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 (PNa) 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 PNa (153 mmol/L) and P_Osm (315 mOsm/kg H2O). After admission, persistently high volumes of dilute urine were noted (20–23 ml/kg/h; U_Osm 80 mOsm/kg H2O). 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 PNa and P_Osm normalized. When seen at 24 months, the patient was doing well, PNa 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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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 [5, 4 and 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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