CLINICAL GENETICS An International Journal of Genetics, Molecular and Personalized Medicine

Clin Genet 2012
Printed in Singapore. All rights reserved

CLINICAL GENETICS
doi: 10.1111/j.1399-0004.2012.01949.x

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

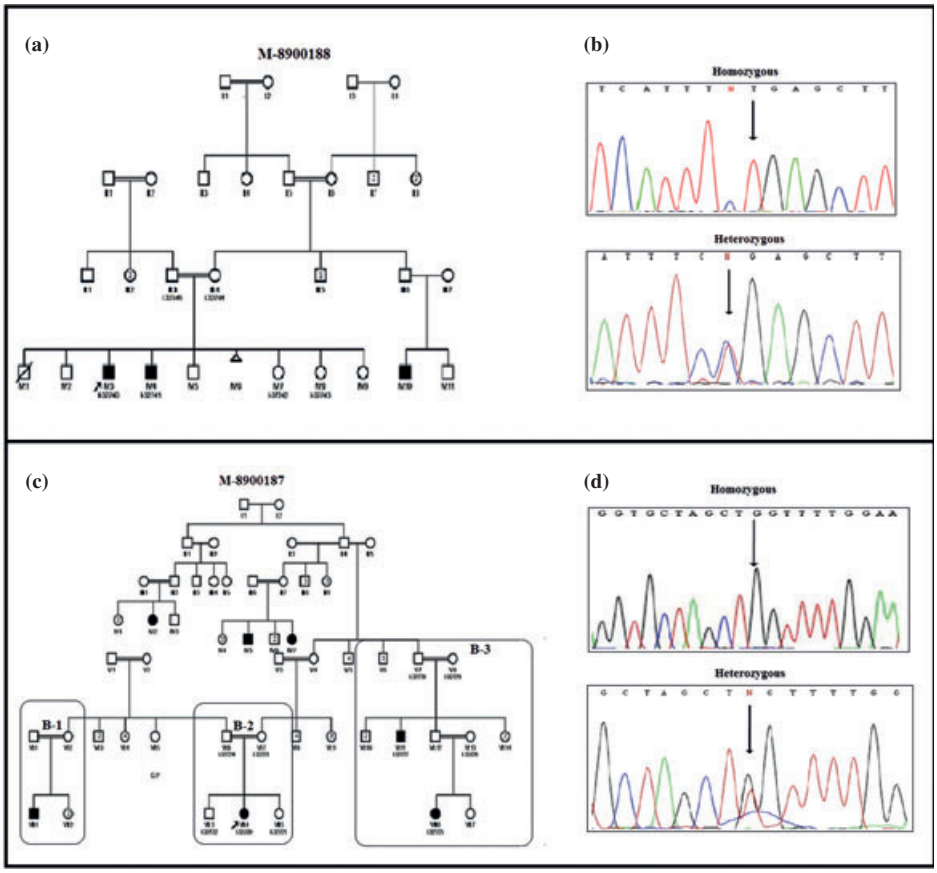

Fig. 1. The families linked to the autosomal recessive primary microcephaly 5 (MCPH5) and MCPH7 loci. (**a, c**) Pedigrees of the families with primary microcephaly. (**b**) Sequencing chromatograms of exon 18 of *ASPM* gene from this family, indicating a C to T transition at nucleotide position 4849 (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.

Table 1. Results of clinical examination and linkage analysis in 14 MCPH families

Number	Family	Affected number	Intellectual disability severity	Degree of consanguinity	Linkage results	Additional feature
1	M-8900117	2	Moderate	4	Unlinked	Long toes in one affected individual
2	M-8900185	3	Mild–moderate	5	Unlinked	Not present
3	M-8900186	4	Severe	5	Unlinked	Syndactyly, short stature, strabismus in one affected individual
4	M-8900187	7	Mild	5	MCPH7	Not present
5	M-8900188	3	Moderate–severe	5	MCPH5	Not present
6	M-8900189	3	Severe	5	Unlinked	Not present
7	M-8900199	7	Mild–severe	5	Unlinked	Not present
8	M-8900213	4	Moderate	–	Unlinked	Not present
9	M-8900219	4	Mild–severe	–	Unlinked	Not present
10	M-8900225	3	Severe	5	Unlinked	Seizure, spasticity, autism in one affected individual
11	M-8900226	3	Mild–moderate	–	Unlinked	Not present
12	M-8900229	2	Moderate	5	Unlinked	Not present
13	M-8900235	2	Severe	–	Unlinked	Strabismus in 2 affected individual
14	M-8900241	4	Moderate	5	Unlinked	Not present

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 tryptophan (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.

Acknowledgements

We are grateful to all affected individuals and their families for their participation in the study. We would like to have special thanks to Mr. Gargori, the head of Bushehr Social Welfare and Rehabilitation Organization, for his help to communicate with patient families. We

also thank Mrs. Susan Banihashimi, Mrs. Sanaz Arghanghi and our team at the Genetics Research Center. This work was supported by the EU-FP7 project GENCODYS.

E Papari[a]
M Bastami[a]
A Farhadi[b]
SS Abedini[a]
M Hosseini[a]
I Bahman[a]
M Mohseni[a]
M Garshasbi[a,c]
L Abbasi Moheb[a,c]
F Behjati[a]
K Kahrizi[a]
H-H Ropers[c]
H Najmabadi[a]

[a]Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, [b]Bushehr Social Welfare and Rehabilitation Organization, Bushehr, Iran, and [c]Department of Human Molecular Genetics, Max Planck Institute for Molecular Genetics, Berlin, Germany

References

1. Darvish H, Esmaeeli-Nieh S, Monajemi G et al. A clinical and mole cular genetic study of 112 Iranian families with primary microcephaly. J Med Genet 2010: 47 (12): 823–831.
2. Thornton GK, Woods CG. Primary microcephaly: do all roads lead to Rome? Trends Genet 2009: 25 (11): 501–511.
3. Mahmood S, Ahmad W, Hassan MJ. Autosomal recessive primary microcephaly (MCPH): clinical manifestations, genetic heterogeneity and mutation continuum. Orphanet J Rare Dis 2011: 6 (1): 39.
4. Marchal JA, Ghani M, Schindler D et al. Misregulation of mitotic chromosome segregation in a new type of autosomal recessive primary microcephaly. Cell Cycle 2011: 10 (17): 2967–2977.
5. Nicholas AK, Swanson EA, Cox JJ et al. The molecular landscape of ASPM mutations in primary microcephaly. J Med Genet 2009: 46 (4): 249–253.
6. Kumar A, Girimaji SC, Duvvari MR et al. Mutations in STIL, encoding a pericentriolar and centrosomal protein, cause primary microcephaly. Am J Hum Genet 2009: 84 (2): 286–290.

Correspondence:
Hossein Najmabadi, PhD
National Reference Laboratory for Prenatal Diagnosis
Genetics Research Center, University of Social Welfare and Rehabilitation Sciences
Koodakyar Ave., Evin, Tehran 1985713834, Iran
Tel/Fax: +98 21 22180138
e-mail: hnajm12@yahoo.com