TCTN3 Mutations Cause Mohr-Majewski Syndrome

Sophie Thomas,[1,2,19] Marine Legendre,[1,19] Sophie Saunier,[2,3,19] Bettina Bessières,[4] Caroline Alby,[2,5] Maryse Bonnière,[4] Annick Toutain,[6] Laurence Loeuillet,[7] Katarzyna Szymanska,[8] Frédérique Jossic,[9] Dominique Gaillard,[10] Mohamed Tahar Yacoubi,[11] Soumaya Mougou-Zerelli,[12] Albert David,[13] Marie-Anne Barthez,[14] Yves Ville,[2,5] Christine Bole-Feysot,[15] Patrick Nitschke,[16] Stanislas Lyonnet,[1,2,4] Arnold Munnich,[1,2,4] Colin A. Johnson,[8] Férechté Encha-Razavi,[1,2,4] Valérie Cormier-Daire,[1,2,4] Christel Thauvin-Robinet,[17,18] Michel Vekemans,[1,2,4] and Tania Attié-Bitach[1,2,4,*]

Orofaciodigital syndromes (OFDSs) consist of a group of heterogeneous disorders characterized by abnormalities in the oral cavity, face, and digits and associated phenotypic abnormalities that lead to the delineation of 13 OFDS subtypes. Here, by a combined approach of homozygozity mapping and exome ciliary sequencing, we identified truncating *TCTN3* mutations as the cause of an extreme form of OFD associated with bone dysplasia, tibial defect, cystic kidneys, and brain anomalies (OFD IV, Mohr-Majewski syndrome). Analysis of 184 individuals with various ciliopathies (OFD, Meckel, Joubert, and short rib polydactyly syndromes) led us to identify four additional truncating *TCTN3* mutations in unrelated fetal cases with overlapping Meckel and OFD IV syndromes and one homozygous missense mutation in a family with Joubert syndrome. By exploring roles of TCTN3 in human ciliary related functions, we found that TCTN3 is necessary for transduction of the sonic hedgehog (SHH) signaling pathway, as revealed by abnormal processing of GLI3 in patient cells. These results are consistent with the suggested role of its murine ortholog, which forms a complex at the ciliary transition zone with TCTN1 and TCTN2, both of which are also implicated in the transduction of SHH signaling. Overall, our data show the involvement of the transition zone protein TCTN3 in the regulation of the key SHH signaling pathway and that its disruption causes a severe form of ciliopathy, combining features of Meckel and OFD IV syndromes.

Orofaciodigital syndrome (OFDS) is characterized by malformations of the face, oral cavity, digits, and central nervous system, and 13 clinical subtypes are delineated. OFD I can be easily distinguished from other subtypes by its X-linked dominant inheritance pattern and is caused by mutations of *OFD1* encoding a centrosomal protein involved in ciliary function.[1,2] More recently, a homozygous mutation in the ciliary gene *TMEM216* (MIM 613277) was reported in two unrelated cases presenting a phenotype reminiscent of OFD VI (MIM 277170), with tongue tumors or multiple oral frenula and a molar tooth sign on brain MRI, a characteristic feature of Joubert syndrome (JS [MIM 213300])[3] and related syndromes. The molecular basis of the other OFDS subtypes remains unknown. OFD II (Mohr syndrome [MIM 252100]) was defined by hallucal and postaxial polysyndactyly. The fourth type of orofaciodigital syndrome (Mohr-Majewski [MIM 258860]) was first proposed by Baraitser in 1986,[4] following the report by Temtamy and McKusick in 1978[5] of two cases with features of both OFD II and short rib polydactyly with tibia dysplasia (SRP II/Majewski syndrome [MIM 263520]) and subsequent reports of individuals with OFD and tibia dysplasia but no short ribs.[6,7] Severe forms of OFDIV have been subsequently reported in children or fetuses presenting additional features of cystic dysplastic kidneys and brain malformation that include occipital encephalocele.[8–10] This phenotype has a high degree of overlap with Meckel syndrome (MKS [MIM 249000]), an autosomal-recessive lethal disease associated with severe cystic kidney disease, occipital encephalocele, polydactyly, and bile duct proliferation of the liver. Thus, OFD IV shows significant overlap with other ciliopathies, in particular SRP II/Majewski syndrome or MKS, and it was unclear whether OFD IV was a separate clinical entity. Therefore, we aimed to identify the molecular basis of OFD IV and to study the clinical spectrum of this syndrome.

We examined a male fetus (10047) born to a consanguineous family from Senegal whose pregnancy was terminated at 19 gestational weeks (gw) because of brain anomalies, cystic kidneys, and severe skeletal dysplasia. He had facial dysmorphism with a lobulated tongue (Figure 1A),

[1]INSERM U781, Hôpital Necker-Enfants Malades, 75015 Paris, France; [2]Université Paris Descartes, 75006 Paris Sorbonne, France; [3]INSERM U983, Hôpital Necker-Enfants Malades, 75015 Paris, France; [4]Département de Génétique, Hôpital Necker-Enfants Malades, Assistance Publique Hôpitaux de Paris (AP-HP), 75015 Paris, France; [5]Service de Gynécologie-Obstétrique, Hôpital Necker-Enfants Malades, AP-HP, 75015 Paris, France; [6]Service de Génétique, CHRU de Tours - Hôpital Bretonneau, 37044 Tours, France; [7]Service d'Anatomie et de Cytologie Pathologiques, CHI Poissy, 78100 Saint Germain en Laye, France; [8]Section of Ophthalmology and Neurosciences, Leeds Institute of Molecular Medicine, St. James's University Hospital, Leeds LS9 7TF, UK; [9]Service d'Anatomie Pathologique, CHU de Nantes, 44093 Nantes, France; [10]Service de Génétique et Biologie de la Reproduction, CHU Reims, 51092 Reims, France; [11]Service d'Anatomie et de Cytologie Pathologiques, CHU Farhat Hached, 4001 Sousse, Tunisie; [12]Service de Cytogénétique, Génétique moléculaire et Biologie de la reproduction, CHU Farhat Hached, 4001 Sousse, Tunisie; [13]Service de Génétique Clinique, Hôpital Hôtel Dieu, CHU de Nantes, 44093 Nantes, France; [14]Service de Neuropédiatrie, CHRU de Tours, Hôpital Clocheville, 37044 Tours, France; [15]Plateforme de génomique, Fondation IMAGINE, Hôpital Necker-Enfant Malades, 75015 Paris, France; [16]Plateforme de Bioinformatique, Université Paris Descartes, 75015 Paris, France; [17]Centre de Génétique, Hôpital d'Enfants, CHU de Dijon, 21079 Dijon, France; [18]EA GAD, IFR Santé – STIC, Université de Bourgogne, 21070 Dijon, France
[19]These authors contributed equally to this work
*Correspondence: tania.attie@inserm.fr
http://dx.doi.org/10.1016/j.ajhg.2012.06.017.

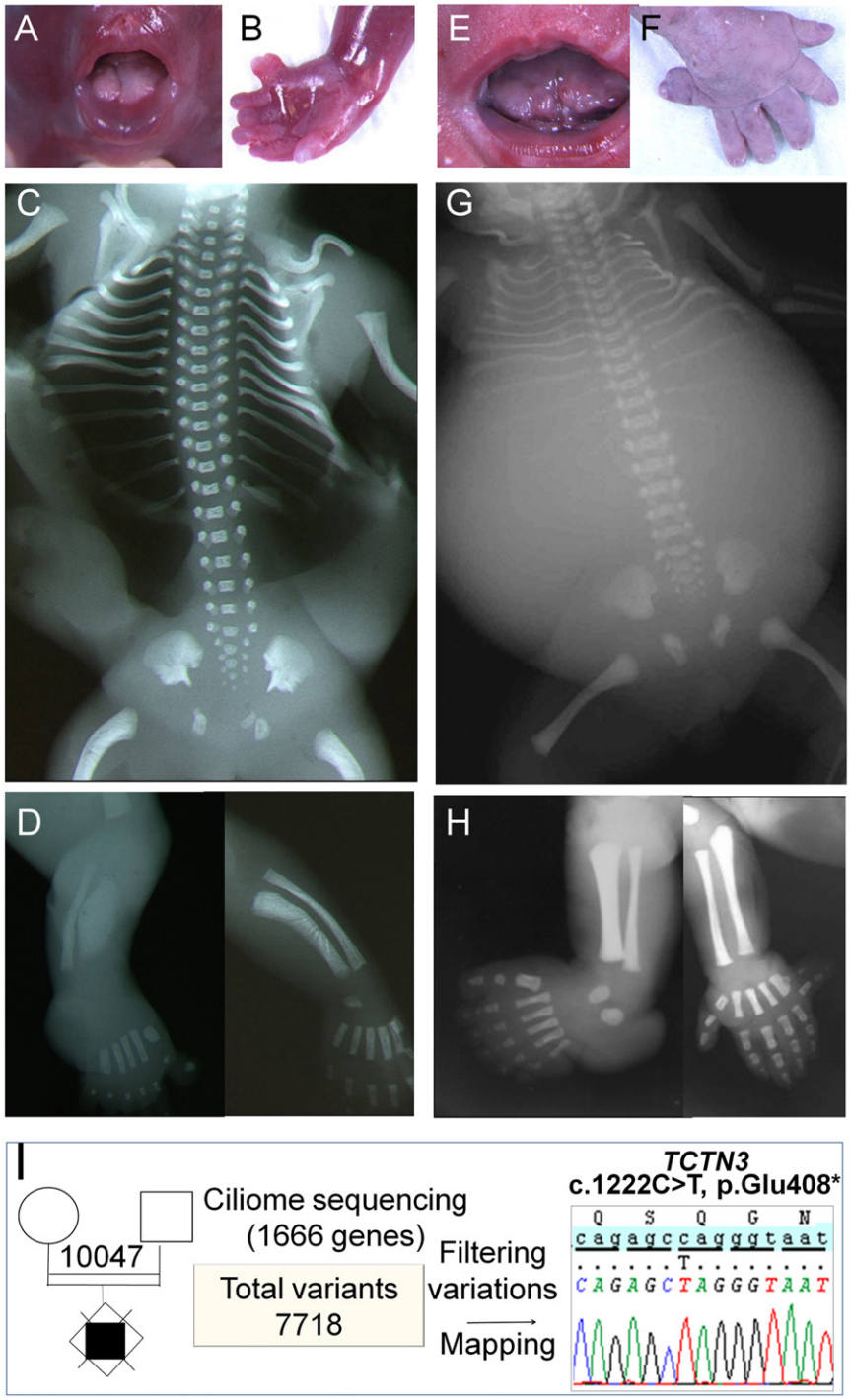

Figure 1. Phenotype of Fetuses and *TCTN3* Mutation Identification

(A–D) Pictures of fetus 10047, showing lobulated tongue (A), postaxial polydactyly of the hand (B), bowing of long bones, a trident appearance of acetabular bones but no short ribs (C), and severe tibia hypoplasia (D).

(E–H) Pictures of fetus 11385, showing lingual hamartoma (E), postaxial polydactyly of the hand (F), moderate bowing of long bones (G), and slightly thickened tibia bones (H).

(I) After homozygosity mapping with Affymetrix 250 SNP microarrays, a ciliary gene exome sequencing and filtering of the data identified a unique nonsense mutation in one of the targeted homozygous regions.

polydactyly of four limbs (Figure 1b), severe cystic kidney disease, ductal plate proliferation in the liver, and occipital encephalocele. Neuropathological assessment disclosed absent olfactory system, corpus callosum agenesis, and vermian hypoplasia. X-rays showed bowing of long bones with severe tibia hypoplasia (Figures 1C and 1D). There was a trident appearance of the acetabular margin but no short ribs (Figure 1C). Therefore, we concluded that this fetus fulfilled the diagnostic criteria for OFD IV.

In order to identify the molecular defect underling this condition, we performed genome-wide homozygosity mapping by using the Affymetrix 250K *Nsp*I SNP genotyping microarray. This analysis identified 14 regions of homozygosity (Table S1, available online), but because of the high number of genes present in these regions, we used a targeted capture strategy combined with next-generation sequencing. We used a 5.3 Mb customized Agilent SureSelect Target Enrichment library to capture 32,146 exons of 1,644 ciliary candidate genes (listed in Table S2). A total of 7,718 variants were identified, and after data were filtered by removal of known SNPs, synonymous coding sequence variations, and intersection with regions of homozygosity, a unique nonsense mutation, c.1222C>T (p.Glu408*) remained in tectonic-3 (*TCTN3* [MIM 613847]) (Table S3 and Figure 1I).

We then performed additional homozygosity mapping analysis by using Affymetrix SNP microarrays for 18 consanguineous cases presenting with lethal ciliopathies with various combinations of brain, renal, skeletal and orofacial abnormalities and polydactyly. In total, six individuals had regions of homozygosity at the *TCTN3* locus. Direct sequencing of the 14 coding exons (NM_015631.5, Table S4) revealed three additional fetuses with *TCTN3* truncating mutations in two families from Pakistan (c.650_653del [p.Tyr217Serfs*6]) and a family originating from Tunisia (c.1327C>T [p.Gln443*]). These mutations segregated in all available family members with the expected patterns of autosomal-recessive inheritance.

Table 1. Clinical and Molecular Data of Individuals with *TCTN3* Mutations

Case	Age	Origin	Oral	PD	CK	BDP	Tibia	Trident	Brain	Other	Nucleotide Changes	Exon	Predicted Effect in ORF	Inheritance
10047	19 gw	Senegalese	+	+	+	+	++	+	OE, MTS	bowing of all long bones	c.1222C>T	11	p.Gln408*	homozygous
11385	31.5 gw	Pakistani	+	+	+	+	+/−	-	OE, MTS	femoral bowing, IUGR	c.650_653del	5	p.Tyr217Serfs*6	homozygous
752	14.5 gw	Pakistani	-	+	+	+	++	+	OE	retrognathism, IUGR	c.650_653del	5	p.Tyr217Serfs*6	homozygous
860	23 gw	Tunisian	-	+	+	+	?	?	OE, CCA, Arh	bilateral club foot, no X-rays	c.1327C>T	12	p.Gln443*	homozygous
842a	23 gw	French	-	+	+	+	+/−	-	OE	microglossia, microretrognathism, bicornate uterus, femoral bowing	c.566_567del	4	p.Glu189Valfs*52	paternal
											c.1348_1349del	12	p.Leu450Serfs*14	maternal
842b	14 gw	French	-	+	+	+	++	+	OE?	cleft palate, bowing of long bones, bilateral club foot and hand	c.566_567del	4	p.Glu189Valfs*52	paternal
											c.1348_1349del	12	p.Leu450Serfs*14	maternal
JS-02a	13 yr	Turkish	-	+	-	-	-	?	VA, MTS	camptodactyly, abnormal eye movements, breathing anomalies, severe mental retardation, joint laxity, cyphoscoliosis	c.940G>A	8	p.Gly314Arg	homozygous
JS-02b	6 yr	Turkish	+	-	-	-	-	?	VA, MTS	IUGR, micrognathism horseshoe kidney, scoliosis, ventricular septal defect	c.940G>A	8	p.Gly314Arg	homozygous

Age is given in gestational weeks (gw) for fetuses and years (yr) for children. Abbreviations are as follows: Arh, arhinencephaly; BDP, bile duct proliferation of liver; CCA, corpus callosum agenesis; CK, cystic kidneys; IUGR, intrauterine growth retardation; MTS, molar tooth sign; OE, occipital encephalocele; ORF, open reading frame; PD, polydactyly; VA, vermis agenesis.

Interestingly, all three cases also presented with osteochondrodyplasia with short lower limbs, talipes, and variable severity of long bone bowing and tibia hypoplasia. A trident appearance of the acetabular margin was present in one patient and multiple tongue hamartomae in another, suggesting variable expressivity of *TCTN3* mutations. Sequencing of an additional 82 nonconsanguineous fetal cases with a severe ciliopathy identified compound-heterozygous frameshift mutations (c.566_567del [p.Glu189Valfs*52] and c.1348_1349del [p.Leu450Serfs*14]) in two siblings (cases 842a and 842b), both with skeletal anomalies including bowing of long bones and severe tibia hypoplasia. Clinical data and mutations are summarized in Table 1.

In view of the tibia anomalies and trident aspect of acetabular bones in two cases, we wondered whether this condition could be allelic with SRP, in particular SRP II and SRP IV (Beemer-Langer Syndrome [MIM 269860]), which also include tibia hypoplasia.[6] We therefore genotyped six SRP II and two SRP IV consanguineous cases, but all were heterozygous at the *TCTN3* locus.

We then asked whether a nonlethal phenotype could be ascribed to *TCTN3* mutations. Sequencing of 18 OFD cases of various types, without *OFD1* mutation, found no additional *TCTN3* mutations. Although all cases had encephalocele and cystic kidneys, two fetal cases had additional vermis agenesis and therefore belonged to the allelic MKS/JS phenotypic spectrum.[11,12] We therefore analyzed genome-wide linkage scans for consanguineous JS families that were excluded by linkage to known JS loci. In family JS-02 from Turkey in which there were two affected siblings, three homozygous regions were identified, the largest of which was 50 Mb long, located on chromosome 10 and containing *TCTN3* (Figure S1). Direct sequencing of *TCTN3* identified a homozygous missense mutation, c.940G>A [p.Gly314Arg], absent in SNP databases, the 1000 Genomes Project, and 150 ethnically matched control chromosomes. This mutation changes a highly conserved amino acid and is predicted as probably damaging by Polyphen 2 (score: 1). Interestingly, in this family, additional signs included severe cyphoscoliosis in both affected siblings, camptodactyly and joint laxity in the elder, and horseshoe kidney and ventricular septal defect in the younger (Table 1). Sequencing of an additional 57 JS cases revealed no additional mutations. Table 2 summarizes the total number and phenotypes of individuals screened, as well as the number of cases with *TCTN3* mutations.

In order to explore possible roles of *TCTN3* in human cilia-related function, we first examined the presence of

Table 2. Phenotypes Screened for *TCTN3*

Phenotypes	Number of Mutations/ Total Number of Cases
Fetal ciliopathies with overlapping Meckel syndrome features	5/100
Short ribs polyactyly II	0/6
Short ribs polydactyly IV	0/2
Non fetal orofaciodigital syndrome	0/18
Joubert	1/58
Total	**6/184**

In all fetuses in this study, pregnancies were terminated after genetic counseling, in accordance with local legislation. Chromosome analysis and clinicopathological examination were performed for all cases. Informed consent was obtained for all participating families, and the study was approved by the Ethical Committee of Paris Ile de France II.

cilia in the kidneys of two affected fetuses, aged 14.5 and 19 gestation weeks (gw), respectively (cases 860 and 10047). We found that primary cilia costained for acetylated alpha tubulin (a ciliary axoneme marker; Sigma) and pericentrin (a basal body marker; AbCam) protruded normally from the surface of epithelial cells in the affected fetal kidney tubules as compared to age-matched controls (Figure 2A), indicating that TCTN3 is not necessary for cilia biogenesis in the kidney.

In the mouse, TCTN1 and TCTN2 have been shown to be required for normal SHH signaling transduction.[13,14] To determine whether TCTN3 is important for SHH transduction, we assayed the expression of *PTCH1* and *GLI1*, general transcriptional targets of SHH signaling, as well as the processing of GLI3 in *TCTN3*-mutated patient fibroblasts and controls. After pathway activation by the addition of Smoothened Agonist (SAG; Tebu-Bio), both *PTCH1* and *GLI1* were induced in control fibroblasts, whereas patient fibroblasts displayed negligible responsiveness (Figure 2B). Moreover, *TCTN3*-mutated fibroblasts exhibited decreased amounts of full-length, unprocessed GLI3 protein (GLI3-FL) and increased amounts of the cleaved repressor form (GLI3-R), indicating that TCTN3 is essential for GLI3 processing and function (Figure 2C). Thus, *TCTN3* mutated cells fail to respond to SHH agonists, suggesting that at least some of the defects in affected individuals with *TCTN3* mutations may be secondary to reduced SHH signaling.

In this study, on the basis of the combination of homozygozity mapping and exome sequencing of ciliary genes, we identified *TCTN3* mutations in autosomal-recessive OFD IV syndrome characterized by OFD anomalies with tibia dysgenesis but without short ribs. We identified a total of five different *TCTN3* truncating mutations in five families: four homozygous and one compound heterozygous. All affected fetuses had skeletal dysplasia with long bone bowing and tibia hypoplasia, with associated orofaciodigital anomalies in two affected individuals. Although none had short ribs, two cases had a trident appearance of the acetabular margin, usually observed in SRP. The three cases that lacked any orofaciodigital anomalies suggest either phenotypic variability of OFD IV or a difference in the onset of orofacial manifestations during development. Whatever the mechanism, this phenotypic variability has until now left many cases with a nonspecific "ciliopathy" phenotype with a clinical spectrum in between OFD IV (with no OF) and SRP II or SRP IV (with no SR). Our results therefore confirm the variable spectrum of OFD IV and show that this entity is distinct from SRP II/Majewski syndrome.

We also identified a missense mutation in a JS family with two severely affected siblings. Interestingly, *TCTN2* mutations were also reported in JS[15] and in a lethal phenotype described as MKS with hypoplastic nose, anophthalmia, and cleft lip and palate.[16] It would be of interest to determine whether this case also had skeletal dysplasia. A *TCTN1* mutation was reported in one family with JS,[13] but a lethal phenotype has not yet been described in humans. Overlap between JS and the other ciliopathies has often been discussed and has even led to the classification of such "JS plus" cases in a pleiotropic group of so-called "JS-related disorders," or JSRD, such as COACH or OFD VI syndromes.[17] Interestingly, vermis agenesis is observed in several other severe ciliopathies such as MKS or SRP, and more recently, it has been shown to be a constant feature of acrocallosal syndrome[18,19]. This might reflect that the vermis defect and accompanying brainstem molar tooth sign (MTS) is the minimal brain anomaly observed in the various disorders that affect primary cilia biogenesis or function. Along this line, since 2006, mutations in six genes have been shown to cause both JS and MKS. This suggests that the JS phenotype is the viable and/or unique clinical manifestation of otherwise multisystemic disorders. In some instances, genotype-phenotype correlations explain the milder effect of apparently hypomorphic mutations,[11,12,20,21] as observed here for *TCTN3*. For other loci, modifier alleles seem the best hypothesis for explaining phenotypic variability, as shown in Bardet-Biedl syndrome, in which pathogenic mutations in *BBS* genes can both drive recessive phenotypes and contribute likely second-site modifiers.[22–24] Cumulative phenotypic and genotypic data from the known causal ciliopathy loci in nephronophtisis have also indicated significant enrichment of pathogenic variations in ciliary genes.[24,25] Common variants in ciliary genes might also drive a specific phenotype in individuals with nephronophtisis, such as the retinal degeneration associated with the c.685 G>A (p.Ala229Thr) change in *RPGRIP1L*[26] (NM_015272.2, MIM 610937) or the c.2488C >T (p.Arg830Trp) variation in *AHI1* (NM_017651.4, MIM 608894).[27] The latter variant is also associated with a neurological phenotype in individuals with *NPHP1*(MIM 607100) mutations.[24] Ciliopathies therefore represent a useful model for understanding the proposed mechanism of total mutational load in a biological system and provide insights into the molecular basis of genotype-phenotype correlations.

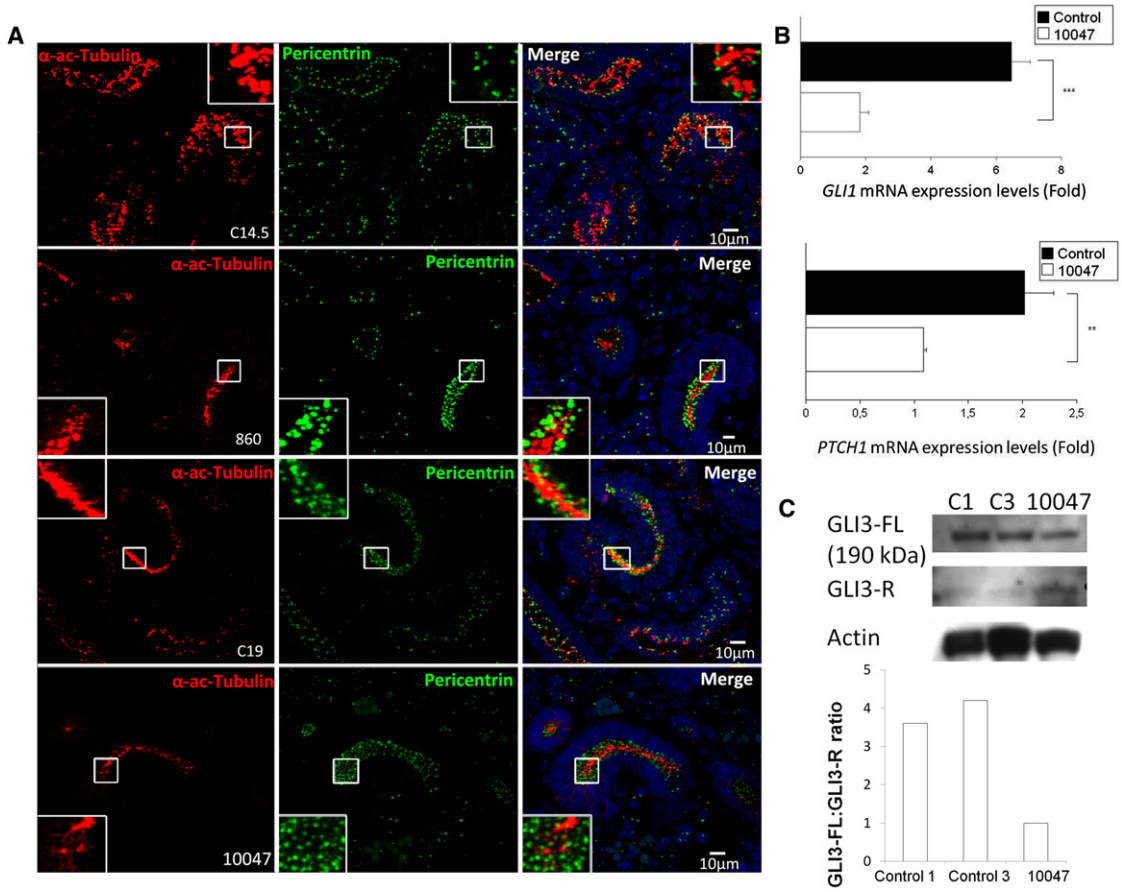

Figure 2. Analysis of Ciliogenesis and SHH Pathway in *TCTN3* Mutants
(A) Analysis of the primary cilia in kidneys of two affected fetuses aged 14.5 and 19 gestation weeks (gw), respectively (cases 860 and 10047), compared to a matched control, costained with acetylated α tubulin (red; Sigma) and pericentrin (green; AbCam). Confocal images were taken with a Leica SP5 confocal microscope.
(B) *TCTN3*-mutated patient fibroblasts failed to upregulate *GLI1* and *PTCH1* after stimulation with Smoothened Agonist (SAG) for 18 hr, as compared to controls. Primers used for quantitative RT-PCR are listed in Table S5. Values were normalized to *GAPDH* and presented as relative expression levels ± SEM. $^{**}p < 0.05$, $^{***}p < 0.001$ (Student's two-tailed t test).
(C) Immunoblot analysis with the use of a GLI3 antibody (Santa Cruz Biotechnology) showed that *TCTN3* processing of GLI3 into the repressor form, GLI3R, is increased in *TCTN3*-mutated fibroblasts as compared to controls. A graphical evaluation of the GLI3-FL:GLI3-R ratio using actin as a loading control and ImageJ software for densitometry is presented.

TCTN3 belongs to a family of genes with three members. *Tctn1*$^{-/-}$ and *Tctn2*$^{-/-}$ mice exhibit neural tube defect and polydactyly.[14,15] Both TCTN1 and TCTN2 were shown to regulate SHH signaling[13,14] and to be essential for ciliogenesis in some but not all tissues. No *Tctn3* mouse model is reported to date, but TCTN3 was shown to form a complex with multiple ciliary proteins colocalizing at the transition zone, including TCTN1, TCTN2, and all known MKS proteins except RPGRIP1L.[13]

While *TCTN3* is not necessary for ciliogenesis in kidney, we show disruption of the SHH signaling transduction in patient fibroblasts associated with abnormal GLI3 processing. In the *Tctn1*$^{-/-}$ mouse, GLI3 processing is also altered but in an opposite way to what we found in *TCTN3*-mutated fetuses. Indeed, *TCTN3*-mutated fibroblasts exhibit decreased amounts of unprocessed GLI3 and increased amounts of the repressor form of GLI3, suggesting an increased SHH signal repression by GLI3. By contrast, *Tctn1*$^{-/-}$ embryo extracts had markedly increased levels of full-length GLI3.[13] These results indicate that TCTN3 is important for GLI3 processing and function in humans and suggest a complementary rather than redundant function for TCTN proteins in mammals.

Overall, we report the identification of *TCTN3* mutations in an unusually broad clinical spectrum that extends from a viable phenotype, characterized by JS with digital anomalies and axial skeletal anomalies, to a severe OFD IV phenotype that is characterized by long bone bowing, tibia hypoplasia, cystic kidney, and encephalocele. Our data further confirm the implication of the transition zone complex proteins in the key SHH signaling pathway. We suggest that a disruption in this functional module leads to severe forms of ciliopathies that share phenotypic overlap with Meckel syndrome and that now include OFD IV.

Supplemental Data

Supplemental Data include five tables and one figure and can be found with this article online at http://www.cell.com/AJHG.

Acknowledgments

We thank the families for their participation. We also thank Leila Hakkakian, Judite De Oliveira, Céline Huber, and Nadège Gigot for technical assistance and Stéphane Romano and Pascale de Lonlay for patient referral, and we are grateful to the French Society of Fetal Pathology (SOFFOET) for participating in the study. This work was supported by grants from ANR (2010 FOETOCILPATH N° BLAN 1122 01 to T.A.B.) and Fondation pour la Recherche Médicale (FRM N° DEQ20071210558 to S.S).

Received: March 5, 2012
Revised: May 15, 2012
Accepted: June 27, 2012
Published online: August 9, 2012

Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), is http://www.omim.org
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2
UCSC Genome Browser, http://genome.ucsc.edu/

References

1. Giorgio, G., Alfieri, M., Prattichizzo, C., Zullo, A., Cairo, S., and Franco, B. (2007). Functional characterization of the OFD1 protein reveals a nuclear localization and physical interaction with subunits of a chromatin remodeling complex. Mol. Biol. Cell *18*, 4397–4404.
2. Singla, V., Romaguera-Ros, M., Garcia-Verdugo, J.M., and Reiter, J.F. (2010). Ofd1, a human disease gene, regulates the length and distal structure of centrioles. Dev. Cell *18*, 410–424.
3. Valente, E.M., Logan, C.V., Mougou-Zerelli, S., Lee, J.H., Silhavy, J.L., Brancati, F., Iannicelli, M., Travaglini, L., Romani, S., Illi, B., et al. (2010). Mutations in TMEM216 perturb ciliogenesis and cause Joubert, Meckel and related syndromes. Nat. Genet. *42*, 619–625.
4. Baraitser, M. (1986). The orofaciodigital (OFD) syndromes. J. Med. Genet. *23*, 116–119.
5. Temtamy, S.A., and McKusick, V.A. (1978). The genetics of hand malformations. Birth Defects Orig. Artic. Ser. *14*, i–xviii, 1–619.
6. Baraitser, M., Burn, J., and Fixsen, J. (1983). A female infant with features of Mohr and Majewski syndromes: variable expression, a genetic compound, or a distinct entity? J. Med. Genet. *20*, 65–67.
7. Burn, J., Dezateux, C., Hall, C.M., and Baraitser, M. (1984). Orofaciodigital syndrome with mesomelic limb shortening. J. Med. Genet. *21*, 189–192.
8. Adès, L.C., Clapton, W.K., Morphett, A., Morris, L.L., and Haan, E.A. (1994). Polydactyly, campomelia, ambiguous genitalia, cystic dysplastic kidneys, and cerebral malformation in a fetus of consanguineous parents: a new multiple malformation syndrome, or a severe form of oral-facial-digital syndrome type IV? Am. J. Med. Genet. *49*, 211–217.
9. Digilio, M.C., Giannotti, A., Pagnotta, G., Mingarelli, R., and Dallapiccola, B. (1995). Joint dislocation and cerebral anomalies are consistently associated with oral-facial-digital syndrome type IV. Clin. Genet. *48*, 156–159.
10. Toriello, H.V., Carey, J.C., Suslak, E., Desposito, F.R., Leonard, B., Lipson, M., Friedman, B.D., and Hoyme, H.E. (1997). Six patients with oral-facial-digital syndrome IV: the case for heterogeneity. Am. J. Med. Genet. *69*, 250–260.
11. Baala, L., Romano, S., Khaddour, R., Saunier, S., Smith, U.M., Audollent, S., Ozilou, C., Faivre, L., Laurent, N., Foliguet, B., et al. (2007). The Meckel-Gruber syndrome gene, MKS3, is mutated in Joubert syndrome. Am. J. Hum. Genet. *80*, 186–194.
12. Delous, M., Baala, L., Salomon, R., Laclef, C., Vierkotten, J., Tory, K., Golzio, C., Lacoste, T., Besse, L., Ozilou, C., et al. (2007). The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. Nat. Genet. *39*, 875–881.
13. Garcia-Gonzalo, F.R., Corbit, K.C., Sirerol-Piquer, M.S., Ramaswami, G., Otto, E.A., Noriega, T.R., Seol, A.D., Robinson, J.F., Bennett, C.L., Josifova, D.J., et al. (2011). A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition. Nat. Genet. *43*, 776–784.
14. Reiter, J.F., and Skarnes, W.C. (2006). Tectonic, a novel regulator of the Hedgehog pathway required for both activation and inhibition. Genes Dev. *20*, 22–27.
15. Sang, L., Miller, J.J., Corbit, K.C., Giles, R.H., Brauer, M.J., Otto, E.A., Baye, L.M., Wen, X., Scales, S.J., Kwong, M., et al. (2011). Mapping the NPHP-JBTS-MKS protein network reveals ciliopathy disease genes and pathways. Cell *145*, 513–528.
16. Shaheen, R., Faqeih, E., Seidahmed, M.Z., Sunker, A., Alali, F.E., AlQahtani, K., and Alkuraya, F.S. (2011). A TCTN2 mutation defines a novel Meckel Gruber syndrome locus. Hum. Mutat. *32*, 573–578.
17. Valente, E.M., Brancati, F., and Dallapiccola, B. (2008). Genotypes and phenotypes of Joubert syndrome and related disorders. Eur. J. Med. Genet. *51*, 1–23.
18. Putoux, A., Thomas, S., Coene, K.L., Davis, E.E., Alanay, Y., Ogur, G., Uz, E., Buzas, D., Gomes, C., Patrier, S., et al. (2011). KIF7 mutations cause fetal hydrolethalus and acrocallosal syndromes. Nat. Genet. *43*, 601–606.
19. Dafinger, C., Liebau, M.C., Elsayed, S.M., Hellenbroich, Y., Boltshauser, E., Korenke, G.C., Fabretti, F., Janecke, A.R., Ebermann, I., Nürnberg, G., et al. (2011). Mutations in KIF7 link Joubert syndrome with Sonic Hedgehog signaling and microtubule dynamics. J. Clin. Invest. *121*, 2662–2667.
20. Khaddour, R., Smith, U., Baala, L., Martinovic, J., Clavering, D., Shaffiq, R., Ozilou, C., Cullinane, A., Kyttälä, M., Shalev, S., et al.; SOFFOET (Société Française de Foetopathologie). (2007). Spectrum of MKS1 and MKS3 mutations in Meckel syndrome: a genotype-phenotype correlation. Mutation in brief #960. Online. Hum. Mutat. *28*, 523–524.
21. Mougou-Zerelli, S., Thomas, S., Szenker, E., Audollent, S., Elkhartoufi, N., Babarit, C., Romano, S., Salomon, R., Amiel, J., Esculpavit, C., et al. (2009). CC2D2A mutations in Meckel and Joubert syndromes indicate a genotype-phenotype correlation. Hum. Mutat. *30*, 1574–1582.
22. Katsanis, N., Ansley, S.J., Badano, J.L., Eichers, E.R., Lewis, R.A., Hoskins, B.E., Scambler, P.J., Davidson, W.S., Beales, P.L., and Lupski, J.R. (2001). Triallelic inheritance in Bardet-Biedl

syndrome, a Mendelian recessive disorder. Science *293*, 2256–2259.

23. Badano, J.L., Kim, J.C., Hoskins, B.E., Lewis, R.A., Ansley, S.J., Cutler, D.J., Castellan, C., Beales, P.L., Leroux, M.R., and Katsanis, N. (2003). Heterozygous mutations in BBS1, BBS2 and BBS6 have a potential epistatic effect on Bardet-Biedl patients with two mutations at a second BBS locus. Hum. Mol. Genet. *12*, 1651–1659.
24. Tory, K., Lacoste, T., Burglen, L., Morinière, V., Boddaert, N., Macher, M.A., Llanas, B., Nivet, H., Bensman, A., Niaudet, P., et al. (2007). 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. *18*, 1566–1575.
25. Davis, E.E., Zhang, Q., Liu, Q., Diplas, B.H., Davey, L.M., Hartley, J., Stoetzel, C., Szymanska, K., Ramaswami, G., Logan, C.V., et al.; NISC Comparative Sequencing Program. (2011). TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum. Nat. Genet. *43*, 189–196.
26. Khanna, H., Davis, E.E., Murga-Zamalloa, C.A., Estrada-Cuzcano, A., Lopez, I., den Hollander, A.I., Zonneveld, M.N., Othman, M.I., Waseem, N., Chakarova, C.F., et al. (2009). A common allele in RPGRIP1L is a modifier of retinal degeneration in ciliopathies. Nat. Genet. *41*, 739–745.
27. Louie, C.M., Caridi, G., Lopes, V.S., Brancati, F., Kispert, A., Lancaster, M.A., Schlossman, A.M., Otto, E.A., Leitges, M., Gröne, H.J., et al. (2010). AHI1 is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis. Nat. Genet. *42*, 175–180.