CLINICAL STUDY

Clinical and molecular analysis of three families with autosomal dominant neurohypophyseal diabetes insipidus associated with a novel and recurrent mutations in the vasopressin–neurophysin II gene

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Abstract

Objective: To test further the hypothesis that autosomal dominant neurohypophyseal diabetes insipidus (adFNDI) is caused by heterozygous mutations in the vasopressin–neurophysin II (AVP-NPII) gene that exert a dominant negative effect by producing a precursor that misfolds, accumulates and eventually destroys the neurosecretory neurons.

Methods: Antidiuretic function, magnetic resonance imaging (MRI) of the posterior pituitary and AVP-NPII gene analysis were performed in 10 affected members of three unreported families with adFNDI.

Results: As in previously studied patients, adFNDI apparently manifested after birth, was due to a partial or severe deficiency of AVP, and was associated with absence or diminution of the hyperintense MRI signal normally emitted by the posterior pituitary, and with a heterozygous mutation in the AVP-NPII gene. In family A, a transition 275G → A, which predicts replacement of cysteine 92 by tyrosine (C92Y), was found in the index patient, but not in either parent, indicating that it arose de novo. The six affected members of family B had a transversion 160G → C, which predicts replacement of glycine 54 by arginine (G54R). It appeared de novo in the oldest affected member, and was transmitted in a dominant manner. In family C, six of 15 living affected members were tested and all had a novel transition, 313T → C, which predicts replacement of cysteine 105 by arginine (C105R). It, too, was transmitted in a dominant manner. As in other patients with adFNDI, the amino acids replaced by the mutations in these three families are known to be particularly important for correct and efficient folding of the precursor.

Conclusions: These findings are consistent with the malfolding/toxicity hypothesis underlying the pathogenesis of adFNDI. Moreover, they illustrate the value of genetic analysis in all patients who develop idiopathic diabetes insipidus in childhood, even if no other family members are affected.

European Journal of Endocrinology 146 649–656

Introduction

Autosomal dominant familial neurohypophyseal diabetes insipidus (adFNDI) is a rare disorder characterized clinically by the excretion of abnormally large volumes of dilute urine (1). It results from a deficiency of the antidiuretic hormone, arginine vasopressin (AVP), which develops several months to years after birth and usually progresses from partial to severe by late childhood or early adolescence.

adFNDI has now been associated with more than 35 different heterozygous mutations in the coding sequence of the AVP–neurophysin II (AVP-NPII) gene (1–18). This gene is located on chromosome 20p13 and contains three exons that encode (a) a signal peptide, AVP and the N-terminal portion of NPII, (b) the central portion of NPII, and (c) the C-terminal portion of NPII and a glycopeptide of unknown function, called copeptin (19). The mutations identified in adFNDI are varied in type and affect all moieties of the precursor except copeptin and the peptide links between them. However, all of them share the unique property of altering one or more amino acid residues known or reasonably presumed to be critical for proper folding and dimerization of the precursor (1, 20). This distinct pattern has led to the hypothesis that adFNDI is caused by production of a mutant precursor that is retained in the endoplasmic reticulum.
(ER) of the neuron because it cannot assume the three-dimensional conformation necessary to pass the ER quality-control mechanisms and transit through the Golgi to the neurosecretory granules where final processing, packaging and storage of the various components normally occur (1). Expression in vitro of several adFNDI mutations has confirmed that mutant precursor does in fact accumulate in the ER and is processed inefficiently to AVP and NPII in cells transfected with mutant cDNAs (21–27).

To further investigate the current theories about the pathogenesis of adFNDFI, we have characterized the phenotypes and identified the AVP-NPII gene mutations in three additional families.

**Methods**

**Clinical studies**

Questionnaires concerning the symptoms and signs of diabetes insipidus were sent to all known relatives of the proband in each of the three kindreds. Those who responded in the affirmative and gave written informed consent were admitted to the General Clinical Research Center of Northwestern University Medical School to verify the diagnosis of neurohypophyseal diabetes insipidus according to a standard procedure approved by the Institutional Review Board. This procedure included a careful history as to the age of onset of symptoms or signs of diabetes insipidus, in addition to measurements of fluid intake, urine output, urine osmolality, body weight, plasma osmolality, plasma electrolytes and plasma AVP under basal conditions with fluid available *ad libitum* and during a complete fluid deprivation test of duration sufficient to increase plasma osmolality and sodium above the normal range (28). If fluid deprivation resulted in urinary concentration before the requisite level of hypertonic dehydration was achieved, it was supplemented by an infusion of 3% saline (0.1 ml/kg per min for 80 min) and the measurement of plasma AVP was repeated when plasma osmolality and sodium concentration had increased beyond the normal range. In all cases, the type of diabetes insipidus was determined by analyzing the relationship of plasma AVP to the concurrent plasma and urine osmolality (Table 1, Fig. 1) (28).

Sagittal and coronal T1- and T2-weighted high-resolution magnetic resonance imaging (MRI) of the pituitary gland was performed before and after administration of gadolinium, to exclude structural alterations, and to establish the presence or absence of the characteristic ‘bright spot’ signal of the posterior pituitary seen on T1-weighted images.

**Genetic analysis**

After informed consent had been obtained from the participants, genomic DNA was extracted from peripheral white blood cells using the Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN, USA). The entire coding sequence of the AVP-NPII gene was amplified by PCR as described previously (12). After purification of the PCR products, both strands were sequenced directly using a dRhodamine terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequencing products were analyzed on a 373 DNA sequencer (Applied Biosystems).

For haplotype analysis, five microsatellite markers flanking the AVP locus on chromosome 20p13 were amplified using fluorescently labeled primers (ABI). PCR was performed according to conditions suggested by the manufacturer. The samples were electrophoresed on 6% denaturing gels (0.4 mm) at 800 V/40 mA/28 W on a DNA sequencer (ABI 373 A, Applied Biosystems) and analyzed using Genescan 672 Software.

**Table 1 Clinical data**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal range</th>
<th>A 3</th>
<th>B 3</th>
<th>B 6</th>
<th>B 11</th>
<th>C 1</th>
<th>C 2</th>
<th>C 3</th>
<th>C 4</th>
<th>C 5</th>
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<td>91.2</td>
<td>70</td>
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<td>84</td>
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</table>

Uvol, urine volume; Uosm, urine osmolality; Posm, plasma osmolality; Pavp, plasma vasopressin; Basal, fluids available *ad libitum*; FD, fluid deprivation; AB, at birth.

1 Posm and Pavp after 3% saline infusion were 307 mosmol/kg and 0.7 pg/ml respectively.
2 Also has partial empty sella.
Results

Family A

Only one member of the family (Fig. 2, panel I) reported symptoms and signs of diabetes insipidus. By history, the symptoms began when he was about 10 months of age. When evaluated at 19 years of age, he excreted abnormally large volumes of very dilute urine and had a low plasma AVP under basal conditions with fluid available ad libitum (Table 1, Fig. 1). When he was deprived of fluid for 3 h, his plasma osmolality and sodium concentration increased to 301 mosmol/kg and 145 mmol/l, but his urine osmolality and plasma AVP remained inappropriately low, indicating severe neurohypophyseal diabetes insipidus. The hyperintense posterior pituitary signal was absent on T1-weighted magnetic resonance imaging.

Genetic analysis of the index patient revealed a heterozygous transition of 275G → A in exon 2, predicting a substitution of cysteine at position 92 by tyrosine (C92Y; Fig. 2, panel II) (nucleotide and amino acid numbering according to references (12) and (29)). The remainder of the coding sequence of the AVP-NPII gene showed no deviation from the wild-type sequence. The C92Y mutation, also referred to as C61Y within NPII, has been described previously in a family with adFNDI (10). The proband’s sister and both parents showed the wild-type sequence in all three exons. Comparison of the haplotypes in the AVP-NPII gene region clearly demonstrated that the patient, A/3, is the offspring of the clinically and genetically normal individuals, A/1 and A/2 (Fig. 2, panel I). Thus the C92Y germline mutation has occurred de novo.

Family B

Six members from three generations of this kindred reported symptoms and signs of diabetes insipidus and the pattern of inheritance was consistent with an autosomal dominant mode (Fig. 3, panel I). Of the three who agreed to further evaluation, one (B3) reported his diabetes insipidus was first noted when he was about 1.5 years of age and the other two (B6 and B11) recalled being told only that their diabetes insipidus began sometime soon after birth. With fluid available ad libitum, all three excreted abnormally large volumes of dilute urine and had low to undetectable concentrations of plasma AVP (Table 1, Fig. 1). When they were fluid-deprived for 1–2 h, their plasma osmolality and sodium increased to more than 300 mosmol/kg and 145 mmol/l, but their urine osmolality and plasma AVP remained inappropriately low (Table 1, Fig. 1), indicating severe neurohypophyseal diabetes insipidus.

Studies at the molecular level were undertaken in these three patients and 11 other members of the family. All three members with documented neurohypophyseal diabetes insipidus, in addition to three others who were symptomatic by history, showed a heterozygous transversion, 160G → C, in exon 2 (Fig. 3, panel II). This missense mutation substitutes arginine for glycine at position 54 (G54R) in the prohormone (position 23 in the neurophysin moiety). Substitution of glycine 54 by valine (G54V) has been identified in another adFNDI family and indicates that this residue is critical for normal processing of the AVP-NPII precursor (7). Haplotype analyses were consistent with paternity and indicated that the mutation occurred de novo in B3 (Fig. 3, panel I). This mutation was not found in eight other members of the kindred who denied signs and symptoms of diabetes insipidus (B1, B2, B5, B7, B10, B12, B13 and B14).

Family C

Twenty-one members from four generations of kindred C reported symptoms and signs of diabetes insipidus and the pattern of inheritance also was consistent with an autosomal dominant mode (Fig. 4, panel I). Of the six affected members who agreed to further evaluation, five reported that their diabetes insipidus was noted shortly after birth and one knew only that it had been present for as long as he could recall (Table 1). With fluid available ad libitum, all six of them, including a 60-year-old female, excreted abnormally large volumes of very dilute urine and had low plasma AVP under basal conditions with fluid available ad libitum (Table 1, Fig. 1). When they were fluid-deprived for 1–2 h, their plasma osmolality and sodium increased to more than 300 mosmol/kg and 145 mmol/l, but their urine osmolality and plasma AVP remained inappropriately low (Table 1, Fig. 1), indicating severe neurohypophyseal diabetes insipidus.

Figure 1 Relationship of plasma AVP to plasma osmolality in the affected individuals from families A, B and C. Values at baseline are filled; open symbols indicate values after dehydration. In individual C4, plasma osmolality and AVP were within the normal range after dehydration, but after infusion of 3% saline, plasma osmolality was 307 mosmol/kg and AVP was 0.7 pg/ml (*) (Table 1). The dashed line marks the limit of detection (LD) of the AVP radioimmunoassay.
large volumes of dilute urine and had very low to undetectable concentrations of plasma AVP. When fluid-deprived, five of the six increased their plasma osmolality and sodium concentration to at least 294 mosmol/kg and 143 mmol/l, but their urine osmolality and plasma AVP remained inappropriately low (Table 1, Fig. 1); the sixth (C4), however, increased his urine osmolality to 307 mosmol/kg when his plasma osmolality and sodium had increased only to 284 mosmol/kg and 139 mmol/l and his plasma AVP had increased minimally, if at all to 0.7 pg/ml (Table 1, Fig. 1). Repeat measurements after an infusion of 3% saline confirmed a plasma osmolality and sodium of 304 mosmol/kg and 147 mmol/l and his plasma AVP had increased minimally, if at all to 0.7 pg/ml (Table 1, Fig. 1). Repeat measurements after an infusion of 3% saline confirmed a plasma osmolality and sodium of 304 mosmol/kg and 147 mmol/l, but revealed no increase in plasma AVP, confirming that he had a marked but incomplete deficiency of the hormone.

In all six patients with documented pituitary diabetes insipidus, direct sequence analysis revealed the presence of a novel mutation in exon 2 (Fig. 4, panel II). The heterozygous transition, 313T -> C, results in the replacement of cysteine 105 by arginine (C105R) in the AVP-NPII precursor (C73R within NPII). C105 is involved in the formation of a disulfide bridge with C92, a residue that, if mutated, also results in the adFNDI phenotype (C92S/X/Y) (2, 10). In this family, no DNA was available from unaffected individuals.

Discussion

The genetic mutations identified in these three previously unreported families confirm and extend the pattern observed in other kindreds with adFNDI (1). Thus each alters an amino acid residue known or reasonably presumed to be particularly important for correct folding, oligomerization and transit of the prohormone through the endoplasmic reticulum in vasopressinergic neurons. The cysteine residues at positions 61 and 73...
in the neurophysin II moiety of the wild-type precursor (positions 92 and 105 in the preprohormone) normally form one of the seven intrachain disulfide bridge that serve to stabilize its three-dimensional conformation (30). Removal of the cysteine at either end of this bridge, as in the mutations identified in family A or C (Fig. 2, panel II; Fig. 4, panel II), would destabilize the folded prohormone and permit the formation of abnormal disulfide bridges with some of the other cysteine residues, either within the same or in other precursor molecules (1, 24). Such incomplete or incorrect formation of disulfide bridges appears to be a common sub-motif in the pathogenesis of adFNDI, as numerous other mutations identified in adFNDI also eliminate or create other cysteine residues in the precursor (1–3, 6, 8, 9, 12, 14–18).

The glycine residue at position 23 in the neurophysin moiety of the wild-type precursor (position 54 in the preprohormone) also has an important role in folding and dimerization, because it is part of a tight pocket that binds the N-terminus of the AVP moiety (30–32). Thus its replacement by the mutation identified in family B (Fig. 3, panel II) would also be expected to disrupt folding and dimerization by interfering with the binding process. Interference with the binding of AVP to neurophysin also appears to be a relatively
common sub-motif in the pathogenesis of adFNDI, because mutations that replace this glycine with arginine or valine have been identified in two other adFNDI families (7, 9). Other mutations identified in adFNDI also appear to impair binding by altering other residues in the binding pocket or the N-terminal part of the AVP moiety that normally fits in the pocket (1, 20).

The mechanism by which retention of misfolded mutant AVP-NPII precursor in the ER exerts a dominant negative effect on expression of the normal allele has not been completely established. Because the deficiency of AVP develops after birth and postmortem studies have consistently shown gliosis and a deficiency of magnocellular neurons in the hypothalamic–neurohypophyseal tract (33–37), we and others have postulated that the large accumulations of misfolded mutant precursors eventually destroy the neurons that produce them. This neurotoxicity hypothesis also receives some support from in vitro expression studies of several adFNDI mutations (21, 23–27). It has also been proposed that some mutant precursors impair expression of the normal allele by forming heterodimers with the wild-type precursor (26) – an effect that we have not been able to demonstrate in our own expression studies (J. Rutishauser and M. Speiss, unpublished observations). In this context, it should also be noted that another mutation that alters the C-terminal part of the AVP moiety is recessive rather than dominant, and produces diabetes insipidus in the homozygous state, not by destroying the neurohypophysis, but by directing the production of a biologically inactive hormone (38). This recent discovery serves as an important negative control for the misfolding/neurotoxicity hypothesis, in that it provides the first evidence that mutations of the AVP-NPII gene that do not affect residues critically involved in folding of the prohormone also do not destroy the neuron or otherwise exert a dominant negative effect on the normal allele.

Our findings of a de novo AVP-NPII gene mutation in one member of both families A (Fig. 2) and B (Fig. 3) illustrates that genetic testing in patients with early onset neurohypophyseal diabetes insipidus may be worthwhile even if a family history of the disease is absent. The discovery of a mutation, as in these individuals, permits genetic counseling, in addition to preclinical diagnosis and early detection and treatment of the diabetes insipidus in affected children. Moreover, as the diabetes insipidus and AVP deficiency usually do not develop for months or years post-natally, identification of mutations at birth eventually may permit an intervention that can prevent progression to clinically overt disease.

Acknowledgements

We thank Leah Sabacan for expert technical assistance and Dr. Masafumi Ito for helpful discussions on molecular aspects of adFNDI. This work was supported by Northwestern University New Investigator Award from the Howard Hughes Medical Institute to PK, a fellowship grant from the Kantonale Nachwuchs-Kommission des Kanton Zürichs and of the Swiss Foundation of Biomedical Research to JR, and the General Clinical Research Center of Northwestern University Medical School (NIH MO1-RR0048).

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Received 26 October 2001
Accepted 14 January 2002