CORRESPONDENCE

A novel splice site mutation in *CEP135* is associated with primary microcephaly in a Pakistani family

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Autosomal recessive primary microcephaly (MCPH; MIM251200) is a rare congenital neurodevelopmental condition characterized by reduced brain and skull size with sloping forehead, a largely normal brain architecture, and mild to severe intellectual disability.¹ Pathogenic mutations in the 13 known MCPH genes (*MCPH1*, *WDR62*, *CDK5RAP2*, *CASC5*, *ASPM*, *CENPJ*, *STIL*, *CEP152*, *ZNF335*, *PHC1*, *CDK6*, *CEP135* and *hSAS-6*) affect centrosome assembly, duplication and microtubule organization during cell division resulting in reduced growth of the cerebral cortex.² However, for some of these genes only one mutation has been discovered so far, including *CEP135* where only one mutation, (c.970delC, p.Gln324Serfs*2) in exon 8 have been reported to underlie MCPH in a Northern Pakistani family.³ Herein, we report the second mutation in *CEP135*, in a consanguineous Pakistani family with MCPH in two affected individuals (IV-1 and IV-2; Figure 1a) with severe learning disabilities, speech impairment, but no seizures. Their head circumferences at the age of 10 and 7 years were –14 and –12 s.d. below the population age and sex mean, respectively. The healthy parents are first cousin. The study was approved by the ethics review board at the National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan and informed consent was obtained from the parents. Direct sequencing of the *ASPM* gene (the most common MCPH disease gene) did not reveal any pathogenic variant and linkage was excluded for *ASPM* and five additional MCPH loci (*MCPH1*, *WDR62*, *CDK5RAP2*, *CASC5* and *CENPJ*) by the use of STS markers. Whole-exome sequencing of patient IV-1 (Nextera Rapid Capture Expanded Exome Enrichment Kit; 2×100 bp paired-end sequencing on an

Illumina Hi-seq 2000 (Illumina, San Diego, CA, USA)), gave an average 100× coverage. Analysis of the exome sequencing data was performed using Burrows Wheel Alignment and the Genome Analysis Toolkit.^{4,5} Common variants in dbSNP138 and 1KG with minor allele frequency >0.01 were excluded. Only one of the 14 known MCPH genes contained a rare mutation: a novel homozygous point mutation (c.1473+1G>A) affecting the first nucleotide of intron 11 in *CEP135*. Sanger sequencing of the exon 11-intron 11 boundary confirmed homozygosity in both patients and heterozygosity in their parents (Figure 1b). The mutation was neither present in 200 healthy Pakistani controls nor reported in the Exome Variants Server (<http://evs.gs.washington.edu/EVS/>), or in the Exome Aggregation Consortium database (ExAC), Cambridge, MA, USA (URL: <http://exac.broadinstitute.org/gene/ENSG00000174799>) (September 2015). Although it was not possible to obtain a new blood sample for functional analysis of the mutation, it is well established that mutations in the conserved GT donor splice-site recognition motif often lead to aberrant splicing,⁶ and the NetGene2 Server (<http://www.cbs.dtu.dk/services/NetGene2/>) predicted that the mutation would abolish the splice donor site at the exon 11-intron 11 boundary of *CEP135*. To further investigate the splicing error caused by the c.1473+1G>A mutation, we constructed mini-gene vectors by cloning a part of *CEP135* gene (including the sequence between exon10 to exon 12) using the genomic DNA of the patient and a control as a template (Figure 1c). The following primers were used for PCR amplification of the genomic region (forward primer; CEP135-E10F-BamHI:

5'-AAAGGATCCGAATTGAACCTATGCC AGAAAG-3', and a reverse primer; CEP135-E12R-XhoI: 5'-AAACTCGAGCTTAGTTTAT CTCTTTCTGCTGTC-3'). The amplified PCR products were cloned into the pCMV-script mammalian expression vector at the *Bam*HI and *Xho*I restriction sites. Transfection was done in HEK293T cells using FuGENE 6 transfection reagent (Promega, Madison, WI, USA). Total RNA was extracted from the transfected cells using a NucleoSpin RNA extraction kit (MACHEREY-NAGEL GmbH & Co. KG., Düren, Germany), and reverse transcription was performed using oligo-dT primers and Superscript II (Invitrogen, Carlsbad, CA, USA). To detect the transcript and possible splicing events, RT-PCR was performed using a vector-specific forward primer (RT-F), and CEP135-E12R-XhoI (reverse primer). Agarose gel electrophoresis of the PCR products showed a 224-bp shorter transcript produced from the mutant mini-gene vector, compared with the normal control (data not shown). Direct sequencing of the RT-PCR products revealed normal splicing of the wild-type allele, whereas the mutant allele showed aberrant splicing leading to complete skipping of exon 11 (Figure 1c). Skipping of exon 11 of the *CEP135* gene leads to a frameshift (at codon 417) and a premature stop codon after incorporation of one amino acid (p.E417Gfs*2). Thus, we expect that the resultant transcripts would lead to nonsense-mediated decay and/or be translated into a truncated protein of 417 amino acids (Figures 1c and d). However, the possibility of alternate splicing event due to a cryptic donor site cannot be excluded *in vivo*.

CEP135 located at 4q12 consists of 26 exons, which encode an 1140 amino acids long coiled-coil centriolar protein, which acts as a scaffolding protein during centriole

Figure 1 Identification of the mutation in *CEP135*. (a) Pedigree of the Pakistani family with two affected individuals born to consanguineous parents. (b) Identification of a homozygous mutation (c.1473+1G>A) affecting the first nucleotide of intron 11 (vertical arrows). (c) Schematic representation of mini-gene vectors of the *CEP135* gene, and splicing events. In case of wild-type vector, normal splicing events as shown by chromatograms (upper panel), whereas in case of mutant vector, loss of splice donor site leads to skipping of exon 11 as shown by chromatograms (lower panel). Primers positions used for RT-PCR are indicated by arrows. (d) Schematic representation of *CEP135* and the full length *CEP135* protein having six coiled-coil domains. The *CEP135* regions involved in interactions with microtubules, CPAP and hSAS-6 proteins are indicated. Both *CEP135* mutations reported so far (including this study) lead to truncated protein products which lack the hSAS-6 interacting domain. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

biogenesis.⁷ Functionally, CEP135 consists of an N-terminal microtubule interacting domain (amino acid residues 1–190), a central CPAP interacting domain (50–460), and a C-terminal hsSAS-6 interacting domain (416–1140; Figure 1c).⁸ Morphologically centrioles are composed of nine triplets of microtubules structured around an hsSAS-6 based cartwheel structure. It has been shown that CEP135 directly interacts via its carboxyl terminal with hsSAS-6.⁸ The mutation p.E417Gfs*2 in *CEP135* would lead to complete loss of its C-terminus hsSAS-6 interacting domain (Figure 1c). Thus, similar to the previously reported mutation p.E417Gfs*2 would most likely lead to multiple and fragmented centrosomes with disorganized microtubules.³ In summary, we have identified the second mutation in *CEP135*, confirming the role of CEP135 during embryonic brain development, and in the pathophysiology of human primary microcephaly.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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