

Case Report

Novel Variant of *AVPR2* Giving Rise to X-Linked Congenital Nephrogenic Diabetes Insipidus in a 7-Month-Old Danish Boy

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Keywords

X-linked congenital nephrotic diabetes insipidus · Antidiuretic hormone receptor 2 · *AVPR2* · Genetic testing

Abstract

Patients affected with congenital nephrogenic diabetes insipidus (CNDI) have reduced ability to concentrate urine. Early diagnosis of CNDI is important to avoid recurrent episodes of severe dehydration. We present a Danish male suffering from typical symptoms and diagnosed with CNDI at the age of 7 months. Gene sequencing of this proband and his mother revealed a novel variant in the gene encoding the antidiuretic hormone receptor (*AVPR2*). The variant is a deletion of nucleotide c.151 in exon 2 of *AVPR2* (GenBank NM_000054.4:c.151del). This 1bp deletion is predicted to cause a frameshift that results in tryptophan replacing valine at position 51 in *AVPR2* and a premature stop codon three codons downstream (p.Val51Trpfs*3) likely resulting in faulty expression of the receptor. Identification of disease-causing variants such as the one described here contributes to precise diagnosis, especially in carriers and newborns,

thus preventing the long-term physical and intellectual disability observed in some CNDI-patients.

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Introduction

Congenital nephrogenic diabetes insipidus (CNDI) is an inherited disease characterized by excessive thirst and polyuria from birth. The kidneys of subjects suffering from the condition respond improperly to diuretic hormone and fail to reabsorb water. The condition can arise either from a non-functional receptor of vasopressin, the antidiuretic hormone receptor 2 (*AVPR2*), or from dysfunction of an aquaporin (*AQP2*), the dedicated water channel of the principal cell of the collecting duct [1]. Patients with CNDI are at risk of severe dehydration and hypernatremia. The defect is present from birth, and the initial symptoms of the child are typically irritability, poor feeding, and failure to thrive [2]. Constipation, intermittent hyperpyrexia, and vomiting are common symptoms of the condition. If left untreated, the repeated episodes of dehydration can lead to brain edema, seizures, and risk of intellectual disability. The chronic polyuria may cause bladder dysfunction and reflux nephropathy in the most severe cases.

The majority of patients with CNDI carry variants in the *AVPR2* gene [1]. The *AVPR2* gene consists of 3 exons. To date, over 287 disease-causing variants of the *AVPR2* gene have been identified (Human Genome Mutation Database, www.hgmd.org). Based on the mechanism of dysfunction, the variants have been divided into five classes [3]. Class I variants either truncate the receptor protein or create unstable mRNA. Class II variants include missense or nonsense variants, leading to a misfolded protein not able to egress the endoplasmic reticulum. Class III and IV variants alter the amino acids in a way that either impair the receptor's affinity for vasopressin or disturb the downstream signal from the vasopressin receptor. Class V variants yield a fully translated receptor, but the receptor is routed to an improper cellular compartment.

The *AVPR2* gene is located on chromosome X (regionXq28), and the variants follow an X-linked recessive inheritance pattern mostly affecting male individuals. Female carriers can experience varying degrees of polyuria and polydipsia. This might resolve from skewed X-inactivation [1, 4, 5]. Some patients present with a partial resistance to vasopressin and can, with high levels of vasopressin, still concentrate the urine [6, 7]. Infrequently, when CNDI arises from variants in the gene encoding aquaporin 2 (*AQP2*), the condition is transmitted in an autosomal recessive manner.

If left untreated, CNDI can be lethal, and it is of uttermost importance to identify the disease at an early stage. Identification of CNDI-causing variants can help with prompt and secure diagnosis without the need of fluid deprivation tests. In this study, we present a novel nonsense mutation in *AVPR2* in a Danish patient suffering from CNDI with a phenotype of complete diabetes insipidus.

Case Presentation

A 7-month-old male was admitted to the hospital referred with failure to thrive and dehydration. From the age of 5-months to admission, his weight had stagnated at 6,500 g. The laboratory workup revealed severe hypernatremia (serum sodium 171 mmol/L), high serum osmolality (355 mmol/kg) and elevated plasma vasopressin (10.3 pmol/L). Initial 24-h urine

collection confirmed the polyuria (750–820 mL/day) and the hyposthenuria (specific gravity of 1.004). Administration of vasopressin (Minirin® iv 2.5 µgr × 2) had no effect on urine output or urine specific gravity. This constellation of findings led to the diagnosis of nephrogenic diabetes insipidus. A formal fluid deprivation test was not performed due to the state of the patient with long-term dehydration and young age. No other family members reported symptoms of polyuria or polydipsia. The mother of the patient admitted having a higher urine output than others, but this was well tolerated, and she declined further testing.

Gene Sequencing Analysis

Years later, on request from the family, the patient was referred to genetic analysis. Blood samples were collected from the proband and his mother. Genomic DNA was extracted from leukocytes, and the three exons of *AVPR2* were amplified by PCR as described previously [8]. The PCR products were sequenced bi-directionally using BigDyeTerminator v1.1 Cycle Sequencing Kit and AB1310 analyzer (Applied Biosystems, Foster City, CA, USA). Sequence data was compared to the *AVPR2* cDNA sequence (GenBank NM_000054.4) with the Sequencer v.3.1.1 software (Gene Codes Corporation, Ann Arbor, MI, USA). The genetic analysis of the proband revealed a deletion of guanine at nucleotide c.151 in exon 2 of *AVPR2* (GenBank NM_000054.4:c.151del). This deletion is predicted to cause a frameshift p.Val51Trpfs*3, where tryptophan replaces valine at position 51 in *AVPR2*, creating a premature stop codon three codons downstream. The mother was heterozygous for the same variant (Fig. 1). The variant was inferred with Alamut Visual software (<http://www.interactive-biosoftware.com/alamut-visual/>) and appears to be a novel CNDI-causing variant, not registered in the Human Genome Mutation Database (HGMD, www.hgmd.org) or gnomAD (<http://gnomad.broadinstitute.org>).

Treatment

At the time of diagnosis, a combination therapy of hydrochlorothiazide (25 mg) + amiloride (2.5 mg) was started in addition to adequate fluids and a low sodium diet.

Outcome and Follow-Up

During hydrochlorothiazide and amiloride treatment, the serum sodium of the patient normalized (135 mmol/L). Renal ultrasound was normal. At 15 years of age, the patient is still on hydrochlorothiazide (50 mg) + amiloride (5 mg × 2) treatment and a low sodium diet. The patient is thriving and reports a slightly increased water intake and nocturia 1–2 times/night. Psychomotor development has been normal. Due to low height, the patient was tested positive for growth hormone deficiency, and growth hormone substitution therapy was commenced.

Discussion

We herein describe a novel variant in the *AVPR2* gene in a child with CNDI. The deletion of a nucleotide at position 51 in *AVPR2* results in a premature termination codon three codons downstream. The variant fits to be a class I type of mutation. Conceivably, the mutation may affect protein production in two possible ways. One possible outcome is that the mRNA is degraded by the non-sense mediated decay mechanism (NMD), a pro-survival mechanism of the cell which removes faulty mRNAs from the cell [9]. NMD is initiated if the premature termination codon is more than 50 nucleotides upstream from a 3' exon-exon junction [10]. The mutation of our proband is located 930 nucleotides upstream from a 3' exon-exon junction, and

NMD is therefore a plausible pathophysiological explanation. An alternative possibility is that the translated protein escapes NMD control, but is degraded by the protein quality control system as an incomplete protein is produced [11]. Either way, the genetic variant prevents the principal cell of the collecting duct to produce normally functional *AVPR2* protein.

There are several benefits in mapping out CNDI-causing variants in *AVPR2* and *AQPR*. Primarily, it permits a precise diagnosis of the patients. Secondly, newborns at risk of getting CNDI can be easily identified when a carrier status of the X-linked variant is known. In such subjects, treatment can be initiated prior to the development of severe dehydration and hypernatremia. Early intervention might prevent the long-term physical and intellectual disability as is observed in some CNDI patients. Genetic testing of CNDI can also prevent misdiagnosis of patients. Generally, the diagnosis of nephrogenic diabetes insipidus is based on symptoms, a 24-h urine collection, and a fluid deprivation test. However, such tests can be challenging especially in infants and may lead to severe dehydration. Furthermore, the clinical characterization of partial diabetes insipidus forms and other polyuric conditions can be challenging. A genetic test can confirm the diagnosis as well as give a further understanding to the genotype-phenotype relation and a better understanding of the pathogenesis behind CNDI. Finally, in the future, if and when gene therapy becomes reality, conditions caused by gene variants such as the one described here may become amendable for curation.

In conclusion, we describe a novel deletion of guanine that occurs at nucleotide c.151 in exon 2 of *AVPR2*. The deletion likely results in faulty expression of the receptor, explaining the X-linked CNDI of our proband.

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Statement of Ethics

The Regional Committee on Biomedical Research Ethics Denmark exempts this study for approval as the patient was referred for clinical diagnostic testing by clinicians. The patient and his mother gave informed consent to genetic testing and to the conception of this article. They both read and approved the final version of the manuscript for publication and gave written informed consent.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contribution

M. Pulczynska Wason and J. Hvarregaard Christensen contributed substantially to the conception of this work. J.E. Sollid, S. Joshi, M. Pulczynska Wason, S. Rittig, J. Hvarregaard Christensen, and K. Kamperis contributed substantially to data collection and data analysis. J.E. Sollid drafted the manuscript together with S. Joshi, J. Hvarregaard Christensen, and K. Kamperis. All authors have revised the manuscript critically for important intellectual content. The final version of the manuscript has been approved by all authors.

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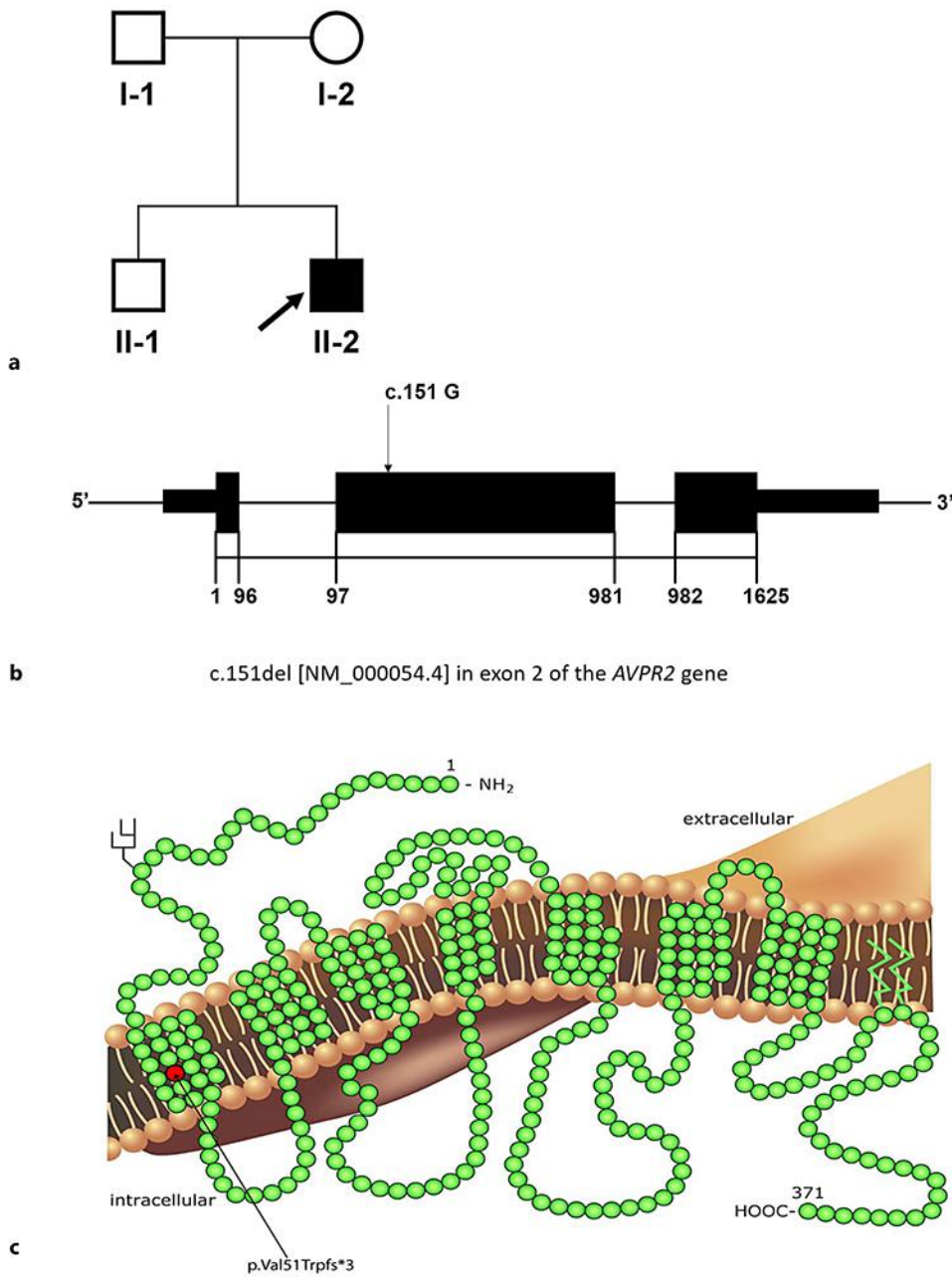


Fig. 1. Illustration of inheritance and location of described genetic variant. **a** Pedigree of family to patient with congenital nephrogenic diabetes insipidus. Squares, male; circle, female; filled symbol with arrow, affected proband. The proband and his mother were genetically tested. **b** Location of c.151del in exon 2 of the *AVPR2* gene. Wide black boxes represent the three exons with cDNA numbering for respective exons, and narrow black boxes represent the untranslated regions of *AVPR2*. The black line represents the untranslated region of *AVPR2*. The location of the variant c.151del is illustrated by a black arrow. **c** Illustration of *AVPR2* with location of affected amino acid illustrated in red. The variant, p.(Val51Trpfs*3), is predicted to cause a frameshift where tryptophan replaces valine at position 51 in *AVPR2*, creating a premature stop codon 3 codons downstream. Illustration adapted with permission from Faerch et al. [6].