EXCLUSION MAPPING OF FAMILIES SEGREGATING AUTOSOMAL RECESSIVE MICROCEPHALY FROM DISTRICT MIRPUR AZAD JAMMU AND KASHMIR

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ABSTRACT: Autosomal recessive primary microcephaly is due to genetic causes but several environmental factors also play role in disease development. It is heterogeneous disorder and seventeen loci for autosomal recessive primary microcephaly have been identified. Pakistani population has strong history of consanguineous marriages. For the present study two families A and B were ascertained from different localities of District Mirpur Azad Jammu and Kashmir depicting autosomal recessive primary microcephaly. Evaluation was done on the basis of phenotypic observation and pedigree construction. Blood samples of all affected and normal individuals of both families were analyzed for linkage analysis by microsatellite markers application. Genotyping data showed heterozygous banding pattern regardless of affected or normal individuals. This family may involve some novel loci or mutation. The linkage at MCPH5 locus was established in family B, associated earlier in causing microcephaly with mutation in *ASPM* gene. Further research is needed to refine the results to find out the actual reason of MCPH by genome wide scan techniques in family A.

Keywords: Autosomal recessive primary microcephaly, genotyping and *ASPM*.

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INTRODUCTION

Changes in the genes or chromosomal structures are main cause of genetic disorders. Individuals who are born in kindred union share homozygous segments of DNA. Consequently, there is increased risk of recessive diseases. Conspicuous phenotype of patients having microcephaly is the presence of small head size (Jackson*etal.,* 1998; Woods *et al.,* 2002). In addition to reduced head circumference, the patients typically show sloping forehead, but no other facial or physical abnormalities (Wang *et al*., 2017). The structure of brain remains normal, whereas cerebral cortex shows major size reduction (Roberts *et al.,* 1999)**.** it is evident by mutations in majority of the reported genes disrupt centrosomal/spindle pole proteins and components of DNA repair mechanism in NPCs, without affecting other organs (Kraemer *et al*., 2015). The human brain is nearly 2% as compared to whole body mass (Ponting and Jackson, 2002; Roth and Dicke, 2005). Due to MCPH the size of central nervous system is reduced and affects greatly on the cerebral cortex (Bond *et al.,* 2002)**.** Microcephaly genes play essential role in cellular pathways which help in regulation of human brain. They affect mitotic activity during neurogenesis because these genes have diverse role (Cox *et al*., 2006).

Human brain size increased during evolution, due to molecular changes in the microcephaly genes (Woods *et al.,* 2005). This genetic problem occurs due to mutation in genes which are responsible for this disorder and are located on chromosome number 1, 8, 9, 13, 15, and 19 (Kumar *et al.,* 2004). Microcephaly is heterogeneous problem having seventeen loci (MCPH1-MCPH17) and thirteen genes have been known yet. *Microcephalin* which refers to MCPH1 (Jackson *et al.,* 2002; Darvish *et al.,* 2010). Second gene is WD repeat protein 62 (*WDR62*) at MCPH 2 (Roberts *et al.,* 1999; Kausar *et al.,* 2011). CDK5 regulatory subunit-associated protein 2 (*CDK5RAP2*) is third gene mapped at MCPH3 (Ng R.K. and J.B. Gurdon 2008; Evans *et al.,* 2006). Cancer susceptibility candidate 5 (*CASC5*) at MCPH4 (Jameison *et al.,* 1999; Genin *et al.,* 2012). Abnormal spindle like microcephaly associated protein (*ASPM*) at MCPH5 (Fish *et al.,* 2006). Centromeric protein J (*CENPJ*) is mapped at MCPH6 (Leal *et al.,* 2006). The seventh gene involved in microcephaly is SCL/TAL1 interrupting locus (*STIL/SIL*) at MCPH7 loci (Kumar *et al.,* 2009). Centrosomal protein135kD (*CEP135*) is eighth gene mapped at MCPH8 (Hussain *et al.,* 2012). Ninth gene involved in microcephaly is centrosomal protein, 152KD (*CEP152*) at MCPH9 (Guernsey *et al.,* 2010). Zinc finger protein335 (*ZNF335*) was mapped at MCPH10 (Yang *et al.,* 2012). Polyhomeotic like protein 1 (*PHC1*) at MCPH11 (Awad *et al.,* 2013). Cyclin dependent kinase 6 (*CDK6*) at MCPH12 (Hussain *et al.,* 2013). Clinically, at all of these corresponding loci mutation analysis is indistinguishable (Mahmood *et al.,* 2011). This study was designed to search for linkage of

any known or novel loci/genes involved in hereditary microcephaly from two families residing in AJK.

MATERIAL AND METHODS

For present study two families affected with microcephaly were selected. These families were ascertained from different localities of district Mirpur, Azad Jammu and Kashmir (AJK). The elders of the families were interviewed to gather information about family history. Almost all affected members were born to closely related parents. Informed consents of affected members were also obtained regarding the medical condition of patients and blood samples of total 14 individuals including both normal (n=8) and affected (n=6) were taken.

Pedigree construction: For pedigree construction (Fig 1 and 2) detailed information of families' history was obtained from grandparents, parents, relatives and neighbors. Pedigrees were drawn according to standard method (Bennett *et al*., 1995). After construction of pedigrees it was shown that mode of inheritance in families was autosomal recessive.

Blood sampling: Blood samples of all affected (n=6) and normal (n= 8) individuals were taken in 10mL syringes and transferred to ethylene diamine tetra acetic acid (BIO-VAC, EDTA.K3) vacationer tubes and transferred to Human Molecular Genetics laboratory of Mirpur University of Science and Technology. Blood samples were stored in refrigerator at 4ºC.

The extraction of DNA from human blood samples was performed by using standardized chloroform- phenolic method (Sambrook and Russel, 2006).

Amplification of microsatellite markers: Extracted genomic DNA samples were resolved on 1% agarose gel. Amplified products were loaded on 2% agarose gel. Finally samples were loaded on Poly acrylamide gel electrophoresis (PAGE) to analyze the genotyping data. **(**Guilliatt, 2002). Gel was prepared by using gel plates which were separated by spacers clipped at 1.5mm distance. Between the plates, the gel solution was poured and polymerized at room temperature for 40 min. 1X TBE buffer was added into the vertical gel tank and the gel plates were set into the tank. Then the mixtures were loaded into the wells followed by running samples at 115 voltage for 2 to 3 hrs till the proper running of samples.

To carry out microsatellite markers amplification (Table 1) on substrate families PCR tubes (0.2ml) containing 25 of reaction mixture were used. The reaction mixture was prepared by using (40 ng) I µL of genomic DNA, Primers (10µm) 1µL each, 2.5 µL of 10X Taq Buffer $(NH4)_2SO_4$ (Thermo Scientific); 2.5µL of $MgCl_2$, 1 µL dNTPs mixture (10mM), 0.5µL DNA polymerase (Thermo Scientific),15.5ml of PCR water. The whole reaction mixture was kept in centrifuge for appropriate mixing at 8,000 rmp for 30 seconds. Samples were placed in thermo cycler machine for amplification of substrate families DNA segments. Initial denaturation at 95ºC for 5 minutes followed by 40cycles of 1 minute , denaturation at 95ºC, annealing at 60ºC for 1minute. Extension of template strand at 72ºC for 1 minute. Final extension of template strand at 72ºC for 5-10 minutes to ensure that DNA polymerase completely synthesizes any remaining single stranded DNA.

RESULTS AND DISCUSSION

The Phenotypic characteristics of both families were typical of microcephaly including slopping forehead and reduced head circumferences. Moreover, the patient from both families also showed some signs of intellectual disability, and mild to moderate seizures, along with reduced speech. No medical reports of the patients were available from both families as due to poor socioeconomic background the parents could not continue their proper treatment. All affected individuals were unable to read and write but one individual from family B (IV.2) had learning skill and well adapted to environment (Table 2). The results of genotyping data were analyzed on PAGE and allelic pattern was determined from the gel image. The allelic banding pattern in family A showed hetrozygosity which is an indication of involvement of any other known or novel loci hence linkage is excluded in this family. This family will be further screened by application of microsatellite markers of other known gene loci The banding pattern of family B depicted that, all the affected individuals were homozygous confirming that this family is linked to MCPH5. MCPH heterogeneity studies have been performed in a northern Pakistani population and MCPH5 was identified as the most common locus, accounting for linkage of nearly half of the amassed families (24/56 families) (Roberts *et al*., 2002). *ASPM* gene (MCPH5) occupies the most prominent position in disease etiology of MCPH owing to its mutations rate, which is as high as 40% (Nicholas *et al*., 2009). Different ethnic populations show different prevalence for the MCPH5 locus. For instance, in Pakistan this toll falls in the range of 43-86% (Gull *et al*., 2006), in Indian populations it is 33.5% (Kumar *et al*., 2004), while in Iranian population it is reported to be 13.3%(Darvish *et al*., 2010). Homozygosity mapping is based on the hypothesis that rare mutation is inherited from common ancestor to both parents, so that affected individuals are homozygous by decent for polymorphic markers close to disease gene loci. Homozygosity mapping for identifying MCPH genes is the best strategy to identify these MCPH genes in large consanguineous families (Ropers and

Hamel, 2007). Incidence of MCPH in Pakistan is approximately 1 in 10,000 (Woods *et al.*, 2005).

Table-1: Primers with their sequence and genetic location used for genotyping.

Primer Name	Sequence	Genetic location (cM)	Gene
GATA135F02	F :TTCATAGACCAATGCTATTGTAA R : CATAACATCCGAGCATGTGA	**204.64**	
D1S1724	F : TTCCCCAAATGTATTACTGC R : AAAGGAGTACCCAATCCCAG	204.78	
D1S2840	F:GACAAGTCATCTTACACCTCAGTTC R:CCAACATAATTTCTGGGCTG	205.81	*ASPM*
D1S510	F:TGCCAGTTGACCTCAAG R:TCCAGTTGCTCCTCACC	207.96	
D1S306	F:CTGGGACTGGAAACACTTTTGAT R:CCAGAGGGAGCATTGGTG	209.16	
D19S249	F:CACAGACATGGACAGTATAC R:CTGTCTATCTATAATCTATCC	53.11	
D19S1170	F: TTGTGCTGAATTGGAATCAA R: GCAGACAGAGCTGGGAAATA	54.58	
D19S555	F: CCGGAAGGCAGAGCTTGCA R: CACCTCAGCCACACGAGCA	55.06	*WDR62*
D19S416	F: CCTGTCCCAGAGAGACCCTA R: AAGAGAGTGTGCCATTTGCT	56.28	
D13S633	F: ACCTAAAATCTCCCAAATTGT R: ACGCTTTACCCCTGAAATC	3.1	
D13S1275	F:ATCACTTGAATAAGAAGCCATTTG R:CCAGCATGACCTTTACCAG	6.97	*CENPJ*
D13S787	F: ATCAGGATTCCAGGAGGAAA R: ACCTGGGAGTCGGAGCTC	8.75	

Table-2: Clinical and morphological features of affected individuals of family A and B.

Clinical Features	IV.1 (A)	IV.6 (A)	IV.8 (A)	III.1	III.9	IV.2
Sex	Male	Female	Male	Female	Male	Female
HC	16.14inch	18.89inch	15.99inch	16.14 inch	14.96 inch	14.17 inch
Height	57.08inch	48.81inch	50.52inch	53.93 inch	51.96 inch	37.40 inch
Weight	67kg	21kg	58kg	30kg	26kg	13kg
Age	18years	13years	3years	38years	16years	14years
MCPH Type	Primary	-	-	Primary	-	-
Performance IQ	Mild	-	-	Present	Mild	Present
Speech Delay	Few words	-	Absent	No	Present	No
Behaviour Problems	Aggressive	-	-	Aggressive	-	No
Neurologic Delay	present	-	-	present	-	Not sever
Sensory	Normal	-	-	Normal	-	-
Consanguinity	Present	-	-	Present	-	-
Schooling	Absent	-	-	Absent	-	Present

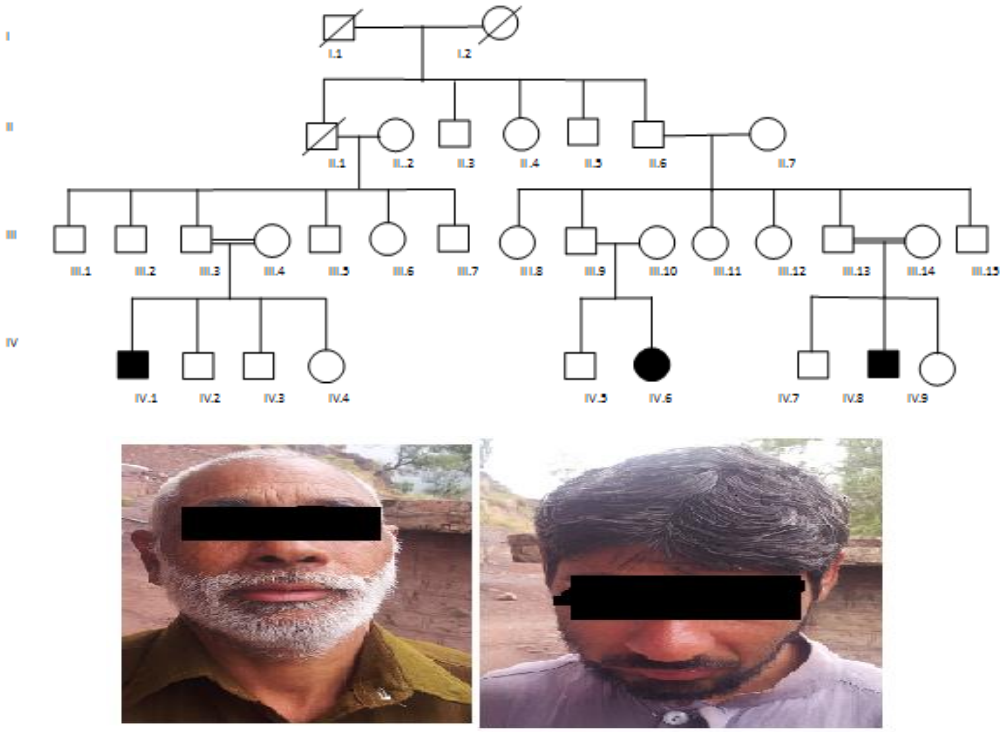

Figure-1: Pedigree and pictures of affected members of Family A segregating Autosomal Recessive Microcephaly. Circles represent females and square represent males. Affected Individuals are presented by filled squares and circles.

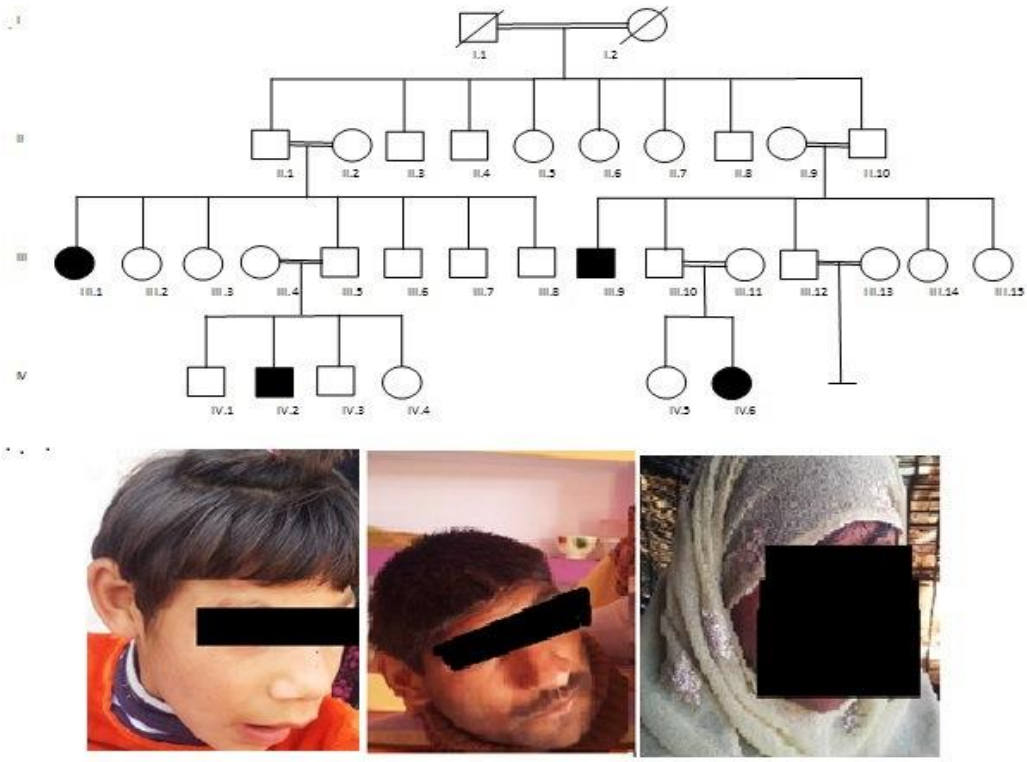

Figure-2: Pedigree and pictures of affected members of Family B Segregating Autosomal Recessive Microcephaly. Circles represent females and square represent males. Affected Individuals are presented by filled squares and circles.

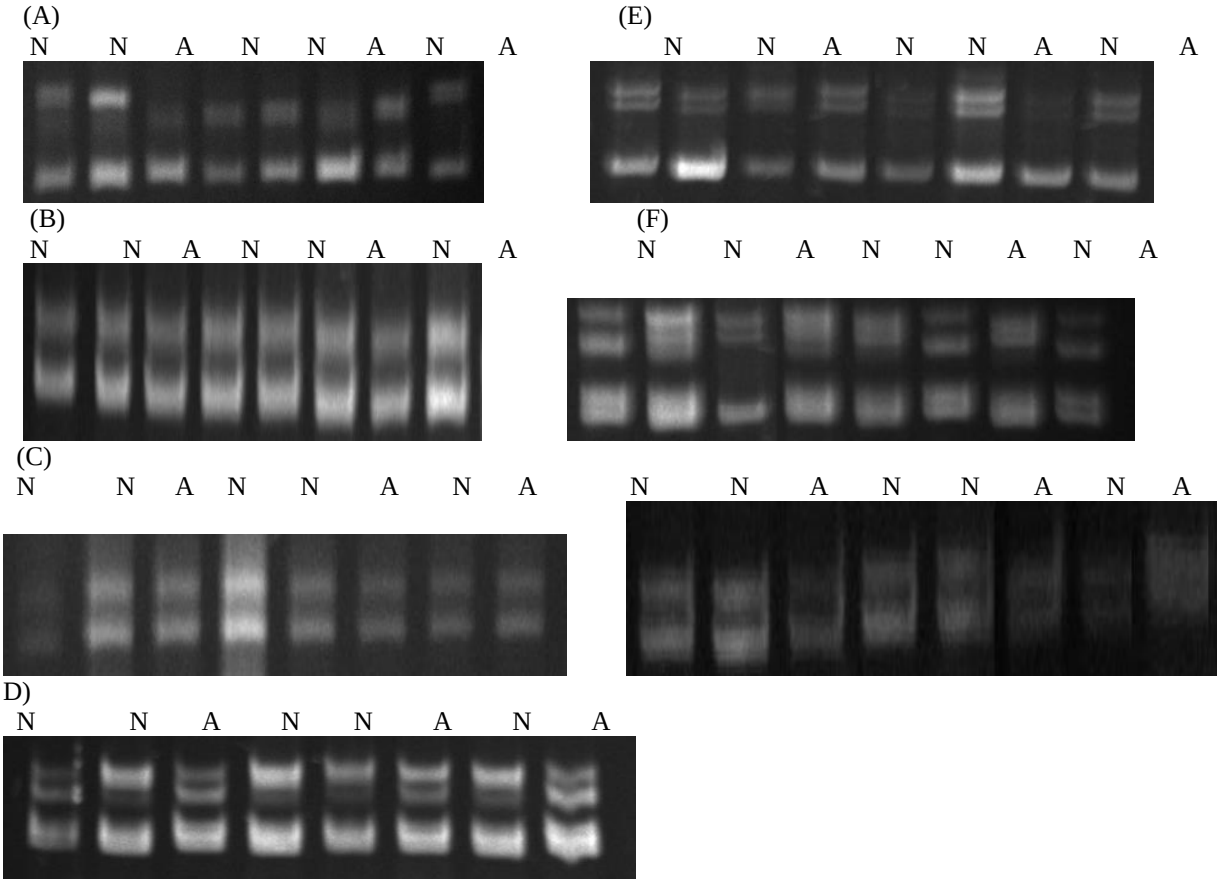

Figure-3: Electropherogram of family A result on 8% non- denaturing gel stained with ethidium bromide, showing allele pattern of microsatellite markers. (A) GATA135FO2 at genetic location 204.64cM. (B) D1S1678 at genetic location 213.76cM. (C) D1S1724 at genetic location 211.52cM. (D) D1S510 at genetic location 214.36cM (E) D1S2840 at genetic location 205.81cM. (F) D1S306 at genetic location 209.16cM (G) D13S787 at genetic location 209.16cM. Heterozygous banding pattern of all the affected and normal individuals is depicted by all microsatellite markers (A-G).

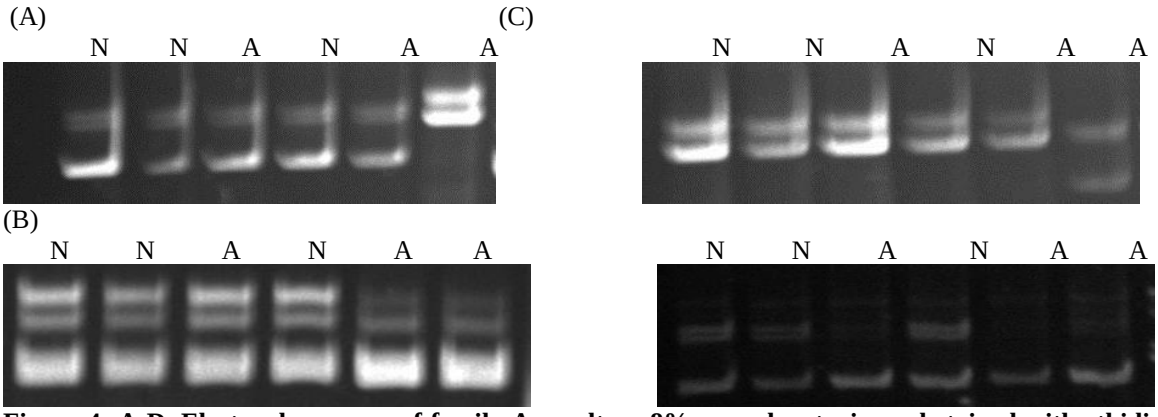

Figure-4: A-D. Electropherogram of family A result on 8% non- denaturing gel stained with ethidium bromide, showing allele pattern of microsatellite markers. (A) D1S2840 at genetic location 205.81cM. (B) D1S510 at genetic location 214.36cM (C) D1S1678 at genetic location 213.76cM (D) GATA135FO2 at genetic location 204.64cM.

Conclusions: Linkage of one family to *ASPM* gene whereas second family did not show linkage to any of the applied primer and it could be further screened for any other novel or known loci.

REFERENCES

Awad, S., M.S. Al-Dosari, N. Al-Yacoub, D. Colak, M.A. Salih, F.S. Alkuraya and C. Poizat (2013). Mutation in *PHC1* implicates chromatin remodeling in primary microcephaly pathogenesis. Hum. Mol. Genet. 22(11): 2200–2213.

Barkovich, A.J., D.M. Ferriero, R.M. Barr, P. Gressens, W.B. Dobyns, C.L. Truwit and P. Evrard (1999). Microlissencephaly a heterogeneous malformation of cortical development. Neuropediat. 29(3):113-119.

Bennett, R.L., Steinhaus, K.A., Uhrich, S.B., O'Sullivan, C.K., Resta, R.G., Lochner-Doyle, D., Markel, D.S., Vincent, V. and Hamanishi, J. 1995. Recommendations for standardized human pedigree nomenclature. J. Genet. Counsel. 4(4): 267-279.

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(2): 316–320.

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(5):1170–1177.

Cox, J., A.P. Jackson, J. Bond and C.G. Woods (2006). What primary microcephaly can tell us about brain growth? Tre. Mol. Med. 12(8): 358-366.

Darvish, H., S. Esmaeeli-Nieh, G.B. Monajemi, M. Mohseni, S. Ghasemi- Firouzabadi, S.S. Abedini, I. Bahman, P. Jamali, S. Azimi, F. Mojahedi, A. Dehghan, Y. Shafeghati, A. Jankhah, M. Falah, M.J. Soltani, M.J. Banavandi, M. Ghani-Kakhi, M. Garshasbi, F. Rakhshani, A. Naghavi, A. Tzschach, H. Neitzel, H.H. Ropers, A.W. Kuss, F. Behjati, K. Kahrizi and H.A. Najmabadi (2010). A clinical and molecular genetic study of 112 Iranian families with primary microcephaly. J. Med. Genet. 47: 823-828.

Evans, P.D., E.J. Vallender and B.T. Lahn (2006). Molecular evolution of the brain size regulator genes *CDK5RAP2* and *CENPJ* Gene. Cell Cycle. 375: 75–79.

Fish, J.L., Y. Kosodo, W. Enard, S. Paabo and W.B. Huttner (2006). Aspm specifically maintains symmetric proliferative divisions of neuroepithelial cells. Proc. Natl. Acad. Sci. USA. 103(27): 10438–10443.

Genin, A., J. Desir, N. Lambert, M. Biervliet, N. Van Der Aa, G. Pierquin, A. Killian, M. Tosi, M. Urbina, A. Lefort, F. Libert, I. Pirson and M. Abramowicz (2012). Kinetochore KMN network gene CASC5 mutated in Primary Microcephaly. Hum. Mol. Genet. 21(24): 5306–5317.

Guilliatt, A.M., (2002). Agarose and polyacrylamide gel electrophoresis. *PCR Mutati. Detect. Prot.*, Humana Press. (pp. 1-12).

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 microcephalyfamilies linked to MCPH4. Am. J. Hum. Genet. 87(1): 40–51.

Gul, A., Hassan, M.J., Mahmood, S., Chen, W., Rahmani, S., Naseer, M.I., Dellefave, L., Muhammad, N., Rafiq, M.A., Ansar, M. and Chishti, M.S., (2006). Genetic studies of autosomal recessive primary microcephaly in 33 Pakistani families: novel sequence variants in ASPM gene. Neurogen 7(2): 105-110.

Hussain, M.S., S.M. Baig, S. Neumann, G. Nür nberg,. M.Farooq. I. Ahmad, T. Alef, H.C. Henies, M. Technau, J. Altmüller, P. Frommolt, H. Thiele, A.A. Noegel and P. Nürnberg (2012). A truncating mutation on *CEP135* causes primary microcephaly and disturbed centrosomal function. Am. J. Hum. Genet. 90(5): 871-878.

Hussain, M.S., S.M. Baig, S. Neumann, V.S. Peche, S. Szczepanski, G. Nürnberg, M. Tariq, M. Jameel, T.N. Khan, A. Fatima, N.A. Malik, I. Ahmad, J. Altmüller, P. Frommolt, H. Thiele, W. Höhne, G. Yigit, B. Wollnik, B.A. Neubauer, P. Nürnberg and A.A. Noegel (2013). *CDK6* associates with centrosome during mitosis and is mutated in large Pakistani family with primary microcephaly. Hum. Mol. Genet. 22(25): 5199-5214.

Jackson, A.P., D.P. McHale, D.A. Campbell, H. Jafri, Y. Rashid, J. Mannan, G. Karbani, P. Corry, M.I. Levene, R.F. Mueller, A.F. Markham, N.J. Lench and C.G. Woods (1998). Primary autosomal recessive microcephaly (MCPH1)

maps to chromosome 8p22-pter. Am. J. Hum. Genet. 63(2): 541–546.

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.

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(5): 1465-1469.

Kraemer N, Picker-Minh S, Abbasi AA, Fröhler S, Ninnemann O, Khan MN, Ali G, Chen W, Kaindl AM. Genetic causes of MCPH in consanguineous Pakistani families. Clin. Genet. 2016; 89(6):744-45.

Kousar, R., M.J. Hassan, B. Khan, S. Basit, S. Mahmood, A. Mir, W. Ahmad and M. Ansar (2011). Mutations in *WDR62* gene in Pakistani families with autosomal recessive primary microcephaly. BMC neurol. 1: 111-119.

Kumar, A., S.H. Blanton, M. Babu, M. Markandaya and S.C. Girimaji (2004). Genetic analysis of primary microcephalyin Indian families novel *ASPM* mutations. Clin. Genet. 66(4): 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(2): 286-290

Leal, G.F., E. Roberts, E.O. Silva, S.M.R. Costa, D. Hampshire and C.G. Woods (2006). A novel locus for autosomal recessive primary microcephaly (MCPH6) maps to 13q12.2. J. Med. Genet. 40(7): 540-542.

Mahmood, S., W. Ahmad and J.M. Hassan (2011). Autosomal recessive primary microcephaly (MCPH) clinical manifestations, genetic heterogeneity and mutation continuum. Orph. J. Rare. Dis. 6:39 .

Ng, R.K and J.B. Gurdon (2008). Epigenetic inheritance of cell differentiation status. Cell Cycle. **7**(9): 1173–1177.

Nicholas, A.K., Swanson, E.A., Cox, J.J., Karbani, G., Malik, S., Springell, K., Hampshire, D., Ahmed, M., Bond, J., Di Benedetto, D. and Fichera, M., (2009). The molecular landscape of ASPM mutations in primary microcephaly. J. Med. Genet. 46(4): 249-253.

Opitz, J.M and M.C. Holt (1992). Microcephaly: general considerations and aids to nosology. J. Craniofac Genet. Dev. Bio. 10:175–204.

Ponting, C. and A.P. Jackson (2002). Evolution of primary microcephaly genes and the enlargement of primate brains. Curr. Opin. Genet. Dev.15(3): 241- 248.

Roberts, E., A.P. Jackson, A.C. Carradice, V.J. Deeble, 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**(7): 815-820.

Roberts, E., D.J. Hampshire, K. Springell, L.Y. Pattison, Y.J. Crow, H. Jafri, P. Corry, G. Kabani, J. Mannon, Y. Rashid, J. Keen, J. Bond and C.G. Woods (2002). Autosomal recessive primary microcephaly: an analysis of locus heterogeneity and phenotypic variation. J. Med. Genet. 39(10): 718– 721.

Ropers, H.H., (2007). New perspectives for the elucidation of genetic disorders. Am. J. Hum. Genet. 81(2): 199-207.

Roth, G. and U. Dicke (2005). Evolution of the brain and intelligence. Tre. Cogn. Sci. 9(5): 250-257.

Sambrook, J. and Russell, D.W., (2006). Purification of nucleic acids by extraction with phenol: chloroform. Col. Spri. Harb. Proto., 2006(1): 4455.

Wang, D., Chen, C., Liu, S., Zhou, H., Yang, K., Zhao, Q., Ji, X., Chen, C., Xie, W., Wang, Z. and Mi, L.Z., (2017). A mutation identified in neonatal Microcephaly destabilizes Zika virus NS1 assembly in vitro. Sci. Report, 15.7: 42580.

Woods, C.G. (2002). Human Microcephaly. Curr. Opin. Neurobiol.14:112–117.

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(5): 717-728.

Yang, Y.J., A.E. Baltus, R.S. Mathew, E.A. Murphy, G.D. Evrony, D.M. Gonzalez, E.P. Wang, C.A. Marshall-Walker, B.J. Barry, J. Murn, A. Tatarakis, M.A. Mahajan,, H.H. Samuels, Y. Shi, J.A. Golden, M. Mahajnah, R. Shenhav and C.A. Walsh (2012). Microcephaly gene links trithorax and *REST/NRSF* to control stem cell proliferation and differenation. Cell 151(5):1097-1112.