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The morbid genome of ciliopathies: an update

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Purpose: Ciliopathies are highly heterogeneous clinical disorders of the primary cilium. We aim to characterize a large cohort of ciliopathies phenotypically and molecularly.

Methods: Detailed phenotypic and genomic analysis of patients with ciliopathies, and functional characterization of novel candidate genes.

Results: In this study, we describe 125 families with ciliopathies and show that deleterious variants in previously reported genes, including cryptic splicing variants, account for 87% of cases. Additionally, we further support a number of previously reported candidate genes (*BBIP1*, *MAPKBP1*, *PDE6D*, and *WDPCP*), and propose nine novel candidate genes (*CCDC67*, *CCDC96*, *CCDC172*, *CEP295*, *FAM166B*, *LRRC34*, *TMEM17*, *TTC6*, and *TTC23*), three of which (*LRRC34*, *TTC6*, and *TTC23*) are supported by functional assays that we performed on available patient-derived fibroblasts. From a phenotypic perspective, we expand the phenomenon of allelism that characterizes ciliopathies by describing novel associations including *WDR19*-related Stargardt disease and *SCLT1*- and *CEP164*-related Bardet–Biedl syndrome.

Conclusion: In this cohort of phenotypically and molecularly characterized ciliopathies, we draw important lessons that inform the clinical management and the diagnostics of this class of disorders as well as their basic biology.

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INTRODUCTION

In humans, the centrioles of postmitotic cells reorganize microtubules to seed the outgrowth of antenna-like structures known as the cilia, abnormalities of which manifest in a wide range of clinical presentations depending on the type of cilia that are affected. Cilia are classified on the basis of three fundamental features: number (mono vs. multi), motility (motile vs. immotile), and ultrastructure arrangement of axoneme, specifically the presence or lack of two central microtubule singlets ($9+2$ vs. $9+0$, respectively).[1] The term "ciliopathies" is usually reserved for clinical disorders of the primary cilium, an immotile $9+0$ monocilium, with or without involvement of the motile $9+0$ monocilium of the embryonic node, whereas clinical disorders of the motile

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9 + 2 cilia are usually referred to as primary ciliary dyskinesia.[2]

The past two decades have witnessed vast changes in our understanding of the clinical and molecular aspects of ciliopathies, a trend that has only accelerated over the past decade with the advent of next-generation sequencing. Several themes have emerged from these studies. For example, the ubiquitous distribution of the primary cilium is consistent with the observation that no organ system is spared in ciliopathies, although different ciliopathies have variable tissue and organ predilection.[3] This can be seen in the conspicuous central nervous system (CNS) involvement in Joubert syndrome (characteristic posterior fossa malformation known as molar tooth sign), acrocallosal syndrome (strong predilection to hydrocephalus and agenesis of corpus callosum), and Meckel–Gruber syndrome (strong predilection to neural tube defect in the form of occipital encephalocele) as part of their multisystemic nature. Similarly, the multisystem phenotype of Bardet–Biedl syndrome has a strong predilection to eye involvement (retinitis pigmentosa), nephronophthisis to kidneys (cysts formation), oral–facial–digital to oral cavity (cleft and hamartoma), and cranioectodermal dysplasia to skeleton (skeletal dysplasia and craniosynostosis). It has also become clear that the delineation of specific ciliopathy syndromes overlooks the remarkable variability of phenotypic presentation that often blurs the clinical boundaries between these syndromes or highlights their allelism.[4] Lastly, earlier speculations about the non-Mendelian inheritance of these disorders have given way to the rigorous analysis of the role of modifiers of disease severity within the widely accepted context of Mendelian inheritance of these disorders.[4–6]

Despite this progress, our knowledge of ciliopathies is far from complete. New clinical phenotypes are still being described and are shedding new light on the role of the primary cilium in the development and function of the human body. Thus, there remains a need to define the clinical manifestations of abnormal primary cilia, not only through the study of patients with "compatible" phenotypes, but also through the reverse phenotyping of those who are found to have deleterious variants in established ciliopathy genes. This also highlights the need to accelerate the discovery of clinically relevant members of the "ciliome," i.e., genes that encode proteins with ciliary function. We have recently estimated the contribution of "known" genes to >85% of all cases with ciliopathies.[4] Although this suggests that the overwhelming majority of patients with ciliopathies should be able to receive a genetic diagnosis, it also shows that disease gene discovery should continue to serve the unmet needs of a significant minority of patients.

In this study, we attempt to address the above research questions by describing the clinical and molecular findings in a large cohort of 125 families. These families include 12 who were labeled "negative" in the previous cohort to provide updates on their genetic diagnosis. These also include 51 families who underwent a "genomics-first" analysis of various clinical phenotypes (regardless of their compatibility with ciliopathies) and were found to have deleterious variants in known ciliopathy genes.

MATERIALS AND METHODS

Human subjects

Two types of patients were eligible for enrollment in this study. First, we included all cases whose phenotype was consistent with any of the established ciliopathies syndromes. Second, we included all cases with biallelic pathogenic/likely pathogenic variants in established ciliopathies genes from a large cohort of patients who underwent exome sequencing (ES).[7] Informed consent was obtained from all subjects, as was permission to publish photos and the study was approved by the local institutional review board (IRB) (King Faisal Specialist Hospital and Research Centre Research Advisory Council [KFSRHC RAC] #2070023, 2080006, and 2121053). Referring physicians were asked to fill out a standard data collection form as described previously.[4] Other family members were recruited whenever possible with informed consent to verify the segregation of the variants.

Next-generation sequencing and variant interpretation

The technical details of ES are described elsewhere.[4] Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[8] Consistent with the guidelines, variants in genes we propose as novel candidates are classified as variants of unknown significance (VUS) regardless of their supporting level of evidence (see below). Importantly, each exome was fully analyzed for additional variants of potential relevance to the phenotype even when a likely causal biallelic variant is identified.

Functional characterization of cilia

Fibroblasts derived from punch skin biopsies obtained from affected and control individuals were propagated in RPMI media (Thermo Fisher Scientific, 22400–089) supplemented with 15% v/v heat inactivated fetal bovine serum (Thermo Fisher, cat. number 16140071), 1% v/v L-glutamine, and 1% v/v penicillin and streptomycin and incubated in a humidified, 5% CO_2 atmosphere at 37 °C.

For immunostaining, fibroblasts were grown to 85% confluence, cells were fixed with 3.6% formaldehyde, permeabilized in 0.1% 100× Tritonx (T9284, Sigma), and blocked in 1% bovine serum albumin. The following primary and their compatible secondary antibodies (TTC6 antibody PA5–64406, Thermo Fisher; TTC23 antibody PA5–58937 and LRRC34 antibodies NBP1–81146, Novus Biologicals) were used to test the localization of the protein in control and patient fibroblasts.

For ciliogenesis potential and to assess ciliary length, patient and control fibroblasts were cultured to 85% confluence on a coverslip, starved for 48 hours in Opti-MEM™ (Thermo Fisher), and then used for immunostaining with acetylated α-tubulin antibody T7451 (Sigma-Aldrich) and DAPI.

For sonic hedgehog (SHH) signaling comparison, sets of affected and control fibroblasts were treated with either smoothened agonist (SAG) at 100 nM or DMSO for 18–21 hours. *GLI1* was used as readout for SHH signaling on quantitative reverse transcription polymerase chain reaction (PCR). The experiment was run in triplicates on serum-starved cells.

RESULTS

Cohort characteristics

In total, we describe 125 families in this study: 12 were reported negative in our previous cohort,[4] while 113 were included after the freeze date of that cohort. The latter include 52 families who were included in a large clinical ES paper in which variants and clinical categories were listed for 2219 families with various clinical phenotypes,[7] and are described here in full detail.

Clinical phenotypes

Table S1 lists the clinical features of the patients in our cohort, while Fig. 1 shows the breakdown of clinical syndromes as follows: 17 families with Bardet–Biedl syndrome (BBS, MIM PS209900), 28 families with Joubert syndrome (JBTS, MIM PS213300), 4 families with acrocallosal syndrome (ACLS, MIM 200990), 17 families with Meckel–Gruber syndrome (MKS, MIM PS249000), 4 families with oral–facial–digital syndrome (OFD, MIM PS311200), 28 families with polycystic kidney disease (PKD, PS173900)/nephronophthisis (NPHP, MIM PS256100), 2 families with Caroli disease (MIM 600643), 4 families with short rib thoracic dystrophy (SRTD, MIM PS208500), 2 families with Ellis–van Creveld syndrome (EVCS, MIM PS208500), and 15 families with other diseases. Representative clinical images for typical features are shown in Fig. S1.

Several phenotypic aspects are worth highlighting, as discussed in the next sections.

Phenocopies

Our cohort contains one family initially suspected as having BBS but later found to have Alstrom syndrome, which we consider a ciliopathy phenocopy since the role of ciliary dysfunction in its pathophysiology has not been clearly established.[9] Three families of suspected MKS turned out to have *FKRP*- and related muscular dystrophy–dystroglycanopathy, *CEP55*-related MARCH (multinucleated neurons, anhydramnios, renal dysplasia, cerebellar hypoplasia, and hydranencephaly) syndrome, and *CPT2*-related lethal neonatal CPT II deficiency (Table S1). We also identified three cases of suspected PKD/NPHP who were found to have *ACE*- and *AGT*-related renal tubular dysgenesis, and *ETFA*-related glutaric acidemia IIA. A case with *LAMA1*-related Poretti–Boltshauser syndromes and three others with *SIL1*-related Marinesco–Sjogren syndrome were initially diagnosed as Joubert syndrome. Finally, we show that several patients presented with a constellation of clinical findings consistent with ciliopathies but did not fit a specific syndrome and were found to have variants in novel candidate genes (see Genetic and Allelic Heterogeneity of Ciliopathies).

Phenotypic expansion

A very unusual case of a homozygous *PKD1* pathogenic variant presented prenatally with enlarged polycystic kidneys, enlarged cisterna magna, and hypoplastic cerebellum, raising suspicion for MKS (16DG0393, Fig. 2). The carrier first cousin parents were subsequently found to have adult-type polycystic kidney disease typical of *PKD1* dominant variants (Fig. 2). We also identified a case whose renal agenesis is most likely linked to their pathogenic variants in *NPHP3* (19DG0819, Fig. 2). We have previously published case 17DG0098 as part of a congenital hydrocephalus cohort,[10] but we note here that the renal agenesis represents an expansion of the established *TTC21B*-related ciliopathy (Fig. 2). We also note that even though Caroli disease is typically defined histopathologically (polycystic dilatation of intrahepatic bile ducts with cholestasis), the majority of patients in this cohort with cholestasis in the setting of liver fibrosis and sclerosing cholangitis had no evidence of Caroli disease.[11] Another interesting phenotypic expansion observation in this cohort is our finding of at least three BBS patients with overgrowth (macrocephaly and tall stature) (16DG0944, 19DG2175, and 19DG2152; see Fig. 2).

Expansion of allelism in ciliopathies

We identified a founder *WDR19* variant in three apparently unrelated families all presenting with nonsyndromic Stargardt disease (Fig. 2). Although isolated retinal dystrophy has been documented for several ciliopathies genes,[12,13] this is the first time to our knowledge that a ciliopathy gene is linked to Stargardt disease phenotype. We also report for the first time *SCLT1*-related BBS (*SCLT1* is currently only linked to OFD IX) in two girls originally thought to have nonsyndromic pituitary hypoplasia with resulting growth hormone deficiency[14] but later found to have obesity, retinitis pigmentosa, and history of removed extra digits. Another case of BBS is worth highlighting. Case 19DG0403 was originally referred as suspected primary ciliary dyskinesia (PCD) because of chronic unexplained cough and bronchiectasis at a young age (Fig. 2). However, the finding of a homozygous *CEP164* truncating variant prompted reverse phenotyping that revealed classical features of BBS (Table S1, Fig. 2). Not only is this the first instance of *CEP164*-related BBS to our knowledge, but it represents a very rare co-occurrence of PCD and a ciliopathy.

Blended phenotypes

Two cases of dual molecular diagnosis in our cohort are highlighted here. The first case (19DG1316) presented with syndromic PKD/NPHP (congenital hepatic fibrosis, end stage renal disease, left subclavian artery, atrioventricular canal [AVC] malformation, epilepsy, intellectual disability, scoliosis, mandibular fracture, and thyroid cancer) and has a family history of a father who died of brain cancer

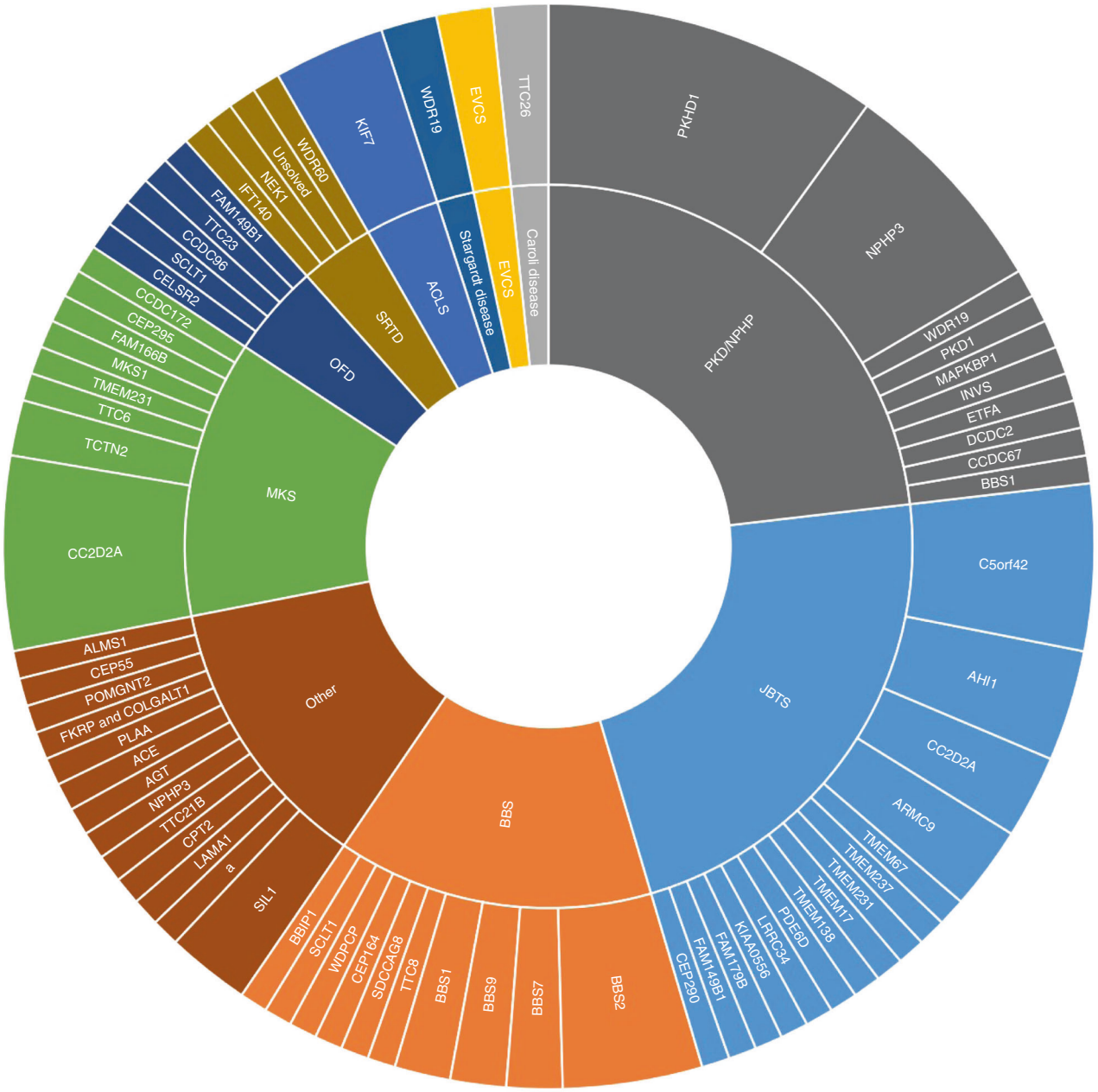

Fig. 1 Pie chart showing the ciliopathies categories in our cohort and the relative contribution of specific genes to each category: Bardet–Biedl syndrome (BBS), Joubert syndrome (JBTS), acrocallosal syndrome (ACLS), Meckel–Gruber syndrome (MKS), oral–facial–digital syndrome (OFD), polycystic kidney disease (PKD), nephronophthisis (NPHP), Caroli disease, short rib thoracic dystrophy (SRTD), Ellis–van Creveld syndrome (EVCS).

(Fig. 2). In addition to the homozygous *INVS*:NM_014425:c.875C > T:p.(Pro292Leu) variant, she was found to have a likely deleterious variant in *SUFU*:NM_001178133:c.1297-3C > A, which suggests the diagnosis of Gorlin hereditary cancer syndrome. The second is a terminated fetus (19DG0238) with classical MKS but who also has clinical features of fetal akinesia (Fig. 2). ES revealed the likely underlying etiology as dual homozygosity for *TMEM231*:NM_001077416:c.597+1G>A and *CHRNG*:NM_005199:c.1495C > T:p.(Arg499Trp), the latter being consistent with fetal akinesia syndrome.

Genetic and allelic heterogeneity of ciliopathies

In total, 97 variants were identified that potentially explain the phenotype, 16.3% of which are novel (Table S1). Some of the founder variants were especially common such as *NPHP3*:NM_153240.4:c.2694–2_2694–1delAG, which was seen in nine of the study families. Splicing variants accounted for 22.6% of all variants, consistent with our prior experience of a high percentage of splicing, including noncanonical splicing, variants in ciliopathies.[4] This may have contributed to the large number of cases referred to us with "negative" clinical ES, but were found by our analysis to have likely underlying

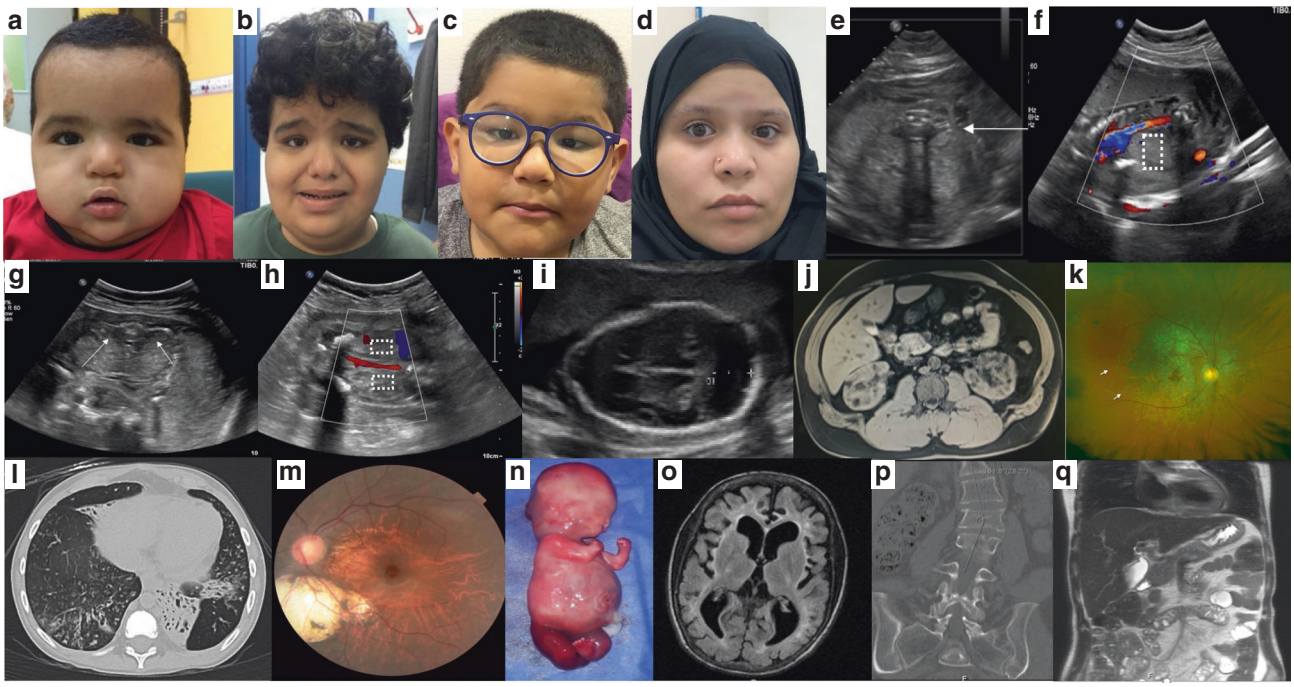

Fig. 2 Clinica images of unusual phenotypes observed in this cohort. (**a**–**c**) Facial images of three Bardet–Biedl (BBS) patients—16DG0944 (*BBS9*: NM_198428.3:c.1789+1G>A), 19DG2152 (*BBS9*:NM_024649:c.363C > A:p.(Tyr121*), and 19DG2175 (*BBS7*:NM_018190:c.1217_1218del:p.(Asn406-Serfs*6), respectively—with the atypical feature of macrocephalic overgrowth. (**d**) *SCLT1*-related BBS (17DG1007 with *SCLT1*:NM_144643.2:c.290+2T > C: p.[Lys79Valfs*4]). (**e**,**f**) Antenatal ultrasound of *TTC21B*-related renal agenesis (17DG0098 with *TTC21B*:NM_024753.4:c.1176_1185+1del:p.[Lys393fs]), arrow points to empty renal fossa, indicating renal agenesis, and absence of the renal artery is indicated in (**f**) by dotted box. (**g**,**h**) Antenatal ultrasound of *NPHP3*-related renal agenesis (19DG0819 with *NPHP3*:NM_153240.4:c.2694-2_2694-1delAG), arrow points to empty renal fossa, indicating renal agenesis, and the absence of the renal artery is indicated in (**h**) by dotted boxes. (**i**) Antenatal fetal ultrasound showing atypical presentation of a fetus (16DG0393) homozygous for a pathogenic dominant variant *PKD1*:NM_000296:c.4349_4351del:p.(Asn1450del), showing enlarged posterior fossa and hypoplastic cerebellum. (**j**) Computed tomography (CT) scan for mother of 16DG0393 showing polycystic kidney and liver. (**k**) Atypical phenotypic expression of *WDR19* in a patient (19DG1441 with *WDR19*:NM_025132.4:c.2777G > T:p.[Ser926Ile]) with nonsyndromic Stargardt disease with a color fundus photo of the right eye showing chorioretinal atrophy in the central macula and residual flecks in the midperiphery (arrows). (**l**) An atypical BBS case (19DG0403 with *CEP164*: NM_001271933.1:c.1735C > T:p.[Arg579*]) with a phenotype of primary ciliary dyskinesia (PCD) in the lungs, and (**m**) color fundus photo of the left eye for the same patient showing macular atrophy, diffuse retinal pigment epithelial (RPE) atrophy, and chorioretinal coloboma. (**n**) An aborted fetus (19DG0238 with *TMEM231*:NM_001077416:c.597+1G > A and *CHRNG*:NM_005199:c.1495C > T:p.[Arg499Trp]) with a blended phenotype of MKS and fetal akinesia. (**o**–**q**) A case (19DG1316) with a homozygous variant *INVS*:NM_014425:c.875C > T:p.(Pro292Leu), and a heterozygous variant *SUFU*:NM_001178133: c.1297-3C > A, images show brain atrophy, liver cirrhosis, and levoscoliotic deformity of the lumbar spine centered at L2 with Cobb angle of 28 degrees.

variants. A very high percentage of our cohort harbored biallelic pathogenic or likely pathogenic variants in previously reported ciliopathies genes (81.4%, or 87% if nonciliopathies genes were also included; see Table S1). A breakdown of the "solved" cases for each of the ciliopathies categories is shown in Fig. 1. BBS, EVCS, and ACLS had the highest solve rate of 100% followed by JBTS (92.85%, or 100% if novel candidate genes are included), OFD (50%, or 100% if novel candidate genes are included), PKD/NPHP, (92.85%, or 96.4% if novel candidate genes are included), MKS (64.7%, or 88% if novel candidate genes are included), SRTD (75%), and Caroli disease (50%). The remaining 18.6% represent those with (1) VUS in known ciliopathies genes (4.4%), (2) VUS in novel candidate genes identified in this study (8.8%), and (3) families who remain negative with no candidate variants in known or candidate genes (5.3%).

We identified variants in nine genes not previously linked to human diseases and are proposed here as novel candidate ciliopathies genes. Based on the level of evidence, they are grouped into two groups: high confidence and low confidence novel candidates.

High confidence candidate genes

LRRC34: In a child with Joubert syndrome (Fig. S2), we identified a novel homozygous NM_001172779:c.199A > T:p.(Lys67*) variant. To investigate a potential link to the disease, we first analyzed its subcellular localization and found it to localize along the ciliary shaft (Fig. 3). Patient cells displayed reduced ciliogenesis potential (Fig. 4) and muted response of SHH signaling to SAG compared with controls ($p = 0.001$, Fig. 3). In addition, LRRC34 in patient cells showed abnormal localization characterized by accumulation at the base of the cilia with only minimal extension into the shaft (Fig. 3 and Fig. S2). It should not be surprising that LRRC34 is not completely abrogated by the homozygous truncating variant since the mutated exon is alternatively spliced (www.ensembl.org) such that there remain other transcripts that do not contain this exon.

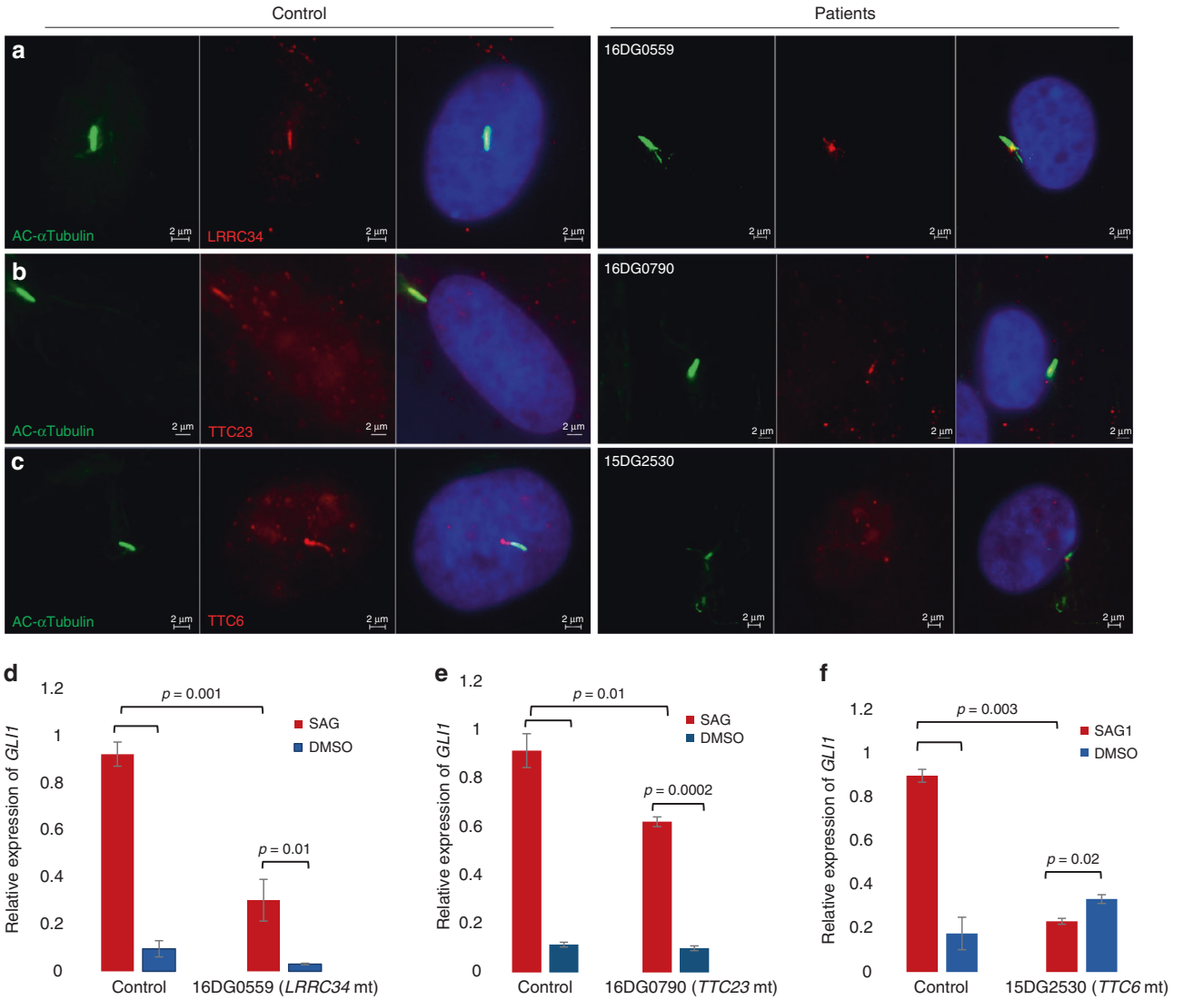

Fig. 3 Characterization of three high confidence candidate ciliopathies genes and the impact of their mutation on SHH signaling. (**a**) LRRC34 localizes along the shaft of the cilia in controls but is restricted to the base of the cilia in patient fibroblasts. (**b**) Similarly, TTC23 localizes to the shaft of the cilia in controls but is restricted mainly to the base of the cilia in patient fibroblasts. (**c**) TTC6 localizes to the centrosome and ciliary shaft in controls but only to the centrosome in patient fibroblasts. (**d**–**f**) Relative quantification of *GLI1* expression as a readout of SHH signaling at baseline (DMSO) and after induction (SAG) using RNA derived from controls and patients (each experiment was performed in triplicates; unpaired *t* test was used for statistical analysis).

TTC23: The encoded TPR domain-containing protein has been found by affinity purification and mass spectrometry to strongly associate with IQCE and EFCAB7,[15] components of the Ellis–van Creveld (EvC) zone, which represents a proximal region of the cilium and is important for SHH signaling but dispensable for cilium assembly.[16,17] We identified a novel homozygous *TTC23*:NM_001288616:c.456-1G>T variant in a newborn with OFD (Fig. S3). To investigate a potential link to the disease, we first analyzed its subcellular localization and found it to localize along the ciliary shaft (Fig. 3). Patient cells displayed normal ciliogenesis potential but had significantly longer cilia ($p = 0.0001$, Fig. 4), and weaker response to SHH signaling induction by SAG compared with controls ($p = 0.01$, Fig. 3). As with the other two high confidence candidate genes (*LRRC34* [see above] and *TTC6* [see below]), the alternatively spliced nature of the gene (www.ensembl.org) rendered the variant hypomorphic rather than null, but TTC23 in patient cells showed abnormal localization characterized by discontinuous (beaded) staining along the cilia or restricted to the lower part of ciliary shaft (Fig. 3 and Fig. S3).

TTC6: In a fetus with MKS (Fig. S4), we identified a homozygous variant NM_001310135:c.3322C > T:p.(Arg1108*). We first analyzed its subcellular localization and found it to localize to the centrosome and the shaft of the cilia (Fig. 3). Patient cells displayed impaired ciliogenesis potential, shorter cilia ($p = 0.003$, Fig. 4), and reduced response to SHH signaling stimulation by SAG compared with control ($p = 0.003$, Fig. 3).

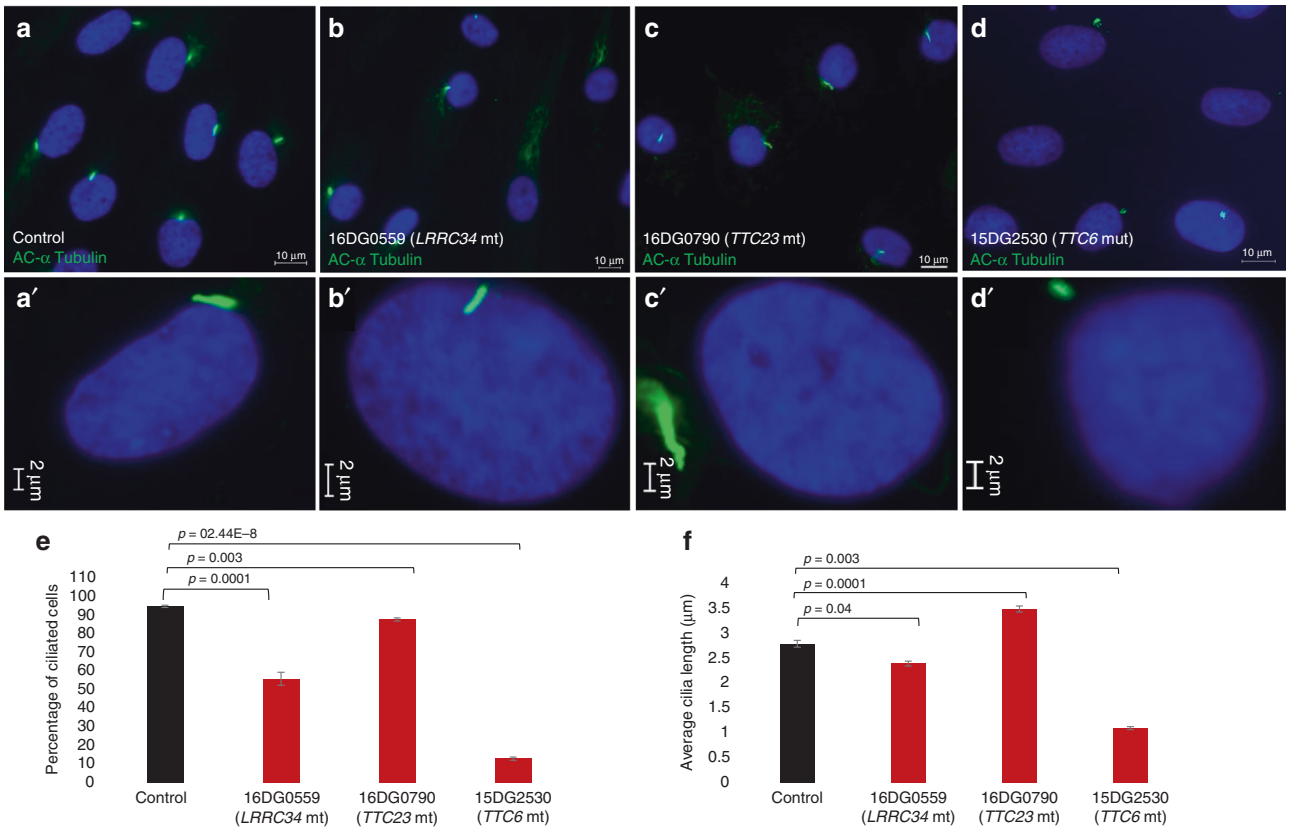

Fig. 4 Impact on ciliogenesis by mutations in three high confidence candidate ciliopathies genes. Immunofluorescence (IF) experiments on control (**a**,**a′**) and patient serum-starved fibroblasts (**b**–**d**) and (**b′**–**d′**) using the ciliary marker acetylated alpha tubulin (AC-α-tubulin). (**b**,**b′**) Patient 16DG0559 with *LRRC34*:NM_001172779:c.199A > T:p.(Lys67*) showing reduction in the ciliogenesis potential, but comparable ciliary length to control. (**c**,**c′**) Patient 16DG0790 with *TTC23*:NM_001288616.3:c.456-1G > T showing normal ciliogenesis but increased length of cilia compared with control. (**d**,**d′**) Patient 15DG2530 with *TTC6*:NM_001310135:c.3322C > T:p.(Arg1108*) showing marked reduction in ciliogenesis and ciliary length compared with control (200 cells were counted for each sample). (**e**) Percentage of ciliated cells in patient serum-starved fibroblasts compared with the control and (**f**) their average ciliary length. Patient 16DG0559 shows reduction in ciliogenesis potential and marginally significant reduction in ciliary length. Patient 16DG0790 shows normal ciliogenesis potential but significantly longer cilia. Patient 15DG2530 shows significant reduction in ciliogenesis potential and ciliary length (all experiments were performed in triplicates, and unpaired *t* test was used for statistical analysis).

Despite the truncating nature of the variant, the variant-containing exon is normally absent in other transcripts (www.ensembl.org). Indeed, TTC6 could be detected in patient cells but showed abnormally restricted localization to the base of the cilia, and could not be detected along the shaft in the few ciliated cells that could be observed (Fig. 3 and Fig. S4).

Low confidence novel candidates

CCDC172: We identified a homozygous *CCDC172*:NM_198515:c.291_294del:p.(Lys98Asnfs*3) variant in a newborn with posterior encephalocele. The gene is interesting because it has been suggested to link the outer dense fiber (ODF) with the middle piece of the sperm flagellum.[18]

FAM166B: In a consanguineous couple that presented with history of intrauterine fetal demise (IUFD) in a previous pregnancy with suspected MKS, we found that both parents carry *FAM166B*:NM_001287239:c.794_795insTAAG; p.Phe267Glnfs*37. FAM166B was previously found to be strictly localized to the ciliary axoneme.[19]

TMEM17: The encoded protein localizes to the ciliary transition zone and interacts with *B9D1* (a known Joubert syndrome gene).[20,21] Thus, the homozygous variant NM_198276:c.302G > T:p.Gly101Val in a child with Joubert syndrome was considered a plausible candidate.

CEP295: In a fetus with MKS, we identified a homozygous novel variant NM_033395:exon15:c.2603delA:p.Gln868Argfs*25. This is a highly conserved protein that acts very early in ciliogenesis by promoting the formation of the mother centriole.[22]

CCDC67 (*Depu1* in mouse): A recent study showed that CCDC67 and its paralog CEP63 interact with centriolar satellite proteins, and another showed that it is involved in de novo centriole biogenesis in multiciliated cells.[23,24]

CCDC96: The encoded protein contains DUF4201 (a coiled-coil domain exclusive to ciliated and flagellated phylogenetic groups[25]) and was reported to localize to the centrosome.[26] We identified a homozygous novel variant NM_153376:c.120_121insCGAACCC:p. Gly41Argfs*68 in a child with OFD.

In addition to the above genes, we encountered variants in genes that were previously reported as candidates thus further supporting their candidacy as follows:

WDPCP: The link between this gene and BBS was based on a single family.[27] The homozygous variant NM_001042692: c.1601 + 1G > T we identified in a child with BBS supports this link.

PDE6D: This gene is listed in OMIM with only a tentative link to JBTS based on a JBTS family reported with a homozygous variant in *PDE6D*.[28] We observed a homozygous truncating variant NM_001291018:exon3:c.257delG:p.Cys86Serfs*2 in another JBTS in this study.

MAPKBP1: Our NPHP patient with NM_001265611: exon27:c.3304C > T:p.Arg1102Cys represents the first follow-up to the initial report linking this gene to NPHP in 2017.[29]

BBIP1: In a patient with classical BBS (18DG0012) we identified a homozygous novel variant NM_001195305.1: c.38–6T > C (aberrant splicing confirmed by RT-PCR (Reverse transcription polymerase chain reaction) with 86% nonsense-mediated decay [NMD]), an independent confirmation of the case reported in 2014, which was the first to link *BBIP1* to BBS.[30]

DISCUSSION

Ciliopathies are important Mendelian disorders that involve nearly every organ system. This study is an attempt to further define the phenotypic and variant spectrum of ciliopathies by exploiting the highly consanguineous nature of the Saudi population, which not only facilitates the occurrence of these recessive disorders but also their mapping to the underlying variant through autozygome analysis. The latter was key to the identification of cryptic splicing variants that defied detection by standard clinical testing.

An advantage of conducting comprehensive genomic analysis of large cohorts of patients with a wide phenotypic spectrum is the achievement of unbiased links between variants and phenotypes. This is particularly relevant in the highly heterogeneous field of ciliopathies since these disorders do not always conform to predefined clinical categories and are characterized by a high degree of allelism. Indeed, our study expands the phenotypic expression of many ciliopathies genes. One noteworthy example is our finding that homozygosity for *PKD1* pathogenic variants can cause a severe ciliopathy phenotype resembling MKS, a reminder of the unexpected phenotypic consequences of homozygous variants in genes that are typically linked to dominant diseases.[31] Although PKD1 ciliary localization is well established, its exact function remains unknown. Our finding that biallelic *PKD1* variant results in brain anomalies consistent with a ciliopathies spectrum seems to confirm the previously described mouse phenotype.[32] Posterior fossa involvement and short bones were also described very recently by Al-Hamed et al. in patients with biallelic *PKD1* variant.[33] Other examples include *INVS*-related severe liver disease, *NPHP3*- and *TTC21B*-related renal agenesis, *WDR19*-related nonsyndromic Stargardt disease, and *SCLT1*- and *CEP164*-related BBS. We also note the unusual observation of macrocephalic overgrowth in several children with BBS. Although accelerated linear growth is not an unusual co-occurrence with childhood obesity, this is not typically the case in BBS patients. It is interesting that although macrocephaly was observed in ~11% of the first reported nationwide BBS cohort,[34] this feature is rarely mentioned in subsequent BBS cohorts, and we are aware of a single report of a BBS patient who is macrocephalic and with a height and weight on the 95th centile.[35]

The very high percentage of ciliopathies patients with biallelic candidate variants in our cohort shows that the non-Mendelian model need not be invoked in the overwhelming majority of cases. Furthermore, despite their highly variable expressivity, we found no instances of nonpenetrance for any of the variants we report in this cohort. The family (14DG0045) with a deep intronic *SDCCAG8* variant (NM_006642.2:c.741–152G > A) is illustrative in this regard. This family of two siblings with BBS was reported negative by clinical ES. However, subsequent autozygome mapping highlighted *SDCCAG8* as a likely candidate to be further interrogated by RT-PCR, which did reveal aberrant splicing (r.740_741ins741-202_741-1, p.Arg247Serfs*23) that eventually led to the identification of the deep intronic variant. While this variant was homozygous in the two affected siblings, it was also homozygous in the 3-year-old younger sister who is said to be normal. However, subsequent electroretinograph (ERG) confirmed that she has early stages of retinitis pigmentosa. Thus, it is critical to fully investigate patients with deleterious biallelic variants in ciliopathies genes to avoid the erroneous conclusion of nonpenetrance. Another illustrative example is the BBS patient 17DG0753 in whom clinical ES suggested the possibility of a non-Mendelian inheritance based on double heterozygosity for a truncating *BBS7* variant (NM_018190:c.1471_1472del:p.[Leu491-Phefs*10]) and *INVS*:NM_014425:exon6:c.643A > G:p.N215D. Again, RT-PCR analysis of *BBS7* revealed that this patient was in fact compound heterozygous for another variant in *BBS7* as revealed by the apparent homozygosity of the heterozygous variant on RT-PCR tracing (Fig. S5). Indeed, this is a reminder that given the tremendous genetic heterogeneity of ciliopathies, it is not uncommon to identify heterozygosity for deleterious variants in ciliopathies genes other than the causal one and these should not be mistaken for non-Mendelian inheritance. We and others have previously shown that patients with ciliopathies do not have a higher burden of deleterious variants in ciliopathies genes (beyond their causal variants in single genes) compared with other patients, and that "unsolved" cases are unlikely solved using the non-Mendelian model using a rigorous statistical approach.[4,5]

None of the novel candidate genes we report in this cohort were found to be mutated in more than one family. This should not be surprising since virtually all recently published disease genes account for an increasingly small

percentage of the morbid genome of ciliopathies, such that much larger cohorts are needed than the one described by this study to identify multiple hits. A practical alternative is to share candidate genes with reasonable level of evidence to allow postpublication matchmaking. In the case of the three novel genes for which patient fibroblasts are available to directly demonstrate (1) the ciliary nature of the genes and (2) the abnormal localization of the mutated protein and (3) associated abnormal ciliary structure and/or function, we believe a reasonable case is made for their candidacy especially when one considers the homozygous truncating nature of the variants identified in each of these genes. However, we note that the lower confidence candidate genes we report are also worth pursuing in other ciliopathies cohorts given their established link to ciliary biology and the mostly truncating nature of the variants identified therein.

In summary, we report a large cohort of ciliopathies from which we draw important phenotypic observations concerning the variable presentation of these disorders and their allelism. We also show the potential for a high diagnostic rate based on previously reported genes especially when employing an approach that takes into account the preponderance of noncanonical splicing variants in these genes. Finally, we support the candidacy of a number of previously reported genes and propose the candidacy of additional genes that should be investigated in future studies.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-020-0761-1) contains supplementary material, which is available to authorized users.

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DISCLOSURE

The authors declare no conflicts of interest.

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