Mutations in \textit{RPGRIP1L}: extending the clinical spectrum of ciliopathies* \\

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\textbf{Introduction} \\
Identification of genes causing inherited cystic kidney diseases has triggered a major interest for the concept of ‘ciliopathies’. Indeed, almost all of the proteins involved in human renal cystic diseases are expressed in the primary cilium complex located in renal epithelial cells. Primary cilia are cellular extensions containing a microtubule-based axoneme covered by a specialized plasma membrane [1]. The basal body of the cilia, which templates the assembly of the microtubules, contains a centriole, which itself is part of the centrosome. Primary cilia project into the lumen, where they probably sense a variety of stimuli involved in the regulation of cell proliferation and differentiation [2]. Primary cilia are present on almost all human cells, explaining why ciliopathies affect multiple organs. However, the molecular mechanisms, potential connections and clinical variability of these diseases remain poorly understood. The study by Delous \textit{et al.} gives new insights into the field, by demonstrating that mutations in the \textit{RPGRIP1L} (retinitis pigmentosa GTPase regulator-interacting protein 1-like) gene cause both Joubert syndrome (JBTS) and Meckel syndrome (MKS), two complex diseases with neurological, renal and ocular manifestations [3]. The protein encoded by \textit{RPGRIP1L} is located in the primary cilium, and mutations impair its interaction with nephrocytin-4, a protein involved in nephronophthisis. Furthermore, \textit{RPGRIP1L} knockout mice show a phenotype similar to that observed in foetuses with MKS. These findings, which were also demonstrated in a companion article by Arts \textit{et al.} [4], highlight the importance of ciliary dysfunction in cerebello-oculo-renal syndromes and nephronophthisis. \\

\textbf{Keywords:} cerebello-oculo-renal syndrome; Joubert syndrome; Leber congenital amaurosis; Meckel syndrome; Senior–Loken syndrome \\

\textbf{Clinical and genetic heterogeneity of the Joubert and Meckel syndromes} \\
Joubert syndrome (JBTS) refers to a group of autosomal recessive disorders characterized by a brainstem malformation creating the ‘molar tooth sign’ (MTS) on axial magnetic resonance imaging and neurologic manifestations that include cerebellar ataxia, developmental delay, hypotonia, abnormal eye movements, dysregulation of the breathing pattern and cognitive deficits. Some JBTS patients also have retinal dystrophy and renal abnormalities. In that case, JBTS is included among the cerebello-ocular-renal syndromes (CORS) which, in addition to MTS, include renal (nephronophthisis or cystic renal dysplasia) and retinal abnormalities (chorioretinal colobomas, retinal dystrophy), and occasional hepatic fibrosis and polydactyly [5,6]. Up until now, four genes and two additional loci have been associated with JBTS [7–10] (Table 1). Mutations in two of these genes (\textit{NPHP1} and \textit{NPHP6}) have been associated with nephronophthisis, the most common genetic cause of renal failure in children [8,9]. Meckel (or Meckel–Gruber) syndrome (MKS) is a rare and lethal autosomal recessive disorder characterized by cystic kidney dysplasia and variably associated central nervous system malformations (typically, posterior occipital meningoecephalocele), hepatic ductal changes and cysts, and polydactyly. MKS is caused by mutations in three genes (\textit{MKS1}, \textit{TMEM67/MKS3} and \textit{CEP290/NPHP6}), and a fourth locus has been described [10–12] (Table 1). The identification of mutations in \textit{TMEM67/MKS3} in JBTS patients [10] and, inversely, mutations in \textit{CEP290/NPHP6} in MKS foetuses [13] suggest that JBTS and MKS may represent parts of a spectrum of cerebello-oculo-renal syndromes involving ciliary proteins (Table 1). \\

\textit{RPGRIP1L} mutations cause JBTS and MKS \\
Using genome-wide linkage scans in consanguineous families in which the known JBTS and MKS loci were excluded, Delous \textit{et al.} identified a critical region on chromosome 16q [3]. By examining the syntenic region deleted in the \textit{Ft}
Mutations in RPGRIP1L

Table 1. Loci, causative genes and proteins involved in the overlapping Joubert syndrome (JBTS), cerebello-oculo-renal syndrome (CORS), Meckel syndrome (MKS), nephronophthisis (NPHP), Leber congenital amaurosis (LCA) and Senior–Loken syndrome (SLSN)

<table>
<thead>
<tr>
<th>Loci and related diseases</th>
<th>Gene map</th>
<th>Gene</th>
<th>Protein</th>
<th>Expression in primary cilium</th>
</tr>
</thead>
<tbody>
<tr>
<td>JBTS1, CORS1</td>
<td>9q34.3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>JBTS2, CORS2</td>
<td>1p12–q13.3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>JBTS3</td>
<td>6q23.3</td>
<td>AHI1</td>
<td>Nephrocytin</td>
<td>Ciliary base, transition zone</td>
</tr>
<tr>
<td>JBTS4, NPHP1</td>
<td>2q13</td>
<td>NPHP1</td>
<td>Centrosomal protein 290-kD, nephrocytin-6</td>
<td>Basal body–centrosome complex</td>
</tr>
<tr>
<td>JBTS5, NPHP4, LCA10, SLSN6</td>
<td>12q21.3</td>
<td>CEP290/NPHP6</td>
<td>Primary cilium</td>
<td></td>
</tr>
<tr>
<td>JBTS6, MKS3</td>
<td>8q21.13–q22.1</td>
<td>TMEM67/MKS3</td>
<td>Transmembrane protein-67, meckelin</td>
<td></td>
</tr>
<tr>
<td>JBTS7, CORS3, MKS5, NPHP8</td>
<td>16q12.2</td>
<td>RPGRIP1L</td>
<td>Retinitis pigmentosa GTPase regulator-interacting protein 1-like (RPGRIP1L)</td>
<td></td>
</tr>
<tr>
<td>MKS1</td>
<td>17q23</td>
<td>MKSI</td>
<td>MKSI</td>
<td>Centrosome</td>
</tr>
<tr>
<td>MKS2</td>
<td>11q13</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA: unknown or not available.

(For fused toes) mouse, which shows phenotypic resemblance to MKS, they identified a candidate gene (KIAA1005 or RPGRIP1L) that was known to interact with nephrocystin-4, a ciliary protein defective in nephronophthisis [14]. Sequencing of RPGRIP1L uncovered nine mutations in five families with CORS and two with MKS [3]. Of note, 5/6 of the individuals with CORS had at least one missense mutation, whereas all foetuses with MKS harboured nonsense mutations. The individuals with CORS and RPGRIP1L mutations showed the neurological features of JBTS, with nephronophthisis in 5/6 of the patients and slightly enlarged cystic kidneys in one patient. All individuals with CORS had ocular symptoms (oculomotor apraxia, ptosis, nystagmus), but only one had retinitis pigmentosa, which is present in the majority of individuals with NPHP6 mutations [9,15,16]. The three foetuses with MKS originated from pregnancies terminated at 15–16 weeks of gestation for severe cystic kidney dysplasia, brain malformation and polydactyly, with further examination disclosing bile duct proliferation, encephalocele and microphthalmia [3]. This severe phenotype resembled that of the Rpgrip1l knockout mice, which die in utero with exencephaly, microphthalmia, polydactyly, and show renal proximal tubules cysts and ductal plate malformation [3].

Functional characterization of RPGRIP1L

Expression and functional studies yielded information about the potential role of RPGRIP1L [3,4]. Like other genes involved in JBTS-CORS and MKS, RPGRIP1L is expressed early in the developing eye, brain and kidney. RPGRIP1L encodes the RPGRIP1L protein which shares a significant homology with RPGRIP1, a ciliary protein located in the photoreceptors in the retina where it associates with CEP290/nephrocytin-6 [17]. Mutations in either RPGRIP1 or CEP290/NPHP6 lead to Leber congenital amaurosis (LCA), the most common cause of congenital blindness in infants and children [18,19]. It is known that RPGRIP1L binds directly to nephrocytin-4 (nephroretinin), the product of NPHP4 [14]. Mutations of NPHP4 cause late-onset retinitis pigmentosa in addition to nephronophthisis, an association described as Senior–Loken syndrome [20,21]. Of note, mutations of RPGRIP1L detected in individuals with CORS significantly decreased the binding between RPGRIP1L and nephrocytin-4, potentially contributing to the pathogenesis of the disease [3,4]. Immunolocalization demonstrated that RPGRIP1L co-localizes with nephrocytin-4 and nephrocytin-6 at the basal body–centrosome complex, and is also detected along the axoneme and in the cytoplasm [3,4] (Figure 1).

The identification of disease-causing RPGRIP1L mutations, their functional analysis and the distribution of RPGRIP1L in the basal body were also reported by Arts et al. [4]. Taken together, these data show that RPGRIP1L interacts with ciliary proteins and that mutations disrupting this interaction cause similar phenotypes, supporting the role of ciliary dysfunction in these diseases [3,4]. The signalling events disrupted by defective RPGRIP1L remain essentially unknown. In mice, RPGRIP1L is necessary for the establishment of left–right asymmetry and patterning of the neural tube and limbs, and is potentially involved in the Hedgehog pathway [22]. Since mutations of RPGRIP1L account for a small fraction of JBTS [4], cloning of other causative genes will probably elucidate these signalling mechanisms.

Towards genotype–phenotype correlations?

The pattern of mutations reported by Delous et al. may suggest a genotype–phenotype correlation in these diseases, with residual function of the mutated proteins in patients with CORS contrasting with loss-of-function in foetuses with MKS [3]. However, Arts et al. identified homozygous loss-of-function mutations (both predicted to cause truncation before the interacting domains of RPGRIP1L) in two consanguineous families with JBTS and one missense mutation together with a nonsense mutation in a family with overlapping JBTS–MKS syndrome [4]. Clinical heterogeneity could also reflect epistatic effects caused by heterozygous mutations and/or variants in other
causative genes of JBTS/NPHP, as first reported by Tory et al. [15]. The low prevalence of the retinal disease in CORS individuals harbouring \textit{RPGRIP1L} mutations could be due to partial redundancy of \textit{RPGRIP1} and \textit{RPGRIP1L} proteins in different cell types [3,4]. Alternatively, this complication may not have developed in some of the younger probands, as the age of onset of retinal dystrophy may be variable [21,23].

\textbf{Conclusion and take-home message}

The studies of Delous \textit{et al.} [3] and Arts \textit{et al.} [4] extend the clinical spectrum of ciliopathies, clarify their nosology and highlight the importance of ciliary dysfunction in developmental disorders that include renal manifestations. These results also demonstrate the power of genome-wide linkage scans in carefully selected families, and the usefulness of comparative genomics in mouse models made available through random mutagenesis.

JBTS and MKS represent parts of a continuum of cerebello-oculo-renal syndromes probably linked to primary cilium dysfunction. These studies are relevant for the pathophysiology of nephronophthisis, Senior–Loken syndrome and Leber congenital amaurosis. Mutations of \textit{RPGRIP1L} account for a small fraction of these diseases, and cloning of additional genes will help to understand signalling mechanisms and develop therapeutic approaches.

\textit{Conflict of interest statement.} None declared.

\textbf{References}

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