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Identification of Pathogenic Variants Causes Microcephaly In Sindh Families

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Abstract: Introduction: The study was designed to identify the genetic mutation in families with autosomal recessive primary microcephaly (MCPH). **Methodology**: The present study was cross-sectional and conducted at the Department of Biochemistry, Quaid-e-Azam University, Islamabad in 2017. The two families (A and B) with MCPH phenotype randomly selected from Hyderabad and Tando Adam districts respectively. Informed written consent was taken, physical parameters were measured and blood samples were collected from both families. DNA was extracted from whole blood and PCR was performed. The ASPM gene located on chromosome 1 is known to play a vital role in mitotic spindle fiber regulation during neurogenesis, and also is the most probable causative agent of microcephaly. Therefore targeted Sanger sequencing method for the ASPM gene was selected for variant identification in both families. **Results:** The Sanger sequencing result showed the novel missense variant (c.5841T/C; p. K1862E) in 18 exon of ASPM gene in Family A and this variant predicted as damaging in mutation tester, and provean and also exhibited deleterious in Polyphen 2 and SIFT public database. Similarly in family B we found a previously reported protein pre termination variant (c.3978G/A; p.Trp1326*) (rs137852995) in exon 17 of ASPM gene. The later mutation was most predominant cause of microcephaly in KPK families. **Conclusion**: Therefore it is concluded that mutation in the ASPM gene is the most prominent genetic player of Microcephaly in Pakistani families. The current study aids in the genetic analysis of MCPH phenotype families in Pakistan alongwith the counseling of MCPH families.

Keywords: MCPH, ASPM, DNA isolation, Mutation, Sanger Sequencing.

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Introduction

A rare neurodevelopmental condition called autosomal recessive primary microcephaly (MCPH) can be identified by impaired cognition and a small head circumference (HC) that is less than 3 standard deviations (SD) below the predicted mean [1]. Patients with MCPH who have undergone neuroimaging presented cortical hypoplasia with simplified gyarus pattern and normal architecture of brain [2]. The reduced cortical size is due to the abnormal spindle orientation in neural progenitor cells (NPC) cells that causes defect in mitotic neurogenesis as well as initiate apoptosis [3].

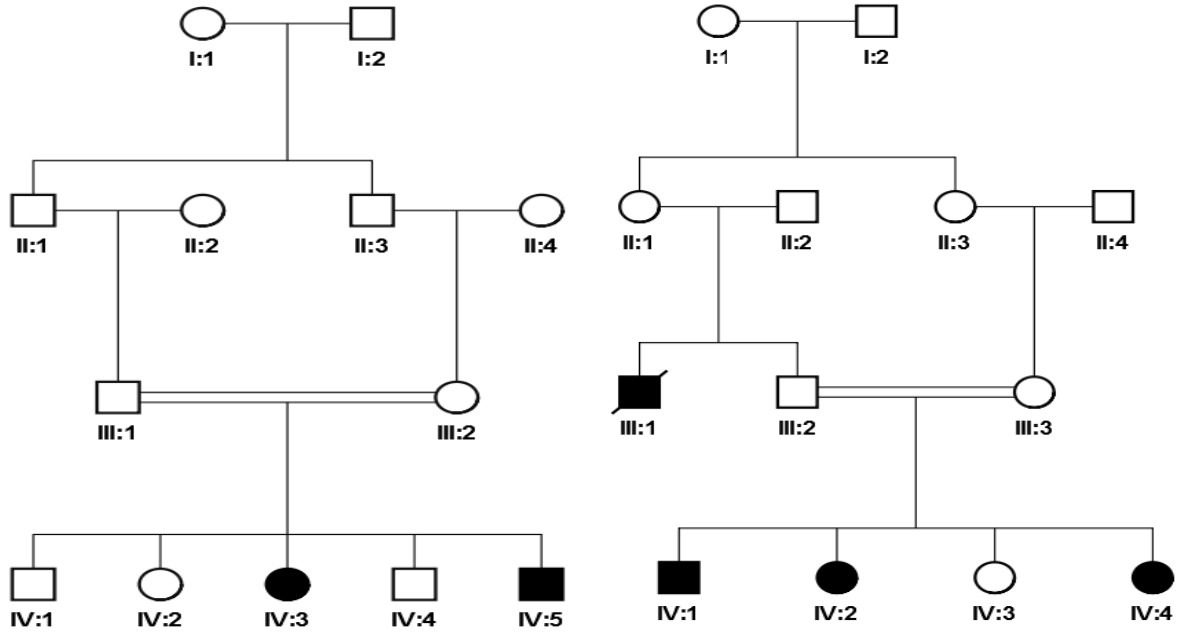
The countries with high consanguinity rates have a high prevalence MCPH; In Pakistan it's prevalent is about 1/10,000 while 1/1,000,000 sporadically detected in European population [4].

The MCPH is heterogeneous disorder, till to date mutations in 28 different genes are reported which were involved in various cellular pathways. Among these genes 68% mutations reported in ASPM gene followed by 14% in WDR62, and 8% in Microcephalin [5].
The significant numbers of MCPH genes has been identified in Pakistani population which includes WDR62 [6], CDK5RAP2 [7], ASPM [8], CEP135 [9], CENPJ [7], CEP152 [10], SASS6 [11], CDK6 [12], KIF14 [13], MFSD2A [14], and NUP37 [15]. However, still the main cause of MCPH in many Pakistani families is undiscovered. Therefore, the current study was design to explore the genetic cause of MCPH in two Pakistani families.

Methods:
The cross sectional study conducted in department of Biochemistry, Qauid e Azam University Islamabad in continuation with June 2017. The two MCPH phenotype families were ascertained randomly from Tando Adam and Hyderabad region of Sindh province. The elders of both families provided their written informed consent in accordance with the Declaration of Helsinki's guidelines. Elders members of the family was interviewed to gather the information about the family history, patient behavior, and disease severity. The head circumferences (HC) of all family associates were also measured. A predetermined proforma as described by Khan *et al*., in 2017 [16] was used to conduct a clinical assessment of the affected members. The pedigree was constructed using the haplopainter software (https: //haplopainter.sourceforge.net/). Blood was collected in Ethylene diamine tetra-acetic acid (EDTA) tubes (BD, Franklin Lakes, NJ, USA) from parents, affected individuals and their siblings. Genomic DNA was isolated by organic method (Phenol-chloroform method), and Colibri Microvolume Spectrometer (Titertek Berthold, Germany) was used for quantification of genomic DNA. The most common MCPH gene is ASPM therefore a targeted ASPM gene Sanger sequencing method was adapted to rule out the both MCPH families (A & B) to uncover the putative genetic aberration. The primers for all the 28 exons of ASPM gene were designed using the Primer 3 software (https: //primer3.ut.ee/) and PCR was performed according to standard protocol. The amplified PCR products were cleanup with ExoSAP-IT™ PCR Product Cleanup Reagent kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States). The Sanger sequencing were performed using the Big Dye Terminator cycling sequencing kit v. 3.1(Applied Biosystems, Foster City, USA) and the products were examined using ABI Analyzer (ABI, USA). The BioEdit software version 7.0.9.0 was used to evaluate the sequencing data. The pathogenicity of variants were identified in different public database include Mutation Tester ((http: //www.mutationtaster.org/), PANTHER (http: //pantherdb.org/), SIFT (https: //sift.bii.a-star.edu.sg), PROVEAN (http: //provean.jcvi.org/), polyphen2 (http: //genetics.bwh.harvard.edu/pph2), and The population frequency of mutations was explored through genomAD (https: //gnomad.broadinstitute.org), Genomes Browser (http: //www.1000genomes.org), and dbSNP (http: //www.ncbi.nlm.nih.gov/SNP/).

Result:
In the study the both families were ascertained from province of Sindh. The four generation family A reside in Hyderabad comprising of two patients with MCPH including one female (IV: 3) and one male (IV: 5). The age of both effected individuals was 20 and 17 years respectively. The pedigree shows clear recessive mode of trait, As both normal and affected individual can be observed in the family (in non sex biased manner). These affected members had reduced OFC occipitofrontal head circumference (OFC) circumferences, moderate intellectual disability, with aggressive behavior and lack of self-care concept. The Family B was recruited from Tando Adam Khan from Sindh province and it was also the four generation family consisting of three affected MCPH individuals in which one was male (IV: 1) and two were females (IV: 2, IV: 4), and pedigree also showing the recessive mendelain trait. The affected members (IV: 2, IV: 4) had reduced head circumferences with severe intellectual disability (ID) and aggressive, hyperactive

behavior, lack of self-care and quantitative concepts. The patient (IV: 1) existing mild ID and depressive behavior. The detailed phenotype spectrum of patients in family A and B showing in Table 1



(A) (B)

Figure 1: MCPH1 family Pedigree having two affected individuals (A). MCPH2 family pedigree shows three patients (B).

Table 1: Phenotypic and clinical features of Family A and Family B

Family ID	**A**		**B**		
Affected Individuals	IV: 3	IV: 5	IV: 1	IV: 2	IV: 4
Gender	Female	Male	Male	Female	Female
Age	20	17	22	8	6
Head Circumference (cm)	44	42	47	36	34
IQ	40	42	50	20	25
Intellectual disability severity	moderate	moderate	mild	severe	severe
Microcephaly	Yes	Yes	Yes	Yes	Yes
Seizures/ fits	No	No	No	No	No
Behavior	Aggressive	Aggressive	Depressive	Aggressive	Aggressive
Motor delay	Yes	Yes	Yes	Yes	Yes
speech disability	No	Yes	No	No	No
Hearing impairment	No	No	No	No	No
Facial dismorphism	Yes	Yes	Yes	Yes	Yes
Skeletal dismorphism	No	No	No	No	No
Self-care concept	No	No	No	No	No

Targeted Sanger Sequencing approach analysis of Family A show the novel missense variant (c.5841T/C; p. K1862E) in exon 18 of ASPM gene (figure 2(A)) and segregate in all affected members of family. This identified variant in family A predicted as damaging in mutation tester,

provean bioinformatics tools and also exhibited deleterious in Polyphen 2 and SIFT public database. The reported variant was neither found in 1000 genome nor in ExAC while 0.0007 minor allele frequency (MAF) in gnom_AD database. The amino acid lysine at 1862 is highly evolutionary conserved among vertebrates.

Sanger sequencing analysis for family B exhibited the previously reported non sense mutation (c.3978G/A; p.Trp1326*) (rs137852995) in exon 17 (figure 2(**B**)) which resulted in premature termination of ASPM protein. This non sense mutation is segregate in all effected individuals of family B.

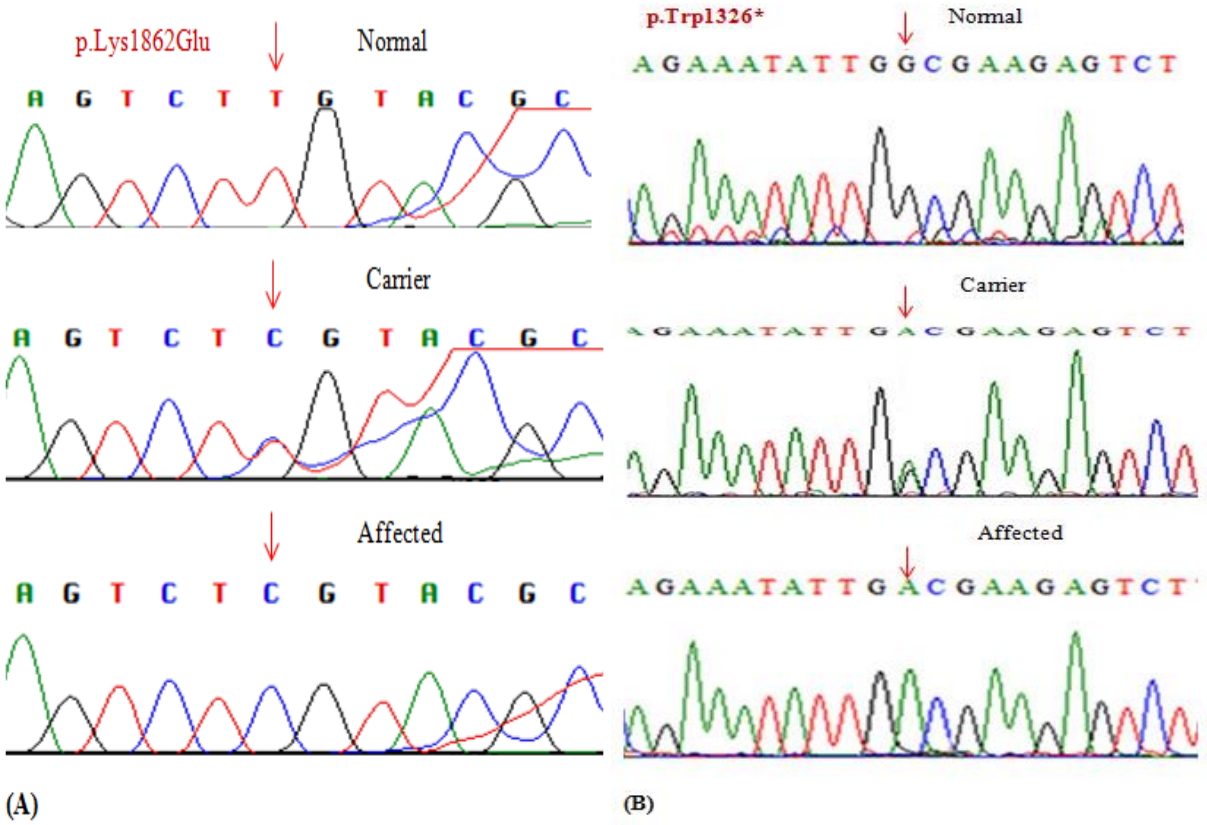


Figure 2: chromatogram showing the ASPM gene variant (c.5841T/C) segregation in family A (A). Chromatogram presented the ASPM gene mutation (c.3978G/A) segregation in family B (B).

Discussion:

MCPH is heterogeneous neurodevelopmental disorder in which head circumferences was reduced due to small size of cerebral cortex [17]. So far twenty eight (28) genes were reported for microcephaly in which mutation in ASPM gene was the main causative player of MCPH phenotype. Assembly factor for spindle microtubules (ASPM) gene mapped on chromosome 1q31.3 which comprising of 28 exons that encode 3477 amino acids protein [8]. ASPM protein is required for the regulation of mitotic spindle fibers functions during neurogenesis [18]. ASPM protein consists of four domains namely N-terminal microtubule binding domain, calponin homology domains, armadillo-type fold domain, and 81 calmodulin-binding repeats IQ motifs [19]. The N- terminal part span on first seven exons and it is essential for the localization of ASPM to the spindle pole [20]. The c terminal domain span on last three exons and during the cell division this domain plays a pivotal role in association of ASPM protein with mid body [21]. Any aberration in ASPM protein causing the premature conversion of mitotic division from symmetric to asymmetric that result in the reduction of neuron numbers in developing cortex [19].

The present study, involving genetic analysis of two MCPH phenotypic families from Sindh, identified one novel missense pathogenic variant (c.5841T/C; p. K1862E) and one earlier reported protein truncating mutation (c.3978G/A; p.Trp1326*) in exon 18 and exon 17 of ASPM gene respectively. These both mutations were found in IQ repeats motifs that paly pivotal role for binding of calmudulin protein or EF-hand protein. The later mutation (c.3978G/A; p.Trp1326*) were earlier identified in Turkish family and many Pakistani families with high prevalent rate in Khyber Pakhtunkhwa MCPH phenotypic families [22-24]. The earlier estimated by Ahmed etal., in 2017[23] the 52 MCPH phenotype families in KPK had exposed association with c.3978G/A; p.Trp1326* mutation in ASPM gene. Subsequently in 2021 Hussain *et al*., [5] identified same mutation in six more KPK resident microcephaly families and considered this mutation as the Pashtun-based founder effect mutation.

Conclusion:

We performed targeted Sanger sequencing of ASPM gene in two consanguineous families recruited from Sindh and we identified one new variation (c.5841T/C; p. K1862E) in family A and one known protein termination mutation (c.3978G/A; p.Trp1326*) in family B. These both variants were found in calmodulin-binding repeats IQ motifs. The present study contributes towards the genetic analysis of MCPH phenotype families in Pakistan and also contributing in counseling of MCPH families in Pakistan. This study further adds to the understanding of the negative consequences of consanguineous marriages and indicates the need for the counseling of affected families as well as the general population.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Department of Biochemistry, Qauid-i-Azam University Islamabad, Pakistan BEC letter Number: BEC-FBS-QAU-59, dated 4-5-2016.

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

None.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Reference:

1. Ponting C, Jackson AP. Evolution of primary microcephaly genes and the enlargement of primate brains. Curr Opin Genet Develop 2005; 15(3): 241-8.
2. Mughal Zu, Zai JA, Ansar M. A Missense variant in LAMA3 gene causes microcephaly and epidermolysis bullosa in a Pakistani family. J Liaquat Univ Med Health Sci 2021; 20(3): 198-203.

3. Woods CG. Human microcephaly. Curr Opin Neurobiol 2004; 14(1): 112-7.
4. Zaqout S, Morris-Rosendahl D, Kaindl AM. Autosomal recessive primary microcephaly (MCPH): an update. Neuropediatrics 2017; 48(03): 135-42.
5. Hussain S, Nawaz A, Hamid M, Ullah W, Khan IN, Afshan M, *et al*. Mutation screening of multiple Pakistani MCPH families revealed novel and recurrent protein-truncating mutations of ASPM. Biotechnol Appl Biochem 2021 Nov 26.
6. Nicholas AK, Khurshid M, Désir J, Carvalho OP, Cox JJ, Thornton G, *et al*. WDR62 is associated with the spindle pole and is mutated in human microcephaly. Nature Genetics 2010; 42(11): 1010-4.
7. Bond J, Roberts E, Springell K, Lizarraga S, Scott S, Higgins J, *et al*. A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size. Nature Genetics 2005; 37(4): 353-5.
8. Bond J, Roberts E, Mochida GH, Hampshire DJ, Scott S, Askham JM, *et al*. ASPM is a major determinant of cerebral cortical size. Nature Genetics 2002; 32(2): 316-20.
9. Hussain MS, Baig SM, Neumann S, Nürnberg G, Farooq M, Ahmad I, *et al*. A truncating mutation of CEP135 causes primary microcephaly and disturbed centrosomal function. Am J Human Genetics 2012; 90(5): 871-8.
10. Guernsey DL, Jiang H, Hussin J, Arnold M, Bouyakdan K, Perry S, *et al*. Mutations in centrosomal protein CEP152 in primary microcephaly families linked to MCPH4. Am J Human Genetics 2010; 87(1): 40-51.
11. Khan MA, Rupp VM, Orpinell M, Hussain MS, Altmüller J, Steinmetz MO, *et al*. A missense mutation in the PISA domain of HsSAS-6 causes autosomal recessive primary microcephaly in a large consanguineous Pakistani family. Human Mol Genet 2014; 23(22): 5940-9.
12. Hussain MS, Baig SM, Neumann S, Peche VS, Szczepanski S, Nürnberg G, *et al*. CDK6 associates with the centrosome during mitosis and is mutated in a large Pakistani family with primary microcephaly. Human Mol Genet 2013; 22(25): 5199-214.
13. Moawia A, Shaheen R, Rasool S, Waseem SS, Ewida N, Budde B, *et al*. Mutations of KIF14 cause primary microcephaly by impairing cytokinesis. Annals Neurol 2017; 82(4): 562-77.
14. Alakbarzade V, Hameed A, Quek DQ, Chioza BA, Baple EL, Cazenave-Gassiot A, *et al*. A partially inactivating mutation in the sodium-dependent lysophosphatidylcholine transporter MFSD2A causes a non-lethal microcephaly syndrome. Nature Genet 2015; 47(7): 814-7.
15. Braun DA, Lovric S, Schapiro D, Schneider R, Marquez J, Asif M, *et al*. Mutations in multiple components of the nuclear pore complex cause nephrotic syndrome. J Clin Invest 2018; 128(10): 4313-28.
16. Khan MA, Windpassinger C, Ali MZ, Zubair M, Gul H, Abbas S, *et al*. Molecular genetic analysis of consanguineous families with primary microcephaly identified pathogenic variants in the ASPM gene. J Genet 2017; 96(2): 383-7.
17. Abuelo D. Microcephaly syndromes. In Seminars in pediatric neurology 2007; 14(3): 118-27.
18. Do Carmo Avides M, Glover DM. Abnormal spindle protein, Asp, and the integrity of mitotic centrosomal microtubule organizing centers. Science 1999; 283(5408): 1733-5.
19. Kouprina N, Pavlicek A, Collins NK, Nakano M, Noskov VN, Ohzeki JI, *et al*. The microcephaly ASPM gene is expressed in proliferating tissues and encodes for a mitotic spindle protein. Human Mol Genet 2005; 14(15): 2155-65.
20. Saunders RD, Avides MD, Howard T, Gonzalez C, Glover DM. The Drosophila gene abnormal spindle encodes a novel microtubule-associated protein that associates with the polar regions of the mitotic spindle. The Journal of cell biology. 1997 May 19; 137(4): 881-90.
21. Tsang WY, Spektor A, Luciano DJ, Indjeian VB, Chen Z, Salisbury JL, *et al*. CP110 cooperates with two calcium-binding proteins to regulate cytokinesis and genome stability. Mol Biol Cell 2006; 17(8): 3423-34.
22. Kousar R, Nawaz H, Khurshid M, Ali G, Khan SU, Mir H, *et al*. Mutation analysis of the ASPM gene in 18 Pakistani families with autosomal recessive primary microcephaly. J Child Neurol 2010; 25(6): 715-20.
23. Ahmad I, Baig SM, Abdulkareem AR, Hussain MS, Sur I, Toliat MR, *et al*. Genetic heterogeneity in Pakistani microcephaly families revisited. Clin Genet 2017; 92(1): 62-8.
24. Wang R, Khan A, Han S, Zhang X. Molecular analysis of 23 Pakistani families with autosomal recessive primary microcephaly using targeted next-generation sequencing. J Human Genet 2017; 62(2): 299-304.