Mutations in hepatocyte nuclear factor-1 β and their related phenotypes

E L Edghill, C Bingham, S Ellard, A T Hattersley

J Med Genet 2006;43:84–90. doi: 10.1136/jmg.2005.032854

Background: Hepatocyte nuclear factor-1 beta (HNF-1 β) is a widely distributed transcription factor which plays a critical role in embryonic development of the kidney, pancreas, liver, and Mullerian duct. Thirty HNF-1 β mutations have been reported in patients with renal cysts and other renal developmental disorders, young-onset diabetes, pancreatic atrophy, abnormal liver function tests, and genital tract abnormalities.

Methods: We sequenced the HNF-1 β gene in 160 unrelated subjects with renal disease, 40% of whom had a personal/family history of diabetes.

Results: Twenty three different heterozygous HNF-1 β mutations were identified in 23/160 subjects (14%), including 10 novel mutations (V61G, V110G, S148L, K156E, Q176X, R276Q, S281fsinsC, R295P, H324fsdelCA, Q470X). Seven (30%) cases were proven to be due to de novo mutations. Renal cysts were found in 19/23 (83%) patients (four with glomerulocystic kidney disease, GCKD) and diabetes in 11/23 (48%), while three other families had a family history of diabetes. Only 26% of families met diagnostic criteria for maturity-onset diabetes of the young (MODY) but 39% had renal cysts and diabetes (RCAD). We found no clear genotype/phenotype relationships.

Conclusion: We report the largest series to date of HNF-1 β mutations and confirm HNF-1 β mutations as an important cause of renal disease. Despite the original description of HNF-1 β as a MODY gene, a personal/family history of diabetes is often absent and the most common clinical manifestation is renal cysts. Molecular genetic testing for HNF-1 β mutations should be considered in patients with unexplained renal cysts (including GCKD), especially when associated with diabetes, early-onset gout, or uterine abnormalities.

Hepatocyte nuclear factor-1 β is a widely distributed transcription factor that is vital for embryonic survival.^{1,2} Early expression of hepatocyte nuclear factor-1 beta (HNF-1 β) is seen in the kidney, liver, bile ducts, thymus, genital tract, pancreas, lung, and gut.^{3,4} It can act either as a homodimer or as a heterodimer with HNF-1 α .⁵

The HNF-1 β (TCF2) gene is located on chromosome 17q21.3. The first description of HNF-1 β mutations associated with disease was in 1997.⁶ A total of 30 heterozygous mutations in the HNF-1 β gene have been described including missense, nonsense, frameshift, insertion/deletions, and splice site mutations.^{7–28} The majority of these mutations are familial, but eight spontaneous mutations^{7–9,13,15,17,23} and one germline mosaic mutation¹⁶ have been described.

The first HNF-1 β mutation identified was in the autosomal dominant, young onset, subtype of diabetes known as maturity-onset diabetes of the young (MODY). Horikawa

and colleagues showed that 1/57 Japanese MODY subjects had a mutation in the HNF-1 β gene.⁶ A potential role for HNF-1 β in MODY was suggested after mutations in the gene encoding its heterodimer partner HNF-1 α were found to be a common cause of MODY.²⁹ Although HNF-1 β gene mutations are not a common cause of MODY in the UK,³⁰ diabetes is frequently associated with HNF-1 β mutations. It is usually of early-onset (median age 20 years (range 15 days–61 years) and insulin treatment is common.³¹ Consistent with HNF-1 β having a key role in the development of the pancreas,³² pancreatic atrophy and exocrine dysfunction are common¹⁵ and neonatal diabetes has been reported.¹⁶

Extra-pancreatic disease was rapidly recognised with the description of renal cystic disease¹⁹ and since then the involvement of multiple organs has been described. The pattern of clinical features follows the expression of HNF-1 β which is primarily in the kidney, pancreas, liver, and genital tract.^{3,4} The most consistent feature has been renal disease (seen in all probands and 78/88 (89%) mutation carriers). The renal disease described is very heterogeneous but is always due to aberrant renal development and includes: renal cysts,^{6–15,17–19,21–26,28,33} familial hypoplastic glomerulocystic kidney disease (GCKD),^{8,23,27} renal malformations (for example, single and horseshoe kidney),^{9,21} and atypical familial hyperuricaemic nephropathy.¹⁰ Other clinical features include genital tract malformations,^{9,18,20,26} abnormal liver function tests,^{6,15,24,26} pancreatic atrophy and exocrine insufficiency,¹⁵ biliary manifestations,²⁵ gout and hyperuricaemia.^{10,12}

Understanding the pathogenic mechanisms of HNF-1 β mutations was delayed as homozygous HNF-1 β knockout mice were not viable and heterozygous HNF-1 β knockout mice had no phenotype.^{1,2} However, organ specific mouse knockouts have been created for the liver,³⁴ kidney,³⁵ and pancreas,³⁶ and all of these have organ specific phenotypes similar to those seen in patients with HNF-1 β mutations. Functional studies have shown mutations with either loss of function,^{17,18} dominant negative actions,^{17,25,37} or gain of function.²²

The majority of patients reported in the literature have been described in case reports or belong to small cohorts (n<3). The largest previous series to date is a study of 20 subjects with diabetes diagnosed before 40 years of age (who did not have obesity or pancreatic autoantibodies) who also had renal disease. Eight of these patients (40%) were found to have HNF-1 β mutations.¹⁵ In this study, we report a series of 160 patients with unexplained renal disease (with or without diabetes) and describe the genetics, phenotypes, and genotype-phenotype relationships in the 23 subjects with mutations in the HNF-1 β gene.

Abbreviations: FJHN, familial juvenile hyperuricaemic nephropathy; GCKD, glomerulocystic kidney disease; HNF-1 β , hepatocyte nuclear factor-1 beta; MODY, maturity-onset diabetes of the young; RCAD, renal cysts and diabetes

Table 1 Summary of clinical findings in the renal disease cohort

Renal disease	Renal disease, n (%)	Diabetes	FH diabetes only	Genital tract abnormalities	No diabetes or genital tract abnormalities
Renal cystic disease and cystic dysplasia	90 (57%)	32	12	3	55
GCKD	13 (8%)	2	1	0	11
Atypical FJHN	7 (4%)	2	0	0	5
Renal dysplasia	21 (13%)	6	2	4	11
Renal malformations	29 (18%)	5	2	12	12
Total	160	47 (29%)	17 (10%)	19 (12%)	94 (59%)

Five groups of renal disease are shown, with personal or family history of diabetes indicated. Classifications of individual renal categories are all defined in the text and are exclusive. Diabetes and genital tract abnormalities may coexist in a single proband. FH, diabetes only, family history of diabetes but no diabetes in probands; FJHN, familial juvenile hyperuricaemic nephropathy; GCKD, glomerulocystic kidney disease.

METHODS

Subjects

A total of 160 Caucasian subjects were recruited from the UK, Europe, and the USA. The inclusion criterion was renal disease without a defined aetiology. Renal disease was classified into five groups: (1) GCKD diagnosed on the basis of histological or radiological appearance; (2) atypical familial juvenile hyperuricaemic nephropathy (FJHN) (subjects with young-onset hyperuricaemia, gout, renal impairment, and also disorders of renal development including renal cysts); (3) unexplained renal cystic disease in patients who had renal cysts but did not meet the criteria for GCKD or atypical FJHN (subjects in groups 1 and 2 may have renal cysts but are not included in this category); (4) renal dysplasia in which the kidneys have severe disturbance of differentiation and complete or partial lack of normal nephrons; and (5) renal malformations which are defined as gross renal developmental abnormalities and include single kidney ($n=20$), horseshoe kidney ($n=1$), and hypoplastic kidney(s) ($n=8$). Details regarding renal disease, the presence of diabetes in the proband or a family member, and other clinical features were obtained from the referring clinician or the patient's hospital records. Summary clinical characteristics for all 160 subjects are shown in table 1.

HNF-1 β genetic analysis

Genomic DNA was extracted from peripheral lymphocytes using standard procedures. Exons 1–9 including intron-exon boundaries and the minimal promoter of *HNF-1 β* were amplified by the polymerase chain reaction.⁶ Sequence specific primers for each exon¹⁰ were tagged with 5' M13 tails to allow sequencing to be performed with a universal M13 primer.

Both strands were sequenced in forward and reverse directions using the Big Dye Terminator Cyclor Sequencing Kit (Applied Biosystems, Warrington, UK) according to the manufacturer's instructions. Reactions were analysed on an ABI 3100 Capillary DNA sequencer (Applied Biosystems). Sequences were compared to the published sequence (NM_000458) using Sequence Navigator Software (Applied Biosystems, Warrington, UK). Changes in the sequence were checked against published polymorphisms and mutations and for conservation across species.

Microsatellite analysis

Family relationships were confirmed using three microsatellites on chromosome 17: D17S800, D17S927, and D17S1718.⁷

RESULTS

Genetics

We identified 23 heterozygous *HNF-1 β* gene mutations (shown in fig 1) in 23/160 subjects (14%). Ten of these subjects have previously been reported (H69fsdelAC, E101X,

S151P, P159fsdelT, IVS2nt+1G>T, IVS2nt+2insT, Q243fsdelC, P328L329fsdelCCTCT, Y352fsinsA, and A373fsdel29).^{7–14} The 13 unreported subjects include 10 novel mutations (V61G, V110G, S148L, K156E, Q176X, R276Q, S281fsinsC, R295P, H324S325fsdelCA, and Q470X) and three patients with previously reported mutations (R181X, IVS2nt+1G>A, and IVS2nt+2delAAGT).^{15–21–33} The 10 novel mutations include missense, nonsense, and frameshift mutations, none of which were found in 100 normal chromosomes. All the missense mutations affect residues which are conserved in both rat and mouse. Details of the novel mutations are shown in table 2.

In addition to pathogenic mutations we also found two rare variants (V25L (GTG>CTG) in 1/159 subjects, and IVS4-22C>T in 1/159 subjects), but it is uncertain whether or not these variants represent aetiological mutations. The V25L carrier inherited this *HNF-1 β* variant from his father who is not known to be affected by renal dysfunction or diabetes but has not had a renal ultrasound. As it was not possible to follow up this family, we therefore report V25L as a rare variant. The intron 4 variant -22C>T does not affect the conserved splicing branch site, but we were not able to check co-segregation within this family.

To investigate co-segregation in the families of the 13 newly reported probands, we tested 27 family members (10 affected and 17 unaffected) from 11 pedigrees. This and previously reported family studies allowed us to classify 39% of mutations as (9/23) as familial, where family members were affected and had an *HNF-1 β* mutation. In the unreported families, three sets of parental DNA did not carry a mutation; microsatellite analysis confirmed these three cases as spontaneous de novo mutations (fig 2), in addition to the four previously reported spontaneous mutations.^{7–9–13} In the remaining pedigrees there was no known family history, suggesting that these were isolated cases.

Clinical phenotypes

The clinical characteristics of the family members from previously unreported pedigrees with *HNF-1 β* mutations are shown in table 3. The commonest renal manifestation in patients with *HNF-1 β* mutations was renal cysts which were present in 19/23 (83%). Four of these subjects had a specific diagnosis of GCKD. Diabetes was present in 11/23 (48%) and three (13%) had an affected first degree relative but no diabetes themselves. Only 6/23 families (26%) met the minimal MODY criteria (families with at least two generations of diabetes with at least one subject diagnosed under the age of 25 years). The common manifestation of both renal cysts and diabetes (RCAD) was found in 9/23 (39%) of probands (table 4).

In addition to renal disease and diabetes, 2/23 (9%) probands had genital tract malformations (table 4). These probands had uterus didelphys and a single ovary (DUK448) and hemi-uterus (DUK1094), respectively. Another subject

Table 2 Molecular genetics characteristics of the 10 novel *HNF-1β* mutations

	Mutation V61G	V110G	S148L	K156E	Q176X	R276Q	S281fsinsC	R295P	H324S325fsdelCA	Q470X
Family number	DUK1094	DUK739	DUK1285	DUK671	DUK1080	DUK802	DUK1027	DUK972	DUK952	DUK950
Nucleotide change*	c.376T>G	c.523T>G	c.637C>T	c.660A>G	c.720C>T	c.1021G>A	c.1034^1035insC	c.1078G>C	c.1166_1167delCA	c.1602C>T
Amino acid change	GTC>GGC	GTG>GGG	TCG>TTG	AAG>GAG	CAA>TAA	CGA>CAA	S281fsinsC	CGT>CCT	H324S325fsdelCA	CAG>TAG
Mutation type	Missense	Missense	Missense	Missense	Nonsense	Missense	Frameshift	Missense	Frameshift	Nonsense
Conservation of amino acid in mouse and rat	Yes	Yes	Yes	Yes	NA	Yes	NA	Yes	NA	NA
De novo mutation	NA	Yes	Yes	Yes	No	No	No	NA	No	No

NA, not applicable.
*Nomenclature is based on reference sequence NM_000458 where nucleotide 1 is the first nucleotide of the promoter which is non-coding. The coding sequence starts at nucleotide 195 in exon 1.

(DUK972) had unexplained primary infertility. It was also noted that in three families a first degree family member had a malignancy although in no cases was it possible to establish that these family members had an *HNF1β* mutation. The tumours arose in biliary tract, uterine tract, and bowel, all tissues with high expression of HNF-1β.

None of our mutation carriers had clinical liver disease, although on biochemical testing the serum levels of liver enzymes (alanine aminotransferase and γ glutamyl transpeptidase) exceeded the upper limit of normal in 5/9 tested. Alkaline phosphatase alone was tested in a further seven patients and was abnormal in four, but this may represent renal bone disease rather than hepatic dysfunction as other liver enzymes were not measured.

Genotype-phenotype correlation

This cohort included four subjects with mutations of the intron 2 splice donor site: IVS2nt+1G>T, IVS2nt+2insT, IVS2nt+1G>A, and IVS2nt+2delAAGT. This is the most

frequent site of mutations in the *HNF-1β* gene with a total of seven unrelated families (19 mutation carriers) reported to have one of these mutations.^{10 12 15 21 33} These mutations are predicted to result in an abnormally spliced variant (either exon loss or intron retention).¹² We examined the clinical characteristics of these patients in order to investigate the possibility of a genotype-phenotype relationship. This analysis was not consistent with there being a specific phenotype associated with this mutation site. All of the 19 subjects with an intron 2 splice donor site mutation had renal disease: 11 had renal cysts (including one with GCKD), seven had FJHN (including two with FJHN and renal cysts), and one had a horseshoe kidney. Twelve of the 19 had diabetes and two had genital tract abnormalities.

DISCUSSION

We report the results of screening a large series of subjects with unexplained renal disease for *HNF-1β* mutations. We have identified 23 different *HNF-1β* mutations, of which 10

Figure 1 *HNF-1β* gene structure illustrating the 40 different mutations identified to date which span the first seven exons of the gene; those identified in this study (n = 20) are in italics. The exons and protein domains are indicated; the exons are relative to their sequence length. The HNF-1β protein contains 557 amino acids with three distinct domains: the dimerisation domain (1–32 amino acids), the DNA binding domain (90–311 amino acids), and the transactivation domain (312–557 amino acids).

Figure 2 Pedigrees of 11 families with unreported *HNF-1 β* mutations. Individuals with renal cysts are identified by a symbol with a filled upper left quarter, those with diabetes with a filled lower left quarter, those with other forms of renal disease with a filled upper right quarter, and those with other non-renal, non-pancreatic abnormalities with a filled lower right quarter.

Table 3 Clinical characteristics of the 13 previously unreported *HNF-1 β* mutation positive probands and their relatives

Family number	ID	<i>HNF-1β</i> mutation	Present age, years	Primary renal disease			Diabetes			Other features
				Primary renal disease	Age at diagnosis, years	Renal failure	Diabetes	Age of diagnosis, years	Treatment	
DUK1094	I:I	V61G	14	SK	3	N	N	–	–	Single ovary and hemi-uterus
DUK739	II:I	V110G	9	GCKD	6.5	N	N	–	–	Autoimmune thyroid disease
DUK1285	II:I	S148L	13	RD	Birth	N	Y	13	Insulin	N
DUK671	II:I	K156E	8	RC	Antenatal US	Y	Y	–	–	N
DUK1080	I:I	Q176X	29	RC	–	N	N	–	–	N
DUK1080	I:I	Q176X	8	RC	–	N	N	–	–	N
DUK1080	II:II	Q176X	1	RC	–	N	N	–	–	N
DUK859	II:II	R181X	37	RC	–	N	Y	28	Insulin	N
DUK859	III:I	R181X	8	RC	–	N	N	–	–	N
DUK859	III:II	R181X	6	RC	–	N	N	–	–	N
DUK859	III:III	R181X	2	RC	–	N	N	–	–	N
DUK971	I:II	IVS2nt+1G>A	62	FJHN	–	Y	Y	57	OHA	Gout
DUK971	II:I	IVS2nt+1G>A	38	FJHN	–	N	N	–	–	Gout
DUK971	II:II	IVS2nt+1G>A	23	NI	–	NI	NI	–	–	NI
DUK1208	I:I	IVS2nt+2delAAGT	–	GCKD	7	N	N	–	–	N
DUK802	II:I	R276Q	15	RC	–	N	Y	–	Insulin	N
DUK802	II:II	R276Q	–	RC	–	N	Y	–	Insulin	N
DUK1027	I:I	S281fsinsC	34	RC	–	N	Y	20	Insulin	N
DUK1027	II:I	S281fsinsC	2	RC	–	N	N	–	–	N
DUK1027	II:II	S281fsinsC	4	RC	–	N	N	–	–	N
DUK972	II:I	R295P	52	RC	39	N	Y	21	Insulin	Breast cancer
DUK952	I:I	H324S325fsdelCA	31	RC	–	N	N	–	–	N
DUK952	II:I	H324S325fsdelCA	2	RC	Birth	N	N	–	–	N
DUK950	I:I	Q470X	30	RI	30	N	N	–	–	N
DUK950	II:I	Q470X	3	RC	Birth	N	N	–	–	N

FJHN, familial juvenile hyperuricaemic nephropathy; GCKD, glomerulocystic kidney disease; NI, not investigated; OHA, oral hypoglycaemic agents; RC, renal cysts; RD, renal dysplasia; RI, renal impairment (raised creatinine levels); SK, single kidney; US, ultrasound.

Table 4 Renal disease and extra-renal phenotypes of all 23 probands with an *HNF-1 β* mutation

Renal disease	Renal disease, n (%)	Diabetes	FH diabetes only	Genital tract abnormalities	No diabetes or genital tract abnormalities
Renal cystic disease and cystic dysplasia	14 (61%)	6	3	1	7
GCKD	4 (17%)	2	0	0	2
Atypical FJHN (with renal cysts n=1)	2 (9%)	2	0	0	0
Renal dysplasia	1 (4%)	1	0	0	0
Renal malformations (single kidney n=2)	2 (9%)	0	0	1	1
Total	23 (100%)	11 (48%)	3 (13%)	2 (9%)	10 (43%)

Classifications of individual renal categories are all defined in the text and are exclusive. Diabetes and genital tract abnormalities may coexist in a single proband. FJHN, familial juvenile hyperuricaemic nephropathy; FH, diabetes only, family history of diabetes but no diabetes in probands; GCKD, glomerulocystic kidney disease.

are novel. These novel mutations co-segregate with renal disease. The mutated residues are conserved across species and were not found in 100 normal chromosomes.

A total of 40 *HNF-1 β* gene mutations have now been identified in 46 families.^{6–28} The majority (37/40) of these mutations are confined to the first four exons. Exons 1–4 encode the dimerisation and DNA binding domains of *HNF-1 β* and mutations in these domains are predicted to alter the ability of hetero/homodimer formation or to change the DNA targeting sequence. There are no missense mutations in the transactivation domain, but the functional significance of this is not known. In this report we describe the most 3' mutation to date: Q470X in exon 7. The majority of mutations are private, but there is a known hotspot at the intron 2 splice donor site (7/46 families). The most commonly mutated region of the gene is exon 2 where 16/40 mutations occur. Functional studies of exon 2 mutations have shown that this domain is critical for transcriptional activity. Studies looking at the effect of the exon 2 *HNF-1 β* mutation H153N on the transcriptional activity of *HNF-1 α* have shown that it has a repressive effect.²⁵ Other mutations in the homeodomain result in a severe reduction in target gene activation³⁸ or do not bind DNA²⁷; therefore mutations in this region will alter the levels of *HNF-1 β* gene targets.

There are limitations to our study. Firstly, the cohort was centre rather than population based and patients were referred on the basis of their phenotype. Therefore, we cannot use the data to determine how common *HNF-1 β* mutations are in the general population with undetected renal disease. Secondly, clinical assessment was not standardised as patients were referred from multiple centres and assessment of potential phenotype features varied. Clinicians varied in the extent to which they tested for diabetes and uterine abnormalities in asymptomatic patients especially if they were young. All clinical features reported were confirmed and are robust, but some features may have been present but not clinically detected especially as regards uterine abnormalities and abnormal liver function tests. However, the features present would reflect the likely clinical features of a case being considered for genetic testing.

The identification of 23 *HNF-1 β* mutations in subjects with renal disease has allowed us to refine the clinical phenotype associated with *HNF-1 β* mutations. The first report of an *HNF-1 β* gene was in a family with MODY, but within our series only six families met minimal MODY criteria. Overall, only 11/23 probands had diabetes and only three probands without diabetes had a family member with diabetes. This is in contrast to the series described by Bellanne-Chantelot *et al* who only screened subjects with diabetes and renal disease.¹⁵ Our study emphasises that diabetes is not an essential requirement for the identification of an *HNF-1 β* mutation. In addition, a family history of diabetes or renal disease may not be present as de novo mutations are common in 32% of the

families where parental DNA is available. Therefore, *HNF-1 β* mutation analysis should not be restricted to subjects with a family history of diabetes and/or renal disease.

Our study confirms that *HNF-1 β* mutations are a fairly common cause of renal disease and that the presentation of renal disease includes a wide spectrum of renal phenotypes. Renal cysts are present in most *HNF-1 β* mutation carriers including those with the histologically proven form of cystic kidney disease, GCKD. Renal malformations are the second most common form of renal disease associated with *HNF-1 β* mutations and include single and horseshoe kidneys. In our study, 9/23 (40%) probands had RCAD. This is the most common phenotype; together with reports from the literature, 24/46 (52%) probands with *HNF-1 β* mutations have RCAD.^{6, 15, 17–22, 24–28}

Genital tract abnormalities, in particular those affecting the Mullerian tract, have previously been reported in 8/23 index cases with an *HNF-1 β* mutation. In our series, only 2/23 probands had a genital tract abnormality. The low detection rate of genital tract abnormalities could be due to incomplete clinical investigation. Therefore, systematic screening of subjects with Mullerian tract abnormalities is required to determine the significance of these findings.

From published data and our data presented here, it is clear that the phenotype of *HNF-1 β* mutation carriers is variable; phenotypes vary between and within families, and there is also little evidence to correlate mutation type or position and the clinical characteristics. We previously reported a family where the mother had diabetes and mild renal dysfunction, but her 19 week fetus had grossly enlarged cystic kidneys incompatible with life.⁷ Yorifuji *et al* have reported a family where two siblings both have a S148W missense mutation but different phenotypes; one had neonatal diabetes with no renal disease, whereas his brother had severe renal disease but no diabetes.¹⁶ These examples illustrate how the same mutation can result in a varied phenotype, suggesting that other genetic or environmental factors can play a considerable role in the pattern and severity of the disease associated with an *HNF-1 β* mutation.

It is apparent that extra-pancreatic features are more common than initially thought in subjects with an *HNF-1 β* mutation. Hence this study and others are limited by incomplete extra-pancreatic clinical investigations. The majority of *HNF-1 β* mutations associated with renal disease and genital tract abnormalities are located within the intron 2 splice donor site or exon 2 (8/10 mutations). Exon 2 encodes the pseudo-POU domain which is involved in DNA binding specificity and is essential for the development of a normal kidney and genital tract.

This study also highlights the fact that that *HNF-1 β* mutations are only one of many causes of renal cysts, as 82% of patients with renal cysts did not have a mutation. Polycystic kidney disease (OMIM 173900) is genetically

heterogeneous with multiple loci including chromosome 16p (polycystic kidney disease 1, PKD1), 4q (PKD2), and 6p (autosomal recessive polycystic kidney disease) where mutations have been reported in the genes encoding polycystin-1, polycystin-2, and fibrocystin, respectively. There is a third autosomal dominant form of PKD (PKD3) which is linked to chromosome 2q. Subjects with PKD present from birth to late adulthood. Therefore, some patients in our cohort are likely to harbour a mutation in one of these PKD genes or in a novel gene.

In conclusion, mutations in the *HNF-1 β* gene are a fairly common cause of renal disease and mutation carriers have a variety of extra-renal phenotypes. We report the largest series to date of *HNF-1 β* mutations which includes three de novo mutations. As de novo mutations represent up to a third of cases, a family history of renal disease or diabetes should not be considered a prerequisite for molecular genetic testing. Following this study, a large number of *HNF-1 β* mutations have now been reported but there is still no clear evidence for a genotype-phenotype relationship. This study highlights the fact that although *HNF-1 β* was initially described as a MODY gene, patients usually present with renal disease or RCAD rather than with MODY.

ACKNOWLEDGEMENTS

The authors thank all the families for their participation, and their referring doctors for supplying the clinical information and making this study possible. These include: Dr N Gordjani, Dr C J Burton, Dr N Albers, Dr A Nicholls, Dr H Leslie, Dr C M Taylor, Professor G Rizzoni, Dr Arikoski, Dr Nadar, Dr M McGraw, Dr S Rigden, Dr S Arslanian, Dr Kuan, Dr T Cole, Dr R Davidson, Professor A Woolf, Dr P Williams, Dr Van't Hoff, Dr Rees, Dr R Jones, Dr Trompeter, Dr K Jones, and Dr Skinner. We thank Dr Ewan Pearson for clinical expertise and Dr Lorna Harries and Lisa Allen for help with molecular genetic analysis.

Authors' affiliations

E L Edghill, C Bingham, S Ellard, A T Hattersley, Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, UK

We thank the National Kidney Research Fund (grant TF13/2000), Exeter Kidney Unit Development Fund, Royal Devon and Exeter NHS Foundation Trust R&D Directorate, British Medical Association, and Wellcome Trust who all supported this work. ATH is a Wellcome Trust research leave fellow and CB Bingham was an NKRf clinical research fellow

Competing interests: none declared

Ethics approval was given for this study by the South West Multi-Centre Research Ethics Committee

Correspondence to: Professor Andrew T Hattersley, Diabetes and Vascular Medicine, Peninsula Medical School, Barrack Road, Exeter, EX2 5AX, UK; A.T.Hattersley@exeter.ac.uk

Revised version received 23 May 2005

Accepted for publication 24 May 2005

Published Online First 8 June 2005

REFERENCES

- Barbacci E, Reber M, Ott M-O, C Breillat, F Huetz, S Cereghini. Variant hepatocyte nuclear factor1 is required for visceral endoderm specification. *Development* 1999;**126**:4795-805.
- Coffinier C, Thepot D, Babinet C, Yarniv M, Barra J. Essential role for the homeoprotein vHNF1/HNF1beta in visceral endoderm differentiation. *Development* 1999;**126**(21):4785-94.
- Coffinier C, Barra J, Babinet C, Yaniv M. Expression of the vHNF1/HNF1beta homeoprotein gene during mouse organogenesis. *Mech Dev* 1999;**89**(1-2):211-3.
- Reber M, Cereghini S. Variant hepatocyte nuclear factor 1 expression in the mouse genital tract. *Mech Dev* 2001;**100**(1):75-8.
- Rey-Campos J, Chouard T, Yaniv M, Cereghini S. vHNF1 is a homeoprotein that activates transcription and forms heterodimers with HNF1. *EMBO J* 1991;**10**(6):1445-57.
- Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinokio Y, Cockburn BN, Lindner T, Yamagata K, Ogata M, Tomonaga O, Kuroki H, Kasahara T, Iwamoto Y, Bell GI. Mutation in hepatocyte nuclear factor-1b gene (TCF2) associated with MODY. *Nat Genet* 1997;**17**:384-5.
- Bingham C, Ellard S, Allen L, Bulman M, Shepherd M, Frayling T, Berry PJ, Clark PM, Lindner T, Bell GI, Ryffel GU, Nicholls AJ, Hattersley AT. Abnormal nephron development associated with a frameshift mutation in the transcription factor hepatocyte nuclear factor-1 beta. *Kidney Int* 2000;**57**(3):898-907.
- Bingham C, Bulman M, Ellard S, Allen L, Lipkin GW, Hoff WG, Woolf AS, Rizzoni G, Novelli G, Nicholls AJ, Hattersley AT. Mutations in the HNF1-beta gene are associated with familial hypoplastic glomerulocystic kidney disease. *Am J Hum Genet* 2001;**68**:219-24.
- Bingham C, Ellard S, Cole TR, Jones KE, Allen L, Goodship JA, Goodship TH, Bakalnova-Pugh D, Russell GI, Woolf AS, Nicholls AJ, Hattersley AT. Solitary functioning kidney and diverse genital tract malformations associated with hepatocyte nuclear factor-1beta mutations. *Kidney Int* 2002;**61**(4):1243-51.
- Bingham C, Ellard S, van't Hoff WG, Simmonds HA, Marinaki AM, Badman MK, Winocour PH, Stride A, Lockwood CR, Nicholls AJ, Owen KR, Spyer G, Pearson ER, Hattersley AT. Atypical familial juvenile hyperuricemic nephropathy associated with a hepatocyte nuclear factor-1beta gene mutation. *Kidney Int* 2003;**63**(5):1645-51.
- Harries LW, Bingham C, Bellanne-Chantelot C, Hattersley AT, Ellard S. The position of premature termination codons in the hepatocyte nuclear factor-1 beta gene determines susceptibility to nonsense-mediated decay. *Hum Genet* 2005 Aug **25**:1-11 [Epub ahead of print].
- Harries LW, Ellard S, Jones RW, Hattersley AT, Bingham C. Abnormal splicing of hepatocyte nuclear factor-1 beta in the renal cysts and diabetes syndrome. *Diabetologia* 2004;**47**(5):937-42.
- Kolatsi-Joannou M, Bingham C, Ellard S, Bulman MP, Allen L, Hattersley AT, Woolf AS. Hepatocyte nuclear factor-1beta: a new kindred with renal cysts and diabetes and gene expression in normal human development. *J Am Soc Nephrol* 2001;**12**(10):2175-80.
- Waller SC, Rees L, Woolf AS, Ellard S, Pearson ER, Hattersley AT, Bingham C. Severe hyperglycemia after renal transplantation in a pediatric patient with a mutation of the hepatocyte nuclear factor-1beta gene. *Am J Kidney Dis* 2002;**40**(6):1325-30.
- Bellanne-Chantelot C, Chauveau D, Gautier JF, Dubois-Laforgue D, Clauin S, Beaufils S, Wilhelm JM, Boitard C, Noel LH, Velho G, Timsit J. Clinical spectrum associated with hepatocyte nuclear factor-1beta mutations. *Ann Intern Med* 2004;**140**(7):510-7.
- Yorifuji T, Kurokawa K, Mamada M, Imai T, Kawai M, Nishi Y, Shishido S, Hasegawa Y, Nakahata T. Neonatal diabetes mellitus and neonatal polycystic, dysplastic kidneys: phenotypically discordant recurrence of a mutation in the hepatocyte nuclear factor-1beta gene due to germline mosaicism. *J Clin Endocrinol Metab* 2004;**89**(6):2905-8.
- Furuta H, Furuta M, Sanke T, Ekawa K, Hanabusa T, Nishi M, Sasaki H, Nanjo K. Nonsense and missense mutations in the human hepatocyte nuclear factor-1 beta gene (TCF2) and their relation to type 2 diabetes in Japanese. *J Clin Endocrinol Metab* 2002;**87**(8):3859-63.
- Lindner TH, Njolstad PR, Horikawa Y, Bostad L, Bell GI, Sovik O. 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-1beta. *Hum Mol Genet* 1999;**8**(11):2001-8.
- Nishigori H, Yamada S, Kohama T, Tomura H, Shio K, Horikawa Y, Bell GI, Takeuchi T, Takeda J. Frameshift mutation, A263fsinsGG, in the hepatocyte nuclear factor-1 beta gene associated with diabetes and renal dysfunction. *Diabetes* 1998;**47**(8):1354-5.
- Iwasaki N, Okabe I, Momoi MY, Ohashi H, Ogata M, Iwamoto Y. Splice site mutation in the hepatocyte nuclear factor-1 beta gene, IVS2nt+1G>A, associated with maturity-onset diabetes of the young, renal dysplasia and bicornuate uterus. *Diabetologia* 2001;**44**(3):387-8.
- Carbone I, Cotellessa M, Barella C, Minetti C, Ghiggeri GM, Caridi G, Perfumo F, Lorini R. A novel hepatocyte nuclear factor-1beta (MODY-5) gene mutation in an Italian family with renal dysfunctions and early-onset diabetes. *Diabetologia* 2002;**45**(1):153-4.
- Yoshiuchi I, Yamagata K, Zhu Q, Tamada I, Takahashi Y, Onigata K, Takeda J, Miyagawa J, Matsuzawa Y. Identification of a gain-of-function mutation in the HNF-1beta gene in a Japanese family with MODY. *Diabetologia* 2002;**45**(1):154-5.
- Macie CJ, Preisegger KH, Kopp S, Ratschek M, Ring E. De novo HNF-1 beta gene mutation in familial hypoplastic glomerulocystic kidney disease. *Pediatr Nephrol* 2002;**17**(12):1021-6.
- Montoli A, Colussi G, Massa O, Caccia R, Rizzoni G, Civati G, Barbetti F. Renal cysts and diabetes syndrome linked to mutations of the hepatocyte nuclear factor-1 beta gene: description of a new family with associated liver involvement. *Am J Kidney Dis* 2002;**40**(2):397-402.
- Kitanaka S, Miki Y, Hayashi Y, Igarashi T. Promoter-specific repression of hepatocyte nuclear factor (HNF)-1 beta and HNF-1 alpha transcriptional activity by an HNF-1 beta missense mutant associated with type 5 maturity-onset diabetes of the young with hepatic and biliary manifestations. *J Clin Endocrinol Metab* 2004;**89**(3):1369-78.
- Shihara N, Horikawa Y, Onishi T, Ono M, Kashimada K, Takeda J. Identification of a new case of hepatocyte nuclear factor-1beta mutation with highly varied phenotypes. *Diabetologia* 2004;**47**(6):1128-9.
- Barbacci E, Chalkiadaki A, Masdeu C, Haumaitre C, Lokmane L, Lohr C, Cloarec S, Talianidis I, Bellanne-Chantelot C, Cereghini S. HNF1[beta]/TCF2 mutations impair transactivation potential through altered co-regulator recruitment. *Hum Mol Genet* 2004;**13**(24):3139-49.
- Wang C, Fang Q, Zhang R, Lin X, Xiang K. Scanning for MODY5 gene mutations in Chinese early onset or multiple affected diabetes pedigrees. *Acta Diabetol* 2004;**41**(4):137-45.

- 29 **Yamagata K**, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Bell GI. Mutations in the hepatocyte nuclear factor 1alpha gene in maturity-onset diabetes of the young (MODY3). *Nature* 1996;**384**:455–8.
- 30 **Beards F**, Frayling T, Bulman M, Horikawa Y, Allen L, Appleton M, Bell GI, Ellard S, Hattersley AT. Mutations in hepatocyte nuclear factor 1 beta are not a common cause of maturity-onset diabetes of the young in the U.K. *Diabetes* 1998;**47**:1152–4.
- 31 **Pearson ER**, Badman MK, Lockwood CR, Clark PM, Ellard S, Bingham C, Hattersley AT. Contrasting diabetes phenotypes associated with hepatocyte nuclear factor-1 alpha and -1 beta mutations. *Diabetes Care* 2004;**27**(5):1102–7.
- 32 **Maestro MA**, Boj SF, Luco RF, Pierreux CE, Cabedo J, Servitja JM, German MS, Rousseau GG, Lemaigre FP, Ferrer J. Hnf6 and Tcf2 (MODY5) are linked in a gene network operating in a precursor cell domain of the embryonic pancreas. *Hum Mol Genet* 2003;**12**(24):3307–14.
- 33 **Iwasaki N**, Babazono T, Tomonaga O, Ogata M, Yokokawa H, Iwamoto Y. Mutations in the hepatocyte nuclear factor-1beta (MODY5) gene are not a major factor contributing to end-stage renal disease in Japanese people with diabetes mellitus. *Diabetologia* 2001;**44**(1):127–8.
- 34 **Coffinier C**, Gresh L, Fiette L, Tronche F, Schutz G, Babinet C, Pontoglio M, Yaniv M, Barra J. Bile system morphogenesis defects and liver dysfunction upon targeted deletion of HNF1beta. *Development* 2002;**129**(8):1829–38.
- 35 **Wild W**, Pogge von Strandmann E, Nastos A, Senkel S, Lingott-Frieg A, Bulman M, Bingham C, Ellard S, Hattersley AT, Ryffel GU. The mutated human gene encoding hepatocyte nuclear factor 1beta inhibits kidney formation in developing *Xenopus* embryos. *Proc Natl Acad Sci U S A* 2000;**97**(9):4695–700.
- 36 **Wang L**, Coffinier C, Thomas MK, Gresh L, Eddu G, Manor T, Levitsky LL, Yaniv M, Rhoads DB. Selective deletion of the Hnf1beta (MODY5) gene in {beta}-cells leads to altered gene expression and defective insulin release. *Endocrinology* 2004;**145**(8):3941–9.
- 37 **Tomura H**, Nishigori H, Sho K, Yamagata K, Inoue I, Takeda J. Loss-of-function and dominant-negative mechanisms associated with hepatocyte nuclear factor-1beta mutations in familial type 2 diabetes mellitus. *J Biol Chem* 1999;**274**(19):12975–8.
- 38 **Thomas H**, Senkel S, Erdmann S, Arndt T, Turan G, Klein-Hitpass L, Ryffel GU. Pattern of genes influenced by conditional expression of the transcription factors HNF6, HNF4alpha and HNF1beta in a pancreatic beta-cell line. *Nucleic Acids Res* 2004;**32**(19):e150.