Letter to the Editor

Investigation of primary microcephaly in Bushehr province of Iran: novel STIL and ASPM mutations

To the Editor:

Microcephaly is a condition which is defined by head circumference 3 SDs below the age- and sex-matched mean because of the reduced brain size (1, 2). Affected individuals suffer intellectual disability (ID) from mild to severe with any abnormalities or dysmorphic features. (1, 2).

So far, eight autosomal recessive primary microcephaly (MCPH) loci (MCPH1–MCPH8) have been mapped and MCPH genes have been identified for seven of them (3, 4).

This study was designed to identify the genetic causes of primary microcephaly in Bushehr province, southern Iran, where consanguineous marriage is common.

In total, samples from 14 families with two or more autosomal recessive ID patients associated with primary microcephaly after obtaining informed consent form have been collected.

Genomic DNA was extracted from peripheral blood samples by a standard salting out method. The standard 450G-band karyotyping for MCPH patients was

Fig. 1. The families linked to the autosomal recessive primary microcephaly 5 (MCPH5) and MCPH7 loci. (a, c) Pedigrees of the families with primary microcephaly. (b) Sequencing chromatograms of exon 18 of ASPM gene from this family, indicating a C to T transition at nucleotide position 4849 (c.4849C>T). (d) Sequencing chromatogram of the STIL gene from this family, indicating a T to G change (c.2392T>G). Arrows show the position of nucleotide change.
performed in order to exclude chromosomal aberration as the cause of microcephaly in these families (1).

Consanguineous marriage was observed in 10 families (71.4%); the degree of microcephaly in the investigated patients ranged from 3 to 13 SD below the age and sex-related mean of populations. Among the total cases, four families (28.6%) were associated with additional features, such as syndactyly, short stature, strabismus, seizure, spasticity, autism, and long toes (Table 1).

All families were genotyped by using a panel of 70 microsatellite markers for seven MCPH loci (1). Two families (14.3%) showed linkage to two MCPH loci; one to MCPH5 [abnormal spindle-like microcephaly associated (ASPM)] and the other to MCPH7 (STIL). Both families had consanguineous marriage and severity of ID varied from mild to severe in MCPH patients. None of the remaining families showed homozygosity for seven MCPH loci.

Subsequent Sanger sequencing revealed two novel variants in two families. Direct DNA sequencing analysis of ASPM gene showed a homozygous C to T transition at nucleotide position 4849 (c.4849C>T) which leads to a truncating mutation at codon position 1617(R1617X) in exon 18 (Fig. 1). The parents and one of the healthy sibs were heterozygous for this variant.

Sequencing of the 18 exons and splice-junction of STIL gene showed a homozygous T to G change at nucleotide position 2392 (c.2392T>G) in exon 14. This variant results in a missense mutation at codon position 798 (L798W). Unaffected individuals in all three branches (B-1, B-2, and B-3) were heterozygous for this change (Fig. 1). These two changes were not found in 100 Iranian controls.

Mutations in ASPM gene at MCPH5 locus are responsible for majority of autosomal recessive primary microcephaly families accounting for up to 40% in both consanguineous and non-consanguineous families (5). ASPM protein is composed of an N-terminal microtubule-binding domain, a calponin-homology domain, 81 isoleucine–glutamine (IQ) motifs, and a carboxy terminal region with no characterized domains. The number of IQ domains is conserved among different organisms and its number increase with organism’s complexity. So, it has been suggested that it has a role in cerebral cortex size (3, 5).

The reported nonsense mutation in this study was detected in the IQ repeat motif of the ASPM which may disrupt the interaction of ASPM with calmodulin and calmodulin-related proteins (1), and this may be the cause of primary microcephaly and ID in these patients.

STIL has three amino acid residues for phosphorylation and a PIN1 binding site (WW domain). The protein is phosphorylated in mitosis and then interacts with PIN1, a regulator of mitosis and activation of the spindle checkpoint (6).

In this study, the missense variant (c.2392T>G) converts the conserved basic amino acid lysine to a neutral tryptphan (L798W). This substitution is a deleterious mutation and may disrupt the WW domain as well as other parts of the STIL protein (http://bioinf.cs.ucl.ac.uk/psipred/ and http://sift.jcvi.org/www/SIFT_BLink_submit.html).

Only two families had mutations in two known MCPH genes which indicates that further studies on molecular causes of primary microcephaly are needed for the remaining families.

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