

## A Linkage Study in 8 Pakistani Families Segregating as Autosomal Recessive Primary Microcephaly

<sup>1</sup>M. Hassanullah, <sup>2</sup>S.M. Ibrahim, <sup>3</sup>S. Ahmad, <sup>2</sup>N. Muhammad, <sup>2</sup>Rafiullah,  
<sup>2</sup>M. Asif and <sup>2</sup>Shahabuddin

<sup>1</sup>Quaid-i-Azam University, Islamabad, Pakistan

<sup>2</sup>Balochistan University of Information Technology, Engineering and Management Sciences  
(BUIITEMS), Quetta, Pakistan

<sup>3</sup>University of Malakand, Malakand, Pakistan

**Abstract:** The current study was designed to find the most frequent MCPH phenotype in inbred Pakistani families. Primary microcephaly is marked by small brain size and is usually inherited as recessive trait. In the present study, we performed linkage analysis on 8 Pakistani families with autosomal recessive primary microcephaly (MCPH) and linked 6 of them to known MCPH genes/loci like MCPH1 (*Microcephalin*), MCPH3 (*CDK5RAP2*) and MCPH5 (*ASPM*). Majority of the families showed linkage with MCPH5, the most common MCPH locus in Pakistan. The linked families were then subjected to mutational analysis, revealing a previously known G to A transition at nucleotide position 3978 in exon 17 of *ASPM* gene in three of the families. To decrease its incidence, it is indispensable to train the people of the possible devastating outcome of cousin marriages and to find the carriers through carrier screening programs.

**Key words:** *ASPM*, consanguineous marriages, linkage analysis, primary microcephaly

### INTRODUCTION

Primary microcephaly inherited in an autosomal recessive manner (MCPH), a rare human neurodevelopment disorder, is marked by diminished brain size with head circumference of at least 4 standard deviations below the age- and sex- related population specific means (Roberts *et al.*, 2002). Affected individuals exhibit a small but architecturally normal cerebral cortex having symptoms of mental retardation from mild to severe with no other associated abnormalities or dysmorphic features. The expected rate ranges from 1 in 30,000 to 1 in 2,000,000 individuals in non-consanguineous populations across the world as compared to the highly inbred families residing in northern Pakistan, where it is 1 in 1000 individuals (Woods *et al.*, 2005).

To-date only seven MCPH loci (MCPH1–MCPH7) have been reported (Jackson *et al.*, 1998; Roberts *et al.*, 1999; Moynihan *et al.*, 2000; Jamieson *et al.*, 1999; Pattison *et al.*, 2000; Leal *et al.*, 2003; Kumar *et al.*, 2009). Out of seven loci, MCPH genes have been identified at six of them: *Microcephalin*, which functions in the DNA damaged

response pathway, (Jackson *et al.*, 2002) at MCPH1; *CDK5RAP2*, *CDK5* regulatory subunit-associated protein 2, (Bond *et al.*, 2005) at MCPH3; *CEP152*, a putative mammalian ortholog of *Drosophila* asterless and mutations in which affect mitosis in the fly, (Guernsey *et al.*, 2010) at MCPH4; *ASPM*, abnormal spindle-like microcephaly associated gene, (Bond *et al.*, 2002) at MCPH5; *CENPJ*, centromeric protein J, (Bond *et al.*, 2005) at MCPH6; and *SCL/TAL1* interrupting locus (*STIL*) at MCPH7 (Kumar *et al.*, 2009) have been identified by autozygosity mapping and positional cloning. Most of MCPH genes code for centrosomal proteins and are known to be playing roles in mitosis and cell division (Thornton and Woods, 2009). Mutations in *ASPM* gene are responsible for majority of MCPH cases accounting up to 40% in both consanguineous and non-consanguineous families (Roberts *et al.*, 2002; Nicholas *et al.*, 2009).

As a part of strict social practice, consanguineous marriages have become an integral part of the life of most of the people living in the rural areas of Pakistan. As a consequence, the diseased alleles in the form of recessive trait have greater chances of being inherited together in the next generation. In the present study, we ascertained

Table 1: List of microsatellite markers for linked MCPH loci/genes

Locus	Cytogenetics location	Marker	Distance (cM)*	Length (bp)	Nucleotide repeats
MCPH1	8p23.2	D8S1099	15.08	253	Tri
	8p23.1	D8S277	17.64	148-180	Di
	8p23.1	D8S1706	19.19	257-281	di
	8p23.1	D8S503	22.06	212-226	di
MCPH3	9q32	D9S930	119.02	283	tetra
	9q32	D9S289	121.32	75-87	di
	9q33.2	D9S1116	131.02	399t	etra
	9q33.2	D9S2155	132.42	256-282	tetra
MCPH5	1q23.3	D1S2628	177.42	99-123	di
	1q31.2	D1S3468	201.3	300-320	tetra
	1q31.2	D1S2625	201.7	185-191	di
	1q31.3	D1S1660	205.81	226-250	tetra
	1q32.1	D1S1726	206.62	61-279	di
	1q32.1	D1S2716	207.96	196-206	di
	1q32.1	D1S1678	213.76	297-313	tetra

\*: Average-sex distance in centiMorgan (cM) according to Rutgers combined linkage-physical human genome map

8 families from different regions of Pakistan affected with autosomal recessive primary microcephaly (MCPH). This study was designed to find the most frequent MCPH phenotype prevailing in these families which might help in making a suitable plane for carrier screening in highly inbred families with a history of primary microcephaly to find the persons at a high risk, which will help to minimize MCPH incidence in the country.

#### MATERIALS AND METHODS

This study was performed at Quaid-i-Azam University, Islamabad from August to December, 2010. Eight families with autosomal recessive primary microcephaly were ascertained from different areas of Pakistan. The families were visited and after confirming the consanguineous marriages, pedigrees were drawn following a standard protocol (Bennett *et al.*, 1995). The mode of inheritance of primary microcephaly was inferred by observing segregation of disease within family. The families were also questioned of the presence of any other associated disorders (genetic or environmental). Blood samples of study subjects were taken for DNA extraction after consent signature. Head circumference and height of each affected individual was also measured at the time of enrollment. Photographs were taken to document physical finding.

#### Linkage analysis:

**DNA extraction:** Genomic DNA was extracted from blood of study subjects following standard phenol-chloroform procedure (Sambrook *et al.*, 1989) and after extraction DNA was dissolved in appropriate amount (150-200 µL) of Tris-EDTA (TE) buffer.

**PCR amplification:** Amplification of microsatellite markers of known MCPH loci (Table 1) was performed

using Gene Amp PCR system 9600 (Perkin Elmer, USA) and T3 thermocyclers (Biometra, Germany). Here only the list of markers for linked loci/genes is shown.

**Genotyping:** After amplification, the PCR products were loaded on 2% agarose gel (horizontal gel electrophoresis) and analyzed through UV transilluminator (Biometra, Germany) for confirmation. In the next step, genotyping was performed by first resolving the amplified PCR products in 8% standard non-denaturing polyacrylamide gel, performing the electrophoresis in a vertical gel tank (vertical gel electrophoresis) of Whatman Biometra (Biometra, Germany) and then visualization of markers through UV transilluminator (Biometra, Germany). Photographs were then taken with the help of a Digital camera DC 290 (Kodak, USA) and genotypes were assigned by visual inspection of the gels. Markers were numbered according to Microsatellite markers mapped by Cooperative Human Linkage Centre (CHLC) and were obtained from Invitrogen Genelink (USA). Information about the cytogenetic locations, size (in base pairs) and cM distance of the markers was obtained from UCSC Genome Browser (<http://genome.ucsc.edu/cgi-bin/hgGateway?org=Human&db=hg19&hgside=175211401>) and Rutgers's map (Kong *et al.*, 2004).

**DNA Sequencing:** Six out of eight families initially showed linkage to markers for known MCPH loci/genes including MCPH1/*Microcephalin*, MCPH3/*CDK5RAP2* and MCPH5/*ASPM*. The linked families were then subjected to sequencing according to standard DNA sequencing protocol. For sequencing of *CDK5RAP2* (38 exons), *ASPM* (28 exons) and *Microcephalin* (14 exons) gene, all the exons including intron-exon boundaries were amplified by Gene Amp PCR system 9600 (Perkin Elmer, USA) from genomic DNA of affected and normal individuals of family A, C, D and F for *ASPM*, family B

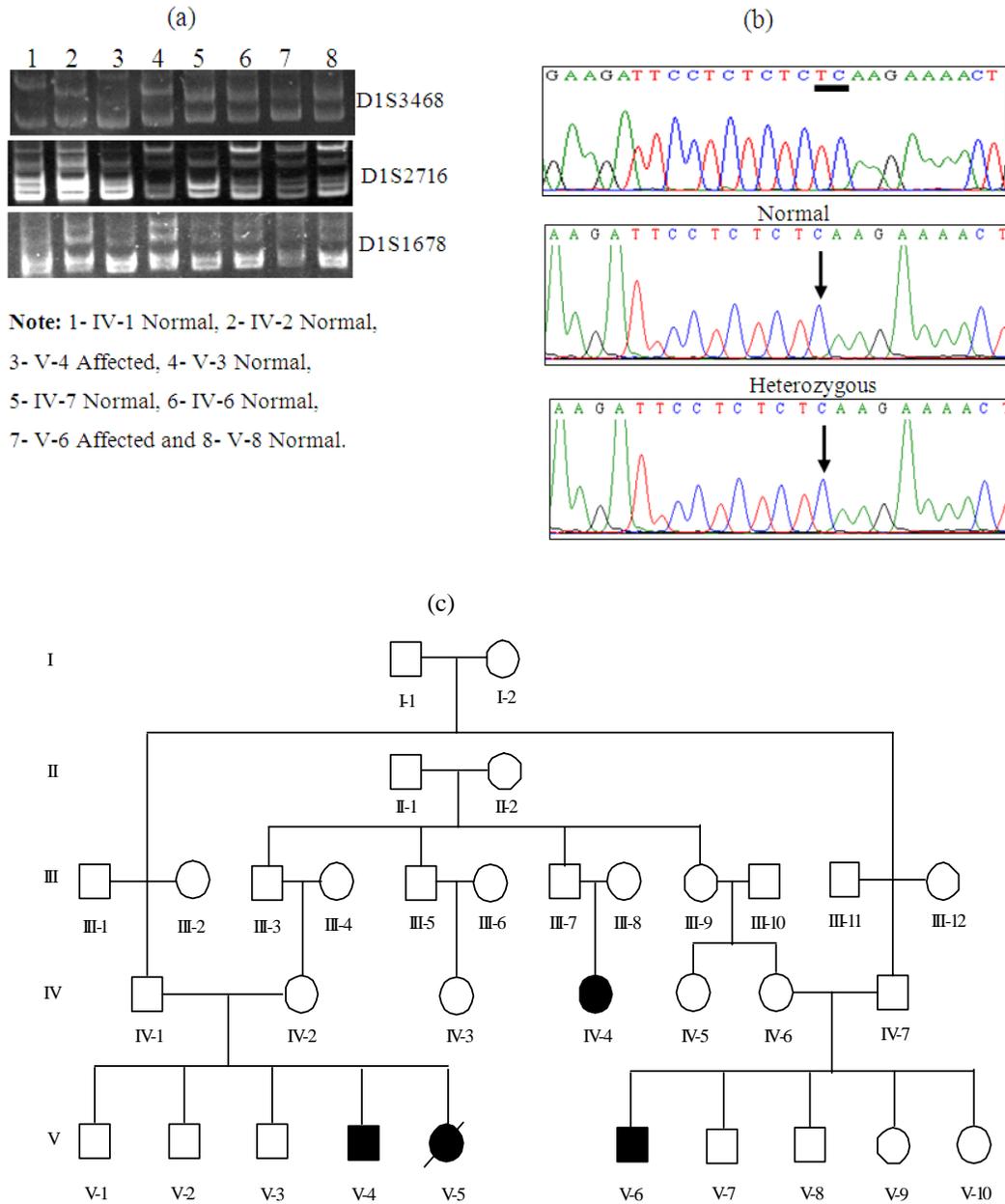


Fig. 1: (a) Electropherograms showing allele pattern obtained with markers D1S3468, D1S2716 and D1S1678 in family A for MCOPH5 locus, (b) Sequencing chromatograms of exon 18 of ASPM gene in normal, carrier and affected, indicating 2 bp deletions at nucleotide position 6854-6855, producing frame shift and resulted in premature stop codon 92 bp downstream (6854delTC). Arrows indicate the position of deletion in the sequence, (c) Pedigree of family A with Primary microcephaly

for *Microcephalin* and individuals of family G for *CDK5RAP2* gene. The amplified products were purified with commercially available kits (Fermentas Life Sciences) and sequenced directly on a CEQ8800 using the DTCS Quick Start sequencing kit (Beckman Coulter,

USA) together with CEQ8800 DNA sequencer (Beckman Coulter, USA).

**Mutation screening:** Mutation screening was performed by comparing the gene sequences of normal and affected

individuals with the corresponding control gene sequences from Ensemble Genome Browser database (<http://www.ensembl.org/index.html>). Sequence variants were ascertained via BIOEDIT sequence alignment editor version 6.0.7.

## RESULTS

### Linked families:

**Family A:** This family was enrolled from Bahawalnagar district of the Punjab Province. Consanguineous marriages are a common trend here. The pedigree consists of five generations (35 individuals) with 2 affected males (V-4, V-6) and 2 affected females (IV-4, V-5) (Fig. 1c). Affected individuals were mild to moderate mentally retarded, but they had no other associated abnormality. Ages ranged from 14-28 years at the time of enrollment and their facial features were normal except an indistinct slopping forehead (Fig. 8a). Clinical examination of affected individuals of the family A was strongly suggestive of presence of a non-syndromic microcephaly in the family.

Eight DNA samples including six normal (IV-1, IV-2, IV-6, IV-7, V-3, V-8) and two affected (V-4, V-6) individuals were typed for genotyping and microsatellite markers D1S3468, D1S2716 and D1S1678 were found homozygous in all the affected individuals but heterozygous in their parents (Fig. 1a). The family A was thus found to be linked with MCPH5 locus on chromosome 1q31. DNA Sequence Analysis of *ASPM* gene revealed 2 bp deletion at nucleotide position 6854, producing frame shift and resulted in premature stop

codon 92 bp downstream (6854delTC) in exon 18 (Fig. 1b). The sequence variant was also identified in normal and carrier individual therefore further characterization of this mutation is required.

**Family B:** Family B resides in Bannu district of Khyber Pakhtunkhwa Province (formerly known as NWFP). Owing to strict social custom, the family members rarely marry outside the community. This is a two-generation pedigree (Fig. 2b), composed of six individuals including one affected female individual (II-3). Clinical findings confirmed that the disease was congenital autosomal recessive microcephaly and was not associated to any environmental causes or part of a syndrome. The affected subject had normal facial features with the exception of an indistinct slopping forehead (Fig. 8b).

Three DNA samples including two normal (I-2, II-1) and one affected (II-3) were used for genotyping and family showed linkage with MCPH1 locus on chromosome 8p23 as markers D8S1099, D8S277, D8S1706 and D8S503 (Fig. 2a) were found homozygous in all affected individuals but heterozygous in their mother. Sequencing of the 14 exons and splice-junction of *Microcephalin* gene in family B failed to detect pathogenic sequence variant suggesting that the mutation is probably present in the regulatory sequences of the gene.

**Family C:** Family C with primary microcephaly also belongs to Bannu district and the cultural values were found to be strictly tied to consanguineous marriages

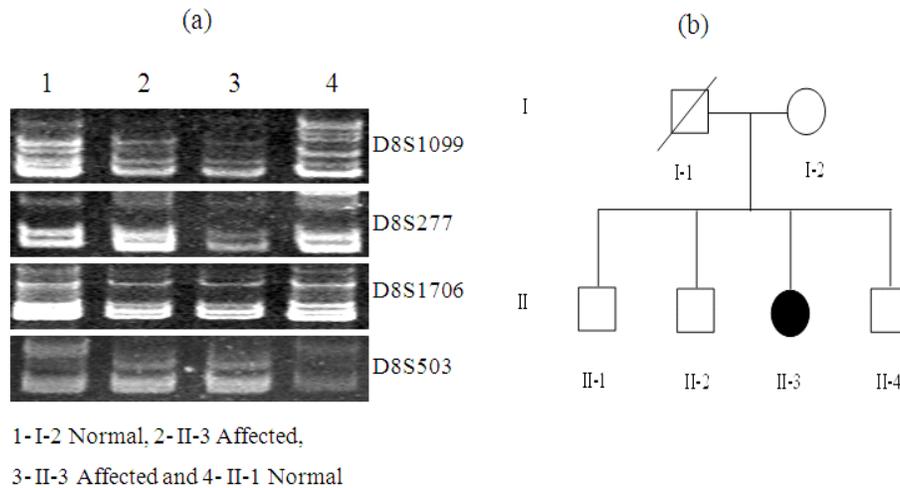


Fig. 2: (a) Electropherograms showing allele pattern obtained with markers D8S1099, D8S277, D8S1706 and D8S503 in family B for MCPH1 locus, (b) Pedigree of family B with Primary microcephaly

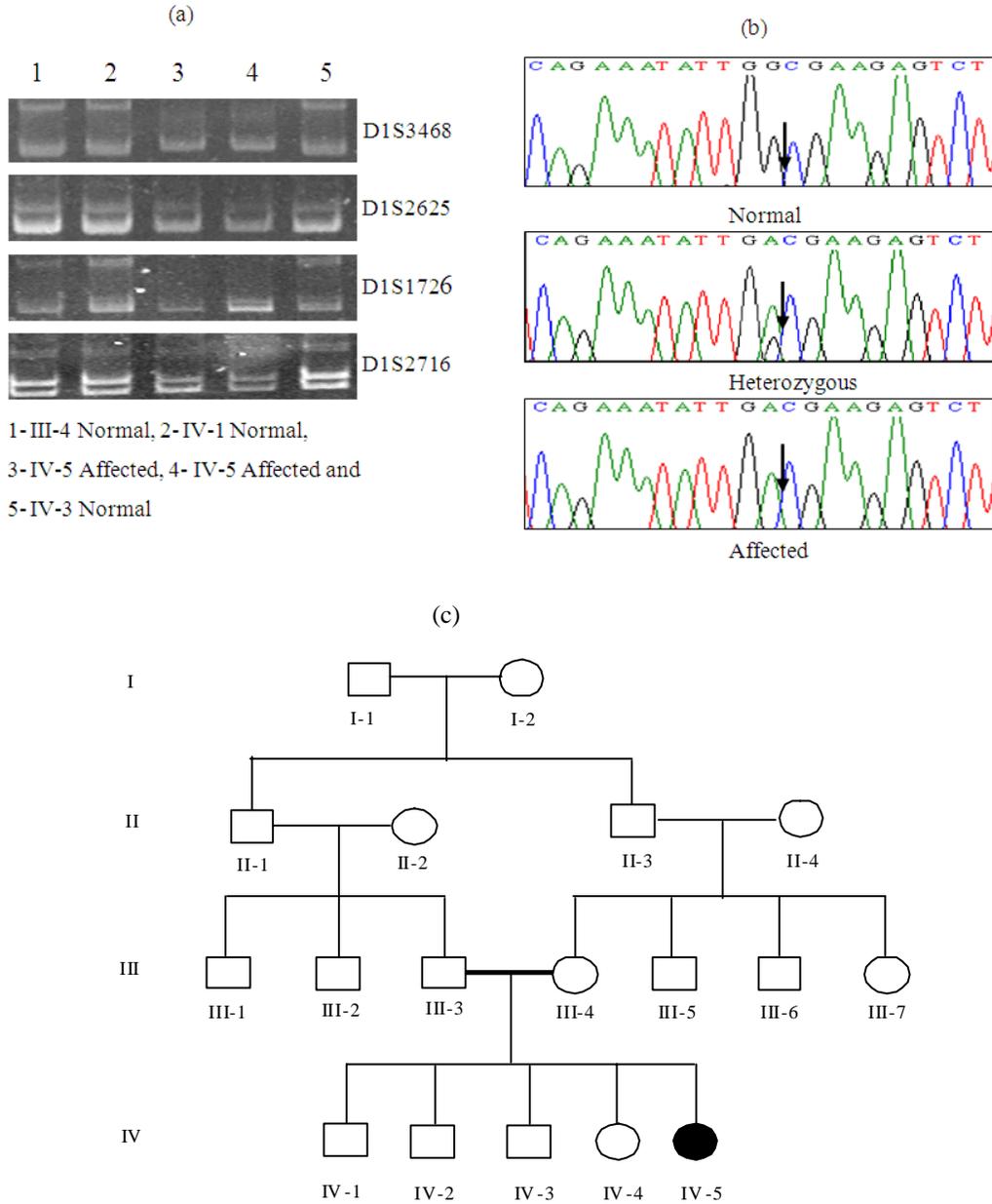


Fig. 3: (a) Electropherograms showing allele pattern obtained with markers D1S3468, D1S2625, D1S1726 and D1S2716 in family C for MCPH5 locus, (b) Sequencing chromatograms of exon 17 of *ASPM* gene, indicating a G to A transition at nucleotide position 3978 (c. 3978G>A). Arrows indicate the position of nucleotide change in the sequence, (c) Pedigree of family C with Primary microcephaly

in the family. The four-generation pedigree with 18 individuals including an affected female (IV-5) is shown in Fig. 3c. The pedigree strongly favors autosomal recessive type of inheritance. The affected individual had normal facial features with the exception of an indistinct

sloping forehead (Fig. 8c). Affected individual was mild to moderate mentally retarded, with no associated abnormality. Average head circumferences of affected and normal individuals were 37 and 48 cm, respectively.

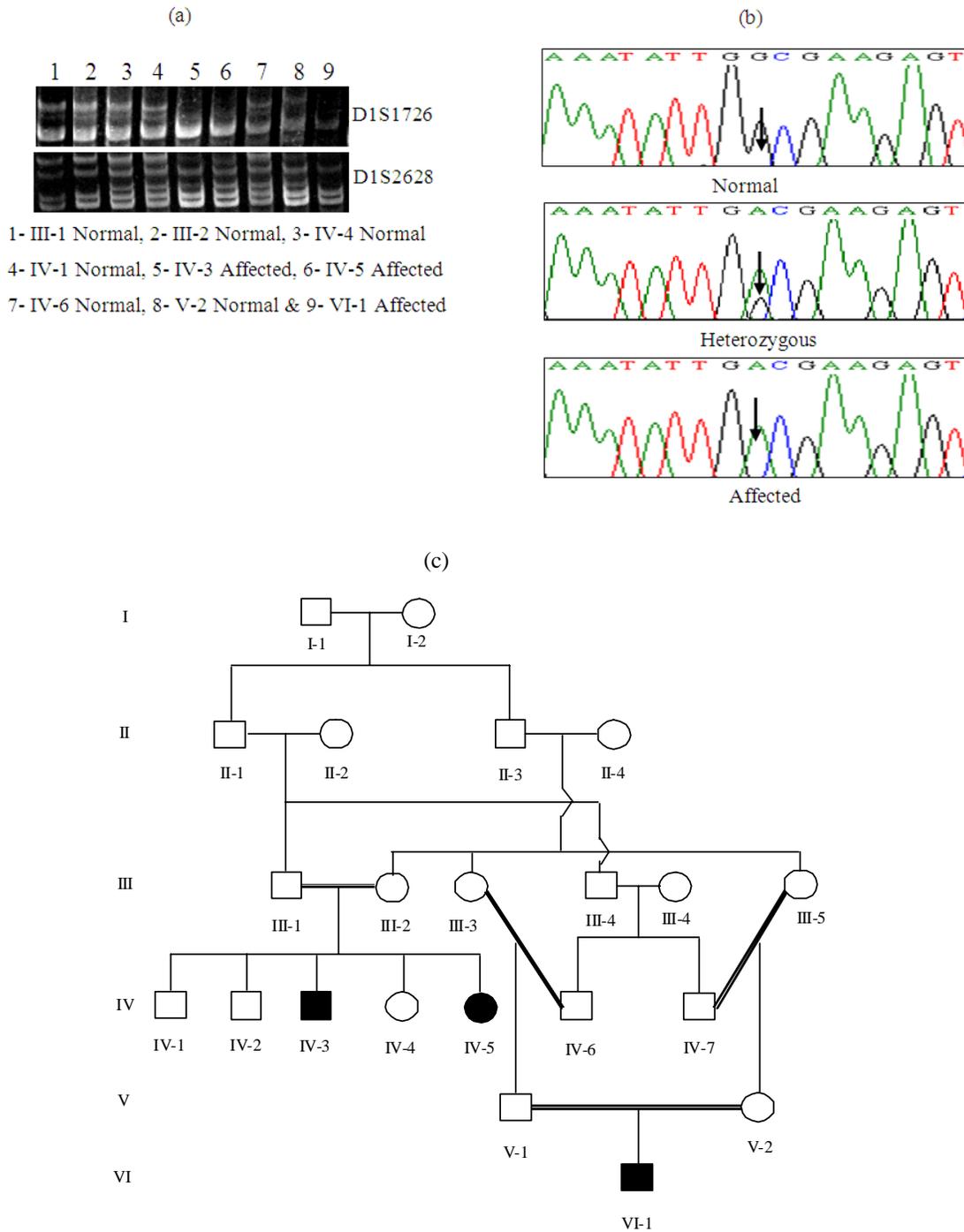


Fig. 4: (a) Electropherograms showing allele pattern obtained with markers DS1726 and D1S2628 in family D for MCPH5 locus, (b) Sequencing chromatograms of exon 17 of *ASPM* gene, indicating a G to A transition at nucleotide position 3978 (c. 3978G>A). Arrows indicate the position of nucleotide change in the sequence, (c) Pedigree of family D with Primary microcephaly

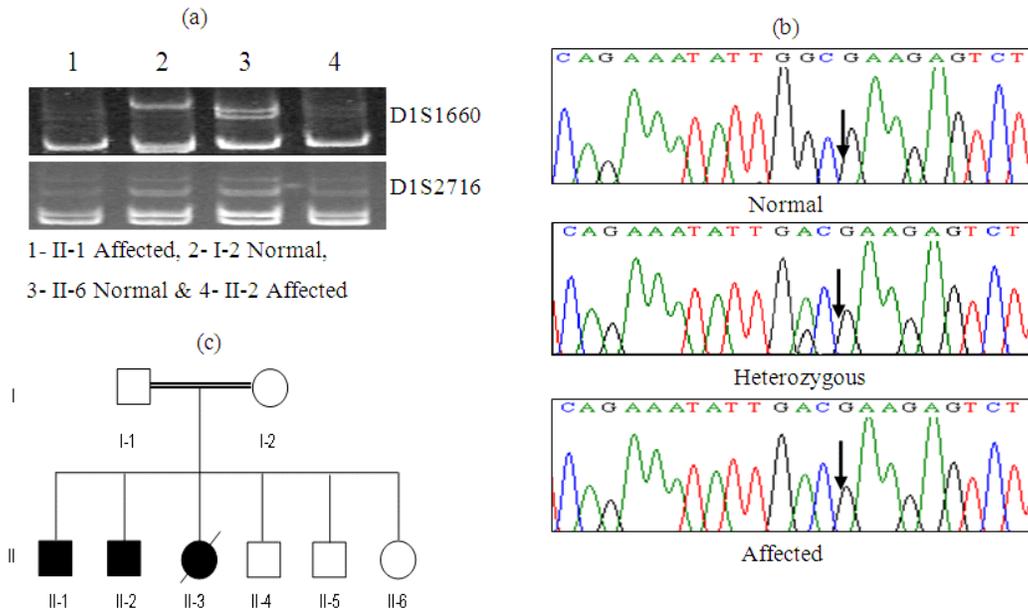


Fig. 5: (a) Electropherograms showing allele pattern obtained with markers D1S1660 and D1S2716 in family F for MCPH5 locus, (b) Sequencing chromatograms of exon 17 of *ASPM* gene, in family F. Arrows indicate the position of nucleotide change in the sequence, (c) Pedigree of family F with Primary microcephaly

For genotyping, four DNA samples of three normal (III-4, IV-1 and IV-3) and one affected (IV-5) individuals were typed and polymorphic microsatellite markers D1S3468, D1S2625, D1S1726 and D1S2716 (Fig. 3a) were found homozygous in the affected female individual but heterozygous in her mother giving a clear indication of family A linkage with MCPH5 locus on chromosome 1q31. DNA sequence analysis of *ASPM* showed a G to A transition at nucleotide position 3978, producing immediate premature stop codon (W1326X) in exon 17 of the gene (Fig. 3b).

**Family D:** Family D with primary microcephaly resides in Bannu district. Consanguineous marriages are quite common here. This is a six generation pedigree (Fig. 4c), which shows autosomal recessive mode of transmission of the disease. Pedigree analysis shows the presence of affected individuals in the fourth and sixth generation. Mental retardation was mild to moderate in all of the affected individuals. The affected individuals had normal facial features with the exception of an indistinct slopping forehead (Fig. 8d). Average head circumferences measured were 37 cm and 50 cm for affected and normal individuals, respectively.

Analysis of the results obtained for polymorphic microsatellite markers in MCPH5 candidate linkage interval, revealed that markers D1S1726 and D1S2628

(Fig. 4a) were homozygous in the affected individuals (IV-3, IV-5 & VI-1) but heterozygous in the parents (III-1, III-2 & V-2), thus showed the linkage in family D to MCPH5 locus on chromosome 1q31. DNA sequence analysis of *ASPM* gene showed a G to A transition at nucleotide position 3978, producing immediate premature stop codon (W1326X) in exon 17 of the gene (Fig. 4b).

**Family F:** This family is a two generation pedigree (Fig. 5c) with six individuals including two affected males enrolled from Bannu district of Khyber Pakhtunkhwa Province. Consanguineous marriages were noted to be a common practice in the family. Clinical findings confirmed that the disease was congenital autosomal recessive microcephaly and was not associated to any environmental cause or part of a syndrome.

Linkage analysis was performed on DNA samples of two normal (I-2, II-6) and two affected (II-1, II-2) individuals and family F showed linkage with two polymorphic microsatellite markers D1S1660 and D1S2716 (Fig. 5a), which were homozygous in all the affected individuals (II-1 & II-2) but heterozygous in parents, confirming that family F is linked with MCPH5 locus on chromosome 1q31. DNA sequence analysis of *ASPM* gene showed a G to A transition at nucleotide position 3978, producing immediate premature stop codon (W1326X) in exon 17 of the gene (Fig. 5b).

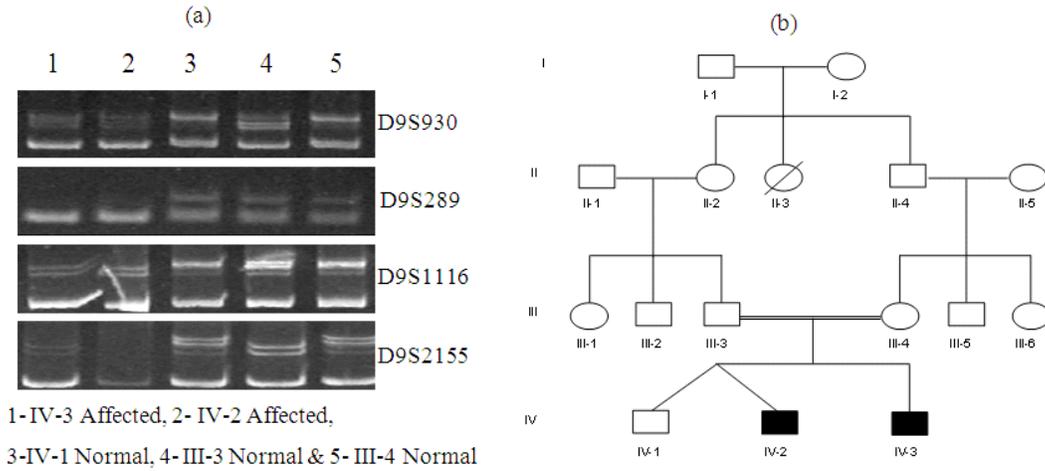


Fig. 6: (a) Electropherograms showing allele pattern obtained with markers D9S930, D9S289, D9S1116 and D9S2155 in family G for MCPH3 locus, (b) Pedigree of family G with Primary microcephaly

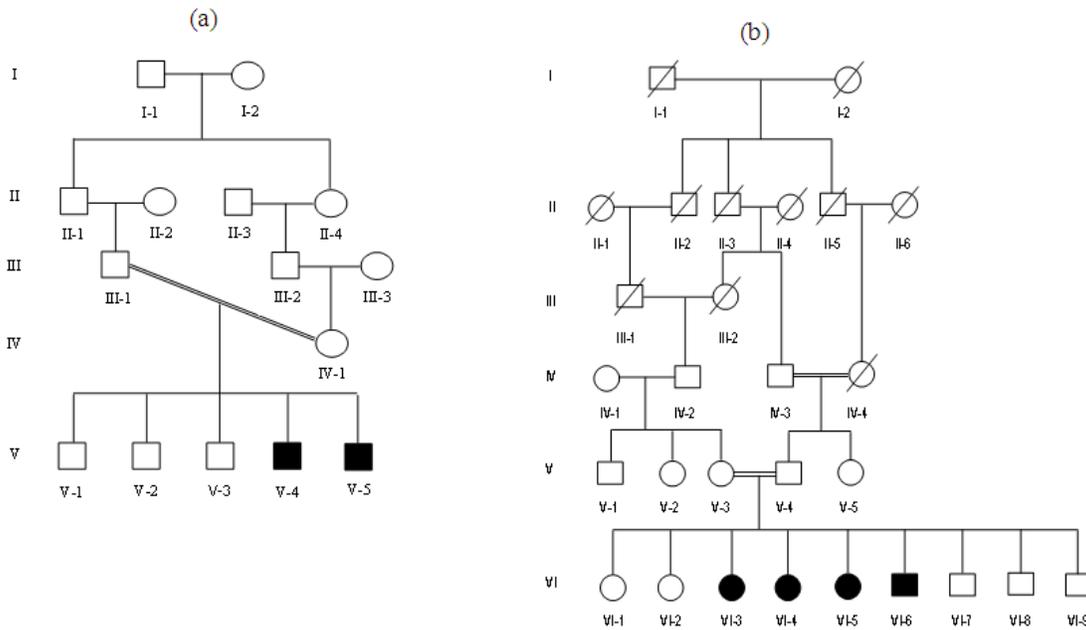


Fig. 7: Pedigree of (a) family E and (b) family H with Primary microcephaly

**Family G:** Family G resides in district Kasoor of Punjab Province with four generation pedigree (Fig. 6b) consisting of 15 individuals including 2 affected males (IV-2, IV-3). Traditionally, family members rarely marry outside of the family. Affected individuals were mild to moderate mentally retarded, but had no other associated abnormality. Ages of the affected individuals varied between 3 months and 3 years at the time of study. The

affected individuals had normal facial features with the exception of an indistinct sloping forehead (Fig. 8e). Average head circumferences measured were 34 and 38 cm for affected and normal individuals, respectively.

Genotyping was performed on DNA samples of three normal (III-3, III-4, IV-1) and two affected (IV-2, IV-3) and markers D9S930, D9S289, D9S1116 and D9S2155 (Fig. 6a) were found homozygous in all affected

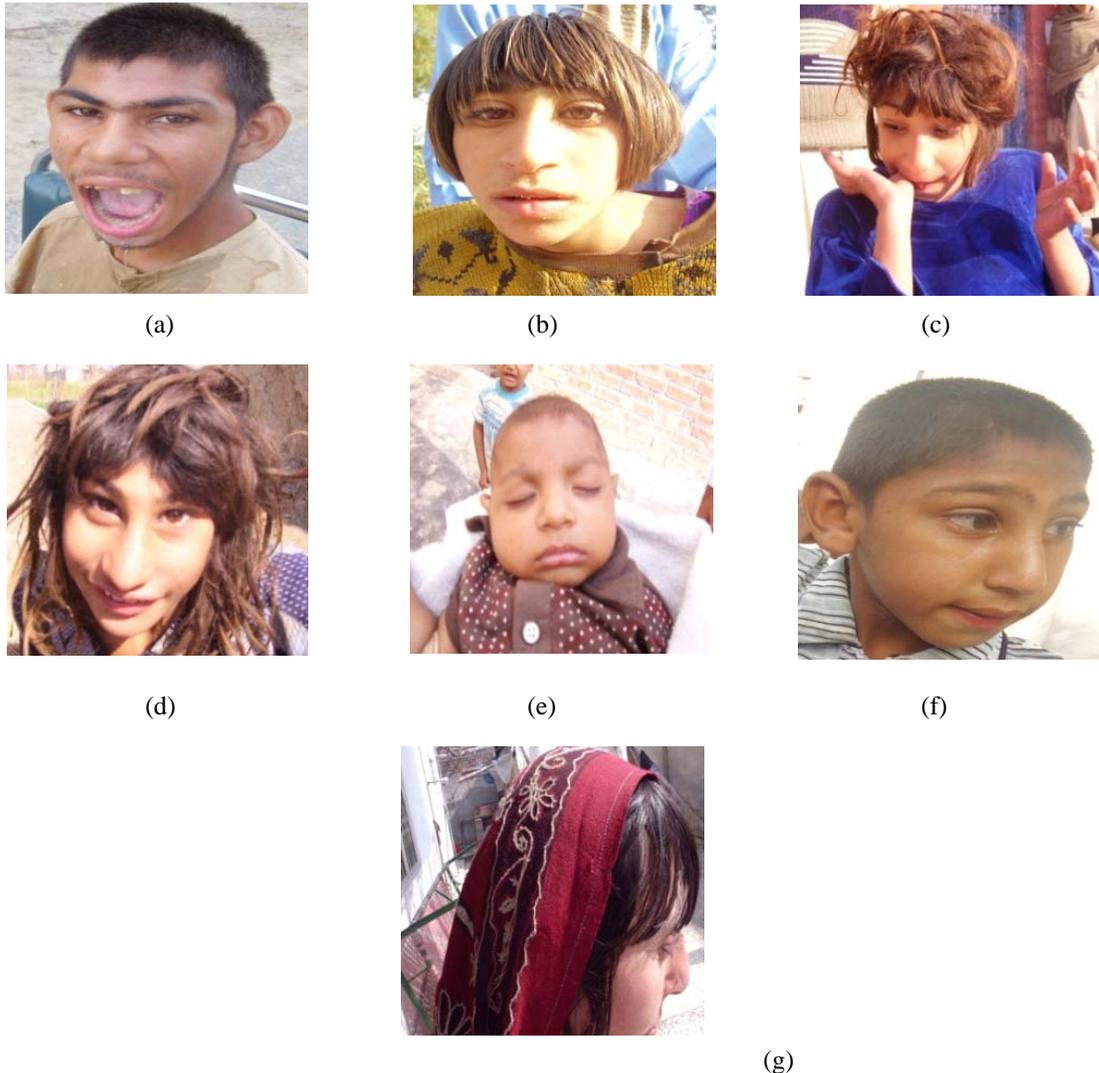


Fig. 8: Pictures of affected individuals of (a) Family A, (b) Family B, (c) Family C, (d) Family D, (e) Family G, (f) Family E and (g) Family H with primary microcephaly

individuals but heterozygous in their parents (III-3 & III-4), thus establishing linkage of family G to MCPH3 locus at 9q34. DNA sequence analysis of all 30 exons of *CDK5RAP2* gene in family G failed to detect pathogenic sequence variant.

**Unlinked families:**

**Family E:** The family E was enrolled from internal Sindh Province, where consanguineous marriages are one of the hallmarks of tradition there. The five-generation pedigree (Fig. 7) shows 15 individuals including two affected male (V-4, V-5) in the fifth generation. Microcephaly was confirmed to be present by birth. The affected males had normal facial features with the exception of an indistinct

sloping forehead and they were mild to moderate mentally retarded, with no associated abnormality (Fig. 8f).

**Family H:** Family H with congenital primary microcephaly resides in Nowshehra district of Khyber Pakhtunkhwa Province. Consanguineous marriages were noted to be a common practice in this family. The six-generation pedigree (Fig. 8) shows 30 individuals including three affected females (VI-3, VI-4, VI-5) and one affected male (VI-6) in the sixth generation. The affected females had normal facial features with the exception of an indistinct sloping forehead (Fig. 8g) and they were having no mental retardation. Ages of the

affected individuals varied between 20 and 33 years. Average head circumference ranged between 45 and 47 cm.

Genotyping performed on families E and H with microsatellite markers for known loci (MCPH1-MCPH7) gave negative results, thus excluding them from linkage to any of the known loci. This is a clear indication of the involvement of novel loci/genes in causing the disease in families E and H.

## DISCUSSION

Whites seems to have rare MCPH cases because of non-consanguineous marriages, whereas in all Arab and Asian populations consanguineous marriages are widely practiced and are culturally preferred, which result in the high incidence of MCPH cases in these populations (Woods *et al.*, 2005). First cousin amalgamation is the most familiar type of consanguinity in the Arab and Pakistani population. Five of the seven MCPH loci (MCPH1, MCPH2, MCPH3, MCPH5 and MCPH6) were first found in families with northern Pakistani origin, with two loci (MCPH1 and MCPH3) remaining exclusive to this population (Woods *et al.*, 2005).

MCPH1, the first locus for primary microcephaly, was first reported through homozygosity mapping in two consanguineous Pakistani families on chromosomal region 8p23 with a maximum multipoint LOD score of 8.1 for marker D8S277 (Jackson *et al.*, 1998). Four years later, mutational analysis in two Pakistani families on two candidate genes *angiopoietin-2* and *BRIT1* (also known as *Microcephalin*) lying within 13-cM linkage interval for MCPH1 revealed a nonsense mutation (ser25-to-ter) in *Microcephalin* gene (Jackson *et al.*, 2002). *Microcephalin* is a centrosomal protein, which appears to be important for regulating the brain size and maintaining genome integrity by participating in cell cycle control and DNA damage response (Bartek, 2006). In the present study, only one family, family B enrolled from district bannu, was found linked with MCPH1 locus by genotyping markers D8S1099, D8S277, D8S1706 and D8S503. Mutational analysis on all 14 exons and splice-junction of *Microcephalin* gene in family B didn't show any mutation, suggesting that the mutation is probably present in the regulatory sequences of the gene.

Family G enrolled from district Kasoor of Punjab Province containing 2 affected males (IV-2, IV-3) through linkage analysis showed linkage with MCPH3 locus causing autosomal recessive primary microcephaly. Affected individuals were found homozygous for markers D9S930, D9S289, D9S1116 and D9S2155 and their parents as heterozygous. MCPH3 locus was first mapped

in a consanguineous family of northern Pakistani origins with four affected individuals at 9q34 chromosomal region with a maximum two-point LOD score of 3.76 for marker D9S290 (Moynihan *et al.*, 2000). The MCPH3 linkage interval harbors a gene called *CDK5RAP2*, a centrosomal protein which inhibits *CDK5* during neurogenesis (Andersen *et al.*, 2003; Bond *et al.*, 2005). It is also thought to be involved in MT nucleating and organizing function of centrosome. Bond *et al.* (2005) through positional cloning reported the first mutation in *CDK5RAP2* gene, a 243T-A transversion in exon 4 resulting in a ser81-to-ter (S81X) substitution. In our study, sequencing of the 30 exons of *CDK5RAP2* gene in family G failed to detect any pathogenic sequence variant.

Mutations in *ASPM* gene are known to be responsible most frequently for autosomal recessive primary microcephaly in humans and normally this gene appears to be an integral part of normal mitotic spindle function in embryonic neuroblasts (Bond *et al.*, 2002). It is a centrosomal protein which plays an important role in cell proliferation during fetal brain development. *ASPM* locates to an 8-cM region on chromosome 1q31 known as MCPH5 locus, which was first mapped in a consanguineous family of city Multan, Pakistan containing three affected individuals with a maximum multipoint LOD score of 3.7 for marker D1S1726 (Pattison *et al.*, 2000). In the present study, four families A, C, D and F were found linked with microsatellite markers D1S3468, D1S1660, D1S2716, D1S2625, D1S1726, D1S2628 and D1S1678 for MCPH5 locus. Mutational analysis in family A revealed 6854delTC mutation (not previously reported) in exon 18 of *ASPM* gene in one of the affected individuals resulting in premature stop codon 92 bp downstream. The sequence variant was also identified in normal and carrier individual therefore further characterization of this mutation is required. DNA sequence analysis in families C, D and F showed a previously reported mutation, a G to A transition at nucleotide position 3978, producing immediate premature stop codon (W1326X) (Kumar *et al.*, 2004) in exon 17 of *ASPM* gene. Till now, majority of the *ASPM* mutations have been reported in families from Pakistan (Bond *et al.*, 2002, 2003; Gul *et al.*, 2006; Gul *et al.*, 2007). A recent study performed by Kousar *et al.* (2010) on 21 Pakistani families with autosomal recessive primary microcephaly linked 18 families with MCPH5, thus indicating MCPH5 to be the most common locus in Pakistani population.

In family E and H, genotyping data revealed that affected individuals were heterozygous for different combinations of parental alleles, thus excluding the linkage in these families to the established primary

microcephaly candidate regions. This signifies that a novel gene or genes to be responsible for autosomal recessive microcephaly (MCPH) in these families. The high number of MCPH cases found in Pakistani population is probably attributed to consanguineous marriages (Woods *et al.*, 2005).

### CONCLUSION

This study is helpful in delineating consanguineous marriages as a common cultural practice to be an important factor in causing high number of MCPH cases in Pakistani population. In the current scenario, MCPH5 (ASPM) is found to be the most common locus prevailing in highly consanguineous Pakistani families, which is consistent with the previous studies performed on the same population. Pakistan is a multicultural country with diverse ethnic groups and the presence of highly inbred families harboring different MCPH cases is suggestive of the involvement of novel genes/loci causing autosomal recessive primary microcephaly. The need is to further characterize the disease at molecular level in these families in order to have a progress towards its cure. To decrease the incidence of primary microcephaly in Pakistan, it is crucial to train the people to avoid cousin marriages. Furthermore, Government agencies and research institutions need to launch carrier screening programs in order to find people at a higher risk, which may help to minimize MCPH cases in the country. It is also suggested that genetic counseling facility be available in different cities at an easy access which will help the parents with information about their child with microcephaly and to plan for future pregnancies.

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### REFERENCES

Andersen, J.S., C.J. Wilkinson, T. Mayor, P. Mortensen, E.A. Nigg and M. Mann, 2003. Proteomic characterization of the human centrosome by protein correlation profiling. *Nature*, 426: 570-574.

Bartek, J., 2006. Microcephalin guards against small brains, genetic instability, and cancer. *Cancer Cell.*, 10: 91-93.

Bond, J., E. Roberts, G.H. Mochida, D.J. Hampshire, S. Scott, J.M. Askham, K. Springell, M. Mahadevan, Y.J. Crow, A.F. Markham, C.A. Walsh and C.G. Woods, 2002. ASPM is a major determinant of cerebral cortical size. *Nat. Genet.*, 32: 316-320.

Bond, J., E. Roberts, K. Springell, S.B. Lizarraga, S. Scott, J. Higgins, D.J. Hampshire, E.E. Morrison, G.F. Leal, E.O. Silva, S.M. Costa, D. Baralle, M. Raponi, G. Karbani, Y. Rashid, H. Jafri, C. Bennett, P. Corry, C.A. Walsh and C.G. Woods, 2005. A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size. *Nat Genet.*, 37: 353-355.

Bond, J., S. Scott, D.J. Hampshire, K. Springell, P. Corry, M.J. Abramowicz, G.H. Mochida, R.C. Hennekam, E.R. Maher, J.P. Fryns, A. Alswaid, H. Jafri, Y. Rashid, A. Mubaidin, C.A. Walsh, E. Roberts and C.G. Woods, 2003. Protein-truncating mutations in ASPM cause variable reduction in brain size. *Am. J. Hum. Genet.*, 73: 1170-1177.

Guernsey, D.L., H. Jiang, J. Hussin, M. Arnold, K. Bouyakdan, S. Perry, T. Babineau-Sturk, J. Beis, N. Dumas, S.C. Evans, M. Ferguson, M. Matsuoka, C. Macgillivray, M. Nightingale, L. Patry, A.L. Rideout, A. Thomas, A. Orr, I. Hoffmann, J.L. Michaud, P. Awadalla, D.C. Meek, M. Ludman and M.E. Samuels, 2010. Mutations in centrosomal protein CEP152 in primary microcephaly families linked to MCPH4. *Am. J. Hum. Genet.*, 87(1): 40-51.

Gul, A., M.J. Hassan, S. Mahmood, W. Chen, S. Rahmani, M.I. Naseer, L. Dellefave, N. Muhammad, M.A. Rafiq, M. Ansar, M.S. Chishti, G. Ali, T. Siddique and W. Ahmad, 2006. Genetic studies of autosomal recessive primary microcephaly in 33 Pakistani families: Novel sequence variants in ASPM gene. *Neurogenetics*, 7: 105-110.

Gul, A., M. Tariq, M.N. Khan, M.J. Hassan, G. Ali and W. Ahmad, 2007. Novel protein truncating mutations in the ASPM gene in families with autosomal recessive primary microcephaly. *J. Neurogenet.*, 21: 153-163.

Jackson, A.P., H. Eastwood, S.M. Bell, J. Adu, C. Toomes, I.M. Carr, E. Roberts, D.J. Hampshire, Y.J. Crow, A.J. Mighell, G. Karbani, H. Jafri, Y. Rashid, R.F. Mueller, A.F. Markham and C.G. Woods, 2002. Identification of microcephalin, a protein implicated in determining the size of the human brain. *Am. J. Hum. Genet.*, 71(1): 136-142.

Jackson, A.P., D.P. McHale, D.A. Campbell, H. Jafri, Y. Rashid, J. Mannan and G. Karbani, 1998. Primary autosomal recessive microcephaly (MCPH1) maps to chromosome 8p22-pter. *Am. J. Hum. Genet.*, 63: 541-546.

Jamieson, C.R., C. Govaerts and M.J. Abramowicz, 1999. Primary autosomal recessive microcephaly: Homozygosity mapping of MCPH4 to chromosome 15. *Am. J. Hum. Genet.*, 65: 1465-1469.

- Kong, X., K. Murphy, T. Raj, C. He, P.C. White and T.C. Matise, 2004. A combined linkage- physical map of the human genome. *Am. J. Hum. Genet.*, 75: 1143-1148.
- Kousar, R., H. Nawaz, M. Khurshid, G. Ali, S.U. Khan, H. Mir, M. Ayub, A. Wali, N. Ali, M. Jelani, S. Basit, W. Ahmad and M. Ansar, 2010. Mutation analysis of the ASPM gene in 18 Pakistani families with autosomal recessive primary microcephaly. *J. Child Neurol.*, 25(6): 715-720.
- Kumar A, Blanton S, Babu M, Markandaya M and Girimaji S, 2004. Genetic analysis of primary microcephaly in Indian families: novel ASPM mutations. *Clin Genet.*, 66: 341-348.
- Kumar, A., S.C. Girimaji, M.R. Duvvari and S.H. Blanton, 2009. Mutations in STIL, encoding a pericentriolar and centrosomal protein, cause primary microcephaly. *Am. J. Hum. Genet.*, 84: 286-290.
- Leal, G.F., E. Roberts, E.O. Silva, S.M. Costa, D.J. Hampshire and C.G. Woods, 2003. A novel locus for autosomal recessive primary microcephaly (MCPH6) maps to 13q12.2. *J. Med. Genet.*, 40: 540-542.
- Moynihan, L., A.P. Jackson, E. Roberts, G. Karbani, I. Lewis, P. Corry, G. Turner, R.F. Mueller, N.J. Lench and C.G. Woods, 2000. A third novel locus for primary autosomal recessive microcephaly maps to chromosome 9q34. *Am. J. Hum. Genet.*, 66: 724-727.
- Nicholas, A.K., E.A. Swanson, J.J. Cox, G. Karbani, S. Malik, K. Springell, D. Hampshire, M. Ahmed, J. Bond, D. Di-Benedetto, M. Fichera, C. Romano, W.B. Dobyns and C.G. Woods, 2009. The molecular landscape of ASPM mutations in primary microcephaly. *J. Med. Genet.*, 46: 249-253.
- Pattison, L., Y.J. Crow, V.J. Deebble, A.P. Jackson, H. Jafri, Y. Rashid, E. Roberts and C.G. Woods, 2000. A fifth locus for primary autosomal recessive microcephaly maps to chromosome 1q31. *Am. J. Hum. Genet.*, 67: 1578-1580.
- Roberts, E., D.J. Hampshire, L. Pattison, K. Springell, H. Jafri, P. Corry, J. Mannon, Y. Rashid, Y. Crow, J. Bond and C.G. Woods, 2002. Autosomal recessive primary microcephaly: an analysis of locus heterogeneity and phenotypic variation. *J. Med. Genet.*, 39: 718-721.
- Roberts, E., A.P. Jackson, A.C. Carradice, V.J. Deebble, J. Mannan, Y. Rashid, H. Jafri, D.P. McHale, A.F. Markham, N.J. Lench and C.G. Woods, 1999. The second locus for autosomal recessive primary microcephaly (MCPH2) maps to chromosome 19q13.1-13.2. *Eur. J. Hum. Genet.*, 7: 815-820.
- Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. *Molecular Cloning: A Laboratory Manual*. Irwin, N., N. Ford, C. Nolan, M. Ferguson and M. Ockler, (Eds.), 2nd Edn., Cold Spring Harbor Laboratory Press, CSH, USA.
- Thornton, G.K. and C.G. Woods, 2009. Primary microcephaly: Do all roads lead to Rome? *Trend. Gen.*, 25(11): 501-510.
- Woods, C.G., J. Bond and W. Enard, 2005. Autosomal recessive primary microcephaly (MCPH): A review of clinical, molecular, and evolutionary findings. *Am. J. Hum. Genet.*, 76: 717-728.