Research Article: Clinical Report**Prenatal identification of a novel Mutation in the *MCPH1* Gene associated with autosomal recessive primary microcephaly (MCPH) using Next Generation Sequencing (NGS).**

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Short title: Papoulidis et al. PRENATAL IDENTIFICATION OF A NOVEL MUTATION IN THE *MCPH1* GENE

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Established Facts and Novel Insights

Established Facts

- The Microcephalin protein is a pleiotropic factor playing an important role in neurogenesis; it regulates the division of neuroprogenitor cells preventing them to exhaust.
- There are two categories of microcephaly: the first one is obvious during childbirth called primary microcephaly (MCPH) while the second one develops later in life and is called secondary microcephaly.

Novel Insights

- Since the mutation c.348del has never been associated with microcephaly phenotypes, we reveal new findings of the disease spectrum linked to *MCPH1* gene mutations and its prenatal diagnosis.
- Using Next Generation Sequencing (NGS) analysis, the present study reveals a new homozygous mutation of *MCPH1* gene, thus expanding the disease spectrum of *MCPH1* gene.

Abstract

MCPH1, otherwise known as the microcephalin gene (*607117) and protein, is a basic regulator of chromosome condensation (BCRT-BRCA1 C-terminus). The Microcephalin protein is made up of three BCRT domains and conserved tandem repeats of interacting phospho-peptide. There is a strong connection between mutations of the *MCPH1* and reduced brain growth. Specifically, individuals with such mutations have underdeveloped brains which means smaller size, varying levels of mental retardation, delayed speech and poor language skills, individuals with mild microcephaly and normal intelligence notwithstanding. In this case, a fetus with novel homozygous mutation of the *MCPH1* gene ((c.348del)), whose parents were recessive heterozygous for (c.348del), displayed severe microcephaly at 22 weeks of gestation. Due to the effect on splice sites in introns, this mutation causes forming of dysfunctional proteins which lack crucial domains of the C-terminus. Our findings portray an association between the new *MCPH1* mutation ((c.348del)) and the clinical features of autosomal recessive primary microcephaly (MCPH) contributing to a broader spectrum related to these pathologies.

Keywords: microcephalin gene, homozygous, next generation sequencing, microcephaly. *MCPH1*

Abbreviations: NGS, next generation sequencing; MCPH1, Microcephalin protein

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Introduction

Autosomal recessive primary microcephaly (MCPH) is an uncommon genetically heterogeneous disorder of neurogenic brain development. Its characteristics are decreased head circumference during childbirth with no severe irregularities of the cerebrum and varying intellectual impairment. There are two categories of microcephaly: the first one is evident during childbirth and is called primary microcephaly (MCPH) while the second one develops later in life and is called secondary microcephaly. The main difference between the two is that while MCPH is most of the times a static irregularity of development, secondary microcephaly is a dynamic neurodegenerative disorder. Patients with primary microcephaly have not severe mental issues and also do not show syndromic, neurologic or significant dysmorphias.

Whole genome (WGS) or whole exome (WES) studies identified 17 OMIM genes associated with MCPH and those are: WDR62 (*613583), CDK5RAP2 (*608201), CASC5 (*609173), ASPM (*605481), CENPJ (*609279), STIL (*181590), CEP135 (*611423), CEP152 (*613529), ZNF335 (*610827), PHC1 (*602978), CDK6 (*603368), CENPE (*117143), CENPF (*600235), PLK4 (*605031), TUBGCP6 (*610053), CEP63 (*614724), NDE1 (*609449) [1]. However, the first gene known to cause MCPH is MCPH1(*607117), making 18 genes in total associated with MCPH [2-3].

The location of MCPH1/microcephalin gene (*607117) is chromosome 8p23. Cellular responses caused by DNA damage and chromosome condensation are dependent on microcephalin, the encoded protein [4]. This protein is involved in G2/M checkpoint arrest maintaining the inhibitory phosphorylation of cyclindependent kinase 1. The ability to maintain normal brain size is lost when microcephalin is absent because it causes premature mitotic entry of neuroprogenitor cells [4-6].

In this study, we reported a case of a homozygous in MCPH1 gene (c.348del) fetus diagnosed at 22 weeks of gestation. The fetus was growth restricted showing microcephaly and partial agenesis of the corpus callosum.

Clinical Report

A 30-year-old woman was referred to our center in her second pregnancy at 24 weeks of gestation, complicated with microcephaly (GW), just as her first one had previously. In the first pregnancy, during ultrasound examination (U/S) at 12 weeks of gestation the nuchal translucency measurement was within normal limits (NT = 1.3 mm) indicating low risk for Down syndrome (1/9052). The second trimester ultrasound examination (U/S) at 24 weeks of gestation revealed Fetal Growth Restriction (FGR), microcephaly and partial agenesis of the corpus callosum, dilatation of the third ventricle, right ureter dilatation and presence of a simple unilocular cyst at the lower lobe of the right kidney. After counselling the parents opted for fetal karyotyping and an amniocentesis was performed. Standard G-banding karyotyping revealed normal karyotype and chromosomal microarray analysis did not detect any pathogenic copy number variations. After genetic counselling the pregnancy was terminated at 26 weeks of gestation and an intact male fetus was delivered. The parents decided against autopsy and no further analysis was performed at this point. The parents were of Greek origin, healthy and nonconsanguineous.

In the second pregnancy, ultrasound examination (U/S) at 12 weeks of gestation revealed high risk for Down syndrome due to increased nuchal translucency measurement (NT =3.1 mm). After counselling the parents opted for fetal karyotyping and chorionic villi sampling (CVS) was performed. Standard G-banding karyotyping revealed normal karyotype and chromosomal microarray analysis did not detect any pathogenic copy number variations. During the second trimester of pregnancy, ultrasound assessment showed severe microcephaly and FGR. Following pregnancy termination, fetal autopsy, standard G-banding karyotyping, chromosomal microarray and NGS analysis were performed.

Material and Methods

Molecular karyotyping

High-resolution molecular karyotyping was performed with an aCGH platform of 60,000 oligonucleotides (Agilent technologies). Briefly, DNA was extracted from the chorionic villus sample with Promega Maxwell 16, and it was hybridized with the human reference DNA of the same gender (Promega Biotech). The statistical test used as a parameter to estimate the number of copies was ADM-2 (provided by the DNA analytics software, Agilent Technologies) with a

window of 0.5 Mb and a threshold of 6. Only those copy number changes that affected at least 5 consecutive probes with identically oriented change were considered as copy number variations. Consequently, for most of the genome, the average resolution of this analysis was 200 kb.

Next Generation Sequencing (NGS)

DNA isolation from the fetus and the parents was performed. Following, Oligonucleotide-based target capture analysis and nucleotide sequencing was performed using TruSight One kit (Illumina) and Next Generation Sequencing (Illumina NextSeq), respectively, examining 99 disease-causing genes that were related with the clinical features of the fetus (Table 1). The software SOPHIA DDM® (SOPHIA GENETICS) was used for the data analysis [reference genome UCSC hg19 and reference database Human Gene Mutation Database (HGMD v.2017.1)].

Table 1: 99 disease-causing genes that were related with the clinical features of the fetus.

List of studied genes
<i>AP3B2, AP4B1, AP4E1, AP4M1, APEX1, ARFGEF2, ASNS, ASPM, ATR, ATRX, BMPR1A, BRWD3, C2, CDK5RAP2, CDON, CDT1, CENPJ, CEP152, COL2A1, CYP21A2, CZ1P-ASNS, DICER1, DPP6, EFTUD2, EHMT2, EMG1, F10, F2, F5, FAM20A, FAM20C, FLNA, FLT1, FN3K, GLI2, GNAS, HUWE1, HYMAI, IGF1R, KARS, KNL1, LIG4, LMNB2, LOC100287042, LOC102724058, MCM5, MCPH1, MCPH1-AS1, MED17, MMP2, MRE11, MTHFR, MTNR1A, MYH11, NBN, NCAPD2, NDE1, NELFE, NPC1, ORC4, ORC6, OSGEP, PACERR, PCNT, PEX2, PHB2, PLAGL1, PRKAR1A, PRKDC, PTGS1, PTGS2, PTPRJ, REV3L, RPTOR, SCAP, SCARNA10, SCN10A, SCN1A, SERPINA3, SKIV2L, SLC16A2, SLC25A19, STIL, STK4, TBCD, TCOF1, TERT, TP53, TUBA1A, TUBGCP6, VPS13B, VPS35, VRK1, WDR62, WDR81, XRN1, ZNF335, ZNF592, ZNF750.</i>

Results

Standard G-banding karyotyping and high-resolution molecular karyotyping revealed a normal chromosome chart of the second foetus. NGS analysis showed an inherited homozygosity for mutations of *MCPH1* gene (c.348del) which has not been reported in generalist polymorphism databases (ExaC or exome variant server (EVS)) (shown in Fig. 1). The results were confirmed by

Sanger sequencing in this fetus and targeted sequencing on the parents showed that they were both heterozygous for the detected mutation (shown in Fig. 2). Following the diagnosis of the c.348del mutation of the second fetus, targeted sequencing of the DNA of the first fetus was performed showing homozygosity of the same (c.348del) mutation.

Figure 1: IGV screenshot for mutations in MCPH1 gene The mutation c.348del in MCPH1 gene in Homozygous state. (fetus 2)

Figure 2.A: Sanger sequencing data of the mutation c.348del in MCPH1 gene in heterozygous state (mother).
Figure 2.B: Sanger sequencing data of the mutation c.348del in MCPH1 gene in heterozygous state (father).

Histopathologic results

Fetal necrotomy of fetus 2 included mild symmetric growth retardation, mild dysmorphic craniofacial features, microcephaly trend without true microencephaly, partial corpus callosum agenesis without histologic cerebral dysplasia. Placental weight was normal, but histologic examination revealed extended inflammatory lesions of maternal origin dating earlier in pregnancy (acute chorionitis and villitis, karyorrhexis, fibrin deposition), multifocal chronic villitis of unknown origin. There were no indications of a specific syndromic microcephaly and partial corpus callosum agenesis. The possibility of “microcephaly-corpus callosum agenesis syndrome” or other cases of isolated genetic microcephaly could not be excluded. The inflammatory lesions of the placenta could imply the possibility of intrauterine teratogenic effect due to an infectious agent, although no analogous histologic findings of the cerebrum were observed.

Discussion

The mode of inheritance of the genetically heterogenous disorder primary microcephaly (MCPH) is autosomal recessive. MCPH causes dysmorphia of the brain with the characteristics of congenital small cranium with reduced occipito-frontal head diameter (OFD) of more than 2 standard deviations (SD) below the mean for ethnicity, age and sex (heavy microcephaly OFD < 3 SD). MCPH is further characterized by simplified gyral pattern, mild to moderate intellectual disability and mild seizures, polymicrogyria, periventricular neuronal heterotopias, hyperactivity and attention deficit disorder, speech delay, focal or generalized seizures, aggressiveness, and delay of developmental milestones and pyramidal signs [7-8].

The incidence of MCPH is about 1:30000-1:250000 live-births [9]. MCPH is less common in whites than in Arab and Asian populations where consanguineous marriages are mutual [10]. More than 300 families worldwide have been reported to manifest MCPH.

241905bp is the genomic size of MCPH1/microcephalin gene. 835 aminoacids are encoded by 14 exons which are in the 8032bp open reading frame; until today, 3 isoforms are known [3]. The Microcephalin protein is a pleiotropic factor and plays an important role in neurogenesis; it regulates the division of neuroprogenitor cells preventing them to exhaust. It basically regulates the telomere integrity and associates with the DNA damage repair mechanism. The protein is involved in G2/M checkpoint arrest maintaining the inhibitory phosphorylation of cyclindependent kinase 1 [4-6]. It also operates as tumour suppressor in many human cancers, in germline functions and regulates the cerebral cortex size as well as the development of the brain

[11-12]. Normal brain growth is dependent on MCPH1 gene that also acts as a positive selector for primate lineage [13].

Two types of mutations in MCPH1 have been described. Mild microcephaly and cellular phenotype are manifested in patients who were identified with a missense mutation [14-16]. Furthermore, there are reported deletions in MCPH1. An Iranian family with mild microcephaly and intellectual disability was identified with a deletion of the first 6 exons of the gene; at least 10% to 15% of cells had early chromosome condensation in this family [16]. Moreover, an Asian-Indian patient was identified with a deletion of the first 11 exons [17].

Hereditary hearing loss, facial dysmorphism and seizures as well as mild to severe mental retardation may be manifested in microcephaly patients [18]. There are two categories of microcephaly; the primary microcephaly when it is evident at birth and the secondary microcephaly when the disease is identified later in life [19]. Prenatal developmental neurogenic disorder relates to primary microcephaly while dynamic neurodegenerative disease relates to secondary microcephaly.

In this report, we present two consecutive pregnancies complicated by severe microcephaly caused by a homozygous mutation of the MCPH1 gene. Both parents of the embryo carried the recessive c.348del and were heterozygous. This mutation leads to the replacement of phenylalanine amino acid for leucine amino acid at position 116 of the protein altering the reading frame which cause an early stop codon at position 145 of the protein leading to protein forms which not function. The *MCPH1* mutation (c.348del) has been revealed by our findings and appears to involve in microcephaly's clinical features contributing to *MCPH1* spectrum's expansion associated to these pathologies. The mutations of MCPH1 gene were those which have been firstly identified in MCPH [8]. Furthermore, there have been reported few mutations in MCPH1 gene [18].

Conclusion

The diagnosis of primary microcephaly is based on the identification of *MCPH1* mutations by molecular techniques. Although, we have found a novel mutation in homozygosity associated with fetal abnormalities, additional studies are necessary to verify the pathogenicity of this mutation and its definite association with fetal microcephaly. We present this case of the novel mutation c.348del to share information and expand our limited knowledge on the spectrum of disease linked to *MCPH1* gene mutations and its prenatal diagnosis.

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Statement of Ethics

The study was approved by the Ethics Committee of “Aretaieio” Hospital, Athens, Greece, and performed with respect to the ethical standards of the Declaration of Helsinki, as revised in 2008. The date of ethical approval was the 19th of June, 2018 with code 60/19-06-18. Also, a written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Conflict of Interest Statement

The authors declare no conflicts of interest. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions Statement

All authors made substantive intellectual contributions, read and approved the final version of the manuscript. I. Papoulidis was in charge of overall patient management and prepared the initial draft of the manuscript. M. Eleftheriades, SM Liappi and Maria Papamichail were in preparation, and finalization of the manuscript providing important intellectual inputs. E. Manolakos, I. Papoulidis and SM Liappi performed and analyzed the WES data and the analysis of the identified variant. A. Konstantinidou participated in the histopathology assessment. M. Eleftheriades, V. Papadopoulos, A. Garas and S. Sotiriou interpreted clinical data and critically read the manuscript. I. Papastefanou and G. Daskalakis, performed ultrasound examination, cvs and amniocentesis respectively. A. Ristic supervised patient work-up and management.

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