

A novel splice-site variant in the *MCPH1* gene manifests with autosomal recessive primary microcephaly

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Abstract

Autosomal recessive primary microcephaly is a rare neurodevelopmental disorder that results in severe microcephaly, reduction of brain volume, and mental retardation. Mutations in *MCPH1*, which encodes the protein microcephalin, have been detected in primary microcephaly. In this report, we describe an infant girl from a consanguineous Turkish family who is affected by autosomal recessive primary microcephaly. This patient also has craniofacial dysmorphic findings, intellectual disability, and developmental delay. Neuroimaging revealed a reduction in cranial volume and delayed myelination. We performed whole exome sequencing to find the genetic defects. A novel splice-site variant (NM_024596.5; *MCPH1*: c.321+5G > A) was identified in a homozygous state in intronic 4 of the *MCPH1* gene. The parents of the proband were heterozygous carriers for this variation. This new splice site homozygous mutation in our patient will help to establish a database of genetic variants for populations. This study also enlarges the mutation spectrum of the *MCPH1* gene and points out the importance of splice-site variants by sequencing.

Keywords: Autosomal recessive primary microcephaly, *MCPH1* gene, microcephalin, splice-site variant, whole exome sequencing.

INTRODUCTION

Autosomal recessive primary microcephaly (MCPH, OMIM251200) is a rare neurodevelopmental disorder that results in severe microcephaly at birth with a pronounced reduction in brain volume and intellectual disability. Homozygous or compound heterozygous mutations in the *MCPH1* gene are known to cause MCPH. The *MCPH1* gene is located at chromosome 8p23 and consists of 14 exons that encode 835 amino acids.¹ *MCPH1* functional domains bind to condensin II, TopBP1, Chk1 and three BRCA1 C-terminus (BRCT) domains, which are frequently found in proteins involved in DNA damage response and cell cycle control. The *MCPH1* gene has been found to have frame shift, nonsense, missense, and splice site mutations, all of which result in the loss of MCPH1 protein.²⁻⁶ Several mouse models with *MCPH1* mutations showed a microcephaly phenotype with a thinner neocortex, a reduction of the neuroprogenitor pool and premature neuronal differentiation during brain development.⁴ Here we present an infant girl from a consanguineous Turkish family affected by microcephaly and neurodevelopmental

delay. A novel splice-site homozygous mutation (c.321+5G > A) has been detected in intron 4 of the *MCPH1* gene by whole exome sequencing.

CASE REPORT

The patient was a 2-year-old female who was the second child of Turkish consanguineous parents. She was referred to our outpatient clinic because of microcephaly and developmental delay. Prenatally, microcephaly and oligohidramnios were noted on ultrasonography. The history of pregnancy was negative for potential environmental factors such as alcohol exposure and infectious diseases. The blood tests for TORCH infection and plasma and urine amino acids were normal. The patient was born by cesarean section at 38 weeks of gestation. The patient's head circumference (occipitofrontal circumference) was measured at 31.0 ± 0.1 cm (<3 SD, standard deviations (SD)) at birth. While the patient's length was reported as normal, her weight was low at birth (2.7 ± 0.1 kg; Z score: -1.4) but gradually became normal. The patient had an 8-year-old brother who had bilateral grade 3 hydronephrosis due to vesicoureteral reflux. Her brother showed no signs of developmental delay.

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Her parents and brother had a normal head size and were intellectually normal. From the manual patient records, it was learned that the head circumference was 36 cm (<3rd percentile) at the age of 4 months. She had a head circumference of 42 cm (<3rd percentile) at one year of age.

Craniofacial dysmorphic features including curved eyebrows, up-slanting palpebral fissures, epicanthus, depressed nose root, high-arched palate, short philtrum, and upper thin lips were noted (Figure 1B). Sleep electroencephalography (EEG) was normal. Neuroimaging showed a reduction in the size of their cranial volume and myelination appropriate for age (Figure 1A). The Denver II development test revealed that there was delayed motor development. She had a speech delay and a minor delay in fine motor skills. She could not make short or long sentences, but she could speak only some words. She started walking at one year of age.

Routine laboratory investigations and metabolic analyses was in normal ranges. Pedigree analysis demonstrated an autosomal recessive model of disease segregation (Figure 2A). Karyotype analysis of G-banding indicated a normal 46, XX female. An agilent oligonucleotide microarray to investigate copy number variants was done using the 8X60K probe. When the agilent cytogenomic 5.0.0.38 (GRCH 37/hg 19) analysis program was used, there was no detection of deletion or duplication. Whole-genome sequencing of a DNA sample from our patient was performed by

MGI (DNBSEQ-G400). The data analysis using the Genemaster analysis programme revealed a novel homozygous mutation (NM_024596.5; c.321+5G>A) in the *MCPHI* gene (Figure 2C). This identified splice-site pathogenic variant is located in the fourth intron. Neither of these variants has previously been reported in control databases, such as the 1,000 Genomes Project, Exome Variant Server, Exome Aggregation Consortium, or the dbSNP Database. The variant disrupts the splice site and is “likely pathogenic” according to the in silico prediction by HSF (Human Splicing Finder). The variant (c.321+5G>A) was heterozygous in unaffected parents. Written informed consent to participate in this study was provided by the patient’s parents.

DISCUSSION

Microcephaly is defined as a child’s head being smaller than expected for their age, sex, and ethnicity (head circumference below the <3 percentile, or more than 2 or 3 SD below the mean value). The known causes of microcephaly include genetic conditions, metabolic diseases, teratogens (thalidomide), severe malnutrition (extreme placental insufficiency), and transplacental infections (such as Zika). The estimated prevalence of microcephaly in European countries is 1.53 per 10000 births, in the United States range from 2 to 12 per 10,000 live births, and 2 to 6 per 10,000 total births in Australia.⁷⁻⁹ The



Figure 1. Brain MRI showed a decreased cranial volume and delayed myelination (A). A facial view (B).

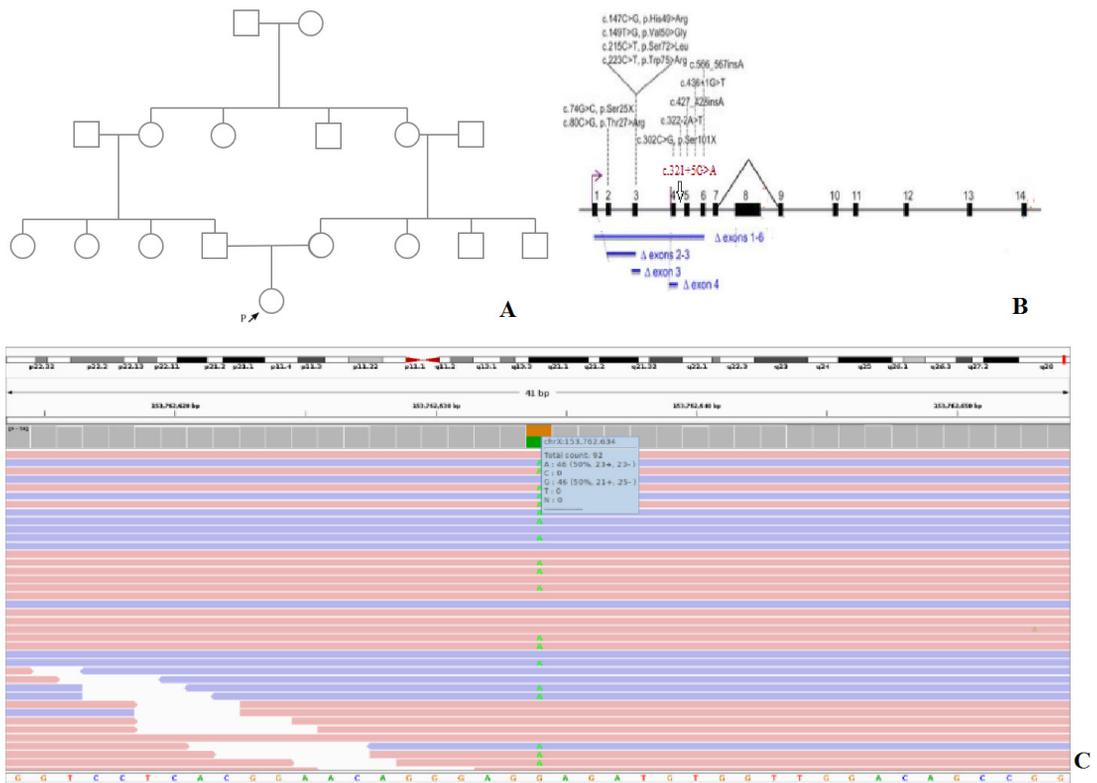


Figure 2. Pedigree analysis of patient's family (A). The structure of the MCPHI gene and the location of the c.321+5G > A variant (Our case was shown in red font) (B). The homozygous variant (c.321+5G > A) in the MCPHI gene was identified by whole exome sequencing (C).

prevalence in Turkey is unknown since there has not been a study in this direction.

MCPH is a neurodevelopmental disorder characterized by a marked reduction in brain size and a congenital small cranium more than 2 standard deviations (SD) below the mean (severe microcephaly occipito-frontal head circumference < 3 SD). Furthermore, the other clinical features include mild to moderate intellectual disability, seizures, speech delay, hyperactivity, attention deficit, delayed developmental milestones, and pyramidal signs.¹⁰⁻¹² MCPH is a genetically heterogeneous disorder and the following genes are related to this phenotype; (MCPH1-27): *Microcephalin*, *WDR62*, *CDK5RAP2*, *CASC5*, *ASPM*, *CENPJ*, *STIL*, *CEP135*, *CEP152*, *ZNF335*, *PHC1*, *CDK6*, *CENPE*, *SASS6*, *MFS2A*, *ANKLE2*, *CIT*, *WDFY3*, *COPB2*, *KIF14*, *NCAPD2*, *NCAPD3*, *NCAPH*, *NUP37*, *MAP11*, *LMNB1*, and *LMNB2*. We detected a homozygous intronic mutation in the MCPHI gene in an infant girl with microcephaly and developmental delay. Functional and cellular studies show that MCPHI plays a pleiotropic role in DNA damage response, cell cycle control, chromosome

condensation, and apoptosis. The MCPHI gene product, microcephalin, is a protein expressed in the fetal brain, and in particular around the lateral ventricles of the developing forebrain, the site of neurogenesis of cells destined to populate the cerebral cortex.^{13,14} Kaindl *et al.* identified that the MCPHI gene was the first gene to cause MCPH.¹⁵ In our patient, we identified a new homozygous splice-site mutation (c.321+5G>A) in MCPHI that was supposed to disrupt the N-BRCT domain. A silico tool, Human Splice Finder, predicted an alteration of splicing due to this variant (<http://www.umd.be/HSF3/>). However, functional reports are needed to assess this hypothesis. The patient was diagnosed with MCPH caused by a novel splice-site mutation of the MCPHI gene in a homozygous state based on the results of this molecular diagnosis. A few intronic mutations have been reported in the MCPHI gene. Darvish *et al.* detected homozygosity at MCPHI in eight families from a cohort study of 112 Iranian families with primary microcephaly. They found a case with an intronic mutation (5th intron; c.436+1G > T) in the MCPHI gene.¹⁶ A novel pathogenic splice-acceptor site homozygous mutation (c.322-

2A > T) in intron four of the *MCPH1* gene has been detected by Ghafouri *et al.* Despite the severe mental retardation observed in the male patient, the female patient had normal intelligence and there was no delay in motor milestones or speech.¹⁷ Interestingly, the pathogenic mutation reported by Ghafouri *et al.* was quite close to our variant.¹⁷ As a result, this variant is thought to be a pathogenic or likely pathogenic variant that contributed to our patient's primary microcephaly. Most patients with *MCPH1* mutations lack notable phenotypic findings other than microcephaly. In fact, our patient did not have any other clinical findings other than severe microcephaly and developmental retardation. Pavone *et al.* reported a novel missense (c.2180C > T) *MCPH1* variant found in twin sisters affected by microcephaly and Hashimoto's thyroiditis.¹⁸ There are no genotype-phenotype correlations that have been identified to date. However, only one study has suggested that larger genomic changes in the *MCPH1* gene may be associated with more severe phenotypes than in patients with point mutations.¹² Once the *MCPH1* pathogenic variants have been identified in an affected family member, carrier testing for at-risk parents is very essential. As in this case, parents carrying the *MCPH1* gene may be offered reproductive options such as prenatal diagnosis or preimplantation genetic diagnosis. Our patient's family preferred the preimplantation genetic diagnosis. Genetic counseling through carrier detection/preimplantation genetic diagnosis or prenatal diagnosis in MCPH families can help reduce the incidence of this autosomal recessive disorder. This case report presents a homozygous splice-site mutation of the *MCPH1* gene that is associated with primary microcephaly and neurodevelopmental retardation; to our knowledge, this is the first such report in a Turkish family. In the future, animal models and functional analysis will be needed to determine the functional effect of this variant and its possible contribution to the reported phenotype.

In conclusion, our case expands the spectrum of mutations in the *MCPH1* gene, demonstrate the rapid and cost-effectiveness of whole exome sequencing for the molecular diagnosis of genetically heterogeneous disorders such as MCPH. This report also supports whole exome sequencing as an effective diagnostic tool in families presenting with genetically heterogeneous disorders like MCPH.

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DISCLOSURE

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Conflict of interest: None

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