

## Genetic study of Khyber-Pakhtunkhwa resident Pakistani families presenting primary microcephaly with intellectual disability

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### Abstract

**Objectives:** To investigate the genetic factor responsible for causing microcephaly and determine allelic heterogeneity of Abnormal spindle microtubule gene.

**Method:** The genetic study was conducted at the Kohat University of Science and Technology, Kohat, and Gomal University, D.I.Khan, Pakistan, during 2017-18, and comprised 5 consanguineous families from South Waziristan, Kurram Agency, Karak, Bannu and Dera Ismail Khan regions of the country's Khyber Pakhtunkhwa province. Blood samples from all available and cooperative family members (including normal and affected) were obtained, and molecular analysis was carried out through whole genome single nucleotide polymorphisms genotyping, exome sequencing and Sanger sequencing.

**Results:** Of the 15 patients, 9(60%) were males and 6(40%) were females. Genetic mapping revealed linkage to the MCPH5 locus which harbours the microcephaly-associated abnormal spindle-like microcephaly gene. Mutation analysis of the gene identified missense mutation c.3978G>A (p.Trp1326\*) in families A, B and C, a deletion mutation c.7782\_7783delGA (p.(Lys2595Serfs\*6)) in family D, and a splice site defect c.2936+5G>A in family E.

**Conclusion:** There was suggestion of strong founder effect of mutation c.3978G>A (p.Trp1326\*).

**Keywords:** MCPH family, ASPM, p.Trp1326\*, Founder mutation. (JPMA 69: 1812; 2019) DOI:10.5455/JPMA.300681

### Introduction

Autosomal recessive primary microcephaly, also called microcephaly primary hereditary (MCPH), is a rare genetic disorder of brain development that is characterised by small volume of cranium due to reduced cerebral cortex, and intellectual disability. Microcephalic patients have reduced occipito-frontal head circumference (>-3 standard deviation [SD]) with sloping forehead.<sup>1,2</sup> The incidence of MCPH is estimated to be 1 in 10,000 newborn from northern area of Pakistan as well as in Khyber Pakhtunkhwa (KP) province due to traditional consanguineous marriages.<sup>3</sup> Aetiologically, microcephaly may be caused due to either germline mutations in MCPH genes or environmental complications (infections, toxins etc.) during pregnancy. Among the infectious agents, congenital infection of zika virus is a recently reported epidemic disorder that can be transmitted to the foetus and causes microcephaly. It has been shown that these

infections disrupt the normal biological process of proliferation and differentiation of neuronal progenitor cells during brain development, and, hence, lead to microcephalic condition.<sup>4</sup> A demographic study in Brazil<sup>5</sup> has shown that 5.2% of microcephaly is caused due to congenital infections of zika virus. To date, 23 MCPH genes have been reported, including microcephalin 1 (MCPH1), WD repeat domain 62 (WDR62), CDK5 regulatory subunit associated protein 2 (CDK5RAP2), abnormal spindle microtubule (ASPM) assembly, centromere protein J (CENPJ), kinetochore scaffold 1 (KNL1), STIL centriolar assembly protein (STIL), centrosomal protein 135 (CEP135), centrosomal protein 152 (CEP152), zinc finger protein 335 (ZNF335), polyhomeotic homolog 1 (PHC1), cyclin dependent kinase 6 (CDK6), centromere protein E (CENPE), SAS-6 centriolar assembly protein (SASS6), major facilitator superfamily domain containing 2A (MFSD2A), ankyrin repeat and LEM domain containing 2 (ANKLE2), Citron Rho-Interacting Serine/Threonine Kinase (CIT), Wd Repeat-And-Fyve Domain-Containing Protein 3 (WDFY3), Coatomer Protein Complex, Subunit Beta-2 (COPB2), Kinesin Family Member 14 (KIF14), Non-Smc Condensin I Complex Subunit D2 (NCAPD2), Non-Smc Condensin Ii Complex Subunit D3 (NCAPD3) and Non-Smc Condensin I Complex Subunit H (NCAPH).<sup>3,6</sup> Most of these genes produce microtubule-associated proteins and are

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involved in the cell division and its regulation. Previous genetic studies on cohort of MCPH families revealed ASPM and WDR62 as the most prevalent genetic factors causing primary microcephaly in Pakistan.<sup>7</sup>

The current study was planned to analyse consanguineous families from KP province and to conduct genetic analysis regarding ASPM gene (c.3978G>A, c.7782\_7783delGA and c.2936+5G>A).

## Materials and Methods

The genetic study was conducted at the Kohat University of Science and Technology (KUST), Kohat, and Gomal University, D.I. Khan, Pakistan, during 2017-18, and comprised 5 consanguineous families from South Waziristan, Kurram Agency, Karak, Bannu and Dera Ismail (DI) Khan regions of the KP province. All the families had multiple affected individuals. The study was approved by the research ethics committees of KUST and Gomal University, DI Khan. Blood samples were obtained from each family member (including normal and affected) after taking informed written consent from the respective family elder. Clinical assessment of the affected members was performed using a proforma.<sup>7</sup>

Selective strategy of genetic dissection was performed on each recruited MCPH family. Among the five recruited families, genetic mapping of families A, B and C were

performed through short tandem repeat (STR) markers (GATA135F02, D1S2816, D1S2840, D1S1660 and D1S2738) genotyping and linkage analysis, followed by Sanger sequencing of ASPM gene. The genotyping of STR markers involved allele amplification through polymerase chain reaction (PCR) and its resolution on 8% polyacrylamide gel electrophoresis (PAGE). After genotyping STR markers present in the vicinity of the ASPM gene, haplotype was developed to determine the segregation of defective homozygous block carrying ASPM among the affected individuals.

Families D and E were analysed through whole exome sequencing and positional cloning approach (genome-wide homozygosity mapping and subsequent Sanger deoxyribonucleic acid [DNA] sequencing). Whole exome data was analysed for the presence of pathogenic variant, using Phe nIX software.<sup>8</sup> The analysis strategy reported in literature<sup>7</sup> was followed for exome sequence analysis. The identified pathogenic variant was sequenced in other family members through Sanger sequencing. After sequence analysis, mutation status (novelty or recurrence) was investigated by surveying the Human Gene Mutation Database (HGMD, URL: <http://www.hgmd.cf.ac.uk/>) and Pakistan GMD (PGMD).<sup>9,10</sup>

## Results

Of the 15 patients, 9(60%) were males and 6(40%)

Table: Phenotypic spectrum of Pakistani patients with various mutations in gene ASPM causing primary microcephaly.

Family ID	A			B			C			D			E		
Region	Kurram Agency			Waziristan Agency			Karak			Bannu			Chashma, D.I.Ikhan		
Ethnicity [Mutation cDNA(NM_018136.4)]	Pukhtun [c.3978G>A (p.Trp1326*)]			Pukhtun [c.3978G>A (p.Trp1326*)]			Pukhtun [c.3978G>A (p.Trp1326*)]			Pukhtun [c.7782_7783delGA (p.Lys2595Serfs*6)]			Saraiki [c.2936+5G>A (IVS9+5G>A)]		
Mutation effect	Nonsense			Nonsense			Nonsense			Frameshift and protein truncation			Frameshift and protein truncation		
Analysis Strategy	Genotyping and Sanger sequencing			Genotyping and Sanger sequencing			Genotyping and Sanger sequencing			Whole Exome Sequencing			Microarray and Sanger sequencing		
Patient's Pedigree ID	IV-3	IV-4	IV-5	IV-6	IV-1	IV-2	IV-3	IV-6	IV-7	IV-9	IV-1	IV-2	IV-3	IV-1	IV-2
Gender	Male	Female	Female	Male	Male	Female	Male	Female	Female	Male	Male	Male	Female	Male	Male
Age at last assessment (years)	15	13	05	18	22	15	09	22	25	27	26	10	8	16	13
Occipito-frontal circumference (Inch)	15	14.8	14	17	16	14.7	15	15.6	16	17.2	15.8	17	16	17	15
Head standard deviation	-11.3	-12.6	-10.7	-8	-9.7	-15.1	-11.3	-10.4	-12.9	-10.7	-10.1	-7.5	-9	-8	-10.8
Seizures	No			No			No			No			No		
Intellectual disability	Moderate			Moderate -Severe			Moderate -Severe			Moderate			Severe		
Speech ability	Under-developed Speech			Under-developed Speech			Under-developed Speech			Under-developed Speech			Under-developed Speech		
Motor Delay	No			No			No			No			No		
Neurological Symptoms	Normal			Normal			Normal			Normal			Normal		
Epileptic Fitz	No			No			No			No			No		
Facial Dysmorphism	No			No			No			No			No		

ID: Identification.

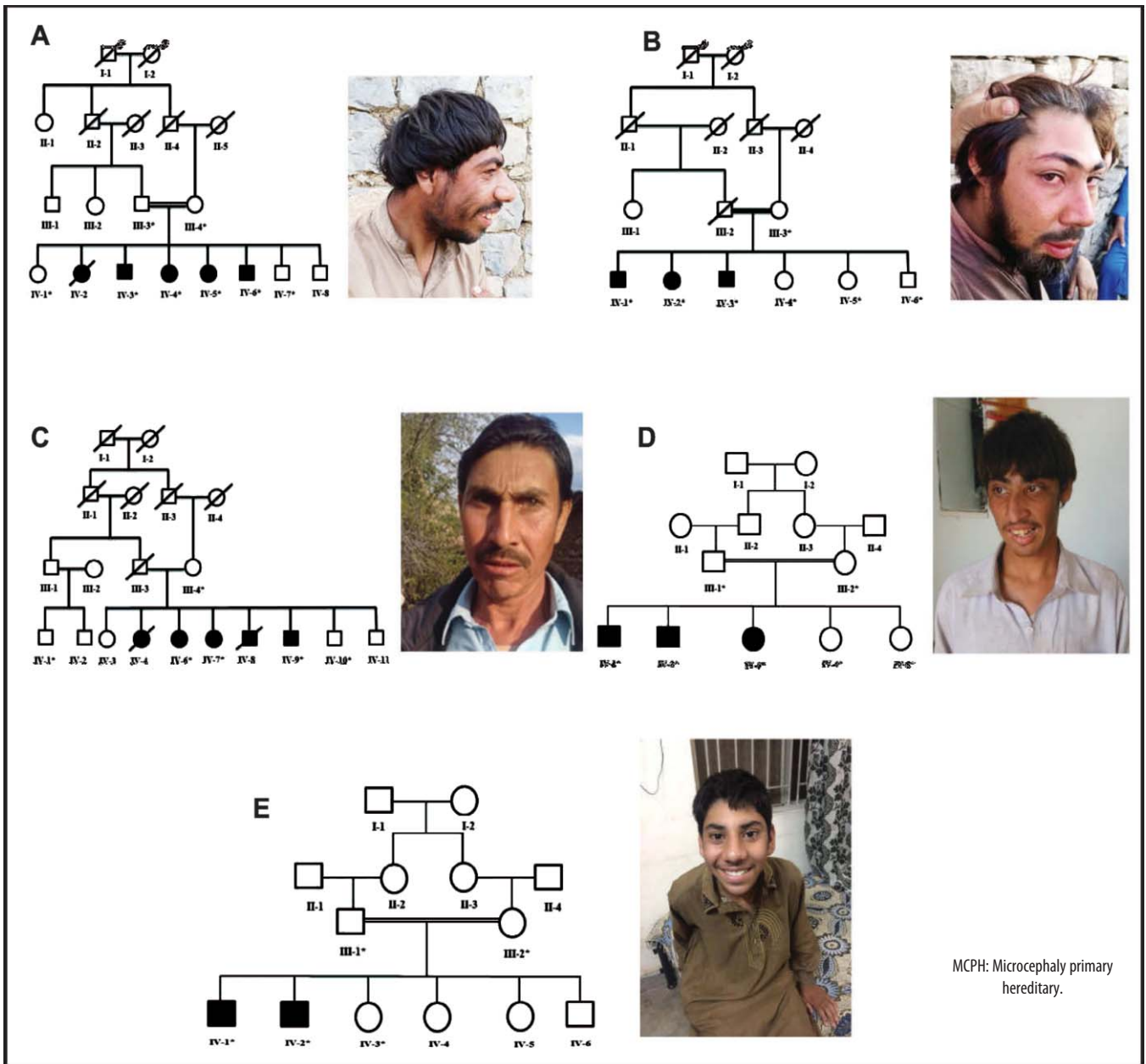


Figure-1: A) Pedigree of consanguineous MCPH families showing autosomal recessive mode of disease segregation. B) Patients photograph showing reduce head circumference and exclude facial dismorphism.

were females. All patients from the five families were born of asymptomatic parents. The patients exhibited severely reduced head circumference with sloping forehead as a prominent feature of microcephaly. The patients had under-developed speech ability and were unable to communicate perfectly. None of the patients from any family exhibited facial dismorphism (Figure-1). Although ambulation was delayed, none of the patients had

muscular dystrophy or skeletal deformity, and no additional signs of neurological, ophthalmological or visceral organ defect was noted. Additional phenotypic features were noted for each patient (Table).

Genotyping and mutation analysis in families A, B and C was found to be missense mutation c.3978G>A in ASPM gene. Genetic analysis in families D and E also identified recurrent mutations of ASPM

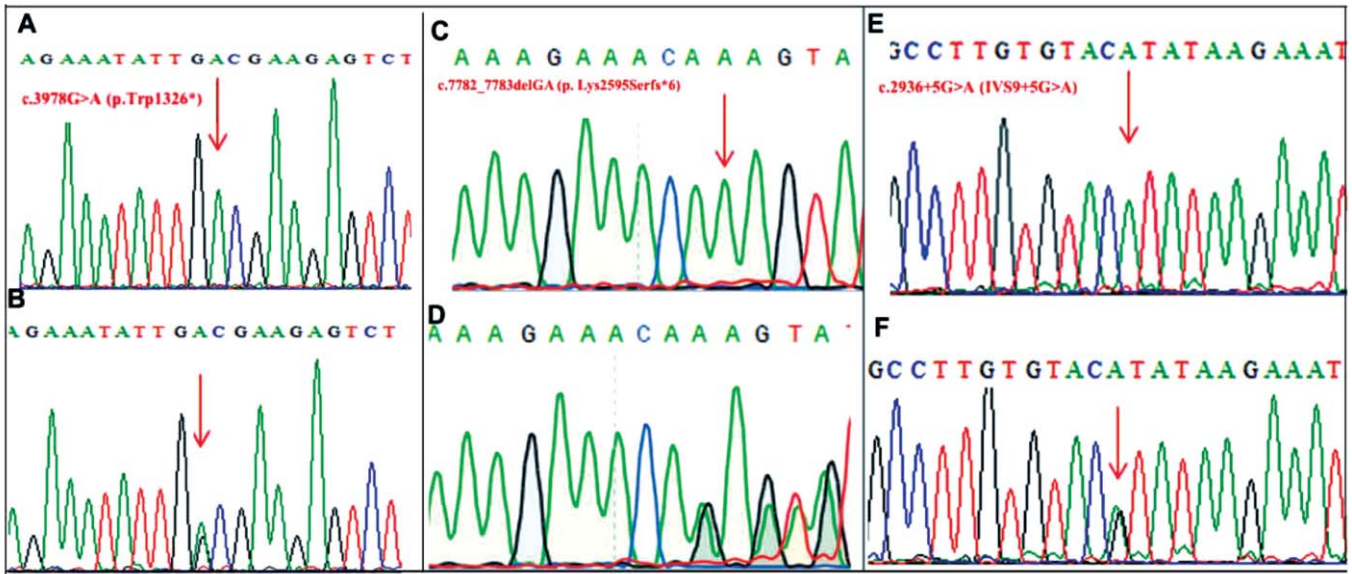


Figure-2: Sequence chromatograms showing mutation in families A, B and C. Upper panel indicate sequence of affected, while lower panel shows carrier individuals and arrow is pointing at the site of mutation.

c.7782\_7783delGA and c.2936+5G>A, respectively (Figure-2).

## Discussion

MCPH is a genetic form of neuro-developmental disorder that is characterised by small head circumference. Among the 23 reported MCPH genes, ASPM (68.6%), WDR62 (14.1%) and MCPH1 (8%) have the highest incidental rate, and their mutations account for about 50% of all MCPH cases.<sup>11</sup> Most of the MCPH gene products are involved in centrosome or spindle fibre, the defect of which lead to disrupted cell cycle. The evidence of premature chromosome condensation in the patient's lymphocyte and fibroblast cell line has explained the patho-mechanism of microcephaly.<sup>12</sup> ASPM gene is located on chromosome 1q31.3, and comprises 28 exons and 62567 bases. Its encoded protein consists of 3477 amino acids that fold to make four functional domains i.e., calponin homology domains, P-loop containing nucleoside triphosphatase hydrolase domains (P-loop NTPase), armadillo-type fold domain, and intelligence quotient (IQ) motifs.<sup>7,13,14</sup> Proteomic studies have revealed that the N-terminal part (encoded by the initial seven exons) of ASPM protein is required to provoke ASPM localisation to the spindle pole during metaphase, while the C-terminal domain (encoded by the terminal three exons) is essential for its localisation to the midbody during cytokinesis.<sup>13</sup> ASPM protein is important for the normal functioning of the mitotic spindle in embryonic neuroblasts. This protein resides in the nucleus, but, during mitosis, it relocates to the spindle pole.<sup>15-17</sup> Aberration of ASPM severely decreases the brain

size by affecting the orientation of mitotic spindles and thus decreases the number of neuron cells by affecting the ratio of symmetrical-to-asymmetrical cell division.<sup>18</sup> ASPM knocked-out mouse models have shown a remarkable decrease in cortical surface area, as is the case in humans.<sup>19</sup> The patho-mechanisms underlying ASPM-induced microcephaly in mice include an increase duration of cell cycle interval in brain progenitor cells, thus leading to enhanced production of neurons at the lower cortical layers along with a decline of neuron in upper layer. However, this process has not been explained in humans yet.<sup>19</sup>

The present study, involving genetic analysis on a cohort of five consanguineous MCPH families from KP, reported previously mapped ASPM gene mutations. All of these mutations were protein-truncating by nature and produced short ASPM peptide that deleted one or more functional domains. The clinical features of all patients from these five families were similar to previous findings in literature according to which ASPM gene mutation was the most prevalent genetic entity in KP resident families of Pakistan.<sup>20-22</sup> A study analysed 35 MCPH families in which 27 showed linkage to ASPM gene.<sup>22</sup> Of them, 17 families revealed p.Trp1326\* mutation. According to the study, 49 families had shown association with this mutation,<sup>22</sup> while the current study has raised this number to 52, suggesting its strong founder effect in Pashtun-origin families.

As such, the present study will definitely contribute towards the genetic counselling of consanguineous as

well as non-consanguineous MCPH families of KP. Hence, developing ethnicity-specific ASPM targeted molecular diagnostic test would be cost-effective.

## Conclusion

The identification of ASPM gene mutations in the cohort of five families clearly suggests the prominent role of ASPM gene involvement in Pashtun-origin MCPH families. The founder effect of mutation c.3978G>A in Pashtun families was also a matter of concern, making out a case for pre-marital screening of ASPM gene in KP resident families.

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**Conflict of Interest:** None.

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