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ARTICLE

Genomic analysis of Meckel–Gruber syndrome in Arabs reveals marked genetic heterogeneity and novel candidate genes

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Meckel–Gruber syndrome (MKS, OMIM #249000) is a multiple congenital malformation syndrome that represents the severe end of the ciliopathy phenotypic spectrum. Despite the relatively common occurrence of this syndrome among Arabs, little is known about its genetic architecture in this population. This is a series of 18 Arab families with MKS, who were evaluated clinically and studied using autozygome-guided mutation analysis and exome sequencing. We show that autozygome-guided candidate gene analysis identified the underlying mutation in the majority ($n = 12$, 71%). Exome sequencing revealed a likely pathogenic mutation in three novel candidate MKS disease genes. These include *C5orf42*, Ellis–van-Creveld disease gene *EVC2* and *SEC8* (also known as *EXOC4*), which encodes an exocyst protein with an established role in ciliogenesis. This is the largest and most comprehensive genomic study on MKS in Arabs and the results, in addition to revealing genetic and allelic heterogeneity, suggest that previously reported disease genes and the novel candidates uncovered by this study account for the overwhelming majority of MKS patients in our population.
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INTRODUCTION

Meckel–Gruber syndrome (MKS, OMIM #249000) is a multiple congenital malformation syndrome characterized mainly by early lethality, occipital encephalocele, dysplastic kidneys, and polydactyly, but the phenotype also includes liver fibrosis, microphthalmia, cleft palate, and a wide-array of associated brain anomalies.[1] MKS tends to be a rare disease in most populations ($<1{:}20\,000$) with the notable exception of Finland where a birth prevalence of 1:9000 was estimated, and Arabia where Teebi and Teebi reported a much higher frequency of 1:3500.[2,3]

Our understanding of MKS pathogenesis has improved greatly over the past few years since it was first revealed that *MKS1*, the first gene found to be mutated in MKS, encodes a ciliary protein.[4] Subsequently, nine additional genes have been identified, all similarly encoding ciliary proteins (*TMEM216* (MKS2), *TMEM67* (MKS3), *CEP290* (MKS4), *RPGRIP1L* (MKS5), *CC2D2A* (MKS6), *NPHP3* (MKS7), *TCTN2* (MKS8), *B9D1* (MKS9) and *B9D2* (MKS10)).[4–14] The finding that defective ciliary biology is the core molecular pathology of MKS made it possible to dissect the pathogenesis of each of its manifestations. For instance, the primary cilium plays a critical role in SHH signaling that controls anterior–posterior and dorsal–ventral patterning of the developing limb buds and neural tube, respectively, thus explaining the polydactyly and neural tube defects that characterize MKS at the molecular level. Similarly, the third classical feature of cystic dysplasia of the kidneys is likely caused by abnormal patterning secondary to defective urine flow-induced signaling that is normally transduced by the cilia on the luminal surface of renal epithelium.

Defective ciliary biology is not unique to MKS; an expansive group of disorders commonly known as ciliopathies share this fundamental pathological feature.[15] In fact, mutations in MKS genes have been reported to cause other ciliopathies, for example, *MKS1* and Bardet–Biedl syndrome,[16] *TMEM216* and Joubert syndrome,[17] *TMEM67* and Joubert syndrome and nephrophthisis,[18,19] *CEP290* in non-syndromic retinal dystrophy, Senior–Loken syndrome, nephronophthisis, Joubert syndrome, and Bardet–Biedl syndrome,[20] *RPGRIP1L* and Joubert syndrome,[21] and *CC2D2A* and Joubert syndrome.[22] The factors that determine the ultimate clinical phenotype are not completely understood but there is growing evidence that ciliopathies represent a spectrum of clinical severity that correlates to some extent with the severity of the ciliary defect. Consistent with MKS being at the severe end of the clinical spectrum, most causative mutations are truncating

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in nature while hypomorphic mutations in the same genes cause less severe phenotypes.[23]

The genetic heterogeneity of MKS has yet to be fully captured. Indeed, a recent study showed that only half of the cases can be traced to a mutation in any of the known genes (seven were included in the analysis, so the additional contribution of *TCTN2* could not be inferred).[14] The autosomal recessive nature of MKS, the highly consanguineous nature of the Arab population where MKS is particularly prevalent, and the success of exome sequencing in other recessive disorders encouraged us to undertake a comprehensive genomic study to fully delineate the genetic architecture of MKS in our population by combining the power of autozygome and exome analysis. In this largest genomic MKS case series in Arabs to date, we show that autozygome-guided candidate gene analysis successfully identified the underlying mutation in >70% of the cases. In the remaining cases, we used exome sequencing followed by autozygome filtration to identify three novel candidate genes, one of which was very recently reported to be also mutated in Joubert syndrome. Assuming the candidate MKS genes that we report here are replicated by future studies, it appears that most of the genetic heterogeneity of MKS, at least in Arabs, has been captured.

MATERIALS AND METHODS

Human subjects

For case definition, we used a relaxed definition of occipital encephalocele as a must-have feature plus any combination of cleft palate, dysplastic kidneys, liver fibrosis, polydactyly, and early lethality. All cases were fully evaluated by an experienced dysmorphologist and/or neonatologist. Eligible cases were recruited after obtaining IRB-approved informed written consent from the parents (KFHSRC RAC#2080006). Blood in EDTA and, when possible, PAXGene tubes was collected for DNA and RNA extraction, respectively.

Autozygome analysis

DNA samples from both affected and unaffected family members were genotyped on Axiom Chip platform as per the manufacture's protocol (Affymetrix, Santa Clara, CA, USA). Runs of homozygosity (ROH) >2 Mb and that span 107 SNPs were used as surrogates of autozygosity given the consanguineous nature of the families using autoSNPa (http://dna.leeds.ac.uk/autosnpa/). The entire set of autozygosity blocks (autozygome) was determined for each patient. The shared haplotype between parents as well as relatives with similarly affected children was determined by using IBDelphi (http://dna.leeds.ac.uk/ibdelphi/).

Exome sequencing and analysis

Exome capture was performed using TruSeq Exome Enrichment kit (Illumina, San Diego, CA, USA) following the manufacturer's protocol. Samples were prepared as an Illumina sequencing library, and in the second step, the sequencing libraries were enriched for the desired target using the Illumina Exome Enrichment protocol. The captured libraries were sequenced using Illumina HiSeq 2000 Sequencer. The reads were mapped against UCSC hg19 (http://genome.ucsc.edu/) by BWA (http://bio-bwa.sourceforge.net/). The SNPs and Indels were detected by SAMTOOLS (http://samtools.sourceforge.net/). The resulting variants were filtered by only considering homozygous novel changes (absent in dbSNP, 1000 Genomes and 200 in-house Saudi exomes) within the autozygome followed by *in silico* analysis for potential pathogenicity. Variants that survived these filters were then Sanger sequenced in at least 192 normal Saudi controls, that is, 384 chromosomes.

Workflow

As summarized in Figure 1, cases were first subjected to autozygome-guided mutation analysis of known MKS genes. If negative, cases were exome sequenced. Exomes were first examined for potential compound heterozygous mutations in known MKS genes since these will be missed by the autozygome approach. If negative, we proceeded with the filtration scheme outlined above (see Exome Sequencing).

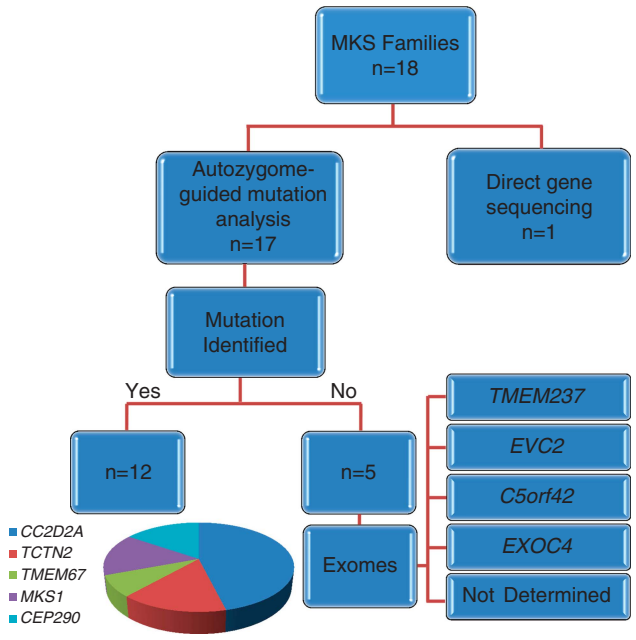

Figure 1 Workflow of the current study.

RESULTS

Human subjects

In total, 18 families that met the inclusion criteria were recruited, all consanguineous. All pedigrees are shown in Supplementary Figure S1 and representative clinical pictures for some of the MKS patients are shown in Figure 2. Table 1 summarizes their clinical features. One family was initially recruited despite lack of additional features of MKS besides occipital encephalocele because there were no ultrasonographic features suggestive of another diagnosis and the fetus was unavailable for clinical assessment after delivery. This case was later found to have two base pair deletion in *POMT1* gene (NM_007171.3:c.2179_2180delTC), so it was excluded and is not considered further in this study although it serves as a reminder that encephalocele is a birth defect with a broad differential diagnosis.[24] As expected, our relaxed clinical definition resulted in the recruitment of two cases (MKS_F1 and MKS_F15) that do not meet the classical minimal definition of early lethality, encephalocele, and cystic/dysplastic kidneys. However, we opted to retain these cases in our molecular analysis to explore the extent of variable expressivity in MKS especially when these cases were subsequently found to harbor mutations in genes recently reported to cause Joubert syndrome (see below).

Autozygome-guided mutational analysis

Consistent with all families being consanguineous, all index cases had evidence of autozygosity and the percentage of their genomes represented by the autozygome ranged 2.5–18.4% (Supplementary Table S1). At the time of the study, 10 MKS disease genes were identified (*TMEM67*, *TMEM216*, *CEP290*, *CC2D2A*, NPHP3, *RPGRIP1L*, *TCTN2*, *B9D1*, and *B9D2*). Gene(s) that overlap with autozygome were Sanger sequenced and this led to the identification of a likely pathogenic homozygous mutation in 9 of 17 families (Figure 1). Supplementary Figure S2 shows both the multi-species alignment of orthologs as well as the PolyPhen/SIFT scores for each of

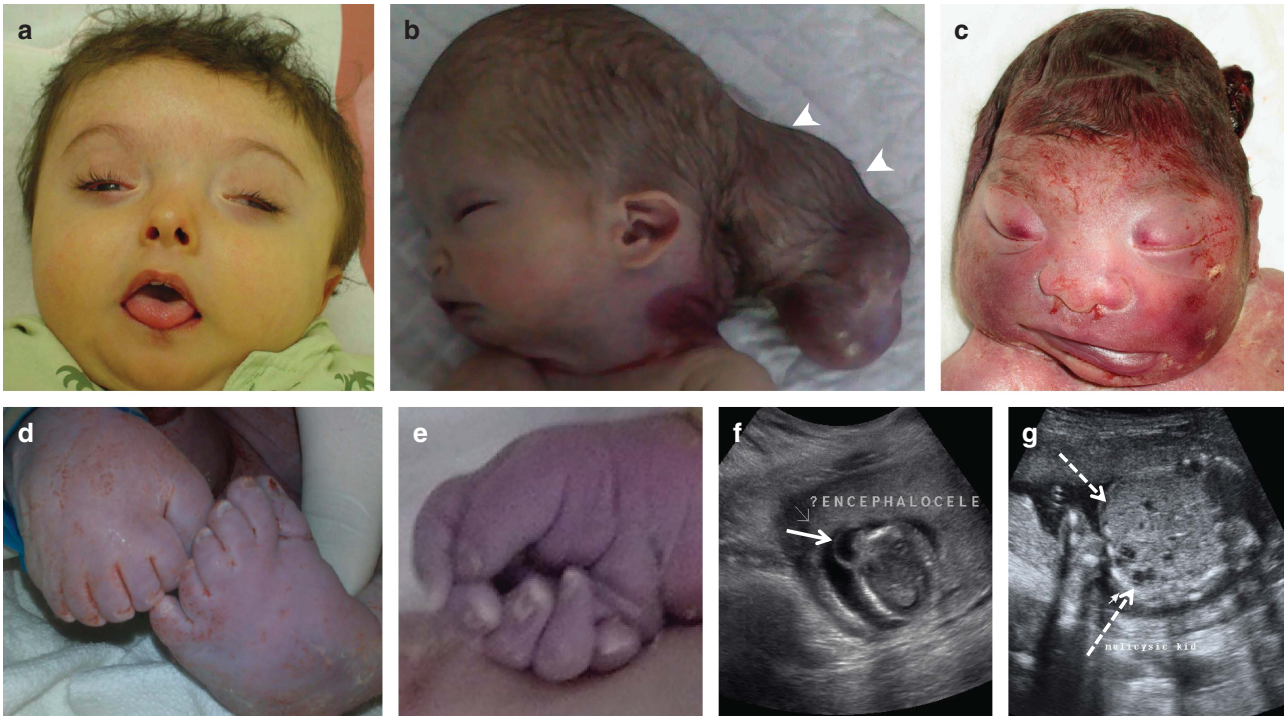

Figure 2 (**a**–**c**) Facial features of some MKS patients MKS_F1, F2, and F8, respectively, showing: microcephaly, sloping forehead, hypertelorism, micrognathia, potter-like facies, and severe occipital encephalocele (black arrows). (**d**, **e**) Representative hand and foot images showing postaxial polydactyly in MKS_F2. (**f**, **g**) Antenatal ultrasound for MKS_F12 patient showed encephalocele (white arrow) and enlarged polycystic kidney (dashes arrows).

the novel missense variants. For three families (MKS_F4, MKS_F11, and MKS_F13), we had no access to the index so we used IBDelphi to infer the shared ancestral haplotype between the parents which helped us identify the following pathogenic mutations: c.1506-2A>G in *TCTN2* (NM_024809.3), c.1855_1858del in *CEP290* (NM_025114) and c.613C>T; p.R205* in *CEP290* (NM_025114.3), respectively. Thus autozygome-guided mutational analysis and analysis to the shared haplotype between parents led to the successful identification of the underlying mutation in 71% of cases (Table 2; Figure 3).

Autozygome-filtered exome sequencing

Five families in which autozygome-guided mutation analysis failed to identify the causative mutation in our initial list of 10 genes were exome sequenced (Figure 1). Summary of the depth of coverage and the amount of exome covered are shown in Supplementary Table S2. Variants were excluded when present homozygous in Saudi controls and heterozygous at a frequency >1% ie present twice in 200 exomes. The filtration steps used in interpreting the exome results are summarized in Supplementary Table S3 and the novel variants that survived the various filters are listed in Table 3. MKS_F1 was found to harbor a novel homozygous mutation that abolished a consensus donor site in *TMEM237* (c.869+1G>A) and RT–PCR confirmed the resulting exonic skipping, which predicts in-frame deletion of >60 amino acids p(Ap.Met227_Arg291del). Mutations in this gene have recently been reported to cause a phenotypic spectrum between Joubert syndrome and MKS.[25] MKS_F15 was found to harbor the same homozygous truncating mutation we recently reported in a gene that causes Joubert syndrome (*C5orf42*).[26,27] Two exomes (MKS_16 and MKS_2) highlighted novel MKS candidate genes: *EXOC4* and *EVC2*, respectively, whereas exome sequencing of MKS_F18 failed to generate any compelling candidate variants after applying the various filters (Figure 1; Supplementary Table S4).

DISCUSSION

Although many MKS genes were mapped using consanguineous Arab families, the mutation distribution of these genes in Arabs is unknown but deserves investigation to inform clinical sequencing and genetic counseling. Furthermore, the success we have had in combining autozygome and exome analysis in delineating the genetic architecture of other genetically heterogeneous disorders, for example, Osteogenesis imperfecta, retinal dystrophy, cataract, mitochondrial diseases, Bardet–Biedl syndrome, and Joubert syndrome[26,28–31] (and Abu Safieh *et al* (2012)[32], Shaheen *et al* (2012)[33]), encouraged us to use a similar approach on MKS to not only determine the mutation distribution in known MKS disease genes but to also potentially identify novel candidate disease genes.

To address the first aim, that is, determination of the mutation distribution in known MKS genes, we took advantage of the consanguineous nature of our study population to trace causative mutations by virtue of the autozygosity signature that typically characterizes identical-by-descent recessive mutations. Indeed, we show that autozygome-guided mutational analysis has efficiently identified the causative variant in each family in which MKS is caused by a mutation in a known gene. Importantly, even though the mutations in *TMEM237* and *C5orf42* were identified by exome sequencing (these were unknown to cause MKS at the time of the analysis), both could have easily been identified using the same approach further increasing the yield of this approach to 88% (15 out of 17) in the setting of MKS. This is consistent with the trend we and others have shown in terms of the power of this approach in the

Table 1 Summery of clinical description of MKS individuals (classical features are underlined)

Patient features	*MKS_F1*	*MKS_F2*	*MKS_F3*	*MKS_F4*		*MKS_F5*	*MKS_F6*	*MKS_F7*	*MKS_F8*	*MKS_F9*	*MKS_F10*	*MKS_F11*	*MKS_F12*	*MKS_F13*	*MKS_F14*	*MKS_F15*	*MKS_F16*	*MKS_F17*	*MKS_F18*
Gene	*TMEM237*	*EVC2*	*CC2D2A*	*TCTN2*		*CC2D2A*	*TMEM67*	*MKS1*	*CC2D2A*	*TCTN2*	*CC2D2A*	*CEP290*	*CC2D2A*	*CEP290*	*CC2D2A*	*C5orf42*	*EXOC4*	*MKS1*	–
Early death	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Microcephaly	–	–	+	?	?	+	+	+	+	+	–	+	–	+	+	–	+	–	–
Variable prenatal growth deficiency	?	–	+	–	–	+	+	?	?	?	–	+	+	+	?	–	?	?	–
Head and neck																			
Potter-like facies	–	+	–	?	+	–	?	+	+	+	–	?	–	+	+	–	+	+	–
Sloping forehead	–	+	+	?	?	+	?	+	+	+	–	?	–	+	+	–	+	+	–
Microphthalmia	+	–	?	?	?	–	?	+	+	+	–	?	–	+	–	?	+	+	–
Hypertelorism	+	+	–	?	?	–	?	+	+	+	–	?	–	–	+	–	+	+	–
Low-set ears	+	+	–	?	?	–	?	+	+	+	+	?	–	+	+	–	+	+	–
Cleft lip	–	–	–	?	?	–	?	–	–	–	–	?	–	–	–	+	–	–	–
Cleft palate	+	–	+	?	?	–	?	–	–	–	–	?	–	–	–	+	–	–	–
Macrostomia	?	–	–	?	?	–	?	+	+	+	–	?	–	–	+	–	+	+	–
Lobulated tongue	–	–	–	?	?	–	?	+	?	–	–	?	–	–	–	?	–	+	–
Natal teeth	?	–	–	?	?	–	?	+	?	–	–	?	–	–	–	–	–	–	–
Short neck	+	–	–	?	?	–	?	+	+	+	+	?	–	+	–	–	+	+	–
Webbed neck	–	–	–	?	?	–	?	+	+	+	–	?	–	+	–	–	–	+	–
CNS																			
Occipital encephalocele	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anencephaly	–	–	–	?	?	+	?	–	–	–	–	+	–	–	–	–	–	–	–
Hydrocephalus	+	–	+	?	?	–	?	–	–	+	+	?	–	–	+	+	?	?	–
Dandy-Walker malformation	+	?	–	?	?	–	?	+	?	?	–	?	–	–	?	?	?	?	–
Arnold-Chiari malformation	–	?	?	?	?	+	?	?	?	?	+	?	–		+	–	?	?	–
Cerebral hypoplasia	+	?	–	?	?	–	?	?	+	?	+	?	–	–	?	?	?	?	–
CVS																			
Patent ductus arteriosus	–	–	–	?	?	+	?	?	?	?	–	?	–	–	?	+	?	?	–
Lung																			
Pulmonary hypoplasia	–	?	+	?	+	+	+	?	+	?	–	+	–	–	?	–	?	?	–
Urinary system																			
Polycystic kidneys	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+
Ambiguous genitalia	–	–	–	?	+	–	?	–	–	–	–	?	–	+	–	–	–	–	–
Extremities																			
Syndactyly	–	–	–	?	?	–		–	+	–	–		–	+	–	–	–	–	–
Polydactyly	–	+	–	+	+	–	–	+	+	+	+	–	–	+	+	–	+	+	+
Bowed long bones	–	–	–	?	?	–	?	–	+	–	–	?	–	+	–	–	–	–	–
Talipes	–	–	–	?	+	–		+	+	+	–		–	+	+	–	+	+	–
Oligohydramnios	?	?	+	+	+	+	+	+	+	+	–	?	–	+	+	?	+	+	+
Prenatal diagnosis by ultrasound	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2 Summary of autozygome-guided mutation analysis

Family ID	No. of affected used for analysis	MKS genes within autozygome or within shared haplotype of the parents	Sequence analysis results				Reference
MKS_F1[a]	1	*TMEM237*	*TMEM237*	NM_001044385.2	c.869+1G>A	p.Met227_Arg291del	Current study
MKS _F2[a]	1	*TMEM67*	*EVC2*	NM_147127.4	c.3870_3893dup	p.Lys1293_Lys1300dup	Current study
MKS _F3	1	*CC2D2A*	*CC2D2A*	NM_001080522.2	c.3084delG	p.Lys1029Argfs[b]3	12
MKS _F4[b]	–	*CEP290, TCTN2, TMEM237*	*TCTN2*	NM_024809.3	c.1506-2A>G		13
MKS _F5	1	*CC2D2A*	*CC2D2A*	NM_001080522.2	c.3084delG	p.Lys1029Argfs[b]3	12
MKS _F6	1	TMEM67	*TMEM67*	NM_153704.5	c.2306del T	p.Leu769Tyrfs[b]4	Current study
MKS _F7	1	*NPHP3, MKS1, CC2D2A*	MKS1	NM_017777.3	c.1126duplA	p.Thr376Asnfs[b]3	Current study
MKS _F8	1	*CC2D2A*	*CC2D2A*	M_001080522.2	c.4531T>C	p.Trp1511Arg	Current study
MKS _F9	1	*TCTN2*	*TCTN2*	NM_024809.3	c.1506-2A>G		13
MKS _F10	1	*CC2D2A*	*CC2D2A*	NM_001080522.2	c.3084delG	p.Lys1029Argfs[b]3	12
MKS _F11[b]	–	*TMEM67, TCTN2, CEP290*	*CEP290*	NM_025114.3	c.1860_1863delAAGA	p.Arg621Ilefs[b]2	Current study
MKS _F12	1	*TMEM67, CC2D2A*	*CC2D2A*	NM_001080522.2	c.3084delG	p.Lys1029Argfs[b]3	12
MKS _F13[b]	–	*CEP290, TMEM67, MKS1, TCTN2*	*CEP290*	NM_025114.3	c.613C>T	p.R205[b]	9
MKS _F14	1	*B9D1, TMEM216, CC2D2A, NPHP3*	*CC2D2A*	NM_001080522.2	c.4531T>C	p.Trp1511Arg	Current study
MKS _F15[a]	1	*CEP290*	*C5orf42*	NM_023073.3	c.7988_7989delGA	p.G2663Afs[b]40	Current study
MKS _F16[a]	1	–	*EXOC4*	NM_021807.3	c. 1733A>G	p.Gln578Arg	Current study
MKS _F17[c]	–	–	*MKS1*	NM_017777.3	c.1126dupA	p.Thr376Asnfs[b]3	Current study
MKS _F18	1	*C5orf42, B9D1*				–	

[a]Uncovered using exome capture.
[b]Analysis was done on parents using IBDelphi.
[c]Analysis was done by direct sequencing.

setting of genetically heterogeneous autosomal recessive diseases.[28,29,34]

In order to explore the potential of revealing novel disease genes, all cases in which autozygome-guided mutational analysis was negative were exome sequenced. As we have shown previously, autozygome served as an extremely powerful filter of the resulting variants.[35,36] In Family MKS_F1, two variants survived filtration but *TMEM237* was recently described as a novel Joubert gene.[25] Some of the original reported cases had occipital encephalocele and cystic kidney so it seems likely that our mutation is the causal variant.

In Family MKS_F15, only two variants survived the various filters (*C5orf42*, NM_023073.3; c.7988_7989delGA and *FAM48A*, NM_001014286; c.236A>G). We could not determine which of the two is likely to be causal because while the former is truncating in nature, the latter missense mutation affected a gene that is clearly linked to neural tube defects in mice.[37] During the preparation of this manuscript, we and others identified several truncating mutations in *C5orf42* in the context of Joubert syndrome.[26,27] Since MKS is a more severe phenotype, it is tempting to speculate that the additional allele in *FAM48A* may have influenced the severity of the phenotype. However, we caution against the overinterpretation of the presence of the *FAM48A* allele because occipital encephalocele/meningocele has been previously reported in the setting of *C5orf42* mutations.[26,27] Indeed, we note that case MKS_F15, while meeting our operational clinical definition of MKS, did not have polydactyly or renal involvement so it can be argued that even if *C5orf42* is indeed an MKS disease gene, it causes atypical forms of the disease. It is worth highlighting that the only two cases in our series that did not meet the classical definition of MKS are those with mutations in *TMEM237* and *C5orf42*, which have been shown to cause both classical Joubert syndrome as well as cases that are Meckel-like as we explained above. Only through analysis of the full extent of *C5orf42* and *TMEM237* contribution to MKS using large case series will it be apparent if classical MKS can also be caused by mutations in these genes.

Families MKS_F2 and MKS_F16 deserve special emphasis. In Family MKS_F2, the child had all the classical features of MKS and yet his exome sequencing revealed a novel variant in *EVC2*, a known disease gene for Ellis–van-Creveld syndrome. Although the mutation is in-frame, it inserts eight amino acids p.Lys1293_Lys1300dup. Interestingly, mutations in *EVC2* have been shown to modulate SHH signaling. Furthermore, EVC2 has been found recently to localize at the basal body of the primary cilium so it is tempting to speculate that this may be the link between *EVC2* and MKS.[38–40] Thus, our results are build on recent data to suggest that EVC is a ciliopathy. However, we note that lack of EVC findings on skeletal survey and fetal echocardiography in this case strongly argue that this is a bona fide MKS phenotype rather than a simple clinical overlap between EVC and MKS as was once reported.[41] In Family MKS_F16, the variant we identified in *EXOC4* (also known as *SEC8*) is particularly interesting. The gene encodes one of the several exocyst proteins that are recruited to the basal body of budding cilia along with the members of disheveled family of proteins in order to dock the basal body to the membrane and allow the nascent cilia to form.[42,43] Knockdown of another exocyst gene *SEC10* was found to impair ciliogenesis, raising the possibility that defective *SEC8* may lead a similar cellular phenotype. Furthermore, another exocyst gene *EXOC8* was recently found mutated in a patient with Joubert syndrome.[44] Of note, the variant we identified is conserved down to Fugu and zebrafish.

One of the cases (MKS_F18) was not solved by Exome sequencing. In our experience, only around 70% of autosomal recessive cases are solved by this techniques and this could be explained by various reasons. First, the mutation may not be in the exon or exon/intron boundary. Second, the mutation may be in an exon that has not been annotated and thus not included in the capture design. Third, the mutation may belong to a class that is particularly difficult to assay using next-generation sequencing (eg, genomic rearrangements, repeats, etc). Fourth, the mutation may simply have been skipped by either the capture or sequencing reaction.

Of the three proposed novel MKS candidate genes, *C5orf49* has been independently confirmed through recent reports of Joubert syndrome patients with biallelic mutations in these genes.[26,27] *EVC2* and *EXOC4*, however, will have to await future confirmation as bona fide MKS loci. If independently confirmed, our study shows that, at

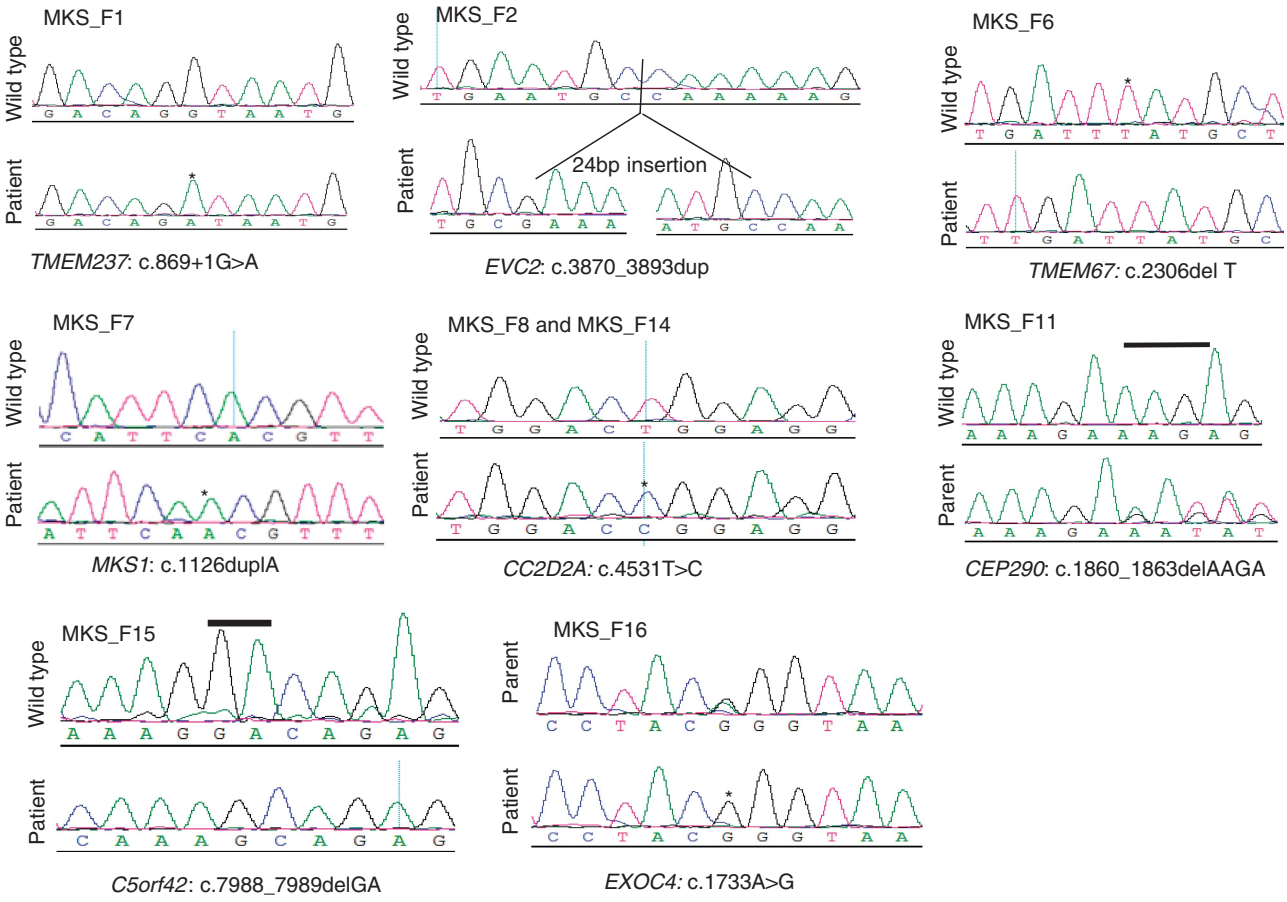

Figure 3 Sequence chromatograms of novel mutations listed in Table 2. Mutation sites are denoted with asterisks or lines.

Table 3 Variants that survived filtration in each exome

Family	*Name of the gene*	*Reference sequence*	*Nucleotide level*	*Protein level*	*Comments*
MKS_F1	*TMEM237*	NM_001044385.2	c.869 + 1G > A	p.Met227_Arg291del	*TMEM237* was recently described as a novel Joubert gene
	NCOA6	NM_014071.3	c.822_830del	p.Gln283_Gln285del	
MKS_F2	*EVC2*	NM_147127.4	c.3870_3893dup	p.Lys1293_Lys1300dup	*EVC2* has been found to localize at the basal body of the primary cilium and modulate SHH signaling
MKS_F15	*C5orf42*	NM_023073.3	c.7988_7989delGA	p.Gly2663Alafs[a]40	*C5orf42* was recently described as a novel Joubert gene
	FAM48A	NM_001014286.2	c.236A > G	p.Asn79Ser	*FAM48A* is linked to neural tube defect in mice
MKS_F16	*TXNDC15*[a]	NM_024715.3.	c.673_687del	p.Ser225_his229del	*TXNDC15*: Two stop gain mutations are reported in the Exome Variant Server
	ETV1[a]	NM_001163149.1	c.1234C > G	p.Gln412Glu	*ETV1*: Has incompatible mouse model
	EXOC4[a]	NM_021807.3	c.1733A > G	p.Gln578Arg	*EXOC4*: Encodes exocyst proteins that are recruited to the basal body of budding cilia in order to dock the basal body to the membrane and allow the nascent cilia to form. There are no reported truncating mutation in the Exome Variant Server

[a]Present in the shared haplotype between the two parents who have affected children with MKS within the same family.

least in Arabs, most of the genetic heterogeneity of this disorder has been captured and that the contribution of future MKS loci is likely to be minimal. One previous study showed that seven MKS genes (*TMEM67*, *TMEM216*, *CEP290*, *CC2D2A*, *B9D1*, and *RPGRIP1L*) were found to be biallelically mutated in 54% of their cohort, a higher contribution for these particular genes than what we observed in our study sample (32%). Thus, it seems possible that the higher percentage of mutation-positive MKS cases (94% by including *TCTN2*, *TMEM237*, *C5orf42*, and if *EVC2* and *EXOC4* are confirmed by future reports) may potentially be relevant to other populations as well.

In summary, we show in the largest genomic study on an MKS cohort the mutation distribution among Arabs where the disease is particularly prevalent. This genomic approach revealed interesting novel candidates that may potentially bring, along with recently described MKS disease genes, the quest to unravel the genetics of

MKS to its final stages making it possible for the overwhelming majority of these patients to receive genetic counseling that is informed by molecular confirmation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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