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A Novel Deletion Mutation in ASPM Gene in an Iranian Family with Autosomal Recessive Primary Microcephaly

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Abstract

Objective

Autosomal recessive primary microcephaly (MCPH) is a neurodevelopmental and genetically heterogeneous disorder with decreased head circumference due to the abnormality in fetal brain growth. To date, nine loci and nine genes responsible for the situation have been identified. Mutations in the ASPM gene (MCPH5) is the most common cause of MCPH. The ASPM gene with 28 exons is essential for normal mitotic spindle function in embryonic neuroblasts.

Materials & Methods

We have ascertained twenty-two consanguineous families with intellectual disability and different ethnic backgrounds from Iran. Ten out of twenty-two families showed primary microcephaly in clinical examination. We investigated MCPH5 locus using homozygosity mapping by microsatellite marker.

Result

Sequence analysis of exon 8 revealed a deletion of nucleotide (T) in donor site of splicing site of ASPM in one family. The remaining nine families were not linked to any of the known loci. More investigation will be needed to detect the causative defect in these families.

Conclusion

We detected a novel mutation in the donor splicing site of exon 8 of the ASPM gene. This deletion mutation can alter the ASPM transcript leading to functional impairment of the gene product.

Keywords: Autosomal Recessive Primary Microcephaly; ASPM; MCPH5; Homozygosity Mapping

Introduction

Autosomal recessive primary microcephaly (MCPH; MIM 251200) is clinically characterized by an occipitofrontal head circumference (OFC) of at least three standard deviations (SDs) below the expected mean for age and sex(1-3). The disease is etiologically heterogeneous and environmental and genetic causes have both been identified as responsible causes of it. Environmental causes of MCPH are congenital infections, maternal alcohol consumption, and drug consumption during pregnancy (4,5). The major causes of microcephaly are genetic mechanisms including cytogenetic abnormalities and single gene disorders (5). Microcephaly is divided into primary (present at birth) and secondary microcephaly.
The birth prevalence of primary microcephaly varies from 1.3 to 150 per 100000 live births depending on the population. The birth prevalence of primary nonsyndromal microcephaly is 1:30000 to 1:250000 live births. Until 2010, MCPH was reported in about 100 families worldwide; but, Darvish and colleagues investigated seven MCPH loci in patients with primary microcephaly from 112 consanguineous Iranian families. To date, nine MCPH loci and nine genes have been identified to be responsible for the situation. These genes include Microcephalin at MCPH1, WDR62 at MCPH2, CDK5RAP2 at MCPH3, CASC5 at MCPH4, ASPM at MCPH5, CENPJ at MCPH6, STIL/SIL at MCPH7, CEP135 at MCPH8, and CEP152 at MCPH9. Different mutations have been identified for these loci in Iran and different parts of the world. We have ascertained twenty-two consanguineous families with intellectual disability and different ethnic backgrounds from Iran. Ten out of twenty-two families showed primary microcephaly in clinical examination.

Materials & Methods
Clinical examination
We evaluated 22 families with two or more intellectual disability patients referred to the Genetics Research Center, Tehran, Iran. These families were from various ethnicities and different provinces of Iran. Informed consents were taken from the family members who participated in this study. Ten out of twenty-two families showed primary microcephaly. Consanguinity was observed in 7 out of these 10 families. On examination, head circumferences were -3 to -13 SD below the population age and sex-related mean values. All parents had normal intelligence scores and normal head circumferences (Table 2).

DNA Extraction and Genotyping
DNA was extracted from peripheral blood lymphocytes following a standard protocol. A panel of 70 microsatellite markers was selected from the Genome Databases (http://www.gdb.org/ and http://genome.ucsc.edu/). Population-specific allele frequencies were available for the Iranian population, because of previous studies in Iran. polymerase chain reaction (PCR) amplification of the microsatellite markers are performed (Table 3). Polyacrylamide gel electrophoresis and standard silver stain protocol were used to visualize the PCR products.

Sequencing of ASPM
All 28 exons, and exon/intron splice sites of the ASPM gene (National Center for Biotechnology Information GenBank Accession Number AF509326), of the family linked to the MCPH5 locus on chromosome 1q31, were sequenced using a set of 33 PCR primers (designed with the Primer3 software). Sequences were compared with the reference genomic and cDNA sequence (NM_018136).

Results
Clinical Findings
The consanguineous Iranian family with primary MCPH (9000013) had two affected subjects, including two males (Fig. 1) with ages varying between 14 and 27 years. Head circumference of the two affected individuals was 10 to 13 SD below the expected mean for sex and age and they (V: 1 and V:2) had severe intellectual disability (intelligence quotient [IQ] of 30 to 35). They were unable to read or write and did not have basic self-care skills. With the exception of intellectual disability, there were no other neurological and motor development problems. The parents had normal head circumference and normal intelligence (Table 4).

Genotyping and Mutation Analysis
Linkage was performed using STR markers present within the known MCPH loci. Linkage of a family to MCPH locus was based on the observation that all affected individuals had the same homozygous pattern. One out of ten families showed homozygosity at MCPH5 locus (family [9000013]). Sequence analysis of exon 8 in 2 affected individuals (V: 1, V:2) and in parents (IV: 1, IV:2) revealed a deletion of nucleotide (T) in donor site of splicing site (Fig. 2). This deletion mutation was present in heterozygous state in the parents. We did not find any linkage for the remaining nine families with primary microcephaly.
A Novel Deletion Mutation in ASPM Gene in an Iranian Family with Autosomal Recessive Primary Microcephaly

Discussion

The MCPH5 has been shown to be the most prevalent MCPH loci in Iran with a frequency of 13.3% (2). It was also the most common locus among microcephal populations in Pakistan (accounting for 43% to 86% of the loci) (21), and India (33.5% of the loci) (7, 22).

In this study, one family showed linkage to MCPH5 (ASPM). We detected a novel mutation in the donor splicing site of exon 8 of the ASPM gene. This deletion mutation can alter the ASPM transcript leading to functional impairment of the gene product.

The ASPM gene with 28 exons is the human ortholog of the Drosophila melanogaster ‘abnormal spindle’ gene (asp), which is essential for normal mitotic spindle function in embryonic neuroblasts (15, 23) (Fig.3). ASPM was highly expressed in fetal and adult human tissues with lower levels in adult tissues. The predicted full-length protein contains 3477 amino acids and has a calculated molecular mass of 410 kD. ASPM contains two conserved regions, ASPM N-proximal (ASNP) repeats, and C-terminal calmodulin-binding IQ motifs with variable length. Immuno-staining of cultured human cells revealed that ASPM was localized in the spindle poles during mitosis (24). ASPM gene is essential for symmetric proliferative division of neuroepithelial cells during brain development (16). Postnatally, ASPM expression decreases neurogenesis and upregulation of gliogenesis in the cortex. This expression pattern shows that ASPM is involved in neuron rather than glia production (25). The previously published ASPM mutations comprise deletions of 1–7 base pairs, nonsense mutations, a breakpoint translocation, and intronic splice-donor site mutations (7, 25, 26).

The remaining nine microcephalic families were not linked to any of the known loci. These families will help to refine the mapping of the other MCPH loci or the genes which have not been identified.

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Patient consent: Obtained.

Ethics approval: This study was conducted with the approval of the University of Social Welfare and Rehabilitation Sciences.

Table 1. A Review of The Previous Studies On Loci For Autosomal Recessive Primary Microcephaly

| Locus   | Genomic region | Gene             | Ethnicity                                      | Reference |
|---------|----------------|------------------|-----------------------------------------------|-----------|
| MCPH1   | 8p22-pter      | Microcephalin    | Northern Pakistani, Iranian                   | (27, 28)  |
| MCPH2   | 19q13.1e13.2   | WDR62            | Northern Pakistani, Indian, Pakistani         | (9)       |
| MCPH3   | 9q34           | CDK5RAP2         | Northern Pakistani                            | (29, 30)  |
| MCPH4   | 15q14          | CASC5            | Moroccan, Canada                              | (14)      |
| MCPH5   | 1q31           | ASPM             | Northern Pakistani, Turkish, Jordanian Dutch, | (15, 31)  |
|         |                |                  | Saudi Arabian, Yemeni, Indian                 |           |
| MCPH6   | 13q12.2        | CENPJ            | Northern Pakistani, Brazilian, Pakistani     | (17, 30)  |
| MCPH7   | 1p32.3ep33     | STIL             | Indian                                        | (18)      |
| MCPH8   | 4q12           | CEP135           | Northern Pakistani                            | (19)      |
| MCPH9   | 15q21.1        | CEP152           | Moroccan, Pakistani                           | (20, 31)  |
Table 2. Microcephalic Families

| Number | Family     | Linkage results | Affected number | Additional feature |
|--------|------------|-----------------|-----------------|-------------------|
| 1      | 9000003    | Unlinked        | 2               | -                 |
| 2      | 9000007    | Unlinked        | 3               | -                 |
| 3      | 9000013    | MCPH5           | 2               | -                 |
| 4      | 9000017    | Unlinked        | 3               | -                 |
| 5      | 9000018    | Unlinked        | 2               | -                 |
| 6      | 9000039    | Unlinked        | 2               | -                 |
| 7      | 9000056    | Unlinked        | 3               | -                 |
| 8      | 9000120    | Unlinked        | 2               | -                 |
| 9      | 9000140    | Unlinked        | 3               | -                 |
| 10     | 9000141    | Unlinked        | 2               | -                 |

Table 3. Standard Procedures Of PCR In A Total Volume Of 30 Ul And Thermal Cycling Conditions.

| Cycle step        | Temp   | Time  | Cycle number |
|-------------------|--------|-------|--------------|
| Denaturation      | 95°C   | 5 min | 1            |
| Denaturation      | 94°C   | 40 sec|              |
| Annealing         | Tm     | 30 sec|              |
| Extension         | 72°C   | 40 sec|              |
|                   |        |       | 30           |
| Final extension   | 72°C   | 2 min | 1            |
|                   | 4°C    | hold  |              |

Table 4. Clinical Features of The Microcephalic Family Linked To MCPH5 Locus.

| Patient | Sex  | Age (yrs) | Severity of Intellectual Disability | Height (Cm) | OFC (Cm) | Other features |
|---------|------|-----------|-------------------------------------|-------------|---------|----------------|
| V :1    | Male | 14        | Severe                              | 143         | -13SD(41cm)| -              |
| V :2    | Male | 27        | Severe                              | 153         | -10SD(45cm)| -              |
Fig 1. Pedigree of family (9000013) with novel mutations in the ASPM gene; affected males are indicated by filled squares.

Fig 2. Sequence analysis of the ASPM gene in family (9000013); the upper panel represents the sequence in parents, while the lower panel represents the sequence in the affected individuals.
Fig 3. Diagrammatic representation of the exon/intron structure of the ASPM gene according to Bond et al’s study (15)

References

1. Pattison L, Crow YJ, Deeble VJ, Jackson AP, Jafri H, Rashid Y, et al. A Fifth Locus for Primary Autosomal Recessive Microcephaly Maps to Chromosome 1q31. Am J Hum Genet 2000;67(6):1578-80.

2. Darvish H, Esmaeili-Nieh S, Monajemi G, Mohseni M, Ghasemi-Firouzabadi S, Abedini S, et al. A clinical and molecular genetic study of 112 Iranian families with primary microcephaly. Journal of Medical Genetics 2010;47(12):823-8.

3. Tolmie JL, M M, JB S, D D, JM C. Microcephaly: genetic counselling and antenatal diagnosis after the birth of an affected child. Am J Med Genet 1987;27583-94.

4. Cowie V. The genetics and sub-classification of microcephaly. J Ment Defic Res 1960;4:42-7.

5. Woods C. Human microcephaly. Curr Opin Neurobiol 2004;14(1):112-7.

6. Kaindl AM PS, Kumar P, Kraemer N, Issa L, Zwirner A, Gerard B, Verloes A MS, et al. Many roads lead to primary autosomal recessive microcephaly. Prog Neurobiol 2010;90:363-83.

7. Kumar A BS, Babu M, Markandaya M, Girimaji SC. Genetic analysis of primary microcephaly in Indian families: novel ASPM mutations. Clin Genet 2004;66:341-8.

8. Jackson AP, Eastwood H, Bell SM, Adu J, Toomes C, Carr IM, et al. Identification of microcephalin, a protein implicated in determining the size of the human brain. The American Journal of Human Genetics 2002;71(1):136-42.

9. Roberts E, Jackson AP, Carradice AC, Deeble VJ, Mannan J, Rashid Y, et al. The second locus for autosomal recessive primary microcephaly (MCPH2) maps to chromosome 19q13. 1-13.2. European journal of human genetics: EJHG 1999;7(7):815.

10. Kousar R, Hassan MJ, Khan B, Basit S, Mahmood S, Mir A, et al. Mutations in WDR62 gene in Pakistani families with autosomal recessive primary microcephaly. BMC neurology 2011;11(1):119.

11. Evans PD, Vallender EJ, Lahn BT. Molecular evolution of the brain size regulator genes CDK5RAP2 and CEPN1. Gene 2006;375:75-9.

12. Nagase T, Nakayama M, Nakajima D, Kikuno R, Ohara O. Prediction of the coding sequences of unidentified human genes. XX. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. DNA research 2001;8(2):85-95.

13. Jamieson CR GC, Abramowicz MJ. Primary autosomal recessive microcephaly: homozygosity mapping of MCPH4 to chromosome 15. Am J Hum Genet 1999;65:1465-9.

14. Genin A, Desir J, Lambert N, Biervliet M, Van Der Aa N, Pierquin G, et al. Kinetochore KMN network gene CASC5 mutated in Primary Microcephaly. Human molecular genetics 2012.

15. Bond J, Roberts E, Mochida GH, Hampshire DJ, Scott S, Askham JM, et al. ASPM is a major determinant of cerebral cortical size. Nature genetics 2002;32(2):316-20.

16. Fish JL, Kosodo Y, Enard W, Pääbo S, Huttner WB. Aspm specifically maintains symmetric proliferative
A Novel Deletion Mutation in ASPM Gene in an Iranian Family with Autosomal Recessive Primary Microcephaly

Abramowicz MJ. A translocation breakpoint disrupts the ASPM gene in a patient with primary microcephaly. European journal of Human Genetics 2004;12(5):419-21.

27. Garshasbi M, Motazacker M, Kahrizi K, Behjati F, Abedini S, Nieh S, et al. SNP array-based homozygosity mapping reveals MCPH1 deletion in family with autosomal recessive mental retardation and mild microcephaly. Hum Genet 2006 Feb;118(6):708-15.

28. Jackson A, McHale D, Campbell D, Jafri H, Rashid Y, Mannan J, et al. Primary autosomal recessive microcephaly (MCPH1) maps to chromosome 8p22-pter. Am J Hum Genet 1998 Aug;63(2):541-6.

29. Moynihan L, Jackson A, Roberts E, Karbani G, Lewis I, Corry P, et al. A third novel locus for primary autosomal recessive microcephaly maps to chromosome 9q34. Am J Hum Genet 2000 Feb;66(2):524-7.

30. Bond J, Roberts E, Springell K, Lizarraga S, Scott S, Higgins J, et al. A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size. Nat Genet. 2005 Apr;37(4):353-5. Nat Genet 2005 Apr;37(4):353-5.

31. Jamieson C, Govaerts C, Abramowicz M, J. Primary autosomal recessive microcephaly: homozygosity mapping of MCPH4 to chromosome 15. Am J Hum Genet. 1999;65:1456-9.
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