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***HNF1B* and *PAX2* mutations are a Common Cause of Renal Hypodysplasia in the CKiD Cohort**

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Abstract

Malformations of the kidney and lower urinary tract are the most frequent cause of end stage renal disease in children. Mutations in *HNF1B* and *PAX2* commonly cause of syndromic urinary tract malformation. We searched for mutations in *HNF1B* and *PAX2* in North American children with renal aplasia and hypodysplasia (RHD) enrolled in the Chronic Kidney Disease in Children Cohort Study (CKiD). We identified 7 mutations in this multiethnic cohort (10% of patients). In *HNF1B*, we identified a nonsense (p.R181X), a missense (p.S148L), and a frameshift (Y352fsX352) mutation, and one whole gene deletion. In *PAX2*, we identified one splice site (IVS4–1G>T), one missense (p.G24E), and one frameshift (G24fsX28) mutation. All mutations occurred in Caucasians, accounting for 14% of disease in this subgroup. The absence of mutations in other ethnicities is likely due to limited sample size. There were no differences in clinical parameters (age, baseline eGFR, blood pressure, body mass index, progression) between patients with or without *HNF1B* and *PAX2* mutations. A significant proportion of North American Caucasian patients with RHD carry mutations in *HNF1B* or *PAX2* genes. These patients should be evaluated for complications (e.g. diabetes for *HNF1B* mutations, colobomas for *PAX2*) and referred for genetic counseling.

Keywords

HNF1B; *PAX2*; renal hypodysplasia; chronic kidney disease; children

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<http://uswest.ensembl.org/index.html>

<http://browser.1000genomes.org/index.html>

<http://www.r-project.org/>

Introduction

Renal hypodysplasia (RHD), encompassing the diagnosis of renal aplasia, hypoplasia, and dysplasia, is the second leading cause of chronic renal insufficiency (eCreatinine clearance $\leq 75\text{ml/min per } 1.73\text{m}^2$) in the pediatric population [1]. In the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) database, RHD was the primary diagnosis in 17.3% of the children with chronic kidney disease (CKD), 14% of children on dialysis, and 15.9% of children with renal transplants (<https://web.emmes.com/study/ped/annlrept/Annual%20Report%20-2008.pdf>).

The incidence of renal aplasia is 1 in 1300 [2]. Unilateral dysplastic kidneys occur in 1 in 1000, and bilateral dysplasia in 1 in 5000 of the general population [3]. Infants with severe bilateral kidney disease often die in the neonatal period secondary to Potter's sequence (i.e. pulmonary hypoplasia secondary to inadequate renal function and amniotic fluid during pregnancy, ref.[4]). The likelihood of developing chronic renal failure in patients with bilateral dysplasia has been correlated with a calculated GFR of $<15\text{ml/min per } 1.73\text{m}^2$ at 6 months of age; children with calculated GFR $>15\text{ml/min per } 1.73\text{m}^2$ at that age tend to show improvement in renal function at follow-up [5].

Hereditary factors are partly responsible for RHD, as evidenced by familial aggregation of disease. For example, Roodhooft et al found a 9% incidence rate of asymptomatic renal malformations in parents and siblings of patients with RHD [6]. Moreover, RHD is a feature of at least 73 syndromic disorders, such as renal cysts and diabetes syndrome (RCAD), due to *HNF1B* mutations (OMIM 137920) or renal-coloboma syndrome (RCS), due to *PAX2* mutations (OMIM 120330, ref. [7]). Although most cases of RHD are attributed to sporadic, nonsyndromic disease, studies have discovered mutations in *HNF1B* and *PAX2* in up to 19.9% of European children diagnosed with RHD [8–11]. These data suggest that compared to clinical diagnosis, genetic screening can more accurately identify these syndromes and permit counseling of patients and family members regarding specific renal and extra-renal complications, such as diabetes in *HNF1B* and eye abnormalities in *PAX2*. The prevalence of *PAX2* and *HNF1B* mutations has however not been determined in North American populations.

Methods

Subjects

CKiD is an NIH sponsored prospective observational cohort study of children with chronic kidney disease in the United States [12]. Details of the CKiD study design have been previously published. Briefly, eligible children are aged 1 to 16 years and have a Schwartz-estimated GFR between 30 and 90 mL/min/1.73 m² [13]. Exclusion criteria include: renal, other solid-organ, bone marrow, or stem cell transplantation, dialysis treatment within the past 3 months, cancer/leukemia diagnosis or HIV diagnosis/treatment within past 12 months, current pregnancy or pregnancy within past 12 months, history of structural heart disease, genetic syndromes involving the central nervous system, and history of severe to profound mental retardation. Children were enrolled at 46 participating tertiary care pediatric nephrology programs across the U.S. and 2 sites in Canada. Institutional Review Boards for each participating site approved the study protocol. Of the 586 CKiD participants, at the time of the analysis 87 children were categorized as having RHD. We examined DNA samples from the 73 RHD patients that consented for genetic studies. Data collected at the baseline visit include demographic information including age and race, the medical record recorded diagnosis causing CKD, family history of kidney and cardiovascular disease, blood chemistries, age at diagnosis with CKD, GFR via plasma disappearance of iothexol (iGFR). The CKiD consent did not include permission to contact patients again to obtain additional

clinical data. The study was approved by the CKiD Steering Committee and the Institutional Review Boards (IRB) at Montefiore Medical Center and Columbia University.

Age, eGFR, body mass index, systolic and diastolic blood pressure were compared between mutation carriers and non-carriers by two-sided ttest with equal variance, adjusted in R version 2.12.0 (<http://www.r-project.org/>). P-values less than 0.05 were considered as significant.

Mutation Screening

DNA was extracted from peripheral blood leukocytes using standard protocols. Reference sequences of *HNFI1B* and *PAX2* were downloaded from National Center for Biotechnology Information (NCBI) database (37.1 Build) and an alternatively spliced exon 9 of *PAX2* from the Ensembl genome browser. Primers were designed for the nine exons of *HNFI1B* and 12 exons of *PAX2* (including one alternate exon). Amplified PCR products were subjected to Sanger sequencing (n=73). Sequence analysis was performed using Sequencer 4.8 software. All putative variants were confirmed by bidirectional sequencing.

On mutational screening of *HNFI1B* and *PAX2*, we found a total SNP rate per base pair (bp) of 1 SNP in every 284 bases surveyed (1 SNP/376 bp in coding regions, and 1 SNP/247 bp in introns), which is comparable to the average SNP distribution rate in the human genome.

Evaluation of rare variants

We evaluated all variants for potential pathogenicity using four methodologies. First, we consulted public databases (dbSNP, 1000 genomes) to determine if the variants were previously detected in reference populations. Coding variants that were not present in public databases were further cross-referenced with prior publications and mutation databases, such as Human Gene Mutation Database (HGMD, ref. [14]). Novel missense variants were evaluated for conservation among species using TCOFFEE [15] and for pathogenic potential using standard prediction programs (i.e Polyphen [16], SIFT [17], PhD-SNP [18]). Novel synonymous and noncoding variants were evaluated for conservation among other mammalian species (bl2seq feature); novel synonymous variants were also evaluated for potential aberrant splicing (Human Splice Finder and ESE Finder) [19–21]. Finally, the frequencies of selected new variants were determined in healthy controls (195 Italian Caucasians, 100 North American Caucasians or 74 African Americans) by Restriction Fragment Length Polymorphism (RFLP) or direct sequencing.

Screening for genomic rearrangements in *HNFI1B*

We used Multiplex Ligation-Dependent Probe Amplification (MLPA) assay to look for structural variants in *HNFI1B* [22]; we used the SALSA® MLPA kit P241-B1 MODY (MRC-Holland, Amsterdam, The Netherlands) designed to evaluate genes implicated in Maturity Onset Diabetes of the Young (MODY). Mutations in *HNFI1B* have been implicated in MODY-5, and as such primer pairs for the exons of *HNFI1B* were present in this kit. We also used a second kit (SALSA® MLPA kit P297-B1 Microdeletion-2), with 7 probes on chromosome 17q12 to verify findings. Amplified samples were fractionated on a capillary sequencer (ABI Prism 3130X Genetic Analyzer, Applied Biosystems). MLPA data was normalized to a normal diploid control; a deletion and a duplication in *HNFI1B*, previously characterized in the lab were incorporated into each run as positive controls. Finally, in the patient found to have a whole gene deletion in *HNFI1B*, we verified the 5' and 3' breakpoints of the 17q12 microdeletion region by Quantitative PCR (QPCR). Primers were designed for the left and right flanks of the 1.4Mb microdeletion region, encompassing *HNFI1B* among 19 other genes [23].

Results

We studied 73 CKiD patients with RHD, of whom 22 had family history of kidney disease (Table 1), and discovered pathogenic *HNF1B* and *PAX2* mutations in seven individuals (10% of the cohort, Table 2)

HNF1B Mutations

We detected one novel frameshift mutation in exon 5 where an insertion of an A shifts the reading frame from a tyrosine to a termination signal (c.1054_1055insA, Y352fsX352, Figure 1A). In a second patient, we found a missense mutation in exon 2 where a C>T transition results in a nonconservative amino acid change of a serine to a leucine (c.444C>T, pS148L). Not only is this mutation predicted to be pathogenic by multiple publicly available prediction programs (SIFT, Polyphen and PhD-SNP, Supplementary Table 1) but it has also been previously reported [16–18, 24]. A third patient had a nonsense mutation in exon 2 where a C>T transition results in the change of an arginine to a stop codon (c.543 C>T, pR181X). This mutation has also been previously reported [25]. Finally, one patient harbored ~1.4Mb deletion at the chromosome 17q12 locus, which includes the whole *HNF1B* gene detected by MLPA [22] (Supplementary Figure 1A). This result was confirmed using QPCR of the flanking regions of microdeletion on 17q12 (Supplementary Figure 1B, ref. [23]).

PAX2 Mutations

We discovered one patient with a novel missense mutation in exon 2 where a G>A transition results in a nonconservative amino acid change from glycine to a glutamic acid (c.71G>A, G24E, Figure 1B). This variant, which is located in a highly conserved region encoding the DNA binding domain of *PAX2*, is predicted to be pathogenic by SIFT, Polyphen and PhD-SNP (Supplementary Table 1, and Supplementary Figure 2A. Moreover, this sequence variant was not found in 350 Caucasian control chromosomes.

We also detected a novel splice site variant at the canonical acceptor splice site of exon 5 where a G>T transversion in the intron 4 at position -1 from exon 5 (IVS4–1G>T) is predicted to result in aberrant splicing (Figure 1C, ref. [26].

Finally, we identified a frameshift mutation in a polyguanine tract where an insertion of a guanine results in a shift in the reading frame to a termination signal (c.69_70insG, G24fsX28). This seven base pair polyguanine tract, in the DNA binding region of *PAX2*, has been reported previously to be highly susceptible to mutations due to contractions or expansions, likely due to slippage during DNA replication [14, 23, 27–31].

Rare HNF1B and PAX2 variants of unknown significance

We identified 22 single nucleotide polymorphisms (SNPs). Of these, 12 have been previously annotated (10 noncoding and 2 synonymous coding, Supplementary Table 2) and ten were novel (9 noncoding and 1 synonymous coding, Supplementary Table 3). We determined the frequency of two rare *PAX2* SNPs (IVS1–48G>C and c.889G>C, Leu>Leu) in healthy controls because both produced substitutions at nucleotides that were highly conserved in *Pan troglodytes*, *Canis lupus familiaris*, *Mus musculus*, *Rattus norvegicus*, and *Gallus gallus* (Supplementary Figure 2B and 2C.) Both SNPs were confirmed to be absent or extremely rare in the general population (frequency ≤ 0.003).

Clinical correlations

Of interest, all seven patients with pathogenic mutations were Caucasian (7 of 52), resulting in a 14% mutation rate in this subset. (Fisher exact p-value=0.08, for differences in mutation

prevalence between Caucasians and non-Caucasians). We found no significant differences between the RHD patients with mutations compared to those without mutations, in relation to age, eGFR, systolic or diastolic blood pressure, BMI or progression of these factors at one year (Supplemental Table 4).

Discussion

In this study, we identified pathogenic *HNF1B* or *PAX2* mutations in 14% of Caucasian individuals in a North American cohort of children with RHD. The mutation prevalence is consistent with previous studies of European and Japanese children with RHD [8–10, 27, 32]. Interestingly, we did not identify any mutations among non-Caucasian children. These differences may be due to variation in sampling and ascertainment methods, but may also reflect true differences in the prevalence of *HNF1B* and *PAX2* mutations among different populations.

Horiwaka et al first discovered *HNF1B* mutations in patients with Maturity Onset Diabetes of the Young (MODY5), an autosomal dominant form of diabetes mellitus frequently associated with renal cysts [33–36]. *HNF1B* is expressed in the kidney, pancreas, liver, gonads, gut, lung and thymus. *HNF1B* mutations produce diabetes at a mean age 17 – 25.8 yrs (30– 66%), genital malformations (12–62.5%), RHD, pancreas atrophy, hyperuricemia and abnormal liver function tests [11, 24, 25, 37, 38]. In women, genital malformations include bicornuate uterus, vaginal aplasia, or absent uterus [34, 39]. In men, asthenospermia, bilateral epididymal cysts, and atresia of the vas deferens have been reported [25].

HNF1B is a critical regulator of a genetic cascade that is essential to controlling the proliferation and differentiation of renal tubular epithelial cells. It also controls the expression of the *PKHD1* gene (the gene mutated in recessive polycystic kidney disease), accounting for the cystic renal phenotype in mutation carriers [40, 41]. The 1.4Mb region of chromosome 17 containing *HNF1B* is highly susceptible to copy number variation as it is flanked by areas of segmental duplications, which are sites for recurrent rearrangements [23, 42]. Accordingly, in the one patient in this study who harbored a heterozygous whole gene deletion in *HNF1B*, we found that the entire 1.4Mb critical region was deleted [23].

PAX2, a member of the “paired box” transcription factor gene family, is one of the earliest genes expressed during fetal kidney development, and is mutated in renal-coloboma syndrome (OMIM 120330, ref. [43, 44]). *PAX2* is expressed in the optic and otic vesicles, the mesonephros (which later give rise to the male and female genital tracts), kidney and parts of the central nervous system [45]. *PAX2* mutations lead to multiorgan defects including RHD (68%), ocular abnormalities in nearly 100% of children, high frequency hearing loss (16%) which can be subtle and often missed, and associated vesicoureteral reflux (26%) [30] [27]. The typical ocular association with renal-coloboma syndrome is bilateral optic nerve coloboma; however, ocular manifestations have also included optic nerve or disc dysplasia, retinal coloboma, microphthalmia, morning glory anomaly, optic nerve cysts, scleral staphyloma, myopia, nystagmus and cataracts [46, 47]. Visual acuity is variable, ranging from near normal to severely impaired, with a reduction in vision acuity of one or both eyes in 75% of affected individuals [46, 47].

There are several important clinical implications from our findings. Although *HNF1B* and *PAX2* mutations classically affect multiple organs, many organ defects may be subtle or subclinical, complicating diagnosis by standard clinical methods. As illustrated in this study, the patients with *PAX2* or *HNF1B* mutations were not readily distinguishable from the patients with no mutations. Mutation screening can therefore provide the correct diagnosis, and also motivate surveillance for extra-renal manifestations and potential future

complications. Detailed information about extrarenal manifestations is now being collected in the follow-up phase of the CKiD study to pursue these findings. Our data, in combination with prior studies, provide a strong rationale for mutation screening of all children with RHD. Clinical genetic testing is available for *PAX2* or *HNF1B* (information available at Genetics Home Reference, <http://ghr.nlm.nih.gov/>).

Mutation identification may aid in genetic counseling. Previous reports have indicated that as many as half of the mutations in *HNF1B* and *PAX2* occur *de novo* [8, 9, 11]. Knowing whether the mutation was inherited or occurred *de novo* would therefore have consequences for screening siblings as well as advising parents who would like to conceive further [48]. In this cohort, only one child reported a family history of RHD (in a cousin), but we did not find any pathogenic mutations in this patient. However, it is noteworthy that there was a history of miscarriage in the mothers of two patients with *HNF1B* mutations, suggesting that these mothers may have uterine abnormalities and be mutation carriers [39].

The majority of children (particularly non-Caucasians) in the CKiD cohort did not have mutations in *HNF1B* and *PAX2*; mutations in other genes such as *SALL1*, *SIX1*, *EYA1* are also exceeding rare in all reported studies [8–10, 27, 32]. These data suggest that there are other, yet undiscovered genes that may cause RHD. In the past few years, the introduction of high-density oligonucleotide arrays and Next-gen sequencing methods has enabled detection of rare mutations associated with human disease, leading to the identification of new clinical entities. These methods have been particularly successful in studies of developmental disorders. For example, studying patients with multiple complex malformations, Unger et al found a mutation in the cyclin family member *FAM58A* to be the cause of an X-linked dominant disorder characterized by syndactyly, telecanthus and anogenital and renal malformations (“STAR syndrome”[49]. Similarly, exome sequencing has recently identified mutations in *MLL2* as a cause of Kabuki syndrome, a multiorgan developmental disorder [50]. Our findings thus support application of these novel methodologies to detect novel genes producing RHD in the CKiD cohort.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Chromatograms of novel pathogenic mutations. The corresponding amino-acid sequences are indicated above each tracing. Arrows indicate the mutation.

A. Frameshift mutation: *HNF1B* c.1054_1055insA

B. Missense mutation: *PAX2* c.71G>A

C. Splice site mutation: *PAX2* (IVS4-1G>T)

For= Sequencing in the forward direction. Rev= Sequencing in the reverse direction. WT= wild type.

Table 1

Demographics of CKiD Cohort with diagnosis of aplasia, hypoplasia and/or dysplasia, with DNA samples stored (n=73).

Characteristic	Value
Age at entry into study (Yr, Range)	9.3 (1.1–17.3)
Male (%)	54.8
Race (%)	
Caucasian	71.2
African American	8.2
American Indian	2.7
Asian	1.4
Other	1.4
>1 race excluding AA	5.5
>1 race including AA	9.6
Estimated GFR (ml/min/1.73m ² , Range)*	41.4 (18.7–131.7)
Systolic BP% (mmHg, Range) [#]	59.2 (4.0–100.0)
Diastolic BP% (mmHg, Range) [#]	61.4 (4.3–100.0)
Body mass index (kg/m ² , Range)	18.1 (11.8–28.1)
Family history of kidney disease (%)	30.1
of dialysis (Dx) (%)	12.3
of transplant (Tx) (%)	5.5

* eGFR based on new estimating formula derived by CKiD using Serum Cr, BUN and Cystatin C measured at first visit.

[#] Blood pressure as percentile based on age, gender and height.

Table 2

Mutations discovered in CKiD patients

Gene	Exon	Base Change	Mutation	AA Change	Sex	Race	Age (y)	eGFR	FHx	Miscar
<i>HNF1B</i>	2	c.444C>T	Missense	S148L	M	Cauc	6.7	35	-	+
<i>HNF1B</i>	2	c.543C>T	Nonsense	R181X	F	Cauc	4.2	50	-	-
<i>HNF1B</i>	5	c.1054_1055insA	Frameshift	Y352fsX352	F	Cauc	4.8	51.1	+M	+
<i>HNF1B</i>	ALL	Chrom 17q12	Whole gene deletion	NA	F	Cauc	15.4	36.6	-	-
<i>PAX2</i>	2	c.71G>A	Missense	G24E	F	Cauc	15.4	38	+GP	-
<i>PAX2</i>	2	c.69_70insG	Frameshift	G24fsX28	M	Cauc	7.7	29.6	-	-
<i>PAX2</i>	5	IVS4-1G>T	Splice site	NA	M	Cauc	13.7	42.5	+Cs	+

Cauc= Caucasian. FHx= Family history (M=mother, GP= grandparent, Cs=cousin; kidney disease unknown in 3.) Miscar = Miscarriage in mother.