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A novel syndrome of diabetes mellitus, renal dysfunction and genital malformation associated with a partial deletion of the pseudo-POU domain of hepatocyte nuclear factor-1β

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Mutations in the homeodomain-containing transcription factor hepatocyte nuclear factor (HNF)-1β are the cause of one form of maturity-onset diabetes of the young (MODY), type 5 (MODY5). We have studied a Norwegian family, N5, with a syndrome of mild diabetes, progressive non-diabetic renal disease and severe genital malformations. The sequence of the HNF-1β gene (*TCF2*) revealed a 75 bp deletion in exon 2 (409–483del) which would result in the synthesis of a protein lacking amino acids Arg137 to Lys161 (R137–K161del). This deletion is located in the pseudo-POU region of HNF-1β, a region implicated in the specificity of DNA binding. Functional studies of R137–K161del HNF-1β revealed that it could not bind an HNF-1 target sequence or stimulate transcription of a reporter gene indicating that this is a loss-of-function mutation. The R137–K161del allele co-segregated with diabetes and renal disease in pedigree N5. In addition, two of four female carriers with this mutation had vaginal aplasia and rudimentary uterus (Müllerian aplasia). These studies strongly suggest that heterozygous mutations in the HNF-1β gene are associated with a syndrome characterized by MODY and severe, non-diabetic renal disease. Moreover, the presence of internal genital malformations in two females suggests that additional clinical features may be associated with HNF-1β mutations.

INTRODUCTION

The formation of tissues and organs during development and the maintenance of normal cell function in the adult organism depends on RNA synthesis, the regulation of which is determined by the interactions between the basal transcription machinery and various transcription factors (1,2). The combination of accessory transcription factors specifies the temporal and tissue specific patterns of gene expression. Although the endocrine pancreas and the kidney arise from distinct embryonic structures and serve very different physiological functions (3,4), they both express the homeodomain-containing transcription factors hepatocyte nuclear factor (HNF)-1α and -1β (5–7; Y. Horikawa and G.I. Bell, unpublished data). Heterozygous mutations in the HNF-1α gene (*TCF1*) are associated with an early-onset non-ketotic form of diabetes mellitus, the type 3 form of maturity-onset diabetes of the young (MODY3), a disorder which is characterized by autosomal dominant inheritance, onset of diabetes usually before 25 years of age and pancreatic β-cell dysfunction (7–9). Mutations in the HNF-1β gene are also associated with MODY: MODY5 (10). In addition to effects on β-cell function, deficiency of HNF-1α and -1β affects normal renal function. Patients with HNF-1α mutations appear to have a lower renal threshold for glucose (11) and those with HNF-1β mutations seem especially susceptible to severe renal disease (10,12).

Here we describe a family in which a syndrome of mild diabetes and severe non-diabetic renal disease co-segregates with a deletion mutation in the HNF-1β gene. In addition, two of four females with this mutation had genital malformations with vaginal aplasia and rudimentary uterus (Müllerian aplasia). These findings suggest that a broader spectrum of clinical symptoms may be associated with HNF-1β mutations than previously recognized.

RESULTS

Clinical evaluation

The pedigree of the N5 family is shown in Figure 1 and the clinical features of the patients are summarized in Table 1. The patients are described below in the order in which they came to clinical attention. The karyotypes of patients 2, 3 and 5 were normal.

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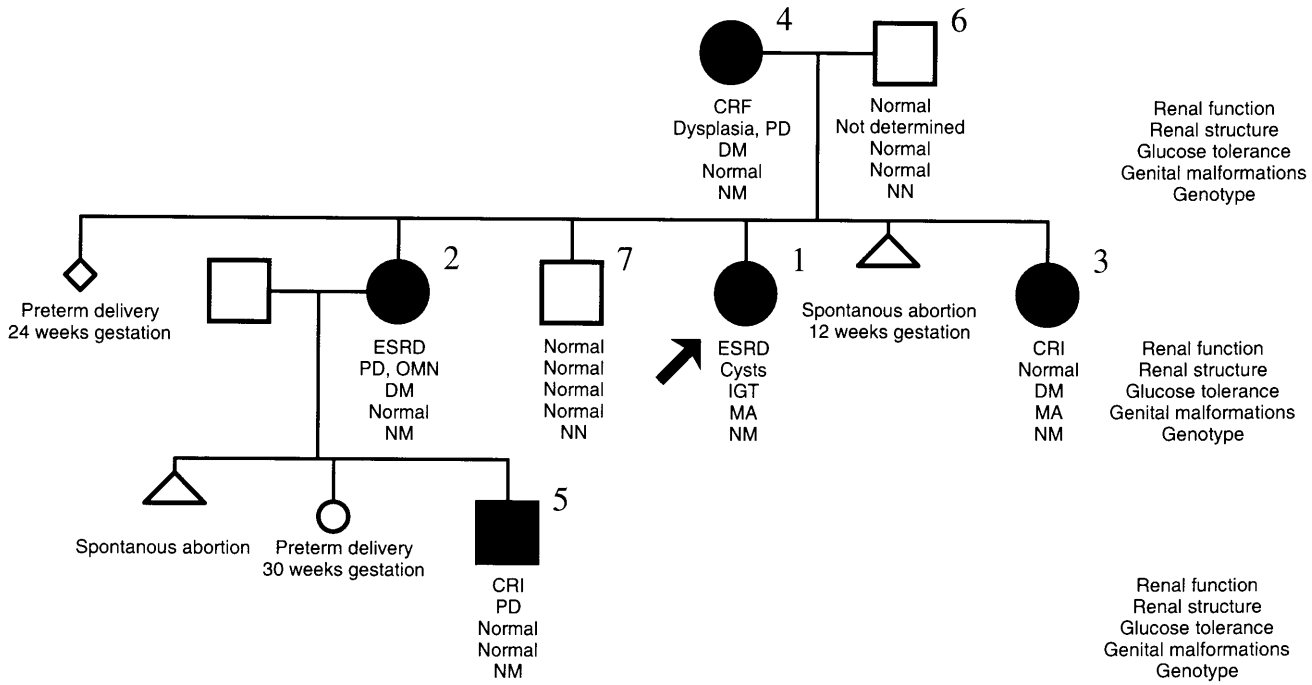

Figure 1. Pedigree of Norwegian family N5. The subjects who were studied are numbered 1–7 and are described in the text and Table 1. Subjects with diabetes are indicated by filled symbols. The arrow indicates the proband. Genotype: N, normal allele; M, R137–K161del HNF-1β allele. CRF, chronic renal failure; CRI, chronic renal insufficiency; DM, diabetes mellitus; ESRD, end stage renal disease; IGT, impaired glucose tolerance; MA, Müllerian aplasia; ND, not determined; OMN, oligomeganephronia; PD, parenchymal disease.

Patient 1. Following a normal gestation, patient 1 presented with retarded motor development and failure to thrive at 10 months. Her physical appearance was normal. Weight and height were at the 2.5th percentile for her age. She had renal failure (serum creatinine, 120 μmol/l; normal range for age, 23–48 μmol/l), and an oral glucose tolerance test (OGTT) indicated impaired glucose tolerance (IGT). At age 2 years, an intravenous glucose tolerance test (IVGTT) confirmed IGT, showing a reduced insulin response to intravenous glucose. Intravenous urography revealed delayed contrast excretion. At age 3 years, she had compensated metabolic acidosis and serum creatinine of 110 μmol/l (normal range for age, 23–60 μmol/l). Blood pressure was normal. Intermittent proteinuria was observed at 4 years of age. During a surgical exploration at age 7 years, a left cystic kidney was noted. At age 14 years, pubertal development had started, but without menarche. Renal function continued to deteriorate with proteinuria and compensated metabolic acidosis, but without glucosuria. Chronic renal failure (CRF) with serum creatinine of 330 μmol/l was diagnosed at age 18 years. A successful renal transplant was performed at 21 years of age. On treatment with glucocorticoids, she developed diabetes treated with small doses of insulin (0.25 U/kg/day). A gynecological examination at age 24 years revealed vaginal aplasia (only 2–3 cm of the lower part of the vagina was present) and rudimentary uterus. Ovaries and external genitalia were normal and there was no virilization or hirsuitism. She had normal serum concentrations of follicle stimulating hormone, luteinizing hormone, prolactin and estradiol.

Patient 2. At 10 years of age, patient 2 had proteinuria, elevated serum creatinine (115 μmol/l; normal range for age, 25–80 μmol/l) and reduced urinary concentration capacity. Intravenous urography showed delayed contrast excretion and ultrasonography revealed parenchymal disease. A right-sided renal biopsy at age 14 years showed cortical tissue with reduced number of glomeruli. However, the glomeruli were markedly enlarged (Fig. 2A), and the proximal renal tubules also appeared hypertrophic with enlarged diameter compared with an age-matched control kidney (Fig. 2B). Transmission electron microscopy showed a glomerulus with only slight irregularities, which were most likely within normal limits (Fig. 2C). There was no evidence of arteriolo- or glomerular scleroses, which are indicative of diabetic nephropathy. During follow-up, serum creatinine continued to increase, although acid–base status, serum potassium, calcium, phosphate and blood pressure were normal. At age 22 years, she gave birth to a premature female infant (30 weeks of gestation) who died at 17 days. During her third pregnancy (22 years of age), she had gestational diabetes treated with insulin and on follow-up developed frank diabetes treated with diet. She was diagnosed with end-stage renal disease (ESRD) at 29 years of age, although she was normotensive.

Patient 3. At 10 years of age, patient 3 had elevated serum creatinine (103 μmol/l; normal range for age, 25–80 μmol/l). There was no proteinuria and renal ultrasonography was normal. At 14 years of age, she was diagnosed with diabetes which was treated with low doses of insulin (0.26 U/kg/day). She had primary amenorrhea, and a gynecological examination at 15 years of age revealed vaginal aplasia and a rudimentary uterus. Serum concentrations of sex hormones and cortisol were normal. At 18 years of age, she was diagnosed with chronic renal insufficiency (CRI): serum creatinine, 134 μmol/l (normal

Table 1. Clinical data for family N5

Family member (Fig. 1)	1	2	3	4	5	6	7
Present age (years)	24	29	18	53	6	52	27
Body mass index (kg/m^2)	18	21	29		14		32
Diabetes mellitus/IGT	IGT[a]	DM	DM	DM	Normal[b]	Normal	Normal
Age at diagnosis (years)	1	22	14	24	5.7	–	–
Treatment	Insulin	Diet	Insulin	Diet	None	–	–
Plasma glucose, fasting (mmol/l)	4.8	7.4	–	10.5	5.4	6	5.9
2 h (mmol/l)	10.5	11.9	–	–	6.1	5.5	5.6
Random (mmol/l)			10.2; 7.3				
HbA1c, recent (%)	6.7	6.9	6.3	7.5	5.1	5.6	5.3
Glucosuria	Y	Y	Y	Y	N[b]	N	N
Ketonuria	N	N	N	N	N	N	N
Micro-/macroangiopathy[c]	N	N	N	N	–	N	N
Polyneuropathy	N	N	N	N	–	N	N
Hyperlipidemia	N	Y	N	N	N	N	–
Creatinine, recent (μmol/l)	603[d]	450	134	207	72	90	71
Blood pressure (mmHg)	105/65[d]	115/75	110/70	133/82	100/70	–	–
Microalbumin positive	Y	Y	Y	Y	N	–	–
Proteinuria	Y	Y	N	Y	Y/N	–	–
Renal ultrasound abnormal	Y	Y	N	Y	Y	–	–
Renal cysts	Y	N	N	N	N	–	–
Renal transplantation	Y	Y[e]	N	N	N	–	–
Vaginal atresia	Y	N	Y	N	–	–	–
Rudimentary uterus	Y	N	Y	N	–	–	–

DM, diabetes mellitus; IGT, impaired glucose tolerance; N, no; Y, yes; –, not determined.
[a]DM on glucocorticoids post-transplantation.
[b]Intermittent glucosuria and borderline oral glucose tolerance test.
[c]Includes retinopathy and coronary heart disease.
[d]Pre-transplantation.
[e]Transplantation planned.

range for age, 55–100 μmol/l); and elevated urine microalbumin of 0.084 g/l (normal range 0.000–0.020 g/l). Blood pressure and renal ultrasonography were normal.

Patient 4. Proteinuria was noted during pregnancy in patient 4. Gestational diabetes was observed during the fourth pregnancy (age 29 years). At 43 years of age, serum creatinine was elevated (153 μmol/l; normal range, 55–100 μmol/l) with normal blood pressure. Ultrasonography and urography revealed renal hypoplasia with parenchymal disease. At 52 years of age, she was diagnosed with CRF (creatinine, 207 μmol/l) and mild diabetes, intermittently treated with oral hypoglycemic agents. Moderate hypertension was treated with losartane.

Patient 5. He presented with neonatal hypoglycemia and failure to thrive. At 10 days of age, metabolic acidosis, glucosuria, generalized amino aciduria and slight proteinuria were observed. At 3 months of age, blood pressure was normal and serum creatinine was elevated (64 μmol/l; normal range for age, 18–35 μmol/l). Ultrasonography revealed a dense renal parenchyma. The acidosis normalized, but proteinuria, glucosuria and elevated serum creatinine persisted. At age 5.8 years, an OGTT revealed glucose tolerance but a reduced insulin value at 30 min compared with normal (23.5 mU/l compared with normal lower level of 26.2 mU/l). Urine microalbumin was normal, but β2-microglobulin (0.7 mg/l; normal, 0–0.3 mg/l) and serum creatinine (72 μmol/l, normal range for age, 27–62 μmol/l) were elevated. Renal ultrasonography showed normal sized kidneys with increased echo density.

Identification of a deletion in the HNF-1β gene

The co-segregation of MODY and renal dysfunction in pedigree N5 suggested that a mutation in the HNF-1β gene might be present. Therefore, the minimal promoter and nine exons of this gene were screened for mutations by PCR amplification of exons and direct sequencing of the PCR product. This analysis revealed a 75 bp deletion (nucleotides 409–483, inclusive: 409–483del) in exon 2 resulting in an in-frame deletion of amino acids Arg 137 to Lys 161 (R137–K161del) (Fig. 3). This deletion is located in the pseudo-POU region of HNF-1β, a region believed to be involved in DNA binding (13). The R137–K161del allele co-segregated with diabetes and renal disease in pedigree N5 (Fig. 1).

Effect of the R137–K161del mutation on HNF-1β function

The functional properties of wild-type and R137–K161del HNF-1β were compared (Fig. 4). Alternative splicing generates three forms of HNF-1β and the R137–K161del mutation affects the structure of all three (14) (Fig. 3). The functional studies were carried out using the 557 amino acid HNF-1β-A (Fig. 3C). DNA

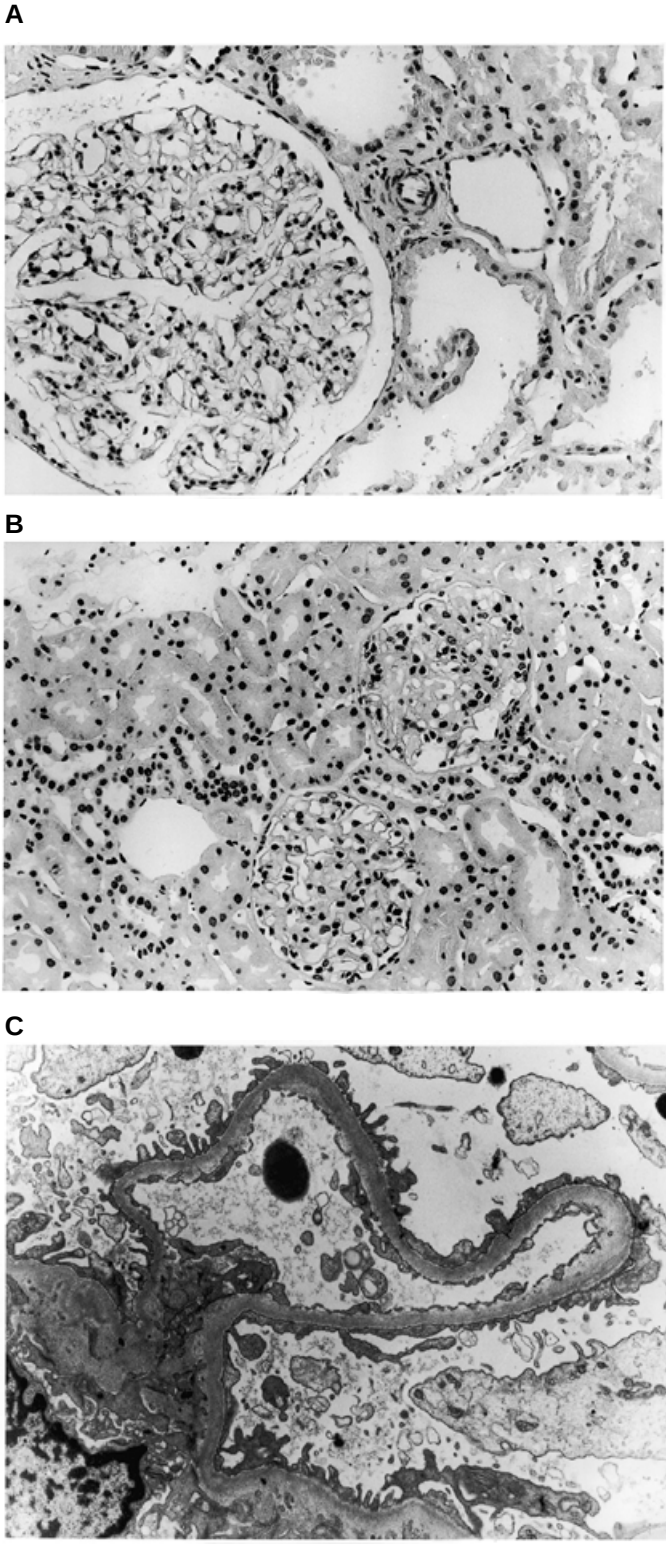

Figure 2. Kidney biopsy from patient 2 at 14 years of age showing dilated proximal tubules and a large glomerulus with an area of 75 483 (**A**) compared with 15 386 μm^2 in an age-matched control (**B**) (both sections stained with periodic acid–Schiff; magnification ×300). A small piece of the biopsy specimen, fixed in glutaraldehyde, post-fixed in osmic acid and embedded in epon for electron microscopy (**C**), shows a representative area of the capillary tuft (magnification ×8800). The epithelial foot processes are effaced in small areas, and there is slight thickening of the basement membrane. The mesangium is normal.

binding studies showed that *in vitro* synthesized R137–K161del HNF-1β was unable to bind DNA (Fig. 4A). Similar results were obtained using nuclear extracts from HeLa cells expressing epitope-tagged wild-type and R137–K161del HNF-1β (data not shown). Western blotting demonstrated the presence of wild-type and mutant protein only in nuclear extracts.

Transfection studies showed that wild-type Xpress-epitope-tagged HNF-1β was able to stimulate transcription of a firefly luciferase reporter gene linked to the HNF-1 binding site of the rat albumin gene by 3.6-fold (Fig. 4B). In contrast, Xpress-epitope-tagged R137–K161del HNF-1β, although expressed at equivalent levels as determined by western blotting, was inactive (Fig. 4A). There was no inhibition of HNF-1β activity when cells were transfected with equivalent amounts of wild-type and mutant HNF-1β expression vectors (Fig. 4B) implying that R137–K161del HNF-1β does not function in a dominant-negative manner. Both wild-type and R137–K161del HNF-1β were correctly targeted to the nucleus in transfected HeLa cells (Fig. 4C), indicating that the inability of R137–K161del HNF-1β to stimulate transcription was not due to its mislocalization within the cell.

DISCUSSION

Here, we report a novel syndrome of mild diabetes mellitus, progressive non-diabetic renal disease and severe genital malformations associated with a heterozygous mutation in the HNF-1β gene. There have been two other reports of families with mutations in the HNF-1β gene (10,12). All three families have been characterized by MODY of variable severity and severe progressive renal dysfunction often leading to ESRD (Table 2). The N5 family described here is similar in many respects to the other families with HNF-1β mutations, with the important additional findings of severe genital malformation in two of four female carriers. In addition, this family has been followed for 25 years, thus providing important clues to the natural history of HNF-1β-related diabetes and renal dysfunction.

Renal dysfunction appears to be a primary feature of this disorder with symptoms evident at 3 months of age and possibly at birth (Table 1). Subsequently, there was compromised glomerular filtration resulting in elevated serum creatinine in all affected subjects, and deteriorating renal function. The reduced number of glomeruli and striking hypertrophy of both glomeruli and proximal renal tubules observed in patient 2 are consistent with oligomeganephronia, a condition characterized by small kidneys with a markedly reduced number of nephrons and hypertrophy of the remaining ones (15,16). Neither the clinical picture nor the histological changes were compatible with diabetic nephropathy.

Two of four female carriers of the R137–K161del HNF-1β mutation had genital tract abnormalities with vaginal aplasia and rudimentary uterus. This region of the female reproductive tract develops from the Müllerian duct (17). HNF-1β is expressed in the uterine epithelium in the adult mouse and thus could function in the normal development of this region of the female reproductive tract (18). If the R137–K161del HNF-1β mutation is the cause of the genital abnormalities, its effects on genital development are incomplete as two female subjects who inherited this mutation had undergone successful pregnancies. However, they were not subjected to detailed studies concerning possible minor genital abnormalities. Patients 2 and 4 each had one

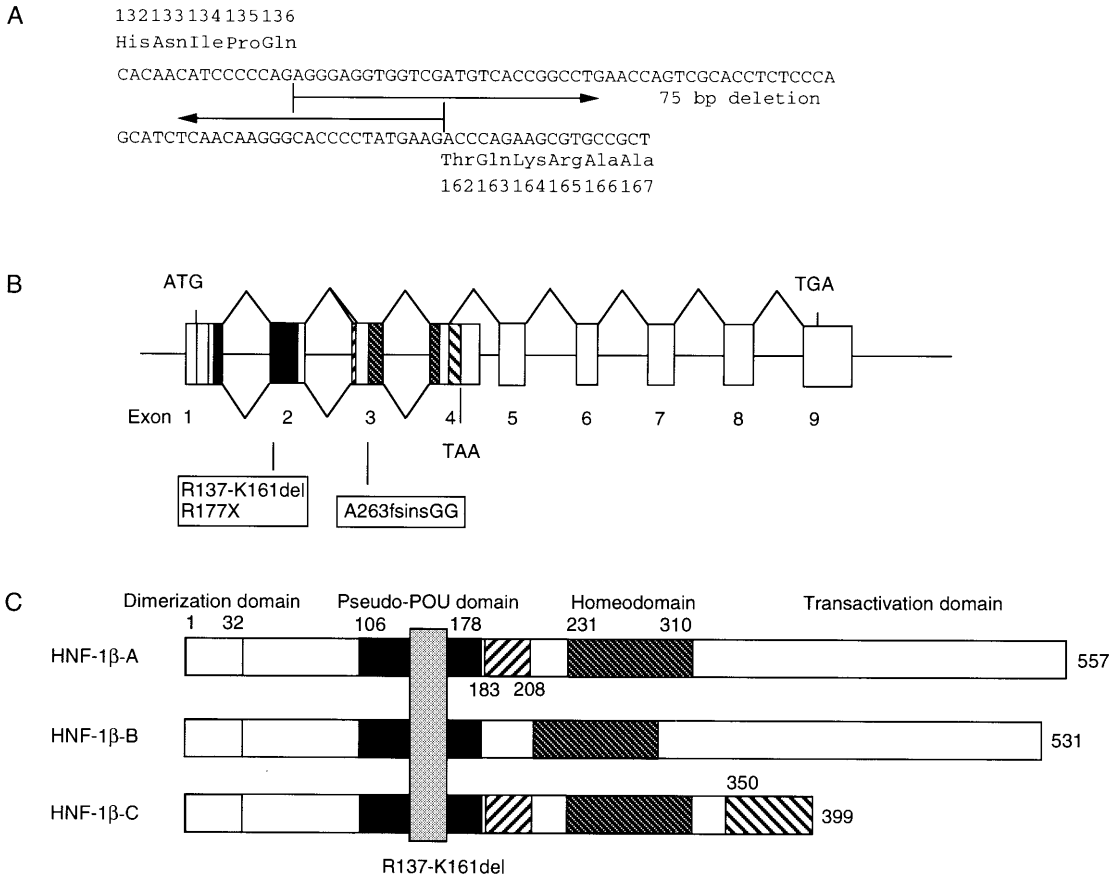

Figure 3. Heterozygous HNF-1β mutation in pedigree N5. (**A**) Nucleotide and amino acid sequence in the region of the 75 bp deletion (409–483del) in exon 2. (**B**) Model of the human HNF-1β gene showing the exon–intron organization and the different patterns of alternative splicing that have been observed in this gene. The exons are numbered 1–9 and the location of the translational start (ATG) and stop (TAA and TAG) codons are shown. The three mutations in this gene that have been described are noted. (**C**) Structural organization of the three forms of HNF-1β that are generated by alternative splicing. The domains of HNF-1β and the amino acids that define their boundaries are indicated. The shading shows the location of each domain in the context of the exon–intron organization (B). The region deleted is shown.

premature delivery and one spontanous abortion (Fig. 1), perhaps consistent with minor uterine abnormalities.

The clinical presentation of the three families with HNF-1β mutations are similar, but not identical (Table 2). Renal dysfunction and diabetes are features of all three families. However, genital malformations were not observed in the other families, one with a nonsense mutation, R177X (10), and the other with a frame-shift mutation, A263fsinsGG (12). This difference may be due to the fact that the R137–K161del mutation found in the N5 family appears to be a loss-of-function mutation, but as it still retains the transactivation domain and could thus interact with other transcription factors and the basal transcription machinery, it may acquire a new function and thereby disrupt uterine and vaginal development. In contrast, the R177X and A263fsinsGG mutations lack the transactivation domain (19) and so would not have this effect on genital development. Further studies are necessary to determine the verity of this hypothesis.

MATERIALS AND METHODS

Subjects

The N5 family was from the coastal area of Northern Norway and has been followed by one of us (O.S.) for 25 years. Informed consent was obtained from all family members or their legal guardians. The studies were approved by a regional ethical committee, and performed according to the Declaration of Helsinki.

Clinical studies

OGTT was carried out following an oral glucose load of 1.75 g/kg body wt (maximum, 75 g). Glucose and insulin values were determined from venous blood (plasma) drawn immediately before and 15, 30, 60, 90, 120 and 150 min after the glucose load. A diagnosis of diabetes or IGT was made according to criteria of the World Health Organisation (20). IVGTT was performed by giving a glucose load of 0.5 g/kg body wt. Glycosylated hemoglobin (HbA_{1c}) was measured using a DAC 2000 instrument (normal range, 4.1–6.0%; Bayer, Leverkusen, Germany). Blood chemistry and hormone measurements were performed using standard methods. Glucosuria and proteinuria were determined in overnight urine using dipsticks.

Mutation analysis of the HNF-1β gene

DNA was isolated from peripheral blood using a QIAamp blood kit (Qiagen, Kebo Lab, Bergen, Norway). The nine exons, flanking introns and minimal promoter region of the HNF-1β gene were amplified using PCR and specific primers, and the PCR products were sequenced directly (10). Mutations

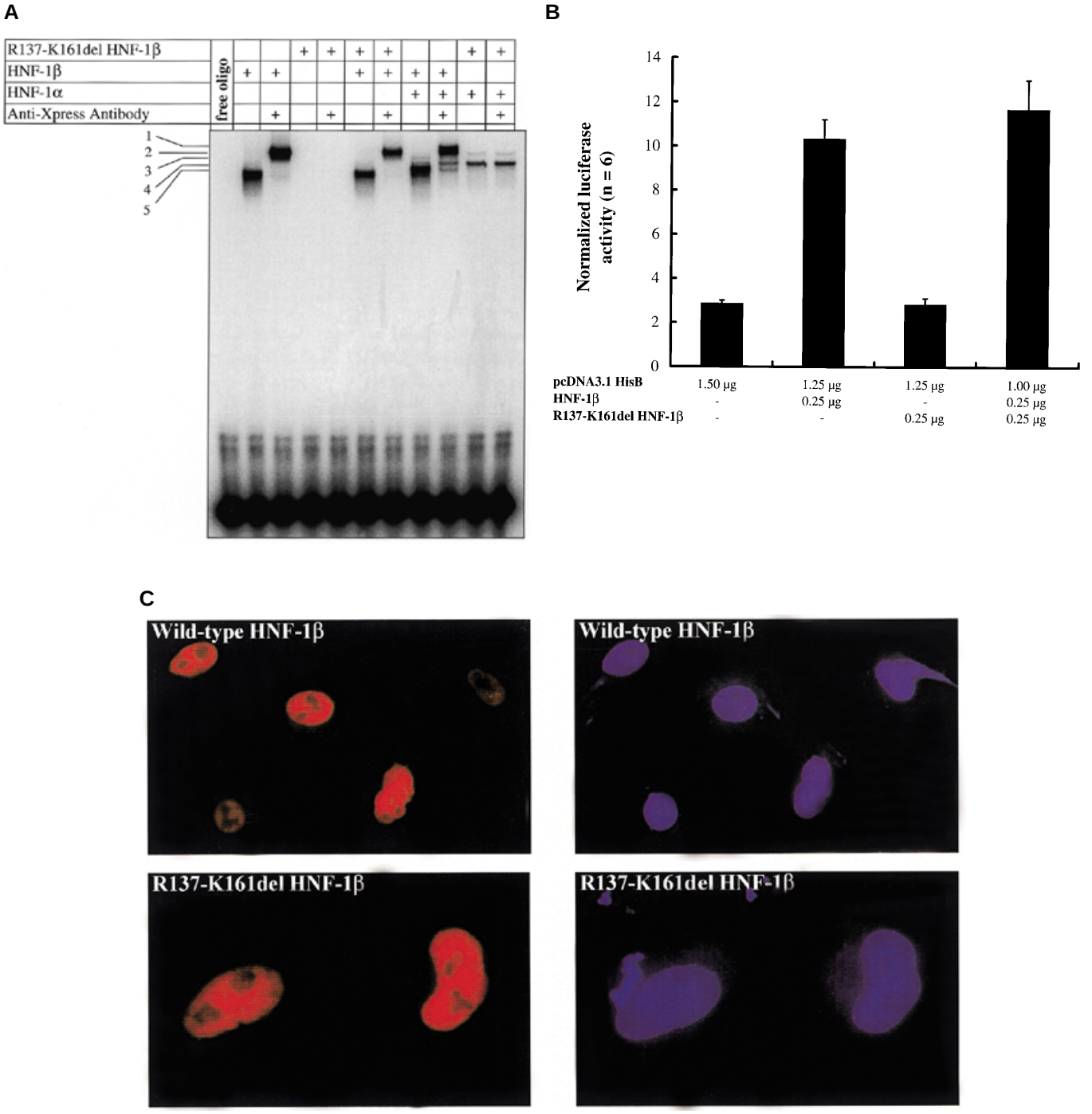

Figure 4. Functional studies of wild-type and R137–K161del HNF-1β. (**A**) Binding of *in vitro*-synthesized Xpress-epitope-tagged wild-type and R137–K161del HNF-1β and untagged HNF-1α to the HNF-1 binding site of the rat albumin promoter. The Anti-Xpress antibody was added to supershift complexes containing the epitope-tagged wild-type and R137–K161del HNF-1β and thereby confirm the identity of the complex. The binding of the *in vitro*-synthesized protein to the ^{32}P-labeled oligonucleotide was effectively competed by 50-fold excess of the unlabeled oligonucleotide (data not shown). In studies to test for the dimerization, one-half the amount of protein was used so that the total amount of protein added was constant. The numbers at the left indicate the different HNF-1 complexes: 1, supershifted wild-type (wt) HNF-1α:wt HNF-1β; 2, supershifted wt HNF-1β:wt HNF-1β; 3, wt HNF-1α:wt HNF-1α (not supershifted); 4, wt HNF-1α:wt HNF-1β (not supershifted); and 5, wt HNF-1β:wt HNF-1β (not supershifted). The identical pattern of binding of epitope-tagged HNF-1β ± epitope-tagged R137–K161del HNF-1β indicates that R137–K161del HNF-1β is unable to form dimers with HNF-1β since wild-type:mutant dimers would be evident as a band with different mobility because of the difference in size between the wild-type and mutant proteins. (**B**) Transcriptional activation by epitope-tagged wild-type and R137–K161del HNF-1β. Data are means ± SEM of six determinations. There was no significant difference in activity between wild-type HNF-1β and wild-type HNF-1β + R137–K161del HNF-1β. (**C**) Localization of epitope-tagged wild-type and R137–K161del HNF-1β in the nucleus of transfected HeLa cells. Wild-type and R137–K161del HNF-1β staining is shown in red and DNA staining in blue. The pictures of the cells expressing R137–K161del HNF-1β were taken at a higher magnification than the cells expressing the wild-type protein.

Table 2. Phenotypes associated with heterozygous mutations in the HNF-1β gene

Mutation	Renal function	Diabetes mellitus[a] Age at diagnosis DM/IGT (years)	Treatment	Genital malformations	Reference
1. R137–K161del	ESRD (2/5) CRF (1/5) CRI (2/5) HT (1/5) Cysts (1/5)	1, 14, 22, 24	Diet (2/4) Insulin (2/4)	Vaginal aplasia, rudimentary uterus (2/4)	This work
2. R177X	CRF (2/3) IRF (1/3) Cysts (0/3)	10, 15, 40	Insulin (3/3)	No	6
3. A263fsinsGG	ESRD (1/5) CRF (2/5) IRF (2/5) HT (3/5) Cysts (5/5)	14, 19, 61	Diet (1/3) Insulin (2/3)	No	7

CRF, chronic renal failure; CRI, chronic renal insufficiency; DM, diabetes mellitus; ESRD, end-stage renal disease; HT, hypertension; IGT, impaired glucose tolerance; IRF, impaired renal function.
The numbers in parentheses indicate the number of subjects who have the indicated clinical feature.
[a]One and two patients with R137–K161del and A263fsinsGG mutations, respectively, are <25 years of age. While not presently diabetic, they are not beyond the at-risk age range.

were confirmed by cloning the PCR product into the vector pCR2.1-TOPO (Invitrogen, Carlsbad, CA) and sequencing clones derived from both alleles.

Plasmids

A construct encoding the type A form of human HNF-1β, pcDNA3.1-HNF-1β, was prepared by cloning a cDNA fragment (nt 195–2783 inclusive; GenBank accession no. X58840) into the *Kpn*I/*Eco*RI site of pcDNA3.1$^+$ (Invitrogen). The 409–483del mutation was introduced by ligating a DNA fragment containing the deletion in place of one encoding the wild-type sequence to generate pcDNA3.1-R137–K161del/HNF-1β. A pair of primers were designed, one forward primer (5'-GGGGTCACCA-AGGAGGTGCTGGTTCAGGCCTTGGAGGAGTTGCTGCC-ATC-3') containing complementary sequences upstream of the mutation and the unique restriction enzyme site *Xcm*1, and a reverse primer (5'-AGGTGTACAGAGCGGCACGCTTCT-GGGTCTGGGGGATGTTGTGTTGCTGC-3') containing complementary sequences upstream and downstream of the mutation and the unique restriction enzyme site *Bsr*G1 site. A PCR fragment containing the mutation was produced from the plasmid including the full-length wild-type cDNA in pcDNA3.1$^+$. Plasmid vector and the PCR fragment were digested with the restriction enzymes in question, and the fragment ligated into the vector. The normal and mutant proteins were also cloned in-frame into the vector pcDNA3.1/HisB which leads to the synthesis of proteins with an N-terminal Anti-Xpress Antibody epitope. The reporter gene construct, pGL3-RA, was prepared by cloning the promoter of the rat albumin gene (nucleotides –170 to +5) (21) into the firefly luciferase reporter vector pGL3-Basic *Sma*I site (Promega, Madison, WI). The sequences of all constructs were confirmed.

DNA-binding studies

Electrophoretic mobility shift analyses (EMSAs) were carried out with normal and mutant HNF-1β proteins with and without epitope tag and normal HNF-1α prepared by coupled *in vitro* transcription and translation (TNT Coupled Reticulocyte Lysate System; Promega). Equivalent amounts of [^{35}S]methionine-labeled proteins, as determined by SDS–PAGE and autoradiography, were incubated with 100 000 c.p.m. of ^{32}P-labeled double-stranded oligonucleotide corresponding to the HNF-1 binding site in the rat albumin promoter (PE56, 5'-TGTGGTTAATGATCTACAGTTA-3') (14) in a 25 µl solution of 100 mM NaCl, 10 mM Tris–HCl pH 7.5, 1 mM dithiothreitol, 1 mM EDTA, 4% glycerol and 80 µg/ml of sonicated salmon sperm DNA at room temperature for 20 min. For supershift assays, 0.25 µl of Anti-Xpress Antibody (Invitrogen) was added and the incubation continued for an additional 20 min. At the end of the incubation, 2.5 µl of loading buffer (40% Ficoll-400, 250 mM Tris–HCl pH 7.5 and 0.2% bromphenol blue) was added to the reaction and the reaction products separated on a 6% polyacrylamide gel in 0.5× TBE buffer. The gel was dried and exposed to two sheets of X-ray film with an intensifying screen at –80°C overnight. The film closest to the gel shows both ^{35}S and ^{32}P signals and the second film only the ^{32}P signal. EMSAs were also performed using 500 ng of protein prepared from cytoplasmic and nuclear fractions from HeLa cells transiently expressing normal and mutant epitope-tagged HNF-1β. The presence of HNF-1β in the fractions was confirmed by western blotting.

Transfection studies

HeLa cells grown in Dulbecco's modified Eagle medium (Gibco BRL, Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL) were transfected using FuGene6 (Boehringer Mannheim, Indianapolis, IN) and 2 µg of DNA including 0.25 µg of pcDNA3.1-HNF-1β or pcDNA3.1-R137–K161del/HNF-1β (with or without epitope-tag), 0.50 µg of the reporter gene pGL3-RA, 25 ng of pRL-SV40 to control for efficiency of transfection. After 24 h, the transactivation activity of the normal and mutant HNF-1β

proteins was measured using the Dual-Luciferase Reporter Assay System (Promega).

Subcellular localization of normal and mutant HNF-1β

HeLa cells transfected with vectors encoding Anti-Xpress Antibody epitope-tagged normal and mutant HNF-1β were grown on poly-L-lysine-coated glass coverslips. After 48 h, cells were washed with cold phosphate-buffered saline (PBS) and fixed with a solution of 4% formaldehyde in PBS. Cells were then permeabilized with a solution of 0.1% Triton X-100 and 0.1% Tween-20 in PBS. After washing three times for 10 min each in a solution of 0.1% Tween-20 in PBS, the cells were incubated with a solution of 5% donkey serum and 0.1% Tween-20 in PBS at room temperature for 2 h, and then with a 1:500 dilution of Anti-Xpress Antibody, in 5% donkey serum and 0.1% Tween-20 in PBS at 4°C overnight. After washing three times for 10 min each with a solution of 0.1% Tween-20 in PBS, the primary antibody was localized and the signal amplified by incubation with a 1:500 dilution of biotinylated donkey anti-mouse secondary antibody and streptavidin-conjugated Cy3 (Jackson ImmunoResearch Laboratories, West Grove, PA). Cells were counterstained with Hoechst 33342 (Molecular Probes, Eugene, OR) to visualize nuclei. Coverslips were wet-mounted with mounting solution (90% glycerol, 10% PBS and 2.5% 1,4-diazabicyclo[2,2,2]octane) and sealed with clear fingernail polish. Cy3 and Hoechst staining were visualized using a Nikon Eclipse E800M microscope (Nikon, Melville, NY) and appropriate filters.

ABBREVIATIONS

CRF, chronic renal failure; CRI, chronic renal insufficiency; EMSA, electrophoretic mobility shift analyses; ESRD, end-stage renal disease; FSH, follicle stimulating hormone; HNF, hepatocyte nuclear factor; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; LH, luteinizing hormone; MODY, maturity-onset diabetes of the young; OGTT, oral glucose tolerance test.

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