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Case Report

A novel mutation in *AVPR2* causing congenital nephrogenic diabetes insipidus with complete resistance to antidiuretic hormone

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Abstract

A 6-month-old male infant presented with failure to thrive. Hypernatraemia and elevated serum osmolality in the presence of low urine sodium and osmolality led to the diagnosis of diabetes insipidus. Administration of 1-deamino-8-D-arginine vasopressin (dDAVP) neither decreased urine volume nor increased urine osmolality indicating congenital nephrogenic diabetes insipidus. Molecular analysis in the arginine-vasopressin receptor-2 gene (*AVPR2*) located on chromosome Xq28 demonstrated a novel 5-base pair deletion (c.962–966delACCCC; g.1429–1433delACCCC) leading to a shift of the reading frame (p.Asn321fs) and a premature termination codon implying an absent or non-functional protein. Treatment with hydrochlorothiazide, amiloride and indomethacin led to a favourable clinical course.

Keywords: *AVPR2*; congenital nephrogenic diabetes insipidus; frameshift mutation; molecular analysis

Introduction

Congenital nephrogenic diabetes insipidus (CNDI) is characterized by a resistance of renal collecting duct cells to antidiuretic hormone (ADH) resulting in a reduced ability to reabsorb water [1]. Leading symptoms are polyuria and polydipsia, typically recognized in infancy. Other presenting symptoms include fever of unknown origin or failure to thrive. An autosomal recessive form and rare dominant forms have been ascribed to mutations in the aquaporin-2 (*AQP2*) gene [2] (OMIM 125800). Approximately 90% of patients are males, the X-linked recessive form (OMIM 304800) caused by mutations in the gene encoding the renal vasopressin V2 receptor (AVPR2, GenBank accession L22206) [3] being most prevalent. The *AVPR2* gene is located on Chr Xq28 and consists of three exons. AVPR2 is expressed in epithelial cells of the renal collecting duct system. It consists of 371 amino acids incorporating seven transmembrane, four extracellular and four cytoplasmatic domains [4]. Mutations may be found throughout the entire coding region, favouring transmembrane domains [5].

Here we report a novel inherited 5-base pair *AVPR2* deletion causing CNDI.

Case report

A 6-month-old male infant was admitted to our hospital for diagnostic workup of failure to thrive. The pregnancy, delivery and postnatal adaptation had been normal. At the age of 8 weeks marked failure to thrive was noticed. Initial treatment included various dietary regimens. The mother supplemented the daily fluid intake with water at the infant's demand. At 6 months of age, despite formula intake of 140–160 ml/kg/day, adequate weight gain was not achieved. Since birth, the body weight had dropped from the 90th to the 3rd percentile.

On admission, the patient's weight was 5710 g (3rd percentile), height was 64.5 cm (3rd percentile) and head circumference was 43 cm (20th percentile). Psychomotor development appeared normal for age. The infant's mother described recurrent episodes of constipation, a persistent moderate increase of body temperature and reported that the child had always been feeding well. During hospitalization with feeds on demand we observed an elevated fluid intake of 260 ml/kg/day and a maximum urine output of 8.9 ml/kg/h.

Laboratory data demonstrated hypernatraemia (Na 160 mmol/L), hyperchloraemia (Cl 127 mmol/L) and a high total serum protein. The serum osmolality was elevated (339 mOsm/kg) while urine analysis showed a low to normal osmolality of 209 mOsm/kg with a low sodium concentration (<20 mmol/L). Clinical and laboratory investigations did not disclose any other abnormalities.

An intravenous challenge test with 0.6 μg 1-deamino-8-D-arginine-vasopressin (dDAVP) was performed. Urine was collected for 6 h. The patient hourly received a fluid volume equal to that of the previous hour's urine production.

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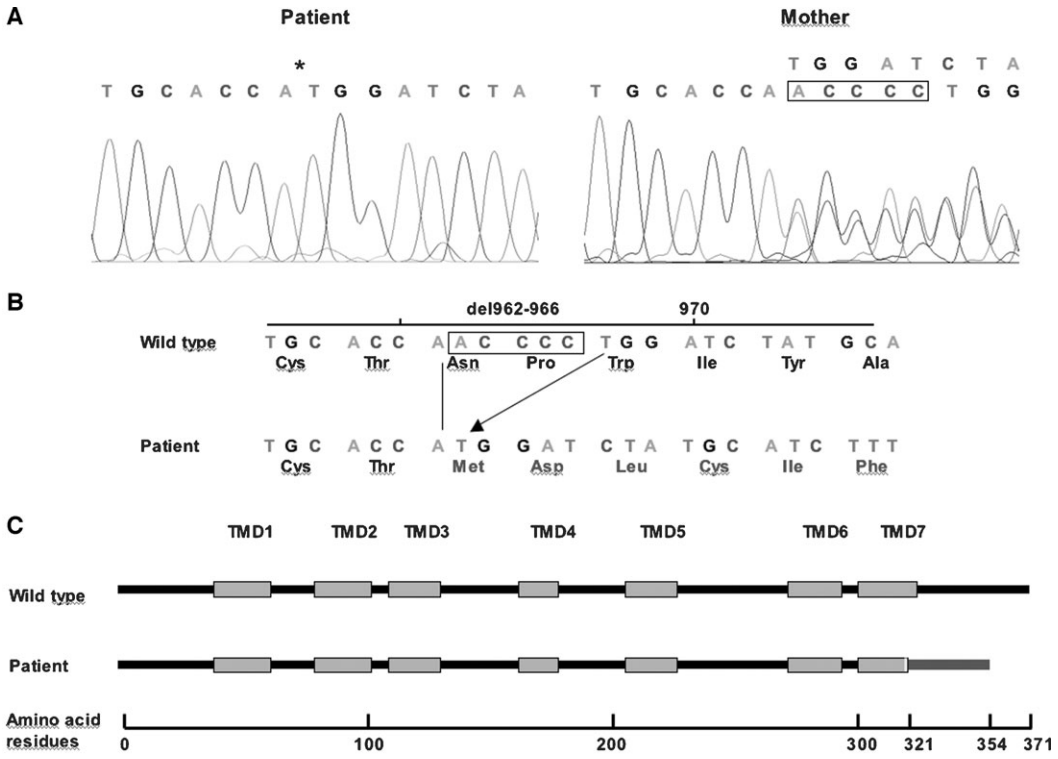

Fig. 1. Results of the AVPR2 DNA analysis. (**A**) The 5 bp deletion in the hemizygous patient is indicated by an asterisk (*). His mother carries the defective (upper line) and a normal allele (lower line). (**B**) As a consequence of the frameshift, all amino acids including and following position 321(Asn) are wrong. However, instability of the altered protein and virtual absence may result. (**C**) The amino acid sequence corresponding to and following the 7th transmembrane domain (TMD) is altered and the deduced protein is truncated due to a termination signal at position 354.

The patient did not reveal a decrease in urinary volume (hourly urine production remained at $\geq$7.5 ml/kg), and urine osmolality remained $<$160 mmol/L despite mild hypernatraemia (153 mmol/L) and hyperosmolality (339 mosmol/L). During the course of the test, serum osmolality and serum sodium concentration remained unchanged. Based on the lack of response to dDAVP, central diabetes insipidus was excluded. An increased serum ADH level of $>$30 ng/L before the dDAVP test supported the diagnosis of CNDI. Coagulation factor VIII and von Willebrand factor did not rise within 4 h after dDAVP administration, indicating impaired functional integrity of AVPR2.

Oral treatment with indomethacin (1.2 mg/kg/day), hydrochlorothiazide (1.6 mg/kg/day) and amiloride (0.15 mg/kg/day) was started as soon as the diagnosis was established. Urine volume and laboratory parameters normalized and a normal weight gain was achieved.

At 2 and a half years of age, the patient has been treated with a low sodium diet (maximum sodium administration 2 mmol/kg/day), hydrochlorothiazide (1.3 mg/kg/day), amiloride (0.1 mg/kg/day) and indomethacin (1.2 mg/kg/day). A normal constant weight gain and a favourable psychomotor development have been observed.

Molecular analyses

Mutation analysis of the *AVPR2* gene in the patient and his mother was performed. Genomic DNA was isolated from blood leucocytes by the salting out method [6]. PCR amplification of the entire coding region in a single PCR-reaction of 1602 base pairs was performed using oligonucleotide primers as previously published [7]. Sequencing of the three exons was performed with exon-specific intronic primers.

All PCR reactions were carried out using the Expand High Fidelity system (Roche Molecular Biochemicals, Mannheim, Germany) and the following conditions: (35 cycles) 96°C for 1 min; 58°C 1 min 40 s; 68°C 1 min 40 s; final extension 68°C 5 min. PCR products were visualized after 1.5% agarose gel electrophoresis, and purified fragments (Qiaquick PCR Purification Kit, Qiagene, Germany) were sequenced using the Big Dye-terminator cycle sequencing kit (Applied Biosystems, Weiterstadt, Germany) on an ABI 3730 automated fluorescence sequencer (Applied Biosystems).

We found a novel deletion of five base pairs c.962-966delACCCC (g.1429-1433delACCCC) beginning at amino acid 321 in exon 3 of the *AVPR2* gene (Fig. 1 A, B). It results in a frameshift (p.Asn321fs). The mutation affects the 7th transmembrane helix. Due to the frameshift, all amino acids from position 321 are altered. In addition, position 354 is altered to a termination codon, the resulting protein consisting of 353 instead of 371 amino acids (Fig. 1C). The mutation was confirmed in the heterozygous state in the patient's mother and grandmother. Cascade screening was offered to the family to identify potential carriers.

Discussion

203 different *AVPR2* mutations have been reported to cause X-linked diabetes insipidus. The identification of disease-causing mutations and–as a consequence–molecular studies of these in appropriate models have led to a more detailed understanding of molecular mechanisms of AVPR2 defects. An article by Fujiwara and Bichet summarizes the molecular biology of hereditary nephrogenic diabetes insipidus [8]. Most mutations (≥50%) are point mutations 85% of which are amino acid exchanges (missense mutations). Small deletions, such as identified in our patient, account for ∼20% of all disease-causing mutations. While nonsense mutations and many missense mutations (due to intracellular trafficking defects) lead to a full functional defect, a genotype–phenotype correlation appears to exist, since a number of missense mutations may be mild in nature and correspond to a partial phenotype with a diminished but not fully absent response to dDAVP. Mutations affecting ligand binding domains tend to retain partial signalling *in vitro*, whereas those introducing a charged residue in a transmembrane domain are inactive [5].

In the family presented here, no response to dDAVP was observed such that a null allele would be expected. Since nonsense mutations at positions 323 [9] and 337 [10] as well as missense mutations at position 321 (N321D, N321K [9]; N321Y [5]) as well as at position 322 (P322H, [11]) show the full phenotype, it may be concluded that the p.Asn321fs mutation completely disrupts the affected allele and causes ADH-unresponsive CNDI. In addition to the confirmation of a diagnosis with immediate specific therapeutic consequences, the molecular diagnosis enables genetic counselling and carrier identification such that affected newborns may be identified immediately after birth in order to avoid developmental sequelae caused by delayed diagnosis and treatment. Therefore, the identification of the underlying molecular defect should be attempted in any newly identified case.

Conflict of interest statement. None declared.

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