Published in final edited form as:
Hum Mutat. 2009 August ; 30(8): E813–E830. doi:10.1002/humu.21057.

Mutation Spectrum of Meckel Syndrome Genes: One Group of Syndromes or Several Distinct Groups?

Jonna Tallila[1], **Riitta Salonen**[2], **Nicolai Kohlschmidt**[3], **Leena Peltonen**[1,4,5], and **Marjo Kestilä**[1]

[1]National Institute of Health and Welfare, Public Health Genomics Unit and FIMM, Institute for Molecular Medicine Finland, Helsinki 00290, Finland [2]Department of Medical Genetics, Väestöliitto, Helsinki 00100, Finland [3]Institut für Klinische Genetik Bonn, Bonn, Germany [4]Department of Medical Genetics, University of Helsinki, Helsinki 00290, Finland [5]The Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK

Abstract

Meckel syndrome (MKS) is a lethal malformation syndrome that belongs to the group of disorders that are associated with primary cilia dysfunction. Total of five genes are known to be involved in the molecular background of MKS. Here we have systematically analyzed all these genes in a total of 29 MKS families. Seven of the families were Finnish and the rest originated from elsewhere in Europe. We found 12 novel mutations in 13 families. Mutations in the MKS genes are also found in other syndromes and it seems reasonable to assume that there is a correlation between the syndromes and the mutations. To obtain some supportive information, we collected all the previously published mutations in the genes to see whether the different syndromes are dictated by the nature of the mutations. Based on this study, mutations play a role in the clinical phenotype, given that the same allelic combination of mutations has never been reported in two clinically distinct syndromes.

Keywords

Meckel syndrome; MKS; ciliopathy; *MKS1*; *TMEM67*; *CEP290*; *RPGRIP1L*; *CC2D2A*

INTRODUCTION

Meckel syndrome (MKS; MIM# 249000) is an autosomal recessive lethal disorder characterized by a combination of malformations. Death occurs already before or shortly after birth. Cystic dysplasia of the kidneys with fibrotic changes in the liver and an occipital encephalocele or other central nervous system malformations are considered as the minimal diagnostic criteria for Meckel syndrome [Salonen, 1984]. In addition, polydactyly is a frequent feature of the phenotype. It is possible to detect and diagnose MKS by ultrasound already at 11^{th} to 14^{th} weeks of gestation [Pachi et al., 1989]. Later during the pregnancy, oligohydramnion might make it more difficult to establish the diagnosis by ultrasound only. The identification of the disease causing genes has enabled the DNA based diagnostic for MKS. Five genes and six loci have been identified in MKS so far [Baala et al., 2007a; Delous et al., 2007; Kyttala et al., 2006; Roume et al., 1998; Smith et al., 2006; Tallila et al.,

*Correspondence to Marjo Kestilä, National Institute for Health and Welfare, Helsinki, Finland, E-mail: marjo.kestila@thl.fi.

Europe PMC Funders Author Manuscripts

2008] and the number will evidently increase since these genes explain only a fraction of known MKS cases. Identification of the disease genes and mutations has demonstrated that they are not restricted to certain populations and that MKS is an allelic disorder with Joubert syndrome, Leber congenital amaurosis, Senior-Loken syndrome, nephronophthisis and Bardet-Biedl syndrome, since all five of the identified genes *MKS1* (*BBS13*), TMEM67 (MKS3, JBTS6), CEP290 (MKS4, NPHP6, JBTS5, SLSN6, *LCA10, BBS14*), *RPGRIP1L (MKS5, JBTS7, NPHP8, CORS3*) and *CC2D2A* (*MKS6, JBTS9*) are known to be mutated also in other syndromes with overlapping clinical features. A common pathogenic pathway provides the molecular basis for the overlap, since all of these disorders are caused by primary cilia dysfunction and therefore termed as ciliopathies.

Joubert syndrome (JS; MIM#s 610688, 610188, 611560) is a clinically and genetically heterogeneous group of disorders characterized by hypoplasia of the cerebellar vermis with the characteristic neuroradiologic ‘molar tooth sign,’ and accompanying neurologic symptoms, including dysregulation of breathing pattern and developmental delay. Additional features sometimes associated with JS include retinal anomalies, polydactyly, hepatic fibrosis, and renal disease. This group of related disorders is often referred to as ‘cerebello-oculo-renal syndromes’ (CORSs). Several features overlap with the classic MKS features including polydactyly, fibrotic changes of the liver and CNS malformation (encephalocele). Seven genes behind Joubert syndrome and related disorders have been identified to date (*NPHP1*, *AHI1*, *CEP290*, *RPGRIP1L*, *TMEM67*, *ARL13B* and *CC2D2A*). In addition, there are two loci, where the disease causing gene is unknown [Gorden et al., 2008; Valente et al., 2008].

Leber congenital amaurosis (LCA; MIM# 204000) is generally inherited in an autosomal recessive manner and is characterized by a severe retinal dystrophy, causing blindness or severe visual impairment at birth or during the first months of life. LCA represents the most common genetic cause of congenital visual impairment in infants and children. Identification of 14 genes mutated in patients with LCA and juvenile retinal degeneration explain approximately 70% of the cases. Several of these genes have also been implicated in other non-syndromic or syndromic retinal diseases, such as retinitis pigmentosa and JS, respectively. *CEP290* is one of the most frequently mutated LCA genes; one *CEP290* mutation (p.Cys998X) is found in approximately 20% of all LCA patients from north-western Europe [den Hollander et al., 2008].

Nephronophthisis (NPHP; MIM# 256100) is a heterogenous group of autosomal recessive cystic kidney disorders and is one of the most common reasons for end stage renal disease in children and adolescents [Hildebrandt and Zhou, 2007]. Nine genes behind NPHP have been identified to date [Salomon et al., 2008]. NPHP may also be associated with other clinical features. Senior-Loken syndrome (SLSN; MIM# 610189) is also known as juvenile nephronophthisis with retinis pigmentosa (Leber amaurosis). It is an autosomal recessive renal-retinal disorder, characterized by progressive wasting of the filtering unit of the kidney, with or without medullary cystic renal disease, and progressive eye disease. Mutations for SLSN have been identified in the *NPHP* genes [Salomon et al., 2008].

Bardet-Biedl syndrome (BBS; MIM# 209900) is an important genetic cause of chronic and end-stage renal disease. It is a multi-system disorder, which consists of obesity, retinal degeneration, cognitive impairment, genitourinary tract malformations, and polydactyly. Correspondent to the variety of malformations, a number of genes behind BBS have been detected, and to date there have been reports of 14 disease causing genes [Leitch et al., 2008; Tobin and Beales, 2007].

Phenotypic overlap of the syndromes caused by mutations in the MKS genes is distinct, but significant differences can also be observed that makes this puzzle very challenging. As a lethal condition MKS represents the most severe end of the syndromes. The aim of this study was to identify disease causing mutations in MKS families where the mutations were not known. We collected all the reported mutations in *MKS* genes (Table 1) as well, since we were interested in seeing whether the different syndromes are associated with specific mutations in these genes.

SUBJECTS AND METHODS

Study Cohort

This study has been approved by the ethical committee of the Joint Authority of the Hospital District of Helsinki and Uusimaa, Finland. We had DNA samples from 22 non-Finnish MKS families in total. In some cases DNA was available only from the parents or from the affected fetus. In addition, we analyzed seven Finnish MKS families without *MKS1* or *CC2D2A (MKS6)* Fin_{major}-mutations. DNA samples were available from parents and affected fetuses in all these families. It was not possible to obtain exact clinical data from the cases included in this study, but all the samples have been sent to us with MKS diagnosis.

Mutation Analysis

Mutational screening of all the known *MKS* genes (*MKS1;* MIM# 609883; GenBank NM_017777.2, gi: 89242136), *TMEM67* (*MKS3;* MIM# 609884; GenBank NM_153704.5, gi: 214830731), *CEP290* (*MKS4*; MIM# 610142; GenBank NM_025114.3, gi: 109255233), *RPGRIP1L* (*MKS5*; MIM# 610937; GenBank NM_015272.2, gi: 118442833) and *CC2D2A* (*MKS6;* MIM# 612013; GenBank NM_001080522.2, gi: 197209973) was performed by direct sequencing of all the coding exons and exon-intron boundaries. Primers for amplifying the genomic DNA were designed using Primer3 software (http://frodo.wi.mit.edu/) and provided by Sigma-Genosys. Primer sequences are available upon request. The PCR reactions were done with AmpliTaq Gold and PCR products were treated with shrimp alkaline phosphatase (SAP) and Exonuclease I (Amersham) before sequencing. Sequencing was carried out using ABI BigDye chemistry with ABI3730 automated sequencer (Applied Biosystems, Darmstadt, Germany) and sequences were analyzed using Sequencher software (Gene Codes). Computational analyses of novel missense mutations were performed with PolyPhen (http://genetics.bwh.harvard.edu/pph/) and SIFT (http://blocks.fhcrc.org/sift/SIFT.html) softwares. Nucleotide numbering in Table 1 and Supp. Table S1 reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. The initiation codon is codon 1. All mutation descriptions were checked using the Mutalyzer software (http://www.humgen.nl/mutalyzer/1.0.1/) [Wildeman et al., 2008].

RESULTS

We identified mutations in 13 of the 29 families included in the study. Some of the detected mutations have been reported previously in MKS cases, but 12 of them were novel (Table 1). To help further studies and to see whether there is a genotype-syndrome correlation between different syndromes and mutations, we collected all the previously reported mutations in the *MKS* genes (Table 1 and Supp. Table S1). The novel mutations are presented in bold (Table 1). PolyPhen and SIFT predictions of amino acid substitutions are presented in Supp. Table S2.

MKS1

One Finnish MKS case (FIM 48) was a compound heterozygote for two likely pathogenic mutations that segregated in an autosomal recessive fashion in the family and have not been reported previously: maternal mutation is a two base pair deletion that leads to a stop codon (c.392_393delCT, p.Ser131X) and paternal mutation is an Arginine to Tryptophan substitution (c.496C>T, p.Arg166Trp). The amino acid change is predicted to be probably damaging by PolyPhen, but tolerated by SIFT.

TMEM67 (MKS3)

Mutations in the *TMEM67 (MKS3)* gene were present in four non-Finnish families. Altogether eight different mutations were found of which four were novel. In the first family (UM1) the fetus was a compound heterozygote for two novel amino acid changes located in exons 8 and 9 (c.734C>T, p.Ser245Phe and c.888G>T, p.Trp296Cys). Ser245Phe is possibly damaging by PolyPhen and not tolerated by SIFT. Trp296Cys is probably damaging by PolyPhen, but predicted to be tolerated by SIFT. In the second family (UM2) the fetus was a compound heterozygote for two amino acid changes, a novel Tyrosine to Cysteine substitution (c.161A>G, p.Tyr54Cys) and previously reported Tyrosine to Cysteine substitution (c.1538A>G, p.Tyr513Cys). The novel Tyr54Cys is probably damaging by PolyPhen and not tolerated by SIFT. In the third family (UM4) the father carried the Met252Thr change and the mother carried a novel Cys615Arg change (c.1843T>C, p.Cys615Arg), which is predicted to be probably damaging by Polyphen and tolerated by SIFT. In the fourth family (UM5) the fetus was a heterozygote for two previously identified amino acid changes (c.1319G>A, p.Arg440Gln and c.2897T>C, p.Leu966Pro). Except for family UM4, where the DNA sample from the fetus could not be analyzed, the segregation of mutations was confirmed in the families.

CEP290 (MKS4)

In one of the Finnish MKS families (FIM 221) we identified compound heterozygote mutations in the *CEP290 (MKS4)*, which segregated in the family. The first mutation was a novel deletion in exon 29 (c.3444_3445delAA, p.Lys1149fs) and the second mutation was a previously reported AT-deletion in exon 14 (c.1219_1220delAT, p.Met407fs). Furthermore, we detected mutations in *CEP290* in four non-Finnish families. In the first family (UM6) we identified two nonsense mutations, a novel G>T change in exon 5 (c.289G>T, p.Glu97X) and a C>T substitution in exon 20 (c.1984C>T, p.Gln662X) that has been reported previously in a case with CORS [Baala et al., 2007a]. In the second family (UM7) both parents were heterozygous for the AT-deletion observed also in the Finnish family. The father of the third family (UM8) had this same deletion (c.1219_1220delAT, p.Met407fs), the mother having c.5850delT in exon 40 (c.5850delT, p.Phe1950fs). In the fourth family (UM9) the fetus was homozygous for the same deletion (c.5850delT) in exon 40.

RPGRIP1L (MKS5)

The analysis of this gene did not reveal any mutations in the families included in the study.

CC2D2A (MKS6)

We identified three different pairs of compound heterozygote mutations in three non-Finnish MKS families originating from Europe. All except one of the mutations were novel. In the first case the fetus (UM10) had two deletions, one in exon 25 that causes a frameshift and results in an early stop codon (c.3084delG, p. Lys1029fs) and one in the end of exon 32 (c. 4179delG, p.Gly1394fs). In the second family (UM11) we detected an earlier identified deletion locating in the beginning of exon 27 (c.3289delG, p.Val1097fs) and an amino acid substitution in exon 27 (c.3341C>T, p.Thr1114Met). The change is predicted to be possibly

damaging by PolyPhen and tolerated by SIFT, but the fact that it is located in the C2 domain further supports the pathogenic nature of the change. In the third family (UM12) two deletions were found, the one being the same as in the second family (c.3289delG) and a four base pair deletion in the end of exon 30 (c.3975delA+1_3delGTA). This deletion is predicted to destroy a highly conserved splice site residue, since the splice site predictor program NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/) recognizes the normal splice donor site, but due to the deletion the splice site is lost. Unfortunately, the cDNA was not available in order to confirm the splice defects. In conclusion, five different mutations were discovered of which three are located in the functional calcium binding domain of *CC2D2A*.

Analysis of mutation descriptions by Mutalyzer

Mutation Analyzer (Mutalyzer) sequence variation nomenclature checker has been developed for automated analysis and correction of sequence variant descriptions using reference sequences from any organism. Mutalyzer generates variant descriptions at DNA level and the deduce outcome at protein level following recommendations of the Human Genome Variation Society (HGVS) [Wildeman et al., 2008]. We used the program to check all our novel mutations as well as the previously reported mutations and noticed some differences between the reported mutations and the results given by Mutalyzer. In cases where a discrepancy between the Mutalyzer description and the previously reported mutation was observed, we have marked both descriptions in Table 1 and Supp. Table S1. The description of the mutation given by Mutalyzer is marked above the reported mutation and marked with either c. (coding sequence) or p. (protein level).

DISCUSSION

The Mutalyzer program is a very useful and valuable tool in creating and checking correct sequence variant descriptions and should be used when reporting mutations in order to unify the mutation nomenclature. When checking reported mutations by Mutalyzer we observed inconsistencies in the descriptions. Most of the differences were seen in reported insertions and deletions. Many of the previously reported deletions have been determined so, that if there is more than one of the same nucleotide or a repeat, the numbering refers to the first nucleotide of the stretch, whereas Mutalyzer describes the last nucleotide or the repeat to be the deleted one. In most cases this causes the shift of the change on protein level to the next amino acid. Few of the reported insertions were described as duplications by Mutalyzer. We did not succeed in analyzing intronic variants using Mutalyzer, but it should be noticed that *in silico* analyses of intronic variants provide only “good guesses” and studies on RNA level tell the real consequence. In addition to intronic variants, the mutations in first and last nucleotides of exons can *in vivo* result in a splicing error, but Mutalyzer is not able to make this kind of analysis. In cases where RNA is available and can be studied it would be very valuable if researchers reported findings both from DNA (c.) and RNA (r.). As an example when a substitution causes a new splice site instead of changing the amino acid, like the c. 1762C>T in *CC2D2A,* Mutalyzer predicts this mutation to result in an early stop codon on protein level (p.Gln588X), but actually it creates a cryptic splice site that is used in the patient transcript and causes a four bp deletion seen as r.1761_1764delGCAA which leads to p.Val587fs on protein level.

The sequence analysis of all the five *MKS* genes revealed mutations in 13 out of the 29 MKS families included in the study. We identified altogether 20 different mutations of which 12 were novel. When examining all the collected MKS mutations (Table 1), one can see that in the *MKS1* gene the major mutation is the intronic deletion (c.1408-7_35del) that disrupts the splicing in MKS patients. This single mutation appears to be also the most frequent MKS mutation. There is no single major mutation in *TMEM67* except c.

1575+1G>A in the Pakistani population, but some mutations (c.622A>T, c.755T>C, c. 1319G>A) have been reported in several families. In *CEP290* the major MKS mutation is the deletion in exon 14 (c.1219_1220delAT). It is interesting to note how the mutation types vary between genes, since e.g. most of the mutations in *TMEM67* are amino acid changes, whereas in *CEP290* the most common mutation type seems to be small deletions. In *CC2D2A* the MKS mutations are mostly small deletions and interestingly the vast majority of them are located in the Ca^{2+} binding domain region.

In some reports there have been cases where researchers sequenced only one MKS gene, identified only one mutation and reported this gene as a causative gene even if the second mutation was not found (see e.g. Gorden et al., 2008; Frank et al., 2008). Based on our findings, this is not ideal, since while conducting this study we observed that one mutation in one gene might not be enough for the determination of the true causative gene. In one Finnish MKS family both parents are heterozygous and the fetus homozygous for the c. 1762C>T mutation (p.Val587fs) in the *MKS6* gene. Interestingly, the mother carries also the Fin_{major} mutation in the *MKS1* gene. The MKS fetus inherited only the *MKS6* mutations. The Finnish family case demonstrates well the fact that the inheritance pattern of MKS is autosomal recessive and that it is a monogenic disorder. We therefore postulate that two mutations need to be identified in the same gene in order to report the gene in question as a disease causing gene for a MKS case. Because of this we have not marked the cases with only one reported pathogenic mutation in Table 1 and Supp. Table S1.

However, mutations in other genes may modify the phenotype, even if they are not the primary disease mutations. For example, Tory and colleagues found out that patients with NPHP and JS-related neurological symptoms have the homozygous *NPHP1* deletion in combination with a heterozygous truncation mutation in *CEP290* and a heterozygous missense mutation in AHI1 [Tory et al., 2007]. Similarly mutations in the *MKS* genes are also found to modify the BBS phenotype [Leitch et al., 2008]. Five out of six BBS patients who manifested seizures had in addition a heterozygous mutation in the *MKS1* gene. The modifying effect is also possible in Meckel syndrome and might be one explanation for the differences in the clinical presentation. A large study, where all the known MKS genes would be sequenced in numerous samples, would make it possible to draw conclusions about the possible influence of polymorphisms or mutations in other genes on the phenotype.

MKS mutations represent every type of mutations: missense, nonsense, frameshift and splice-site mutations, all except the missense mutations most likely resulting in null alleles. A genotype-phenotype correlation has been hypothesized, but Frank et al. noted that due to the small number of cases, it is currently impossible to predict an individual's phenotype by the mutations' type, location, and/or affected protein [Frank et al., 2008]. Our findings additionally support their assumption. Frank et al. also noted that intrafamilial phenotypic variability argues against the genotype-phenotype correlations [Frank et al., 2008]. However, it is very interesting that there is a clear distinction between the mutations and syndromes, since none of the reported combinations of the mutations in MKS genes have been reported across different syndromes. For example the c.5850delT mutation in *CEP290* has been reported in patients with MKS, JS (MKS-like), CORS and LCA. One of the MKS fetuses in our sample collection (UM9) was homozygous for this mutation, while in the other cases with other syndromes the mutation has been a compound heterozygote with another variant. This indicates that there is a functional role for the type and the location of the mutation. Further understanding of the character of the mutations is needed in order to understand their role in pathogenesis of these related syndromes.

In some studies genotype-phenotype correlation has been observed, depending on the mutated gene, since postaxial polydactyly is rare in *MKS3* mutated cases compared to *MKS1* mutated cases. There is also difference between the type of CNS malformation between *MKS1* and *MKS3* mutated cases, since occipital encephalocele is typically seen in the *MKS1* fetuses, but not in the *MKS3* fetuses [Khaddour et al., 2007]. In contrast, no distinct phenotype differences can be seen between *MKS1* mutated cases and *MKS6* mutated cases. Neither, there is a strong difference between the Finnish cases were the mutation has not yet been identified compared to the *MKS1* and *MKS6* mutated cases. The *MKS4* mutated Finnish case lacked polydactyly, which is also a common feature of *MKS3* mutated cases, but it should be noticed that polydactyly is not found in all *MKS1* or *MKS6* cases either.

Making the right diagnosis can be occasionally rather difficult due to the overlap of clinical features between the different syndromes with similar clinical picture. The identification of the disease causing mutations might be a valuable tool in making the right diagnosis in such cases and it is possible that the reported mutations might help to define the diagnosis in cases where the clinical picture is not apparent. In a paper by Paavola et al. the authors discussed that not all patients with encephalocele, cystic kidneys and polydactyly have MKS, but if strict diagnostic criteria are applied, the diagnosis should be quite unambiguous. They further hypothesized if atypical cases still really represent true MKS or a distinct cerebro-reno-digital syndrome? Or, could MKS itself be considered a very heterogeneous syndrome with a wide spectrum of mutations in different genes resulting in deviating phenotypes [Paavola et al., 1997]? The identification of the disease genes and the realization that several syndromes, earlier reported as distinct clinical entities are actually allelic makes this question again relevant. Since all the syndromes have the same molecular background, should they be called collectively e.g. CORSs or CDKs (cystic diseases of the kidney)? Conversely, one cannot exclude the fact that even though the diseases are caused by mutations in the same genes, there can still be seen a clear difference between the mutations and the syndromes. In addition, most of the syndromes caused by *MKS* genes also have their own specific disease causing genes and characteristics. For example, one of the characteristics that clearly separates MKS from other related disorders with similar phenotypes is inevitable perinatal death. We therefore feel that each syndrome still can and should have their own specific criteria and should not be considered as one group.

Functional studies on the different mutation types will give interesting information on the disease mechanisms, e.g. do all the MKS mutations result in null alleles as expected. Other than the protein co-localization to cilia or basal bodies, there is currently very little known about the genes and proteins encoded by them. The functional relation of the proteins associated with these syndromes and how mutations in one of the genes affect the localization or function of the other, is not known. In the future, functional studies will undoubtedly shed some light on the relationship between the genotype and phenotype and MKS related syndromes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the families for participating in the study and clinicians and genetic counselors for the sample collection. The work was supported by the Research Foundation of the University of Helsinki (grant for young researchers) and Maud Kuistila Memorial Foundation grant to J. Tallila, by the Academy of Finland (grants 211124 and 118468 to M. Kestilä and by the Sigrid Juselius Foundation (M.K.). The Department of Medical Genetics, Väestöliitto is funded by Finland's Slot Machine Association (RAY) (R. Salonen).

REFERENCES

Auber B, Burfeind P, Herold S, Schoner K, Simson G, Rauskolb R, Rehder H. A disease causing deletion of 29 base pairs in intron 15 in the MKS1 gene is highly associated with the campomelic variant of the Meckel-Gruber syndrome. Clin Genet. 2007; 72(5):454–9. [PubMed: 17935508]

Baala L, Audollent S, Martinovic J, Ozilou C, Babron MC, Sivanandamoorthy S, Saunier S, Salomon R, Gonzales M, Rattenberry E, Esculpavit C, Toutain A, Moraine C, Parent P, Marcorelles P, Dauge MC, Roume J, Le Merrer M, Meiner V, Meir K, Menez F, Beaufrere AM, Francannet C, Tantau J, Sinico M, Dumez Y, MacDonald F, Munnich A, Lyonnet S, Gubler MC, Genin E, Johnson CA, Vekemans M, Encha-Razavi F, Attie-Bitach T. Pleiotropic effects of CEP290 (NPHP6) mutations extend to Meckel syndrome. Am J Hum Genet. 2007; 81(1):170–9. [PubMed: 17564974]

Consugar MB, Kubly VJ, Lager DJ, Hommerding CJ, Wong WC, Bakker E, Gattone VH 2nd, Torres VE, Breuning MH, Harris PC. Molecular diagnostics of Meckel-Gruber syndrome highlights phenotypic differences between MKS1 and MKS3. Hum Genet. 2007; 121(5):591–9. [PubMed: 17377820]

Dawe HR, Smith UM, Cullinane AR, Gerrelli D, Cox P, Badano JL, Blair-Reid S, Sriram N, Katsanis N, Attie-Bitach T, Afford SC, Copp AJ, Kelly DA, Gull K, Johnson CA. The Meckel-Gruber Syndrome proteins MKS1 and meckelin interact and are required for primary cilium formation. Hum Mol Genet. 2007; 16(2):173–86. [PubMed: 17185389]

Delous M, Baala L, Salomon R, Laclef C, Vierkotten J, Tory K, Golzio C, Lacoste T, Besse L, Ozilou C, Moutkine I, Hellman NE, Anselme I, Silbermann F, Vesque C, Gerhardt C, Rattenberry E, Wolf MT, Gubler MC, Martinovic J, Encha-Razavi F, Boddaert N, Gonzales M, Macher MA, Nivet H, Champion G, Bertheleme JP, Niaudet P, McDonald F, Hildebrandt F, Johnson CA, Vekemans M, Antignac C, Ruther U, Schneider-Maunoury S, Attie-Bitach T, Saunier S. The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. Nat Genet. 2007; 39(7):875–81. [PubMed: 17558409]

den Hollander AI, Roepman R, Koenekoop RK, Cremers FP. Leber congenital amaurosis: genes, proteins and disease mechanisms. Prog Retin Eye Res. 2008; 27(4):391–419. [PubMed: 18632300]

Frank V, den Hollander AI, Bruchle NO, Zonneveld MN, Nurnberg G, Becker C, Du Bois G, Kendziorra H, Roosing S, Senderek J, Nurnberg P, Cremers FP, Zerres K, Bergmann C. Mutations of the CEP290 gene encoding a centrosomal protein cause Meckel-Gruber syndrome. Hum Mutat. 2008; 29(1):45–52. [PubMed: 17705300]

Frank V, Ortiz Bruchle N, Mager S, Frints SG, Bohring A, du Bois G, Debatin I, Seidel H, Senderek J, Besbas N, Todt U, Kubisch C, Grimm T, Teksen F, Balci S, Zerres K, Bergmann C. Aberrant splicing is a common mutational mechanism in MKS1, a key player in Meckel-Gruber syndrome. Hum Mutat. 2007; 28(6):638–9. [PubMed: 17437276]

Gorden NT, Arts HH, Parisi MA, Coene KL, Letteboer SJ, van Beersum SE, Mans DA, Hikida A, Eckert M, Knutzen D, Alswaid AF, Ozyurek H, Dibooglu S, Otto EA, Liu Y, Davis EE, Hutter CM, Bammler TK, Farin FM, Dorschner M, Topcu M, Zackai EH, Rosenthal P, Owens KN, Katsanis N, Vincent JB, Hildebrandt F, Rubel EW, Raible DW, Knoers NV, Chance PF, Roepman R, Moens CB, Glass IA, Doherty D. CC2D2A Is Mutated in Joubert Syndrome and Interacts with the Ciliopathy-Associated Basal Body Protein CEP290. Am J Hum Genet. 2008

Hildebrandt F, Zhou W. Nephronophthisis-associated ciliopathies. J Am Soc Nephrol. 2007; 18(6): 1855–71. [PubMed: 17513324]

Khaddour R, Smith U, Baala L, Martinovic J, Clavering D, Shaffiq R, Ozilou C, Cullinane A, Kyttala M, Shalev S, Audollent S, d'Humieres C, Kadhom N, Esculpavit C, Viot G, Boone C, Oien C, Encha-Razavi F, Batman PA, Bennett CP, Woods CG, Roume J, Lyonnet S, Genin E, Le Merrer M, Munnich A, Gubler MC, Cox P, Macdonald F, Vekemans M, Johnson CA, Attie-Bitach T. Spectrum of MKS1 and MKS3 mutations in Meckel syndrome: a genotype-phenotype correlation. Mutation in brief #960. Online. Hum Mutat. 2007; 28(5):523–4. [PubMed: 17397051]

Kyttala M, Tallila J, Salonen R, Kopra O, Kohlschmidt N, Paavola-Sakki P, Peltonen L, Kestila M. MKS1, encoding a component of the flagellar apparatus basal body proteome, is mutated in Meckel syndrome. Nat Genet. 2006; 38(2):155–7. [PubMed: 16415886]

Leitch CC, Zaghloul NA, Davis EE, Stoetzel C, Diaz-Font A, Rix S, Alfadhel M, Lewis RA, Eyaid W, Banin E, Dollfus H, Beales PL, Badano JL, Katsanis N. Hypomorphic mutations in syndromic

encephalocele genes are associated with Bardet-Biedl syndrome. Nat Genet. 2008; 40(4):443–8. [PubMed: 18327255]

Paavola P, Salonen R, Baumer A, Schinzel A, Boyd PA, Gould S, Meusburger H, Tenconi R, Barnicoat A, Winter R, Peltonen L. Clinical and genetic heterogeneity in Meckel syndrome. Hum Genet. 1997; 101(1):88–92. [PubMed: 9385376]

Pachi A, Giancotti A, Torcia F, de Prosperi V, Maggi E. Meckel-Gruber syndrome: ultrasonographic diagnosis at 13 weeks' gestational age in an at-risk case. Prenat Diagn. 1989; 9(3):187–90. [PubMed: 2652130]

Roume J, Genin E, Cormier-Daire V, Ma HW, Mehaye B, Attie T, Razavi-Encha F, Fallet-Bianco C, Buenerd A, Clerget-Darpoux F, Munnich A, Le Merrer M. A gene for Meckel syndrome maps to chromosome 11q13. Am J Hum Genet. 1998; 63(4):1095–101. [PubMed: 9758620]

Salomon R, Saunier S, Niaudet P. Nephronophthisis. Pediatr Nephrol. 2008

Salonen R. The Meckel syndrome: clinicopathological findings in 67 patients. Am J Med Genet. 1984; 18(4):671–89. [PubMed: 6486167]

Smith UM, Consugar M, Tee LJ, McKee BM, Maina EN, Whelan S, Morgan NV, Goranson E, Gissen P, Lilliquist S, Aligianis IA, Ward CJ, Pasha S, Punyashthiti R, Malik Sharif S, Batman PA, Bennett CP, Woods CG, McKeown C, Bucourt M, Miller CA, Cox P, Algazali L, Trembath RC, Torres VE, Attie-Bitach T, Kelly DA, Maher ER, Gattone VH 2nd, Harris PC, Johnson CA. The transmembrane protein meckelin (MKS3) is mutated in Meckel-Gruber syndrome and the wpk rat. Nat Genet. 2006; 38(2):191–6. [PubMed: 16415887]

Tallila J, Jakkula E, Peltonen L, Salonen R, Kestila M. Identification of CC2D2A as a Meckel syndrome gene adds an important piece to the ciliopathy puzzle. Am J Hum Genet. 2008; 82(6): 1361–7. [PubMed: 18513680]

Tobin JL, Beales PL. Bardet-Biedl syndrome: beyond the cilium. Pediatr Nephrol. 2007; 22(7):926–36. [PubMed: 17357787]

Tory K, Lacoste T, Burglen L, Moriniere V, Boddaert N, Macher MA, Llanas B, Nivet H, Bensman A, Niaudet P, Antignac C, Salomon R, Saunier S. High NPHP1 and NPHP6 mutation rate in patients with Joubert syndrome and nephronophthisis: potential epistatic effect of NPHP6 and AHI1 mutations in patients with NPHP1 mutations. J Am Soc Nephrol. 2007; 18(5):1566–75. [PubMed: 17409309]

Valente EM, Brancati F, Dallapiccola B. Genotypes and phenotypes of Joubert syndrome and related disorders. Eur J Med Genet. 2008; 51(1):1–23. [PubMed: 18164675]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 1

Mutations in MKS genes. Novel mutations identified in this study are bolded.

		Allele 1			Allele 2				
Family ID	**Syndrome**	**Exon/int.**	**Mutation 1**	**Effect on protein**	**Exon/int.**	**Mutation 2**	**Effect on protein**	**Origin**	**Reference**
MKS1									
FIM 48	MKS	**ex 4**	**c.392_393delCT**	**p.Ser131X**	**ex5**	**c.496C>T**	**p.Arg166Trp**	Europe	*
in 37 families	MKS	int15	c.1408-7_35del	p.Gly470fs^	int15	c.1408-7_35del	p.Gly470fs^	Europe, America	[Auber et al., 2007; Consugar et al., 2007; Khaddour et al., 2007; Kyttala et al., 2006]
F4	MKS	ex1	c.51_55dupCCGGG 50insCCGGG	p.Asp19fs Pro17fs	int1	c.80+2T>C	pot. missplicing	Europe	[Kyttala et al., 2006]
102 and Fam 8	MKS	ex16	c.1448_1449insCAGG 1448_1451dupCAGG	p.Gly484fs Thr485fs	ex16	c.1448_1449insCAGG 1448_1451dupCAGG	p.Gly484fs Thr485fs	n.d.and Pakistan	[Dawe et al., 2007; Khaddour et al., 2007]
M338	MKS	int11	c.959+1G>A	p.Val320_His342delinsD^	int15	c.1408-7_35del	p.Gly470fs^	Europe, America	[Consugar et al., 2007]
M340and 55875	MKS	ex4	c.417G>A	p.Phe88_Glu139del^	int15	c.1408-7_35del	p.Gly470fs^	Europe	[Consugar et al., 2007]
Fam 1	MKS	ex4	c.417G>A	p.Phe88_Glu139del^	ex5	c.424C>T	p.Gln142X	Europe	[Khaddour et al., 2007]
Fam 2	MKS	ex10	c.958G>A	p.Val320Ile	ex16	c.1408-7_35del	p.Gly470fs^	Europe	[Khaddour et al., 2007]
Fam 3	MKS	ex2	c.184_190del	p.Thr62fs Thr61fs	ex16	c.1490G>A	p.Arg497Lys pot. missplicing	Europe	[Khaddour et al., 2007]
Fam 4 and 5	MKS	ex12	c.1048C>T	p.Gln350X	ex12	c.1048C>T	p.Gln350X	Palestina	[Khaddour et al., 2007]
Fam 6	MKS	ex5	c.472C>T	p.Arg158X	ex16	c.1408-7_35del	p.Gly470fs^	Europe	[Khaddour et al., 2007]
850	MKS	int3	c.262-37_179del	missplicing	int3	c.262-37_179del	missplicing	Turkey	[Frank et al., 2007]
937	MKS	int15	c.1407+2delT	pot. missplicing	int15	c.1407+2delT	pot. missplicing	Turkey	[Frank et al., 2007]
951	MKS	int5	c.515+1G>A	pot. missplicing	int5	c.515+1G>A	pot. missplicing	Kuwait	[Frank et al., 2007]
943	MKS	int15	c.1408-7_35del	p.Gly470fs^	ex4	c.417G>A	p.Phe88_Glu139del^	Europe	[Frank et al., 2007]
TMEM67 (MKS3)									
UM1	MKS	**ex8**	**c.734C>T**	**p.Ser245Phe**	**ex9**	**c.888G>T**	**p.Trp296Cys**	Europe	*
UM2	MKS	**ex1**	**c.161A>G**	**p.Tyr54Cys**	ex15	c.1538A>G	p.Tyr513Cys	Europe	*
UM4	MKS	ex8	c.755T>C	p.Met252Thr	**ex18**	**c.1843T>C**	**p.Cys615Arg**	Europe	*
UM5	MKS	ex13	c.1319G>A	p.Arg440Gln	ex27	c.2897T>C	p.Leu966Pro	Europe	*
125	MKS	ex3	c.383_384delAC	p.His128fs	ex3	c.383_384delAC	p.His128fs	Oman	[Smith et al., 2006]

Europe PMC Funders Author Manuscripts

		Allele 1			Allele 2				
Family ID	**Syndrome**	**Exon/int.**	**Mutation 1**	**Effect on protein**	**Exon/int.**	**Mutation 2**	**Effect on protein**	**Origin**	**Reference**
67F	MKS	ex6	c.648delA 647delA	p.Val217fs Glu216fs	ex6	c.648delA 647delA	p.Val217fs Glu216fs	Pakistan	[Smith et al., 2006]
73 and Fam9	MKS	int7	c.870-2A>G	pot. missplicing	int7	c.870-2A>G	pot. missplicing	Pakistan	[Khaddour et al., 2007; Smith et al., 2006]
40T	MKS	ex11	c.1127A>C	p.Gln376Pro	ex11	c.1127A>C	p.Gln376Pro	Pakistan	[Smith et al., 2006]
in 4 families	MKS	int15	c.1575+1G>A	pot. missplicing	int15	c.1575+1G>A	pot. missplicing	Pakistan	[Khaddour et al., 2007; Smith et al., 2006]
M329	MKS	ex6	c.622A>T	p.Arg208X	ex13	c.1351C>T	p.Arg451X	Europe	[Consugar et al., 2007]
M361	MKS	ex13	c.1319G>A	p.Arg440Gln	ex27	c.2897T>C	p.Leu966Pro	Europe	[Consugar et al., 2007]
M376	MKS	int1	c.224-2delA	p.Gly75fs$^{\wedge}$	ex8	c.755T>C	p.Met252Thr	Europe	[Consugar et al., 2007]
68408	MKS	ex6	c.579delA	p.Gly195fs Thr193fs	ex6	c.622A>T	p.Arg208X	Europe	[Consugar et al., 2007]
Fam 1	MKS	ex13	c.1336G>C	p.Asp446His	ex23	c.2439G>A	p. no change $^{\infty}$ pot. missplicing	Morocco	[Khaddour et al., 2007]
Fam 2	MKS	ex10	c.1046T>C	p.Leu349Ser	ex25	c.2557A>T	p.Lys853X	Europe	[Khaddour et al., 2007]
Fam 3	MKS	int10	c.1065+1delG	pot. missplicing	int10	c.1065+1delG	pot. missplicing	Palestina	[Khaddour et al., 2007]
Fam 4/M360	MKS	ex6	c.622A>T	p.Arg208X	ex8	c.755T>C	p.Met252Thr	Europe	[Consugar et al., 2007; Khaddour et al., 2007]
Fam 5	MKS	ex6	c.651+2G>T	pot. missplicing	ex13	c.1319G>A	p.Arg440Gln	Europe	[Khaddour et al., 2007]
Fam6	MKS	ex17-21	c.1675-?_2241+?del	p.Thr559_Glu747del$^{\wedge}$	ex17-21	c.1675-?_2241+?del	p.Thr559_Glu747del$^{\wedge}$	Ivory coast	[Khaddour et al., 2007]
CEP290 (MKS4)									
FIM 221	MKS	**ex29**	**c.3446_3447delAA**	**p.Lys1149fs**	ex14	c.1219_1220delAT	p.Met407fs	Europe	*
UM6	MKS	**ex5**	**c.289G>T**	**p.Glu97X**	ex20	c.1984C>T	p.Gln662X	Europe	*
UM7	MKS	ex14	c.1219_1220delAT	p.Met407fs	ex14	c.1219_1220delAT	p.Met407fs	Europe	*
UM8	MKS	ex14	c.1219_1220delAT	p.Met407fs	ex40	c.5850delT	p.Phe1950fs	Europe	*
UM9	MKS	ex40	c.5850delT	p.Phe1950fs	ex40	c.5850delT	p.Phe1950fs	n.d.	*
in 2 families	MKS	ex40	c.5493delA 5489delA	p.Ala1832fs Gln1830fs	ex40	c.5493delA 5489delA	p.Ala1832fs Gln1830fs	Europeq	[Frank et al., 2008]
Family 1	MKS	ex9	c.613C>T	p.Arg205X	ex9	c.613C>T	p.Arg205X	Morocco	[Baala et al., 2007a]
Family 2	MKS	ex28	c.3175delA	p.Ile1059X	ex28	c.3175delA	p.Ile1059X	Tunis	[Baala et al., 2007a]
Family 3	MKS	ex6	c.384_387delTAGA	p.Asp128fs	ex6	c.384_387delTAGA	p.Asp128fs	Tunis	[Baala et al., 2007a]

Europe PMC Funders Author Manuscripts

		Allele 1			Allele 2				
Family ID	**Syndrome**	**Exon/int.**	**Mutation 1**	**Effect on protein**	**Exon/int.**	**Mutation 2**	**Effect on protein**	**Origin**	**Reference**
Family 4	MKS	int3	c.180+2T>A	pot. missplicing	ex6	c.384_387delTAGA	p.Asp128fs	Tunis/ Europe	[Baala et al., 2007a]
Family 5	MKS	ex14	c.1219_1220delAT	p.Met407fs	ex32	c.4115_4116delTA	p.Ile1372fs	Europe	[Baala et al., 2007a]
Family 6	MKS	ex6	c.381_382delinsT	p.Lys127fs	ex32	c.1860_1861delAA	p.Asp622fs Glu620fs	n.d.	[Baala et al., 2007a]
RPGRIP1L (MKS5)									
F1MKS 03/107, 04/428	MKS	ex4	c.394A>T	p.Arg132X	ex 4	c.394A>T	p.Arg132X	Morocco	[Delous et al., 2007]
F2MKS 05/206	MKS	ex9	c.1033C>T	p.Gln345X	ex17	c.2614C>T	p.Gln872X	Europe	[Delous et al., 2007]
CC2D2A (MKS6)									
UM10	MKS	**ex25**	**c.3084delG**	**p.Lys1029fs**	**ex32**	**c.4179delG**	**p.Gly1394fs**	Europe	*
UM11	MKS	ex27	c.3289delG	p.Val1097fs	**ex27**	**c.3341C>T**	**p.Thr1114Met**	Europe	*
UM12	MKS	**ex27**	c.3289delG	p.Val1097fs	**ex30**	**c.3975delA+1_3delGTA**	**pot. missplicing**	Europe	*
in 11 families	MKS	ex16	c.1762C>T	p.Val587fs^	ex16	c.1762C>T	p.Val587fs^	Europe	[Tallila et al., 2008]

MKS1 (NM_017777.2), *TMEM67* (NM_153704.5), *CEP290* (NM_025114.3), *RPGRIP1L* (NM_015272.2), *CC2D2A* (NM_001080522.2).

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. The initiation codon is 1.

n.d. not determined.

*This study.

^The splice effect of the mutation has been demonstrated on cDNA level.

∞Mutation has no effect on the amino acid, but in view of the fact that the last base of exon 23 is mutated, it most likely affects the splicing according to the authors.