POC1A Truncation Mutation Causes a Ciliopathy in Humans Characterized by Primordial Dwarfism

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Primordial dwarfism (PD) is a phenotype characterized by profound growth retardation that is prenatal in onset. Significant strides have been made in the last few years toward improved understanding of the molecular underpinning of the limited growth that characterizes the embryonic and postnatal development of PD individuals. These include impaired mitotic mechanics, abnormal *IGF2* expression, perturbed DNA-damage response, defective spliceosomal machinery, and abnormal replication licensing. In three families affected by a distinct form of PD, we identified a founder truncating mutation in *POC1A*. This gene is one of two vertebrate paralogs of *POC1*, which encodes one of the most abundant proteins in the *Chlamydomonas* centriole proteome. Cells derived from the index individual have abnormal mitotic mechanics with multipolar spindles, in addition to clearly impaired ciliogenesis. siRNA knockdown of *POC1A* in fibroblast cells recapitulates this ciliogenesis defect. Our findings highlight a human ciliopathy syndrome caused by deficiency of a major centriolar protein.

Primordial dwarfism (PD) is a term used for describing severe human growth deficiency with a prenatal onset and reflects a fundamental postnatally persisting defect in the genetic growth potential of human embryos.[1] Clinically, many subtypes have been described on the basis of the presence of additional features, but perhaps one of the most helpful clinical traits in the differential diagnosis is the degree of involvement of the head circumference. A few conditions are characterized by a largely preserved head circumference despite PD phenotypes such as 3M syndrome (MIM 273750, 612921, and 614145), Russel-Silver syndrome (SRS [MIM 180860]), and Mulibrey nanism (MIM 253250).[2–4] On the other hand, many PD-affected individuals have microcephaly as part of the overall growth-restriction phenotype. Seckel syndrome (SCKL [MIM 210600, 606744, 608664, 613676, and 613823]) is one of the best known because of highly characteristic facies, but other conditions such as microcephalic osteodysplastic primordial dwarfism type I (MOPD I [MIM 210710]), MOPD II [MIM 210720], and Meier-Gorlin syndrome (MGORS1 [MIM 224690, 613800, 613803, 613804, and 613805]) are also recognized clinical entities, although their clinical definitions are less clear.[5–8]

PD is genetically heterogeneous, and an expansive list of genes is linked to this phenotype. As predicted for a condition that is characterized by impairment of one of the most basic biological processes, i.e., growth, the products of these genes represent a wide array of molecular mechanisms that include mitotic mechanics, *IGF2* expression, DNA-damage response, spliceosomal machinery, and replication licensing.[9–14] The identification of the above genetic lesions has provided fresh insights into factors controlling human growth and has propelled renewed interest into PD research, which is likely to unravel additional disease pathways.

Abnormal mitotic mechanics as a pathogenic lesion in PD has been documented for three subtypes. *PCNT* (MIM 605925), encoding pericentrin, is mutated in almost all individuals with MOPD II and causes disorganized mitotic spindles and missegregation of chromosomes.[9,15,16] We have shown that a mutation in *CENPJ* (MIM 609279), encoding another centrosomal protein, causes Seckel syndrome most likely through disruption of centrosome integrity and induction of multipolar spindles, as shown by others.[13,17] Interestingly, mutations in the gene encoding centrosomal protein CEP152 (MIM 613529), known for its role in recruiting CENPJ to the centrosome, have also been shown to cause Seckel syndrome.[18,19] Surprisingly, none of these mutations has been shown to impair ciliogenesis in cells derived from affected individuals, so despite the shared origin of centrosomes and cilia, it remains unclear whether PD represents a ciliopathy phenotype, at least in some cases.

Here, we combine the use of autozygome and exome analysis in one consanguineous family affected by PD to uncover a truncating mutation in *POC1A*, one of two vertebrate paralogs of *POC1*, which encodes one of the most abundant proteins in the *Chlamydomonas* centriole proteome.[20] The distinct clinical phenotype aided us in identifying similarly affected individuals from two other

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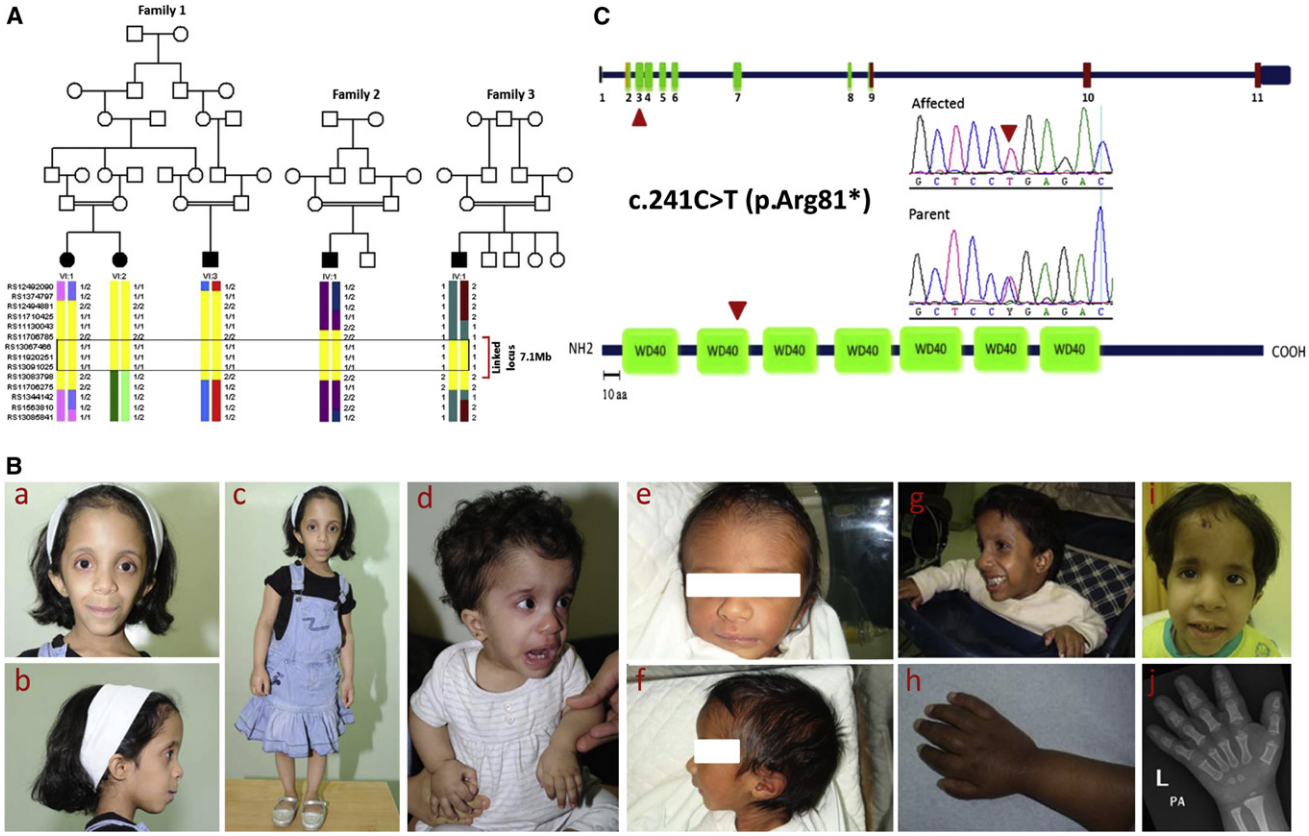

Figure 1. Identification of a POC1A-Related PD Phenotype

(A) Pedigrees of the three PD-affected families included in the study and the haplotype shared between the affected individuals in the three families.

(B) Clinical photographs of the affected individuals (a, b, and c are of VI1 from family 1, d is of VI2 from family 1, e and f are of VI3 from family 1, g and h are of IV1 from family 2, and i and j are of IV1 from family 3).

(C) The upper panel shows a diagram of *POC1A* (the triangle indicates the site of the mutation). The middle panel shows a sequence chromatogram of the nonsense mutation and the parent tracing shown for comparison (the location of the mutation is indicated by a triangle). The bottom panel shows a diagram of POC1A (note that the truncated alteration is upstream of the five WD40 domains).

PD-affected families who we show to harbor the same mutation on a common ancestral disease haplotype. In addition to the multipolar spindle formation, this mutation results in severely impaired ciliogenesis in cells derived from affected individuals. Thus, our study adds PD to a growing list of ciliopathy phenotypes in humans.

The index patient in family 1 (Figure 1A) is a 6-year-old girl with profound growth deficiency (weight of −5 standard deviations [SDs], height of −6.7 SDs, and occipitofrontal circumference [OFC] of −2.3 SDs). Her birth parameters were also severely reduced (weight of −3.9 SDs, length of −4.7 SDs, and OFC of −2.2 SDs). She had relative macrocephaly and distinct facial features in the form of an elongated triangular face, a high forehead, hypertelorism, a depressed nasal bridge, a broad upturned nose, a long philtrum, and posteriorly rotated low-set ears (Figures 1Ba–1Bc). She displayed normal development. One year later, another female child was born to the same parents and clearly had evidence of PD at birth (weight of −3.8 SDs, length of −5.8 SDs, and OFC of −2.2 SDs). When she was 22 months of age, growth retardation persisted (weight −6.3 SDs and height −7.1 SDs) and similar facial features to her sister's became more recognizable (Figure 1Bd). Two years later, a cousin was born with an abnormally low weight and length but a normal head circumference (weight of −2.3 SDs, length of −3 SDs, and OFC at the 35th percentile). Similar to his two cousins, he displayed relative macrocephaly at the age of 3 months (weight of −3.2 SDs, length of −5.1 SDs, and OFC at the 57th percentile) and similar facial features (Figures 1Be and 1Bf).

Family 2 (Figure 1A) consists of first-cousin Saudi parents and one 6-year-old child with profound global developmental delay and PD. His facial features have a striking resemblance to those observed in family 1. The pregnancy was complicated by poor fetal growth necessitating induced delivery at 36 weeks of pregnancy. The birth weight was 1 kg (−4.1 SDs), and the remaining parameters were not available to us. He stayed in the neonatal intensive care unit for 2 months because of his low birth weight. He ate poorly, vomited frequently, and showed significant developmental delay. His clinical examination revealed a failure to thrive, a weight of 6.45 kg (−6.3 SDs), a height of 79.6 cm (−7.1 SDs), and an OFC of 43 cm (−6.4 SDs). He

had a high forehead, deep-set eyes, a prominent columella, dolichocephaly, and stubby fingers (Figures 1Bg and 1Bh). Neurological examination was significant for hypotonia. His thyroid-function test and growth-hormone level were unremarkable.

Family 3 (Figure 1A) consists of first-cousin Saudi parents with two healthy daughters and three sons, one of whom was born at term with a very low birth weight (1.25 kg), but the other parameters were unavailable. He was 32 months of age at the time of his referral for severe failure to thrive (weight of −5.9 SDs, length of −7.1 SDs, and OFC of −3.3 SDs). He had evidence of global developmental delay (he spoke 3–4 words and walked at 24 months). In addition to having facial features similar to those of the previously described individuals, his hands and feet were similar to those of the individuals from family 2. A skeletal survey showed diffuse osteopenia, strikingly hypoplastic epiphyses most notably in the proximal humerus and femur, a 2 year delay in skeletal maturity, and short and broad carpals, metacarpals, tarsals, and metatarsals (Figures 1Bi and 1Bj). A summary of the clinical features of all five affected individuals is provided in Table S2, available online. Of note, typical signs of ciliopathy, such as polydactyly, retinal degeneration, and abnormal liver and kidneys, were specifically ruled out.

When the sister of the index patient was born, it was likely that this form of PD was autosomal recessive. Therefore, they were enrolled after written informed consent was obtained and approved by the King Faisal Specialist Hospital and Research Center Research Advisory Council (2080006). Autozygome analysis was performed on the index patient and her affected sister from family 1 with the Axiom SNP Platform (Affymetrix, Santa Clara, CA, USA) and was followed by autoSNPa genome-wide determination of runs of homozygosity essentially as described before.[21] Autozygome analysis revealed no shared overlap between the two sisters with any of the known autosomal-recessive causes of PD. This was followed by exome sequencing and autozygome filtration as described before.[22,23] The resulting short list of four genes contained a nonsense mutation (c.241C>T [p.Arg81*]; RefSeq accession number NM_015426.4) in *POC1A* (located on chromosome 3, band 3p21.2), which encodes a centriolar protein (Table S1). Despite the clear difference in motor and cognitive developmental profile between the two sisters in family 1 and the affected individuals in families 2 and 3, the strikingly similar facial profile prompted us to sequence this gene in them, and we did indeed uncover the same mutation in all individuals and confirmed that all shared a common ancestral disease haplotype (Figure 1A and Figures S1A and S1B). Reassuringly, exome sequencing revealed that the mutation we identified was the only novel (not present in SNP databases) truncating homozygous mutation in the shared haplotype. Two homozygous missense variants were also identified in the shared haplotype; however, both are present in the reference genome of other species (Table S1). The nonsense mutation in *POC1A* was not present in 194 Saudi exomes or in the Exome Variant Server.

In order to study the effect of this mutation on the transcript stability, we performed real-time PCR, which showed a 65%–80% reduction consistent with nonsense-mediated-decay activation (Figures S2A and S2B) as confirmed by a cyclohexamide rescue assay (Figure S2C). Immunoblot analysis of the cells derived from the index individual consistently showed that apparently normal-sized POC1A was produced at a reduced level (Figure S2D). The nonsense mutation predicted an 8 kDa protein compared with the normal 40 kDa POC1A. We considered the possibility that this might represent a nonspecific 40 kDa band, but two independent POC1A antibodies gave the exact same result. Furthermore, the specificity of the band was confirmed with the antigen protein against which the POC1A antibody was raised (Figure S2D). The possibility that this protein was a product of an alternatively spliced transcript that skipped exon 3 and did not contain the mutation was not supported by RT-PCR analysis, which failed to identify any RNA transcript other than the mutant RNA that harbored the nonsense mutation (Figure S2A). The 40 kDa band is unlikely to be the product of downstream reinitiation because the first possible alternate downstream initiation codon will create a protein of 20 kDa. Therefore, the possibility of a nonsense premature termination codon (PTC) readthrough was the most likely. Naturally occurring PTC readthroughs of a nonsense mutation have been previously reported.[22,24] It is interesting that three independent immunoblots were performed for the same cells derived from the index individual and were harvested at different times and that we found variability in the level of POC1A (50%, 60%, and 85% for each of the three immunoblots), indicating that the PTC readthrough is variable and might account, at least in part, for the variability in phenotype.

We then performed in situ hybridization on embryonic day 10.5 mouse embryos and quantitative RT-PCR (qRT-PCR) on various mouse embryo stages and adult tissues, and we found widespread expression of *Poc1a* (Figure S4). In view of the established role of POC1 in centriole formation and maintenance,[25] we tested whether the mutation we identified affects the function of the centriole during mitosis given that this could explain the PD phenotype on the basis of abnormal mitosis, the same mechanism invoked for at least two other genes linked to PD.[9,14,19] Indeed, we observed multipolar spindle formation at a frequency of 20% in metaphase-stage cells derived from the index individual (none was observed in control cells). Remarkably, we also observed the occurrence of supernumerary centrioles at a frequency of 13% in cells derived from the index individual (none was observed in control cells), although it was not clear whether these represented overduplication or abnormal fragmentation (Figure 2). Repeated experiments in fibroblasts derived from the index individual clearly showed that this phenomenon

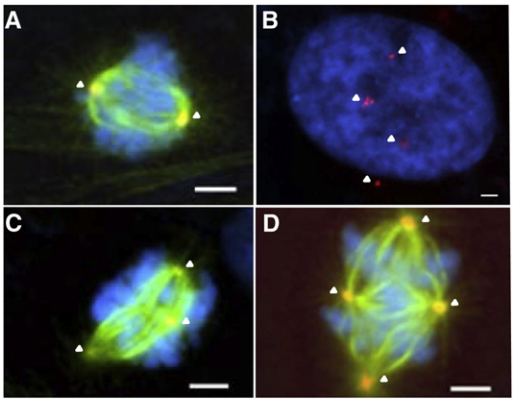

Figure 2. Mutation in *POC1A* Leads to Abnormal Mitotic Spindles and Centrioles
Fibroblast cells stained with anti-PCNT (red), anti-α-tubulin (green), and DAPI.
(A) The control fibroblast cell in the metaphase stage shows the normal number of centrioles and mitotic spindles.
(B–D) Fibroblasts derived from the index individual show an abnormal number of centrioles and the tri- and quadri-polar spindle phenotype (arrowheads). The scale bars represent 5 μm.

of supernumerary centrioles occurred in cells in the prophase and metaphase stages of the cell cycle.

Other than the important role the centrioles play in centrosome-mediated control of mitosis through organization of the mitotic spindles, they are critical for the formation of cilia (motile and immotile). We asked whether the abnormal centriole phenotype we observed in the centrosome extends to the cilia as well. Indeed, whereas control fibroblasts were almost uniformly capable of forming normal cilia after serum starvation, fibroblasts derived from the index individual were severely deficient in that capacity (70% reduction, $p = 5.4 \times 10^{-7}$) (Figures 3A–3C), which strongly suggests an essential role played by POC1A in ciliogenesis in humans and that our mutation severely impairs this function. Furthermore, we observed that the cilia from PD fibroblasts derived from the index individual were significantly shorter ($p = 1.28 \times 10^{-11}$) than the cilia from control cells (all cells were plated on gelatin [Figures 3A, 3B, and 3D]). To reveal whether the few short cilia observed in the fibroblasts derived from the index individual were functional, we stained the cells by using antibodies against IFT-A and IFT-B complex, and we saw a normal IFT-A (represented by THM1) and IFT-B (represented by IFT88) staining pattern (Figures 3F and 3H). One explanation that we hypothesize is that the PTC readthrough mechanism is variable between cells and that once a critical amount of POC1A is made, the cell will manage to form a normal functioning cilium. In line with the data we show on the normal IFT-A and IFT-B, *GLI1* upregulation, as a readout of sonic hedgehog (SHH) signaling, in response to SAG does not appear muted in *POC1A*-knockdown cells (Figure S3). This might explain why our affected individuals lack the more "classical" ciliopathy phenotype.

POC1A-knockdown experiments with RNAi showed that *POC1A* deficiency causes a severe ciliogenesis defect (Figures 4B and 4C). It is important here to note that there was no evidence that *POC1B* was reduced in those knockdown cells, which lends credence to the conclusion that *POC1A* deficiency causes the ciliogenesis defect we see in cells derived from the index individual (Figure 4H). Pearson et al. found that knockdown of *POC1B*, but not of *POC1A*, results in a ciliogenesis defect, but they acknowledged that the efficiency of *POC1A* knockdown was poor and that more significant reduction could result in a ciliogenesis defect,[26] as we were able to achieve here. This raises interesting possibilities about whether *POC1B* mutations in humans could result in a similar phenotype. The supernumerary centriole phenotype observed in prophase- and metaphase-stage cells derived from the index individual was also replicated in cells in which we achieved highly efficient but low-frequency (5%) knockdown of *POC1A* (Figures 4E and 4G). This raises the intriguing possibility that this cellular phenotype might be allele specific.

Centrioles have long been recognized for two physiological roles—microtubule organization and the formation of cilia and flagella—and both roles have been implicated in human diseases, most notably in the setting of developmental anomalies.[27] The MTOC role is known to be disrupted by mutations in four genes known to cause primary microcephaly (*CENPJ*, *CEP215*/*CDK5RAP2* [MIM 608201], *ASPM* [MIM 605481], and *STIL* [MIM 181590]) and in one gene that is linked to Seckel PD (*PCNT*). It is important to highlight that PCNT is a component of the PCM rather than the centriole itself, and yet it has been found in flies and mice to be essential for cilia formation.[28,29] Although the PD phenotype in *PCNT*-related Seckel individuals is presumed to be caused by defective MTOC, it remains unknown whether impaired ciliogenesis might also be part of the pathogenesis. On the other hand, ciliopathies represent a clinically heterogeneous group of disorders in which a ciliary defect is the key pathogenic mechanism.[30] A few ciliopathies, such as Alstrom syndrome (MIM 203800), are caused by defective structural centriolar proteins, but most can be traced to abnormalities in other aspects of ciliary biology. Unlike other forms of centriole-related PD, the families we describe in this study highlight a clinically distinct PD, and the fact that cells derived from affected individuals with this phenotype display defects in both roles of centrioles suggests that this is a bona fide ciliopathy phenotype.

In summary, we report a distinct PD phenotype caused by deficiency of the centriolar protein POC1A. The two basic centriolar functions of MTOC and ciliogenesis are impaired in individuals with this phenotype, suggesting that PD can be added to a growing list of ciliopathy phenotypes in humans.

Supplemental Data

Supplemental Data include four figures and two tables and can be found with this article online at http://www.cell.com/AJHG.

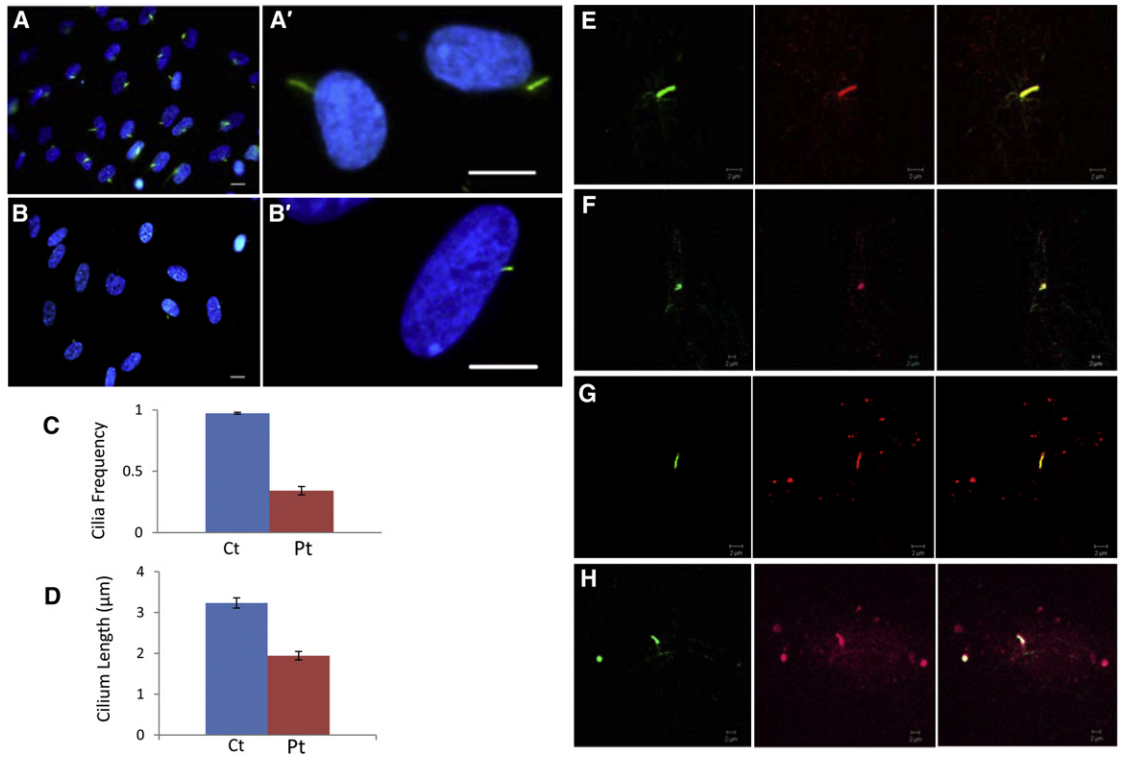

Figure 3. *POC1A*-Related PD Is a Ciliopathy
(A–B′) Control fibroblasts and fibroblasts derived from the index individual were visualized by fluorescent microscopy for acetylated-α-tubulin staining of cilia (green) and DAPI-stained nuclei (blue). Control fibroblasts are uniformly capable of forming normal cilia after serum starvation (A), whereas fibroblasts derived from the index individual show severely deficient cilia formation (B) (the same numbers of cells were seeded on the coverslip for each cell line). Higher magnifications (A′ and B′) show that the axonemal lengths of the cilia from control fibroblasts (A′) and fibroblasts derived from the index individual (B′) are significantly different. The scale bars represent 10 μm.
(C) The cilia frequencies of gelatin-plated control fibroblasts and fibroblasts derived from the index individual are significantly different ($p = 5.4 \times 10^{-7}$). Error bars represent the standard error of the mean (SEM).
(D) The average cilia lengths from control cells and cells derived from the index individual are significantly different ($p = 1.28 \times 10^{-11}$). Error bars represent the SEM.
(E–H) Control fibroblasts and fibroblasts derived from the index individual were visualized by confocal fluorescent microscopy for acetylated-α-tubulin staining of cilia (green) and IFT88 (1:1000; a generous gift from Dr. Bradley K. Yoder) or THM1 (1:500; a generous gift from Dr. Pamela V. Tran) (red). Control fibroblasts show the colocalization of IFT88 (E) and THM1 (G) with acetylated-α-tubulin staining of cilia. Normal IFT88 (F) and THM1 (H) staining patterns are shown in the few short cilia observed in the fibroblasts derived from the index individual.

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Web Resources

The URLs for data presented herein are as follows:

Burrows-Wheeler Aligner, http://bio-bwa.sourceforge.net/
Ensembl Genome Browser, http://www.ensembl.org/index.html
NHLBI Exome Sequencing Project Exome Variant Server, http://evs.gs.washington.edu/EVS/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
SAMtools, http://samtools.sourceforge.net/
UCSC Genome Browser, http://genome.ucsc.edu/

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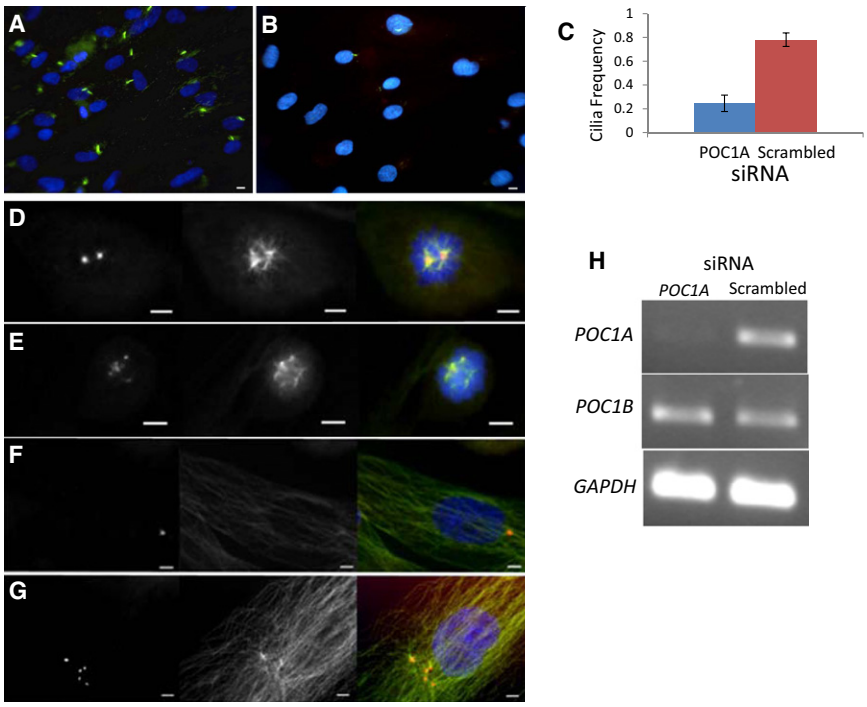

Figure 4. siRNA Knockdown of *POC1A* in Fibroblast Cells Recapitulates the Ciliogenesis Defect

(A and B) siRNA knockdown of *POC1A* in control fibroblasts cells and fibroblasts treated with scrambled siRNA (Ambion Silencer Select 4390846) were visualized by fluorescent microscopy for acetylated-α-tubulin staining of cilia (green) and DAPI-stained nuclei (blue). (One hundred picomoles of RNAi was diluted in 500 μl Opti-MEM I medium without serum in the 6-well tissue culture with the use of Ambion Silencer Select s24676). Fibroblast cells treated with scrambled siRNA are uniformly capable of forming normal cilia after serum starvation (A), whereas siRNA *POC1A* fibroblasts show severely deficient cilia formation (B). The scale bars represent 10 μm.

(C) The average cilia frequency of siRNA-knockdown *POC1A* and the negative control are significantly different ($p = 1.5 \times 10^{-9}$). Error bars represent the SEM.

(D–G) siRNA knockdown of *POC1A* in control fibroblasts and fibroblasts treated with scrambled siRNA were stained with anti-PCNT (red), anti-α-tubulin (green), and DAPI (blue).

(D and F) Scrambled siRNA-treated cells show the normal number of centrioles and mitotic spindles.

(E and F) Cells treated with siRNA-knockdown *POC1A* show abnormal numbers of centrioles and the multipolar spindle phenotype.

(H) A gel image shows the siRNA depletion of *POC1A* and the normal expression of *POC1B*.

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