RESEARCH NOTE

Characterization of V71M mutation in the aquaporin-2 gene causing nephrogenic diabetes insipidus

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Introduction

The aquaporin-2 (*AQP2*) water channel plays an important role in reabsorption of water in the kidney's collecting duct and consequently concentrating urine (Deen *et al*. 1994). Binding arginine vasopressin (AVP) to its V2 receptor (AVPR2), leads to an increase in the intracellular cAMP levels, resulting in phosphorylation of AQP2, and possibly other proteins, by protein kinase A and subsequent redistribution of AQP2 from subapical storage vesicles to the apical plasma membrane. Driven by the interstitial hypertonicity, water reabsorption and urine concentration is thereby initiated. This process is achieved after the dissociation of AVP from its receptor (Nielsen *et al*. 1995; van Os and Deen 1998). Several mutations in the *AQP2* genes have been reported to cause congenital nephrogenic diabetes insipidus (NDI), a disease in which the kidney is unable to concentrate urine in response to AVP. These mutations cause NDI that is inherited as either an autosomal recessive or dominant trait (Rosenthal *et al*. 1992; Deen *et al*. 1994; van Lieburg *et al*. 1994; Mulders *et al*. 1998; Fujiwara and Bichet 2005; Iolascon *et al*. 2007).

In this study, we report a consanguineous family with an autosomal recessive NDI. We have identified a c.211G>A mutation in *AQP2* gene. It is the first mutation in this gene reported in North Africa. Structural modelling of the V71M mutant has shown that the cavity corresponding to the water channel seems to be reduced.

Materials and methods

Patients

The two patients reported in this study (a girl and her brother, aged 13 and 11 years, respectively) belong to a consanguineous family of Magrebine origin. They have a six-year-old healthy sister. They presented a polyuria, polydipsia (estimated at 8–10 l/day) syndrome since birth. Urine specific gravity is lower than 1.005 for the two patients. Biochemical analysis revealed a natremia of 147 and 148 mM, plasmatic osmolality of 290 and 295 mOsm/Kg for the girl and her brother, respectively. Plasma AVP measured by the radioimmunoassay was at the upper-normal limit (7.0 pmol/l and 6.6 pmol/l for the female patient and her brother, respectively). Oral fluid deprivation was not tolerated by both patients. DAVP was then given (intranasal, during one week in a dose of 10 μg twice a day) and no increase in osmolality was observed, indicating that the two affected children suffered from NDI. The occurrence of the disease in both sexes in this family (male and female patients) was suggestive of a defective *AQP2* mechanism. Informed consent for participation in this study was obtained from each subject.

Mutational analysis

Genomic DNA from the family members was extracted from the nucleated cells in the peripheral blood as previously described (Kawasaki and Erlich 1990). Intron-based primers were used in PCR to amplify the four exons of *AQP2* (Deen *et al*. 1994). PCR amplifications were carried out with hot start *Taq* DNA polymerase (GO *Taq* DNA polymerase, Promega). The amplified products were sequenced with ABI big dye terminator kit on an ABI prism 310 DNA Analyzer (Applied Biosystems, California, USA).

Structural modelling of the mutated aquaporin-2

The aquaporin-2 model was built using X-ray structure of lens aquaporin-0 open form (Lens Mip) as template (pdb

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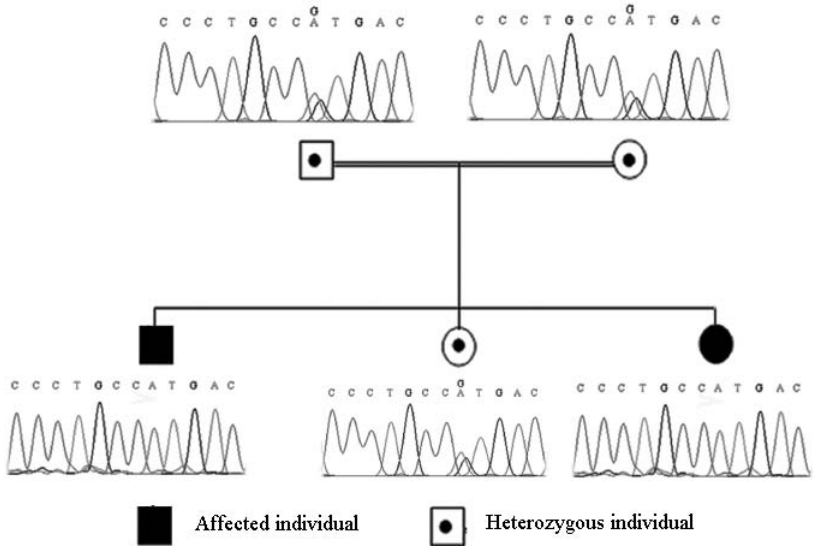

Figure 1. Electrophoregrams corresponding to exon1 sequence of *AQP2* gene in the studied family members.

code 2b6p) and the automated modelling server Swiss-PDB (SPDB) viewer (www.expasy.org/spdbv). The model was then subjected to energy minimization using the Gromo 96 software (http://igc.ethz.ch/gromos) implemented to the Swiss-PDB server. Three minimization steps were carried out containing each 1000 cycles of steepest descent, 1000 cycles of conjugate gradient, and 1000 steps of steepest descent. The use of alternate minimization methods was to increase the performance of the minimization strategy. Upon minimization, total energy was constant. A cut-off of 30 Å and a harmonic constant were used. The quality of the model was found acceptable using PROCHECK.

Results

Mutational analysis

Analysis of the *AQP2* gene in the two patients showed a homozygous G to A transition at position 211 in exon 1 (c.211G>A), leading to a substitution of valine by a methionine at amino acid 71 in the second intracellular loop of the aquaporin-2 water channel. Exon 1 was sequenced using DNA from all nuclear family members (the parents and their healthy daughter). All the three were heterozygous for this mutation, confirming an autosomal recessive inheritance of NDI (figure 1). Investigation of the exon 1 coding region of 100 normal chromosomes from a control group indicated that this mutation was not present in the normal population.

Structural modelling of the V71M mutant of aquaporin-2

To investigate the pathogenetic nature of this mutation in the 3D structure, we have performed its structural modelling. The overall structure of aquaporin-2 was found to be very similar to that of aquaporin-0 (figure 2,a). The root mean squared deviation (rmsd) between the aquaporin-0 structure used as template and the aquaporin-2 model is 1.95 Å for 199 alpha carbon atoms involved. This is not surprising since they share an amino acid identity of 60% over 250 residues. The mutated V71M aquaporin-2 structure was built by replacing *in silico* V71 by M71. A minimization step as described in the materials and methods was then applied. The residue 71 is located closer to the NPA motif and occupies a central position in the water channel (figure 2,b&c). The cavity corresponding to the water channel seems to be reduced due to a steric hindrance caused by a large hydrophobic side chain of M71 as compared to that of V71 in the aquaporin-2 wild-type (figure 2,c). The water channel is narrowed by the side chain of Met71. The diameter of this cavity does not allow the water molecule to get through since the distances of the Cξ of Met71 to the O of the hydroxyl group of Thr149 and to the O of Ala65 are 3.2 Å and 3.4 Å, respectively. The channel diameter in the wild-type molecule is higher than 3.5 Å, allowing the water transport. This fact might explain that this mutation can impair the water transport through the aquaporin water channel in the homozygous mutation.

Discussion

We have reported a consanguineous family with autosomal recessive NDI caused by a mutation in *AQP2* gene. No previous *AQP2* mutations has been described in individuals of North African ethnic origin. Molecular analysis showed a G to A transition at position 211 of the exon 1 of *AQP2* gene leading to a substitution of valine by a methionine at amino acid 71 in the second intracellular loop of the aquaporin-2 water channel. It is the same mutation reported previously in a consanguineous family of Pakistani origin (Marr *et al*. 2002). Results of multiple alignments of aquaporins from different species have shown that the valine amino acid

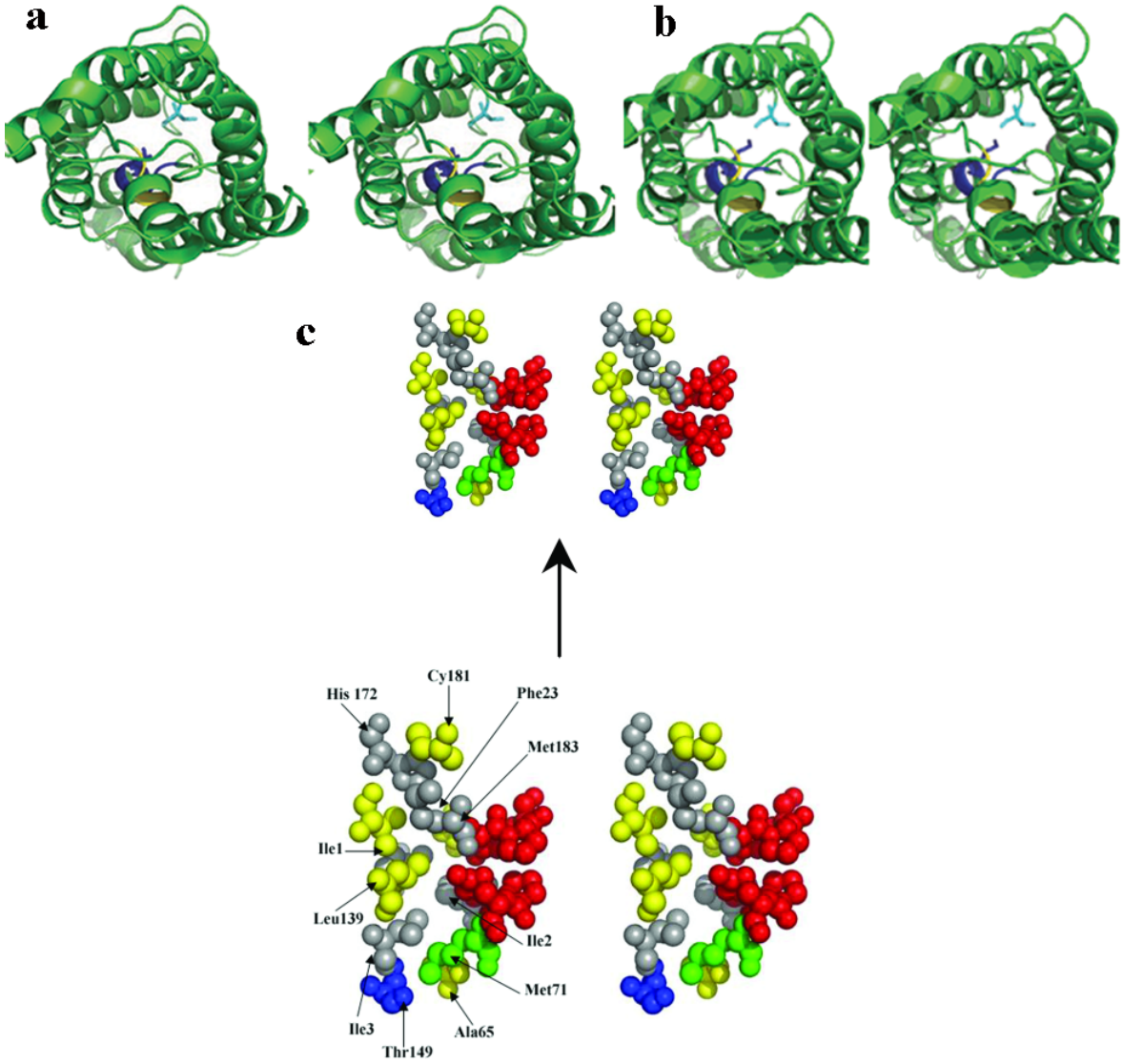

Figure 2. (a) Ribbon representation, in stereo, of aquaporin-2 model built using X-ray structure of lens aquaporin-0 open form (Lens Mip) as template (pdb code 2b6p), showing NPA sequences in blue and yellow. V71 (in blue) and Thr149 (in pink) are represented in sticks. (b) Same as (a), except that V71 is mutated by M71. (c) Spheres representation in stereo of the residues forming the AQ2 water channel. The upper panel is a bottom view of the lower one. The NPA motif is depicted in red. Met71 is shown in green and Thr149 in blue. The upper panel shows that the water channel is blocked by these latter residues.

at position 71 is conserved among all species that are investigated (data not shown). This amino acid (V at position 79 in *AQP1*) lies next to the asparagine-proline-alanine (NPA) (N68P69A70) motif of *AQP2* and belongs to helix HB. Several mutagenesis studies have indicated that mutations near the NPA motif alter aquaporin function, (Verkman and Mitra 2000) suggesting that this conserved region may be located at or near the water channel. Interestingly, in our *AQP2* model, the impaired activity of the *AQP2* M71 mutant might be due to the large methionine side chain, partly or totally blocking the water path in the aquaporin channel, as suggested by our structural modelling studies (figure 2,b). Indeed, the side chain of V71 is directed towards the water channel. On the other hand, alignment of *AQP1* (for which the mechanism of water permeability was well elucidated (Murata *et al*. 2000) and *AQP2* has shown that residues involved in the aqueous pore (deduced from modelling of *AQP1*) are well conserved between the two aquaporins except Ile191 and Leu75, that are replaced by Met183 and Ile67, respectively. Our model showed that Ala65 and Thr149 are involved in *AQP2* aqueous pore. In this model, M71 is located close to Thr149 (figure 2,c). As a consequence, the *AQP2* diameter was reduced and did not allow the water molecule to get through since the distances of the Cξ of Met71 to the O of Thr149 is only

3.2 Å. This finding argues for a direct effect of M71 mutation in reducing the aqueous pore in *AQP2* water channel. This would explain the findings of functional analysis of this mutation in oocytes of the African clawed frog (*Xenopus laevis*) which has demonstrated that the *AQP2* mutated water channel expressed in these oocytes were nonfunctional (Marr *et al*. 2002).

Mechanistic studies, as well as testing of pharmacological and gene therapies for NDI were rendered plausible with the first viable animal model of NDI, which was recently reported through forward genetic screening of ethylnitrosourea-mutagenized mice (Lloyd *et al*. 2005).

To conclude, this is the first report to characterize *AQP2* mutations associated with autosomal recessive NDI in North Africa and to show that the water channel was reduced due to the V71M mutation.

Acknowledgements

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