## ORIGINAL ARTICLE

# A novel deletion mutation in *CENPJ* gene in a Pakistani family with autosomal recessive primary microcephaly

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**Abstract** Autosomal recessive primary microcephaly (MCPH) is a rare human genetic disorder in which the head circumference is reduced because of abnormality in fetal brain growth. To date, six loci and four genes have been identified for this condition. Our study of primary MCPH led to the identification of 33 Pakistani families with different ethnic backgrounds. Most of these families showed linkage to *MCPH5* locus on chromosome 1q31. Only one family with Pashtoon origin from a remote region in Pakistan linked to *MCPH6* locus on chromosome 13q12.12–q12.13. Sequence analysis of exon 11 of *CENPJ* gene, located at *MCPH6* locus, revealed a novel four base pair deletion mutation, which is predicted to be protein truncating.

**Keywords** Autosomal recessive primary microcephaly · *CENPJ* gene · *MCPH6* locus · Pakistan · Sequence analysis

## Introduction

Autosomal recessive primary microcephaly (MCPH) (MIM 251200) is a rare genetic disorder in which the head circumference of an affected individual is >3 SD below the population age and sex-related mean. The affected individuals have significantly small head circumference and are mentally retarded, but have no other abnormal clinical findings.

MCPH is genetically heterogeneous with six loci (*MCPH1–MCPH6*) mapped to date (Jackson et al. 1998; Roberts et al. 1999; Moynihan et al. 2000; Jamieson et al. 1999; Pattison et al. 2000; Leal et al. 2003). For these six loci mutations in four genes including *Microcephalin* (Jackson et al. 2002) at *MCPH1*, *CDK5RAP2* (Bond et al. 2005) at *MCPH3*, *ASPM* (Bond et al. 2002) at *MCPH5* and *CENPJ* (Bond et al. 2005) at *MCPH6* have been reported to cause clinically indistinguishable disorders.

The centromere associated protein J (*CENPJ*; MIM 609279), also known as centrosomal protein 4.1-associated protein (*CPAP*), is one of the more recently identified MCPH genes (Bond et al. 2005). This gene contains 17 exons and spans 39.8 kb on human chromosome 13q. Analysis of cDNA of *CENPJ* revealed a nucleotide sequence of 4,370 bases that contains a single open reading frame (ORF) of 1,338 amino acids (Hung et al. 2000). The *CENPJ* shows high level of expression in brain and spinal cord with primary expression localize to neuroepithelium (Bond et al. 2005).

To date, only two mutations including a homozygous single base deletion 17delC resulting in a premature stop codon (T6fsX3), and a missense mutation (E1235V), have been reported in *CENPJ* gene (Bond et al. 2005).

In the present study, we report a homozygous four base-pair deletion, c.3,243–3,246delTCAG, in a Pakistani family with autosomal recessive primary microcephaly (MCPH).

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## Materials and methods

### Family history

A family (Fig. 1) from a remote region of Pakistan with primary MCPH was located and studied. Prior to start of the study, approval was obtained from the Quaid-i-Azam University Institutional Review Board (IRB). The family members rarely marry outside the family, and consequently consanguineous unions are common. The pedigree provides convincing evidence of autosomal recessive mode of inheritance and consanguineous loops accounted for all the affected persons being homozygous for the mutant allele. After obtaining informed consent, blood samples were collected from three affected (IV-3, IV-4, IV-5) and five normal (III-1, III-6, III-7, IV-6, IV-7) individuals.

### Genotyping

DNA was isolated from venous blood samples following a standard protocol (Sambrook et al. 1989), quantified by spectrophotometry at optical density 260, and stored at –20°C. In order to determine linkage or exclusion of the family to six known MCPH loci, a minimum of five microsatellite markers from each of the candidate region of these loci were genotyped in three affected and five normal individuals of the fam-

ily. Microsatellite markers were purchased from Invitrogen (Calif., USA). Genetic map distances of markers were obtained from the Marshfield genetic map (Broman et al. 1998). PEDCHECK (O'Connell and Weeks 1998) was used to identify Mendelian inconsistencies. Two-point linkage analysis was carried out using the MLINK program of the FASTLINK computer package (Cottingham et al. 1993).

PCR for each primer was performed in 25-μl reaction volumes containing 40 ng of genomic DNA, 20 pmol of primers, 200 μM of each dNTP, 1 U of Taq DNA polymerase (MBI Fermentas, UK), and 2.5 μl reaction buffer (KCl 50 mM, Tris–HCl pH 8.3, MgCl$_2$ 1.5 mM). The thermal cycling conditions used included 95°C for 5 min, followed by 40 cycles of 95°C for 1 min, 57°C for 1 min, 72°C for 1 min, and final extension at 72°C for 10 min. PCR was performed in a thermal cycler T3 obtained from Biometra (Germany). PCR products were resolved on 8% non-denaturing polyacrylamide gel, and genotypes were assigned by visual inspection.

### Sequencing CENPJ

The CENPJ (GenBank accession No. NM_018451.2) gene was sequenced in three affected and five normal members of the family. Primer3 software (Rozen and Skaletsky 2000) was used to design primers for the 17



**Fig. 1** Drawing of the pedigree segregating primary microcephaly. Affected males and females are indicated by *filled squares* and *circles*, respectively. *Double lines* between figures are representatives of consanguineous unions. Haplotypes for the most closely linked markers are shown below each symbol
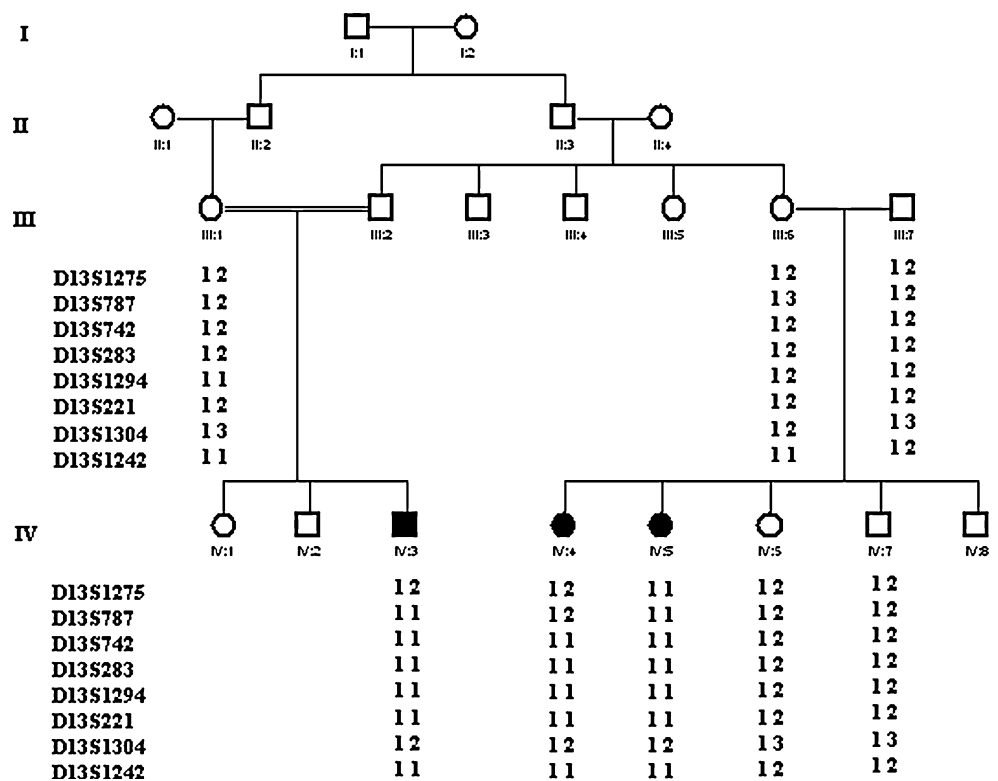
exons and exon/intron splice junctions of the CENPJ gene (Primer sequences and amplifying conditions are available on request). After DNA purification with ExoSAP-IT (USB, Cleveland, Ohio, USA), sequencing was performed with the BigDye Terminator v3.1 Cycle Sequencing Kit, together with an ABI Prism 310 Genetic Analyzer (Applera, Foster City, Calif., USA). Sequence variants were identified via Bioedit sequence alignment editor version 6.0.7. When the identified sequence variant was shown to segregate with the disease status within a family, a minimum 100 unrelated ethnically matched control individuals were also screened for the same exon.

## Results

### Clinical findings

The consanguineous Pakistani family with primary MCPH had three affected subjects, including one male and two females (Fig. 2), with ages varying between 8 and 13 years. The head circumference of all the three affected individuals was 3–5 SD below the expected mean when examined by us. Two affected individuals (IV-3 and IV-4) had mental retardation of moderate severity (IQ range from 45 to 50) whereas the third one (IV-5) had severe mental retardation (IQ 30–35). They were unable to read or write, could not speak simple phrases and did not have basic self-care skills. With the exception of intellectual impairment, there were no other neurological problems and motor development had been normal. No environmental causes could be found to explain the finding of MCPH. The parents had normal head circumference and intelligence. Standard lymphocyte karyotype, and electroencephalogram performed in affected subjects were normal, and brain scans showed no cerebral malformations.

### Genotyping and mutation analysis

Linkage was performed using highly polymorphic markers present within the known MCPH candidate linkage intervals. Linkage of a family to MCPH locus was based on the observation that all affected individuals had the same homozygous pattern (Fig. 1). According to International Human Genome Sequence Consortium 2003 (http://www.genome.ucsc.edu/cgi-bin/hgGateway), a single 2.99 Mb region of shared homozygosity and allele sharing among the three affected individuals was identified at MCPH6 locus on chromosome 13q12.12–q12.13 between markers D13S787 and D13S1304. Analysis of the marker genotypes within this region with PEDCHECK (O'Connell and Weeks 1998) did not elucidate any genotyping errors. The maximum two-point LOD score of 1.45 ($\theta$=0) was obtained with two markers D13S742 and D13S283, which is close to the 1.52 theoretical maximum for this family.

The entire coding region, as well as the intron–exon boundaries of *CENPJ*, a causative gene at MCPH6, was sequenced in all the three affected individuals (IV-3, IV-4, IV-5). Sequence analysis of exon 11 revealed a deletion of four consecutive nucleotides (TCAG) (Fig. 3). The deletion spans from nucleotide position 3,243 to 3,246 (c.3,243–3,246delTCAG). This mutation,
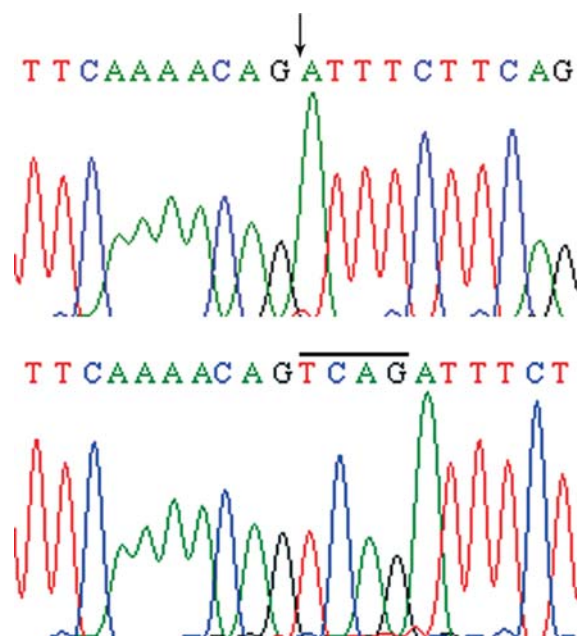


**Fig. 2** Affected individuals (IV-4 and IV-5) of the Pakistani family with typical sloping forehead



**Fig. 3** Sequence analysis of the *CENPJ* gene. *Top panel* Four base-pair deletion (3243delTCAG) in the homozygous state in an affected individual. *Bottom panel* The wild-type sequence of exon 11. The *bar* above the wild-type sequence in the *bottom panel* represents the sequence that is deleted in the homozygous state in the affected individual in the *top panel*

which leads to frameshift and premature termination codon 19 bp downstream in the same exon, is predicted to add six amino acids downstream of the mutation. The deletion mutation was present in heterozygous state in the obligate carriers of the family.

To ensure that the mutation does not represent a neutral polymorphism in this population, a panel of 50 unrelated unaffected ethnically matched control individuals (100 chromosomes) was screened for the mutation and it was not identified outside the family.

## Discussion

Recently, we have collected and studied 33 Pakistani families with primary MCPH. The majority of these families showed linkage to *MCPH5* locus on chromosome 1q31 (Gul et al. 2006). Only one family, belonging to a Pashto-speaking community living in a remote region of Pakistan, showed linkage to *MCPH6* locus on chromosome 13q12.2. Affected individuals had typical features of MCPH including sloping forehead and reduced head circumference. Mental retardation varied from moderate to severe form.

The *CENPJ*, at *MCPH6* locus, is the recently identified gene and so far only two homozygous mutations have been identified in three families (Bond et al. 2005). In the present study, we have identified a novel four base pair deletion mutation (c.3243–3246delT-CAG) in *CENPJ* gene responsible for primary MCPH in a Pakistani family of Pashtoon origin. In this ethnic group, mutations in *Microcephalin* and *ASPM* genes have been reported previously in families linked to *MCPH1* and *MCPH5* loci, respectively (Bond et al. 2003; Jackson et al. 2002).

The *CENPJ*, centromere associated protein J, also known as centrosomal P4.1-associated protein (*CPAP*), is a centrosomal protein localized to centrosomes in interphase and to the spindle poles during mitosis (Hung et al. 2000). Recently, Cho et al. (2006) have observed that depletion of CPAP disrupts centrosome integrity, and cells lacking CPAP arrest in mitosis with multipolar spindles.

The CENPJ protein consists of multiple hydrophobic regions and a C-terminal domain (residues 978–1,338) that shares limited homology with human Tcp-10, which is a t-complex responder gene that plays a role in the sex transmission ratio distortion. The hydrophobic regions in *CENPJ* form a stripe that winds around alpha-helix, which is capable of interacting with the hydrophobic residues of a second molecule to form a coiled-coil structure (Cohen and Parry 1986; Hung et al. 2000). Several recent reports

have shown that *CENPJ* contributes in the RelA, STAT5 and NF-kB mediated transcription, but these seem less likely to influence neurogenesis (Peng et al. 2002; Koyanagi et al. 2005; Bond and Woods 2006).

The centrosome plays a key role in regulating cell division, functioning as a microtubule organizing centre (MTOC). Bond et al. (2005) proposed that centrosomal mechanism is responsible for determining the brain size. The importance of centrosome in each major stage of the development and function of the nervous system is evident from the finding that three of the four MCPH proteins, Asp, CDK5RAP2 and CENPJ, have been found in the centrosome during mitosis (Bond et al. 2005). It has been shown that microtubules (MT) undergo reorganization during the cell cycle (Desai and Mitchison 1997). When cell enters mitosis, the interphase MT network is rapidly disassembled, followed by reorganization of MTs into mitotic spindle. The precise regulation of microtubule assembly and disassembly at centrosomes is thought to be important for the maintenance of spindle structures (Waters et al. 1996). Hung et al. (2000) observed that CPAP is a part of g-tubulin complex that may participate in microtubule nucleation.

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