Prevalence of Mutations in Renal Developmental Genes in Children with Renal Hypodysplasia: Results of the ESCAPE Study


*Division of Pediatric Nephrology, Hospital for Pediatric and Adolescent Medicine, Ruperto-Carola University, Heidelberg, Germany; †Inserm U1574 and 2Pediatric Nephrology, Hôpital Necker-Enfants Malades, Université René Descartes, Paris, France; 3Motol Children’s Hospital Prague, Czech Republic; 4Istituto Gaslini, Genoa, Italy; 5University Children’s Hospital, Vilnius, Lithuania; 6University Children’s Hospital, Izmir, Turkey; †Division of Pediatric Nephrology, Children’s Hospital, University of Padova, Padova, Italy; ††University Children’s Hospital Belgrade, Serbia and Herzegovina; and §§University Children’s Hospital, Gdansk, Poland

Renal hypodysplasia (RHD) is characterized by a reduced nephron number, small kidney size, and disorganized renal tissue. A hereditary basis has been established for a subset of affected patients, suggesting a major role of developmental genes that are involved in early kidney organogenesis. Gene mutations that have dominant inheritance and cause RHD, urinary tract anomalies, and defined extrarenal symptoms have been identified in TCF2 (renal cysts and diabetes syndrome), PAX2 (renal-coloboma syndrome), EYA1 and SIX1 (branchio-oto-renal syndrome), and SALL1 (Townes-Brocks syndrome). For estimation of the prevalence of these events, an unselected cohort of 99 unrelated patients with RHD that was associated with chronic renal insufficiency were screened for mutations in TCF2, PAX2, EYA1, SIX1, and SALL1. Mutations or variants in the genes of interest were detected in 17 (17%) unrelated families: One mutation, two variants, and four deletions of TCF2 in eight unrelated patients; four different PAX2 mutations in six families; one EYA1 mutation and one deletion in two patients with branchio-oto-renal syndrome; and one SALL1 mutation in a patient with isolated RHD. Of a total of 27 patients with renal cysts, six (22%) carried a mutation in TCF2. It is interesting that a SIX1 sequence variant was identified in two siblings with renal-coloboma syndrome as a result of a PAX2 mutation, suggesting an oligogenic inheritance. Careful clinical reevaluation that focused on discrete extrarenal symptoms and thorough family analysis revealed syndrome-specific features in nine of the 17 patients. In conclusion, 15% of patients with RHD show mutations in TCF2 or PAX2, whereas abnormalities in EYA1, SALL1, and SIX1 are less frequent.


Renal hypodysplasia (RHD) is a common congenital anomaly that is characterized by a reduction in nephron number, a small overall kidney size, and/or disturbed organization of the renal tissue with lack of corticomedullary differentiation (1). One in 200 neonates presents with anomalies of the kidneys and/or the urinary tract on renal ultrasound, and RHD is one of the prominent anomalies observed (2). Although RHD is the underlying cause in more than one third of children with chronic kidney disease (3,4), its molecular pathogenesis is only beginning to be unraveled (5). Familial aggregation of renal malformations in a subset of patients suggests that genetic events might be involved. Indeed, mutations in renal developmental genes have been demonstrated in patients with syndromal RHD that follows Mendelian patterns of inheritance, such as TCF2 mutations in autosomal dominant (AD) renal cysts and diabetes syndrome (RCAD) associated with maturity-onset diabetes of the young type 5 (6–8), PAX2 mutations in AD renal-coloboma syndrome (RCS) (9), EYA1 and SIX1 mutations in AD branchio-oto-renal (BOR) syndrome (10,11), and mutations in SALL1 in patients with AD Townes-Brocks syndrome (TBS) (12). Recently, TCF2 mutations were identified in 25 of 80 children with renal hypodysplasia typically with cortical microcyts (13). The genes that underlie these syndromal forms of RHD encode transcription factors or co-factors that regulate critical steps in early renal development: The budding of the ureter toward the metanephric mesenchyme, the induction and con-
denervation of the mesenchyme, and the further growth and branching of the ureteric tree (14–20). Disturbances of these reciprocal interactions lead to a profound impairment of early ureterorenal development, resulting in RHD (19,21). Besides their functions in kidney development, these transcriptional factors have additional extrarenal functions that give rise to the specific syndromal phenotypes, including maturity-onset diabetes of the young type 5; hyperuricemia and liver dysfunction in RCAD (7); ocular coloboma in RCS (9); external ear anomalies, hearing impairment, and cervical cysts and fistulas in BOR syndrome (10,11); and imperforate anus and limb and ear anomalies in TBS (12).

In the majority of children with RHD, neither a syndromal phenotype nor a Mendelian pattern of inheritance is obvious. However, careful evaluation of family history reveals familial clustering of isolated renal and urinary tract malformations in approximately 10% of cases (S.W. personal observation, 2006). Moreover, some of the mutations that were identified in the genes mentioned previously were de novo mutations (10,13,22,23), explaining the sporadic appearance of RHD. Finally, even with the same underlying mutation, the clinical phenotype of syndromal RHD may vary considerably (8,24–26).

These observations suggest that genetic events may be involved in the pathogenesis even of sporadic and seemingly nonsyndromal RHD. However, the prevalence of mutations in the renal developmental genes that are associated with syndromal disorders in the total population of pediatric RHD largely is unknown. In this study, we made use of the large data collection that was obtained by the European multicenter Effect of Strict Blood Pressure Control and ACE Inhibition on CRF Progression in Pediatric Patients (ESCAPE) study group to analyze systematically a cohort of 100 patients with RHD (of 99 unrelated families) and mild to moderate chronic renal insufficiency (CRI) for mutations in TCF2, PAX2, EYA1, SIX1, and SALL1. This unselected, well-characterized group comprised two patients with a priori suspected RCAD, one with RCS, and two with BOR syndrome.

## Materials and Methods

### Patients

A total of 466 children with stages 2 through 4 chronic kidney disease (GFR 15 to 75 ml/min per 1.73 m²) from 33 pediatric nephrology units in 13 European countries were enrolled in a prospective, multicenter trial (ESCAPE Trial [4]). In 175 patients of this cohort, RHD had been diagnosed sonographically by small kidneys (<3rd percentile for length or volume) and/or lack of corticomedullary differentiation with or without cysts. After exclusion of patients in whom RHD was associated with posterior urethral valves or primary bladder abnormalities and of patients with complex syndromes involving the kidneys other than those caused by the genes under investigation (RCS, RCAD, and BOR syndromes), 100 children with RHD from 99 unrelated families were eligible and available for mutation analysis of TCF2, PAX2, EYA1, SIX1, and SALL1. One family had two affected siblings (Figure 1).

Further clinical characteristics of the cohort are given in Table 1. In 23 cases, the renal malformation was associated with anomalies of other organs, including ear, heart, vertebral, limb, gastrointestinal, and cerebral malformations. A positive family history for renal/ureteral malformations and/or other kidney diseases (e.g., recurrent urinary tract infections, CRI, nephrolithiasis) was noted in 12% of the patients. Siblings were affected in three of 12 families, parents in four of 12 families, and grandparents in six of 12 families; in one family, a cousin of the mother was affected.

The study was approved by the ethics committees in all participating centers, and informed assent and/or consent for genetic screening was obtained from the patients and/or parents as appropriate.

### Mutation Screening

Genomic DNA was extracted from peripheral blood leukocytes by standard methods. Overlapping sets of primers that were based on the sequence of the human genes were used to amplify by PCR the coding sequences of the genomic DNA of TCF2, PAX2, EYA1, SIX1, and SALL1. Primer design was accomplished by applying the software
Oligos 5.1 (NBI, Plymouth, MN). Mutation screening was performed by denaturing HPLC (WAVE System, Cheshire, UK), and when abnormal migration patterns were found on denaturing HPLC, direct sequencing on both strands applying the fluorometric method (BigDye Terminator Cycle Sequencing Kit; ABI 3700 DNA sequencer; Applied Biosystems, Foster City, CA) was performed. Primer sequences are available upon request. When mutation analysis was positive for one of the genes under study in a patient, genetic studies were extended to family members whenever possible. All of the variants were screened in 100 unrelated white control subjects using direct sequence analysis.

**Deletion Screening of TCF2 and EYA1**

Patients with no mutation in the TCF2 and EYA1 coding sequence were screened further for gene deletions by quantitative multiplex PCR of short fluorescence fragments analysis as described previously (24,27) with slight modifications. For the TCF2 gene, we screened exons 3 and 7 considering that most of the deletions include the whole gene (13,27). For the EYA1 gene, we first studied exons 1, 5, 10, and 15 by this method. Because exons 1, 5, and 10 but not exon 15 seemed to be deleted in patient ESS3, we delineated more precisely the deletion with the multiplex ligation-dependent probe amplification (MLPA) method using the SALSA MLPA kit P153EYA1 (MCR-Holland, Amsterdam, the Netherlands) in the conditions suggested by the manufacturer. Amplified samples were denatured and separated by capillary electrophoresis on an ABI 3130 sequencer (Applied Biosystems).

**Statistical Analyses**

Statistical analyses of protein structure and amino acid composition, sequence alignments, and similarity searches were conducted using software and databases that were provided by Infobiogen (www.info-biogen.fr), ENSEMBL (www.ensembl.org), and the National Center for Biotechnical Information (www.ncbi.nlm.nih.gov).

**Results**

In the 100 patients with RHD in 99 families, sequence variants in renal developmental genes were identified in 18 patients of 17 (17%) unrelated families (Table 2). One mutation, two different variants, and four complete deletions of TCF2 were found in eight patients; four different PAX2 mutations were found in seven patients of six families; one EYA1 mutation and one EYA1 partial gene deletion were found in the two patients with BOR syndrome; one SALL1 mutation was found in a patient with isolated RHD; and one SIX1 sequence variant was found in two siblings with RCS as a result of a PAX2 mutation. None of the mutations was identified in DNA samples of 100 white control subjects.

**TCF2 Gene Mutations**

One splice-site mutation and two different, hitherto undescribed heterozygous variants in TCF2 (encoding Hnf1β) were identified in four unrelated patients, and heterozygous gene deletions were detected in another four patients (Table 2). The splice-site mutation is an A>G transition at position −2 of intron 4, which is predicted to cause aberrant splicing. The first variant is a missense mutation that replaces a cysteine that is highly conserved among different species by a tyrosine (C273Y) in the important functional DNA binding domain of the Hnf1β protein. The second variant that replaces a histidine by an aspartic acid (H336D) was found in two unrelated patients. This nonconservative amino acid change involves a highly conserved amino acid residue and is located in the activation domain of the Hnf1β protein.

Cystic lesions (cystic dysplasia or multicystic dysplastic kidneys [MCDK]) were present in six of the eight patients with genetic anomalies in TCF2, corresponding to a prevalence of 22% (six of 27) for cystic and 3% (two of 73) for noncystic RHD in the total cohort. A biopsy performed in one case demonstrated glomerulo- and tubulocystic changes, and both kidneys were hypoplasic in renal ultrasound. Diabetes was present since age 13 yr in one and hyperuricemia in two patients.

Parental DNA was available in four of eight families, and mutation screening in the parents revealed that the causative mutation was transmitted by either father or mother in two families and occurred de novo in another two families (Table 2). The two parental mutation carriers also were affected by RHD, whereas no renal phenotype was detectable by ultrasound in three other carrier relatives. Family history was positive for diabetes in three of the eight families (Table 2).

**PAX2 Gene Mutations**

Four different heterozygous PAX2 mutations were detected in seven patients of six unrelated families (Table 2). Three of the four mutations localize to exon 2, encoding a large part of the paired domain of Pax2 that is important for DNA binding (Table 2). Whereas the 619insG mutation has been reported in numerous patients with RCS (9), the deletion 635–640delGGC–CCC, the splice-site mutation (A>G954–2), and the nonsense mutation R140X have not been described so far (Human PAX2 Allelic Variant Database, http://pax2.hgu.mrc.ac.uk). The sixnucleotide deletion 635–640delGGCCCC results in the ablation of two highly conserved amino acids of the paired domain. The splicing mutation A>G transition at position −2 of intron 3 is predicted to cause aberrant splicing. Parental mutation screening was performed in three of six families and revealed that the mutation was inherited from the father in two families and occurred de novo in one family.

Renal hypoplasia and/or dysplasia was present in five and MCDK in two patients with PAX2 mutation. Contralateral pyelo-ureteral junction (PUJ) obstruction was identified in one of the patients with MCDK. Ocular abnormalities (coloboma and optic disc dysplasia) were found in five of the seven patients and in one of the two parents identified as affected; in five of these eight individuals, the alterations were so subtle that they had not been diagnosed before the detection of the PAX2 mutation. Three patients had mild sensorineural hearing defects, a symptom that was reported previously in a subset of patients with PAX2 mutations (26,28).

**EYA1 and SIX1 Gene Mutations**

EYA1 mutation screening revealed two heterozygous mutations in two unrelated patients. The first mutation was a heterozygous insertion mutation of a glycine (1179insGGG) that locates between the α helices 1 and 2 in the eye homology region of the Eya1 protein, which has not been reported before. This domain is highly conserved among different species and involved in Six1 binding (29). It is interesting that the 1179insGGG mutation was transmitted by the mother, who also
showed deafness, ear tags, and cervical fistulas but no renal abnormalities (BO syndrome). The second mutation was a heterozygous partial deletion of *EYA1*, which encompasses exons 1 through 10, whereas exons 12 through 16 were not deleted (exon 11 was not analyzed by the MLPA kit that we used). DNA from the parents was not available, but it is interesting to note that the father also had deafness, ear tags, and cervical fistulas but no renal abnormalities.

Of note, the analysis of *SIX1*, the second gene involved in BOR syndrome, revealed a heterozygous sequence variant (D227Y) that affects a highly conserved residue of the C-terminal part of the Six1 protein in the *PAX2*-mutated siblings GDA3 and GDA4. Both children presented with early-onset CRI, rapid deterioration of renal function, and optic disc dysplasia, whereas the *PAX2*-affected father, who had isolated RHD with late-onset moderate CRI, did not present with the *SIX1* D227Y variant. This sequence variant was transmitted by the mother, who has no renal or extrarenal symptoms (Figure 1). No other *SIX1* variants were found in the total cohort.

### Table 2. Mutation analysis results

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender/Age</th>
<th>Gene</th>
<th>Nucleotide Exchange (Transmitted from F or M, De novo, or pnt)</th>
<th>Amino Acid Exchange/Functional Effect</th>
<th>Renal Phenotype</th>
<th>Extrarenal Phenotype</th>
<th>Manifestations in Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE3</td>
<td>m/10 yr</td>
<td>TCF2</td>
<td>A&gt;G1240-2 (de novo) Splice-site intron 4</td>
<td>Bilateral cystic dysplasia</td>
<td>Hyperuricemia</td>
<td>No</td>
<td>Affect M mother CRF</td>
</tr>
<tr>
<td>NZM</td>
<td>f/14 yr</td>
<td>TCF2</td>
<td>C&gt;A1012 (M) C237Y (DNA binding domain)</td>
<td>Bilateral hypoplasia</td>
<td>No</td>
<td>Data not available</td>
<td>No</td>
</tr>
<tr>
<td>GDA9</td>
<td>m/23 yr</td>
<td>TCF2</td>
<td>C&gt;G1200 (F) HE36D (activation domain)</td>
<td>Bilateral cystic dysplasia, ectopy, horsehoe fusion, VUR</td>
<td>No</td>
<td>No</td>
<td>Data not available</td>
</tr>
<tr>
<td>FRA6</td>
<td>m/15 yr</td>
<td>TCF2</td>
<td>C&gt;G1200 (pnt) HE36D (activation domain)</td>
<td>Bilateral hypoplasia, VUR</td>
<td>No</td>
<td>Data not available</td>
<td>No</td>
</tr>
<tr>
<td>POR4</td>
<td>m/12 yr</td>
<td>TCF2</td>
<td>Gene deletion (de novo) Loss-of-function</td>
<td>Bilateral cystic dysplasia</td>
<td>No</td>
<td>No</td>
<td>Data not available</td>
</tr>
<tr>
<td>PAD4</td>
<td>m/13 yr</td>
<td>TCF2</td>
<td>Gene deletion (pnt) Loss-of-function</td>
<td>MCDK (f), noncystic dysplasia (r)</td>
<td>No</td>
<td>No</td>
<td>Data not available</td>
</tr>
<tr>
<td>GEN12</td>
<td>f/17 yr</td>
<td>TCF2</td>
<td>Gene deletion (pnt) Loss-of-function</td>
<td>Glomerulocystic dysplasia, bilateral hypoplasia</td>
<td>Hyperuricemia</td>
<td>Data not available</td>
<td>Maternal family diabetes</td>
</tr>
<tr>
<td>GEN17</td>
<td>m/17 yr</td>
<td>TCF2</td>
<td>Gene deletion (pnt) Loss-of-function</td>
<td>Cystic dysplasia (l)</td>
<td>Diabetes (onset age 13 yr)</td>
<td>Data not available</td>
<td>Affect M mother CRF</td>
</tr>
<tr>
<td>GDA3</td>
<td>f/15 yr</td>
<td>PAX2</td>
<td>619insG (F) Frameshift</td>
<td>Bilateral hypoplasia, single cyst</td>
<td>Hypoplastic optic disc (l), coloboma (r)</td>
<td>Affect M father RHD, no ocular phenotype</td>
<td>Affect M father RHD, no ocular phenotype</td>
</tr>
<tr>
<td>GDA4</td>
<td>f/17 yr</td>
<td>PAX2</td>
<td>619insG (F) Frameshift</td>
<td>Bilateral hypoplasia</td>
<td>Optic disc dysplasia, hearing impairment</td>
<td>Affect father RHD, no ocular phenotype</td>
<td></td>
</tr>
<tr>
<td>PRA9</td>
<td>m/14 yr</td>
<td>PAX2</td>
<td>619insG (pnt) Frameshift</td>
<td>Bilateral hypoplasia</td>
<td>Coloboma, optic disc dysplasia</td>
<td>Affect father RHD, no ocular phenotype</td>
<td></td>
</tr>
<tr>
<td>PAR16</td>
<td>f/10 yr</td>
<td>PAX2</td>
<td>619insG (de novo) Frameshift</td>
<td>MCDK, PUJ obstruction</td>
<td>No</td>
<td>No</td>
<td>Data not available</td>
</tr>
<tr>
<td>VIL8</td>
<td>f/11 yr</td>
<td>PAX2</td>
<td>A&gt;G954-2 (F) Splice-site intron 3</td>
<td>MCDK</td>
<td>No</td>
<td>No</td>
<td>Data not available</td>
</tr>
<tr>
<td>PRA1</td>
<td>m/14 yr</td>
<td>PAX2</td>
<td>635–640delGGCCCC (pnt)</td>
<td>Bilateral hypoplasia, VUR</td>
<td>Data not available</td>
<td>Father ESRD, coloboma</td>
<td></td>
</tr>
<tr>
<td>PRA7</td>
<td>f/16 yr</td>
<td>PAX2</td>
<td>G&gt;A961 (pnt) R140X</td>
<td>Hypoplasia (l), noncystic dysplasia (r)</td>
<td>No</td>
<td>No</td>
<td>Data not available</td>
</tr>
<tr>
<td>BEL9</td>
<td>f/18 yr</td>
<td>EYA1</td>
<td>1179insGGG (M) 394insG (Six1-binding region)</td>
<td>Bilateral hypodysplasia oligomeganephronia</td>
<td>Sensorineural hearing defect, external ear anomalies, cervical fistulas</td>
<td>Mother BO syndrome, Father bo syndrome</td>
<td></td>
</tr>
<tr>
<td>ESS3</td>
<td>m/15 yr</td>
<td>EYA1</td>
<td>Partial gene deletion (pnt) Loss-of-function</td>
<td>Bilateral noncystic dysplasia</td>
<td>Preauricular pits, external ear anomalies, cervical fistulas, lacrimal duct stenosis</td>
<td>Father bo syndrome</td>
<td></td>
</tr>
<tr>
<td>GDA3</td>
<td>f/15 yr</td>
<td>SIX1</td>
<td>G&gt;T679 (M) D227Y</td>
<td>See above</td>
<td>See above</td>
<td>Mother carrier, no phenotype</td>
<td></td>
</tr>
<tr>
<td>GDA4</td>
<td>f/17 yr</td>
<td>SIX1</td>
<td>G&gt;T679 (M) D227Y</td>
<td>See above</td>
<td>See above</td>
<td>Mother carrier, no phenotype</td>
<td></td>
</tr>
<tr>
<td>GDA18</td>
<td>f/17 yr</td>
<td>SALL1</td>
<td>3414–3415delAT (pnt)</td>
<td>Bilateral hypoplasia</td>
<td>None</td>
<td>Data not available</td>
<td></td>
</tr>
</tbody>
</table>

*BO, branchio-oto; F, father; M, mother; pnt, parents not tested; PUJ, pyeloureteral junction; VUR, vesicoureteric reflux.

**Partial gene deletion**

**SALL1 Gene Mutation**

A heterozygous frameshift *SALL1* mutation that leads to a premature translation stop at amino acid position 1152 (T1138fs1152X) was identified in one patient with isolated bilateral hypoplasia and no extrarenal symptoms. To our knowledge, this is the first report of a pathogenic *SALL1* mutation associated with an isolated kidney phenotype. Unfortunately, DNA from the parents was not available.
In our study, a group of important renal developmental genes that are associated with syndromal renal malformations were analyzed systematically for mutations in a large and unselected cohort of European children with renal hypoplasia to identify and quantify their role in the pathogenesis of isolated renal malformation. Screening of the coding sequences of TCF2, PAX2, EYA1, SIX1, and SALL1 in 99 unrelated patients with RHD revealed 10 different variants or mutations (three missense variants, two splice mutations, a two–amino acid deletion, a single–amino acid insertion, two frameshift mutations, and a nonsense mutation) in 12 families and heterozygous deletions (one partial deletion of EYA1 and four large gene deletions of TCF2) in another five unrelated children. The missense variants in TCF2 (C273Y and H336D) are thought to be pathogenic because they were not found in 100 control samples, they affect highly conserved amino acid residues, and they locate to important functional domains of the HNF1β protein. However, in two families (IZM3 and GDA9), these variants also were found in seemingly healthy relatives (Table 2), suggesting that they probably are not sufficient to explain the phenotype. Additional genetic events might be associated with the renal phenotype in these patients, because it also is observed in the two affected siblings with one PAX2 mutation and an additional SIX1 variant (D227Y) (see below).

In only five of the 18 children affected, the syndrome that is associated with the mutated gene had been suspected on clinical grounds before the study. Hence, one in six children who presented with RHD can be expected to have a variant in one of the five genes studied here, with detection rates being highest in TCF2 and PAX2. This high prevalence mirrors the diversity of renal and extrarenal manifestations that are associated with genetic variants and the frequent subtleness or lack of extrarenal symptoms, which prevents a clinical diagnosis in many cases.

Mutations in individual renal developmental genes can cause a wide spectrum of kidney and urinary tract malformations as a consequence of discoordinated interactions between the ure-teric bud and the metanephric mesenchyme (CAKUT [congenital malformations of the kidney and the urinary tract] hypothesis) (1). In keeping with this hypothesis, the renal phenotype of patients who were affected by PAX2 mutations encompassed renal hypoplasia, noncystic dysplasia, MCDK, and isolated PUJ obstruction with associated vesicoureteric reflux in 48 patients. It is interesting that we found a de novo PAX2 mutation in a patient with MCDK in the right kidney and PUJ obstruction in the left kidney. This is the second case reported with MCDK in association with a PAX2 mutation (30), supporting a close relationship between MCDK and the RHD complex. This observation also reinforces the hypothesis that PAX2 mutations can lead to early ureteral obstruction during nephrogenesis and consequently to ureteropelvic junction obstruction, MCDK, or renal agenesis.

TCF2 anomalies were associated with global hypoplasia, noncystic or cystic dysplasia, MCDK, or glomerulocystic dysplasia (the last in one patient in whom a renal biopsy was performed). Of note, even patients with identical mutations or large gene deletions exhibited markedly variable renal phenotypes. Three mutation-carrying relatives of two index patients presented without any renal anomaly as detectable by ultrasound and biochemical measurements. This finding is in line with a recent report suggesting that only 70% of patients with TCF2 mutation present with a renal phenotype (8).

Notwithstanding this variability, cystic malformations clearly clustered with abnormalities in the TCF2 gene. Overall, 22% of all children with cystic RHD in the ESCAPE cohort were affected by a mutation or deletion of TCF2. This result is in accordance with the recent report of Ulinski et al. (13), who detected TCF2 gene deletions or mutations in 25 of 80 patients with mainly cystic renal hypoplasia (21 of 25). Taken together, the findings of both studies argue for a systematic TCF2 screening in all children with cystic renal dysplasia, including MCDK, because a positive result would have a major impact for the follow-up of affected patients (development of diabetes, liver dysfunction, and hyperuricemia) and genetic counseling of the families.

The variability of phenotypic penetrance of the observed genetic anomalies was not restricted to the renal manifestations. Three of nine patients with PAX2 mutations did not present with an ocular phenotype, five of eight patients affected in TCF2 had isolated kidney disease at the time of the study, and the patient who carried a SALL1 mutation lacked any of the extrarenal manifestations that are typical for TBS. This is the first time a SALL1 mutation has been found to be associated with an isolated renal malformation. The variability of organ affection might be explained by the summarizing effect of sequence variations in different developmental genes with organ-specific expression. The results of mutation analysis in the family of GDA3 and GDA4 might support this hypothesis: In this family, both children have a severe renal phenotype, a clinical course with early-onset renal failure, and optic disc dysplasia consistent with RCS. The father, sharing the PAX2 mutation 619insG, had isolated RHD, only mild CRI, and no ocular phenotype. It is interesting that only in the siblings was an additional SIX1 variant D227Y present, inherited from the
healthy mother. This variant is not found in the control subjects and concerns a highly conserved aspartic residue in the C-terminal domain of the SIX1 protein. It is tempting to speculate that this additional variant in the SIX1 gene, which is strongly expressed in the kidney and the eye, acts as a genetic modifier and contributes to the more severe renal manifestation and the ocular phenotype limited to the children. Similar intrafamilial variability was observed in the two families that were affected by a dominant EYA1 mutation and deletion, respectively. In both families, the transmitting parent was affected by deafness, ear tags, and cervical fistulas without a renal phenotype, whereas complete BOR syndrome was present only in the index patients. These observations lend strong support to the notion that additional genetic factors modify the expressivity of a renal phenotype even in patients with genetic lesions that are associated with defined syndromes.

An important clinical lesson from this study is that subtle extrarenal symptoms in syndromal RHD easily can be missed: In one patient who was affected by a PAX2 mutation and one transmitting father, coloboma and optic disc dysplasia were detected only after the results of mutation analysis had prompted a thorough ophthalmologic examination. These findings confirm our previous observations in patients with renal oligomeganephric hypoplasia (31,32). Taken together, our systematic analysis of genes that are associated with syndromal RHD suggests that approximately 15% of nonselected patients with RHD and CRI can be genetically characterized by a mutation screening of TCF2 and PAX2. Genetic testing in children with RHD should be preceded by thorough clinical evaluation for extrarenal symptoms, including eye, ear, and metabolic anomalies, which, if positive, increases the likelihood of detecting a specific genetic abnormality. All children with cystic renal malformations should be screened for mutations and deletions in TCF2, because a detection rate of at least 20% can be expected in this subgroup and affected patients are at risk for developing major metabolic derangements in later life. A negative family history does not rule out genetic anomalies in these genes, because de novo mutations do occur. In this study, we identified a patient who had isolated RHD and was affected by a mutation in SALL1, a gene that generally is associated with complex syndromal disease; however, genetic variants in EYA1, SIX1, and SALL1 seem to account only for a minor fraction of children with RHD.

In general, genetic counseling is recommended for all patients who are affected by mutations in the genes TCF2, PAX2, EYA1, SIX1, and SALL1 because the related syndromes are inherited in an AD manner, although with variable expressivity and sometimes incomplete penetrance. This suggests that affected individuals will pass on the mutation to 50% of the descendents. We are convinced that it is important to evaluate all parents, siblings, and descendents of affected individuals clinically for absent signs of organ maldevelopment and, as the case may be, to perform mutation analysis to provide a basis for genetic counseling. It should be emphasized that in >80% of all patients who were enrolled in our study, including 85% of the patients who presented with additional extrarenal anomalies and 75% of patients with a family history of renal disease, we were unable to detect any sequence variants that were identified in the five developmental genes studied. It is highly likely that other, as yet unreported developmental genes will be identified in the future, and especially genes for which renal maldevelopment has been demonstrated in genetically modified animal models may be of interest for further studies.

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ESCAPE trial participants are as follows: A. Anarat (Adana); A. Bakkaloglu, F. Ozaltin (Ankara); A. Peco-Antic (Belgrade); U. Querfeld, J. Gellermann (Berlin); P. Sallay (Budapest); D. Drozdz (Cracow); K.-E. Bonzel, A.-M. Wingen (Essen); A. Zuworska, I. Balasz (Gdansk); F. Perfumo, A. Canepa (Genoa); D.E. Müller-Wiefel, K. Zepf (Hamburg); G. Öffner, B. Enke (Hanover); O. Mehls, F. Schaefer, E. Wühl, C. Hadtstein (Heidelberg); U. Berg, G. Celsi (Huddinge); S. Enre, A. Sirin, I. Bilge (Istanbul); S. Çaliskan (Istanbul-Cerrahpasa); S. Mir, E. Serdaroglu (Izmir); C. Greiner, H. Eichstädt, S. Wyygoda (Leipzig); K. Hofbach-Hohenfellner (Mainz); N. Jeck, G. Klaus (Marburg); A. Appiani, G. Ardissoino, S. Testa (Milano); G. Montini (Padova); C. Antignac, P. Niaudet, M. Charbit (Paris); J. Dusek (Prague); A. Caldas-Afonso, A. Teixeira (Porto); S. Picca, C. Matteucci (Rome); M. Wigger (Rostock); M. Fischbach, J. Terzic (Strasbourg); J. Fdyryk, T. Urasinski (Szczecin); R. Coppo, L. Peruzzi (Torino); A. Jankauskienè (Vilnius); M. Litwin, M. Abuauba, R. Grenda (Warszawa); K. Arbeiter (Vienna); T.J. Neuhaus (Zurich).

References


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