Variable phenotype in HNF1B mutations: extrarenal manifestations distinguish affected individuals from the population with congenital anomalies of the kidney and urinary tract

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ABSTRACT

Background. Mutations in hepatocyte nuclear factor 1B (HNF1B) have been associated with congenital anomalies of the kidney and urinary tract (CAKUT) in humans. Diabetes and other less frequent anomalies have also been described. Variable penetrance and intrafamilial variability have been demonstrated including severe prenatal phenotypes. Thus, it is important to differentiate this entity from others with similar clinical features and perform confirmatory molecular diagnosis.

Methods. This study reports the results of HNF1B screening in a cohort of 60 patients from 58 unrelated families presenting with renal structural anomalies and/or non-immune glucose metabolism alterations, and other minor features suggesting HNF1B mutations.

Results. This study identified a pathogenic variant in 23 patients from 21 families. The most frequent finding was bilateral cystic dysplasia or hyperechogenic kidneys (87% of patients). Sixty percent of them also fulfilled the criteria for impaired glucose metabolism, and these were significantly older than those patients with an HNF1B mutation but without diabetes or prediabetes (14.4 versus 3.3 years, P < 0.05). Furthermore, patients with HNF1B mutations had higher frequency of pancreatic structural anomalies and hypomagnesaemia than patients without mutations (P < 0.001 and P = 0.003, respectively). Hyperuricaemia and increased liver enzymes were detected in some patients as well.
Conclusions. Renal anomalies found in patients with HNF1B mutations are frequently unspecific and may resemble those found in other renal pathologies (CAKUT, ciliopathies). Active searching for extrarenal minor features, especially pancreatic structural anomalies or hypomagnesaemia, could support the indication for molecular diagnosis to identify HNF1B mutations.

Keywords: CAKUT, HNF1B, hypomagnesaemia, MODY, pancreatic structural anomalies

INTRODUCTION

The hepatocyte nuclear factor 1B gene (HNF1B; OMIM * 189907) encodes the transcription factor HNF1B, involved in the embryologic development of several tissues, including kidney, pancreas, lung, liver and gut [1]. Heterozygous mutations of this gene were first described in patients with maturity-onset diabetes of the young (MODY) [2]. Subsequently, several anomalies have also been associated with pathogenic mutations in this gene. Among them, congenital anomalies of the kidney and urinary tract (CAKUT), mainly bilateral cystic dysplasia [3], and MODY-type diabetes [4] are the most common. Less often, pancreatic or genital structural anomalies, mildly elevated liver enzymes, hyperuricaemia and hypomagnesaemia are also present in the affected patients [5–8]. Although in children kidney disease represents the most frequent phenotype at diagnosis, diabetes remains the leading clinical expression in adults [4, 9, 10].

The HNF1B gene has also been linked to ciliopathies through its direct effect on gene regulation. Indeed, HNF1B knockout mice diminish the expression of PKHD1 and PKD2 genes, whose mutations are responsible for autosomal-recessive and -dominant polycystic kidney disease (ARPKD and ADPKD), respectively [11, 12]. Both ARPKD and ADPKD are classical models of ciliopathies, a group of diseases caused by abnormalities of genes coding for proteins expressed at the primary cilium, with progressive development of cysts in several organs.

More specifically, mutations in the HNF1B gene have also been observed in some patients with severe and early-onset ADPKD phenotype presenting with large hyperechogenic kidneys and prenatally severely impaired kidney function, mimicking ARPKD [8, 13]. Seminal vesicle or epididymal cysts, as well as moderately enlarged and cystic kidneys are also other classical findings in patients carrying HNF1B mutations, characteristics that again resemble ADPKD. Different transmission patterns of these diseases underlie the need for confirmatory molecular diagnosis [10, 14].

Mutations in HNF1B are inherited in a dominant way with 40–60% of patients carrying a de novo mutation [3, 4]. About half of the described mutations consist of whole heterozygous gene deletions [15]. Variable penetrance with non-specific clinical manifestations and a high prevalence of de novo cases complicates the diagnosis. However, genetic confirmation is important for patient and relatives due to different implications in disease outcome and impacts on family counselling.

In this study, we aimed to diagnose HNF1B mutations in a group of patients harbouring renal and/or glucose metabolism alterations and described those often associated extrarenal minor clinical features. We focus on those associated phenotypic characteristics that might guide the differential diagnosis with CAKUT of different origin and even with cystic diseases, in order to encourage early patient detection and promote genetic confirmation of HNF1B mutations.

MATERIALS AND METHODS

Patients

This is a retrospective study that included 60 patients from 58 unrelated families. Blood samples were sent from 2006 to 2016 for the analysis of HNF1B, within a research project for the clinical and molecular characterization of the alterations in this gene. Patients’ samples were referred by several Nephrology or Endocrinology Departments (paediatric or adults) from different hospitals in Spain. These patients presented with either renal structural anomalies or non-immune glucose metabolism disturbances, and other minor features suggesting HNF1B mutations: genital or pancreatic structural anomalies, elevated liver enzymes, hyperuricaemia, hypomagnesaemia or family history of any of these anomalies in first- and/or second-degree relatives. Written informed consent and clinical data were required in all cases. The study was approved by the Ethics Committee for Clinical Research of Euskadi.

All clinicians participating in the study were asked to fill out a registration data form with demographic, prenatal and familial history data, and clinical data from abdominal ultrasound (US) or computed tomography scan and biochemical analysis. Collected clinical data included information about the presence and grade of chronic kidney disease (CKD), alterations of carbohydrate metabolism, anomalies in the serum levels of uric acid, liver enzymes and magnesium, and description of renal, genital and pancreatic structural anomalies.

Renal hyperechogenicity was defined as the finding of more intense kidney than liver echogenicity by US. Glomerular filtration rate was estimated using the Schwartz or Cockroft formula, in patients <18 or >18 years, respectively [16, 17].

Diabetes and other carbohydrate metabolism disturbances (impaired fasting glucose (IFG) or impaired glucose tolerance) were defined following the 2014 American Diabetes Association diagnostic criteria [18]. Pancreatic islet cell autoantibodies were tested in all cases in our laboratory and turned out to be negative.

Molecular analysis

After informed consent was given, peripheral blood samples were collected for molecular analysis of the HNF1B gene at the laboratory of the Genetic Department of BioCruces Health Research Institute in Barakaldo, Spain. Genomic DNA was extracted from blood leukocytes according to manufacturer’s instructions (QIAamp® DNA Blood Mini Kit, QIAGEN, Germany). Copy number quantification and identification of gross duplications or deletions at the HNF1B region were performed using the multiplex ligation-dependent probe amplification technique as previously described [19]. When no alteration was found, all nine exons and all exon–intron boundaries of the HNF1B gene...
were analysed by polymerase chain reaction amplification and direct sequencing [2]. Pathogenic effects of previously described DNA variants of the HNF1B gene were assessed using Ensembl (www.ensembl.org) and Human Gene Mutation Database (www.biobase-international.com/product/hgmd) databases. New missense DNA variants were assessed using the following software tools for prediction of pathogenicity: Mutation taster (www.mutationtaster.org), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), Sorting intolerant from tolerant (SIFT) (sift.jcvi.org) and SNPs&GO (www.snpsand-go.biocomp.unibo.it).

Statistical analysis
Qualitative variables were described with percentages and quantitative variables with median and interquartile range (IQR). We compared the clinical features observed in the subgroup of patients with HNF1B mutations versus the one without, using the Fisher’s exact test for categorical variables and the Mann–Whitney U test for continuous variables. All tests were two sided. P < 0.05 was considered significant.

RESULTS
All study group
A total of 60 patients from 58 unrelated families were studied. The main clinical findings in all patients are shown in Table 1. Renal structural anomalies were the most common finding (87%). Unilateral or bilateral cystic dysplasia at kidney US was observed in 33 patients (55%), bilateral hyperechogenic kidneys represented the main finding in eight patients (13%), and non-cystic renal anomalies were present in 18% of the series (four cases of unilateral agenesis and seven cases of hypo-dysplasia without cysts).

Pancreatic anomalies were observed in 29 out of 60 patients (48%). Of those, 27 (93%) suffered from diabetes or pre-diabetes (IFG/impaired glucose tolerance) and 8 (28%) had structural anomalies (partial agenesis or hypoplasia).

Hyperuricaemia was the most frequent minor anomaly detected in the study group (32%), followed by the presence of elevated liver enzymes (27%) and structural reproductive system defects (22%). Finally, hypomagnesaemia was found in 23% of them.

Subgroup of patients with HNF1B mutations
We found HNF1B mutations in 23 patients from 21 families, that is, in 38% of the studied patients. Those were point mutations in 6 cases (26%), whole heterozygous deletions in 16 cases (70%) and partial heterozygous deletions in 1 case (4%). Point mutations c.589A>C and c.513G>C have not been previously reported and were considered pathogenic based on prediction software results. Furthermore, in 14 cases, both parents were additionally studied concluding that in 10 of the families, mutations appeared de novo (71%), while they were inherited from the mother in the 4 remaining cases (29%). Eight out of 10 de novo mutations were partial or whole heterozygous deletions.

Detailed renal and extrarenal features of patients carrying a mutation in HNF1B are shown in Table 2. Male to female ratio was 1.3. The median (IQR) age at diagnosis was 9.8 years (0.1–17.2).

Renal phenotype. All but one patient had kidney anomalies on the US. Among them, bilateral cystic dysplasia and bilateral hyperechogenic kidneys were by far the most frequent findings (20 of 22 patients). There was a positive prenatal history of renal abnormalities detected by renal US during pregnancy in 53% of the patients. The median age at diagnosis of those patients with hyperechogenic kidneys was lower than that in patients with other sorts of renal defect, although not significant (5.07 versus 12.02 years, P = 0.16).

More than one-third of the patients with HNF1B mutations (36%) developed moderate to severe CKD over time (CKD Stage ≥3). Kidney biopsy was performed in three of them, demonstrating glomerulocystic disease and renal dysplasia (two cases) and mild mesangial glomerulonephritis (one case). Furthermore, in six patients, renal dysplasia was also associated with other CAKUT findings: bilateral hydronephrosis in four cases (one ureteropelvic junction obstruction), and unilateral hydronephrosis or vesicoureteral reflux in one case each.

Hyperuricaemia was a common finding and present in 29% of the patients with mutations in HNF1B. Most of them (4/6) also had associated moderate to severely reduced renal function, and baseline uric acid levels at early CKD stages were not available. Finally, 53% of the patients carrying a mutation presented with hypomagnesaemia (preserved glomerular filtration rate in most cases).

Diabetes. More than half of the patients with HNF1B mutations (60%) presented diabetes or prediabetes. Moreover, one diabetic female recalled a previous history of gestational diabetes. The median (IQR) age of onset of diabetes in those patients was 16 years (11–22 years). The median age at diagnosis of patients with HNF1B mutations and permanent disturbances of carbohydrate metabolism was significantly higher compared with those without diabetes or prediabetes (14.4 versus 3.3 years, P < 0.05). Finally, two patients (SOR0042 and DM0729) without clinical evidence of impaired glucose metabolism but with severe pancreatic hypoplasia developed transient diabetes early after renal transplantation and treatment with corticosteroids and tacrolimus, at the age of 7 and 8 years, respectively. In both cases, diabetes resolved spontaneously following those drugs’ dose reductions.

Pancreatic phenotype. More than one-third of patients with an HNF1B mutation (35%) had pancreatic structural anomalies, namely partial hypoplasia and cystic dysplasia. Among them, all except two suffered from permanent diabetes or prediabetes as well: patient DM2483, who had an agenesis of pancreas body and tail and was 11 years old at the time of diagnosis; and patient SOR0042, who was born with a severe cystic dysplasia of
| Patient code | Heterozygous mutation | Age at diagnosis (years) | Prenatal renal US | Renal anomalies (US) | Renal function (CKD stage) | Pancreatic anomalies | Genital structural anomalies | Elevated liver enzymes | Hyperuricaemia | Hypomagnesaemia | Other features |
|--------------|-----------------------|--------------------------|-------------------|---------------------|---------------------------|----------------------|--------------------------|-----------------------|----------------|----------------|------------------|
| DM1555       | c.494G>A; p.Arg165His  | 10                       | Normal            | Bilateral cystic dysplasia | –                       | IFG                   | Epidermoid cysts         | Yes                   | No             | –              | Hyperlipidaemia |
| DM1261       | Whole deletion        | 31                       | Normal            | Bilateral cystic dysplasia | 3                       | Diabetes (gest. diabetes), partial hypoplasia | Yes                   | No             | –              | Chronic diarrhoea |
| DM1261a      | Whole deletion        | 17                       | Normal            | Bilateral cystic dysplasia | 0                       | Diabetes, partial hypoplasia | Yes                   | No             | –              | Hyperlipidaemia |
| DM1261b      | Whole deletion        | 28                       | Normal            | Bilateral cystic dysplasia | 0                       | Diabetes              | Yes                   | No             | –              | Hyperlipidaemia |
| SOR0059      | Whole deletion        | 0.1                      | Dysplasia and hydronephrosis | Bilateral cystic dysplasia | 1                       | No                   | No                      | Yes                   | Yes           | Yes           | No               |
| DM1207       | c.494G>A; p.Arg165His  | 20                       | –                 | Bilateral cystic dysplasia | 4                       | Diabetes              | No                      | Yes           | No             | –              | No               |
| DM1824       | Whole deletion        | 1                        | –                 | Bilateral cystic dysplasia | –                       | Diabetes              | Septated uterus         | No                    | –             | –              | No               |
| DM1815       | c.207215delGCCAG; p.His69fs | 17                    | –                 | Hyperechogenic kidneys    | 3                       | Diabetes, partial hypoplasia | Yes                   | Yes           | Yes           | No               |
| DM2665       | Whole deletion        | 18                       | Normal            | Hyperechogenic kidneys    | 0                       | No                   | Hypospadia              | No                   | No             | No             | No               |
| DM2686       | Whole deletion        | 0.1                      | Hyperechogenic kidneys | Hyperechogenic kidneys    | 0                       | No                   | No                      | No                   | No             | No             | No               |
| DM2686       | Whole deletion        | 0.1                      | Severe bilateral hydronephrosis | Hyperechogenic kidneys    | 3                       | No                   | No                      | No                   | No             | No             | No               |
| DM2089       | c.513G>C; p.Trp171Cys | 13                       | Normal            | Normal                  | 3                       | Diabetes              | No                      | No           | Yes           | Yes           | No               |
| DM1991       | Whole deletion        | 13                       | Hyperechogenic kidneys | Bilateral cystic dysplasia | 0                       | Diabetes, partial hypoplasia | No                   | No             | Yes           | No             | No               |
| DM2413       | Whole deletion        | 0.1                      | Severe oligoamnios  | Hyperechogenic kidneys    | 0                       | No                   | No                      | No                   | Yes           | No             | Hypokalaemia    |
| DM2483       | Whole deletion        | 11                       | Normal            | Bilateral cystic dysplasia | 0                       | Partial hypoplasia    | No                      | No             | No             | No             | No               |
| SOR0042      | c.1144C>T; p.Gln382fs | 0.1                      | Hyperechogenic kidneys | Hyperechogenic kidneys    | 5                       | Post-transitory diabetes, cystic dysplasia | No                   | Yes           | No             | No               |
| DM0729       | Partial deletion      | 0.1                      | Polycystic kidneys  | Bilateral cystic dysplasia | 5                       | Partial hypoplasia, diabetes (post-transitory diabetes) | No                   | No             | Yes           | Yes           | Chronic diarrhoea, solitary liver cyst |
| DM0862       | Whole deletion        | 12                       | Normal            | Bilateral cystic dysplasia | 1                       | Diabetes              | No                      | No           | No             | No             | No               |
| DM2748       | Whole deletion        | 0.1                      | Hyperechogenic kidneys | Hyperechogenic kidneys    | 0                       | No                   | No                      | No             | No             | Yes           | No               |
| DM2817       | Whole deletion        | 1                        | Normal            | Unilateral cystic dysplasia | 0                       | Diabetes              | No                      | Yes           | No             | No             | No               |
| DM2846       | Whole deletion        | 25                       | –                 | Bilateral cystic dysplasia | 4                       | Diabetes, partial hypoplasia | No                   | No             | Yes           | No             | No               |
| DM2961       | Whole deletion        | 0.1                      | Oligoamnios, unilateral hypoplasia | Unilateral agenesis medullary nephrocalcinosis | 2                       | IFG                   | No                      | No             | Yes           | Yes           | No               |

DM1261a & DM1261b: Siblings Gest. diabetes, gestational diabetes; Post-tx transitory diabetes, post-transplant transitory diabetes.
the pancreas and had a transitory diabetes right after renal transplantation.

**Reproductive organs phenotype.** Genital structural anomalies were present in 35% of the patients with mutations in HNF1B; the most frequent finding was the presence of epididymal cysts in three cases and septated uterus in two cases.

**Liver phenotype.** One-third of these patients (35%) had elevated liver enzymes, with a mixed hepatocellular–cholestatic pattern in most of them (6/8). Liver enzymes were only mildly elevated in all cases. Three of these patients also had a hepatic steatosis. A solitary liver cyst was also observed in one patient (DM0729).

**Comparison between cases with and without HNF1B mutations**

In order to establish which clinical features were more specifically associated with HNF1B mutations, we compared the frequency of the following findings between both groups (Table 3).

The presence of bilateral hyperechogenic kidneys at renal US was significantly higher in the group of patients with HNF1B mutations (P = 0.04) although there was no difference in the frequency of unilateral or bilateral cystic dysplasia between both groups. Hypomagnesaemia and the presence of pancreatic structural anomalies were also significantly more frequent in the group with HNF1B mutations (P = 0.003 and P < 0.001, respectively).

We found no differences in the frequency of glucose metabolism disturbances between both groups or in the age of diabetic patients with and without mutations in HNF1B. Finally, we found no differences in the frequency of genital structural anomalies, liver enzymes’ anomalies or hyperuricaemia in both groups.

**DISCUSSION**

In this study, we analysed the prevalence of mutations in HNF1B in a cohort of 60 patients with renal structural anomalies and/or alterations of glucose metabolism, and other minor features suggestive of this syndrome. We described the molecular variants found in this gene and the clinical findings in patients carrying those mutations. Finally, we compared the clinical findings between the group of patients with and without mutations in this gene and suggested which features could be helpful to distinguish this entity from other renal cystic diseases or CAKUT. Therefore, in this study, we report a detailed phenotype description in a large series of affected patients, adding new knowledge of the systemic expression of HNF1B mutations.

A potential limitation of this study was the fact that samples were sent by Endocrinologists or Nephrologists with a clinical suspicion of HNF1B. Thus, the concern for a selection bias could rise as clinical criteria needed for sample submission were wide and depended on the clinician’s diagnosis. This could affect the generalizability of our findings to other non-selected cohorts.

We identified disease-causing mutations in 23 patients from 21 unrelated families, which represent a detection rate of 38% in this cohort. This is consistent with previous studies in which the prevalence of HNF1B mutations in patients with isolated renal structural anomalies was 21%, whereas in those with associated MODY-type diabetes it was 41% [20]. The frequency of complete heterozygous deletions (70%) in our study was higher than that previously published, which is ~50% [15]. These differences should be interpreted with caution due to the small sample size and potential variation of diagnostic methodology.

All except one of the patients in our cohort carrying an HNF1B mutation had renal structural anomalies. Most of those anomalies were bilateral cystic dysplasia or bilateral hyperechogenic kidneys, emphasizing the importance of bilateral occurrence of congenital renal defects of genetic origin. The latter finding was significantly associated with the presence of HNF1B mutations in our cohort. Moreover, patients with hyperechogenic kidneys carrying a mutation were overall younger than those with other renal anomalies. This is consistent with a previous report showing that HNF1B mutations were the most common cause of foetal bilateral hyperechogenic kidneys [13]. This US finding is due to the presence of dysplasia with microscopic cysts, as shown in previous studies where histological examination of these kidneys was performed [8, 21]. In our opinion, it is crucial to consider the variability of the echographic findings of those patients in relation to the age at diagnosis and to take into account that renal cysts usually are not evident in younger patients.

We found permanent glucose metabolism alterations in 60% of patients carrying HNF1B mutations, which is similar to 45–50% prevalence of diabetes shown in previous studies [4, 20]. In addition, there were no differences in the frequency of glucose metabolism alterations between patients with and without HNF1B mutations. This could be explained by the fact that diabetes is very frequent in the general population, especially in patients with CKD, and the only requirement for including diabetes as a major criterion in these patients was the absence of pancreatic antibodies, thus including Type 2 diabetes. However, in the group of patients with HNF1B mutations, diabetic patients
were significantly older than non-diabetic patients. This is not surprising as MODY-type diabetes in these cases usually appears in the second and third decade of life [10, 20]. Again, this variability in the clinical manifestations in relation to the age at diagnosis should be taken into account: younger patients will most probably present with renal anomalies, especially bilateral hyperechogenic kidneys, and older patients will probably suffer additionally from impaired glucose metabolism.

The frequency of pancreatic structural anomalies in the group of patients with HNF1B mutation was higher than that of previous publications [7, 10, 20], and it was significantly higher than that of patients without HNF1B mutations. This could be explained by the fact that information from pancreatic US was actively requested from doctors sending blood samples for these patients. This finding has important implications as the pancreas is an organ easily seen by US in children, even in prenatal US [22], and the presence of a structural anomaly in this organ can help guiding the molecular diagnosis towards HNF1B anomalies. Noteworthy is the case of patient SOR0042 with a severe pancreatic cystic dysplasia: although the presence of pancreatic cysts has rarely been reported before in these patients [8], this finding supports the molecular known relation between HNF1B and polycystic kidney disease genes [11, 12], as pancreatic cysts are found in almost 10% of patients with ADPKD [23].

Although there was a higher frequency of genital structural anomalies in patients with HNF1B mutations, it was not significantly different from that of the patients without mutations. There is a known association between congenital renal and genital anomalies, but as previously reported, it is not specific to HNF1B mutations [24, 25]. This association is probably due to the common embryologic development of renal and genital systems. However, as uterine anomalies are not frequent in patients with polycystic kidney disease, this feature could help guiding the molecular diagnosis in those patients with renal features compatible with both diseases (bilateral big hyperechogenic or cystic kidneys).

The frequency of hyperuricaemia and elevated liver enzymes was not significantly different between patients with and without HNF1B mutations. This is not surprising as the prevalence of these anomalies in the general population is high, especially in adult patients. In addition, hyperuricaemia is more frequent in patients with CKD, as was the case of many of our patients with renal structural anomalies. Thus, we think that the presence of hyperuricaemia and elevated liver enzymes as a marker of HNF1B mutations is probably more significant in younger patients without CKD or other associated pathologies that can be confounding.

Finally, hypomagnesaemia was present in half of the patients carrying a mutation in HNF1B, and it was significantly more frequent in this group. This feature has been reported before in 40% of patients with HNF1B mutations [6]. We think that it should be actively searched for in cases of renal structural anomalies because, although generally asymptomatic, it can help guide the molecular diagnosis in these cases. Hypomagnesaemia is thought to be of renal origin, as HNF1B regulates the transcription of FXYD2, which participates in the tubular handling of magnesium [6].

In conclusion, anomalies associated with mutations in HNF1B support the detection of affected individuals from the general pool of CAKUT and the indication for molecular analysis. Furthermore, some clinical findings in patients with HNF1B mutations can mimic those found in polycystic kidney disease, thus complicating the molecular confirmation of the diagnosis.

This is important for the genetic counselling of those patients, as the two diseases are inherited in different ways and have different prognoses. In our study, we showed that some minor findings, such as pancreatic structural anomalies or hypomagnesaemia, can help guide the molecular diagnosis. Active searching for those anomalies with radiologic studies and analyses is crucial as they are frequently asymptomatic and overlooked in everyday clinical practice. In addition, variability of the clinical findings in HNF1B mutations in relation to the age of the patient must be taken into account especially as family history will not be helpful in de novo cases.

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CONFLICT OF INTEREST STATEMENT
None declared.

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