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Mutations in 3 genes (*MKS3, CC2D2A* and *RPGRIP1L*) cause COACH syndrome (Joubert syndrome with congenital hepatic fibrosis)

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Abstract

Objective—To identify genetic causes of COACH syndrome

Background—COACH syndrome is a rare autosomal recessive disorder characterised by Cerebellar vermis hypoplasia, Oligophrenia (developmental delay/mental retardation), Ataxia, Coloboma, and Hepatic fibrosis. The vermis hypoplasia falls in a spectrum of mid-hindbrain malformation called the molar tooth sign (MTS), making COACH a Joubert syndrome related disorder (JSRD).

Methods—In a cohort of 251 families with JSRD, 26 subjects in 23 families met criteria for COACH syndrome, defined as JSRD plus clinically apparent liver disease. Diagnostic criteria for JSRD were clinical findings (intellectual impairment, hypotonia, ataxia) plus supportive brain imaging findings (MTS or cerebellar vermis hypoplasia). *MKS3/TMEM67* was sequenced in all subjects for whom DNA was available. In COACH subjects without *MKS3* mutations, *CC2D2A, RPGRIP1L* and *CEP290* were also sequenced.

Results—19/23 families (83%) with COACH syndrome carried *MKS3* mutations, compared to 2/209 (1%) with JSRD but no liver disease. Two other families with COACH carried *CC2D2A* mutations, one family carried *RPGRIP1L* mutations, and one lacked mutations in *MKS3, CC2D2A, RPGRIP1L* and *CEP290*. Liver biopsies from three subjects, each with mutations in one of the three genes, revealed changes within the congenital hepatic fibrosis/ductal plate

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malformation spectrum. In JSRD with and without liver disease, *MKS3* mutations account for 21/232 families (9%).

Conclusions—Mutations in MKS3 are responsible for the majority of COACH syndrome, with minor contributions from *CC2D2A* and *RPGRIP1L*; therefore, *MKS3* should be the first gene tested in patients with JSRD plus liver disease and/or coloboma, followed by *CC2D2A* and *RPGRIP1L.*

COACH syndrome (MIM 216360) was proposed in 1989 by Verloes and Lambotte to describe the combination of **C**erebellar vermis hypoplasia (CVH), **O**ligophrenia (developmental delay/mental retardation), **A**taxia, **C**olobomas, and **H**epatic fibrosis in three individuals including two siblings.[1] Since that initial description, more than 40 individuals have been described with that constellation of findings or a subset thereof. In 1999, Satran *et al*[2] proposed that COACH syndrome represents a subtype of the autosomal recessive disorder Joubert syndrome (MIM 213300) manifesting liver disease. In 2004, Gleeson *et al*[3] established that the CVH in COACH syndrome was accompanied by elongation of the superior cerebellar peduncles and a deep interpeduncular fossa on brain magnetic resonance imaging (MRI), all features that constitute the molar tooth sign (MTS), the key feature of Joubert syndrome and related disorders (JSRD). As a JSRD subtype, COACH syndrome manifests core neurological features including the MTS, hypotonia in infancy with later development of ataxia, developmental delay/mental retardation, abnormal breathing pattern (tachypnoea and/or apnoea) and abnormal eye movements (typically, nystagmus, oculomotor apraxia (OMA) or difficulty with smooth visual pursuit).[4–6] Other features variably reported in JSRD include dysmorphic facial features, polydactyly, retinal dystrophy, chorioretinal colobomas, renal disease (cysts or juvenile nephronophthisis), hepatic fibrosis, and tongue papules or oral frenulae.[7–9]

The autosomal recessive disorder Meckel (Gruber) syndrome (MKS; MIM 607361) typically manifests prenatal or perinatal lethality and is characterised by the triad of posterior fossa brain malformation (90% occipital encephalocele), cystic renal dysplasia, and congenital liver disease (the ductal plate malformation (DPM)).[10–13] These features place MKS within a wider group of congenital hepato-renal fibrocystic diseases that include autosomal recessive polycystic kidney disease (ARPKD), nephronophthisis with liver fibrosis, Bardet–Biedl syndrome and Jeune asphyx-iating thoracic dystrophy. The DPM (figure 1A) is thought to result from incomplete remodelling of the embryonic ductal plate which normally leads to the mature structure of the portal triad (figure 1B). This developmental arrest is associated with the presence of an excess of dilated bile ducts, often arranged in a circular profile, as well as portal fibrosis. It is often referred to as congenital hepatic fibrosis (CHF), especially after infancy when the ducts typically become less conspicuous and dense fibrosis predominates.[14–16]

Marked genetic heterogeneity exists for JSRD and MKS. Mutations in seven genes (NPHP1, AHI1, CEP290, RPGRIP1L, MKS3/TMEM67, CC2D2A and ARL13B) and two additional loci (9q34, pericentromeric chromosome 11) have been associated with JSRD,[17–30] but these account for only approximately 50% of patients. *MKS1* and *MKS3* are each estimated to contribute to a variable percentage of total MKS patients, highly dependent on ethnic background.[31–34] Several of the genes responsible for JSRD (*CEP290, RPGRIP1L, MKS3/TMEM67, CC2D2A*) also cause MKS.[20 22 35–38] This overlap is not completely unexpected given that the genes responsible for JSRD and MKS are implicated in primary cilium/basal body function, and both physical and genetic interactions between these proteins/genes have been demonstrated.[38] Given the shared genetic causes of MKS and JSRD, the underlying pathophysiology of liver disease in COACH syndrome is likely to lie within the same spectrum as MKS.

Recently, Brancati *et al*[39] identified *MKS3* mutations in more than half of probands with COACH syndrome, defined as JSRD with CHF; however, the contribution of *MKS3* mutations to JSRD as a whole was not reported. In this work, we demonstrate that *MKS3* mutations account for 9% of families in a large JSRD cohort, and that *MKS3* mutations occur almost exclusively in the JSRD plus clinically apparent liver disease (COACH) subtype. In addition, we found *RPGRIP1L* and *CC2D2A* mutations in families with COACH, and collectively, these three genes account for up to 96% of families with COACH in our cohort.

SUBJECTS AND METHODS

Subjects

In cooperation with the Joubert Syndrome Foundation & Related Cerebellar Disorders and clinical collaborators throughout the world, the University of Washington (UW) Joubert Center has enrolled >250 families with JSRD under an approved protocol through the Human Subjects Division at the University of Washington. All subjects in the cohort have clinical findings of Joubert syndrome (intellectual impairment, hypotonia, ataxia) and supportive brain imaging findings (MTS on MRI or cerebellar vermis hypoplasia on computed tomography (CT) scan). For subjects without brain MRI studies, vermis hypoplasia was considered a sufficient imaging criterion. Diagnosis of CHF was based on medical record review using any one of the following criteria: histopathological evidence of hepatic fibrosis from either biopsy or autopsy, hepatic ultrasound/MRI imaging findings consistent with fibrosis or bile duct dilatation, otherwise unexplained elevated serum transaminases, hepatomegaly, portal hypertension, portosystemic shunting, or liver transplantation. Patients with an alternative cause for liver disease (medication, infection, inflammation) were excluded. Other features including coloboma, retinal disease, renal disease, polydactyly, and encephalocele were identified based on parent/physician report or medical record review. Ocular coloboma was not required for the COACH diagnosis. Renal disease was collated and assigned to the following categories using clinical, imaging and histopathological data, as available: (1) nephronophthisis, or (2) macrocystic renal disease, or (3) other renal disease as reported. These criteria were also applied to interpretation of historical reports of COACH syndrome.

Mutation identification

The known JSRD and MKS loci were excluded using micro-satellite and single nucleotide polymorphism (SNP) genotyping as previously described.[38] The remaining probands were sequenced for *MKS3*,[37 40] *CC2D2A*,[41] and *RPGRIP1L*[19] as previously described. Sequencing of forward and reverse strands of all exons plus 30–50 bp of flanking intronic sequence was performed using BigDye v3.1 (Applied Biosystems, Foster City, California, USA) terminator chemistry and reactions purified with BigDye XTerminator (Applied Biosystems) technology. Extension fragments were separated by capillary electrophoresis on a 3730×l DNA Analyzer (Applied Biosystems). Sequence variants were identified using the software package, Variant Reporter (Applied Biosystems). Confirmation of segregation of mutations with disease status within a family was determined for all mutations when parental and/or sibling DNA was available. Determination of the likelihood that missense mutations would be deleterious was made using the PolyPhen program.[42] NetGene2 (www.cbs.dtu.dk/services/NetGene2/), Genie (www.fruitfly.org/seq_tools/splice.html), Human Splicing Finder (www.umd.be/HSF/), SPL (linux1.softberry.com), and SpliceView (http://zeus2.itb.cnr.it/~webgene/wwwspliceview.html) were used to model the effects of splice site mutations.[43 44]

Literature review

Review of all papers published in the medical literature with reference to COACH syndrome was performed recording the age of onset of hepatic disease, presenting signs/symptoms of liver involvement, other associated features (MTS, colobomas in particular), evidence of renal cystic disease, medical and/or surgical interventions, and outcomes with regard to morbidity and mortality associated with liver and/or kidney disease. In some cases, direct contact with the authors yielded updated clinical information. Patients without evidence of liver disease or cerebellar vermis hypoplasia were excluded, as well as reports of Dekaban–Arima syndrome, characterised by congenital amaurosis and polycystic kidneys.[2 45 46]

RESULTS

Patients with COACH syndrome due to mutations in *MKS3*, *CC2D2A* or *RPGRIP1L*

Patient 1: *MKS3*/*TMEM67*—This 9-year-old male (UW65) was initially evaluated in his first year of life for macrocephaly, overgrowth, hypotonia and developmental delay (figure 2A). At 16 months, ophthalmologic evaluation revealed rotatory nystagmus, OMA, reduced visual acuity and bilateral inferior chorioretinal colobomas, sparing the macula. A brain MRI demonstrated the MTS (figure 2B–C), and he manifested developmental delay and an ataxic gait (walking independently from 8 years of age). At 9 years, he uses two words, follows complex commands, reads and types at a basic level, and can do multiplication (figure 2D).

By 11 months of age, palpable hepatosplenomegaly with fourfold elevated liver enzymes (aspartate transaminase (AST) 250 IU/l, alanine aminotransferase (ALT) 236 IU/l) prompted liver biopsy which demonstrated minimal portal fibrosis and excess of ductal elements (figure 1C). At age 6 years, he had normal serum transaminases but “slightly coarsened” hepatic echogenicity on abdominal ultrasound. At 9 years, mildly elevated serum transaminases (AST 73 IU/l, ALT 92 IU/l) and borderline splenomegaly prompted a second liver biopsy which demonstrated progression of hepatic fibrosis with numerous duct profiles in portal triads (figure 1D). Currently, his renal function remains intact. Testing for the genes associated with JSRD revealed compound novel missense mutations in the *MKS3* gene (figure 2E), with one mutation confirmed to be inherited from his mother (paternal sample not available).

Patient 2: *CC2D2A*—This 22.5-year-old female (UW49), briefly reported in Gorden *et al* (2008), has speech dyspraxia, reads at the 6th grade level, and uses a wheelchair for mobility (figure 2F). She has moderate intellectual disability (full scale IQ = 41) and completed high school in a regular classroom with a full time aide. She had apnoea and tachypnoea as an infant, with episodic events persisting into maturity. Ophthalmological evaluation revealed OMA, strabismus, and bilateral small chorioretinal colobomas inferior to the optic nerves. Cerebellar vermis hypoplasia, agenesis of the corpus callosum and hydrocephalus were identified on head CT scans. Ventriculoperitoneal (VP) shunt, strabismus, ptosis, bilateral inguinal hernia and tongue reduction surgeries were required.

At 5.9 years of age, hepatosplenomegaly and elevated serum transaminases led to a liver biopsy which showed portal fibrosis and an excess of bile ducts. Progression of her liver disease led to a second biopsy at 8.5 years of age, revealing mild-moderate portal fibrosis with focal portal–portal bridging. By 10 years of age, a transjugular intrahepatic portosystemic shunt was placed for treatment of massive ascites and splenomegaly. Subsequently, she received a paternal partial liver transplant for end stage liver failure. The native liver showed moderate to severe dense portal fibrosis with variably thick septa bridging portal regions and focal nodule formation (figure 1E). Some portal triads contained

an excess of ducts, including a few irregularly complex forms (figure 1F). At 17 years, she developed mild chronic renal failure, hypertension and renal tubular acidosis, medically treated. Compound heterozygous mutations (one missense, one frame-shift) were identified in the *CC2D2A* gene (table 1).[38]

Patient 3: *RPGRIP1L*—This 17.5-year-old male (UW04) presented at age 15 months with hypotonia, developmental delay, alternating hyperpnoea/apnoea and OMA. Brain MRI at 9 months of age demonstrated the MTS. His younger brother had a very similar neonatal course with milder OMA, plus unilateral ptosis, strabismus and autistic behaviours. By the age of 5 years, he manifested ataxia and was diagnosed with chronic anaemia and progressive renal insufficiency (blood urea nitrogen (BUN) 96 mg/dl and creatinine (Cr) 6.7 mg/dl). A renal ultrasound showed diffusely echogenic kidneys of normal size. Combined renal and liver biopsies were performed. The liver demonstrated mild to moderate portal fibrosis with focal portal–portal bridging without inflammation or cholestasis (figure 1G); many triads contained an excess number of ducts (figure 1H). Neither biliary stasis nor an inflammatory process was present. The renal biopsy showed marked interstitial fibrosis and inflammation with tubular atrophy consistent with nephronophthisis. Peritoneal dialysis was required followed by renal transplantation at 6 years of age. Compound heterozygous mutations (nonsense and missense) in the *RPGRIP1L* gene were identified in this patient (table 1) and his affected brother who required kidney transplantation but has no clinical evidence of liver disease at 16 years of age (table 2).

COACH syndrome cohort

In our JSRD cohort of 251 families, 26 subjects (14 male, 12 female) in 23 families met criteria for COACH syndrome, defined as JSRD plus clinically apparent liver disease (see Methods). Most of the subjects were ascertained in the USA (European, Asian, African, and Native American descent), with one family each from Italy, the Netherlands, Germany, the UK, and Turkey. No families were reported to be consanguineous. The mean age of last ascertainment was 9 years with a range of 0–22 years. Developmental delay/intellectual disability, hypotonia and abnormal eye movements were invariant features. Assignment of CHF was made by histopathological means in 18 of 26 cases (63%). The DPM was reported in 1 of 24 subjects (4%), with cirrhosis or chronic hepatitis observed in two of 24 subjects (8%). Pathological features of cholangitis were not seen in any subjects. Portal hypertension developed in four subjects (17%) and hypersplenism in one (4%) with portosystemic shunting required in one (4%), and liver transplantation in two (8%). No deaths thus far have resulted from liver disease.

Colobomata were present in 17 subjects (71%) and renal disease was present in 10 subjects (42%): five with nephronophthisis (or likely nephronophthisis) and six with macrocystic kidney disease. Chronic renal insufficiency or end stage renal disease (ESRD) occurred in three (13%) and renal transplantation was required in two (8%), with no deaths from renal complications. Additional features included encephalocele (4%), abnormal respiratory control (80%), hypoplasia/agenesis of the corpus callosum (8%), ptosis (25%) and intestinal malrotation (8%). No subjects had polydactyly or retinal dystrophy.

Mutation analysis

The identification of *MKS3, CC2D2A* and *RPGRIP1L* mutations in JSRD patients with liver fibrosis prompted us to evaluate all of our COACH patients for mutations in these three genes. In subjects who did not have conclusive *MKS3* mutations, we sequenced *CEP290*, since *CEP290* mutations have been associated with both MKS and JSRD. These probands (UW52, UW58, UW59 and UW72) did not have mutations in *CC2D2A, RPGRIP1L,*

CEP290, AHI1 nor deletion of the *NPHP1* gene. One proband (UW66) lacked mutations in *MKS3, CC2D2A, RPGRIP1L, CEP290, AHI1* and deletion of the *NPHP1* gene.

MKS3

Affected subjects in 16 pedigrees (70%) had two mutations in the *MKS3* gene, while one family carried two possible mutations (see below), and single mutations were found in an additional three subjects in two families (table 1). The 17 subjects with two mutations had either: (1) two missense mutations (n=8); or combinations of (2) one missense and one nonsense mutation (n=4); (3) one missense and one splice site mutation (n=4); or (4) one nonsense mutation and one possible splice site mutation (n=1). The splice site mutation in UW05 (c.1674+3A>G) results in the use of a cryptic splice site 124 nucleotides within intron 16, creating a stop codon one amino acid after exon 16. We were not able to directly assess the functional significance of the splice site changes in UW59 (intron 25, c. 2661+5G>A) and UW57 (intron 9, c.978+3A>G) because we do not have cell lines from these subjects; however, the mutations greatly decrease the predicted strength of the donor sites in multiple splice site modelling programs (see Methods).

Several subjects did not have conclusive mutations in both copies of the *MKS3* gene. In UW58, the K99N change is not a known polymorphism, is not seen in >192 control chromosomes, and is conserved throughout mammals, platypus and opossum; however, it is predicted to be benign by PolyPhen.[42] The splice site change did not alter the splicing of exon 22 in a lymphoblastoid cell line, raising additional concern that loss of *MKS3* function is not the cause in this proband. In the two probands with single mutations, we identified a frameshift mutation that truncates the last 61 amino acids of the protein (UW52) and a M252T change (UW72), previously reported in three MKS pedigrees, two of which carried a second truncating mutation and manifested polydactyly.[31 33]

To determine whether *MKS3* mutations were more prevalent in COACH subjects compared to JSRD subjects without liver disease, we sequenced *MKS3* in probands from our entire cohort of JSRD families without liver disease, excluding 41 families with causal mutations in other JSRD genes (n=168). We identified mutations in four subjects from two families for a prevalence of ~1% in JSRD families lacking evidence of CHF (table 2). Previously unreported changes in residue 82 (p.P82R and p.P82S) were seen in both of these families in *trans* with p.M252T in UW85 and a frameshift p.T193Tfs14X in UW86. In our entire JSRD cohort, regardless of the presence or absence of liver disease, 9% (21/232) of families had *MKS3* mutations. In contrast to the high correlation between liver disease and *MKS3* mutation status, *MKS3* mutations were identified in 16/30 (53%) families with coloboma, irrespective of liver disease: 15/16 (94%) COACH families with coloboma carried at least one *MKS3* mutation, while only 1/14 (7%) families with coloboma but lacking clinically apparent liver disease carried at least one *MKS3* mutation.

MKS3 mutations were distributed throughout the protein (figure 3), with the majority of the missense mutations located in the N-terminal extracellular domain. Of the 20 missense mutations identified in *MKS3* probands, nine were novel (P82R, P82S, K99N, R172Q, M257V, R441C, P485S, Q841P, F942C). Although Q376E is a unique substitution, Q376P has been reported earlier.[37] These novel missense mutations were predicted to be either possibly or probably damaging by PolyPhen[42] and were excluded as rare polymorphisms based on testing at least 210 control chromosomes. When possible, familial segregation of mutations (n=7) to exclude *in cis* inheritance of a variant were confirmed (table 1). Five missense mutations, M252T (n=2), L349S (n=2), Y513C (n=2), C615R (n=2), and I833T (n=4) were recurrent within the cohort. The phenotype of subjects with *MKS3* mutations does not differ markedly from the subjects without *MKS3* mutations in our cohort.

CC2D2A and RPGRIP1L

The affected subjects in UW49 and UW67 had truncating *CC2D2A* mutations in combination with missense mutations (table 1). Discordance for liver disease between siblings was observed in UW04, where a combination of missense and nonsense mutations in *RPGRIP1L* was identified as being causative; however, only one sibling had a liver biopsy as part of his evaluation before renal transplantation. The phenotype of these subjects is indistinguishable from the subjects with *MKS3* mutations; however, all three COACH subjects with *CC2D2A* and *RPGRIP1L* mutations manifested renal disease. In contrast to *MKS3*, the prevalence of *CC2D2A* mutations was similar in the COACH cohort (2/23–9%) and the entire JSRD cohort (17/183–9%). Likewise, the prevalence of *RPGRIP1L* mutations was similar in the two cohorts (1/23–4% *vs* 4/174–2%).

Phenotypic spectrum of COACH patients

A comprehensive review of the medical literature, using the same criteria for COACH syndrome, revealed 43 patients with COACH syndrome from 26 families, including 21 males, 13 females, three fetuses of unknown gender, and 13 sets of siblings (table 3).[1 3 22 26 39 47_61] Two terminated fetuses assigned a Joubert syndrome diagnosis by Baala *et al* had cerebellar vermis hypoplasia, enlarged microcystic kidneys and hepatic fibrosis determined by autopsy.[22] The mean age at last follow-up of patients in the literature was 14.5 years (range 0–46 years) versus 8.9 years (range 0–22 years) for our cohort. *MKS3* mutations were reported in 19 subjects from 11 families, with all but two subjects from one family having two mutations. Additionally, *RPGRIP1L* mutations were reported in two subjects (only one mutation was identified in one of the two subjects). Mutation testing was not reported in the remaining 22 subjects in 16 families.

We compared the frequency of clinical findings/complications in our cohort of COACH patients (this work) to all reported COACH patients (literature) and all reported COACH patients, with and without *MKS3* mutations (table 4). Despite the substantial ascertainment bias and differential reporting of clinical features, this analysis gives some sense of the frequency of complications seen in COACH syndrome. The results for the literature as a whole are biased toward COACH syndrome caused by *MKS3* mutations, since Brancati *et al*[39] describe their eight families with *MKS3* mutations and not the six families without *MKS3* mutations. Combining our COACH patients with *MKS3* mutations with the similarly ascertained patients described by Brancati *et al*[39] provides estimates of features/complications in this select group. Our cohort provides the only estimate of complications in JSRD patients with *MKS3* mutations independent of liver disease status, since Brancati *et al*[39] did not report *MKS3* mutation analysis in their JSRD patients without clinically apparent liver disease.

The liver disease in our cohort encompassed multiple outcomes ranging from asymptomatic elevation of liver enzymes to organomegaly and portal hypertension and is comparable to patients described in the literature. The renal disease in patients with and without *MKS3* mutations ranged from prenatal onset MKS-like cystic renal dysplasia[31 33] to an infantile onset ARPKD-like presentation,[62] to early childhood onset cystic dysplasia,[39] and to late childhood/adult onset nephronophthisis.[22] Severe liver and renal complications (death, porto-systemic shunt, dialysis or transplantation) were some-what more frequent in the literature, possibly due to the older age at last ascertainment, preferential reporting of more severe cases with renal disease,[50 53 56 58 59] differences in medical practice, and/or the highly variable nature of disease progression.[16] The frequency of coloboma (64% *vs* 71%), encephalocele (0% *vs* 7%), polydactyly (0% *vs* 5%), and ptosis (16% *vs* 25%) were similar across groups.

DISCUSSION

Summary of main findings

We have identified the genetic bases of COACH syndrome (JSRD with liver disease) in the largest cohort of such patients published to date, finding mutations in all but one of 23 families. Mutations in *MKS3* account for the vast majority of COACH subjects, while *CC2D2A* and *RPGRIP1L* mutations account for a minority. By contrast, *MKS3* mutations were uncommon in JSRD subjects lacking clinically apparent liver disease. Furthermore, only 2/20 families (4/23 subjects) with JSRD due to *MKS3* mutations did not have liver disease, confirming this highly specific genotype-phenotype correlation. Brancati *et al*[9] described a 57% prevalence of at least one *MKS3* mutation in their cohort of 14 similarly ascertained families, giving a prevalence of 73% in our combined cohorts which were ascertained in a similar manner. Based on our observations, mutations in *MKS3* are rarely seen in JSRD patients without liver disease; however, the full phenotypic spectrum of *MKS3* related ciliopathy may be even broader, given the report of a patient with a homozygous *MKS3* mutation but minimal neurological involvement.[62] Although patients with liver involvement may be somewhat over-represented in our cohort, *MKS3* mutations account for 9% of our JSRD subjects irrespective of liver involvement, on par with the other major JSRD genes *AHI1, CC2D2A* and *CEP290.*

The striking association of *MKS3* mutations with liver disease in JSRD contrasts with the less clear-cut genotype–phenotype correlations observed for other JSRD genes that have hindered selective gene testing. Among these correlations, retinal dystrophy is seen in most subjects with *AHI1* mutations, many with *CEP290* mutations and few with *RPGRIP1L* mutations.[17 19–21 29 63 64] Renal disease is seen in virtually all subjects with *NPHP1* deletions, many subjects with *RPGRIP1L* and *CEP290* mutations, but very few with *AHI1* mutations.[25 65–68] Other features may help distinguish patients with *MKS3* mutations, since we observed coloboma and renal disease in substantial subsets of our subjects, while polydactyly and retinal disease were absent. These findings have clear clinical implications for JSRD patients with liver disease: *MKS3* should be tested first, followed by *CC2D2A* and possibly *RPGRIP1L.*

MKS3 mutations in JSRD versus MKS

The spectrum of *MKS3* mutations differs between JSRD and MKS subjects. We and others have identified single *MKS3* mutations in both JSRD and MKS, consistent with the presence of second non-coding mutations in *MKS3* versus the possibility of oligogenic inheritance seen in other ciliopathies such as Bardet–Biedl syndrome and nephronophthisis.[69–72] Many subjects with JSRD/COACH carry compound missense mutations, while this has been reported in only one subject with MKS.[22] Both families with JSRD due to *MKS3* mutations, but no clinically apparent liver disease, have mutations that alter the proline at residue 82; however, the significance of this finding is unclear and will need to be evaluated in additional patients. MKS is typically caused by combinations of missense and truncating mutations or homozygous splice site mutations.[22 31 33 37] No JSRD and MKS subjects have the same combination of mutations and only two nonsense mutations (R208X, R451X) and a single missense mutation (L349S) are shared between the cohorts. These data suggest that MKS may be caused by more severe loss of *MKS3* function than JSRD.

COACH phenotypes

We report clinical features of all patients with COACH syndrome in a cohort of JSRD patients ascertained only by brain imaging findings, in contrast to case series in the literature that ascertained patients specifically for brain, liver and sometimes renal involvement. Brancati *et al*[9] report a relatively large number of families ascertained by brain imaging

findings, but only subjects with *MKS3* mutations are described in detail. The reported complications from portal hypertension with potentially fatal variceal bleeding highlight the need for close monitoring of these patients.[3 50 52 55 57 73] The presence of liver disease associated with mutations in multiple JSRD genes (*MKS3, CC2D2A, RPGRIP1L* and *CEP290*) raises the question of whether subclinical liver involvement is present in many more subjects with JSRD, and if present, whether subclinical disease ever becomes clinically significant.

Pathological findings in the livers of the three subjects with *MKS3, CC2D2A* and *RPGRIP1L* mutations were compatible with the variable changes seen in CHF, including an excess of bile duct profiles and progressive portal fibrosis in the subject for whom serial biopsies were available. Although cirrhosis/chronic hepatitis is not typical for CHF the cirrhosis reported in 2/40 postnatal COACH subjects may be due to prior cholangitis, a known complication of CHF, (described in 4/40 COACH patients) versus misclassification of the clinical biopsy findings. The proposed ciliary function for *MKS3, CC2D2A* and *RPGRIP1L* supports a unified underlying pathophysiology for liver disease in JSRD and MKS.

Coloboma is not an invariant feature of COACH patients defined by the presence of the MTS and liver disease, and in this regard diverges from the original specification of COACH syndrome.[1] Nonetheless, in our cohort, coloboma was associated with liver disease (50% of the time) and *MKS3* mutations (53% of the time). These associations are also present in the cohort described by Brancati *et al.*[39] Therefore, monitoring for liver disease and *MKS3* testing are indicated in all JSRD subjects with coloboma.

Nephronophthisis and larger, mixed size renal cystic disease appear indistinguishable at the histopathological level,[74] consistent with a renal *MKS3* spectrum. Direct comparison with the literature cohort is also problematic as juvenile nephronophthisis manifests with renal failure at a mean age of 13 years,[75] close to the mean age of the literature cohort, versus our UW-MKS3 cohort mean (8.9 years). Overall, 12% of the combined cohorts of comparably ascertained *MKS3* cases had chronic renal failure or ESRD, with one subject receiving a renal transplant (3% prevalence). Due to the high risk of renal disease, COACH syndrome patients should be closely monitored for renal disease regardless of mutation status.

The prevalence of ptosis in our study is consistent with the prior reports as well as brainstem abnormalities observed in autopsies of JSRD subjects.[4 46] The low prevalence of encephalocele in COACH patients contrasts with the 60–90% reported in MKS patients.[10–13] The surprising lack of polydactyly in COACH syndrome may reflect the stronger association of polydactyly with *MKS1* mutations (versus *MKS3* mutations) seen in MKS.[31 33]

Conclusion

We have identified the genetic cause for the majority of JSRD patients with liver involvement, also known as COACH syndrome. Pre-symptomatic, gene based diagnosis of *MKS3* mutations should make it possible to deliver improved medical and surgical care for the liver and renal complications associated with substantial morbidity and mortality. Assuming that our JSRD cohort reflects JSRD in the population, patients with known liver involvement should be tested first for *MKS3* mutations, followed by *CC2D2A* and *RPGRIP1L*.

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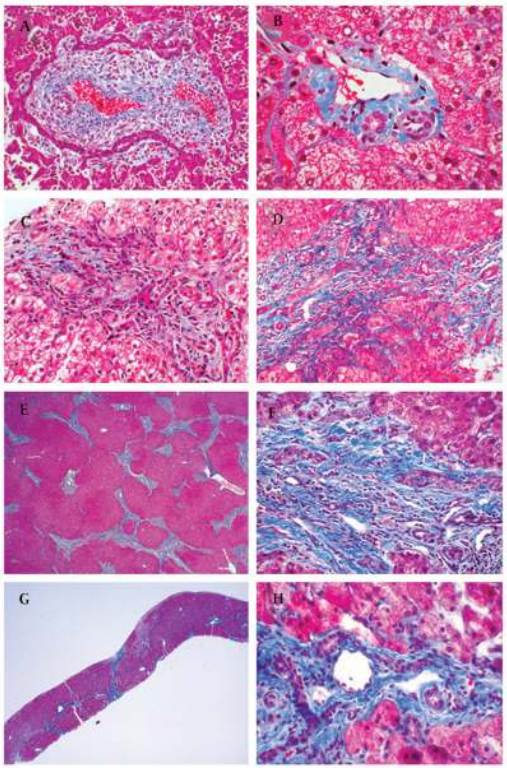

Figure 1.

Liver biopsy findings in patients with COACH syndrome. (A) Ductal plate malformation, fetus with MKS: the portal triad with ductal plate malformation has a ring of irregularly shaped and dilated bile duct structures that parallel the limiting plate. Trichrome, 150×. (B) Normal liver, 4 years: the portal triad contains a bile duct (lower left lumen), hepatic artery (lower right lumen) and portal vein (upper lumen) within a minimal amount of supporting fibrocollagen. Trichrome, 300×. (C) UW65, 11 months: the portal triad is expanded by minimal fibrosis and too many duct profiles, located both at the limiting plate and within the connective tissue. Trichrome, 200×. (D) UW65, 9 years: the portal triad has moderate fibrosis and an excess of bile ducts and hepatic artery branches with only minimal inflammation. Trichrome, 200×. (E) UW49, 10 years: the explanted liver has dense portal fibrosis that extends between triads and focally forms nodules. Trichrome, 20×. (F) UW49, 10 years: a portal triad in the explant shows moderate fibrosis and several irregular bile duct profiles. Trichrome, 300×. (G) UW04-3, 5 years: the liver biopsy has intact lobular architecture with portal fibrosis and occasional septa bridging portal areas. Trichrome, 20×. (H) UW04-3, 5 years: a representative portal area shows several profiles of bile ducts and hepatic artery branches within dense fibrocollagen; central clear lumen is a normal portal vein branch. Trichrome, 300×.

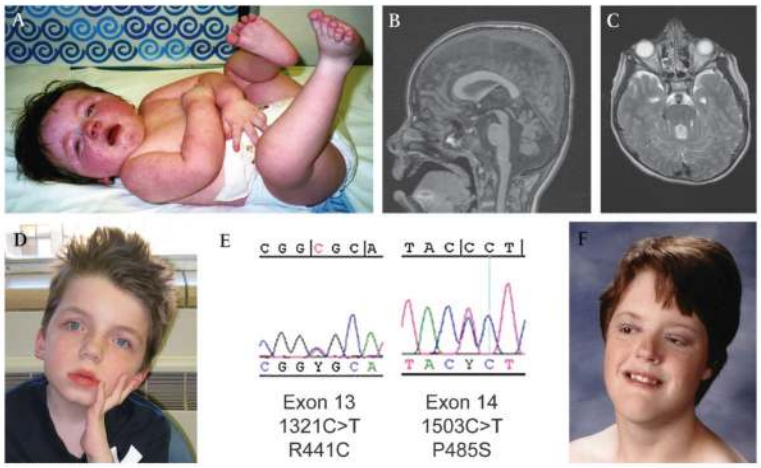

Figure 2.

Facial features, brain imaging and mutations for UW65 and UW49. (A) UW65 at 11 months of age. (B and C) Sagittal T1 weighted and axial T2 weighted brain magnetic resonance images demonstrating the molar tooth sign. (D) UW65 at 9 years of age. (E) *MKS3* sequence tracings for UW65. (F) UW49 at 20 years of age. Photographs printed with permission from the families.

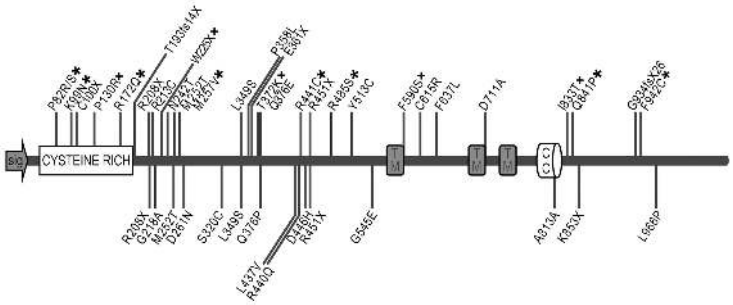

Figure 3.

MKS3 mutations in COACH and Meckel syndromes. *MKS3* encodes a 995 amino acid predicted protein with a signal peptide (sig), cysteine rich region, three transmembrane (TM) domains and a coiled-coil (CC) domain. Mutations identified in JSRD/COACH patients are listed above the protein diagram while mutations found in MKS patients are listed below. Previously unreported mutations are indicated with an asterix (*) and mutations also observed by Brancati *et al*[9] are indicated with a plus sign (+).

Table 1

Phenotypes of COACH subjects with *MKS3, RPGRIP1L* and *CC2D2A* mutations

Pedigree	Mutations	Amino acid	Age/sex	CNS	Liver	Col	RD	Renal	Other
MKS3									
UW51	c.1046T>C c.2498T>C	p.L349S p.I833T	21y/M	MTS (CT)	ELE(18 mo), PH(12y) Bx(1y,12y): BDA, prog CHF *Tx(17y)*	+	–	–	AB, ptosis
UW54	c.675G>A c.389C>G	p.W225X p.P130R	11y/F	MTS	ELE(9y)	+L	–	–	AB, ptosis, small stature
UW56	c.515G>A c.769A>G	p.R172Q p.M257V	17y/M	MTS	ELE(10y), PH(15y) Bx(12y): BDA, CHF *ursodiol*	+R	–	–	–
UW57	c.2825T>G c.978+3 A>G	p.F942C Spl	2y/M	MTS	ELE(<2y)	+	–	–	AB Micropenis
UW60	c.1046T>C c.1843T>C	p.L349S p.C615R	3y/F	MTS	ELE(3y)	–	–	–	AB
UW61	c.2522A>C c. 622A>T	p.Q841P p.R208X	5y/F	MTS	ELE(3y) Bx(3y): CHF	+	–	MKD	AB
UW62	c.1115C>A c.1115C>A c.2322+3 Ins(T) [*†]	p.T372K Spl	10y/F	MTS	HSM(6 m) Bx(6 m): BDA, DPM, CHF	–	–	NPH	AB
UW63	c.2498T>C c.1351C>T	p.I833T p.R451X	14y/F	MTS	Bx(14y): BDA, CHF	+	–	CRF, NPH, *ESRD(13y)* *Tx(14y)*	AB, anterior anus
UW64	c.300C>A c.2498T>C	p.C100X p.I833T	4y/F	MTS	HSM(2y) Bx(2y): BDA,CHF *ursodiol*	+	–	–	AB
UW65	c.1321C>T c.1453C>T	p.R441C p.P485S	9y/M	MTS	ELE, HSM(11 mo), SM(9y) Bx(11 mo, 9y): BDA, CHF (fig 1C,D)	+	–	–	Hypogonadism
UW05	c.1126C>G c.1674+3A>G [‡]	p.Q376E Spl	14y/F	MTS	ELE, abn US, PH(13y) Bx(14y): CHF *PSS(14y)*	+	–	–	AB Fetal sibling: MKD
UW30-4	c. 1073T>C c. 1911A>C	p.P358L p.F637L	Birth/F dec	–	Ax: HSM, BDA	NA	NA	MKD	Dysmorphic
UW30-3	c. 1073T>C c. 1911A>C	p.P358L p.F637L	1 m/M dec	MTS	NA (Ax not done)	+	_ [§]	MKD	AB
UW30-5	c. 1073T>C	p.P358L	18 w/M	CVH	Ax: immature portal fields without evident ductal plate malformation	NA	NA	MKD	Dysmorphic
	c. 1911A>C	p.F637L	fetus	EC					
UW83	c.1438A>G c.2497T>C	p.Y513C p.I833T	8y/F	CVH	ELE(4y)	+L	–	–	AB, ptosis

Pedigree	Mutations	Amino acid	Age/sex	CNS	Liver	Col	RD	Renal	Other
									sib: 20 wk M fetus CVH
UW84	c.725A>G c.725A>G	p.N242T p.N242T	21y/M	MTS	HSM(7y), PH, HSP(8y) Bx(7y): CHF *Rx*	–	–	CRF(16y) Bx(16y): ?NPH *Rx*	Ptosis
UW73	c.1538A>G c.1843T>C	p.Y513C p.C615R	4y/M	MTS	ELE, HSM(3y) Bx(4y): BDA, CHF	–	–	–	familial (paternal) balanced translocation
UW59	c.108G>T c.2661+5 G>A[*]	p.E361X Spl	4y/F	MTS	ELE(1y), Abn US(4y) Bx(3y): CHF	+	–	–	AB, ptosis, IM
UW58	c.297G>T[*¶] c.2322+3 Ins(T)[*†]	p.K99N Spl	9y/M	MTS	ELE(4y) Bx(5y): CHF	+	–	–	AB Seizures, dystonia
UW52-3	c.2802delA ?	p.G934GfsX26 ?	9y/M	MTS	ELE(18 mo)	+R	–	–	–
UW52-4	c.2802delA ?	p.G934GfsX26 ?	4y/M	MTS	ELE, HSM(3y)	+	–	MKD	AB, ASD/VSD
UW72	c.755T>C ?	p.M252T ?	5y/M	MTS	ELE(4y), abn US(5y)	+	–	MKD, CRF *HTN*	AB, small stature, hypothyroid
CC2D2A									
UW67	c.3145 C>T c.3347 C>T	p.R1049X p.T1116M	3y/M	MTS	ELE(2y) Bx(3y): CHF	–	–	EK *HTN*	AB
UW49[**]	c.3289delG c.4582C>T	p.V1097FfsX1 p.R1528C	22y/F	CVH/ACCHC	HSM (5y) Bx(6, 8, 10y): prog CHF (fig 1E,F) *Tx(11y)*	+	–	EK, NPH *HTN*	AB, ptosis
RPGRIP1L									
UW04-3[††]	c.2413C>T c.1975T>C	p.R805X p.S659P	17 y/M	MTS	Bx(5y): BDA, CHF (fig 1G, H)	–	–	EK Bx(5y):NPH *Tx(5y)*	AB
No mutations									
UW66	Negative	Negative	7.5y/F	CVH/ACC (CT)	Bx(>1y): CHF	–	–	–	AB, abn ears, IM, clubfoot

abn, abnormal; AB, abnormal respiratory control; ACC, agenesis of the corpus callosum; AT, cerebellar ataxia; Ax, autopsy; BDA, bile ductule abnormality (proliferation/persistence/dilation) or bile duct dilation on imaging/MRI; Bx, biopsy; C, cirrhotic features including hepatocellular damage/necrosis and evidence of regeneration; CB, cerebellum; CD, choreoathetosis/dystonia; CG, cholangitis; CH, chronic hepatitis; CHF, congenital hepatic fibrosis; CNS, central nervous system; Col, chorioretinal coloboma (bilateral unless otherwise stated); CRF, chronic renal insufficiency; CT, computed tomography scan; CVH, cerebellar vermis hypoplasia; dec, deceased; DPM, ductal plate malformation (classic ring); EC, encephalocele; EK, echogenic kidneys on US; ELE, elevated liver enzymes; ESRD, end stage renal disease; HC, hydrocephalus; HCV, hepatitis C; HD, haemodialysis; HM, hepatomegaly; HR, hyperreflexia; HSM, hepatosplenomegaly; HSP, hypersplenism; IM, intestinal malrotation; LK, large kidneys; MCM, mega cisterna magna; MKD, macrocystic kidney disease; MTS, molar tooth sign on brain MRI; MRI, magnetic resonance imaging; NA, not available/not specified/not known; NC, nephrocalcinosis; NPH, nephronophthisis; PD, polydactyly; PH, portal hypertension (includes complications of PH such as gastrointestinal bleeding/varices); PSS, portosystemic shunt (includes splenorenal, transjugular intrahepatic portosystemic shunts); RD, retinal dystrophy; RMC, renal microcystic disease; Rx, medications; Spl, predicted splicing defect; SZ, seizures; SK, small kidneys; SM, splenomegaly; Tx, transplant; US, ultrasound; V, ventricle(s).

All UW subjects had developmental delay/mental retardation, hypotonia and/or ataxia, oculomotor apraxia.

Age refers to age at last contact in years (y), months (m) or weeks gestation (w).

Laterality of manifestations is stated as either; R, right; L, left; or bilateral unless otherwise stated.

Outcome details are italicised and appear in the lower portion of the cell.

[*] Not seen in >192 control chromosomes.

[†] splicing of exon 22 was not affected in a lymphoblastoid cell line from UW58.

[‡] results in the use of a cryptic splice within intron 16 and a premature termination codon one amino acid after exon 16.

[§] fundus flavus noted on retinal exam.

[¶] predicted to be benign by Polyphen and SIFT (see text).

[**] previously published in Gorden *et al* (2008).

[††] brother without liver disease is listed in table 2.

Table 2

Phenotype of Joubert syndrome related disorder (JSRD) subjects with *MKS3* and *RPGRIP1L* mutations lacking clinically apparent liver disease

Pedigree	Mutations	Amino acid	Age/sex	CNS	Liver	Col	RD	Renal	Other
MKS3									
UW85-1	c.245C>G c.755T>C	p.P82R p.M252T	26y/M	MTS	–	+R	–	–	Transient ELE (valproate)
UW85-2	c.245C>G c.755T>C	p.P82R p.M252T	22y/M	MTS	–	+R	–	–	Ptosis
UW85-3	c.245C>G c.755T>C	p.P82R p.M252T	19y/M	MTS	–	+R	–	–	–
UW86	c.579_80delAG c. 244C>T	p.T193Tfs14X p.P82S	2y/F	MTS	–	–	–	–	–
RPGRIP1L									
UW04-4 *	c.2413C>T c.1975T>C	p.R805X p.S659P	16y/M	MTS	–	–	–	EK Bx(7y):NPH *Tx(7y)*	AB, ptosis

See table 1 for key to abbreviations.

All UW subjects had developmental delay/mental retardation, hypotonia and/or ataxia, oculomotor apraxia.

Age refers to age at last contact in years (y), months (m) or weeks gestation (w).

Laterality of manifestations is stated as either; R, right; L, left; or bilateral unless otherwise stated.

Outcome details are italicised and appear in the lower portion of the cell.

* Brother with liver disease is listed in table 1.

Table 3

Phenotype and mutations in COACH subjects reported in the literature

Reference	Mutations*	Amino acid*	Age/sex	CNS	Liver	Col	RD	Renal	Other
Hunter 1974, case 1	NA	NA	12y/F	AT	HM(4y) PH(11y) Bx(4y): BDA, CHF, CG PSS(>11y)	+	–	NPH(11y)	AB, post-axial PD, CD
Hunter 1974, case 2	NA	NA	9y/M	AT	HSM(14 m) PH(6y) Bx(14 m): CHF	+	–	NPH, CRF	AB, CD
Thompson 1986, case 1	NA	NA	23y/F	NA	PH(8 m) Bx(14y): BDA, CHF PSS, splenectomy(14y)	+	–	abn US: SK, CRF, ? NPH(21y)	AB, CD
Thompson 1986, case 2	NA	NA	18m/F	EC, MCM lg CB lg 4th V	Bx/Ax(18 m): CHF	–	–	Ax(18 m): NPH	AB, SZ, HR dec:pneumonia(18 m)
Verloes 1989, case 1†	c.2498T>C c.G2556+1G>T	p.I833T Spl	32y/M	CVH(CT)	HSM(<1y) ELE Bx(7,11y) : BDA, CHF, CG PSS(11y), dec: PH(32y)	–	–	CRF, NPH Bx(11y) Diet	AB, HR, club foot Small stature
Verloes 1989, case 2†	c.2498T>C c.G2556+1G>T	p.I833T Spl	37y/F	CVH(CT)	HSM(13y), PH(36) Bx(11y): CHF, CH PSS(30y)	+L	–	CRF, NPH *HD* Tx (20's)	HR
Verloes 1989, case 3	NA	NA	8y/M	Dilated 4th V, MCM(CT)	HSM(15 m) Bx(1,5y): BDA, CHF, CH	+	–	CRF(8y), ?NPH	–
Wiesner 1992, case 1	NA	NA	34y/M	CVH	ELE(34y) Bx(34y): BDA, CHF Rx: ursodiol	+	–	–	Deaf
Wiesner 1992, case 2	NA	NA	46y/F	AT	PH(NA) Bx/Ax(46y): BDA, CHF dec: PH(46y)	NA	NA	Ax: NPH	–
Lewis 1994, case 1	NA	NA	5y/F	MTS(CT)	HM(3 m),HSM(3y) PH(4y) Bx/Ax(3y): BDA, CHF, focal DPM dec: PH(5y)	+	+(4 m)	CRF(5y) Ax; NPH	dec: PH + CRF(5y)
Lewis 1994, case 2	NA	NA	17m/M	CVH(CT)	HM(6 m) Bx(1y): BDA, CHF	+	+ (12 m)	EK ?NPH(17 m)	AB, HR
Gentile 1996, case 1†	c.1769T>C c.G1961-2A>C	p.F590S Spl	16y/M	CVH	HSM(2y), ELE, PH(10y)	–	–	–	–

Reference	Mutations*	Amino acid*	Age/sex	CNS	Liver	Col	RD	Renal	Other
					Bx(7y, 10y): BDA, CHF Tx(12,16y), dec: HCV hepatitis(16y)				
Gentile 1996, case 2†	c.1769T>C c.G1961-2A>C	p.F590S Spl	17y/M	MTS	HM(18 m), ELE, PH, HSP Bx(30 m): CHF platelet transfusion PH, Rx	–	–	–	–
Kumar 1996, case 1	NA	NA	23y/F	AT	HSM(21y), PH(22y), HSP(23y) Bx(23y): BDA, CHF PSS	+	–	CRF(22y) Bx: NPH(23y)	–
Kumar 1996, case 2	NA	NA	6y/F	CVH, elevation 4th V	HM, ELE(5y)	+	–	–	–
Barzilai 1998	NA	NA	19y/F	MTS	ELE, HSM, PH, HSP(7y) Bx(7y): BDA, CHF	+	–	NPH(7y)	CD
Kirchner 2002	NA	NA	20y/F	CVH	ELE, HM(9y), PH, HSP(20y) US(20y): liver nodules/fibrosis Bx(11y): CHF, C regenerative nodules	+	–	–	–
Coppola 2002, case 1†	c.579_80delAG c.1769T>C	p.G195IfsX13p.F590S	10y/M	MTS	ELE(8y), abn US(10y) Bx(10y): BDA, CHF Rx: ursodiol	–	–	–	–
Coppola 2002, case 2†	c.579_80delAG c.1769T>C	p.G195IfsX13 p.F590S	3y/F	MTS	Abn US Bx(3y): CHF Rx: ursodiol	–	–	–	–
Coppola 2002, case 3	NA	NA	18y/M	CVH(CT)	PH(13y) Bx(13y): CHF, CG sclerotherapy	–	–	EK(13y), ?NPH	dec: PH(18y)
Herzog 2002	NA	NA	18y/F	CVH	ELE, PH, HSP(8y) Bx(8y): BDA, CHF Tx(12y)	+	–	CRF(12y)	–
Gleeson 2004, case 3	NA	NA	7y/M	MTS	ELE(3y), PH(5y) Bx(5y): BDA, CHF sclerotherapy	+	–	EK, NC, NPH(5y)	AB
Dieterich 1980, case 1	NA	NA	6y/F	CVH(CT)	HM(5y) Bx(5y): BDA, CHF	+	–	CRF Bx(5y): NPH dec: ESRD(6y)	–

Reference	Mutations*	Amino acid*	Age/sex	CNS	Liver	Col	RD	Renal	Other
Dieterich 1980, case 2	NA	NA	6y/F	AT	PH(6y) ?Ax/Bx: CHF(6y) dec: PH(6y)	+	–	CRF, ?NPH	–
Uemura 2005, case 1	NA	NA	38y/F	AT	PH, ELE(18y), PH(22y), Bx(21y): BDA, CHF, *PSS,* *splenectomy, Tx(28y)*	+	–	CRF, ?NPH	–
Uemura 2005, case 2‡	NA	NA	21y/F	MTS	SM(10y) Bx(10y): CHF PSS	+	–	EK, NC, ?NPH	–
Brinkman 2004§	NA	NA	28y/M	MTS	ELE, US(22y): fibrosis	+	–	NPH(22y) ESRD(25y)	AB, HR, Burkitt lymphoma (ileal)
Graber 2001, case 2	NA	NA	15m/M	MTS	HM(birth) Bx(>1y):CHF	+	+	abn US(>15 m), NPH	–
Baala 2007 JS-661	c.1538A>G c.2315_23+4 del13insGG	p.Y513C Spl	28w fetus	CVH(Ax)	Ax(28w EGA): BDA, CHF	NA	NA	RMC, ?NPH	Termination
Baala 2007 JS-660	c.1538A>G c.2315_23+4 del13insGG	p.Y513C Spl	30wfetus	CVH(Ax)	Ax(30w EGA): BDA, CHF	NA	NA	RMC, ?NPH	Termination
Baala 2007 JS-786	c. 637C>T c. 2132A>C	p.R213C p.D711A	7y/M	CVH(CT)	“severe liver disease”	–	–	EK, RMC, ?NPH	AB
Wolf 2007 A1183 II-1	RPGRIP1L:	Spl	7y/M	CVH	PH(7y)	–	–	EK	AB, pituitary agenesis, abdominal myofibroblastic tumour
	c.3497-2A>G ?	?			Bx(7y): CHF			Bx: NPH	
Wolf	*RPGRIP1L*: c.1177G>A	p.E393K	29y/M	–	Bx(29y): CHF	+	–	EK, SK, NPH	–
2007 F631 II-1	?	?						ESRD(21y)	
Brancati 2009 COR32-1	c.1115C>A c.2345A>G	p.T372K p.H782R	6y/M	MTS	HM, BDA(MRI)	+	–	–	AB Unilateral renal agenesis
Brancati 2009 COR32-2	c.1115C>A c.2345A>G	p.T372K p.H782R	1y/M	MTS	ELE	+	NA	–	–
Brancati 2009 COR71	c.389C>G c.675G>A	p.P130R p.W225X	20y/F	MTS	HM, ELE Bx: CHF	+	–	NPH ESRD	HR, small stature
Brancati 2009 COR94	c.1319G>A c.2182A>G	p.R440Q p.S728G	3y/M	MTS	HM, ELE Bx: CHF	+	–	–	AB, CD, HR, small stature
Brancati 2009 COR190	c.G312+5G>A c.2498T>C	Spl p.I833T	9y/F	MTS EC	HM, ELE, BDA	–	–	NPH, CRF	AB

Reference	Mutations*	Amino acid*	Age/sex	CNS	Liver	Col	RD	Renal	Other
Brancati 2009 COR191-1	c.2460A>C ?	p.R820S ?	9y/M	MTS	HM, ELE Bx: CHF	–	–	NPH, ESRD	AB, HR
Brancati 2009 COR191-2	c.2460A>C ?	p.R820S ?	fetus	CVH EC	Ax: CHF	–	NA	MKD	PD
PKD238 Gunay-Aygun 2009	c.224-2A>T c.1843T>C	p.C615R	8y/M	MTS	ELE(5y), HSM(7y)	–	–	LK, EK(fetal)	–
		Spl			Bx(7y): BDA, CHF			RMC(birth) CRF, HTN	
PKD271 Gunay-Aygun 2009	c.1843T>C c.1843T>C	p.C615R p.C615R	6y/F	AT	ELE	–	–	MKD(fetal), CRF, HTN	–
PKD272 Gunay-Aygun 2009	c.1843T>C c.1843T>C	p.C615R p.C615R	10y/M	MTS	ELE Bx(5y): BDA, CHF CG(5y)	–	–	HTN, CRF, NPH Bx(8y): RMC Tx(8y)	–

See table 1 for key to abbreviations.

All UW subjects had developmental delay/mental retardation, hypotonia and/or ataxia, oculomotor apraxia.

Age refers to age at last contact in years (y), months (m) or weeks gestation (w).

Laterality of manifestations is stated as either; R, right; L, left; or bilateral unless otherwise stated.

*All mutations are in MKS3 except for the two families in Wolf 2007 as noted.

†also in Brancati *et al.*[39].

‡unpublished information C Clericuzio.

§also in Lindhout *et al.*[60]

Table 4

Comparison of clinical features reported in COACH patients: our cohort versus all patients in the literature versus COACH patients with and without known *MKS3* mutations

	This work	Literature	Known *MKS3* mutations	No known *MKS3* mutations
Liver complications	21% (5/24)	40% (16/40)	22% (7/32)	47% (15/32)
Coloboma	71% (17/24)	64% (25/39)	66% (21/32)	68% (21/31)
Renal disease	46% (12/26)	77% (33/43)	39% (13/33)	88% (30/34)
Renal complications	8% (2/24)	22% (9/40)	12% (4/32)	22% (7/32)
Encephalocele	4% (1/26)	7% (3/42)	6% (2/33)	3% (1/33)
Ptosis	25% (6/24)	18% (7/40)	16% (5/32)	25% (8/32)
Polydactyly	0% (0/24)	5% (2/43)	3% (1/33)	3% (1/34)

Liver complications included porto-systemic shunt, transplantation and death, while renal complications included transplantation, dialysis and death. The denominators differ in some cases because liver complications, coloboma, renal complications and ptosis were not scored in all cases, particularly in fetuses.