Familial neurohypophyseal diabetes insipidus in 13 kindreds and 2 novel mutations in the vasopressin gene

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Abstract

Background: Autosomal dominant neurohypophyseal diabetes insipidus (adNDI) is caused by arginine vasopressin (AVP) deficiency resulting from mutations in the *AVP-NP11* gene encoding the AVP preprohormone.

Aim: To describe the clinical and molecular features of Italian unrelated families with central diabetes insipidus.

Patients and methods: We analyzed *AVP-NP11* gene in 13 families in whom diabetes insipidus appeared to be segregating.

Results: Twenty-two patients were found to carry a pathogenic *AVP-NP11* gene mutation. Two novel c.173 G>C (p.Cys58Ser) and c.215 C>A (p.Ala72Glu) missense mutations and additional eight different mutations previously described were identified; nine were missense and one non-sense mutation. Most mutations (eight out of ten) occurred in the region encoding for the NP11 moiety; two mutations were detected in exon 1. No mutations were found in exon 3. Median age of onset was 32.5 months with a variability within the same mutation (3 to 360 months). No clear genotype–phenotype correlation has been observed, except for the c.55 G>A (p.Ala19Thr) mutation, which led to a later onset of disease (median age 120 months). Brain magnetic resonance imaging (MRI) revealed the absence of posterior pituitary hyperintensity in 8 out of 15 subjects, hypointense signal in 4 and normal signal in 2. Follow-up MRI showed the disappearance of the posterior pituitary hyperintensity after 6 years in one case.

Conclusion: adNDI is a progressive disease with a variable age of onset. Molecular diagnosis and counseling should be provided to avoid unnecessary investigations and to ensure an early and adequate treatment.

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Introduction

Central diabetes insipidus (CDI) is a heterogeneous condition characterized by polyuria and polydipsia due to a deficiency of arginine vasopressin (AVP). In many patients, especially children and adolescents, it is caused by the destruction or degeneration of the neurons that originate in the supraoptic and paraventricular nuclei of the hypothalamus. Germinoma (1, 2, 3, 4) craniopharyngioma (1, 2, 3, 4), Langerhans'-cell histiocytosis (1, 2, 3, 4, 5), inflammatory, autoimmune (1, 2, 3, 4, 5, 6)/vascular diseases (6), brain malformations (7), trauma resulting from surgery or an accident (1, 2, 3, 4, 5) and in rare cases autosomal dominant neurohypophyseal DI (adNDI) (8, 9, 10, 11, 12) or X-linked recessive traits (13, 14, 15) can cause permanent damage to supraoptic and paraventricular nuclei. The differential diagnosis of the above reported conditions, all presenting with hypotonic polyuria and polydipsia, is challenging and requires a long-term follow-up, including endocrine and imaging evaluations (2, 3) suggesting that early diagnosis of genetic conditions could avoid unnecessary investigations.

The frequency of adNDI due to AVP-neurophysin II (*AVP-NP_{II}*) gene mutation is currently unknown, although autosomal dominant forms account for between 3.5 and 8% among reported series of patients with CDI (1, 3, 9), but these data are skewed by differing inclusion criteria and the availability of genetic testing. Among 79 children with CDI of different etiologies, five of 41 patients (6%) with isolated AVP deficiency and without other evolving pituitary dysfunctions over time, with a normal pituitary stalk size and normal central nervous system appearances were diagnosed with autosomal dominant CDI in one study (1), while three patients with autosomal dominant CDI (3.5%) were identified among 85 CDI patients in a more recent one (3).

More than 70 disease-causing mutations resulting in a defective pro-hormone and a deficiency of AVP have been described in adNDI (8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30). The *AVP-NP_{II}* gene consists of three exons where exon 1 encodes the signal peptide, the AVP peptide and the first nine amino acids of NP_{II}; exon 2 encodes the majority of NP_{II} and exon 3 encodes the carboxyl-terminal 17 amino acids of NP_{II} and copeptin. The pathophysiology could be explained by a progressive postnatal degeneration of the magnocellular neurons producing AVP (8, 10, 11, 12, 16). The severity of polyuria may vary considerably between different families and even among siblings carrying the same mutation. In fact, in adNDI, clinical disease onset

typically ranges from the first to the sixth year of life, but various cases of early or delayed onset have also been reported (31). Symptoms usually worsen with time in patients with early onset of mild polyuria and polydipsia, especially before 10 years of age, but it is also possible that complete CDI is expressed from the neonatal period in patients with brain malformations (32).

The wide variability in the age of onset and the severity of the AVP deficiency among patients with the same mutation may be ascribed to individual differences among such patients, like the rate of production of the mutant precursor, the intensity of neurohypophyseal stimulation, individual susceptibility to the toxic effect of the mutant precursor, the ability to degrade mutant precursors and variations in secretory reserve capacity or in the development of the gland itself (8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30). Recent data demonstrated that although autophagy should primarily be a protective mechanism, continuous autophagy leads to gradual loss of organelles including endoplasmic reticulum (ER), resulting in autophagy-associated cell death of AVP neurons in mice with familial NDI (33).

Aim of this study was to investigate the presence of mutations in the *AVP-NP_{II}* gene in 13 families with clinical diagnosis of CDI. Establishing the genetic etiology of CDI enables early appropriate management and family counseling and could be useful to avoid unnecessary neuroimaging follow-up.

Patients and methods

We studied 22 members of 13 Italian families, in whom CDI appeared to be segregating. The family history was described by a pedigree covering the indicated number of generations (Fig. 1). Patients were referred by eight Italian Pediatric Endocrine Units (Istituto Giannina Gaslini Genova, Federico II University Napoli, Ospedale Bambino Gesù Roma, Policlinico San Donato Milan, Policlinico Verona, Policlinico S. Orsola-Malpighi Bologna, Policlinico Modena, Ospedale Careggi Firenze) between January 2003 and April 2016.

The diagnosis of CDI was based on a standard protocol as reported previously (3); the criteria for the enrolment of patients were (1) isolated AVP deficiency and (2) family history of CDI. Brain MRI studies in T1-weighted sequences were performed in 15 subjects. MRI was performed at the time of diagnosis and was repeated during follow-up in one patient.

Figure 1

Pedigrees of the 13 families (A-M). Affected individuals who underwent genetic testing are indicated by the arrows; circles denote females, squares denote males.

The pituitary–thyroid–adrenal axis was assessed by measuring serum free thyroxine, thyrotropin and serum cortisol once every 6–12 months. Growth hormone deficiency was ruled out based on the presence of normal growth. This study was approved by the Institutional Review Board of ‘Istituto Giannina Gaslini’, Genova, and written informed consent was obtained from the patients and/or their parents.

Two-hundred healthy individuals as well as the unaffected family members of the respective kindreds were screened for all the *AVP-NP11* gene mutations.

Nucleotide sequences of exons of *AVP-NP11* gene

Genetic analyses were performed at the Pediatric Unit of the ‘Department of Woman, Child and General and Specialized Surgery’ of the University of Campania ‘L. Vanvitelli’.

Detection of gene mutation: PCR amplification of *AVP-NP11* exons

Genomic DNA was isolated from peripheral blood leukocytes using Extraction DNA Kit (Qiagen) in accordance with the manufacturer’s instruction. To identify genetic variants, all three exons of the *AVP-NP11* (OMIM ID: 192340; ENSEMBL ID: ENSG00000101200) were amplified in 25 µL reactions containing 100 ng of genomic DNA. Intronic PCR primer pairs lying about 100 bp external to each intron/exon boundary were designed to amplify each exon using Primer3 design package. Exons were PCR amplified using standard conditions. Briefly, reactions comprised 10× buffer, dNTP stock containing 2.5 mM dNTP, 25 mM magnesium chloride, 5 µM forward primer stock and reverse primer, 1 U Taq Super Yield, 100 ng genomic DNA template and sterile distilled water to 25 µL (final volume). The thermal cycling was 95°C for 5 min followed by 35 cycles of 95°C, 30 sec, 60°C, 45 sec, 72°C 45 sec and a final extension at 72°C for 5 min (7).

Direct DNA sequencing of PCR products

The reaction products were then purified using a QIAquick Gel Extraction Kit (Qiagen GmbH) and sequencing was performed using the Big Dye Terminator (Applied Biosystems) on automated DNA capillary sequencer (ABI PRISM 310, Applied Biosystems).

Primers:

- AVP_Ex1F TGCAGTCACAGTAGAGGCAA
- AVP_Ex1R TACCACCACCCATGACTTCC

- AVP_Ex2F GGGTTCTGGATGGGTAGGG
- AVP_Ex2R GGAGTTCTCCTCCTGGCAG
- AVP_Ex3F CTTCGGGGCCAGCATCTG
- AVP_Ex3R TTTATTGTCCGTGCTGCAGG

Summary of genetic and clinical findings

We analyzed 22 members of 13 Italian families with CDI and a median age of onset of 32.5 months (3–360 months), who were found to carry a pathogenic *AVP-NP11* gene mutation. Two novel mutations were detected in two families, and eight other different mutations, all known to be associated with adNDI, were documented in the other ones (Fig. 2). The new mutations were not found in 200 healthy controls and in the unaffected family members of the respective patients. Sequencing chromatograms containing the identified variants are shown in Fig. 3. Nine were missense mutations and 1 a nonsense mutation with 8 of 10 occurring in the gene regions encoding the NP11 moiety; 2 mutations were detected in exon 1 and no mutations were found in exon 3. Subjects carrying c.55G>A – (p.Ala19Thr) and c.173 G>C (p.Cys58Ser) mutations were found to have a later onset of disease at a median age of 120 months (120–132 months). Clinical and biochemical evaluations demonstrated that polyuria and polydipsia were the only symptoms at disease onset, and none of the patients showed either severe hyponatremia or growth delay. Neither hyponatremia (Na <135 mEq/L) nor seizures were reported under desmopressin treatment.

Family 1 A

The index case is a 13-year-old-boy with polyuria and polydipsia from the age of 32 months. Six members from three generations (the father, paternal grandmother, two paternal aunts and a paternal cousin) reported symptoms and signs of DI, and the pattern of inheritance was consistent with an autosomal dominant mode (Fig. 1). The patient enjoyed relatively good health and his growth was normal. MRI showed an absent posterior pituitary hyperintensity (PPI). Genetic analysis performed in the index case and in his father revealed a previously described c.164 C>T (p.Pro55Leu) mutation (Fig. 3 and Table 1).

Family 2 B

The index case is a 16-year-old girl with a history of polyuria and polydipsia from the age of 30 months. Water deprivation test and desmopressin (dDAVP) test were performed confirming the diagnosis of CDI. Her father was

Figure 2

Schematic diagram of the coding regions of the vasopressin-neurophysin II gene. The location and type of mutations associated with familial central diabetes insipidus identified are represented and indicated by the arrows.

also affected by CDI and showed polyuria and polydipsia from the age of 6 years (72 months) (Fig. 1). Brain MRI was performed that showed a normal anterior pituitary and pituitary stalk with absent PPI (Table 2). Genetic analysis revealed a c.322G>T (p.Glu108X) nonsense mutation (Fig. 3 and Table 1).

Family 3 C

Two siblings, a 3-year-old male and a 4-year 9 month-old-female showed polyuria and polydipsia from the age of 9 months and 16 months, respectively. They had a family history of CDI. Their mother had onset of polyuria and polydipsia from the age of 24 months, whereas their grandmother had clinical manifestations of DI in adulthood (30 years) (Fig. 1). The parents were not consanguineous. Water deprivation test and dDAVP test confirmed the diagnosis of CDI. Brain MRI performed in the boy and in his mother showed a lack of PPI in both patients and anterior pituitary hypoplasia in the boy. The direct sequencing of the *AVP-NPII* gene showed a point mutation (c.287G>A, p.Gly96Asp) in a heterozygous state, confirming the diagnosis of adNDI (Fig. 3 and Table 1). In addition, the boy showed hypokalemia, metabolic alkalosis, hyperreninemia and hyperaldosteronism. Plasma magnesium concentration was slightly low.

Urine analysis showed hypercalciuria, hyposthenuria and high excretion of potassium. Since adNDI did not explain the boy's phenotype, genetic analysis for Gitelman syndrome was performed that confirmed the combination of Gitelman syndrome and adNDI.

Family 4 D

The index case is a 17-year-boy with polyuria from the age of 33 months and a family history of CDI in his paternal lineage (Fig. 1). He had a sister with polyuria and polydipsia from the age of 22 months. The two siblings underwent water deprivation and the dDAVP tests that confirmed the diagnosis of CDI. Genetic analysis performed in the index case showed a c164C>T (p.Pro55Leu) missense mutation (Fig. 3 and Table 1). Brain MRI was normal in the boy, whereas the PP signal was hypointense in the girl (Table 2).

Family 5 E

The index patient is a 7-year-old girl with a history of polyuria, nycturia and polydipsia since she was aged 2 years. Blood examinations showed hyponatremia and urinary hypo-osmolality with OsmU/OsmP ratio <1, both corrected after dDAVP administration confirming the diagnosis of CDI.

Figure 3

Sequencing chromatograms obtained by automated dye-terminator sequencing of the vasopressin-neurophysin II gene in the patients. A full colour version of this figure is available at <https://doi.org/10.1530/EJE-19-0299>.

Her growth had always been in the normal range, with a height at the 50th centile according to her target height. Her father had a history of polyuria and polydipsia since his first years of life (Fig. 1). Genetic analysis showed a previously described c.56C>T (p.Ala19Val) mutation (Fig. 3 and Table 1). Interestingly, while the first brain MRI that was performed in the girl at the time of diagnosis showed the presence of normal PPI, a second MRI performed 6 years later showed a disappearance of the PP signal (Table 2 and Supplementary material, see section on supplementary data given at the end of this article).

Family 6 F

A 19-year-old female showed polyuria and polydipsia from the age of 3 years. A water deprivation test demonstrated a defect in urine concentrating ability, with a peak plasma osmolality of 300 mosmol/kg and urine osmolality below 300 mosmol/kg; dDAVP led to resolution of symptoms, confirming the diagnosis of CDI. Her mother and her 13-year-old brother also had symptoms of DI (Fig. 1). The water deprivation test was stopped in the brother because of a significant weight loss >5%. Brain MRI revealed the absence of PPI in the three patients (Table 2). Genetic analysis performed in the index case showed a previously

described c.287G>T (p.Gly96Val) missense mutation (Fig. 3 and Table 1).

Family 7 G

The index case is an 11-year-old girl with polyuria and polydipsia from the first years of life. Laboratory test showed hypernatremia, high serum osmolality and low urinary osmolality, which did not increase after water deprivation test. She showed a good response to desmopressin treatment, which confirmed the diagnosis of CDI. The neuroimaging study revealed a normal anterior pituitary and a decreased PP signal intensity. Because of her family history of familial DI (father, paternal grandfather and her paternal uncle) (Fig. 1), genetic analysis was performed showing a c.262G>C (p.Gly88Arg) missense mutation in exon 2 (Fig. 3 and Table 1).

Family 8 H

Two male siblings showed polyuria and polydipsia from the age of 17 months and 19 months, respectively. Their mother, their maternal uncle as well as two maternal cousins had CDI (Fig. 1). The water deprivation test and the dDAVP tests confirmed diagnosis of CDI.

Table 1 Age of onset in index cases and family members with vasopressin-neurophysin II gene mutations ($n = 22$).

Families	Age of onset (months)	Mutation/exon	Exon
Family 1 A			Exon II
Index case	32	c.164C>T - (p.Pro55Leu)	
Father	nd	c.164C>T - (p.Pro55Leu)	
Family 2 B			ExonII
Index case	30	c.322G>T - (p.Glu108X)	
Father	72		
Family 3 C			ExonII
Index case	16	c.287 G>A - (p.Gly96Asp)	
Index case	12	c.287 G>A - (p.Gly96Asp)	
Mother	24	c.287 G>A - (p.Gly96Asp)	
Grandmother	360	c.287 G>A - (p.Gly96Asp)	
Family 4 D			ExonII
Index case	33	c.164C>T - (p.Pro55Leu)	
Sister	22		
Family 5 E			Exon I
Index case	24	c.56C>T (p.Ala19Val)	
Father	18	c.56C>T (p.Ala19Val)	
Family 6 F			ExonII
Index case	36	c.287G>T - (p.Gly96Val)	
Brother	36		
Mother	72		
Family 7 G			ExonII
Index case	NA*	c.262G>C - (p.Gly88Arg)	
Family 8 H			ExonII
Index case	17	c.160G>C - (p.Gly54Arg)	
Index case	19	c.160G>C - (p.Gly54Arg)	
Mother	NA**	c.160G>C - (p.Gly54Arg)	
Family 9 I			Exon I
Index case	120	c.55G>A - (p.Ala19Thr)	
Index case	120		
Family 10 J			Exon I
Index case	120	c.55G>A - (p.Ala19Thr)	
Family 11 K			Exon I
Index case	132	c.55G>A - (p.Ala19Thr)	
Family 12 L			ExonII
Index case	132	c.173 G>C - (p.Cys58Ser)	
Brother	120	c.173 G>C - (p.Cys58Ser)	
Family 13 M			Exon II
Index case	3	c.215 C>A - (p. Ala72Glu)	
Father	6	c.215 C>A - (p. Ala72Glu)	

*, first years of life; **, childhood.
NA, not applicable.

Brain MRI showed in both patients a normal anterior pituitary with absent posterior pituitary hyperintensity. The c.160G>C (p.Gly54Arg) missense mutation was detected (Fig. 3 and Table 1).

Family 9 I

Two female twins had clinical signs suggestive of DI from the age of 10 years. Their father and paternal grandmother had the same condition (Fig. 1). Genetic analysis showed

a c.55G>A (p.Ala19Thr) mutation in exon1 (Fig. 3 and Table 1).

Family 10 J

The index case is a 49-year-old woman with clinical signs of DI from the age of 10 years. Five family members (sister, mother, maternal grandfather, uncle and cousin) (Fig. 1) showed the same clinical signs. The patient enjoyed good health and had two unaffected children. She had also Hashimoto's thyroiditis and was treated with levothyroxine. Genetic analysis showed c.55G>A (p.Ala19Thr) mutation (Fig. 3 and Table 1).

Family 11 K

This girl was a second child, born after 39-week gestation via vaginal delivery, her birth weight (3.300kg) and length (49cm) were appropriate for gestational age. She showed polyuria and polydipsia from the age of 9 years. Her mother and maternal grandfather showed the same symptoms (Fig. 1) (age of onset was not available). Brain MRI was normal, although PP data are not available (Table 2). Genetic tests showed c.55G>A (p.Ala19Thr) heterozygous mutation (Fig. 3 and Table 1).

Family 12 L

The index case is a girl with history of polyuria and polydipsia from the age of 11 years. Clinical evaluation at the age of 11 years showed a weight of 45.2kg (25°–50° centile), height 144.1cm (3°–25° centile) with a normal growth velocity. Diagnosis of CDI was performed at the age of 12 years. Her brother had the same history with polyuria and polydipsia from the age of 10 years and diagnosis of CDI was performed at the age of 11 years. Clinical evaluation showed a normal height: cm 141.5 (25°–50° centile) and weight: kg 36.9 (50°–75° centile). Genetic tests revealed a new c.173G>C (p.Cys58Ser) missense mutation in exon 2 (Fig. 3 and Table 1). The family tree is shown in Fig. 1.

Family 13 M

This boy was a second child, born at term; his birth weight (4kg) and length (52cm) were appropriate for gestational age. He showed polyuria and polydipsia from the first months of life. Diagnosis of CDI was performed at the age of 9 years. Brain MRI showed an undetectable PP signal. His father had the same history with polyuria

Table 2 MRI findings in patients with vasopressin-neurophysin II gene mutations.

Families/Members	Anterior pituitary	Pituitary stalk	Posterior pituitary signal
1 A			
Index case III1	Normal	Normal	Undetectable
2 B			
Index case II1	Normal	Normal	Undetectable
3 C			
Index case IV1	Hypoplasia	Normal	Undetectable
Mother-III2	Normal	Normal	Undetectable
4 D			
Index case III3	Normal	Normal	Normal
Sister III4	Normal	Normal	Hypointense
5 E			
Index case IV1	Hypoplasia	Normal	Normal
Index case MRI follow-up	Hypoplasia	Normal	Undetectable
6 F			
Index case IV2	Normal	Normal	Hypointense
Brother IV1	Normal	Normal	Undetectable
Mother III2	Empty sella	Normal	Hypointense
7 G			
Index case III1	Normal	Normal	Hypointense
8 H			
Index case II1	Normal	Normal	Undetectable
11 K			
Index case III2	Normal	Normal	Not available
12 L			
Index case III2	Normal	Normal	Hypointense
13 M			
Index case III2	Normal	Normal	Undetectable

and polydipsia from the age of 6 months. Diagnosis was performed at the age of 30 years. Genetic tests revealed a new c.215C>A (p.Ala72Glu) missense mutation in exon 2 (Fig. 3 and Table 1). The family tree is shown in Fig. 1.

MRI results

Brain MRI was performed in 15 patients and the results are shown in Table 2. MRI results were not available for kindreds 9 and 10. The anterior pituitary was normal in 12 subjects, hypoplastic anterior pituitary/empty sella was found in 3; the pituitary stalk was normal in all patients and none displayed midline/central nervous system malformations. The PPI was undetectable in eight subjects, hypointense in four and normal in two. Follow-up MRI performed in one case showed a disappearance of the PP signal after 6 years. Anterior pituitary function was normal at the time of the first evaluation and during the follow-up in all patients. A diagnostic flow chart for genetic CDI is represented in Fig. 4.

Discussion

The present study defines clinical and molecular characteristics of 22 affected patients with familial central

DI from 13 different Italian families. Eight of the identified mutations were known to be associated with adNDI of the *AVP-NP_{II}* gene and have previously been reported by others (8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 34, 35, 36, 37, 38, 39), while two novel mutations are here described for the first time. Genetic analyses revealed that all affected members were heterozygous for the mutations as expected from autosomal dominant mode of inheritance.

According to the literature, most of our affected patients carried a missense mutation, 8 of 10 involving the NP_{II} which is an intracellular binding protein for AVP confirming that most mutations occur in gene regions encoding the NP_{II} moiety (10). Since the first report of an *AVP* gene mutation causing adNDI (34), over 70 mutations have been listed in the Human Gene Mutation Database, most of them single base missense/nonsense substitutions, several small deletions (22, 35, 36) or indels (22) and one splice-site mutation (26). The mutations are predicted to interfere with protein folding of the pre-prohormone, for example, by impaired binding of AVP to NP_{II} (26), replacing (22, 29, 37) or introducing (21, 22, 38) cysteine residues or introducing premature stop codons (22, 25, 39).

In our cohort, families 1 and 4 showed c.164C>T (p.Pro55Ileu) mutation that might alter the proper folding

of the pro-hormone (34), while c.322G>T mutation detected in family 2 should produce an AVP mutant with a different C-terminus (starting at codon 108) and/or a truncated protein (p.Glu108X) (35). Families 3, 6 and 7 showed c.287G>A, c.287G>T and c.262G>C mutations, which seem to cause a severe impairment of the AVP pro-hormone processing and secretion likely due to trapping in the ER (22, 36), and c.56C>T (p.Ala19Val) mutation identified in family 5 causes possible abnormal pre-hormone processing (22). Family 8 showed the c.160G>C (p.Gly54Arg) missense mutation, acting as the one described in family 2 (37). These two mutations alter the correct folding of the protein. The AVP mutants complex with the wild-type proteins to form heterodimers which are retained in endoplasmic reticulum of secreting neurons. The mutant WT heterodimers lead to cytotoxic effects with gradual and progressive loss of viable neurosecretory neurons (35, 37). c.55G>A (p.Ala19Thr) had been detected in families 9, 10 and 11 (38). This latter mutation involves a residue of the signal peptide and could produce an abnormally processed precursor that gradually destroys vasopressinergic neurons (38).

Two novel mutations in Exon 2 of the *AVP-NP2* gene were found in families 12 and 13, one being a single heterozygous missense mutation with a change G-to-C

transversion at nucleotide position 173; as a consequence of this mutation, at position 58, cysteine is replaced by serine. The other mutation determines a change from C-to-A transversion at nucleotide position 215; due to the latter, at position 72 of the protein, alanine is predicted to be replaced by glutamic acid. These two novel mutations were not observed in the alleles of the unaffected members of the analyzed kindred and in 200 healthy controls. The late onset of the disease in family 12 may be explained by the fact that this new mutation causes a switch among neutral amino acids, which potentially does not severely affect the biochemical features of the mature protein. The hypothesized mechanisms underlying the phenotype of the two novel mutations could be explained by one of those extensively investigated in the cell lines of patients with adNDI (8, 16, 17, 33). Mutant AVP precursors reportedly accumulate and aggregate in the ER, which leads to defective intracellular trafficking of wild-type AVP protein and a decrease in cell viability. Furthermore, autophagy has been suggested to have a bifunctional role in cell survival and death in a transfected cell line-expressing mutant NP2 (40, 41), and recently, Hagiwara *et al.* demonstrated that although autophagy should primarily be a protective mechanism, continuous autophagy leads to gradual loss of organelles including ER,

*Genetic analysis in the presence of at least one affected family member with no need to further investigations.

Figure 4

Diagnostic flow chart of familial central diabetes insipidus.

resulting in autophagy-associated cell death of AVP neurons in mice with familial NDI mice (33).

In autosomal dominant CDI, clinical disease onset can be expressed during the neonatal period (8, 10), but typically polyuria and polydipsia in adNDI occur during the first months to years of life and progress as AVP deficiency becomes increasingly severe (8). In addition, the onset of disease and the severity of polyuria may vary considerably between different families and even among siblings who carry the same mutation (31). In our study, subjects carrying c.55G>A (p.Ala19Thr) or c.173 G>C (p.Cys58Ser) mutations have a later onset of disease with the c.55G>A (p.Ala19Thr) encoding for a mutation of the signal peptide, while the c.173 G>C (p.Cys58Ser) affecting NPII; both changes are among neutral amino acids, and they potentially will only have a mild effect on the biochemical features of the mature proteins, thus inducing a late onset of the inherited condition; c.55G>A (p.Ala19Thr) mutation causing impaired signal cleavage has been associated with later onset of clinical signs (30, 39). The median age of disease onset in our patients was found to be slightly higher than the average age reported in the literature (24 months) for several reasons, above all because of the inaccurate time of diagnosis, since the first symptoms of polyuria and polydipsia may lead to disease underestimation, especially in families with many affected members where such symptoms can be considered 'physiological'. We believe that the rarity and heterogeneity of the mutations published so far produces a variety of clinical features that would require a systematic analysis of the genotype–phenotype correlation in larger cohorts.

In the present study, the PPI was undetectable in eight subjects, hypointense in four and normal in two in agreement with our previous findings in small cohorts and with the literature (1, 3, 5, 7). Although 10% of normal individuals do not display PPI (40), its absence in patients with CDI is always associated with hypothalamic–neurohypophyseal axis lesion and correlates closely with undetectable plasma AVP (41). This suggests that evidence of PPI does not rule out diagnosis of CDI, as release of stored AVP may be impaired in some cases of autosomal dominant DI. Thus, the identification of PP signal does not necessarily indicate that the functional integrity of the hypothalamic–neurohypophyseal axis is preserved and – when initially present – the signal may disappear at follow-up. Similar findings were also reported by our group in patients with *de novo* mutations of AVP gene who were classified as affected by 'idiopathic' CDI (7).

In conclusion, we describe the clinical, molecular and imaging characteristics of 22 members of 13 Italian

families with adNDI, with 8 previously reported mutations and two novel mutations in two different families. aFNDI is a progressive disease with a variable age of onset and severity. Posterior pituitary hyperintensity may be present or hypointense, with a loss of the MRI signal over time suggesting that persistence of the hyperintensity in isolated and early-onset CDI should favor the diagnosis of adNDI. Molecular analysis of AVP-NPII gene and counseling should be provided in selected cases to avoid unnecessary investigations and to ensure an early and adequate treatment.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-19-0299>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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