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Short Communication

Homozygous Nonsense Mutation in the *ASPM* Gene Causes MCPH in Consanguineous Pakistani Families

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Authors' Contributions
AAA designed the study and wrote the manuscript. KB presented and designed the concept and analysis and interpretation of data. AG, ZL, IA, KH and AR collected blood samples and did clinical examination. HH and SPM obtained exome sequencing data MNK and AMK supervisoed the study.

Key words
Autosomal recessive primary microcephaly, *ASPM*, Pakistan, MCPH, WES.

ABSTRACT

Autosomal recessive primary microcephaly (MCPH) is a rare neurodevelopmental disorder characterized by severe reduction in the brain volume that affects especially the neocortex, and intellectual disability. To date 17 genes have been linked with this disease. Biallelic mutations in the abnormal spindle-like, microcephaly associated gene *ASPM* causes MCPH type 5, the most common MCPH subtype. In the current study we recruited two families from Sialkot region of Pakistan which were assessed by whole exome sequencing and cosegregation analysis. We identified a previously described mutation c.4802C>G (p.S1601*) in the *ASPM* gene in both families. This study further underlines that mutations in the *ASPM* gene are a common cause for microcephaly in the Pakistani population and updates our knowledge regarding the frequent involvement of *ASPM* in microcephaly.

Autosomal recessive primary microcephaly (MCPH) is a genetically heterogeneous neurodevelopmental disorder with a prevalence of 1.3 to 150 per 100 000 births depending on the level of consanguinity and ethnicity. MCPH is characterized by a reduction of the occipitofrontal head circumference (OFC) of more than two standard deviations below the mean for age, sex and ethnicity, and intellectual disability (ID). To date, 19 genes (MCPH1-19) have been linked to MCPH (Abdel-Hamid *et al*., 2016) that encode proteins involved in cell cycle regulation, DNA damage response, centriole duplication, neurogenesis and spindle pole organization and orientation (Bond *et al*., 2002; Faheem *et al*., 2015). Mutations in the *ASPM* gene, linked to MCPH5 (#605481), are reported to be the most common genetic cause for MCPH (68.6%) (Zaquot *et al*., 2017). Up to now, 71 different nonsense mutations in the *ASPM* gene are reported, all predicted to produce truncated proteins, while no correlation between the position of the mutation within the *ASPM* gene and the degree of microcephaly and ID exists (Khan *et al*., 2018). The *ASPM* gene encodes for a 3477 amino acids protein involved in mitotic spindle function in neuronal progenitors. A knockdown of ASPM results in alterations in cleavage plane orientation, leading to a premature

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switch from symmetric to asymmetric cell division (Fish *et al*., 2006). Thereby, mutations in the *ASPM* gene cause a substantial reduction in the number of post mitotic neurons reflected in a reduced brain size, and make ASPM a candidate gene for the regulation and evolution of brain size in the primate lineage.

Here, we report a previously reported homozygous nonsense mutation c.4802C>G in the *ASPM* gene (rs199422189) in two Pakistani families and provide clinical signs of patients with an *ASPM* mutation.

Materials and methods

The study was reviewed and approved by the local ethical committee and the ethical committee of the Charité University of Medicine, Berlin Germany (EA1/212/08). Informed written consent was obtained from the patients and guardians of the families. Patients were recruited from Sialkot region of Pakistan by field survey. All the patients were examined by medical experts for clinical assessment. Blood samples were taken from all available individuals of affected pedigrees, and DNA was extracted using standard laboratory protocols.

For whole exome sequencing (WES), genomic DNA libraries were prepared through an Agilent Sure SelectXT Human All Exon V6 kit following the manufacturer's protocol. The sequencing libraries were sequenced on an Illumina HiSeq 2000 platform. The preliminary whole exome data analysis was performed through BWA and GATK software to generate a BAM and a VCF file, respectively. Annotation of the VCF files were carried out through the wANNOVAR software and the data was manually analyzed for the presence of candidate pathogenic variant.

For segregation analysis, identified mutations in the *ASPM* gene (NM_018136) were verified through Sanger sequencing and segregation analysis. Sanger sequencing was performed on all available patients and one parent was used as control. Primer3web (version 4.0.0) tool was used to design the primers in the flanking region of pathogenic variants. Sequencing primers F5′- GTGCTTATGAGTTATTCTACCGGC -3′, R5′- TTTGCACCAAGTGGAGTGGG -3′, were used for Sanger sequencing.

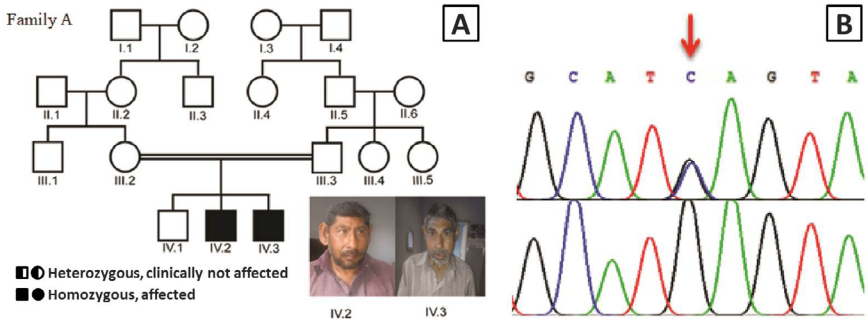

Fig. 1. A, Pedigree of the consanguineous family A of Pakistani descent with two patients affected by microcephaly (□, male; ○, female; ═, consanguineous marriage); B, Sanger sequencing results of patient IV.2 from family A showing a homozygous mutation c.4802C>G in the *ASPM* gene that leads to a nonsense mutation p.S1601* (red arrow). The parental control III.2 is heterozygous for the ASPM mutation.

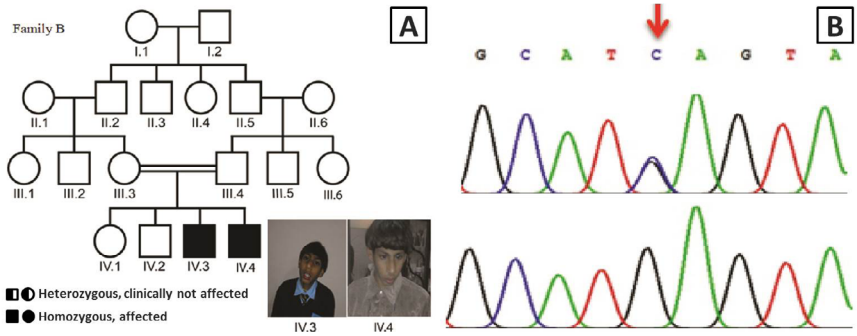

Fig. 2. A, Pedigree of the consanguineous family B of Pakistani descent with two patients affected by microcephaly, speech delay and motor delay (□, male; ○, female; ═, consanguineous marriage); B, Sanger sequencing results of patient IV.2 from family B showing a homozygous mutation c.4802C>G in the *ASPM* gene that leads to a nonsense mutation p.S1601* (red arrow). The parental control III.3 is heterozygous for the ASPM mutation.

Table I.- Clinical and morphological features of affected patients from Family A and B with *ASPM* gene mutation.

Family	Pedigree ID	Gender	Age	Phenotype	OFC	Weight (kg)	Height (cm)
A	IV.2	Male	55 years	Microcephaly, Aggressiveness	-6.4 SD	40	161.5
	IV.3	Male	45 years	Microcephaly, Cognitive delay	-5.5 SD	55	152.4
B	IV.3	Male	12 years	Microcephaly, Speech delay, Motor delay	-8.8 SD	25	145
	IV.4	Male	12 years	Microcephaly, Speech delay, Motor delay	-8.8 SD	24	143

Results

All affected individuals of family A had primary microcephaly with occipital frontal head circumference of 45.72 cm and 46.99 cm (IV.2 at 55 years; -6.4 SD and IV.3 at 45 years; -5.5 SD) and moderate ID, while patient IV.2 also showed aggressive behavior (Fig. 1A). In family B both patients had primary microcephaly with occipital frontal head circumferences of 40.64.cm (IV.3 at 12 years; -8.8 SD) and 40.64cm (IV.4 at 12 years; -8.8SD) and mild ID (Fig. 2A). In addition, both patients displayed a speech and motor delay. Affected members in both families were born at complete term with normal delivery. Clinical features of patients are summarized in (Table I).

In both families affected members showed small head with frontal narrowing. However, patients in family A with ASPM mutations had facial features in the form of prominent glabella, arched, thin eyebrows, and prominent eyes in infancy. With age this facial phenotype became subtle. Both the families had no relationship with each other. The forehead became sloping and the prominent eyes became less apparent. Both the families were recruited from two different regions and had no previous history of common ancestry.

We have identified via WES a previously reported nonsense mutation c.4802C>G located in exon 22 of the *ASPM* gene transcript 1 (NM_018136) in both families. The mutation was confirmed via Sanger sequencing and cosegregates within the pedigrees (Figs. 1B, 2B). The reported variant is not present in ExAC, 1000 genomes browser, or exome variant server, but reported in NCBI variation viewer and dbSNP as known disease mutation (rs199422189) and only pathogenic for primary autosomal recessive microcephaly type 5. The homozygous nonsense mutation (c.4802C>G) in the *ASPM* gene leads to a truncated protein, or nonsense mediated mRNA decay, resulting in the complete loss of the ASPM protein.

Discussion

We identified a previously reported nonsense mutation c.4802C>G (p.S1601*; rs199422189) in the *ASPM* gene in two Pakistani families showing clinical signs of primary microcephaly and ID. The mutation cosegregates with the disease phenotype in both families.

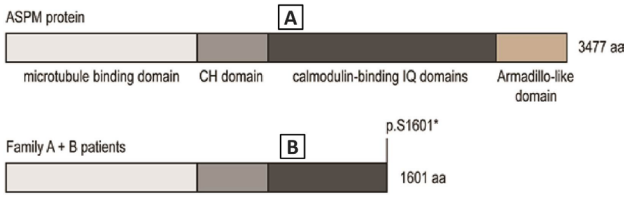

Fig. 3. A, the *ASPM* gene codes for a 3477 aa protein with four protein domains: microtubule binding domain (light grey), CH domain (grey), calmodulin-binding IQ domains (dark grey) and the Armadillo-like domain (brown); B, the nonsense mutation p.S1601* in patients of family A and B lead to a truncated protein, which is missing the Armadillo-like domain and part of the calmodulin-binding IQ domain.

The *ASPM* gene is mapped on chromosome 1q31.3 and encodes for a 10887 bp transcript (NM_018136) containing 28 exons that is translated into a 3477 amino acid protein (Fig. 3). Fetal expression pattern of *ASPM* mRNA in mouse brain revealed an expression at sites of active neurogenesis in the neuroepithelium (Bond *et al*., 2002). Thereby, the ASPM protein is involved in cleavage plane orientation, regulating the mitotic spindle function in neuronal progenitors. The loss of ASPM leads to a premature switch from symmetric to asymmetric cell division, decreasing the number of postmitotic neurons in the developing cortex, reflected in a reduced cortical surface area and a simplified gyral pattern. Therefore, the *ASPM* gene is involved in determining cerebral cortical size during development and linked to primary autosomal recessive microcephaly type 5 (MCPH5; (#605481)). To date 171 mutations in the *ASPM* gene have been identified comprising 73 nonsense, 4 missense and 14 splice site mutations along with 68 microdeletions 8 duplications and 1 insertion leading to frame shift mutations (Khan *et al*., 2018). Moreover one complex rearrangement along with two additional microdeletions has also been reported (Tan *et al*., 2014). In this study, we identified a homozygous nonsense mutation p.S1601* located in exon 22 that encodes for the calmodulin-binding IQ domains that serves as binding site for EF-hand proteins like calmodulin and PKC phosphorylation site (Baudier *et al*., 1991). In a previous study we have reported mutation in CRB1 gene

causing blindness in Kashmiri families (Latif *et al*., 2017). Calmodulin is known to regulate cell proliferation via the interaction with ASPM to regulate spindle assembly (Berchthold and Villalobo, 2014). We hypothesize that our nonsense mutation leads to a truncated protein or nonsense-mediated mRNA decay, resulting in the complete loss of the ASPM protein. Several publications support this notion by showing that no correlation between the severity of the disorder and the length of the truncated protein exist (Bond *et al*., 2003) and that the lack of the C-terminal domain of ASPM, which mediates mid body localization, seems to be sufficient to cause microcephaly in humans (Paramasivam *et al*., 2007).

Conclusion

We report two Pakistani families with a homozygous nonsense mutation in the *ASPM* gene and expand the knowledge about ASPM related microcephaly cases in the Pakistani population. This updates our knowledge regarding the frequent involvement of ASPM in microcephaly. Thus, ASPM should be included in an NGS panel to study microcephaly cases in a Pakistani cohort.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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