

## Detection of mutations of the HNF1B gene in children with congenital anomalies of the kidney and urinary tract

### Detección de mutaciones del gen de HNF1B en niños con malformaciones congénitas renales y del tracto urinario

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#### Abstract

**Introduction:** Congenital anomalies of the kidney and urinary tract are caused by genetic alterations mostly unknown. Mutations in the gene that codes for hepatocyte nuclear factor 1B (HNF1B) are the most frequently described monogenic causes. Data are unknown in Chile and Latin America. **Objective:** To determine the presence of variants of the HNF1B gene in Chilean children with congenital anomalies of the kidney and/or the urinary tract and their clinical characteristics. **Patients and Method:** Descriptive study with children aged 10 months to 17 years, patients of the Calvo Mackenna Hospital Nephrology Unit, with cystic renal dysplasia, non cystic renal dysplasia/hypoplasia, horseshoe kidney between April and December 2016. HNF1B variants were determined by sequencing of exons 1, 2, 3 and 4 after DNA extraction and amplification. Restriction enzymes were used to define if the variants were homo or heterozygous. Direct family members of index cases were studied with sequencing of the affected exon. **Results:** 32 patients were included, 43.75% males, median age 11 years. 65.6% of them had non-cystic renal dysplasia, 31.25% cystic renal dysplasia, and 3.15% horseshoe kidney. In two patients (6.25%) the same heterozygous genetic variant was detected in exon 4, position 1027 (C1027T), not previously described. The study of relatives found the same variant in three out of five individuals, all without congenital nephro-urological anomalies. **Conclusions:** We confirmed the presence of a not previously described heterozygous genetic variant of the HNF1B gene. This work initiates the search for this type of mutations in our region which allows us to approach the knowledge of causality, determination of extrarenal involvement, and genetic counseling.

#### Keywords:

Urinary tract;  
CAKUT;  
mutations;  
Congenital anomalies;  
Kidney;  
renal dysplasia;  
renal cysts

## Introduction

Congenital anomalies of the kidney and urinary tract (*CAKUT*) have an incidence of 1 in 500 live newborns and are currently the most common cause of chronic kidney disease (*CKD*) in childhood. Mutations in the *TCF2* gene, which codes for hepatocyte nuclear factor 1B (*HNF1B*), are the most common monogenic cause of impaired renal development in *CAKUT* patients<sup>1</sup>.

The transcription factor *HNF1B* regulates the expression of genes involved in the early organs development such as the kidney, pancreas, liver, intestine, and lung<sup>2</sup>. It was originally identified as one of those responsible for type *MODY5* diabetes (maturity-onset diabetes of the young), a monogenic disease that is transmitted in an autosomal dominant manner. The observation of the high frequency of renal malformations in these patients made it possible to determine their association with *CAKUT*<sup>1,2</sup>.

The *TCF2* gene is located in the q12 region of chromosome 17 and contains nine exons. To date, more than 50 different mutations have been reported, such as missense, nonsense, frame-shift, and splicing. A complete deletion of the gene has been detected in a significant percentage of patients. Out of the reported mutations, most are in exons 1, 2, 3, and 4. Due to the high number of described mutations, the study of the association between this gene and renal pathology is carried out mainly through sequencing techniques<sup>1</sup>.

Renal malformations caused by deletion and/or mutations of the *TCF2* gene are very heterogeneous, with uni- or bilateral cystic renal dysplasia as the most frequent finding. Renal aplasia or hypoplasia, and horseshoe kidney<sup>1-3</sup> have also been found.

Extrarenal involvement may include elevated liver enzymes, endocrine, and exocrine pancreatic involvement, genital tract malformations causing infertility, hypomagnesemia, hyperuricemia, and early-onset gout. From a clinical point of view, the presence of extrarenal involvement does not contribute significantly to early diagnosis since it is usually expressed later in life<sup>1,2,4</sup>.

Studies on the prevalence of mutations in the *TCF2* gene in *CAKUT* carrier patients have been carried out in Europe, the United States, and Japan<sup>5-7</sup>, however, data are unknown in Latin America. The objective of this work is to determine the presence of variants of the gene coding for *HNF1B* (*TCF2*) in Chilean children with congenital anomalies of the kidney and/or urinary tract and their phenotypic characteristics.

## Patients and Methods

### Patients

Prospective, observational, descriptive study with patients of the Luis Calvo Mackenna Hospital Nephro-

logy Unit for nine months, between April and December 2016. The presence of at least one of the following phenotypes was considered as inclusion criteria: uni- or bilateral cystic renal dysplasia, uni- or bilateral renal dysplasia or hypoplasia, and horseshoe kidney. The ultrasound evaluation was performed by pediatric radiologists at the Luis Calvo Mackenna Hospital. From the ultrasound point of view, renal dysplasia was defined as the finding of alteration of the corticomedullary differentiation and/or diffuse hyperechogenicity of the renal parenchyma; hypoplasia due to a renal length less than 2 SD for age<sup>8</sup>. Patients with obstructive uropathies and/or moderate to severe vesicoureteral reflux (grade III to V) that could generate secondary renal damage were excluded, and those with clinical and/or molecular evidence of other genetic anomalies that explained the malformations they presented in the nephroureteral tract, such as, for example, autosomal dominant or recessive polycystic kidney disease.

Molecular and ultrasound studies were carried out on the first-degree relatives of the index cases.

### Detection of *TCF2* gene variants

Genomic DNA was obtained from peripheral blood using the MagNa Pure Compact kit (Roche) according to the manufacturer's protocol. The first four exons of the *TCF2* gene were amplified through Polymerase Chain Reaction (PCR), specific primers were used for exons 1, 2, 3, and 4 (Promega) previously described in the literature<sup>9</sup>. The purity of the obtained amplicon was evaluated using a 2% agarose gel. The PCR product was sequenced through the Sanger technique, using the services of Macrogen (Korea) with 100% sequencing coverage. Bioinformatic analysis of the sequences was performed with Sequencher 5.4.5 (Gene Codes Corp.). As a reference, the NM\_00458.2 sequence of the *TCF2* gene was used.

To determine if the found variant affected one or both alleles (homo or heterozygous), the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique was used. Exon 4, which presented the variant under study, was amplified by PCR, and the amplified product was digested with the *EarI* enzyme (Promega), capable of recognizing the DNA segment that incorporates the nucleotide variant position. The enzymatic digestion products were analyzed through Electrophoresis in polyacrylamide gels.

### Biochemical Study

All patients included in the study were evaluated for plasma biochemical parameters, including: *Creatinine* (mg/dl), *urea nitrogen* (mg/dl), *blood glucose* (mg/dl), *GPT-GOT transaminases* (UI/L), *uric acid* (mg/dl), and *magnesium* (mEq/L). The samples were processed in the Luis Calvo Mackenna Hospital Central Laboratory. The

calculation of the glomerular filtration rate was performed using the Schwartz Formula<sup>10</sup>.

A molecular and renal ultrasound study was carried out on the first-degree relatives of the index cases.

### Ethical Aspects

The study was approved by the Human Research Ethics Committee of the School of Medicine, University of Chile, and the Luis Calvo Mackenna Hospital Management. Informed Consent was obtained from the responsible adult in all cases and Assent in patients over 12 years of age.

### Results

32 patients were included, 18 female (56.25%), the median age was 11 years, ranging from ten months to 17 years. 21 out of the 32 had non-cystic renal dysplasia (65.6%); from this group, 76.2% had bilateral involvement. Ten patients (31.25% of the sample) presented cystic dysplasia, bilateral in three of them. One patient was admitted to the study with a horseshoe kidney diagnosis (Figure 1).

Regarding renal function, 12 patients had CKD in stage 1, six patients in stage 2, and 14 patients in stage 5. Out of the latter group, two patients were on peritoneal dialysis and 12 patients were transplanted (Figure 2).

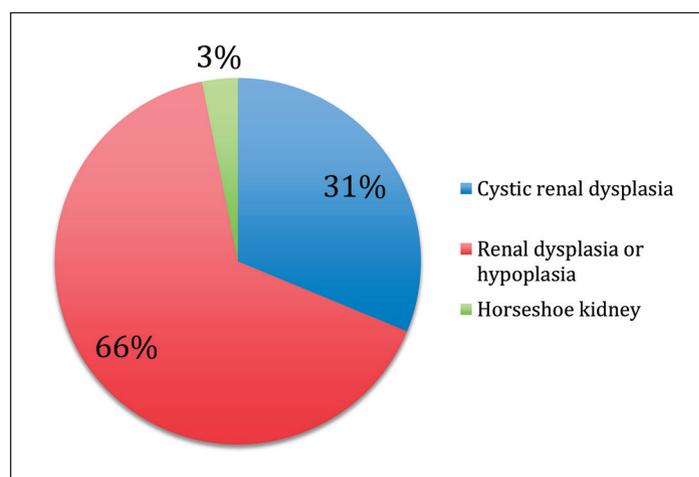
The blood glucose, transaminases, magnesium, and uric acid levels of the 32 patients were within normal ranges.

The same heterozygous variant (Figure 3) was found in two of the 32 studied patients, corresponding to 6.25% of the sample. This variant is located in exon 4, at position 1027, determines a change in the nitrogenous base (cytosine-to-thymine - C1027T), which results in a change in the amino acid encoded at position 343 of proline-to-serine.

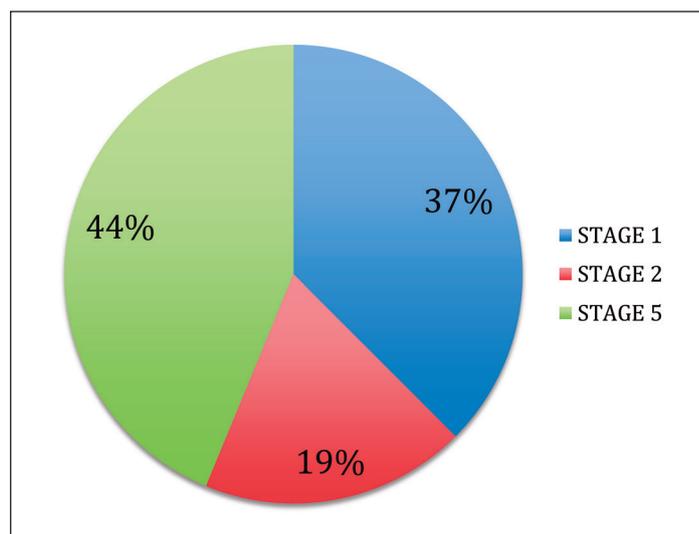
One of the patients affected by this variant is a 17-year-old adolescent with left cystic renal dysplasia and normal renal function. The other index case is an 8-year-old male patient with bilateral non-cystic renal dysplasia who is currently transplanted (Table 1). These patients have no known blood relationship.

The presence of this variant was studied in the fa-

mily of one of the index cases since the other patient has adoptive parents and the history of his/her biological parents is unknown. The same heterozygous variant (C1027T) was found in three out of the five studied



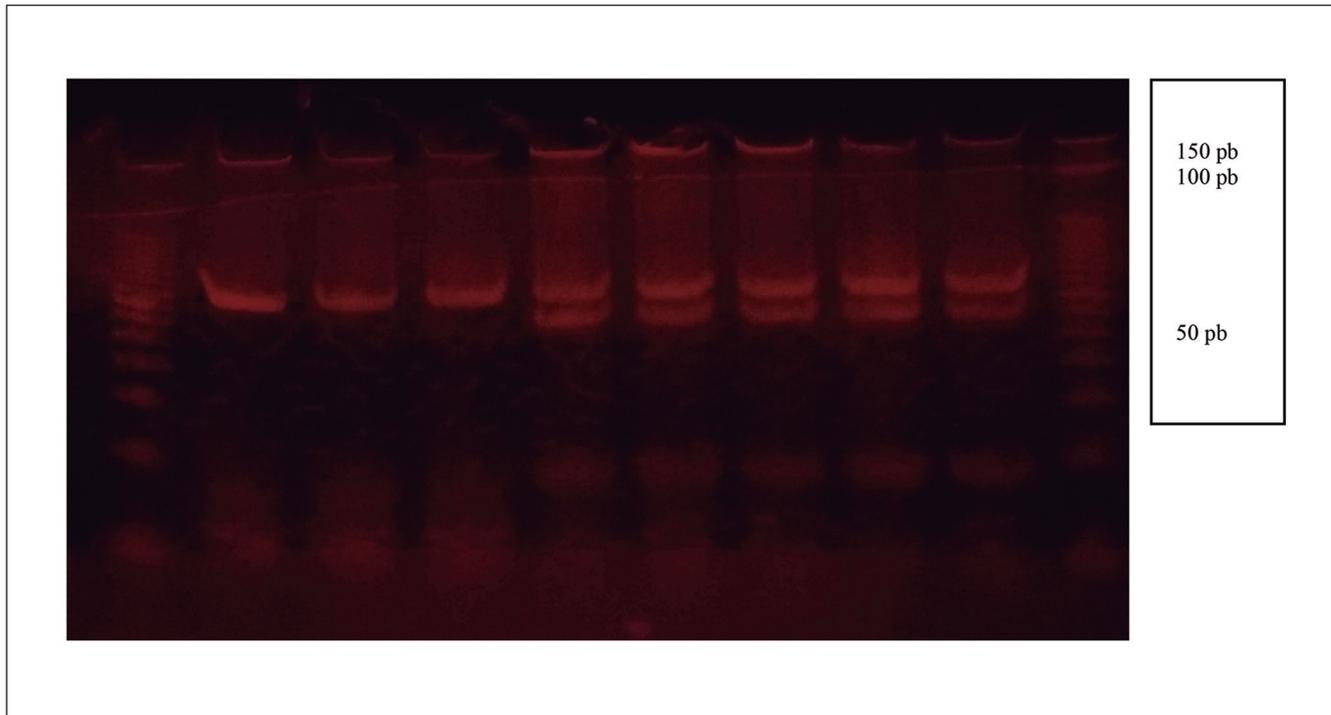
**Figure 1.** Characterization of the sample according to type of Nephrourological Malformation



**Figure 2.** Characterization of the sample according to the Chronic Kidney Disease Stage.

**Table 1. Características generales de los casos índices**

ID paciente	Edad (a)	Sexo	CAKUT	Etapa ERC	Exon	Mutación
10	17	F	Displasia quística izquierda	1	4	C1027T
24	8	M	Displasia no quística bilateral	5	4	C1027T



**Figure 3.** Polyacrylamide gel electrophoresis showing products of the exon 4 digestion with restriction enzyme. Exon 4, previously amplified through PCR, is digested using Earl restriction endonuclease, which recognizes the DNA segment that incorporates the variant. Eight samples were analyzed, the first 3 on the left are from control patients and the 5 on the right correspond to the 2 index cases and their 3 relatives variant carriers. It can be observed that in the controls the enzyme does not fragment the DNA, and in the carriers of the variant one of the alleles is fragmented and the other remains intact, which allows us to show that it is a heterozygous variant. The fragmentation by the enzyme originate smaller pieces of DNA that migrate in the gel with greater speed and can be observed.

family members (one of the parents and two of his/her three siblings), which shows that it is not a *de novo* variant (Figure 3). These studied relatives are asymptomatic, with normal renal and urinary tract ultrasound study.

## Discussion

This is the first time that a study has been carried out in Chile, which tries to find an association between CAKUT and variants of the gene that encodes for HNF1B.

We analyzed 32 non-consanguineous patients with CAKUT phenotype and identified two of them with the same heterozygous variant in exon 4 of the gene coding for *HNF1B*. This variant is not previously described in the literature, therefore its pathogenic role is unknown, although finding an identical alteration in both patients could suggest it. In order to determine its pathogenicity, an *in silico* analysis (computer simulation) was used with three available software: Polyphen-2, SIFT, and Mutation Taster. The first two predict a benign and tolerable effect; the third indicates that this variant could be the cause of illness.

The first index case is a 17-year-old adolescent with history of physiological pregnancy, without prenatal ultrasound abnormalities, born by vaginal delivery at 38 weeks, birth weight 2,850 g. She was admitted in the Polyclinic of Nephrology in February 2012, at 13 years of age, due to the ultrasound finding of a left kidney with signs of cystic dysplasia during a study of images due to low-back pain. A DMSA renal scintigraphy showed left kidney scintigraphic exclusion. Within her examination, it highlights blood creatinine 0.68 mg/dl and normal urine test. The patient evolves asymptomatic and normotensive. In April 2015, she is referred to adult medicine for further follow-up. The second index case, a 10-year-old male, has history of irregularly controlled pregnancy, with no known maternal pathology or abnormal ultrasound findings. Born by vaginal delivery at 35 weeks, birth weight 2,020 g. He was admitted to the Polyclinic of Nephrology in November 2010 referred from another pediatric center, with diagnoses of bilateral renal dysplasia and end-stage chronic renal disease in peritoneal dialysis, to start a pre-transplant study. In his history, the situation of social vulnerability due to family abandonment stands out. He receives a kidney transplant from a donor who died in February 2014, at the age of five. The patient is currently five

years post-transplant, under adoptive family care, with an estimated 70 ml/min/1,73m<sup>2</sup> filtration rate.

The literature describes cases of patients with inherited or de novo variants in *HNF1B* in about 50% of cases, respectively<sup>1</sup>. We studied the family of one of the index cases, finding that three family members carried the same heterozygous variant of exon 4. The very different phenotypes of the two index patients and the absence of renal malformations in the relatives carrying the variant reaffirms the lack of genotype-phenotype correlation, which is already described in the literature and suggests an inheritance with incomplete penetrance<sup>6</sup>. Therefore, the pathogenic role of this genetic variant is uncertain. An indirect way of approaching this could be to determine its relative absence in a significant sample of healthy population, which could be done in a cost-effective way through PCR-RFLP.

The variants frequency of the gene coding for *HNF1B* in our study was 6.25%, which is consistent with results described in other series, ranging from 5 to 31%<sup>1</sup>. In 2006, Ulinski<sup>11</sup> published a descriptive study of 80 French patients carrying *CAKUT*, showing *TCF2* alterations in 31% of them, either mutation or complete deletion of the gene. Thomas<sup>12</sup>, in 2011 published a US series of patients carrying renal aplasia and/or hypoplasia, finding 5% of patients with alterations of the gene coding for *HNF1B*. Different publications have shown that the variants frequency of this gene is higher in patients with *CAKUT* who present uni- or bilateral renal cysts, reaching 50% of the studied children in this group<sup>11,13</sup>.

No alterations were observed in uric acid or plasma magnesium levels, nor transaminases or blood glucose elevation. This can be explained because much of the extrarenal manifestations of the *HNF1B* variants manifest late in life.

We cannot fail to mention the limitations of our study. In the first place, due to cost reasons, only the variants of exons 1, 2, 3 and 4 were analyzed, with a total of nine. In addition, no search was made for gene deletions, which would have required other technique types. This explains the relatively low frequency of variants in the *TCF2* gene found in our series. On the other hand, the fact that none of our patients have presented abnormal basal blood glucose does not allow us to rule out diabetes or prediabetic states since there was no glucose tolerance test and/or blood insulin measurement. In this study, no imaging tests were performed to rule out anatomical abnormalities of the pancreas or internal genitalia, which may be extrarenal manifestations of *HNF1B* variants. Finally, we cannot rule out in our patients abnormalities in other involved genes in renal development, which have also been associated with *CAKUT*: *Ret*, *GDNF*, *Pax2*, *Six2*, *UMOD*, *BMP4*, among others<sup>14</sup>.

## Conclusions

This work is the first study carried out in Chile that looks for gene variants coding for *HNF1B*, whose alterations are recognized as the most frequent monogenic cause of *CAKUT*, and has allowed us to recognize a new variant whose pathogenic value we do not know.

The genotypic characterization of this group of patients is far from being a reality in clinical practice, however, in selected groups could be very useful for genetic counseling, prevention, and search for extrarenal manifestations.

Scientific research that allows us to know the genotypic characteristics of patients with nephrourological malformations provides a basis for a thorough understanding of the origin of these diseases and is the starting point for future studies.

## Ethical Responsibilities

**Human Beings and animals protection:** Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

**Data confidentiality:** The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

**Rights to privacy and informed consent:** The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

## Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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## References

1. Clissold RL, Hamilton AJ, Hattersley AT, Ellard S, Bingham C. HNF1B-associated renal and extra-renal disease—an expanding clinical spectrum. *Nat Rev Nephrol* 2015; 11:102-12.
2. Bockenbauer D, Jaureguiberry G. HNF-1B associated clinical phenotypes: the kidney and beyond. *Pediatr Nephrol* 2015; 8 [Epub ahead of print].
3. Igarashi P, Shao X, McNally BT, Hiesberger T. Roles of HNF-1b in kidney development and congenital cystic diseases. *Kidney Int* 2005;68: 1944-7.
4. Adalat S, Woolf AS, Johnstone KA, et al. HNF1B Mutations Associate with Hypomagnesemia and Renal Magnesium Wasting. *J Am Soc Nephrol* 2009;20:1123-31.
5. Nakayama M, Nozu K, Goto Y, et al. HNF1B alterations associated with congenital anomalies of the kidney and urinary tract. *Pediatr Nephrol* 2010;25:1073-9.
6. Heidet L, Decramer S, Pawtowski A, et al. Spectrum of HNF1B Mutations in a Large Cohort of Patients Who Harbor Renal Diseases. *Clin J Am Soc Nephrol* 2010;5:1079-90.
7. Edghill EL, Bingham C, Ellard S, Hattersley AT. Mutations in hepatocyte nuclear factor-1b and their related phenotypes. *J Med Genet* 2006;43:84-90.
8. Han BK, Babcock DS. Sonographic measurements and appearance of normal kidneys in children. *AJR Am J Roentgenol.* 1985;145(3):611-6.
9. Beards F, Frayling T, Bulman M, et al. Mutations in hepatocyte nuclear factor 1 beta are not a common cause of Maturity-onset Diabetes of the young in the UK. *Diabetes* 1998; 47:7.
10. Schwartz GJ, Muñoz A, Schneider MF, et al. New Equations to Estimate GFR in Children with CKD. *J Am Soc Nephrol.* 2009;20(3): 629-37.
11. Ulinski T, Lescure S, Beaufile S, et al. Renal Phenotypes Related to Hepatocyte Nuclear Factor-1β (TCF2) Mutations in a Pediatric Cohort. *J Am Soc Nephrol* 2006;17: 497-503.
12. Thomas R, Sanna-Cherchi S, Warady BA, Furth SL, Kaskel FJ, Gharavi AG. HNF1B and PAX2 mutations are a Common Cause of Renal Hypodysplasia in the CKiD Cohort. *Pediatr Nephrol* 2011; 26(6):897-903.
13. Weber S, Moriniere V, Knüppel T, et al. Prevalence of Mutations in Renal Developmental Genes in Children with Renal Hypodysplasia: Results of the ESCAPE Study. *J Am Soc Nephrol* 2006; 17:2864-70.
14. Song R, Yosypiv IV. Genetics of congenital anomalies of the kidney and urinary tract. *Pediatr Nephrol* 2011; 26: 353-64.