Kidney International, Vol. 61 (2002), pp. 1243–1251

Solitary functioning kidney and diverse genital tract malformations associated with hepatocyte nuclear factor-1β mutations

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Background. Renal tract malformations are, on occasion, associated with uterine malformations. The transcription factor hepatocyte nuclear factor (HNF)-1β is expressed from the earliest stages of development of the Wolffian duct, the mesonephros and metanephros, and the Müllerian ducts in the mouse. In adult mice HNF-1β is expressed in the kidney tubules, collecting ducts, and in the oviducts and uterus in the female (Müllerian duct derivatives) and in the epididymis, vas deferens and seminal vesicles (Wolffian duct derivatives) in the male. HNF-1β mutations have been reported in two families where affected members have renal abnormalities, female genital tract malformations and early-onset diabetes. Renal and uterine abnormalities have not been described in families without early-onset diabetes.

Methods. We sequenced the *HNF-1β* gene in nine subjects with renal abnormalities and a personal or family history of female genital tract malformations, but no history of diabetes.

Results. Two families were identified with novel HNF-1β mutations: a missense mutation in exon 2 with conversion of serine to proline at codon 151 (S151P) and a frameshift mutation in exon 3 with a 1 base pair deletion at codon 243 (Q243fsdelC). The S151P mutation proband has cystic kidneys and uterus didelphys. Her affected second son has renal cysts and hypospadias. The Q243fsdelC proband has a single functioning kidney and her two children have renal dysplasia. Histology in one child shows cystic dysplasia with a lack of glomeruli. The proband's sister is a mutation carrier and has a bicornuate uterus. Diabetes is not a feature in either family.

Conclusions. This study confirms an association between HNF-1β mutations and renal and Müllerian anomalies. The hypospadias may be coincidental. This study describes the first HNF-1β mutations that are associated with a single functioning kidney and the absence of diabetes. This study further reinforces the variability of the renal and non-renal phenotypes associated with HNF-1β mutations.

Key words: genital malformation, transcription factors, HNF-1β mutation, hypospadia, renal tract malformation, heredity, Müllerian duct abnormality, urogenital adysplasia.

Received for publication August 20, 2001
and in revised form November 12, 2001
Accepted for publication November 15, 2001

Renal tract malformations are associated with uterine malformations. Hereditary urogenital adysplasia (OMIM 191830) is an autosomal dominant disorder of Müllerian anomalies and renal agenesis, aplasia or dysplasia first described by Schimke and King [1]. The Müllerian anomalies include fusion malformations or vaginal atresia, which may be associated with absence of the uterus in the Rokitansky-Kuster-Hauser syndrome (OMIM 277000). Opitz has suggested that variable expression of a single autosomal dominant gene may give rise to developmental defects in the mesonephric (Wolffian) and paramesonephric (Müllerian) ducts [2]. The molecular genetic basis of these disorders, which have been described using clinical criteria, has not been established.

Recently, mutations in the gene encoding hepatocyte nuclear factor (HNF)-1β have been associated with diabetes, abnormal renal development and genital tract malformations. Lindner and colleagues described a family where an HNF-1β mutation was associated with young onset diabetes and non-diabetic renal disease (histology showed oligomeganephronia). Two of the four affected female subjects with the mutation had genital tract malformations with vaginal aplasia and rudimentary uterus (Mül-

lerian aplasia) [3]. One further subject with an HNF-1β mutation, from a different family, has been noted to have a bicornuate uterus, a Müllerian duct fusion abnormality, renal cysts and renal dysplasia on ultrasound examination and diabetes [4].

HNF-1β mutations have been reported in eight families in total and all have early-onset diabetes and severe non-diabetic renal disease [3–10]. The renal phenotype is variable both within and between families. Renal cysts have been seen in some members of all of the eight families described [3–10]. The histology is variable and a clinical syndrome characterized by renal cysts and diabetes (RCAD) is common [9]. Two families have familial hypoplastic glomerulocystic kidney disease with the affected subjects having small kidneys with abnormal calyces and papillae together with renal histology showing glomerulocystic kidney disease [9]. A 17-week fetus from a third family showed an absence of normal nephrogenesis with histology consistent with cystic renal dysplasia [8].

HNF-1β is a member of the homeodomain-containing superfamily of transcription factors. HNF-1β functions as a homodimer or a heterodimer with the structurally related HNF-1α [11, 12]. Heterozygous HNF-1α mutations are the most common cause of maturity-onset diabetes of the young (MODY), a dominantly inherited sub-group of young onset non–insulin-dependent diabetes mellitus [13]. However, disorders of renal development and genital tract malformations are specific to HNF-1β. These clinical features have not been seen in over 100 UK families with HNF-1α mutations (M. Shepherd, S. Ellard, A. Hattersley, personal observation). Studies of LacZ expression controlled by HNF-1β regulatory regions in mice have allowed detailed analysis of the HNF-1β expression pattern from 4.5 to 5.5 days post coitum. These studies have shown that HNF-1β is expressed in the Wolffian duct, the developing meso- and metanephros and in the Müllerian ducts from the earliest stages of differentiation. In adult mice HNF-1β is expressed in the kidney tubules and collecting ducts and in females in the inner epithelial layer of the oviduct and the uterus, which are derived from the Müllerian ducts. In adult males HNF-1β is expressed in the epididymis, vas deferens and seminal vesicles, which are derived from the Wolffian duct and in the prostate and testes. HNF-1β is also expressed in the pancreas, liver, intestine, lungs and thymus [14–16]. Reverse transcription-polymerase chain reaction (RT-PCR) has been used to show that human metanephroi express HNF-1β at preglomerular stages (54 to 56 days gestation) through to 91 days gestation. In situ hybridization studies have shown that HNF-1β transcripts are prominently expressed in fetal collecting duct branches with lower mRNA levels in metanephric mesenchyme and nephron precursors such as S-shaped bodies [10].

Our study describes members of two families with some features of urogenital adysplasia, but no history of diabetes, who have mutations in the *HNF-1β* gene.

METHODS

Subjects

The study population consisted of members of nine unrelated families with renal abnormalities and a personal or family history of genital tract malformations (Table 1). None of the subjects tested had diabetes and there was no history of diabetes in their first degree relatives. The probands in these families were screened for *HNF-1β* gene mutations. The study was approved by the local Ethics Committee and informed consent was obtained from each subject.

Mutation analysis of the *HNF-1β* gene

Genomic DNA was extracted from peripheral lymphocytes using a Nucleon DNA extraction kit (Scotlab, Coatbridge, UK).

The entire promoter and coding regions of the nine exons and the intron-exon boundaries of the *HNF-1β* gene were amplified by PCR using genomic DNA from a single proband and sequence specific primers [17]. PCR was performed in a 25 μL volume containing 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1 to 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTPs, 2.5 pmol of each primer, 0.25 U Amplitaq Gold Taq polymerase (Perkin-Elmer, Warrington, UK) and 100 ng DNA. The cycling conditions were 15 minutes at 95°C followed by 35 cycles consisting of one minute at 94°C, one minute at 60°C, and two minutes at 72°C.

Polymerase chain reaction products were purified using a QIAquick column (Qiagen, Crawley, UK) and both strands sequenced using a BigDye Terminator Cycle Sequencing kit (Perkin-Elmer Applied Biosystems) according to the manufacturer's recommendations. Reactions were analyzed on an ABI Prism™ 377 DNA Sequencer (Perkin-Elmer Applied Biosystems).

Clinical studies

When an HNF-1β mutation was found in the proband, genetic testing and additional clinical studies were performed in all consenting family members and the medical notes were reviewed.

Glucose tolerance. A standard 75 g oral glucose tolerance test (OGTT) was given after a 12-hour overnight fast. Blood samples for glucose were taken at 0 and 120 minutes.

Biochemical tests of renal function. Blood samples were taken for measurement of serum sodium, potassium, urea, creatinine and HbA_{1c}.

Radiological investigations

Renal ultrasound and intravenous pyelogram (IVP) studies, which had previously been performed on a number of subjects, were reviewed.

Table 1. Clinical characteristics of the probands

Family proband	Mutation	Renal malformation	Genital malformation	Family history of renal/ genital malformations	Other features
DUK339	None	Single kidney	Bicornuate uterus	None	Gestational diabetes. Maternal grandmother type 2 diabetes
DUK396	None	Single kidney	Bicornuate uterus and cervix	Mother - single kidney, bicornuate uterus	
DUK417	None	Single kidney	Absent uterus and fallopian tubes	Sister - single kidney, absent uterus and fallopian tubes Father - duplex urinary system	
DUK448	S151P	Renal cystic disease	Uterus didelphys	Son - hypospadias	Recurrent shoulder and patella dislocations. Hearing loss
DUK486	None	Enlarged kidneys	Bicornuate uterus	Mother - cystic kidneys and bicornuate uterus	Short fingers Mother - short stature, small hands, narrow chest, short clavicles
DUK487	None	Horseshoe kidney	Bicornuate uterus and biseptate vagina	Daughter - horseshoe kidney	Shoulder dysplasia and subluxation of patellae. Hearing loss
DUK505	None	Single kidney	Uterus didelphys	Brother - cystic renal dysplasia with atrophy of 1 kidney	Maternal uncle ESRF
DUK506	None	Hypoplastic right kidney	Absent right ovary and fallopian tube	Cousin - unilateral renal aplasia 2nd cousin - unilateral renal dysplasia	
DUK507	Q243fsdelC	Single kidney	None	Son and daughter - cystic renal dysplasia Sister - bicornuate uterus Mother - 2 small kidneys	

Renal histology

Renal histology was available from subject IV1 in family DUK507 and this was reviewed.

RESULTS

Identification of mutations in the *HNF-1β* gene

Heterozygous mutations were identified in two of the nine probands. A novel missense mutation in exon 2 of the *HNF-1β* gene was identified in the proband (II2) from family DUK448. The mutation was also present in the proband's affected second-born son III2 (Fig. 1A), however, other members of the family declined testing. This mutation is the conversion of TCC to CCC at codon 151 with the conversion of serine (uncharged polar) to proline (non-polar), mutation designated S151P.

A novel frameshift mutation in exon 3 of the *HNF-1β* gene was identified in the proband III5 from family DUK-507. This mutation was also present in the proband's son (IV1), daughter (IV2) and sister (III2) (Fig. 1B). The 1 base pair deletion at codon 243 (mutation designated Q243fsdelC) resulted in a frameshift, which was predicted to cause premature termination at codon 264.

Neither the S151P nor the Q243fsdelC mutation was present in 100 normal chromosomes analyzed by sequencing.

Clinical characteristics

Family DUK448. The clinical characteristics of the two members of family DUK448 with the HNF-1β mutation S151P are shown in Table 2. Cystic kidney disease was diagnosed in the proband II2 at the age of 26 years on ultrasound scanning. She has renal impairment with a serum creatinine of 168 μmol/L (Table 2). II2 has uterus didelphys and double vagina. Her first pregnancy resulted in a male infant III1 who is in good health (not genotyped). Her second pregnancy resulted in a miscarriage at 8 weeks gestation. There was no measurement of her glucose tolerance during her first two pregnancies. During her third pregnancy glucosuria was detected on routine antenatal screening and she had an OGTT performed at 27 weeks gestation [fasting plasma glucose (fpg) 5.4 mmol/L and a two hour glucose 7.5 mmol/L] with an HbA_{1c} of 5.5%. The glucose tolerance test was repeated at 33 weeks gestation and showed an impaired glucose tolerance (fpg 5.1 mmol/L, 2 h 9.1 mmol/L). She did not require any treatment for her impaired glucose tolerance during the remainder of the pregnancy. Since delivery she has had a normal OGTT (fpg 5.2 mmol/L, 2 h 5.9 mmol/L) aged 35 years. There is no known history of diabetes in the rest of the family but they have declined investigation.

Her third pregnancy resulted in a male infant III2 who was noted on antenatal ultrasound at 32 weeks gestation to have enlarged echogenic kidneys with loss of the normal corticomedullary differentiation. Postnatally he has been noted to have multiple small cortical and corticomedullary cysts in both kidneys. He has glandular hypospadias and a hooded appearance to the prepuce.

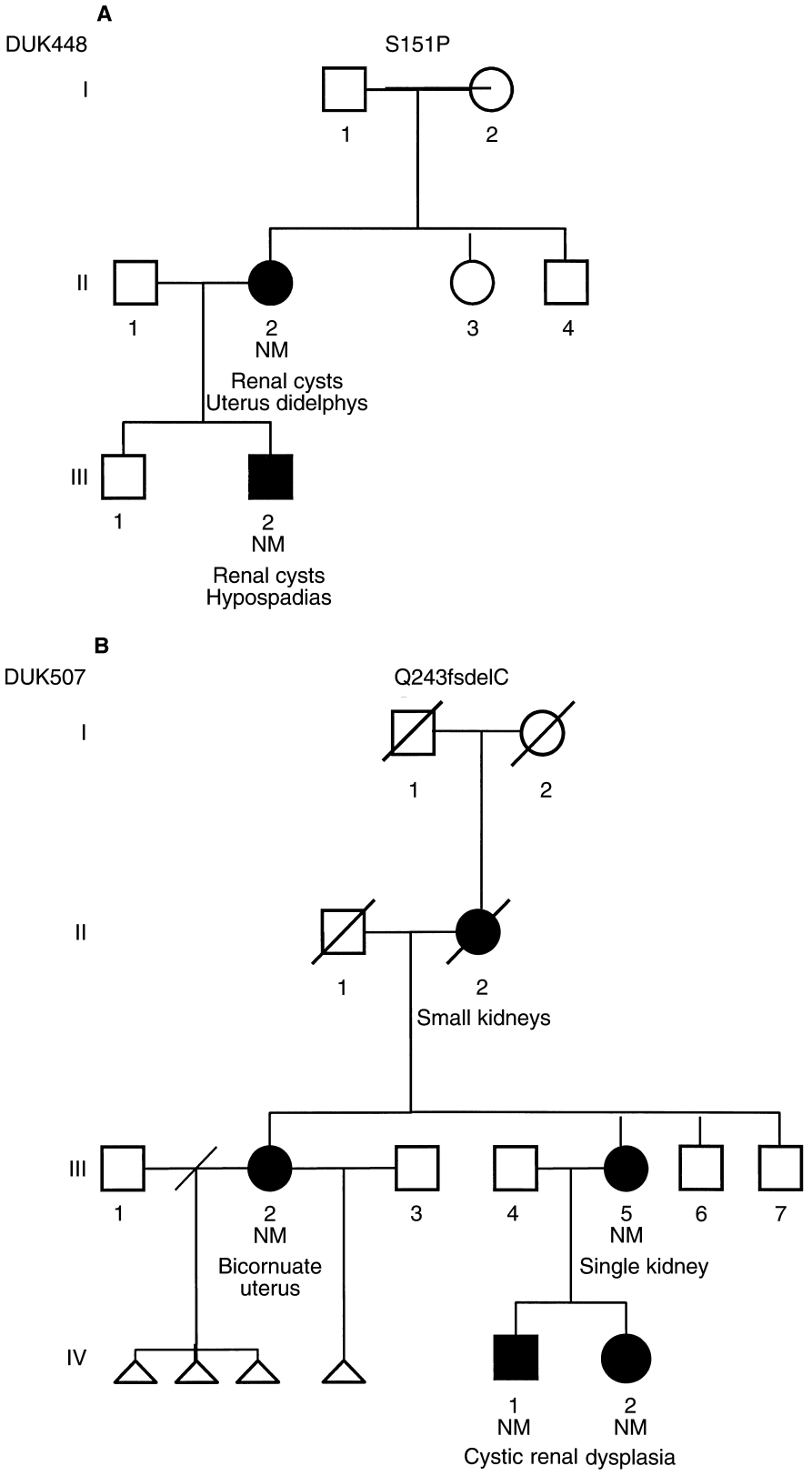

Fig. 1. (*A*) Pedigree of family DUK448 with the S151P mutation in the *HNF-1β* gene. (*B*) Pedigree of family DUK507 with the Q243fsdelC mutation in the *HNF-1β* gene. Roman numerals on the left of the figure indicate generation number, and the numbers below the symbols indicate individuals within that generation. The *HNF-1β* genotype of each individual tested is indicated below the symbol: N, normal; M, mutated allele. Subjects with renal and/or genital manifestations are shaded with the details summarized in the text beneath.

Table 2. Clinical characteristics of subjects with HNF-1β mutations

Family	DUK448	DUK448	DUK507	DUK507	DUK507	DUK507
Subject	II2	III2	III2	III5	IV1	IV2
Mutation	S151P	S151P	Q243fsdelC	Q243fsdelC	Q243fsdelC	Q243fsdelC
Present age	35	1	44	30	10	7
Creatinine *μmol/L*	168 (55–108)	NA	98 (55–95)	102 (55–95)	154 (40–80)	63 (35–70)
Calculated creatinine clearance *mL/min*	66	NA	65	61	45/1.73 m^2	63/1.73 m^2
IVP	NA	NA	NA	Single left kidney with abnormal blunted calyces	NA	NA
Renal ultrasound	Multiple cysts	Multiple cysts	Normal kidneys	NA	Bilateral cystic renal dysplasia	Bilateral cystic renal dysplasia
Renal histology	NA	NA	NA	NA	Cystic dysplasia	NA
Genital malformations	Uterus didelphys + double vagina	Hypospadias	Bicornuate uterus with single cervix	Nil known	Nil known	Nil known
Other features	Recurrent shoulder and patella dislocations Hearing loss		4 spontaneous abortions			

Abbreviations are: IVP, intravenous pyelogram; NA, not available.
Reference ranges are in parentheses.

The proband's parents and two siblings have no history of renal disease, genital tract malformations, or diabetes but they declined further testing.

Family DUK507. The clinical characteristics of the members of family DUK507 with the HNF-1β mutation Q243fsdelC are shown in Table 2. The proband III5 was diagnosed with a single left kidney of normal size on an IVP at the age of 22 years. On review of the IVP the calyces have been noted to be abnormal and blunted (Fig. 2). She has a serum creatinine of 102 μmol/L aged 30 years (Table 2) and normal glucose tolerance (fpg 4.8 mmol/L, 2 h 6.1 mmol/L) with an HbA_{1c} of 5.5%. She has no known uterine abnormality.

The proband's son IV1 was diagnosed with bilateral renal dysplasia on antenatal ultrasound at 20 weeks gestation. Ultrasound examination four days postnatally showed bilateral cystic renal dysplasia and hydronephrosis. At one month of age a DMSA scan showed nonfunction of the right kidney and poor function of the left kidney. The collecting system of the left kidney was noted on an IVP to be dilated with a pelviureteric junction obstruction. There was no evidence of reflux on a micturating cystogram. At two months of age a right nephrectomy and left pyeloplasty were performed. Subject IV1 has chronic renal failure with a glomerular filtration rate (GFR) of 45 mL/min/1.73 m^2. There is no known genital tract malformation. Blood glucose has not been recorded in subject IV1.

The proband's daughter IV2 was found to have bilateral cystic renal dysplasia on ultrasound screening at one month of age. She has mild renal impairment with a GFR of 63 mL/min/1.73 m^2. There is no known uterine malformation. Blood glucose has not been recorded in subject IV2.

The proband's sister III2 has a bicornuate uterus with a single cervix and normal ovaries and fallopian tubes. She has a history of four spontaneous abortions at 24, 26, 12 and 21 weeks. The spontaneous abortion at 26 weeks was of a male fetus and at 21 weeks of a female fetus, both had a normal karyotype and kidneys and genitalia that appeared normal. No details are available on the other two fetuses. Subject III2 had an ultrasound scan at the age of 37 years that showed normal kidney appearances. She has a serum creatinine of 98 μmol/L (Table 2) and a non-diabetic fasting plasma glucose of 6.1 mmol/L and HbA_{1c} of 5% aged 44 years.

The proband's mother, II2, (not genotyped) died aged 59 years of metastatic breast carcinoma. She had a history of chronic pyelonephritis. On an IVP she had bilateral small kidneys with loss of calyceal cupping. She had chronic renal failure but no history of diabetes. The proband's other two siblings are not known to have a history of renal disease, genital tract malformations or diabetes, and they have declined genotyping.

Renal histology

The renal histology on subject IV1 showed typical features of cystic dysplastic kidney with a lack of glomeruli. There were dysplastic tubules surrounded by poorly differentiated stroma (Fig. 3A), larger cysts without evidence of glomerular tufts (not shown), as well as metaplastic cartilage (Fig. 3B).

DISCUSSION

The novel HNF-1β mutations Q243fsdelC and S151P have been identified in two out of nine families with renal and genital tract malformations. This confirms previous

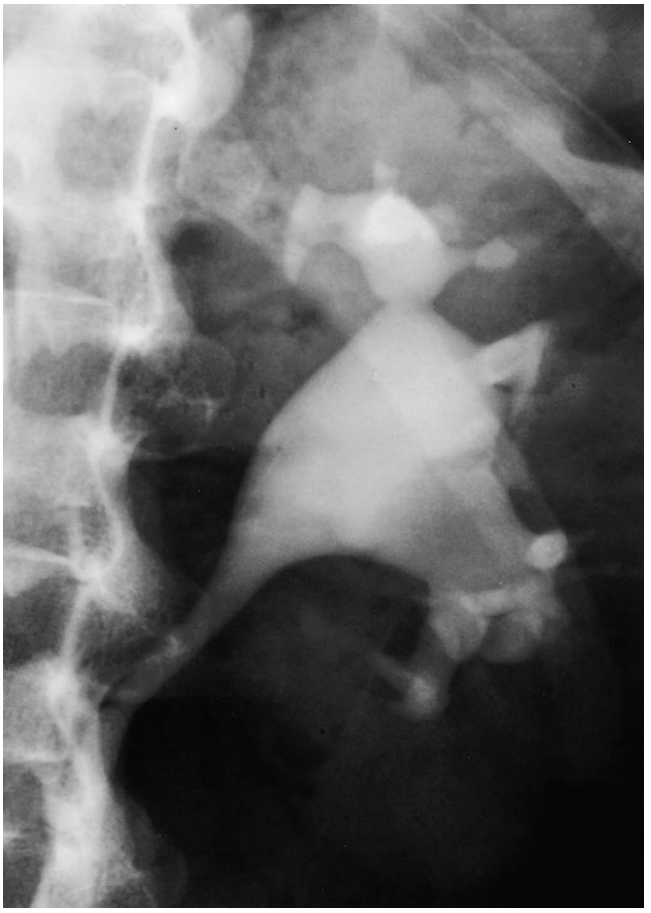

Fig. 2. Intravenous pyelogram (IVP) of left kidney from subject III5 in family DUK507 showing the renal pelvis and blunted calyces.

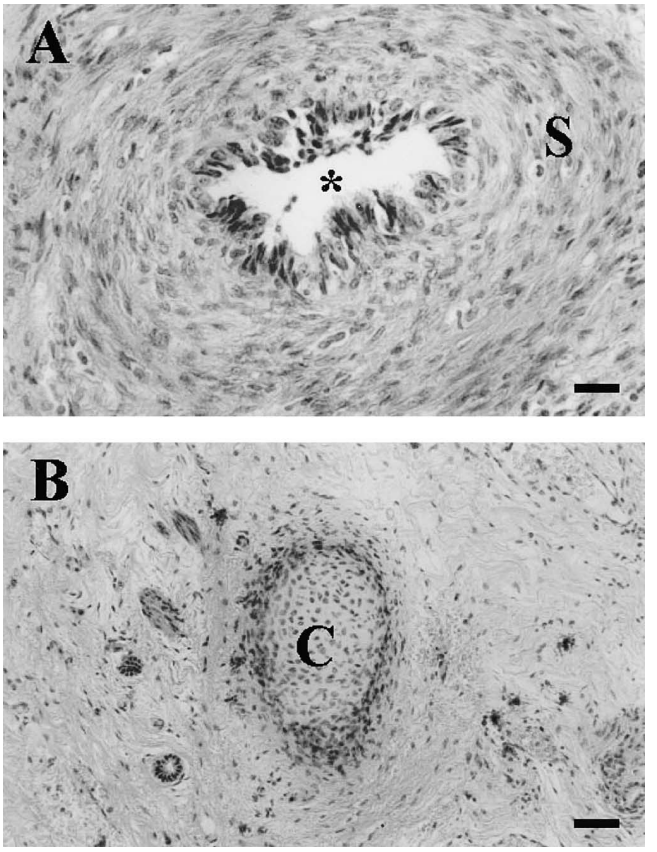

Fig. 3. Histology sections of dysplastic kidney stained with hematoxylin and eosin. (*A*) Dysplastic tubule (*marks the lumen) surrounded by poorly differentiated stromal tissues(s). (*B*) Metaplastic cartilage (c). Note the absence of normal nephrons and glomeruli. Bars are 15 μ in A and 150 μ in B.

reports of an association between HNF-1β mutations and renal and genital tract malformations and suggests it may be a relatively common molecular genetic diagnosis. The new features associated with the HNF-1β mutations in the families described in this report are the absence of diabetes, a single functioning kidney and an association in one subject of a male genital tract malformation, hypospadias.

These are the first families with HNF-1β mutations to be described where early-onset diabetes has not been a feature in some affected subjects. In the other reported families diabetes typically develops after the renal disease, mean age at diagnosis 26.2 years (Table 3), the oldest subject at diagnosis is 61 years [6]. However, hyperglycemia may be mild in some patients and hence undiagnosed for a long period of time. The age of diagnosis with diabetes may not therefore be the age of onset and the diagnosis of diabetes may only be made on an OGTT. Impaired glucose tolerance (2 hour glucose 10.4 mmol/L) was detected in an OGTT in a subject with an HNF-1β mutation aged 17 years [9]. The two probands represent the oldest mutation carriers who do not have

Table 3. Phenotype summary for all reported subjects with HNF-1β mutations

Clinical feature	Number of affected subjects (*% of total*)
Renal cysts	24/32 (75) 1 not examined
Renal impairment	29/32 (91) 1 not examined
Renal histology	Oligomeganephronia 1 Renal dysplasia 2 Glomerulocystic kidney disease 3
Diabetes	18/32 (56) Mean age of diagnosis 26.2 years 2 impaired glucose tolerance aged 1 year and 17 years 8 not examined
Uterine malformations	5/23 (22)
Male genital tract malformations	1/9 (11)
Other features	1 pyloric stenosis 1 prognathism 1 learning difficulties 1 ligament laxity 1 hearing loss

Data are from [3–10].

diabetes after testing. Although they are not diabetic at present they may be at increased risk of developing diabetes in the future.

The renal phenotype is variable both between and within the two families with mutations. The proband, III5 in family DUK507 has a single kidney of normal size with abnormal, blunted calyces (Fig. 2). This is the first subject with an HNF-1β mutation with a single functioning kidney. A single kidney may be the result of unilateral renal agenesis, a condition in which the kidney fails to develop. The incidence of unilateral renal agenesis is 1 in 1000 [18]. Alternatively, pre- or postnatal regression of a multicystic dysplastic kidney will produce an identical radiological appearance of a single kidney [19, 20]. Subject III5 has no features of multiorgan disorders such as branchio-oto-renal and Kallmann syndromes, which are associated with unilateral renal agenesis [21]. Calyceal abnormalities are a feature of subjects with HNF-1β mutations associated with familial hypoplastic glomerulocystic kidney disease [9]. The calyces are derived from the ureteric bud in the developing embryo and this has been shown to be a site of HNF-1β expression [10]. Calyceal abnormalities were not a feature in the subjects examined with a single kidney who did not have an HNF-1β mutation. Subjects IV1 and IV2 in family DUK507 have bilateral cystic renal dysplasia and impaired renal function. Bilateral renal dysplasia was noted on antenatal ultrasound scanning at 20 weeks gestation in subject IV1. Renal abnormalities were noted at 32 weeks gestation in subject III2 from family DUK448. Renal abnormalities have been detected in utero in a number of other reported subjects with HNF-1β mutations [6, 8, 10]. This supports a major role for HNF-1β in human kidney development. Renal histology consistent with cystic renal dysplasia has previously been reported in one affected subject, a 17-week female fetus [8]. In the current study renal histology has been examined from the non-functioning right kidney in subject IV1 in family DUK507 and it shows changes characteristic of cystic dysplasia with a lack of glomeruli (Fig. 3). Since subject IV1 has impaired renal function, but not renal failure, it may be assumed that his dysplastic left kidney does contain glomeruli. Cystic glomeruli were a feature of the histology in the 17-week fetus [8]. Subject II2, the proband's mother, who is deceased, was known to have bilateral small kidneys with loss of calyceal cupping. This had been attributed to chronic pyelonephritis but it may have been a manifestation of an HNF-1β mutation. The proband, II2 in family DUK448 has renal cystic disease, the renal histology has not been examined. There is a cystic component to the renal malformations in the majority of the affected subjects in the two families. This is consistent with the previous reports of HNF-1β mutations where the presence of renal cysts has been one of the cardinal features (Table 3).

The Müllerian (paramesonephric) ducts develop into the main genital duct in the female embryo. The caudal, vertical parts of the two Müllerian ducts fuse to form the corpus and the cervix of the uterus and the upper third of the vagina. HNF-1β expression has been demonstrated in the Müllerian ducts in the mouse embryo and in the inner epithelial layer of the uterus in the adult mouse [14]. The previous reports of uterine malformations associated with HNF-1β mutations have included both a Müllerian duct fusion abnormality giving rise to a bicornuate uterus and vaginal aplasia with rudimentary uterus as in the Rokitansky-Kuster-Hauser syndrome [3, 4]. Subject III2 in family DUK507 has a bicornuate uterus, a Müllerian duct fusion abnormality. She has previously had a normal renal ultrasound. This is the first subject to be described with an HNF-1β mutation and an isolated genital tract malformation. There is no known history of genital tract malformations in the other affected subjects in this family. In family DUK448, subject II2 has uterus didelphys and double vagina. This is a more severe phenotype of a Müllerian duct fusion abnormality. In addition, subject III2 has hypospadias, the first male genital tract malformation to be described in a subject with an HNF-1β mutation. Hypospadias is present in 1 in 300 male children, so this may be a coincidental finding in this child [22]. In animal studies HNF-1β expression has not been demonstrated in the urethra [14, 16]. There were no features suggestive of either the Denys-Drash or Townes-Brocks syndromes, which have defined genetic associations and include renal abnormalities and hypospadias [23, 24].

Hereditary urogenital adysplasia describes the combination of anomalies of the urinary tract and the Müllerian duct [1]. A number of kindreds have been described with affected members in more than one generation consistent with autosomal dominant transmission. The family reported by Battin supports autosomal dominant inheritance with incomplete penetrance and variable expressivity [25]. In the families reported by Biedl three males were described with renal agenesis. This indicates that the condition is not sex limited [26]. Opitz suggested that hereditary urogenital adysplasia may be the result of variable expression of a single autosomal dominant gene [2]. The transcription factor PAX2 has been considered a candidate gene for this syndrome [27]. PAX2 is expressed in the human mesonephric (Wolffian) duct and paramesonephric (Müllerian) duct [28]. PAX2 mutations may cause the renal-coloboma syndrome, but female genital tract malformations have not been described in association with this syndrome [29]. The PAX2 gene was not evaluated for mutations in the seven probands in the current series who did not have HNF-1β mutations. HNF-1β is expressed in the Wolffian and Müllerian ducts in the mouse and also may be considered as a candidate gene. A conserved pattern of HNF-1β expression has been demon-

strated from the earliest stages of kidney development in rat, mouse and human [10, 14, 30]. Our work suggests that hereditary urogenital adysplasia is a heterogeneous condition in which HNF-1β mutations may be associated with some cases, particularly those with a cystic component to the renal malformations. Hypospadias may not be part of this disease spectrum, it was only present in one affected subject and the incidence of hypospadias is high in the general population.

The Q243fsdelC mutation is predicted to result in premature termination at codon 264, resulting in a truncated protein that lacks part of the DNA-binding region and all of the transactivation domain. Alternatively, the mutant mRNA transcript may be subject to nonsense mediated decay and consequent absence (or reduced level) of mutant protein [31]. The Q243fsdelC mutation is therefore likely to result in loss of function or haploinsufficiency. The A263fsinsGG mutation reported in a Japanese family with renal cysts and early-onset diabetes but no genital tract malformations also results in premature termination at codon 264 [6]. The renal cysts in this family were noted on ultrasound examination, but no renal histology was available [6]. If these mutant proteins are expressed, we speculate that the different phenotypes reflect the variation of the C-terminal 21 amino acids that are predicted to be abnormal in the Q243fsdelC mutant protein. However, since the penetrance of the genital malformations within families is incomplete, both mutations may result in haploinsufficiency (or truncated proteins with similar functional capacity) and the phenotypic differences between families reflect the general phenotypic variability associated with HNF-1β mutations. The timing of the development of diabetes may depend on the available β cell functional mass and this may vary between the two families. The S151P mutation results in a significant amino acid change with serine, an uncharged polar amino acid being substituted by the non-polar proline. The serine at position 151 is evolutionarily conserved in both the *HNF-1α* and *HNF-1β* genes. Further evidence that this is a significant mutation is that serine is conserved in rat, mouse, pig, and *Xenopus*, and the change is not present in 100 normal chromosomes. Evidence of co-segregation is difficult to assess, as it has only been possible to genotype two members of the DUK448 family. It is intriguing to note that the subject with uterus didelphys, the more severe uterine malformation, has the missense mutation. Functional studies and site-specific mutation experiments in animals will need to be performed on both mutations to allow further genotype phenotype correlations.

Functional studies have shown that the R137-K161 mutation, which resulted in oligomeganephronia and genital tract malformations, is a loss-of-function mutation. There is deletion of part of the pseudo-POU domain that is involved in the specificity of DNA binding, but the homeodomain and the transactivation domain are intact. Lindner and colleagues suggested that genital development may be altered by the mutant transcription factor with its intact transactivation domain interacting with other transcription factors and the basal transcription machinery [3]. Functional studies are not available on the IVS2nt + 1G > A mutant, which also resulted in renal and genital malformations [4]. This mutation would be predicted to result in defective splicing. The other reported case of cystic renal dysplasia, in a fetus, was a result of the frameshift mutation P328L329fsdelCCTCT [8]. This has been shown to be a gain-of-function mutation and overexpression of this mutation in the *Xenopus* embryo leads to defective development and agenesis of the pronephros, the first kidney form of amphibians [32].

HNF-1β mutations are clearly not the only cause of renal and genital tract malformations as HNF-1β mutations were not identified in seven out of the nine families tested. In four out of the seven probands the renal malformation has been a single kidney, in one unilateral renal hypoplasia, in one a horseshoe kidney and in one enlarged kidneys with a family history of renal cysts. In family DUK486 there were other dysmorphic features present (Table 1). Bilateral renal changes are more commonly associated with HNF-1β mutations.

In conclusion, we report two families with renal and genital tract malformations that cosegregate with mutations in the *HNF-1β* gene. The renal malformations frequently involve a cystic component and both male and female genital tract malformations occur. Unlike the other reported cases of HNF-1β mutations, early-onset diabetes was not a feature in these families. This study reinforces the variability of the renal and the non-renal phenotype both within and between families with HNF-1β mutations.

ACKNOWLEDGMENTS

We thank the National Kidney Research Fund (grant TF13/2000), the European Union funding for the GIFT consortium, Diabetes UK, the British Medical Association and the Exeter Kidney Unit Development Fund, who all supported this work. The authors thank the families and their clinicians who made this study possible. The assistance of Ms. Susan Brittain-Jones is appreciated.

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REFERENCES

1. Schimke RN, King CR: Hereditary urogenital adysplasia. *Clin Genet* 18:417–420, 1980
2. Opitz JM: Vaginal atresia (von Mayer-Rokitansky-Kuster or MRK anomaly) in hereditary renal adysplasia (HRA). *Am J Med Genet* 26:873–876, 1987
3. Lindner TH, Njolstad PR, Horikawa Y, *et al*: 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. *Hum Mol Genet* 8:2001–2008, 1999

4. Iwasaki N, Okabe I, Momoi MY, *et al*: Splice site mutation in the hepatocyte nuclear factor-1β gene, IVS2nt + 1G>A, associated with maturity-onset diabetes of the young, renal dysplasia and bicornuate uterus. *Diabetologia* 44:387–388, 2001
5. Horikawa Y, Iwasaki N, Hara M, *et al*: Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY. *Nat Genet* 17:384–385, 1997
6. Nishigori H, Yamada S, Kohama T, *et al*: Frameshift mutation, A263fsinsGG, in the hepatocyte nuclear factor-1 beta gene associated with diabetes and renal dysfunction. *Diabetes* 47:1354–1355, 1998
7. Iwasaki N, Ogata M, Tomonaga O, *et al*: Liver and kidney function in Japanese patients with maturity-onset diabetes of the young. *Diabetes Care* 21:2144–2148, 1998
8. Bingham C, Ellard S, Allen L, *et al*: Abnormal nephron development associated with a frameshift mutation in the transcription factor hepatocyte nuclear factor-1β. *Kidney Int* 57:898–907, 2000
9. Bingham C, Bulman MP, Ellard S, *et al*: Mutations in the hepatocyte nuclear factor-1β gene are associated with familial hypoplastic glomerulocystic kidney disease. *Am J Hum Genet* 68:219–224, 2001
10. Kolatsi-Joannou M, Bingham C, Ellard S, *et al*: Hepatocyte nuclear factor-1β: A new kindred with renal cysts and diabetes, and gene expression in normal development. *J Am Soc Nephrol* 12:2175–2180, 2001
11. Mendel DB, Hansen LP, Graves MK, *et al*: HNF-1 alpha and HNF-1 beta (vHNF-1) share dimerisation and homeo domains, but not activation domains, and form heterodimers in vitro. *Genes Dev* 5:1042–1056, 1991
12. Rey-Campos J, Chouard T, Yaniv M, Cereghini S: vHNF1 is a homeoprotein that activates transcription and forms heterodimers with HNF1. *EMBO J* 10:1445–1457, 1991
13. Hattersley AT: Maturity-onset diabetes of the young: Clinical heterogeneity explained by genetic heterogeneity. *Diabetic Med* 15: 15–24, 1998
14. Coffinier C, Barra J, Babinet C, Yaniv M: Expression of the vHNF/HNF1β homeoprotein gene during mouse organogenesis. *Mech Dev* 89:211–213, 1999
15. Barbacci E, Reber M, Ott M-O, *et al*: Variant hepatocyte nuclear factor 1 is required for visceral endoderm specification. *Development* 126:4795–4805, 1999
16. Reber M, Cereghini S: Variant hepatocyte nuclear factor 1 expression in the mouse genital tract. *Mech Dev* 100:75–78, 2001
17. Beards F, Frayling T, Bulman M, *et al*: Mutations in hepatocyte nuclear factor-1β are not a common cause of maturity-onset diabetes of the young in the UK. *Diabetes* 47:1152–1153, 1998
18. Kiprov DD, Colvin RB, McCluskey RT: Focal and segmental glomerulosclerosis and proteinuria associated with unilateral renal agenesis. *Lab Invest* 46:275–281, 1982
19. Dungan JA, Fernandez MT, Abbit PL, *et al*: Multicystic dysplastic kidney. Natural history of prenatally detected cases. *Prenat Diagn* 10:175–180, 1990
20. Sukthar S, Watson AR: Unilateral multicystic dysplastic kidney disease; defining the natural history. *Acta Paediatr* 89:811–813, 2000
21. Woolf AS: The single kidney, in *Pediatric Surgery and Urology: Long-term Outcomes*, edited by Stringer MD, Mouriquand PDE, Oldham KT, Howard ER, London, Saunders, 1998, pp 625–631
22. Sweet RA, Schrott HG, Kurland R, *et al*: Study of the incidence of hypospadias in Rochester, Minnesota 1940–1970, and a case control comparison of possible etiologic factors. *Mayo Clin Proc* 49:52–59, 1974
23. Little MH, Williamson KA, Mannens M, *et al*: Evidence that WT1 mutations in Denys-Drash syndrome patients may act in a dominant-negative fashion. *Hum Mol Genet* 2:259–264, 1993
24. Powell CM, Michaelis RC: Townes-Brocks syndrome. *J Med Genet* 36:89–93, 1999
25. Battin J, Lacombe D, Leng JJ, *et al*: Familial occurrence of hereditary renal adysplasia with Müllerian anomalies. *Clin Genet* 43:23–24, 1993
26. Biedel CW, Pagon RA, Zapata JO: Müllerian anomalies and renal agenesis: Autosomal dominant urogenital adysplasia. *J Pediatr* 104:861–864, 1984
27. Woolf AS: Solitary kidney and bicornuate uterus in mother and child. *Nephrol Dial Transplant* 14:960–961, 1999
28. Winyard PJD, Bao Q, Hughes RC, Woolf AS: Epithelial galectin-3 during human nephrogenesis and childhood cystic diseases. *J Am Soc Nephrol* 8:1647–1657, 1997
29. Sanyanusin P, McNoe LA, Sullivan MJ, *et al*: Mutation of *PAX2* in two siblings with renal-coloboma syndrome. *Hum Mol Genet* 4:2183–2184, 1995
30. Lazzaro D, de Simone V, de Magistris L, Lehtonen E, *et al*: LFB1 and LFB3 homeoproteins are sequentially expressed during kidney development. *Development* 114:469–479, 1992
31. Frischmeyer PA, Dietz HC: Nonsense-mediated mRNA decay in health and disease. *Hum Mol Genet*: 8 1893–1900, 1999
32. Wild W, Pogge von Strandmann E, Nastos A, *et al*: The mutated human gene encoding hepatocyte nuclear factor-1β inhibits kidney formation in developing *Xenopus* embryos. *Proc Natl Acad Sci USA* 97:4695–4700, 2000