Am. J. Hum. Genet. 68:219–224, 2001

Report

Mutations in the Hepatocyte Nuclear Factor-1β Gene Are Associated with Familial Hypoplastic Glomerulocystic Kidney Disease

Coralie Bingham,[1] Michael P. Bulman,[1] Sian Ellard,[1] Lisa I. S. Allen,[1] Graham W. Lipkin,[2] William G. van't Hoff,[3] Adrian S. Woolf,[3] Gianfranco Rizzoni,[4] Giuseppe Novelli,[5] Anthony J. Nicholls,[1] and Andrew T. Hattersley[1]

[1]Department of Vascular Medicine and Diabetes Research, School of Postgraduate Medicine and Health Sciences, University of Exeter, Exeter; [2]Department of Nephrology, Queen Elizabeth Hospital, Birmingham; and [3]Nephro-Urology Unit, Institute of Child Health, University College London, London; [4]Division of Nephrology and Dialysis, Bambino Gesu Children's Hospital and Research Institute, and [5]Dipartimento di Biopatologia e Diagnostica Per Immagini, Sezione di Genetica, Università di Roma "Tor Vergata," Rome

Familial glomerulocystic kidney disease (GCKD) is a dominantly inherited condition characterized by glomerular cysts and variable renal size and function; the molecular genetic etiology is unknown. Mutations in the gene encoding hepatocyte nuclear factor (HNF)–1β have been associated with early-onset diabetes and nondiabetic renal disease—particularly renal cystic disease. We investigated a possible role for the *HNF-1β* gene in four unrelated GCKD families and identified mutations in two families: a nonsense mutation in exon 1 (E101X) and a frameshift mutation in exon 2 (P159fsdelT). The family members with *HNF-1β* gene mutations had hypoplastic GCKD and early-onset diabetes or impaired glucose tolerance. We conclude that there is genetic heterogeneity in familial GCKD and that the hypoplastic subtype is a part of the clinical spectrum of the renal cysts and diabetes syndrome that is associated with *HNF-1β* mutations.

Familial glomerulocystic kidney disease (GCKD [MIM 137920]) is a rare, inherited renal cystic disorder that is characterized by autosomal dominant inheritance, variable renal size and renal function, and histology showing cortical glomerular cysts with dilatation of the Bowman spaces and primitive glomerular tufts in at least 5% of the cysts (Bernstein 1993). No gene has been identified for familial GCKD. The genes for autosomal dominant polycystic kidney disease, *PKD1* (MIM 601313) and *PKD2* (MIM 173910), have been excluded by linkage studies in Italian (Mesoraca et al. 1996) and US (Sharp et al. 1997) families.

Glomerulocystic kidneys, as defined by the histological features, may be divided into three major categories (Bernstein 1993): (1) GCKD, which comprises nonsyndromal, inherited, and sporadic forms of cystic kidneys in children and adults and includes all subtypes of familial GCKD; (2) glomerulocystic kidneys as a feature of inherited malformation syndromes—for example, X-linked dominant oral-facial-digital syndrome type 1 (Feather et al. 1997) (cysts are inconsistently expressed in these syndromes); and (3) glomerular cysts in dysplastic kidneys, which may be sporadic or syndromal and may be associated with fetal lower urinary tract obstruction. The glomerular cysts are minor in comparison with the dysplastic features. Glomerular cysts may be generated experimentally after urinary flow impairment, by ureteric obstruction, in fetal sheep (Attar et al. 1998).

Mutations in the gene encoding hepatocyte nuclear factor (HNF)–1β are a cause of renal cystic disease. 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 (Mendel et al. 1991; Rey-Campos et al. 1991). Heterozygous *HNF-1α* mutations are the most common cause of maturity-onset diabetes of the young (MODY) (Frayling et al. 1997). MODY is a form of non–insulin-dependent diabetes mellitus characterized by autosomal dominant inheritance and a young age at

Received September 27, 2000; accepted for publication November 2, 2000; electronically published November 20, 2000.

Address for correspondence and reprints: Prof. Andrew Hattersley, Department of Diabetes and Vascular Medicine, School of Postgraduate Medicine and Health Sciences, Barrack Road, Exeter, EX2 5AX, Devon, United Kingdom. E-mail: A.T.Hattersley@exeter.ac.uk

0002-9297/2001/6801-0022$02.00

onset, usually diagnosed at age <25 years (Hattersley 1998). *HNF-1β* mutations have been reported in four families with early-onset diabetes (MIM 604284) (Horikawa et al. 1997; Nishigori et al. 1998; Lindner et al. 1999; Bingham et al. 2000), but they are a rare cause of MODY (Beards et al. 1998, Weng et al. 2000). Nondiabetic renal disease was reported in all families, with renal cysts described in three families. Renal histology has only been available from two subjects with *HNF-1β* mutations. A subject, aged 14 years, from one family has been shown to have oligomeganephronia with a reduced number of glomeruli and hypertrophy of both the glomeruli and proximal renal tubules (Lindner et al. 1999). Another member of the same family has renal cystic disease, but histology is not available. A 17-wk fetus from a second family showed an absence of normal nephrogenesis, with replacement of the renal parenchyma by cysts and occasional cystic glomeruli and primitive tubules, consistent with cystic renal dysplasia (Bingham et al. 2000). In the original description of familial GCKD, one family member was reported as having early-onset diabetes (Rizzoni et al. 1982). We therefore hypothesized that *HNF-1β* mutations might be associated with familial GCKD.

Subjects with familial GCKD were recruited from the published literature on familial GCKD, including the Italian family from the original report (Rizzoni et al. 1982). Additional subjects were recruited from adult and pediatric nephrology clinics in the United Kingdom. DNA was collected from members of four unrelated families; the details of the probands are given in table 1. Direct sequencing of the entire promoter and coding regions of the nine exons and the intron-exon boundaries of the *HNF-1β* gene was performed using an ABI Prism 377 DNA Sequencer (PE Biosystems). When a mutation was found, the mutational status was examined in other available family members.

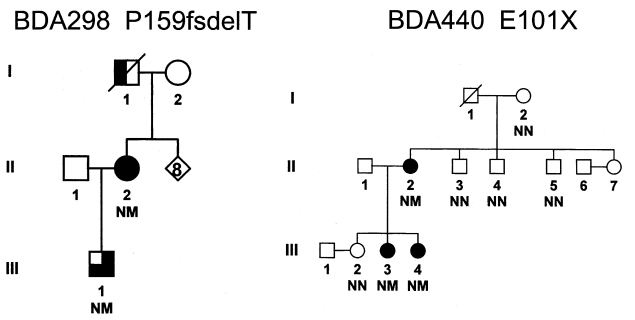

Figure 1 Pedigrees of two families with *HNF-1β* mutations. Roman numerals on left of the figure indicate generation number, and the numbers below the symbols indicate individuals within that generation. The *HNF-1β* genotype of each individual tested is indicated below the symbol: N, =normal, M = mutated allele. Blackened lower left quadrant = impaired glucose tolerance; blackened lower and upper left quadrants = diabetes; blackened upper right quadrant = renal cysts; blackened lower right quadrant = renal impairment; diamond symbol containing a number = no. of siblings unavailable for testing.

Two novel heterozygous mutations in the *HNF-1β* gene were identified. A nonsense mutation in exon 1,

Table 1

Clinical Characteristics of the Probands

	Proband			
Characteristic	1	2	3	4
Family	BDA395	BDA430	BDA440	BDA298
Mutation	None	None	E101X	P159fsdelT
Age at diagnosis of renal disease	30 wk gestation	39 years	9 mo	2 mo
Present age (years)	9	62	34	40
Creatinine (μmol/l)[a]	80 (39–70)	ESRF	164 (35–105)	ESRF
Creatinine clearance (ml/min)[a]	44/1.73 m^2 (89–165)	NA	NA	4 (80–120)
Diabetes	No	No	Yes	Yes
Renal size	Large kidneys	Normal-size kidneys	Small kidneys	Small kidneys
Renal histology	Glomerular and collecting duct cysts	Glomerular cysts and glomerular tufts	Histology in sister: cortical cysts; cystic glomeruli with glomerular tufts	Glomerular cysts and glomerular tufts
Other affected family members and age at diagnosis of renal disease	Brother, 11 years; mother, 30 years	Son, 13 years; son, 11 years; daughter, 7 years	Sister, 1 mo; mother, 35 years	Son, at birth
Other features	Prognathism; partial anomalous pulmonary venous drainage; learning difficulties			Prognathism; pyloric stenosis
Reference	Not reported	Melnick et al. (1984)	Rizzoni et al. (1982)	Kaplan et al. (1989)

Note.—NA = not available; ESRF = end-stage renal failure.

[a] Most recently available values, with reference ranges in parentheses.

E101X, was found in family BDA440 (the Italian family of the original description). A frameshift mutation in exon 2, P159fsdelT, with predicted termination at codon 160, was found in family BDA298. The mutations cosegregate with GCKD in both families (see fig. 1) and were not present in 60 normal chromosomes.

The clinical characteristics are summarized in table 2 and figure 1. Both families with *HNF-1β* mutations have the hypoplastic subtype of familial GCKD. In family BDA440 (Rizzoni et al. 1982), all three affected subjects have small kidneys and renal impairment. Intravenous urogram (IVU) appearances show absent calyces and irregular, enlarged collecting systems. Renal histology on subject III:4 has shown cortical cysts with cystic glomeruli and glomerular tufts. All three subjects also now have early-onset diabetes, with an age at diagnosis ranging from 22 to 39 years. In family BDA298 (Kaplan et al. 1989), both affected subjects have renal impairment and small kidneys, with cortical cysts visualized on ultrasound scanning and calyceal abnormalities on IVU. Renal histology on II:2 has shown cortical glomerular cysts and glomerular tufts. II:2 was diagnosed as having diabetes at age 39 years. After the finding of the mutation, III:1 was found to have impaired glucose tolerance on an oral glucose–tolerance test performed at age 17 years. In contrast, the two families with familial GCKD in which *HNF-1β* mutations were not found did not have the hypoplastic subtype of familial GCKD. The probands from families BDA395 and BDA430 (Melnick et al. 1984) have large and normal-size kidneys, respectively, and calyceal abnormalities were not reported. A further difference was that the proband from family BDA395 had a different histology, since there were collecting-duct as well as glomerular cysts. None of the families reported have features of any recognized syndromes or evidence of urinary tract obstruction.

This is the first description of an etiological gene for familial GCKD. Our results suggest that there is genetic heterogeneity within familial GCKD. The two families with *HNF-1β* mutations that we describe have been described elsewhere as having the hypoplastic subtype of familial GCKD (Rizzoni et al. 1982; Kaplan et al. 1989). Both families have the clinical features of small kidneys and abnormal calyces and papillae seen in this subtype. We were unable to obtain DNA from a third published French pedigree (Rizzoni et al. 1982). Both families without *HNF-1β* mutations have nonhypoplastic familial GCKD, since they have large or normal-size kidneys and normal calyces and papillae. This suggests that *HNF-1β* mutations are an important cause of the hypoplastic subtype but may not have an etiological role in nonhypoplastic familial GCKD. A further gene or genes are likely to be identified in GCKD. The role that *HNF-1β* mutations may have in the other categories of GCKD is currently unknown and warrants investigation, especially in sporadic isolated GCKD, because a spontaneous *HNF-1β* mutation has been described (Bingham et al. 2000).

In addition to hypoplastic GCKD, the other characteristic clinical feature of the two families with *HNF-1β* mutations is early-onset diabetes, which was not seen in the two families without mutations. All three affected subjects in family BDA440 have early-onset diabetes. In the original report on this family, diabetes was only described in one subject but has subsequently developed in her two daughters. This family, with an autosomal dominant inheritance of diabetes and two subjects diagnosed at age <25 years, would meet the diagnostic criteria for MODY (Hattersley 1998). In family BDA298, the mother developed diabetes at age 39 years, and we have established, after identification of an *HNF-1β* mutation, that her son has impaired glucose tolerance on an oral glucose tolerance test at age 17 years. This family does not, at present, reach diagnostic criteria for MODY. In these families, the diabetes has presented years or decades after the renal disease, which explains why this was not previously recognized as a clinical characteristic of their familial hypoplastic GCKD.

The nonsense and frameshift mutations we describe in families with hypoplastic GCKD are predicted to result in truncated proteins, with the loss of part of the DNA-binding region of the protein and all of the transcription-activation domain. They are likely to be loss-of-function mutations. The previously described R137-K161del mutation, which resulted in oligomeganephronia and abnormal uterine and vaginal development, was shown in functional studies to be a loss-of-function mutation (Lindner et al. 1999). However, the resulting in-frame deletion leads to a loss of the pseudo-POU domain, which is implicated in giving specificity to DNA binding, but the transactivation domain was preserved. Lindner and colleagues suggest that the genital abnormalities may result from the mutant transcription factor, with its retained transactivation domain interacting with other transcription factors or basal transcription activity and altering genital development (Lindner et al. 1999). In contrast, the frameshift mutation P328L329fsdelCCTCT, present in the fetus with cystic renal dysplasia, has been shown to be a gain-of-function mutation (Wild et al. 2000). Overexpression of this mutation in the *Xenopus* embryo leads to defective development and agenesis of the pronephros, the first kidney form of amphibians (Wild et al. 2000).

HNF-1β is known to be expressed from the earliest inductory phases of kidney development in the rat. In the newborn rat kidney, HNF-1β transcripts are found in the proximal and distal convoluted tubules, the loop of Henle, and the collecting ducts (Lazzaro et al. 1992). Renal abnormalities have been detected on fetal ultrasound scanning in subjects from the reported families

Table 2

Clinical Characteristics of the Affected Subjects

	Subject				
Characteristic	1	2	3	4	5
Family	BDA440	BDA440	BDA440	BDA298	BDA298
Subject	III 3	III 4	II 2	III 1	II 2
Mutation	E101X	E101X	E101X	P159fsdelT	P159fsdelT
Age at diagnosis of renal disease	9 mo	1 mo	35 y	At birth	2 mo
Present age (y)	34	29	60	18	40
Blood pressure (mmHg)	150/90	120/80	120/80	90/50	110/50
Creatinine (μmol/l)[a]	164 (35–105)	191 (35–105)	282 (35–105)	193 (45–120)	ESRF
Creatinine clearance (ml/min)[a]	NA	NA	NA	23 (90–130)	4 (80–120)
24-hour urine protein (g/24 hr)[a]	No proteinuria	No proteinuria	1.8 (.01–.14)	.18 (.01–.14)	.32 (.01–.14)
IVU	Small kidneys; bilateral enlarged pelvis and absent calyces	Small kidneys; small calyces absent; collecting systems irregular and enlarged	Small kidneys; collecting systems irregular and enlarged	Abnormal calyces	Small kidneys with abnormal calyces
Renal ultrasound	NA	NA	NA	Small kidneys with cysts	Small kidneys with cysts
Renal histology	NA	Cortical cysts; cystic glomeruli with glomerular tufts	NA	Dilatation of Bowman's spaces	Glomerular cysts and glomerular tufts
Age at diagnosis of diabetes (years)	22	23	39	17[b]	39
Treatment for diabetes	Insulin	Insulin	Insulin	None (not diabetic)	Oral hypoglycemic agents
Other features	…	Prognathism	…	Learning difficulties	Prognathism; pyloric stenosis

Note.—NA = not available; ESRF = end-stage renal failure.

[a] Most recently available values, with reference ranges in parentheses.

[b] Impaired glucose tolerance.

with *HNF-1β* mutations (Nishigori et al. 1998; Bingham et al. 2000). Cystic changes associated with *HNF-1β* mutations occur early in fetal development, supporting a major role for HNF-1β in human kidney development. In the studies on rat kidney, HNF-1β was not found to be expressed in the glomeruli (Lazzaro 1992). In view of our finding of *HNF-1β* mutations in cases of GCKD, it is interesting to investigate whether HNF-1β is expressed in the glomeruli in the human kidney. Alternatively, the glomerular cysts may result from an early disruption in nephron development.

This report describes the fifth and sixth families in which an *HNF-1β* mutation has been found that cosegregates with diabetes and/or renal disease. Early-onset diabetes is a constant feature of all families, but the nondiabetic renal manifestations are variable (Horikawa et al. 1997; Nishigori et al. 1998; Lindner et al. 1999; Bingham et al. 2000). Three separate histologies have been described—oligomeganephronia, cystic dysplasia, and hypoplastic GCKD. It is likely that all these renal phenotypes are manifestations of abnormal nephron development. The most constant clinical feature is the presence of renal cysts, which have been seen in members of five of the six families described. In the sixth family, there was no description of renal appearance or histology (Horikawa et al.1997). We therefore propose that *HNF-1β* mutations frequently result in a clinical syndrome characterized by renal cysts and diabetes (RCAD). The recognition of this autosomal dominantly inherited syndrome will assist the identification of patients in whom *HNF-1β* mutation testing is desirable.

We conclude that *HNF-1β* gene mutations are associated with the hypoplastic subtype of familial GCKD. We have established that there is genetic heterogeneity in familial GCKD, which reflects clinical heterogeneity. In the families in which we have identified *HNF-1β* mutations, affected subjects have hypoplastic GCKD and early-onset diabetes. This is the third renal histology to be reported in association with *HNF-1β* mutations, and it reinforces the variability in the renal phenotype associated with these mutations. All renal phenotypes associated with *HNF-1β* mutations are likely to represent abnormal nephron development. These observations allow a further extension of the molecular genetic classification of renal cystic disease that cuts across previous classification by clinical or histological criteria. Familial hypoplastic GCKD forms part of the clinical spectrum of the RCAD syndrome associated with *HNF-1β* mutations.

Acknowledgments

We thank the National Kidney Research Fund (grant TF13/2000), the European Union funding for the GIFT consortium, the British Diabetic Association, and the Exeter Kidney Unit Development Fund, who all supported this work. We also thank Dr. A. Mesoraca, for his help in linkage analysis, and the families and their clinicians, in particular Professors D. B. Brewer and T. M. Barratt.

Electronic-Database Information

The accession numbers and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for familial GCKD [MIM 137920], autosomal dominant polycystic kidney disease, *PKD1* [MIM 601313] and *PKD2* [MIM 173910], and MODY type 5 [MIM 604284]).

References

Attar R, Quinn F, Winyard PJD, Mouriquand PDE, Foxall P, Hanson M, Woolf AS (1998) Short-term urinary flow impairment deregulates PAX2 and PCNA expression and cell survival in fetal sheep kidneys. Am J Pathol 152:1225–1235

Beards F, Frayling T, Bulman M, Horikawa Y, Allen L, Appleton M, Bell GI, Ellard S, Hattersley AT (1998) Mutations in hepatocyte nuclear factor 1b are not a common cause of maturity-onset diabetes of the young in the U.K. Diabetes 47:1152–1154

Bernstein J (1993) Glomerulocystic kidney disease—nosological considerations. Pediatr Nephrol 7:464–470

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 (2000) Abnormal nephron development associated with a frameshift mutation in the transcription factor hepatocyte nuclear factor-1b. Kidney Int 57: 898–907

Feather SA, Winyard PJD, Dodd S, Woolf AS (1997) Oral-facial-digital syndrome type 1 is another dominant polycystic kidney disease: clinical, radiological and histopathological features of a new kindred. Nephrol Dial Transplant 12:1354–1361

Frayling T, Bulman MP, Ellard S, Appleton M, Dronsfield MJ, Mackie ADR, Baird JD, Kaisaki PJ, Yamagata K, Bell GI, Bain SC, Hattersley AT (1997) Mutations in the hepatocyte nuclear factor 1a gene are a common cause of maturity-onset diabetes of the young in the United Kingdom. Diabetes 46:720–725

Hattersley AT (1998) Maturity-onset diabetes of the young: clinical heterogeneity explained by genetic heterogeneity. Diabetes Med 15:15–24

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 (1997) Mutation in hepatocyte nuclear factor-1β gene (TCF2) associated with MODY. Nat Genet 17:384–385

Kaplan BS, Gordon I, Pincott J, Barratt TM (1989) Familial hypoplastic glomerulocystic kidney disease: a definite entity with dominant inheritance. Am J Med Genet 34:569–573

Lazzaro D, de Simone V, de Magistris L, Lehtonen E, Cortese R (1992) LFB1 and LFB3 homeoproteins are sequentially ex-

pressed during kidney development. Development 114:469–479

Lindner TH, Njolstad PR, Horikawa Y, Bostad L, Bell GI, Sovik O (1999) 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

Melnick SC, Brewer DB, Oldham JS (1984) Cortical microcystic disease of the kidney with dominant inheritance: a previously undescribed syndrome. J Clin Pathol 37:494–499

Mendel DB, Hansen LP, Graves MK, Conley PB, Crabtree GR (1991) HNF-1α and HNF-1β (vHNF-1) share dimerisation and homeo domains, but not activation domains, and form heterodimers in vitro. Genes Dev 5:1042–1056

Mesoraca A, Massari A, Novelli G, Dallapiccola B and Rizzoni G (1996) Linkage analysis excludes familial hypoplastic glomerulocystic kidney disease from chromosome 4 and 16. J Am Soc Nephrol 7:1338

Nishigori H, Yamada S, Kohama T, Tomura H, Sho K, Horikawa Y, Bell GI, Takeuchi T, Takeda J (1998) Frameshift mutation, A263fsinsGG, in the hepatocyte nuclear factor-1b gene associated with diabetes and renal dysfunction. Diabetes 47:1354–1355

Rey-Campos J, Chouard T, Yaniv M, Cereghini S (1991) vHNF1 is a homeoprotein that activates transcription and forms heterodimers with HNF1. EMBO J 10:1445–1457

Rizzoni G, Loirat C, Levy M, Milanesi C, Zachello G, Mathieu H (1982) Familial hypoplastic glomerulocystic kidney. A new entity? Clin Nephrol 18:263–268

Sharp CK, Bergman SM, Stockwin JM, Robbin ML, Galliani C, Guay-Woodford LM (1997) Dominantly transmitted glomerulocystic kidney disease: a distinct genetic entity. J Am Soc Nephrol 8:77–84

Weng JP, Lehto M, Forsblom C, Huang X, Li H, Groop LC (2000) Hepatocyte nuclear factor-1β (MODY 5) gene mutations in Scandinavian families with early-onset diabetes or kidney disease or both. Diabetologia 43:131–134

Wild W, Pogge v. Strandmann E, Nastos A, Senkel S, Lingott-Frieg A, Bulman M, Bingham C, Ellard S, Hattersley AT, Ryffel GU (2000) The mutated human gene encoding hepatocyte nuclear factor 1b inhibits kidney formation in developing Xenopus embryos. Proc Natl Acad Sci 97:4695–4700