NDT Plus (2009) 2: 20–22
doi: 10.1093/ndtplus/sfn163
Advance Access publication 31 October 2008

NDT PLUS
Nephrology Dialysis Transplantation

Case Report

A novel disease-causing mutation in *AVPR2*: Q96H

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Abstract

A 4-month-old male infant was diagnosed with nephrogenic diabetes insipidus (NDI). Genetic testing of the arginine vasopressin receptor-2 (*AVPR2*) yielded a novel X-linked mutation, termed Q96H, in both the propositus and his mother; there was no family history. Protein sequence comparison between AVPR subtypes shows that Q96 is part of a highly conserved motif. Many other disease-causing mutations, confirmed with *in vitro* expression studies, map to surrounding residues. Molecular modelling studies showed that the equivalent residue in *AVPR1* is likely critical for vasopressin binding. We posit that Q96 must be important for the integrity of *AVPR2* function.

Keywords: *AVPR2*; DDAVP; nephrogenic diabetes insipidus; vasopressin

Introduction

Diabetes insipidus (DI) is a likely diagnosis for a child presenting with dehydration, high plasma sodium (P_{Na}) and osmolality (P_{Osm}) and inappropriately low urine osmolality (U_{Osm}) [1]. Because infants with DI are only mildly hypovolaemic, the degree of polyuria is often underappreciated on initial history [1]. The two forms of DI (nephrogenic and central) are distinguished by administering exogenous vasopressin (DDAVP): failure to increase urine osmolality is diagnostic for nephrogenic DI (NDI) [1].

The goal of NDI therapy is to promote the overall water balance. This is achieved using liberal administration of water, reduction in dietary salt intake and attenuation of the urine output with adjunct medications (hydrochlorothiazide with or without indomethacin and/or potassium-sparing diuretics) [2]. Mutations in the *AVPR2* are a likely cause for NDI [3].

In this report, we present a case of NDI who harbours a novel missense mutation in the transmembrane domain 2 (TM-2) of *AVPR2*. Based on the results of expression studies done with mutant homologous receptors, Q96 is predicted to be important for the integrity of *AVPR2*'s interaction with vasopressin, its cognate ligand.

Case report

The patient presented at 4 months of age, born at term from a healthy mother, with a prolonged history of lethargy, poor feeding, vomiting, irritability and poor weight gain, all of which were noted during the first month of life. On presentation, heart rate was 140 beats/min, respiratory rate 40 breaths/min, blood pressure 82 mmHg/pulse and temperature 36.7°C. Mucus membranes and capillary refill were normal. Birth and current weights were 3.7 kg (50th percentile for age) and 5.6 kg (10th), respectively. Family history was negative for renal diseases, and the parents were unrelated (Figure 1a). The most salient laboratory abnormalities were high P_{Na} (153 mmol/L) and P_{Osm} (315 mOsm/kg H_2O). After admission, persistently high volumes of dilute urine were noted (20–23 ml/kg/h; U_{Osm} 80 mOsm/kg H_2O). Urine sodium was undetectable. NDI was confirmed after two failed trials of DDAVP. After starting NDI therapy, urine output decreased to 3–4 ml/kg/h, and both P_{Na} and P_{Osm} normalized. When seen at 24 months, the patient was doing well, P_{Na} and P_{Osm} were still in the normal range and his weight was up to 15.6 kg (50th percentile).

Results and discussion

Genetic testing was performed to clarify the etiopathology of this infant's NDI. DNA extraction, amplification and direct sequence analysis from a blood sample were performed according to the standard protocol [4]. Sequence analysis of the *AVPR2* gene revealed a novel missense mutation, referred to as Q96H (glutamine → histidine). The mother carries the same X-linked mutation (Figure 1b). Genetic counselling was provided to the mother and the maternal aunt (who was not tested and has no children).

As of 2008, there are 193 distinct disease-causing *AVPR2* mutations described in 307 NDI families (Figure 2a) [3].

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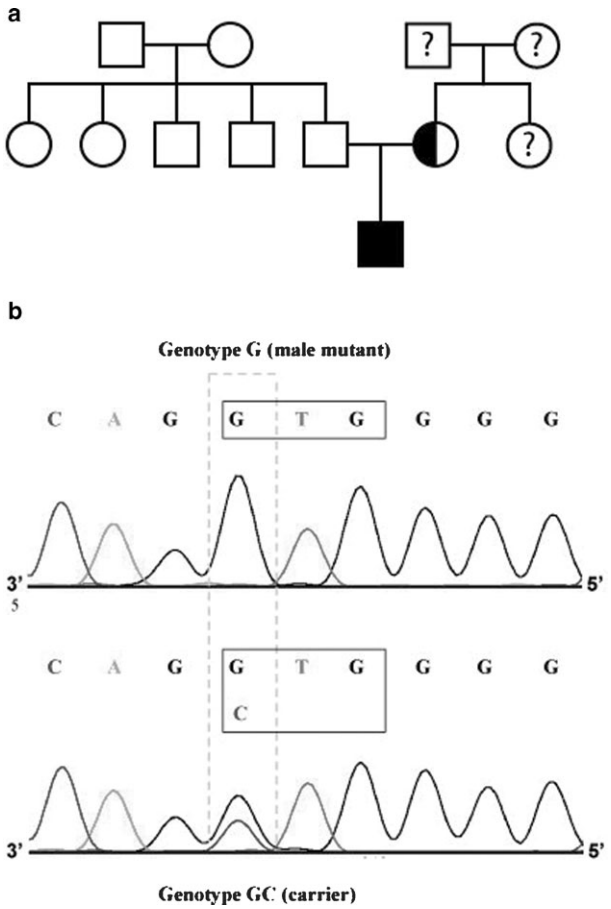

Fig. 1. (**a**) Family tree of the proband (indicated by an arrow). (**b**) DNA chromatogram of the affected proband and his mother, who is a carrier of the mutated (GTG) allele (the normal amino acid is CTG).

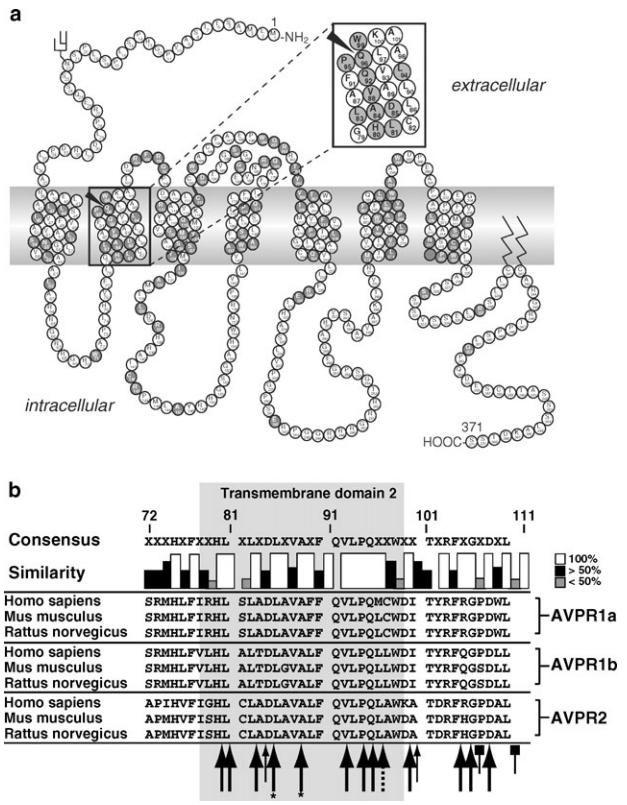

Fig. 2. (**a**) Schematic representation of AVPR2 modified from Bichet [3] to include the new mutation Q96H (arrow). Please see http://www.medicine.mcgill.ca/nephros for a list of mutations. (**b**) Sequence homology in TM-2 (and flanking regions) of various *AVPR* subtypes in human, mouse and rat. The novel mutation (located at residue Q96, dotted arrow) is part of one of the largest and most extensively conserved areas amongst the various proteins of this family. Disease-causing mutations in *AVPR2* have been reported in the residues highlighted at the bottom. Thick arrows indicate mutations in highly conserved regions, thin arrows in less conserved areas and squared arrows refer to regions with poor homology. Sequence accession numbers, in descending order: P37288.1, Q62463.1, P30560.4, Q9WU02.1, P30518.1, O88721.1 and Q00788.2. Sequences were aligned using the software Geneious Pro 3.5.6 (www.geneious.com); residue numbering is based on Homo sapiens *AVPR2*.

The mutation Q96H included herein has not been reported, yet. Residue 96 is part of TM-2, which encompasses residues 78–98 of *AVPR2*. The predicted polypeptide contains 371 amino acids with a structure typical of G-protein-coupled receptors with seven transmembrane domains [3]. The comparison of the sequences of *AVPR2* and its homologues *AVPR1a* and *AVPR1b* in three species (human, mouse and rat) reveals that residue Q96 is part of a unique string of six amino acids representing a highly conserved motif (91–96; Figure 2b). This region may thus play an important role for AVPR2's function. Not surprisingly, *AVPR2* disease-causing mutations have been reported in contiguous residues (92 [5], 94 [4] and 95 [6]), as well as in a large number of other highly conserved TM-2 residues (Figure 2a) [3].

It is instructive to refer to *in vitro* expression studies in COS-7 cells performed with previously identified *AVPR2* mutants to assess the potential impact of Q96H. Three mutations have been reported in TM-2. Expression of *AVPR2* harbouring mutations at residues 83 (lysine → glutamine) and 88 (valine → methionine) showed poor cell surface expression despite appropriate biosynthesis [7]. In contrast, impaired vasopressin-binding ability was demonstrated for AVPR2 mutated at residue 105 (phenylalanine → valine) [8]. Unfortunately, *in vitro* confirmatory studies are unavailable for disease-causing mutations at residues 92, 94 and 95.

Additional indirect evidence for the importance of Q96 for AVPR2 function is derived from studies on AVPR1, a homologue of AVPR2. Substitution of alanine for glutamine at residue 218 resulted in a 290-fold reduction in vasopressin affinity [9]; importantly, Q218 is the structural equivalent to AVPR2's Q96. Molecular modelling of the AVPR1 bound to vasopressin reveals that the C-terminus of AVP is predicted to 'form a strong hydrogen bond with Q218 on TM-2' [9]. The impact of Q218A on the AVPR1 function may help predict that of Q96H on AVPR2 since in both cases a hydrophilic amino acid (glutamine) is replaced by a hydrophobic amino acid (alanine or histidine). However, since histidine is positively charged and alanine is neutral, the consequence of each missense mutation may be distinct.

Conclusion

Herein, we present a case of NDI whose symptomatology appears to be due to a novel disease-causing mutation in TM-2, termed Q96H. Based on comparative analysis of analogous peptides, the expected impact of Q96H on AVPR2 is to reduce significantly the efficacy of the ligand vasopressin in activating the downstream signalling pathway. Further studies of the mutant protein expressed in an *in vitro* system will be required to elucidate in detail the functional relevance of the reported novel *AVPR2* mutation.

Conflict of interest statement. None declared.

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Received for publication: 2.10.08
Accepted in revised form: 3.10.08