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Experimental Eye Research xx (2006) 1–5

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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

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Received 28 November 2005; accepted in revised form 7 March 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).

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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

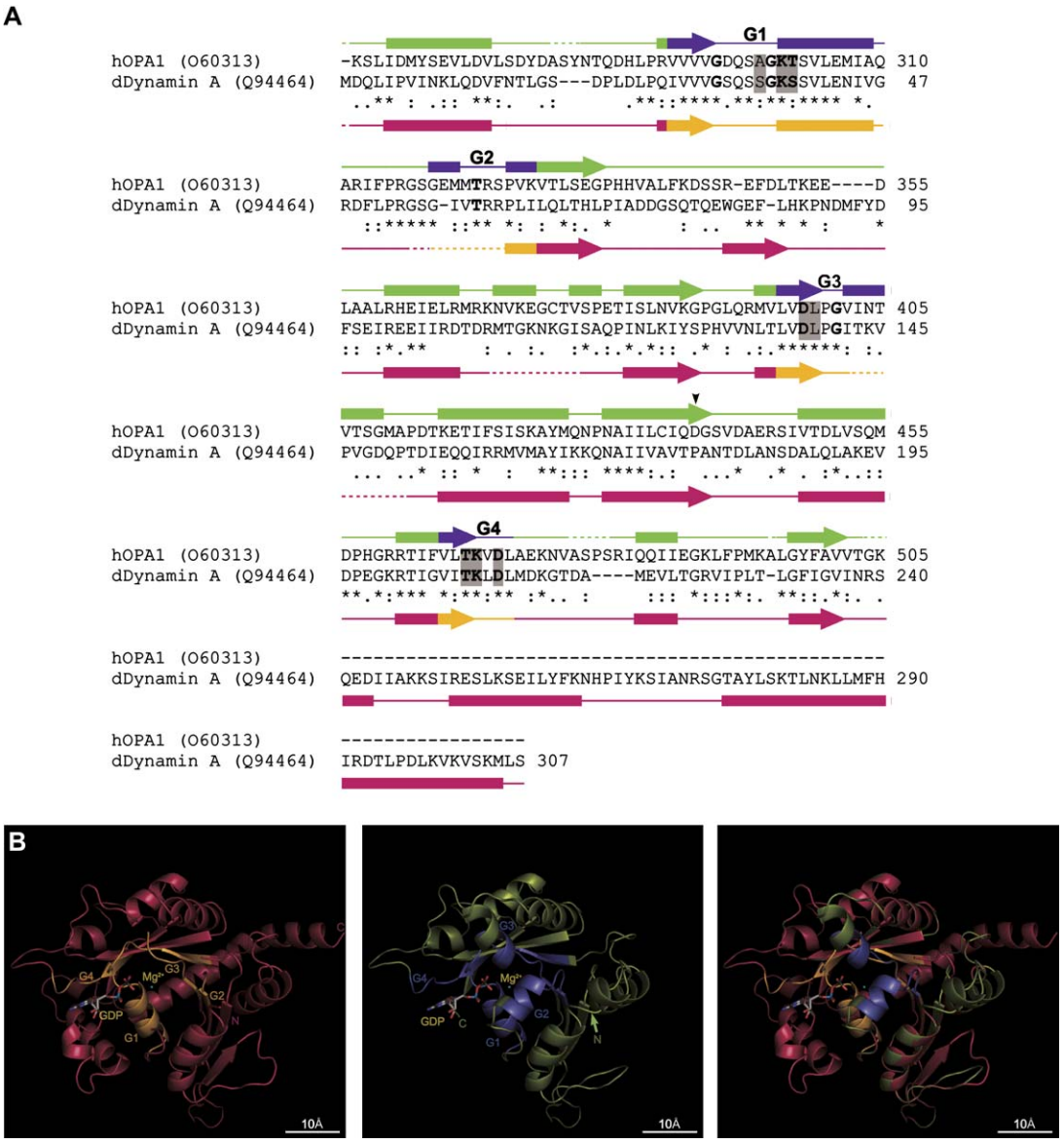

Fig. 1. The GTPase domains of OPA1 and dynA are structurally homologous. (A) Structure-based sequence alignment comparing the human OPA1 and *Dictyostelium* dynamin A GTPase domains. Rectangles represent helices, arrows β-sheets and dashed lines disordered regions. Identities are marked by an asterisk, conserved substitutions by a colon and semi-conserved substitutions by a dot. G1–G4 signatures are indicated, with the G1–G4 motif consensus residues depicted in bold face. Those residues highlighted in Fig. 2 are shaded in gray. D438 is indicated by an arrowhead. Color coding corresponds to the one used in panel B. (B) A rendering of the GDP-bound X-ray crystal structure of the *Dictyostelium* dynamin A GTPase domain (magenta), as reported by Niemann et al. (2001), is depicted in the left panel. A model of the OPA1 GTPase domain (green), based on the dynA^{GTPase} structure, is shown in the middle panel. The extent of homology between both structures can be appreciated from the overlay shown in the right panel. G1–G4 signatures are highlighted in yellow (dynA^{GTPase}) and in blue (OPA1^{GTPase}). N- and C-termini are indicated (N and C, respectively). C, light gray; O, red; N, blue; P_α, cyan-blue; P_β, dark gray; Mg^{2+}, cyan-green. Graphics were generated with PyMOL v.0.98 software (DeLano Scientific LLC, San Francisco, CA).

possibilities are not mutually exclusive. The interactions of D438 with its environment could be based on hydrogen bonding, for example influencing the T467–A299 and K468–ribose hydrogen bonds through charge transfer. Alternatively, the specific Van der Waals volume of Asp (91 Å^3), determining electrostatic repulsion, which is both energetically and structurally the most important interaction in protein folding, may be required to keep the important residues in place. This hypothesis is supported by the fact that dynamin A P178 (90 Å^3), corresponding to OPA1 D438 (Fig. 1A), has an almost identical Van der Waals volume.

Mutation of D438 to glycine (Fig. 2B, middle panel), as reported in this study, or to valine (Fig. 2B, right panel) as reported by Pesch et al. (2001), abolishes the possibility to form hydrogen bonds through their side chains. In addition, the Van der Waals volume of Gly (48 Å^3) is almost half that of Asp and the one of Val (105 Å^3) is markedly bigger than that of Asp. Those differences may affect the position and orientation of nearby side chains, such as that of K468. Both the lack of hydrogen bonding and the disorientation of side chains crucial in GTP coordination may result in suboptimal GTPase function.

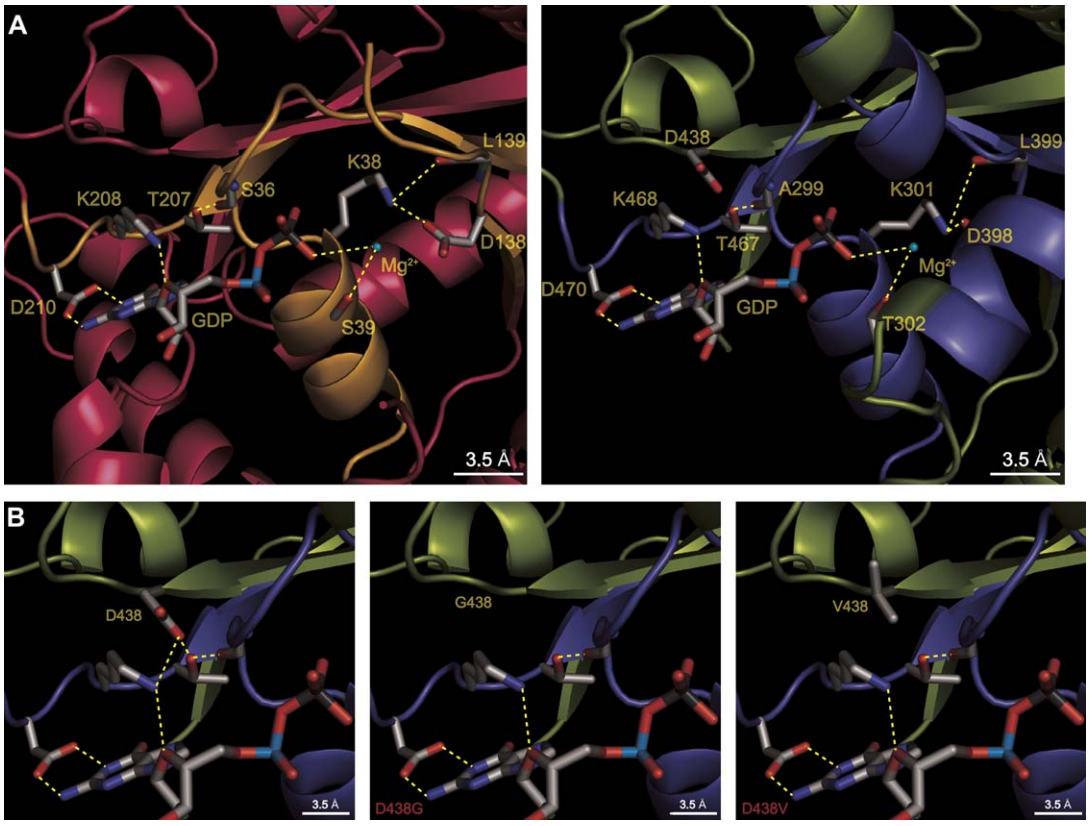

Fig. 2. The structural homology between the GTP-binding pockets of dynA^{GTPase} and OPA1^{GTPase} suggests that mutation of OPA1 D438 may influence GTP coordination. (A) Homology modeling reveals extensive structural similarity between the GTP-binding site of *Dictyostelium* dynA^{GTPase} (left panel) and OPA1^{GTPase} (right panel). A ~15 Å sphere centered around the GDP molecule is represented. Those residues that have been highlighted by Niemann et al. (2001) (left panel) and their counterparts in the nucleotide-binding pocket of OPA1 (right panel) are incorporated. The D438 residue is included in the OPA1 representation to show its localization relative to the nucleotide-binding site. Color coding is as in Fig. 1B, hydrogen bonds are represented by dashed lines. (B) *In silico* mutagenesis shows that both the D438G mutation (middle panel) in the Iranian family and the D438V mutation (right panel) in the German families lead to a loss of hydrogen bonds involving D438 (left panel), possibly influencing GTP coordination. However, other effects may also play a part (see text for explanation). Color coding is as in Fig. 1B.

It has been shown that the mitochondrial network is disorganized in ADOA patients with *OPA1* mutations (Delettre et al., 2000). Mitochondrial network formation and maintenance depends on equilibrium between mitochondrial fusion and fission (Bereiter-Hahn, 1990). Fusion of mitochondria can be dissected in fusion of the inner membrane, needing elevated levels of GTP hydrolysis, and fusion of the outer membrane, requiring low levels of GTP hydrolysis (Meeusen and Nunnari, 2005). In mouse embryonic fibroblasts, overexpression of an OPA1 K301A mutant, which is severely impaired in GTPase activity (Griparic et al., 2004), impedes mitochondrial fusion (Cipolat et al., 2004). Interestingly, postexercise phosphocreatine resynthesis, a measure of mitochondrial ATP production rate, is delayed in ADOA patients with *OPA1* mutations. Such an *in vivo* impairment of ATP synthesis points to the central role of mitochondrial dysfunction in the pathophysiology of *OPA1*-related ADOA (Lodi et al., 2004). Histological examination of a patient's eyes revealed diffuse atrophy of the ganglion cell layer of the retina with a loss of myelin and nerve tissue within the optic nerve (Johnston et al., 1979).

Taken together, we hypothesize that the p.D438G mutation, which causes ADOA in a large Iranian family, leads to misalignment of GTP in the GTP-binding pocket of OPA1's GTPase domain. As a consequence, GTP hydrolysis will be impaired, resulting in reduced mitochondrial function. Initially, carriers cope well with the ensuing energy deficiency. However, the highly energy-demanding and delicate retinal ganglion neurons eventually suffer gradual damage due to the continuous lack of sufficient energy, leading to the insidious onset of visual loss, characteristic for OA.

The next step will be to verify and, if necessary, to adjust our hypothesis by comparing wild-type and mutant OPA1 in GTP-binding and hydrolysis assays.

Acknowledgements

We are indebted to all family members. We are grateful to Kamran Ali-madadi, Omlila Ebrahimzadeh Khorasani, and Yosef Shafeghati for recruiting patients and collection of clinical data, and to Birgit Meyer for microsatellite typing. We thank Hans-Hilger Ropers for financial and moral support, Andreas Kuss for comments on the manuscript, Steffen Lenzner for assistance in initializing the molecular part of this study and Daniel Wilson for competent advice on the homology modeling.

Appendix A. Supplementary data

Supplementary data for this manuscript can be downloaded at doi:10.1016/j.exer.2006.03.004.

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