Molecular and Clinical Identification of A45T Mutation in AQP2 Gene

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OBJECTIVES

Diabetes insipidus (DI) is a disorder which is rarely seen and it is characterized by polydipsia and polyuria. Inadequate secretion of arginine vasopressin (AVP) from hypothalamus or inadequate response of kidney cells to AVP could be causes of DI. Therefore, any mutations in AVPR2, AVP and AQP2 genes which are the parts of that stimulation and response pathyway can cause DI.

In this study, mutational analyse was performed for A45T mutation in AQP2 gene.

METHODS

Histories from affected and unaffected family members were taken. The patient was subjected to sequencing for all exons of *AQP2* gene. PCR products were visualized on 1.5% agarose gel by electrophoresis. PCR products were purified with enzymatic purification (Exo-SAP) method before sequencing. Sequencing of purified PCR products was performed by using the Big Dye Terminator Cycle Sequencing v3.1 kit (Applied Biosystems, Foster City, CA, USA). Then the products were purified using ethanol/sodium acetate precipitation method and electrophoresed on an ABI PRISM 310 Genetic Analyzer. Comparison of three dimensional protein structures for wild type and mutant AVP-NPII were obtained with Swiss-Model. These structures were superimposed using UCSF Chimera 1.9. Ribbon display was obtained, mutant aminoacid was labeled and atomic structure was shown **(Figure 3.)**.

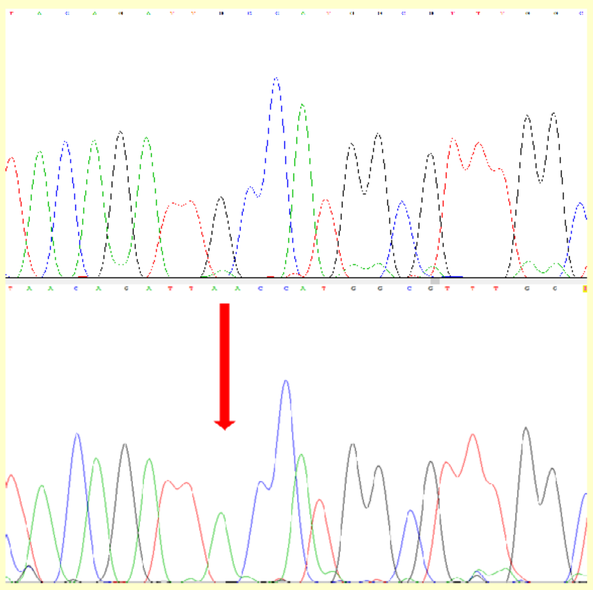

Figure 1.Sequence chromatograms demonstrating the homozygous mutations (p. A45T). Arrow designate the proband.

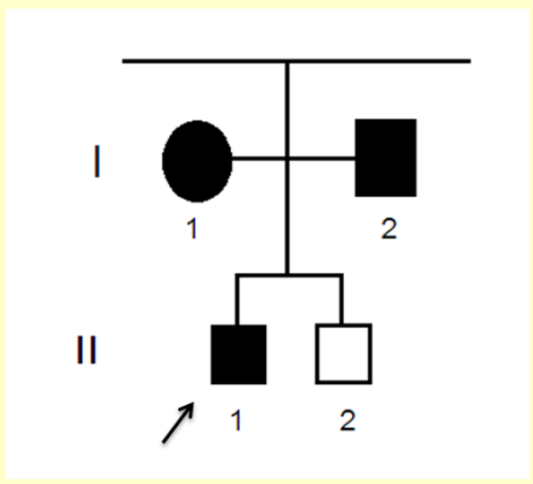

Figure 2. Pedigrees of familiy. The individuals marked with numbers are those who were available for mutation screening of the *AOP2* gene. Black and white symbols represent clinically affected and unaffected individuals, respectively. Arrow designate the proband.

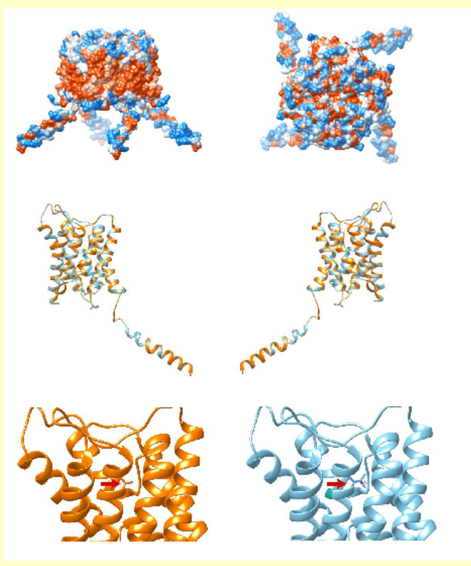

Figure 3. 3-D protein structures for wild type and mutant AQP2 were obtained with computational tools such as Swiss-Model and UCSF Chimera.

RESULTS

A total of three affected and one unaffected individuals were studied. We found a novel homozygous mutation (p. A45T) in exon 1 of *AQP2* gene in proband (case II.1) and affected parents had a heterozygous form this mutation (cases ; I.1, I.2,). Unaffected brother (case II.2) had no mutation **(Figure 2.)** Sequence analyses of the *AQP2* coding region revealed the presence of homozygous missense mutation at codon 45, which causes the the substitution of Ala (GCC) by a Thr (ACC) in exon 1 **(Figure 1.)**.
According to bioinformatics analyses based on DNA sequence, there was no difference between a three-dimensional protein structure prediction of mutant AQP2 protein and wild type protein **(Figure 3.)**.

CONCLUSIONS

In this study, we describe a novel mutation (p.A45T) of *AQP2* gene in Turkish family with NDI. In the literature , there are several hypotheses for the role of *AQP2* mutations in NDI patogenesis. In generally, *AQP2* gene mutations have been identified lead to misdirection of the mutant protein; but it has also been reported in the absence of functional AQP2 water channel.

In our study, we present a novel mutation in exon 1 of the *AQP2* gene. We suggest that future functional investigations of the A45T mutation may provide a basis for understanding the pathophysiology of the NDI. For that reason, in our future studies we are planning to do functional characterization of the A45T mutation. We think that functional characterization of this mutant protein will improve the clinical and theoretical knowledge of this area. The opportunity to manipulate the cellular machinery associated with protein folding and trafficking may provide the tools for novel pharmacotherapeutic strategies that may be use in the treatment of this form of NDI.

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