Hepatocyte nuclear factor-1β gene deletions—a common cause of renal disease

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Abstract

Background. Hepatocyte nuclear factor-1β (HNF-1β) is a critical transcription factor in pancreatic and renal development. Our previous report identified HNF-1β mutations in 23/160 patients with unexplained renal disease. The most common phenotype is renal cysts, which is frequently associated with early-onset diabetes in the renal cysts and diabetes (RCAD) syndrome. HNF-1β gene deletions have recently been shown to cause renal malformations and early-onset diabetes.

Methods. We developed a multiplex ligation-dependent probe amplification (MLPA) assay for HNF-1β gene dosage analysis and tested patients with unexplained renal disease in whom mutations had not been found by sequencing.

Results. Whole HNF-1β gene deletions were detected in 15/133 probands. Renal cysts were present in 13/15, including three with glomerulocystic kidney disease and one with cystic renal dysplasia. Renal function ranged from normal to transplantation aged 3 years. Ten probands had diabetes (nine having RCAD). In addition, four had abnormal liver function tests, two showed pancreatic atrophy and 3/10 female probands had uterine malformations. Whole HNF-1β gene deletions are a common cause of developmental renal disease, particularly renal cystic disease with or without diabetes.

Conclusions. The phenotype associated with deletions or coding region/splicing mutations is very similar suggesting that haploinsufficiency is the underlying mechanism. Patients with features suggestive of the HNF-1β clinical phenotype should be tested for mutations both by sequence and dosage analysis.

Keywords: HNF-1β; renal disease; diabetes; deletion mutation

Introduction

Hepatocyte nuclear factor-1β (HNF-1β) is a member of the homeodomain-containing super family of transcription factors. It is important in the tissue-specific regulation of gene expression in a number of organs including the kidney, pancreas, liver, genital tract and gut [1]. HNF-1β is also involved in the early embryogenesis of these organs and has been shown to be vital for embryonic survival in rodents [2].

Mutations in the HNF-1β gene (HGVS approved gene name TCF2) cause multi-system disease in man. However, renal disease is the most consistent phenotype, present in all reported probands. The renal disease is variable with renal cysts being the most common manifestation [3]. A number of different renal histologies have been described including glomerulocystic kidney disease (GCKD), cystic renal dysplasia and oligomeganephronia. In some cases, there have been morphological renal abnormalities including single functioning kidney and horseshoe kidney (see review [4]). The wide spectrum of renal diseases associated with HNF-1β mutations can all be classified as disorders of renal development. Renal abnormalities are frequently detected on antenatal ultrasound scans from as early as 17 weeks gestation [5]. Patients with a HNF-1β mutation have renal function that ranges from normal to dialysis dependent or transplanted [4].

The majority of HNF-1β mutation carriers have extra-renal phenotypes with diabetes being the most common. The gene was first described as causing maturity onset diabetes of the young type 5 [6], however, it is more commonly associated with renal disease. There is an associated syndrome termed renal cysts and diabetes (RCAD), which is used to describe the phenotype observed most often in HNF-1β mutation carriers [4]. Diabetes usually presents in early adulthood with a median age of 20 years (range 15 days to 61 years) and frequently requires insulin treatment [3]. When HNF-1β mutations are associated with diabetes, there is usually pancreatic atrophy and
We report 15 patients with whole HNF-1 unexplained renal disease (with or without diabetes). We designed synthetic probes for the nine exons of the HNF-1 gene to measure gene dosage. Conventional MLPA assays dependent probe amplification (MLPA) technique was utilized. We identified that in 58% of cases arise spontaneously [3,13].

Gene deletions or, less frequently, the deletion of a single exon [11,12]. These types of genomic rearrangement are not detected by direct sequencing techniques. Mutations and genomic deletions of the HNF-1 gene may show dominant inheritance, although 32–58% of cases arise spontaneously [3,13].

In this study, we utilized the multiplex ligation-dependent probe amplification (MLPA) technique to measure gene dosage. Conventional MLPA assays utilise cloned probes but the use of synthetic oligonucleotide probes has recently been described [14].

We designed synthetic probes for the nine exons of the HNF-1β gene and tested a series of 151 subjects with unexplained renal disease (with or without diabetes). We report 15 patients with whole HNF-1β gene deletions and discuss their clinical phenotypes.

### Methods

#### Subjects

We previously collected 160 subjects with renal disease with an unknown aetiology and identified an HNF-1β mutation in 23 cases by sequencing [3]. For this retrospective study, we obtained DNA from 151 cases with unexplained renal disease (including the 137 negative cases from our previous study and an additional 14 cases). All were negative for a mutation on HNF-1β sequencing (Figure 3). Informed consent was obtained from all subjects and the study was conducted in agreement with the Declaration of Helsinki as revised in 2000. The subjects were classified by the diagnosis of the renal disease, renal cystic disease and cystic dysplasia 82/151 (54%); GCKD 12/151 (8%) (six with histological evidence); atypical familial juvenile hyperuricaemic nephropathy (FJHN) 5/151(3%) (subjects with young-onset hyperuricaemia, gout, renal impairment and also disorders of renal development including renal cysts); renal dysplasia 20/151 (13%); renal malformations 28/151 (19%) are defined as gross renal developmental abnormalities and include single kidney (n = 19), horseshoe kidney (n = 1) and hypoplastic kidney(s) (n = 8) and other diagnoses 4/151(3%) (including Fanconi syndrome, renal agenesis and renal failure).

Diabetes was diagnosed on the basis of receiving treatment, either insulin or oral agents, for diabetes or if they were not on treatment then biochemical evidence of diabetes in line with WHO guidelines [15]. Clinical details were obtained from the referring clinician or patients’ hospital records. Renal disease and other clinical features are outlined in Table 1. The median age of the cohort at time of test was 18 years (1–78), with 84/151 female. Parental samples were requested following the identification of a deletion.

### Multiple ligation-dependent probe amplification (MLPA) HNF-1β dosage assay

We designed an MLPA assay to detect partial or whole HNF-1β gene deletions. MLPA utilizes sequence-specific probes in a fluorescently labelled multiplex ligation/PCR reaction. We designed nine HNF-1β exon-specific synthetic probes (Table 2), which were used in conjunction with the MEN1 MLPA Kit (#P017) (MRC Holland, The Netherlands). The MEN1 exon specific and other control probes from the kit (n = 24) were used as controls for the assay. The procedure was carried out according to manufacturer’s instructions. Briefly, 100–150 ng of genomic DNA was used as template. Following DNA denaturation, probe hybridization, probe ligation and amplification, the products were separated according to size on an ABI3100 (Applied Biosystems, Warrington, UK). The data were analysed using GeneScan and Genotyper version 2 analysis software (Applied Biosystems, Warrington, UK) to define the size and peak heights of the 33 probes. Two control samples, known to be heterozygous for an HNF-1β point mutation, a positive control with a whole gene deletion, and a negative control were included in the assay.

Dosage quotients were calculated by dividing the peak height for each of the HNF-1β probes and control probes by the sum of the peak heights for the exon-specific probe and the six control probe peaks with sizes 146–183 base pairs. This ratio was then normalized to the mean of the two control samples for that specific probe. For sequences that contained two copies of HNF-1β, the dosage quotient would be expected to be 1. Peak height ratios

### Table 1. Clinical characteristics of the cohort

<table>
<thead>
<tr>
<th>Renal disease</th>
<th>Total</th>
<th>Diabetes</th>
<th>Family history of diabetes</th>
<th>Genitourinary tract abnormality</th>
<th>No diabetes or genitourinary tract abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal cysts and cystic dysplasia</td>
<td>82</td>
<td>32</td>
<td>7</td>
<td>5</td>
<td>45</td>
</tr>
<tr>
<td>Glomerulocystic kidney disease</td>
<td>12</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Atypical familial juvenile hyperuricaemic nephropathy</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Renal dysplasia</td>
<td>20</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Renal malformations</td>
<td>28</td>
<td>6</td>
<td>2</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>151</td>
<td>46</td>
<td>13</td>
<td>21</td>
<td>84</td>
</tr>
</tbody>
</table>
Results

Genetic analysis of HNF-1β

A novel HNF-1β MLPA dosage assay was designed and validated using DNA from a patient with a whole gene deletion (Figure 1). A total of 151 DNA samples were tested, of which 133 (88%) tests produced usable data. We identified 15/133 (11%) subjects with a heterozygous HNF-1β gene deletion (p.M1_W557del) subject DUK1915 that has previously been reported by Eller et al. [16]. Parental samples were available from seven families; in three families there was vertical transmission of the HNF-1β gene deletion and in the other four families the deletion had arisen spontaneously (Figure 2).

Microsatellites surrounding the HNF-β gene on chromosome 17 were used to confirm family relationships and to define the size of the deletion. D17S1788 lies within intron 4 of HNF-β and therefore confirms the presence of a deletion mutation (Figure 2). The microsatellites D17S1867, D17S1788 and D17S927 were hemizygous in all informative families, defining a minimal deleted region of at least 1.2 Mb. The size of the chromosome 17 deletion ranges from ≥1.2 Mb (Chromosome 17: 32080457-33179182) to ≥2.3 Mb (Chromosome 17: 30890413-33179182) in family DUK677.

Clinical characteristics

The phenotypic data of the 15 whole HNF-1β gene deletion carriers and their affected family members (n = 4) are displayed in Table 3.

The commonest renal manifestation in the subjects with an HNF-β deletion was renal cysts that were present in 13/15 (87%). In one proband (DUK614), the cysts were unilateral and in kindred DUK395 subject II:II had a single enlarged cystic kidney. In three subjects, renal histology was consistent with GCKD (DUK395, DUK567 and DUK886). Subject DUK893 had a diabetic first degree relative (the father of DUK1915). Diabetes was present in 10/15 (66%), 3/10 had a diabetic first degree relative (the father of DUK1575 is a phenocopy). One other subject had a diabetic first degree relative (the father of DUK1575). Gestational diabetes and a first degree relative affected amongst all the deletion carriers was 17 years (range 6–37). DUK1575 and DUK1915 had pancreatic atrophy demonstrated on CT or MRI scanning. RCAD was found in 9/15 (60%) probands.

In addition to renal disease and diabetes, three subjects had uterine malformations. These subjects had an absent uterus (DUK893), bicornuate uterus (DUK717) or a bilateral hemi-uterus (DUK1826).
None of the subjects had clinical liver disease, although five had abnormalities of liver function on blood tests (DUK531, DUK567, DUK674, DUK717 and DUK1575). One diabetic subject had evidence of steatosis on a liver biopsy (DUK1575).

**Discussion**

We have identified 15 subjects with a heterozygous whole HNF-1β gene deletion (p.M1_W557del) from a large series of 133 patients with unexplained renal
disease (11%). Coding region or splicing mutations were previously identified in 23/160 (14%) probands [3]. An additional 15 probands have now been shown to have whole gene deletions, so the number of positive cases in this updated series is now 38/160 (24%) (Figure 3), with 18/151 failing analysis (12%). Therefore, large genomic rearrangements account for ~39% of HNF-1β mutations in this series.

We report that renal cysts are the most common clinical feature accounting for 87% of deletion carriers, this is similar to subjects with a HNF-1β mutation as previously described (83% of mutation carriers) [3]. Renal histology has been examined in three cases showing GCKD, which has previously been reported in HNF-1β mutation carriers [17]. In contrast to our previous study, no deletions were identified in subjects with atypical FJHN. Moreover, in the 118 subjects, whose MLPA assay was successful, we did not find evidence of either a mutation or a deletion in the HNF-1β gene (Table 4); it is possible that these subjects have abnormalities in UMOD, PKHD1, PKD1, PKD2 or other genes. Our results indicate that patients with unexplained renal cystic disease (including GCKD with histological evidence of this disorder) and to a lesser extent those with developmental renal abnormalities with or without diabetes are the most likely to test positive following an HNF-1β genetic test.

In eight subjects, renal abnormalities were first detected on antenatal ultrasound scans, six with cysts and two with bright kidneys. In both of the cases with antenatal bright kidneys, subsequent post-natal scans revealed the presence of cysts. It is therefore common for this disorder to present initially to obstetricians and neonatologists.

Fig. 2. Partial pedigrees of seven families showing the haplotype data from microsatellites flanking HNF-1β (between D17S1867 and D17S1851), D17S1788 is located in intron 4 of the HNF-1β gene. Solid bars represent affected haplotypes, unfilled bars denote unaffected haplotypes and diagonal striped bars show regions of unknown zygosity. DEL/N, HNF-1β deletion; N/N, no mutation.
<table>
<thead>
<tr>
<th>Family number</th>
<th>Sex</th>
<th>ID</th>
<th>Present age, year</th>
<th>Primary renal diagnosis</th>
<th>Renal disease Histology</th>
<th>Age of diagnosis year (week’s gestation)</th>
<th>GFR (ml/min)</th>
<th>Creatinine (μmol/l)</th>
<th>Diabetes yes/no</th>
<th>Age of diagnosis (years)</th>
<th>Treatment</th>
<th>Family history of diabetes or renal disease</th>
<th>Genital tract abnormalities</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>DUK220</td>
<td>F</td>
<td>43</td>
<td>SK</td>
<td>–</td>
<td>–</td>
<td>61</td>
<td>74</td>
<td>53 (at 7 years)</td>
<td>Yes</td>
<td>32</td>
<td>insulin</td>
<td>Fetal loss with cystic dysplasia</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DUK614</td>
<td>F</td>
<td>17</td>
<td>Small cystic dysplastic left kidney</td>
<td>Antenatal</td>
<td>–</td>
<td>12</td>
<td>Yes</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>OHA</td>
<td>Father RCAD</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DUK395</td>
<td>M</td>
<td>48</td>
<td>RC</td>
<td>–</td>
<td>–</td>
<td>14</td>
<td>Yes</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>No</td>
<td>Hypothyroidism</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DUK674</td>
<td>F</td>
<td>34</td>
<td>RC</td>
<td>–</td>
<td>25</td>
<td>84</td>
<td>GDM</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
<td>Insulin</td>
<td>Mother renal failure and diabetes, three children enlarged bright kidneys</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DUK654</td>
<td>M</td>
<td>4</td>
<td>RC</td>
<td>–</td>
<td>(28)</td>
<td>84</td>
<td>GDM</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
<td>Insulin</td>
<td>–</td>
<td>Loss of corticomedullary differentiation, bright kidneys</td>
<td>–</td>
</tr>
</tbody>
</table>
Unilateral abnormalities were found in two subjects; one with a single cystic and dysplastic kidney detected antenatally (DUK395 II:II) and one with a single kidney, detected in adulthood (DUK220). In the case with a single kidney, it is possible that they had a second cystic dysplastic kidney, which involuted during childhood. Interestingly, in a previous study \( HNF-1\beta \) deletions were only found to be associated with bilateral renal abnormalities [12].

Renal function in our affected subjects ranges from normal to dialysis dependent and one subject, aged 3 years required a renal transplant. A similar variability in renal function has been reported in subjects with mutations [4]. Subject DUK674 I:II is dialysis dependent and has a severe diabetic phenotype with microvascular complications including diabetic retinopathy. Examination of renal histology has not been undertaken in this subject, but it is possible that she has poor renal function as a result of developing diabetic nephropathy, in addition to developmental renal abnormalities.

The mean age of diagnosis of diabetes in our study was 17 years (range 6–37) and in the majority of cases the diabetes developed after the renal disease, in keeping with previous reports [3]. The commonest mode of treatment was with insulin.

Other phenotypic features include uterine malformations and abnormal liver function tests; these have previously been described in \( HNF-1\beta \) mutation and \( HNF-1\beta \) deletion carriers [3,11].

Hemizygous regions flanking the \( HNF-1\beta \) gene define a minimal deleted region of 1.2 Mb (chromosome 17:33179182-31916586) consistent with the original description of large genomic rearrangements [11]. The 1.2 Mb deletion is predicted to result in haploinsufficiency for at least seven other genes (\( AATF, ACACA, LHX1, TADA2L, DUSP14, DDXS2 \) and \( AP1GBP1 \)) [11]. The similarity of the phenotype compared to coding region/splice site mutations suggests that the deletion of these additional genes does not contribute to the clinical phenotype, however further studies are needed. This region of chromosome 17 is susceptible to genomic rearrangement. A duplication of this region has recently been reported in a case with idiopathic mental retardation [18]. The duplicated region is flanked by segmental duplications, which are predicted to cause non-allelic homologous recombination and result in a duplication or deletion of the intervening sequence. It is therefore likely that the deletions identified in subjects with renal disease represent the reciprocal event reported by Sharp et al. [18].

It was possible to test both parents from seven of the probands with an \( HNF-1\beta \) deletion. Three families showed autosomal dominant inheritance, whilst four probands had a spontaneous deletion (57%). A previous study reported a high prevalence (50%) for spontaneous \( HNF-1\beta \) gene deletions [12]. Therefore, deletions as well as coding region/splice site mutations may occur even when there is no family history of renal disease or diabetes.
Whole or partial gene deletions have previously been identified in subjects with early onset diabetes and renal disease (25%) and paediatric renal disease (18%) [11,12]. Our pick-up rate was lower than the previous reports (11%). This could be due to differences in the clinical characteristics of the patients investigated, as our cohort was selected for unexplained renal disease and only 39% (59/151) had a personal or family history of diabetes. In contrast to the previous reports, we did not detect any single exon deletions. However, these are rare as only two of the 24 cases reported to date had a single exon deletion [11,12].

In order to detect a HNF-1β gene deletion, we developed a novel MLPA assay using synthetic probes specific for the HNF-1β gene. This is the only second report to describe the successful incorporation of synthetic oligonucleotide probes (rather than cloned probes) in an MLPA assay [14]. We were unable to obtain a result for 18/151 samples either due to insufficient DNA quantity or concentration. Therefore, for optimal results, this assay should be used with high quality genomic DNA.

The HNF-1β whole gene deletions result in haploinsufficiency and a multi-system disease in man. The disease mechanism of HNF-1β deletions and other mutations have been described using in vivo studies. Renal-specific inactivation of the HNF-1β gene in mice leads to the development of cystic kidneys by a reduction in transcriptional activation of the Umod, Pkhd1 and Pkd2 [19]. Other studies have shown that HNF-1β is vital in the transcription factor hierarchy for mouse pancreatic development [20]. This has been further supported by an hnf-1β-null mouse model, rescued from early lethality by using embryonic stem cells, which had pancreatic agenesis at e13.5 days [21]. In man, children with an HNF-1β mutation born to a mother who is not diabetic during the pregnancy have a reduced birth weight and hence reduced insulin secretion in utero, indicating that HNF-1β is important in human pancreatic development [8].

<table>
<thead>
<tr>
<th>Renal disease</th>
<th>Total</th>
<th>Diabetes</th>
<th>Genitourinary tract abnormality</th>
<th>No diabetes or genitourinary tract abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal cysts and cystic dysplasia</td>
<td>65</td>
<td>22</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Glomerulocystic kidney disease</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Atypical familial juvenile hyperuricaemic nephropathy</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Renal dysplasia</td>
<td>16</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Renal malformations</td>
<td>23</td>
<td>5</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
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<tr>
<td>Total</td>
<td>118</td>
<td>34</td>
<td>10</td>
<td>17</td>
</tr>
</tbody>
</table>

Therefore, for optimal results, this assay should be used with high quality genomic DNA.
In conclusion, HNF-1β whole gene deletions are a common cause of unexplained developmental renal disease, particularly in subjects with renal cystic disease, with or without diabetes. The phenotype associated with whole gene deletions or mutations is very similar suggesting that haploinsufficiency is the underlying mechanism. It is important to screen for HNF-1β gene mutations by both sequencing and gene dosage analysis.

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Conflict of interest statement. None declared.

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