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Mutation analysis of *ASPM* gene in two Pakistani Famillies with autosomal recessive primary microcephaly

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Abstract

Autosomal recessive primary microcephaly (MCPH) is a disorder of fetal brain growth, with reduced head circumference. The affected individuals exhibit reduced occipital-frontal head circumference (>2 SD) and mild-to-severe mental retardation. Autosomal recessive primary microcephaly is genetically heterogeneous and 18 loci have been reported to date. Mutations in *ASPM* gene are the most common cause of MCPH in the majority of the cases. The objective of the study was to search for pathogenic mutations in two Pakistani families with autosomal recessive primary microcephaly. In the current investigation, we have identified a previously reported mutation in *ASPM* gene. DNA samples of all available affected and unaffected individuals were PCR amplified using microsatellite markers and further analyzed by DNA sequencing. DNA sequence analysis revealed a previously reported mutation (p.W1326*) in *ASPM* gene. This study furthur validate that mutations in ASPM are the major cause of autosomal recessive primary microcephaly in the Pakistani population.

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Introduction

Autosomal recessive primary microcephaly (MCPH, MIM#251200) is a disorder of fetal brain growth, representing a reduction in head circumference (HC). HC of at least 2 standard deviations (SD) below ethnically matched, age and sex related mean with variable degree of mental retardation and no other developmental abnormalities or neurological deficits is usually the cut-off for defining microcephaly (Mahmood *et al.,* 2011). The incidence of MCPH is relatively high (approximately 1/10,000), in regions where consanguineous marriages are common, such as Pakistan (Thornton *et al.,* 2009). MCPH is genetically heterogeneous and about 16 gene have been identified, including MCPH1, WDR62, CDK5RAP2, CASC5, ASPM, CENPJ, STIL, CEP135, CEP152, ZNF335, PHC1, CDK6, CENPE, SASS6, MFSD2A and ANKLE2 (Wang *et al.,* 2017). Mutations of abnormal spindle-like microcephaly-associated (ASPM) gene are the most leading cause of MCPH accounting for up to 40% in MCPH cases (Nicholas *et al.,* 2009).

In this study, we report autosomal recessive form of microcephaly in two unrelated consanguineous Pakistani families, exhibiting a recurrent homozygous nonsense mutation in the *ASPM* gene.

Materials and methods

For commencement of this study, approval was obtained from the Institutional Review Board (IRB) of Quaid-i-Azam University, Islamabad and Gomal University D. I. Khan, Pakistan. Two families (A and B), demonstrating autosomal recessive primary microcephaly, were recruited from KPK province of Pakistan (Fig. 1). The affected individuals were clinically examined by local medical doctors and genetic consultants. Informed consents for the genetic analysis were obtained from elders of the two families. Pedigree drawings of the families were obtained after detailed question/answer sessions conducted with guardians of the families.

Genomic DNA was extracted from peripheral blood samples, collected from 6 affected and 7 unaffected members of the two families, by GenEluteTM blood genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA). DNA was quantified by Nanodrop-1000 spectrophotometer (Thermal Scientific, Wilmington, MA, USA) measuring its optical density (OD) at 260nm and diluted up to 40–50ng/µl for amplification by polymerase chain reaction (PCR).

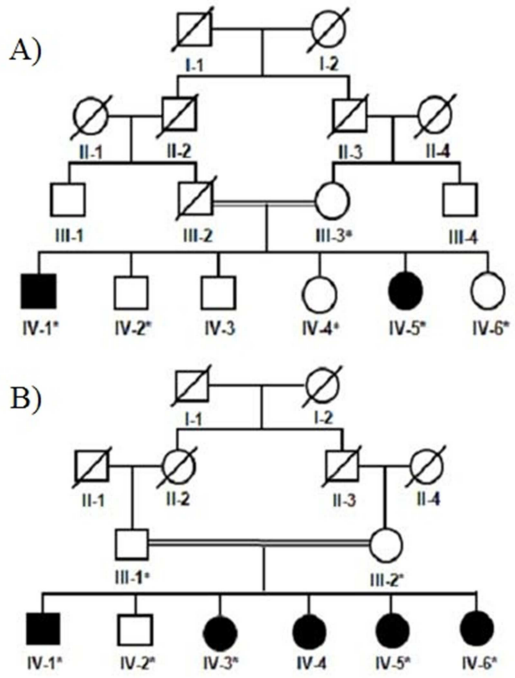

Fig. 1. Pedigree drawings of two Pakistani families (A and B) with autosomal recessive primary microcephaly. Circles and squares represent females and males, respectively. Clear symbols represent unaffected individuals while filled symbols represent affected individuals. Symbols with crossed lines represent deceased individuals. Symbols with a star represent the samples that were available for the study.

In view of the features observed in affected members and mode of inheritance of the phenotype, genetic linkage in two families (A and B) was tested by genotyping microsatellite markers linked to gene *ASPM* on chromosome 1q31.3 (D1S518, D1S3468, GATA135F02, D1S2816, D1S1660, D1S2716, D1S2738, D1S1723, D1S306, D1S2686, D1S1678). PCR-amplification of the microsatellite markers was performed according to standard procedure as described by Mir *et al.,* 2012. The PCR-amplified products were resolved on 8% non-denaturing polyacrylamide gel, stained with ethidium bromide and genotypes were assigned by visual inspection.

Allele size for respective microsatellite markers was determined using 5-, 10- and 20-bp DNA ladders (MBI Fermentas®, Life Sciences, York, UK). Order of markers was based on Rutgers combined linkage-physical map of the human genome (Matise *et al.,* 2007).

DNA sequencing of the *ASPM* gene was performed in both families linked to MCPH5 locus on chromosome 1q31.3. Standard sequence of the gene was obtained from Ensembl Genome Browser (http://www.ensembl.org/Homo_sapiens/Gene). Using Primer3 version 0.4.0 software, forward and reverse primers for PCR amplification of coding exons, splice junction sites, 5' UTR (untranslated region) and 3' UTR of the *ASPM* gene were designed. The PCR amplification conditions used were the same as described by Mir *et al.,* 2012. Amplified PCR products were analyzed on 2.5% agarose gel under UV transilluminator (Biometra, Germany). Fragment size of each amplicon was determined using 100 bp DNA ladder (MBI, Fermentas, UK). Purification of the PCR-amplified products was performed with a commercially available kit (Marligen Bio-sciences, Ijamsville, MD, USA). DNA Sequencing of the amplified PCR products was performed with Big Dye Terminator v3.1 Cycle Sequencing Kit together with an ABI Prism 310 Genetic Analyzer (Applera, Foster City, CA, USA). The sequence of each amplicon was then aligned with reference sequence by using Bioedit sequence alignment tool (editor version 6.0.7, Ibis, Biosciences, CA, USA).

Results

All affected members of the two families (A and B) were clinically examined at the local government hospitals. In both the families, MCPH patients were born to first-cousin parents with a normal pregnancy and delivery. Clinical history showed presence of microcephalic condition by birth in all affected individuals of the two families. All the affected individuals displayed characteristic features of autosomal recessive primary microcephaly, having slopping forehead with reduced head circumference (Fig. 2 a-d). Head circumferences of affected individuals varied from 3 to 9 SD below the population age and sex-related mean.

All the affected children showed speech delay and mild to moderate intellectual disability, although no hearing impairment was observed. The ages of the affected individuals examined were 7 to 30 years at the time of study. Brain CT scan and MRI of the affected individuals were unavailable for our study. Parents of the affected individuals in both families showed normal intelligence and normal head circumferences.

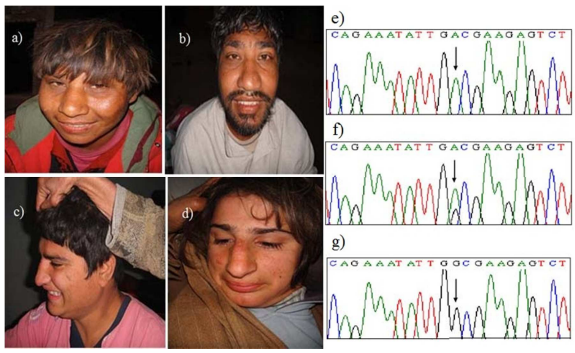

Fig. 2. Clinical presentation of autosomal recessive primary microcephaly in two Pakistani families. The affected members of both the families showed characteristic features of MCPH with sloping forehead, variable head circumference and mild-to-moderate mental retardation (a-d). Sequence analysis of a recurrent homozygous nonsense mutation (p.W1326*) in *ASPM* gene. The upper panel represents the nucleotide sequences in the affected individuals, the middle panel in the heterozygous carrier and the lower panel in the control unaffected individual of the families A and B. Arrows indicate position of mutations (nucleotide change) (e-g).

Linkage in two families (A and B) was tested, using microsatellite markers, specific to *ASPM* gene on chromosome 1q31.3 Haplotype analysis showed linkage in both the families to the *ASPM* gene. Sequence analysis of the *ASPM* gene in the two families revealed a recurrent homozygous nonsense mutation involving G to A transition at nucleotide position 3978 (c.3978G>A), producing immediate premature stop codon (p.W1326*) in exon 17 of the gene (Fig. 2 e-g). The sequence variants, identified here, were found in the heterozygous state in the obligate carriers and segregated with the disease in the respective families. To exclude the possibility that the mutations identified in the present families, do

not represent non-pathogenic polymorphisms, a panel of 100 unaffected unrelated ethnically matched control individuals were screened for the nonsense mutations identified in family A and B.

Discussion

In the present analysis we have described two Pakistani families affected with autosomal recessive primary microcephaly. The affected members of both the families showed characteristic features of MCPH with sloping forehead, variable head circumference and mild-to-moderate mental retardation. These individuals did not reveal any signs of seizures and spasticity but are not able to read or write. Screening of the *ASPM* gene in the two families with MCPH revealed a recurrent homozygous nonsense mutation (c.3978G>A), producing immediate premature stop codon (p.W1326*).

Mutations in the *ASPM* gene account for about 50% of the MCPH cases from Pakistani population. .So far, a total of 174 pathogenic ASPM mutations have been recognized, including 76 nonsense/missense mutations, 15 splicing mutations, 80 frame shift mutations, two gross deletions and one mutation of complex rearrangement (https://portal.biobaseinternational.com/hgmd/pro/gene.php?gene=ASPM). The nonsense mutation c.3978G>A (p.W1326*) identified in this study is present in about 20-25% of the MCPH families linked to the *ASPM* gene, proposes that this might be a founder mutation with a common ancestral origin.

ASPM gene (MIM 605481) at the *MCPH5* locus has 28 exons, spans 62 kb of genomic DNA, with a 10,434 bp long open reading frame (ORF) that codes for a 3477 a.a. long protein. ASPM protein has four main regions, a putative N-terminal microtubule-binding domain, a calponin-homology domain (CH), 81 calmodulin- binding IQ repeats, and a C-terminal region. Most IQ motifs are organized into a higher order trimer repeat (HOR) containing two 23-amino acid residue units and one 27-amino acid residue unit (Bond *et al.,* 2002; Saunders *et al.,* 1997).

ASPM is a centrosomal protein during interphase and localized to the microtubule-organizing center (MTOC) at the spindle poles, and midbody between prophase and telophase of the cell cycle (Zhong *et al.,* 2005). Thus, in the process of neurogenesis, *ASPM* organizes microtubules at the spindle pole during mitosis and at the central spindle during cytokinesis, to ensure symmetric cell divisons (Paramasivam *et al.,* 2007). Mutations in *ASPM* probably caused microcephaly by altering the regulation of mitotic spindle orientation and timing or placement of abscission, in neuronal progenitors (Fish *et al.,* 2006).

Conclusion

In the current study, we have investigated two Pakistani families with MCPH. Homozygosity mapping analysis showed linkage of both the families to MCPH5 locus harboring ASPM gene. Sequence analysis of the ASPM gene identified a previously reported nonsense mutation (p.W1326*), suggesting that this might be a founder mutation with a common ancestral origin.

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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