

A novel mutation in MCPH1 gene in an Iranian family with primary microcephaly

Miss Masoumeh Hosseini,¹ Seyed Hassan Tonekaboni,² Elaheh Papari,³ Idea Bahman,⁴
Farkhondeh Behjati,⁵ Kimia Kahrizi,⁶ Hossein Najmabadi⁷

Genetics Research Center, University of Social Welfare and Rehabilitation Sciences (USWR),^{1,3-7}
Neurology Department, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences (SBMU),² Tehran, Iran.
Corresponding Author: Hossein Najmabadi. Email: hnajm12@yahoo.com

Abstract

Primary microcephaly (MCPH) is a genetic disorder in which affected individuals present with a head circumference 3 standard deviations (SDs) below the age- and sex-related mean and is accompanied by mental retardation without further associated malformations. Here we report a patient with sporadic MCPH from Northwest of Iran who was investigated for MCPH1 locus. Clinical examination and karyotype analyses were performed and microsatellite based mapping was done by using flanking and intragenic short tandem repeat (STR) markers for MCPH1 locus. For these markers the affected individual was homozygote and the parents were heterozygote. According to this pattern of allele sharing and also the cytogenetic findings, mutation screening of Microcephalin gene was performed and subsequent sequencing revealed a novel mutation in Microcephalin gene.

Keywords: Primary Microcephaly, Mental retardation, Novel mutation, Iran.

Introduction

Microcephaly is a neurodevelopmental disorder

which is characterized with a head circumference 3 standard deviations (SD) below the age-related mean.¹ Microcephaly is divided in two categories: primary microcephaly (MCPH), which is present at birth, and secondary microcephaly, which develops postnatally.² The differences between these two groups is that MCPH is usually a static developmental anomaly, whereas secondary microcephaly is a progressive neurodegenerative disorder.² In primary microcephaly affected individuals have no neurologic, syndromic, or significantly dysmorphic features but have mild-to-moderate mental retardation (MR).¹

Different causes have been proposed for microcephaly including intrauterine infections, drugs taken during pregnancy, prenatal radiation exposure, maternal phenylketonuria, and birth asphyxia, but these causes are rare with the exception of birth asphyxia. Actually in most of the cases, genetic mechanisms including cytogenetic abnormalities, single-gene disorders, and undetermined etiology syndromes are the major causes of the disease.³

Autosomal recessive primary microcephaly (MCPH) is a genetically heterogeneous disorder and seven loci

(MCPH1-7) with seven corresponding genes have been identified for this disorder to date.

Microcephalin which corresponds to MCPH1, WDR62 (WD repeat-containing protein 62) at MCPH2, CDK5RAP2 (CDK5 regulatory subunit-associated protein 2) at MCPH3, CEP152 (centrosomal protein, 152-KD) at MCPH4, ASPM (abnormal spindle like microcephaly associated) at MCPH5, CENPJ (centromeric protein J) at MCPH6 and STIL corresponding to MCPH7.⁴ Mutations at any of these loci are indistinguishable clinically.⁴

A hallmark of MCPH1 mutation is detectable in routine cytogenetic analysis in which about 10-20% of the cells have prophase like chromosomes. This is caused by early onset of chromosome condensation in the early G2 of the cell cycle (so it is called premature chromosome condensation or PCC syndrome) and delayed decondensation in early G1-phase of the cell cycle.⁵

Premature chromosome condensation syndrome is a disorder which is characterized by microcephaly, short stature, and misregulated chromosome condensation.⁵

The aim of this study was to determine the molecular basis of microcephaly in a family from Northwest of Iran.

Case report

The case of a 2.4-year-old girl with developmental delay and small head circumference is presented. History didn't reveal any other family member with a similar phenotype (Figure-1).

Consent form was obtained from the parents, according to the guidelines of local ethics committee of University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.

High resolution G-banding conventional cytogenetic analysis was performed⁶ for the patient in order to exclude the possibility of chromosomal aberration as the cause of microcephaly in this family.

DNA was extracted from peripheral blood samples following a standard protocol.⁷ Microsatellite based mapping was performed by using five STR markers covering the Microcephalin gene region on chromosome 8p23.1 (D8S1099, D8S1742, D8S277, D8S561, and D8S1819). These markers and PCR amplification conditions were selected according to Darvish et al's study.⁶ The family were genotyped for microsatellite markers flanking the Microcephalin gene. Polyacrylamide gel

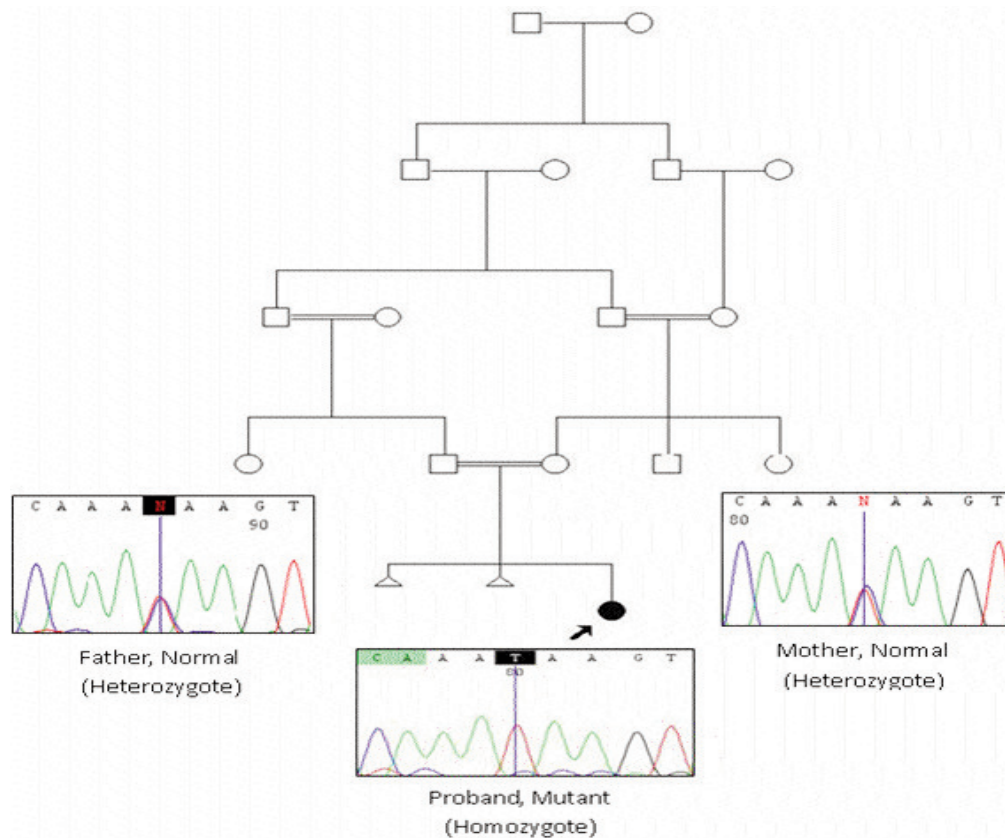


Figure-1: Pedigree structure and sequencing chromatograph of family studied, the proband shows 136C>T mutation.

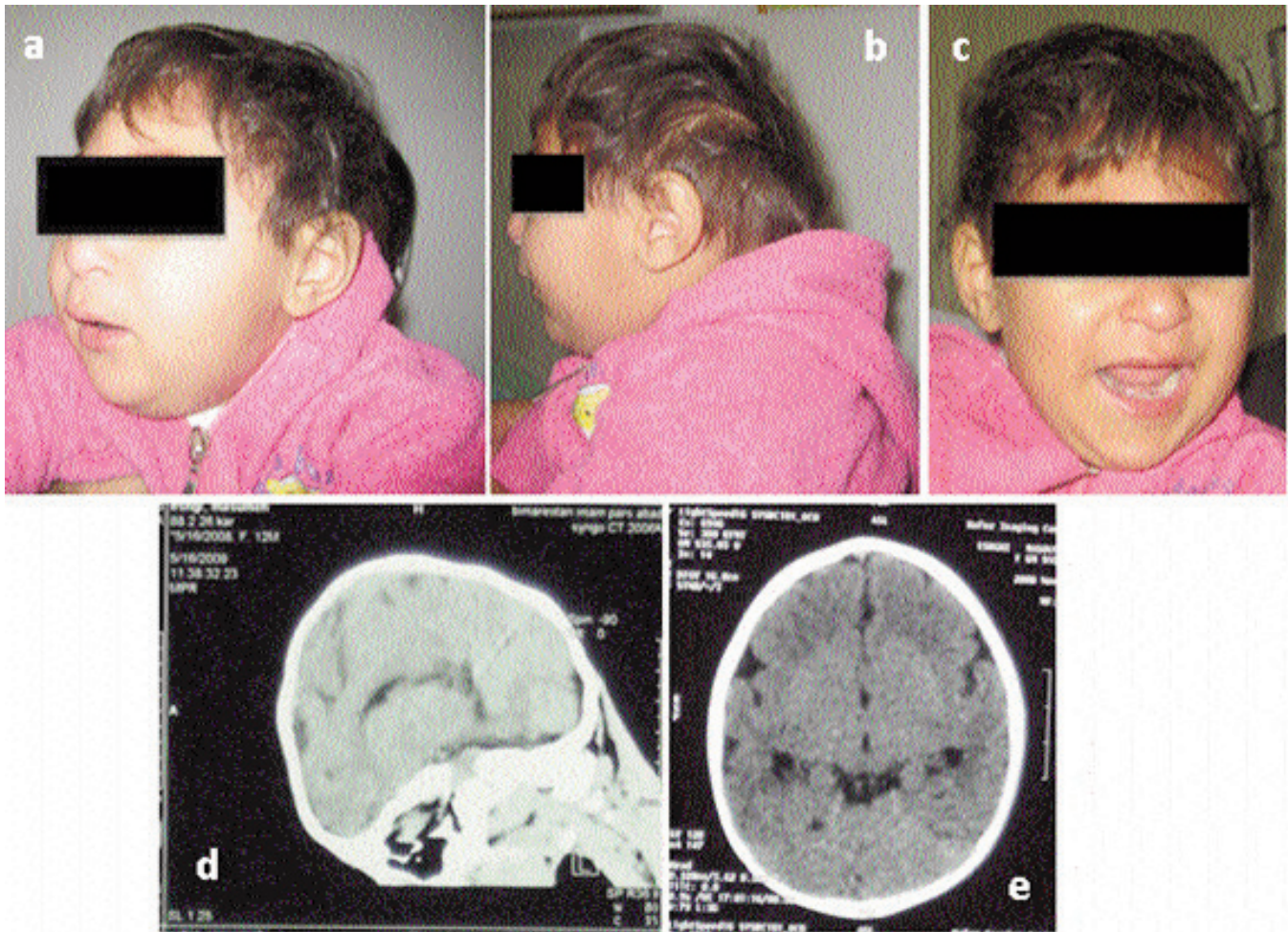


Figure-2: (a, b, c) Front and side view of proposita at 2.4 years of age. (d, e) MRI findings of proposita.

electrophoresis and a standard silver stain protocol were used to visualize the results. Based on the obtained result, mutation screening of Microcephalin gene was initiated.

After PCR amplification the amplicons were screened for mutations by sequencing using BigDye Terminator Cycle Sequencing kits (Applied Biosystems, Foster city, California, USA) and analyzed on ABI 3130 Genetic Analyzer (Applied Biosystems, Foster city, California, USA). Sequencing was performed using the same primers as for PCR amplification.⁶ Sequences were compared with the reference genomic and cDNA sequence (NM_024596).

The affected individual was sequenced for all the 14 exons and exon/intron splice junctions of Microcephalin gene and followed by identification of mutation, for the parents.

The proposita was a 2.4-year-old girl (Figure-1). The parents were first cousin originating from Northwest of Iran

with normal head circumference and appeared to have normal intelligence. There was no history of any genetic disorders including neurological disorder in the family.

The proposita was outcome of an uneventful pregnancy with a birth weight of 3200 g and length of 51 cm. No history of prenatal exposure to environmental causes of microcephaly were detected. There were no dysmorphic features with the exception of a small head circumference at birth (28 cm). There was no history of seizures.

On examination, she had a small head circumference (42 cm), less than 4 of the standard deviation (SD) for her age and sex (Figure-2 a, b, c) with developmental delay, she was not able to stand and walk, she sat at 1.5 years, and suffered from verbal skill disability with the exception of meaningless voice. Other measurements including the weight and height were normal for the age (BW: 12kg and Height: 87 cm).

Brain Magnetic Resonance Imaging (MRI) of the proposita revealed thickening of fronto-parietal and temporal gyri (pachygyria) and mild hypoplasia of corpus callosum (Figure -2d, e).

The chromosome analysis revealed a numerically normal female karyotype. However, in addition to enhanced fraction of prophase stage chromosome (PCC) the morphology of chromosomes was poor, twisted and curly. Both of these features are consistent with mutation in MCPH1 gene.⁵

In this study, microsatellite based mapping was used for MCPH1 locus. The markers were homozygote for the affected individual and heterozygote for parents. According to this pattern of allele sharing and also the cytogenetic findings, mutation screening of Microcephalin gene was performed.

Subsequent mutation screening lead to the identification of one novel nonsense mutation (c.136C>T/Q46X) in exon 3 of Microcephalin gene which leads to truncation of microcephalin.

Discussion

MCPH1 mutation is a known cause of primary microcephaly and has been reported to be associated with a short stature.⁸ Here, we report the identification of a novel nonsense mutation in a patient with microcephaly without short stature. Pachygyria which is a nonspecific finding in patients with MCPH1 mutation was also present in our patient. In this family, we could identify c.136C>T mutation which is located in the BRCT1 domain of Microcephalin gene.

Two other missense mutations have been found previously in two Iranian families (c.215C>T/ S72>L) from southeast and (c.147C>G/ H49>Q), which are also located in the BRCT1 domain of the microcephalin protein.

It is predicted that microcephalin contains three BRCA1 C-terminal (BRCT) domains.⁹ The carboxyl-terminal domain (BRCT) of the Breast Cancer Gene 1 (BRCA1) protein is conserved during evolution and exists in a large number of proteins from prokaryotes to eukaryotes.¹⁰ MCPH1 has a function in the cell cycle regulation and mitotic entry.¹⁰ Bioinformatics analysis with ConSurf Server showed that about 130 amino acids from N-terminus of microcephalin are conserved.

On the other hand the nonsense mutation in our patient, generated a termination codon in exon 3 and thus a truncated protein of 46 amino acids, encoding only the N-terminal BRCT domain of the MCPH1 protein. Thus, the most important and conserved part of microcephalin protein was deleted, which suggests that this mutation can be pathogenic in this patient.

In another Iranian family, a large deletion in exon 4 of MCPH1 has been reported in a microcephalic patient with moderate mental retardation (HC: -10 to -11 SD), leading to truncation of microcephalin with 115 amino acids, on both alleles.

Conclusion

In conclusion, we suggest that this mutation in the Microcephalin gene may account for some cases of primary microcephaly.

Acknowledgments

We are grateful to our patients and their families who kindly consented to join the study.

References

1. 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. *Am J Hum Genet* 2002; 71: 136-42.
2. Woods CG, Bond J, Enard W. Autosomal recessive primary microcephaly (MCPH): a review of clinical, molecular, and evolutionary findings. *Am J Hum Genet* 2005; 76: 717-28.
3. Jackson AP, McHale DP, Campbell DA, Jafri H, Rashid Y, Mannan J, et al. Primary autosomal recessive microcephaly (MCPH1) maps to chromosome 8p22-pter. *Am J Hum Genet* 1998; 63: 541-6.
4. Mahmood S, Ahmad W, Hassan MJ. Autosomal Recessive Primary Microcephaly (MCPH): clinical manifestations, genetic heterogeneity and mutation continuum. *Orphanet J Rare Dis* 2011; 6: 39.
5. Trimborn M, Bell SM, Felix C, Rashid Y, Jafri H, Griffiths PD, et al. Mutations in microcephalin cause aberrant regulation of chromosome condensation. *Am J Hum Genet* 2004; 75: 261-6.
6. Darvish H, Esmaceli-Nieh S, Manajemi GB, Mohseni M, Ghasemi-Firoozabadi S, et al. A clinical and molecular genetic study of 112 Iranian families with primary microcephaly. *J Med Genet* 2010; 47: 823-8.
7. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 1215.
8. Garshasbi M, Motazacker MM, Kahrizi K, Behjati F, Abedini SS, Nieh SE, et al. SNP array-based homozygosity mapping reveals MCPH1 deletion in family with autosomal recessive mental retardation and mild microcephaly. *Hum Genet* 2006; 118: 708-15.
9. Huyton T, Bates PA, Zhang X, Stemberg MJ, Freemant PS, et al. The BRCA1 C-terminal domain: structure and function. *Mutat Res* 2000; 460: 319-32.
10. Yu X, Chini CC, He M, Mer G, Chen J. The BRCT domain is a phospho-protein binding domain. *Science* 2003; 302: 639-42.