CLINICAL STUDY

Autosomal recessive familial neurohypophyseal diabetes insipidus: onset in early infancy

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Abstract

**Background:** Familial neurohypophyseal diabetes insipidus (FNDI), usually an autosomal dominant disorder, is caused by mutations in the arginine vasopressin (AVP)–neurophysin II preprohormone leading to aberrant preprohormone processing and gradual destruction of AVP-secreting cells. Patients typically present between 1 and 6 years of age with polyuria and polydipsia.

**Objective:** Clinical, biochemical, and genetic studies of three new cases of autosomal recessive FNDI presenting in early infancy.

**Patients:** Three Palestinian cousins presented with failure to thrive, vomiting, irritability, and fever. The parents were asymptomatic. Patients developed hypernatremia (154–163 mmol/l) and serum hyperosmolality (>320 mOsm/kg), while urine osmolality remained between 73 and 229 mOsm/kg. Plasma AVP levels were low, and the posterior pituitary bright spot was absent on magnetic resonance imaging (MRI). All patients responded to desmopressin.

**Results:** Patients were homozygous and parents were heterozygous for microsatellite markers flanking the AVP gene. All patients were homozygous for the P26L (proline to leucine) substitution affecting mature AVP. A founder effect with the single original kindred carrying the P26L mutation was confirmed by microsatellite analysis, but patients in that family presented only at 2 years of age. In microsatellite analysis, the new kindred patients were not homozygous and did not share a single allele at the aquaporin 2 and vasopressin receptor-2 genes locuses.

**Conclusion:** This is the first description of autosomal recessive FNDI presenting in the neonatal period. The unusual early clinical and radiological (MRI) presentation argues against gradual destruction of AVP-secreting neurons as the pathophysiological mechanism. Factors beside allelism of AVP-related genes must influence the age of FNDI presentation given the founder effect demonstrated for the P26L mutation.

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Introduction

Neurohypophyseal or central diabetes insipidus is a disorder of water conservation resulting from a deficiency of arginine vasopressin (AVP). It is usually caused by tumors, infection, or trauma (1). Familial neurohypophyseal diabetes insipidus (FNDI) is a rare disorder accounting for only 1% of central diabetes insipidus patients (2); it is caused by mutations in the AVP gene on chromosome 20 (3), usually inherited in an autosomal dominant pattern (4). FNDI typically presents between the ages of 1 and 6 years with polyuria and polydipsia (5–7), and is radiologically characterized by the loss of the posterior pituitary bright spot on T1 magnetic resonance imaging (MRI), indicating dysfunction of the neurohypophysis (8–11).

AVP is synthesized as a preprohormone by specialized hypothalamic neurons with long extensions projecting through the diaphragma sellae to form the neurohypophysis (12). Biosynthesis of AVP starts with tightly regulated transcription of the AVP gene (13), continues with regulated translation and enzymatic cleavage of the AVP–neurophysin II (AVP–NPII) prohormone (14–17), and ends with active AVP secretion into the circulation upon an appropriate stimulus (i.e. hyperosmolality and hypovolemia).

The first of the three exons of the AVP gene (14–15) encodes a signal peptide, AVP, and the amino terminus of NPII; the second encodes the NPII central region, and the third gives rise to NPII’s carboxy terminus and to a glycoprotein (16). Since the first reported AVP–NPII
mutation (18), 56 others have been described, mostly in the signal peptide or the NPII moiety of the preprohormone. These are presumed to interfere with preprohormone processing leading to neuronal damage and gradual destruction of AVP-secreting cells (19). Only one autosomal recessive mutation causing FNDI (P26L, affecting the seventh of nine amino acids of the mature AVP hormone) has been previously described. The three affected children in that kindred all developed polyuria between 1.5 and 2 years of age (20).

The present study describes clinical and molecular studies of three cases of autosomal recessive FNDI in newly ascertained kindred (the second described worldwide) (20), but with an unusual age of presentation, in very early infancy. This distinct presentation strongly suggests the existence of genetic or environmental factors that can modify the phenotype of FNDI.

**Methods**

**AVP assay**

Blood samples were collected into ice-chilled tubes following discontinuation of desmopressin treatment for at least 36 h. Plasma AVP levels were measured by RIA with a preceding extraction procedure as described by Glick et al. (21).

**Microsatellite markers**

Genomic DNA was extracted from whole blood samples from patients 1–3 and their parents, as well as from one patient from the previously described kindred, using the MagNA Pure Compact System and kit (Roche Diagnostics GmbH). Patients were genotyped for microsatellite markers using fluorescence dye-labeled forward primer and unlabeled reverse primer and PCR conditions as described in the ABI manual (ABI, Applied Biosystems, Foster City, CA, USA). The microsatellite markers analyzed were D20S906, D20S842, and D20S193 flanking the AVP gene; D12S85 and D12S368 flanking the aquaporin 2 (AQP2) gene, and DXS8069 and DXS1073 flanking the vasopressin receptor (V2R) gene. Fluorescently labeled PCR products were pooled, and 1–2 µl were sampled into 9 µl loading buffer (formamide with GENESCAN 400HD (ROX) size standard, Applied Biosystems). PCR products were analyzed with the 3700 Automated DNA Analyzer using GENESCAN and GENOTYPER software (Applied Biosystems).

**Sequencing of the AVP gene**

Genomic DNA was amplified using a PTC 225 DNA Engine (MJ Research Hercules, CA, USA). Fifty nanograms of DNA sample were added to 10 pmol of each primer. 1.5 mM MgCl2, 0.2 mM dNTP's, 1×PCR Gold buffer (15 mM Tris–HCl, pH 8.0, 50 mM KCl), and 0.4 unit of AmpliTaq Gold DNA polymerase (both from ABI) in a total volume of 25 µl. PCR conditions included initial 12-min denaturation at 95 °C. 37 cycles of 1 min at 92 °C, 1 min at 55 °C and 1 min at 72 °C. 10 min at 72 °C. Primers for amplification and sequencing of the AVP gene were exon 1: F: gctatggctgccctgagatggccacagtg, R: tggcggccgcgtctcgcctccacgggaaca; exon 2: F: cgcccccccccaggcccgcccccgccgcgc, R: tcgctgcgttccccgtcacaacccctcgactc; exon 3: F: cctctctccctctttcccttcccgccag, R: cccaggcgcccgtgctcacacgtccctcccg.

After PCR cleanup (incubation of 5 µl PCR reaction with 2 µl SAP/Exo (USB) for 1 h at 37 °C followed by 15 min at 80 °C), automated DNA sequencing was performed using Big-Dye terminator cycle sequencing chemistry on the ABI PRISM 3700 DNA Analyzer.

![Figure 1](https://www.eje-online.org)

**Figure 1** Pedigrees of the affected families; (A) new and (B) previously reported (Texas). Affected homozygous cases are indicated by filled squares or circle and numbered. Half-filled squares or circles indicate heterozygotes. Numbers within the boxes indicate number of siblings not individually plotted in the pedigree. Consanguinity is indicated by double lines.
Informed consent was obtained from study participants, and studies were approved by the Institutional Review Boards of Hadassah Hebrew University Medical Center and Southwestern Medical Center.

Results

Clinical presentations and studies

Three affected children, cousins in single extended Palestinian kindred (Fig. 1A, patients 1–3), were studied. Each presented with irritability, vomiting, diarrhea, failure to thrive, and intermittent fever developing gradually during the first few weeks of life. Hypernatremia, high serum osmolality and inappropriately low urine osmolality and inappropriately low AVP levels (0.77, 0.75, and 2.77 pg/ml respectively) were consistent with diabetes insipidus (Table 1). Anterior pituitary hormone levels were normal, but the posterior pituitary bright spot could not be visualized by MRI (performed at 2 months of age at normal serum osmolarity levels of 285–290 mOsm/l) in any of the three patients. All responded well to desmopressin treatment and grew and developed normally. Patients 4–6 (Fig. 1B), from an unrelated Arab kindred described previously (20), presented later in childhood and had relatively high AVP levels. Desmopressin replacement was initiated only at 4.3 years for case 4, 2.3 years for case 5, and 1.7 years for case 6 (Table 1). The mother and

Table 1 Clinical and laboratory characteristics of affected patients.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age of first symptom (years)</th>
<th>Na</th>
<th>Osm blood</th>
<th>Osm urine</th>
<th>AVP (pg/ml)</th>
<th>MRI</th>
<th>Current age (years)</th>
<th>Height centile (cm, percentile)</th>
<th>Other clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>F</td>
<td>0.1</td>
<td>158</td>
<td>320</td>
<td>229</td>
<td>0.77</td>
<td>Absent bright spot</td>
<td>8.75</td>
<td>129, 25</td>
</tr>
<tr>
<td>Case 2</td>
<td>M</td>
<td>0.1</td>
<td>159</td>
<td>283</td>
<td>73</td>
<td>0.75</td>
<td>Absent bright spot</td>
<td>7.5</td>
<td>125, 50</td>
</tr>
<tr>
<td>Case 3</td>
<td>M</td>
<td>0.1</td>
<td>154</td>
<td>315</td>
<td>189</td>
<td>2.77</td>
<td>Absent bright spot</td>
<td>5.2</td>
<td>110.4, 50</td>
</tr>
<tr>
<td>Case 4*</td>
<td>M</td>
<td>4.25</td>
<td>149</td>
<td>312</td>
<td>67</td>
<td>6.8</td>
<td>Absent bright spot</td>
<td>18.9</td>
<td>185.5, 91</td>
</tr>
<tr>
<td>Case 5*</td>
<td>M</td>
<td>2</td>
<td>147</td>
<td>317</td>
<td>81</td>
<td>8.4</td>
<td>Absent bright spot</td>
<td>15.3</td>
<td>180.1, 89</td>
</tr>
<tr>
<td>Case 6*</td>
<td>F</td>
<td>1.25</td>
<td>140</td>
<td>293</td>
<td>428</td>
<td>3.8</td>
<td>Normal</td>
<td>13.2</td>
<td>158.5, 56</td>
</tr>
</tbody>
</table>

Osm, osmolarity.

*Part of the clinical data of cases 4–6 is cited from Ref. (20).

Figure 1 Linkage analysis of the AVP–NPII V2R and AQP2 genes. (A) Map of the AVP gene locus and its flanking regions on chromosome 20 indicating the positions of the microsatellite markers D20S906, D20S842, and D20S193 used for linkage analysis. (B) Example of a microsatellite marker study on cases 1–3 and family members of patient 2. Cases 1–3 are homozygous for short tandem repeats (STRs) length for the D20S193 marker; (one single gray peak at 140) compared to the parents and brother of case 2 who are heterozygous (two gray peaks). Case 4* from kindred B is also homozygous for this marker with the same length of 140 (suggesting a founder effect). (C) A table summarizing the results of the linkage analysis with the three microsatellite markers. Cases 1–3 are homozygous for short tandem repeats (STRs) lengths for all three markers flanking the AVP–NPII gene whereas the parents and a healthy brother (of case 2 shown as an example for the three families) are heterozygous. Case 4* from the previously reported kindred (Fig. 1B) has the same genotype as cases 1–3. (D) Table summarizing the results of linkage analysis with microsatellite markers flanking the aquaporin 2 (AQP2) and V2 receptor (V2R) genes. None of patients 1–3 was homozygous at AQP2, and there was no common shared allele at the AQP2 or V2R loci.

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cases 5 and 6 also had sensorineural deafness. MRI studies revealed no bright spot in patients 4 and 5, but in patient 6 the bright spot was still present at 2 years in spite of clinically apparent diabetes insipidus (DI). The growth rate of patients 4 and 5 was deteriorated during adolescence and following diagnosis of GH deficiency by GH stimulation tests (peak secretion – 0.27 and 3.0 ng/ml respectively) replacement therapy was initiated with good response. Patient 4 was also diagnosed with hypogonadotrophic hypogonadism during adolescence. The consanguineous parents of the affected children in each kindred were unaffected with normal osmolarity and AVP levels, suggesting autosomal recessive inheritance.

Molecular studies
All three new cases had identical homozygous genotypes for the D20S906, D20S842, and D20S193 microsatellite markers flanking the AVP gene on chromosome 20 (Fig. 2B and C), whereas the parents were heterozygous: this finding strongly suggested that a mutated AVP gene caused the DI in our patients.

Sequencing of the AVP gene revealed that all three patients were homozygous for a missense mutation at nucleotide 301 (C to T) (Fig. 3), predicting a substitution of proline to leucine (P26L) at the seventh amino acid residue of mature AVP. The parents were heterozygous for this mutation. No other mutations were found in the AVP gene.

The finding of the same mutation in patients 1–3 in Israel and in the previously reported kindred in Texas (20), despite no known relation between the two Palestinian families, led us to look for a founder effect. The oldest patient in the previous kindred indeed had identical alleles in the microsatellite markers flanking the AVP gene on chromosome X, nor did the three patients share a common allele at this locus.

The dominant negative effect caused by even one out of four aberrant AVP–NPII subunits in a tetramer may account for the typical dominant mode of inheritance (25–27). The location of the P26L mutation, however, has not been considered crucial for folding or processing of the AVP preprohormone (22, 28, 29). Thus, the P26L prohormone, unlike mutations associated with autosomal dominant FNDI, is apparently folded, dimerized, and transported with little effect on cellular integrity in the heterozygous state. Although the processing and actual secretion pattern of the mutant AVP in neuroendocrine cell lines (e.g. Neuro 2A) has not been investigated yet, affected patients are able to secrete mutant AVP. This mutated hormone has a 30-fold decrease in binding to the V2 receptor and a tenfold decrease in ability to stimulate adenylate cyclase (20). Therefore, in the homozygous state, it was thought that the dramatically increased levels of AVP secretion required to maintain antidiuresis could eventually exhaust vasopressinergic neurons, leading to their death. Yet, in the newly ascertained kindred, all patients described here developed DI within weeks of birth, and their AVP levels were not elevated. The unequivocal absence of the bright spot in T1-weighted MRI images in the neonatal period of these patients may suggest prenatal or neonatal neuronal loss or complete failure to synthesize, accumulate, or maintain stores of mutant AVP–NPII prohormone in neurosecretory granules. This contrasts with the persistent bright spot at almost 2 years of age in patient 6 from the original family.

Why would the three patients reported here present so much earlier than patients in the previously reported kindred (1.5–5 years) carrying the same mutation?

Discussion
FNDI is a rare disease caused by mutations in the AVP gene, most of which are located in the region encoding the NPII moiety. We describe the fifth reported kindred worldwide with a mutation affecting mature AVP, and the second with autosomal recessive mode of inheritance (20). All affected infants in our kindred were homozygous for a mutation predicting a substitution of leucine for proline at position 7 of AVP itself (P26L in the AVP–NPII preprohormone).

The unique location of this mutation may be responsible for the unusual recessive mode of inheritance. The presumed pathophysiological mechanism in most cases of FNDI involves a defective tetrameric three-dimensional structure of the prohormone and aberrant intracellular prohormone processing (22–24). Eventually, this results in cellular damage from abnormal accumulation of unprocessed AVP–NPII tetramers.

To identify other mutated proteins contributing to the early clinical presentation of DI in patients 1–3, we looked for common inheritance of alleles for genes encoding two proteins mediating the effects of AVP in the kidney, the AVP type 2 receptor (V2R), and the AQP2 water channel. No patient in our kindred was homozygous for microsatellite markers flanking the AQP2 gene on chromosome 12; moreover, these three cousins did not share a common allele at this locus.

(Fig. 2D). Patient 1, a female, was not homozygous for microsatellite markers flanking the V2R gene on chromosome X, nor did the three patients share a common allele at this locus.
In spite of the known difficulty to accurately determine the exact age of the clinical presentation of DI especially in young patients, the difference in the time of presentation between the two families is major and should be addressed. One explanation could be a second mutation in our kindred, further interfering with AVP function, and decreasing the mutant AVP secretion capacity, but no such mutation was detected by sequencing all three exons of the AVP gene. Moreover, the affected patients in both kindreds share the same microsatellite alleles flanking the AVP gene locus, consistent with a founder effect.

The remaining possibilities include either epistasis with other genes or environmental stressors in the Palestinian family, compared with the first reported family, which lives in Texas. The lack of alleles in common for downstream proteins of the antiurea signaling pathway in the kidney (V2R or AQP2) did not support the involvement of these classical proteins in the pathogenesis of DI in this kindred. Regarding environmental stressors, the new Palestinian kindred comes from a middle class socioeconomic status without shortage in either food supply or housing conditions. The climate is not that different between the two countries, and the three cousins were born in different seasons of the year with no significant variability in the time of clinical presentation between them.

It is also difficult to relate the GH deficiency developing during adolescence in patients 4 and 5 to their FNDI. Although growth retardation has been reported in FNDI (30), our cases were adequately treated by desmopressin and clinically well for years prior to their growth retardation. The anterior and posterior pituitaries are anatomically and functionally distinct structures, and it is difficult to see how cell death affecting neuronal projections into the posterior pituitary could affect the anterior pituitary by triggering, for example, an inflammatory process. Finally, the lack of any other deficiencies in cases 1–3 and 6 (or in typical cases of autosomal dominant FNDI) makes such a local inflammatory process an unlikely pathophysiological explanation for anterior pituitary deficits.

In conclusion, FNDI can present in the neonatal period. A founder effect was demonstrated for the two kindreds with the P26L autosomal recessive mutation in the AVP moiety of the AVP–NPII gene. The early clinical presentation challenges the classical pathophysiological concept that a gradual apoptosis of neurons secreting AVP–NPII in the hypothalamus is responsible for FNDI. Additional factors modulating AVP synthesis, processing, storage, and/or action must be postulated given the discrepant clinical presentations in the two kindreds carrying the P26L mutation.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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