layer thickness by OCT was severely decreased in the 3 patients (range: 46-59 microns) indicating optic nerve fiber loss. Furthermore optic nerve atrophy in its postlaminal region was also appreciated by brain MRI.



Figure 1: Fundoscopic examination showing optic disc pallor, more evident on the temporal side.

OPA1 gene analysis disclosed a new 38 base pair deletion spanning between exon and intron 14 (c1410_1443+4del38), with a predicted frame shift and splice site defect. The localization of this complex mutation is the catalytic GTPase domain of OPA1, which is considered a relative hot spot for OPA1 gene mutations.

In addition to the ophthalmological typical features of ADOA, the 3 patients presented winging scapulae and one also presented *pes cavus* and a mild increase in blood CK (208 U/L, nv 0-160). EMG revealed a severe sensory-motor peripheral neuropathy in one patient, and a mild predominantly demyelinating asymptomatic neuropathy in her sister. Accordingly, histological features of denervated muscle were evident at light microscopy examination of the muscle biopsy (Figure 2).



Figure 2: Muscle biopsy from patient 2 (acid ATPase) showing type grouping, suggestive of denervated muscle.

Additionally mitochondrial granular subsarcolemmal and intermyofibrillar aggregates were appreciated by modified Gomori trichromic staining and oxidative stain reactions. Ultrastructural analysis by electron microscopy showed subsarcolemmal aggregates of

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mitochondria which showed increased size variability and some mild cristae abnormalities (Figure 3). The biochemical study of the respiratory chain enzyme activity in the muscle biopsy tissue did not show significant differences from 29 controls.

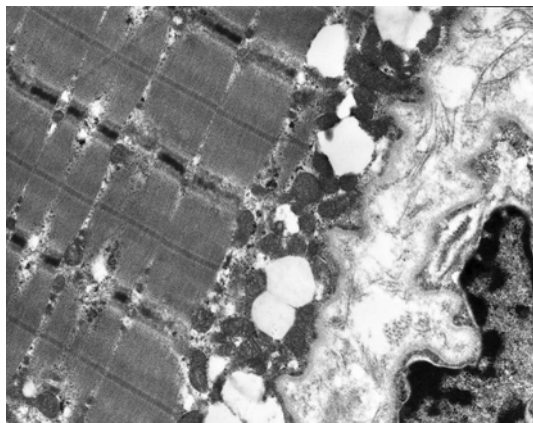


Figure 3: Early degenerative changes in subsarcolemmal mitochondria in muscle biopsy.

Discussion

We examined in this study the clinical, radiological, electrophysiological, biochemical and muscle pathological features of a family affected by a new OPA1 mutation, with a new clinical phenotype characterized by peripheral neuropathy in addition to optic nerve atrophy. This feature was clinically very subtle, manifesting as bilateral pes cavus in only one patient, but it was more evident at the electrophysiological level and at the muscle pathological study with features of denervated muscle in all the 3 patients.

These features could suggest a partially overlapping phenotype of ADOA in this family with CMT2A, even though in CMT2A peripheral neuropathy is much more severe and invariably present, whereas optic atrophy is an additional inconstant feature.

This partial clinical overlap could be hypothetically be related to a functional overlap between OPA1 and MFN2A, both involved in mitochondrial fusion dynamics.

Further studies involving EMG analysis in larger groups of patients with different mutations will be necessary to indicate if subclinical peripheral neuropathy could be a frequent unrecognized feature of ADOA, or rather a unique characteristic to this particular mutation.

Interestingly, audiometric analysis revealed no abnormalities, indicating that sensorineural deafness, a reported feature of “ADOA plus” families, was not present in this family.

Moreover this is the first study of muscle pathology in ADOA patients. The muscle histopathology in these series suggests that mild mitochondrial abnormalities are presents also in the skeletal muscle. This

observation would be in accordance with OPA1 gene ubiquitous expression, which is more prominent in the RGC but, to a minor extent also in the skeletal muscle. We suppose that ADOA could be a more generalized mitochondrial disorder, more prominent but not restricted to the optic nerves. Additional genetic modulators or specific mutations could give explain the rare reports of “ADOA plus” families presenting with sensorineural deafness, ptosis, ophtalmoplegia, nystagmus in addition to bilateral optic atrophy. These supplemental clinical manifestations could be variably observed also in other mitochondrial disorders due to mtDNA mutations (ex. Chronic progressive ophtalmoplegia, MELAS, MERRF).

Respiratory chain enzyme analysis did not show a specific decrease of complex I-IV activity, suggesting that respiratory chain dysfunction should not be the main mechanism leading to optic nerve degeneration. This result would be in agreement with the observation that the high energy dependent pigmented epithelium of the retina is not significantly involved in ADOA patients.

Similar results were found on skin fibroblasts by another group, which however found an ATP synthesis deficiency [3]. As we did not test ATP synthesis and oxygen consumption in our samples, we cannot exclude a subtle energetic failure in ADOA skeletal muscle which could be responsible of the ATP synthesis defect observed in vivo by muscle MR spectroscopy in ADOA patients [15].

If these findings will be confirmed, an additional question to answer will be if these mitochondrial energy defects are primary or rather secondary effects of different pathogenic mechanism.

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Abbreviations

Autosomal dominant optic atrophy (ADOA), Charcot-Marie-Tooth (CMT), optic coherence tomography (OCT), Retinal ganglion cells (RGC).

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