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Exclusion mapping of primary microcephaly in Pakistani kindred

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Abstract: Microcephaly is a disorder of small head in which the head circumference of the individual is less than 3 standard deviation as compared to normal. Primary microcephaly is a condition present by birth and effects 2-2.5% of total population. Microcephaly is frequently present in Asian countries like in Pakistan and India where cousin marriages are commonly practiced. *ASPM* is most common gene which affects 50% of microcephalic population. In this study linkage analysis in two Pakistani kindred MCPA was carried out. DNA of the peripheral blood was isolated after informed consent, pedigree construction and multiple interviews of the family members. This family was screened with all known microsatellite markers of loci MCPH1 to MCPH7. PCR was followed by 8% non-denaturing Polyacrylamide gel electrophoresis. No linkage was observed with any locus previously mapped. Further mapping would be followed by screening with approximately 396 microsatellite markers of human genome at distance of 2 cM apart on all chromosomes of the family. This genome wide search might map the new region involved in this kindred.

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INTRODUCTION

Microcephaly is a rare neurological disorder which leads to non progressive mental retardation and reduces brain size upto 70%[1]. In microcephaly the head circumference of the effected individual is 2 to 3 standard deviation below with age and sex as compared to normal. Microcephaly is heterogenous etiologically as it may be occurred by environmental as well as genetic causes.

Primary microcephaly is a genetic based microcephaly that is caused by inheritance of an autosomal recessive trait while secondary may be caused due to environmental factors[2]. Occurrence of microcephaly is 1/25,000 to 1/30,000 live births in western population[3]. Where as in Pakistan occurrence of microcephalic live birth is 1/10,000[4]. Seven Loci MCPH1 to MCPH7 have been identified uptil now which are involved in microcephaly (Table 1).

Table 1: Loci of microcephaly and their respective genes.

Locus	Chromosomal position	Genes	Location	Reference
MCPH1	8p23.1	*Microcephalin*	Northern Pakistan	(5)
MCPH2	19q13.1-13.2	*WDR62*	Northern Pakistan	(6)
MCPH3	9q33.2	*CDK5RAP2*	Northern Pakistan	(7)
MCPH4	15q21.1	*CEP152*	Morocco/ Canada	(8)
MCPH5	1q31	*ASPM*	Turkey / Pakistan	(9)
MCPH6	13q12.2	*CENPJ*	Brazil	(10)
MCPH7	1p33	*STIL*	India	(11)

MCPH1 is a 13 cM region on chromosome 8p which have 14 exons and 236 kb genome. Encoding gene of this locus named *Microcephalin*. Cytogenetic analysis of MCPH1 shows the increased proportion of cells with pre-mature chromosome condensation that leads to prophase like appearance[5].

Recently *WDR62* gene is found on 19q and as a causative gene of microcephaly located on MCPH2 locus. Gene is associated with the spindle pole and is mutated in human microcephaly[6]. MCPH3 encoded gene named *CDK5RAP-2* (cyclin dependent kinase regulatory associated protein). Mutation in this gene was firstly detected in Pakistani kindered. It consists of 38 exons spanning 191 Kb[7].

MCPH4 was first time observed in moroccan family. Critical region might be 5.3 cM between markers D15S222 to D15S962[8]. *CEP152* gene is recently been identified to be present on locus 4 in chromosome 15. Gene is involved in centrosome duplication and centriole formation[9].

ASPM is the most common gene on locus 5 whose abnormal function causes microcephaly. This gene is responsible for approximately 50% of microcephaly. About 69 mutations have been identified in *ASPM*[9]. MCPH6 encodes gene *CENPJ* (centomere associated protein J) located in 6 MB region on chromosome 13q12.2. This 17 exon gene spans 64 Kb genome that encodes 1338aa protein. *CENPJ* earlier named as *CPAP*.

Gene involves in gama tubulin ring complexes and also acts as inhibitors of microtubule nucleation[10]. *STILL* gene is found to be present on locus 7. It consists of 18 exons and spans 64 Kb. Mutations in this gene cause Protein truncations. *STILL* involves in cell cycle regulation and check points[11].

The aim of the present research work was to study linkage of autosomal recessive primary Microcephaly in Pakistani kindred however the family showed exclusion by not linking with any of the known locus.

MATERIAL AND METHODS

Family MCPA consists of twelve individuals out of which three females are affected. In the pedigree III-1, III-5, III-6 are effected whereas I-1, II-1, II-2, II-3, II-4, II-7, II-8 and II-9 are normal individuals as shown in the (Figure 1). DNA from Peripheral blood of all twelve individuals of family MCPA was extracted through phenol chloroform extraction method. Qualities of the DNA from all individuals were checked on 1% agarose gel (Figure 2). PCR was further followed using specific set of primer for each locus from MCPH1 to MCPH7. Optimized conditions for PCR reaction used were 2.5µl of $MgCl_2$, 2.5µl of 10X PCR buffer, 2µl of dNTPs, 1 µl of forward and reverse primer each from 50pM of stock primers, 1U of *Taq* DNA polymerase, 1.5µl of DNA and 13.5µl of PCR water making final volume 25µl in each PCR tube. PCR was further followed by 8% native page to check the linkage of family MCPA with any known locus.

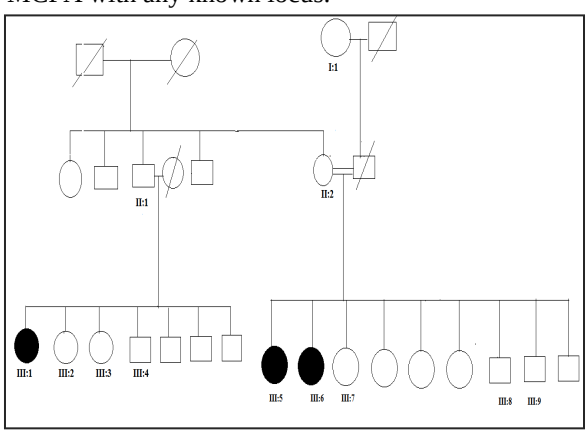

Figure 1: Pedigree of the family MCPA segregating for the autosomal recesive primary microcephaly. Circles represents females, squares represent males, filled circles represent affected individuals, and double line shows cousin marriage.

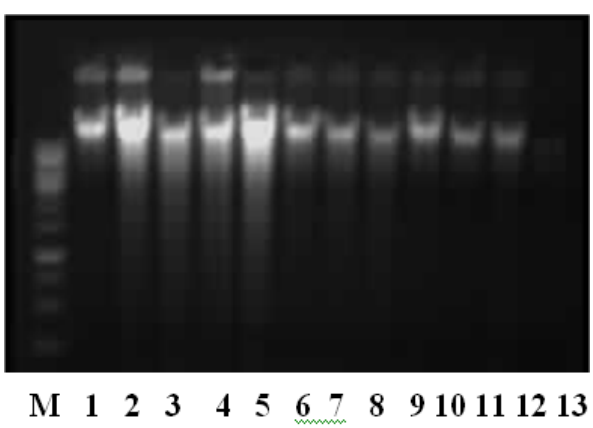

Figure 2: Agarose gel 1% to check the quality of DNA in twelve members of MCPA. M=1 Kb ladder while 1, 2, 5, 6, 7, 8, 10, 11, 12 are normal and 3, 4, 9 are affected individuals.

RESULTS

Family MCPA belongs from KotRadha Kishan, a small city near Lahore, in province of Punjab. Family preferred marriages within cast due to which they have autosomal recessive mode of inheritance. In the pedigree III-1, III-5, III-6 are effected whereas I-1, II-1, II-2, II-3, II-4, II-7, II-8 and II-9 are normal individuals as shown in figure 1. Family consists of twelve individuals out of which three females are affected. DNA that was isolated from the peripheral blood run on 1% agarose gel to check quality of DNA. Bands of DNA appear under UV as in the figure 2. PCR amplification of Family MCPA was done by screening with known avalaible markers of MCPH1 to MCPH7. Markers were attached by giving priority of chance of occurrence in certain populations so MCPH5 was screened first and then MCPH2, MCPH7, MCPH6, MCPH1, MCPH3 and MCPH4 respectively. Electropherogram of one marker of each locus is shown from (Figures 3-9).

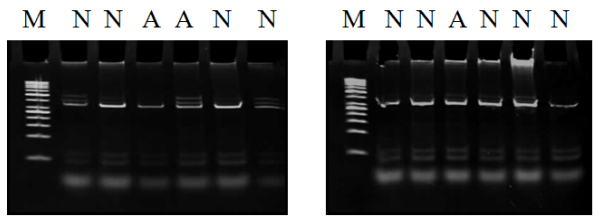

Figure 3: Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel of MCPA for primer D1S1678 (218.5 cM) on chromosome 1q31 showing different banding pattern among all affected (A) and normal (N) individuals.

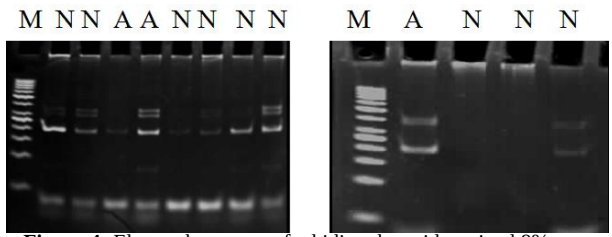

Figure 4: Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel of MCPA for primer D19S245 (58.69 cM) on chromosome 19q13.1 showing different banding pattern among all affected (A) and normal (N) individuals.

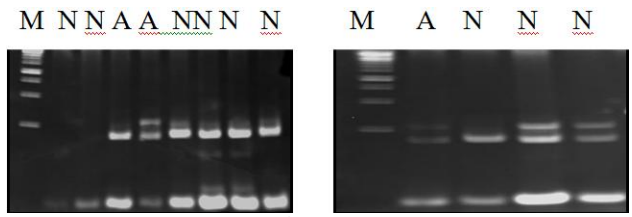

Figure 5: Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel of MCPA for primer D1S417 (79.80 cM) on chromosome 1p33 showing different banding pattern among all affected (A) and normal (N) individual.

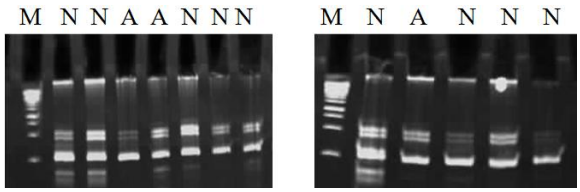

Figure 6: Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel of MCPA for primer D13S787 (8.87 cM) on chromosome 13q12.2 showing heterozygosity among all affected (A) and normal (N) individuals.

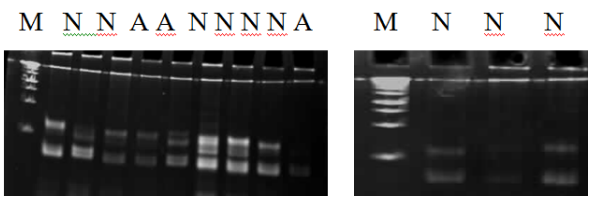

Figure 7: Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel of MCPA for primer D8S277 (8.34 cM) on chromosome 8p21 showing different banding pattern among all affected (A) and normal (N) individuals.

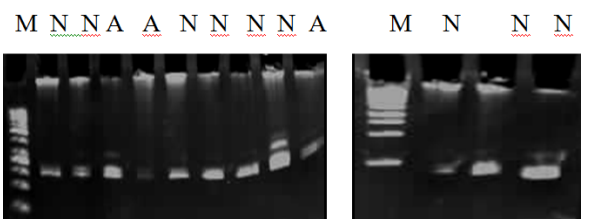

Figure 8: Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel of MCPA for primer D9S1682 (132.09 cM) on chromosome 9q33.2 showing different banding pattern among all affected (A) and normal (N) individuals.

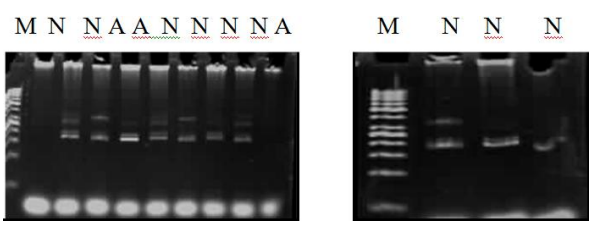

Figure 9: Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel of MCPA for primer D15S222 on chromosome 15q21 showing different banding pattern among all effected and normal individuals.

DISCUSSION

Family MCPA belonged from kotradha kishan Punjab province. DNA of all members was extracted from peripheral blood and used for screening of known loci from MCPH1 to MCPH7. The family MCPA was screened for the known locus of microcephaly. Locus screening was done by giving priority according to its chance of occurrence in particular population. Hence, according to priority MCPH5 is a most common and prevalent locus with almost 45% chance of occurance[3]. Different banding patterns were observed in normal and affected as family was amplified with MCPH5 marker D1S1678 (218.5 cM). Family was excluded with MCPH5 as shown in (Figure 3).

MCPH2 is a second most common cause of microcephaly[12]. So D19S245 (58.69cM) marker for MCPH2 on chromosome 19q13.1 was screened for the linkage. Analysis showed different banding pattern in all individuals regardless of affected. Individual Bands were seen on 8% native PAGE as in (Figure 4). Family MCPA did not linked with MCPH7 as the marker D1S417 (79.80cM) on chromosome 1p33 showed exclusion as the different pattern of bands were observed (Figure 5). Next set of primers D13S787 (8.87 cM) for MCPH6 and D8S277 (8.34 cM) for MCPH1 were screened. MCPH6 and MCPH1 (Figure 6) and (figure7) respectively didn't show any linkage. The possibility of linkage for MCPH1 locus is less than 5%. Results showed that family is going towards exclusion and correlates the work of previous studies[13].

MCPH3 as in (Figure 8) also excluded by showing different banding pattern. Lastly marker for MCPH4 D15S222 was screened with genomic DNA of all individuals of family[15]. This showed that the family is excluded with all known markers of MCPH**.** In this MCPA family there was no evidence of homozygosity at any of five known microcephaly genes *MCPH1*, *CDK5RAP2*, *ASPM*, *CENPJ*, and *STIL*. The family was excluded as it did not show any linkage with any of known loci. Results showed the involvement of a new locus in MCPA and corroborate the work of Bond et al[19] who suggested that MCPH loci await discovery with a number of families as yet unlinked.

The impact of the present study is to understand the knowledge of underlying genetic cause of primary microcephaly. Which would ultimately help to enable the appropriate genetic counseling, prenatal diagnosis and therapeutic interventions of MCPH families.

CONCLUSION

Present study showed the plausibility of the presence of some novel region or locus involved in in this family MCPA. Moreover genome wide search is suggested further for this study.

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