Full Length Research Paper

A nonsense (c.3978G>A) abnormal spindle-like, microcephaly associated (ASPM) gene mutation is a major cause of primary microcephaly in Pashtoon ethnic group of Pakistan

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Primary microcephaly (MCPH) is an autosomal-recessive congenital disorder characterized by smaller-than-normal brain size and mental retardation. MCPH is genetically heterogeneous with six known loci: MCPH1 to MCPH7. The abnormal spindle-like, microcephaly associated (ASPM) gene at MCPH5 locus, which accounts for 37 to 54% of MCPH, appears to be the most common cause of microcephaly. More than 50% of the MCPH families genetically analyzed in Pakistan were mapped to MCPH5 locus including both families in this study. On mutation screening of ASPM gene by PCR amplification and direct DNA sequencing, a common c.3978G>A transition was identified in exon 17 of ASPM gene to be responsible for diseased phenotype in both families. This change results to the substitution of an amino acid residue at position 1326 from tryptophan to a stop codon (p.Trp1326Stop). The same mutation was also identified in several other families of Pakistani origin. Since the disease is both clinically and genetically heterogeneous, the diagnosis of MCPH1–7 is based on clinical findings; brain imaging that shows reduced brain volume with grossly normal architecture, family history consistent with autosomal recessive inheritance and molecular genetic testing when available. The mapping of large number of families to MCPH5 locus and identification of a common mutation, that is, c. 3978A>G of ASPM gene will enable us to formulate future strategies to control and prevent the disease by genetic counseling, prenatal/postnatal diagnosis and carrier testing.

Key words: Primary microcephaly (MCPH), abnormal spindle-like, microcephaly associated (ASPM) mutations, microcephaly, Pakistani families.

INTRODUCTION

Autosomal recessive primary microcephaly (MCPH, MIM 251200) is clinically described as a congenital neurological disorder in which the affected individual have head circumference at least three standard deviations (SDs) below the expected mean for age and sex and mild-to-severe mental retardation. The incidence of MCPH is approximately 1 in 10,000 individuals in Pakistan and 1 in 1,000,000 in the Caucasian population (Woods et al., 2005). MCPH is more common in populations where consanguineous are practiced more frequently (Nicholas et al., 2009; Kousar et al., 2010).

MCPH is a genetically heterogeneous disorder and seven loci (MCPH1–MCPH7) have been mapped to date. Six loci out of seven reported (MCPH1 to MCPH3, MCPH5 and MCPH6) have been identified in families of northern Pakistani origin (Woods et al., 2005) and only a single locus, MCPH4 was reported to be identified in the
Table 1. A summary of clinical findings of affected members of MCPH families.

<table>
<thead>
<tr>
<th>Pedigree code</th>
<th>Age (year)</th>
<th>Head circumference (cm)</th>
<th>Clinical finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1MIC003</td>
<td>19</td>
<td>44</td>
<td>Sloping forehead; Mild mental retardation</td>
</tr>
<tr>
<td>1MIC006</td>
<td>15</td>
<td>44.5</td>
<td>Sloping forehead, rare seizures; Moderate mental retardation</td>
</tr>
<tr>
<td>1MIC008</td>
<td>08</td>
<td>42</td>
<td>Narrow forehead; Moderate mental retardation</td>
</tr>
<tr>
<td>1MIC010</td>
<td>03</td>
<td>43.5</td>
<td>Sloping forehead; Mild mental retardation</td>
</tr>
<tr>
<td>2MIC004</td>
<td>29</td>
<td>42.5</td>
<td>Normal</td>
</tr>
<tr>
<td>2MIC006</td>
<td>26</td>
<td>46</td>
<td>Sloping forehead; Moderate mental retardation</td>
</tr>
</tbody>
</table>

Figure 1. Photographs of few primary microcephaly patients of MCPH Pakistani families.

Moroccan population. Five genes for the 7 known loci are reported to date and they include MICROCEPHALIN at MCPH1, CDK5RAP2 at MCPH3, ASPM (abnormal spindle-like, microcephaly associated) at MCPH5, CENPJ at MCPH6 and STIL at MCPH7. In the Pakistani population, autosomal recessive primary microcephaly is quite frequent and mutations of ASPM gene seem to be the most common cause of autosomal recessive primary microcephaly. About 71 distinct MCPH associated ASPM mutations are reported to date (Nicholas et al., 2009; Kousar et al., 2010). In families of northern Pakistan with primary microcephaly, 43% of them had mutations in ASPM gene (Roberts et al., 1999, 2002). The identification of a large number of recessive ASPM gene mutations in Pakistani families with MCPH probably reflects the effects of consanguinity.

In this study, the MCPH5 locus was mapped in two families with primary microcephaly from Khyber Paktoonkhwa, Pakistan. A common c.3978A>G mutation was identified in exon 17 of ASPM gene to be responsible for disease phenotype in the both families. This change results to the substitution of amino acid residue at position 1326 from tryptophan to a stop codon (p.Trp1326Stop). This mutation was found to segregate within both MCPH families. The mutation identified in these families was also observed in several other families to be responsible for MCPH in northern region of Pakistan (Nicholas et al., 2009). The mapping of large number of families to MCPH5 locus and identification of a common mutation, that is, c. 3978A>G of ASPM gene will enable us to formulate future strategies to control and prevent the disease by genetic counseling, prenatal/postnatal diagnosis and carrier testing.

MATERIALS AND METHODS

Sample collection and DNA preparation

Blood samples from affected individuals, their parents and clinically normal siblings of both families were collected with informed consent. Before sample collection, pedigree was drawn and detailed clinical examination was performed as summarized in Table 1. It was observed that microcephaly is present at birth in all affected individuals (Figure 1). Affected individuals have mild to moderate degree of mental retardation. However, no other neurological findings were observed in any of the affected individual. Genomic DNA was extracted from peripheral blood by following the standard phenol-chloroform extraction procedure (Maniatis et al., 1982).
analyzed by 2 to 2.5% agarose gel. PCR products were then
confirmed by bi-directional sequencing and analyzed by using CLC
sequencing kit in an ABI 3130 genetic analyzer (Applied
U.K.) and sequenced directly using Big Dye® Terminator v3.1 cycle
purified using QIAquick PCR Purification Kit (Qiagen, Crawley,
45 s, primer specific annealing temperature (55 to 65° C) for 45 s
conditions were 95° C for 5 min, followed by 35 cycl es of 95° C for
thermal cycler (Hybaid, Basingstoke, U.K.). The amplification
polymerase chain reaction (PCR). Each PCR reaction was
using microsatellite marker for the known microceph aly loci (Table
2). The microsatellite markers for each locus were amplified by
polymerase chain reaction (PCR). Each PCR reaction was
performed in a 10 µl volume, containing 1.5 mM MgCl2, 0.6 µM of
each forward and reverse primer, 0.2 mM dNTPs, 1U Taq DNA
polymerase and PCR buffer [16 mM (NH4)2SO4, 67 mM Tris-HCl
(pH 8.8), and 0.01% of the nonionic detergent Tween-20] (Bio-line,
London, UK). Amplification was performed with an initial
denaturation for 4 min at 94°C, followed by 35 cycles of
denaturation at 94°C for 35 s, annealing at 55°C for 35 s, extension
at 72°C for 35 s and a final extension at 72°C for 7 min. The PCR
products were separated on 10% non-denaturing polyacrylamide
gels (Protogel; National Diagnostics, Edinburgh, Scotland, UK). The
gel was stained with ethidium bromide and photographed under UV
illumination. Alleles were assigned to individuals and genotypic data
was used to find genotypes of all individuals of both family
members. The phenotype was analyzed as an autosomal recessive
trait.

ASPM gene mutation screening
Polymerase chain reaction (PCR) amplification of twenty eight (28)
exons of ASPM gene was performed with intronic forward and
reverse primers spanning the whole exonic regions. PCR
amplification was performed in a 50 µl reaction volume containing
250 ng of genomic DNA, amplification buffer containing 600 nM of
each primer, 1.5 mM MgCl2, 200 mM of dNTPs and 2.5 U of Taq
DNA polymerase (Applied Biosystems, Warrington, U.K.) in an PxE
thermal cycler (Hybaid, Basingstoke, U.K.). The amplification
conditions were 95°C for 5 min, followed by 35 cycles of 95°C for
45 s, primer specific annealing temperature (55 to 65°C) for 45 s
and 72°C for 45 s. Aliquots (5 µl) of the PCR products were
analyzed by 2 to 2.5% agarose gel. PCR products were then
purified using QiAquick PCR Purification Kit (Qiagen, Crawley,
U.K.) and sequenced directly using Big Dye® Terminator v3.1 cycle
sequencing kit in an ABI 3130 genetic analyzer (Applied
Biosystems, Foster City, CA, USA). Potential mutations were
confirmed by bi-directional sequencing and analyzed by using CLC
viewer software (www.clcbio.com).

RESULTS AND DISCUSSION
Both Pakistani families with MCPH were mapped to
MCPH5 locus on chromosome 1q31 (Figure 2A and B),
which harbors ASPM gene. We have screened both
families for mutations of the ASPM gene. A common
homozygous G>A pathogenic mutation (c.3978G>A) in
exon 17 of the ASPM gene was found in all the affected
individuals of the both families (Figure 3). The change
was identified in homozygous condition only in the
patients and the normal parents were carrier for the
change. The CLC sequence viewer software predicted
the substitution of amino acid residue at position 1326 of
ASPM gene protein product from tryptophan to a stop
codon (p.Trp1326Stop). It resulted to a truncated protein
product of 1325 amino acids, instead of normal 3477
amino acid protein.

The genetic disorders that are strongly associated with
consanguinity are inherited as an autosomal recessive
trait (Hamamy et al., 2007). In Pakistan, consanguineous
marriages are common and 60% of marriages are
reported to be within families and approximately 50% of
marriages are practiced between first cousins (Hussain
and Bittles, 1998). Autosomal recessive primary
microcephaly show considerable locus heterogeneity and
may emerge worldwide in a population as the prevalence
of a deleterious gene or when degree of consanguinity
increases. Molecular genetic analysis of MCPH in
consanguineous families has been instrumental for
mapping disease loci and for identification of causative
genes and mutations.

In this study, we reported a consanguineous family and
a non-consanguineous family from Karak district of
NWFP in Pakistan with autosomal recessive
microcephaly. Khattak tribe lives in Karak district, and the
traditional system of marriage within family/tribe results in
high rate of consanguineous marriages. Analysis of
pedigree is strongly suggestive of autosomal recessive
mode of inheritance and marriage within family/tribe
could account for all affected individuals being
homozygous for the abnormal allele. A common
homozygous missense mutation in the both Pakistani
families is also indicative of an autosomal recessive
inheritance of MCPH either due to deleterious gene or
consanguinity.

The identification of common mutation in ASPM gene in
families with primary microcephaly analyzed in this study
and several other families of same ethnic group, will not
only help to educate people about the anticipated genetic
consequences and genetic counseling but will also help
the patients in prenatal diagnosis, postnatal diagnosis

Table 2. Known loci and list of STR markers used for genotyping in Pakistani families.

<table>
<thead>
<tr>
<th>Locus name</th>
<th>Chromosome</th>
<th>Gene</th>
<th>STR marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCPH1</td>
<td>8p22-pter</td>
<td>Microcephaly</td>
<td>D8S264, D8S1099, D8S277 and D8S1130</td>
</tr>
<tr>
<td>MCPH2</td>
<td>19q13.1-13.2</td>
<td>Unknown</td>
<td>D19S433, D19S178, D19S246, D19S589 and D19S254</td>
</tr>
<tr>
<td>MCPH3</td>
<td>9q34</td>
<td>CDK5RAP2</td>
<td>D9S934, D9S282 and D9S915</td>
</tr>
<tr>
<td>MCPH4</td>
<td>15q15-q21</td>
<td>Unknown</td>
<td>ACTC, D15S659 and D15S643</td>
</tr>
<tr>
<td>MCPH5</td>
<td>1q31</td>
<td>ASPM</td>
<td>D1SS58, D1S1660, D1S1678, D1S1663, D1S2141 and D1S549</td>
</tr>
<tr>
<td>MCPH6</td>
<td>13q12.2</td>
<td>CENPG</td>
<td>D13S5787 and D13S1493</td>
</tr>
<tr>
<td>MCPH7</td>
<td>1p32.3-p33</td>
<td>STIL</td>
<td>D1S2134, D1S1661 and D1S2652</td>
</tr>
</tbody>
</table>
Figure 2. Pedigrees of 1MIC and 2MIC Pakistani families with STR genotyping data for MCPH5 locus on chromosome 1q31.3. Filled circles and squares represent affected females and males, respectively. Double lines between symbols are representatives of consanguineous marriages.

Figure 3. DNA sequence analysis of the ASPM gene in 2 families (1 and 2MIC) with microcephaly. DNA sequencing analysis revealed a homozygous G>A pathogenic mutation (c.1326G>A) in exon 17 of the ASPM gene in all the affected individuals of the both families. The upper electropherogram represents the sequence in the carrier individual, while the lower electropherogram represents the sequence in the affected individual. Arrows indicate the site of mutation.
and carrier testing to reduce the prevalence of MCPH in a particular ethnic group of Pakistan.

REFERENCES


Roberts E; Jackson AP; Carradice AC; Deeble VJ; Mannon J; Rashid Y; Jafri, H; McHale DP; Markham AF; Lench NJ; Woods CG (1999). The second locus for autosomal recessive primary microcephaly (MCPH2) maps to chromosome 19q13.1–13.2. Eur. J. Hum. Genet., 7:815-8201.