Available online at www.sciencedirect.com
SCIENCE DIRECT
Experimental Eye Research 83 (2006) 702–706
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Letter to the Editor

Structural model of the OPA1 GTPase domain may explain the molecular consequences of a novel mutation in a family with autosomal dominant optic atrophy

Sharareh Dadgar [a,1,2], Olivier Hagens [b,2], Seyed Razi Dadgar [c], Ehsan Nobakht Haghighi [a], Simone Schimpf [d], Bernd Wissinger [d], Masoud Garshasbi [b,*]

[a] *Faculty of Medicine, Iran University of Medical Sciences, P.O. Box 14155/5983, Tehran, Iran*
[b] *Department of Human Molecular Genetics, Max Planck Institute for Molecular Genetics, Ihnestrasse 73, D-14195 Berlin, Germany*
[c] *Clinical Skill Lab Center, Iran University of Medical Sciences, Firoozgar Hospital, 15900 Tehran, Iran*
[d] *Molecular Genetics Laboratory, University Eye Hospital, University of Tuebingen, Roentgenweg 11, D-72076 Tuebingen, Germany*

Received 28 November 2005; accepted in revised form 7 March 2006
Available online 12 May 2006

Abstract

Autosomal dominant optic atrophy (ADOA) is the most frequent hereditary optic neuropathy. Three loci have been reported for ADOA: a major locus, harboring all identified mutations to date, maps to 3q28 (*OPA1*), a second locus is linked to 18q12.2–q12.3 (*OPA4*) and a third locus on 22q12.1–q13.1 (*OPA5*) has been reported recently. We describe a six-generation Iranian family in which optic atrophy runs as an autosomal dominant trait with an age of onset at 14–15 years. We performed linkage analysis with markers mapping to 3q28 and 18q12.2–q12.3 and found linkage to 3q28. Subsequent sequencing of *OPA1* identified a novel heterozygous missense mutation (c.1313A>G) replacing aspartic acid by glycine (p.D438G) in the GTPase domain of OPA1. Interestingly, another missense mutation at the same position (c.1313A>T, D438V) has been reported before in two unrelated German families, indicating a possible mutation hot spot. Further evidence supporting the importance of D438 is its conservation from human to acoelomata. OPA1 is believed to be the human orthologue of yeast MGM1, a dynamin-related protein required for the integrity of mitochondrial DNA. Homology modeling of the OPA1 GTPase domain revealed extensive structural similarity to the *Dictyostelium* dynamin A GTPase domain and showed that D438 may interact with residues of the G1 and the G4 motifs, which are crucial in coordinating GTP. Based on this analysis, we propose a mechanism which explains the gradual decline of vision in ADOA patients with OPA1 mutations at position 438.

Keywords: optic atrophy; OPA1; homology modeling; dynamin A

Autosomal dominant optic atrophy (ADOA) (OMIM 165500), the most prevalent form of hereditary optic neuropathy (Kjer et al., 1996; Lyle, 1990), is genetically heterogeneous. It has been linked to *OPA4* on 18q12.2–q12.3 (Kerrison et al., 1999) and to *OPA5* on 22q12.1–q13.1 (Barbet et al., 2005), but the majority of ADOA patients have mutations in the *OPA1* gene on 3q28 (Alexander et al., 2000; Delettre et al., 2000). The autosomal dominant inheritance of ADOA and the absence of neurological, congenital or developmental abnormalities clearly distinguish it from the other OAs. Although considerable inter- and intrafamilial phenotypic variation has been reported, ADOA is characterized by an insidious onset of visual impairment in childhood, moderate to severe loss of visual acuity, temporal optic disc pallor, color vision deficits and centrocecal scotoma of variable density (Votruba et al., 1998a).

* Corresponding author. Tel.: +49 30 8413 1244; fax.: +49 30 8413 1383.
E-mail address: garshasb@molgen.mpg.de (M. Garshasbi).
[1] Present address: Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario, Canada M5G 1L6.
[2] These authors contributed equally to this work.

0014-4835/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2006.03.004

The *OPA1* gene consists of 31 exons coding for eight mRNA isoforms (Delettre et al., 2001). The OPA1 protein is thought to be the human orthologue of yeast MGM1, a dynamin-related protein essential for the maintenance of mitochondrial DNA (Jones and Fangman, 1992; Meeusen et al., 1999). Like MGM1, OPA1 contains an N-terminal basic mitochondrial localization domain, a GTPase domain and a dynamin central region (Delettre et al., 2000). Although mutations are spread throughout *OPA1*, the majority is clustered in the sequence encoding the GTPase domain and in exons 27 and 28.

We describe a six-generation Iranian family from the Western province of Kermanshah in which 38 family members are affected with OA (Fig. S1A). The diagnosis was based on standard ophthalmologic examinations, which comprised visual acuity, visual field and color testing, as well as fundoscopy, electrophysiology and retinal angiography. Our research followed the tenets of the Declaration of Helsinki. A gradual and progressive decrease in visual acuity was noticed from the age of 14–15 years in most patients. These observations, the absence of any other consistent phenotypic characteristics and the pattern of inheritance in this family, strongly support the diagnosis of ADOA.

Due to the diagnosis of ADOA, we performed linkage analysis including individuals IV:14, IV:15, V:23, V:24, V:25, V:27, V:28 and VI:7 (boxed family branch in Fig. S1A) with five microsatellite markers located on 3q28 in the vicinity of *OPA1* (D3S2398, D3S2418, D3S3562, D3S1265 and D3S1311) and four 18q12.2 markers in the vicinity of *OPA4* (D18S57, D18S535, D18S1118 and D18S474). Genomic DNA extraction, genotyping, multipoint linkage analysis and haplotype reconstruction were performed according to standard protocols. We found cosegregation between the chromosome 3 markers (Fig. S1B), but not the chromosome 18 markers (Fig. S1C), and the ADOA running in the family.

Genomic *OPA1* DNA from IV:14, IV:15, V:23 and V:25 was PCR-amplified as reported earlier (Pesch et al., 2001) and amplicons from exons 8–16 and 27 were directly sequenced using Big Dye Terminator chemistry (Applied Biosystems, Weiterstadt, Germany), resulting in the identification of a novel heterozygous missense mutation. This mutation, c.1313A>G (Fig. S2), leads to an aspartic acid to glycine substitution at position 438 in the GTPase domain (p.D438G).

Since the c.1313A>G mutation affects the first codon of exon 14, we investigated it at the mRNA level. A PCR product encompassing the mutation (primers 5′-ATC GTG GAT CTG AAA GTG ACA AGC-3′ and 5′-TGT TGT TCA ACA GAC TCT CGT ACC AT-3′) was amplified from oligo(dT)-primed cDNA synthesized from total lymphocyte RNA from subject V:25 using an RNA RT-PCR Kit (Takara, Shiga, Japan). Subsequently, the amplicon was directly sequenced. We found that c.1313A>G does not cause inappropriate splicing of *OPA1* transcripts.

A recent study in ADOA families with asymptomatic carriers of a c.2708del(TTAG) mutation resulted in penetrance figures of 43% and 62% (Toomes et al., 2001), which is considerably less than the 98% estimated earlier (Kivlin et al., 1983). Hence, genetic testing is of utmost importance to avoid providing incorrect genetic advice (Patel et al., 2002). Because of the variety of *OPA1* mutations and the extensive inter- and intra-familial phenotypic variability, direct assessment of *OPA1* cDNA may be the most straightforward way to conduct such genetic evaluation. To devise a sensible and cost-effective DNA-based screening protocol, it is important to recognize recurring mutations. Founder effects have been suggested (Delettre et al., 2000; Thiselton et al., 2001; Votruba et al., 1998b), but it is now becoming clear that those effects are less prominent than anticipated (Thiselton et al., 2002; Toomes et al., 2001) and that only few mutations occur in more than one family (Ferré et al., 2005). Interestingly, we found a novel missense mutation (c.1313A>G, p.D438G) affecting a position in the OPA1 GTPase domain which has also been affected by a different mutation (c.1313A>T, p.D438V) in two unrelated German families (Pesch et al., 2001), possibly indicating a mutation hot spot. However, seen the hundreds of families that have been screened for *OPA1* mutations, this observation could also be a chance event. Further mutation screening is indispensable to establish the mutation frequency at position c.1313.

Computer-aided alignment of the human OPA1 GTPase domain with homologues of 23 different species, shows a remarkable conservation of D438, ranging from human to the acoelomate *Schistosoma japonicum* (Fig. S3). The residue is not conserved in the yeast orthologues MGM1 (*S. cerevisiae*) and MSP1 (*S. pombe*) (data not shown).

Automated structural homology modeling of the OPA1 GTPase domain ($OPA1^{GTPase}$) on the Phyre (v.0.2) server yielded the *Dictyostelium* dynamin A GTPase domain ($dynA^{GTPase}$, PDB 1JWY) as the most similar structure (E $5.6e^{-26}$) (Niemann et al., 2001). After manual refinement, $OPA1^{GTPase}$ and $dynA^{GTPase}$ could be superposed with an r.m.s.d. of 0.29 Å for 199 common C_α atoms (Fig. 1B). Interestingly, our model shows that the resolved G1, G3 and G4 signatures, involved in the coordination of GDP/GTP and the Mg^{2+} ion (Bourne et al., 1991), essential for GTP hydrolysis, may be structurally conserved between both GTPase domains (Fig. 1).

Assessment of an ~15 Å sphere centered around the GDP molecule, shows that all $dynA^{GTPase}$ side chains reported to be engaged in coordination of the guanine moiety, the ribose sugar and the Mg^{2+} ion (Niemann et al., 2001), may be structurally and functionally mirrored in the $OPA1^{GTPase}$ model. For example, $dynA^{GTPase}$ T207, equivalent to $OPA1^{GTPase}$ T467, stabilizes the GDP pocket by interacting with the S36 ($OPA1^{GTPase}$ A299) carbonyl backbone and $dynA^{GTPase}$ K208 ($OPA1^{GTPase}$ K468) interacts, via a molecule of water (not shown), with the endocyclic ribose oxygen (Fig. 2A).

The affected D438 residue is, at ~5 Å from N7 of the guanine base, within reach for non-covalent interactions, such as electrostatic repulsion and attraction or hydrogen bonding, with the residues making up the G4 and G1 loops (Fig. 2B, left panel). First, D438 may be important in the T467 (G4)–A299 (G1) interaction, which itself aids in shaping the structure of the GTP-binding pocket by linking the G4 and G1 loops. Second, D438 may affect K468 (G4), which forms a hydrogen bond with the endocyclic ribose oxygen. These