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Autosomal Recessive Primary Microcephaly (MCPH) and Novel Pathogenic Variants in *ASPM* and *WDR62* Genes

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Keywords
MCPH · Whole-exome sequencing · Novel variant · Autosomal recessive · *ASPM* · *WDR62*

Abstract
Introduction: Autosomal recessive primary microcephaly (MCPH) is a disorder characterized by congenital microcephaly and intellectual disability without extra-central nervous system malformation. MCPH is a disease with heterogeneity in genotype and phenotype. For this reason, it is important to determine the genetic causes and genotype-phenotype relationship in MCPH, which causes lifelong impairment. In this study, we aimed to evaluate the clinical, genetic, and brain imaging findings of cases diagnosed with MCPH. ***Methods:*** Electroencephalogram and brain magnetic resonance imaging were performed for all cases. We evaluated genetic results of the 39 families including cases with suspected MCPH diagnosis. ***Results:*** Genetic diagnosis related to MCPH was provided in 11/39 (28.2%) of these families including 13/41 cases (31.7%). Variants of the *WDR62* gene were the most common (61.5%) cause, and variants of the *ASPM* gene were the second most common cause (38.5%). We have found 6 novel variants and 4 previously reported variants in *ASPM* and *WDR62* genes. Main brain imaging findings in our cases were lissencephaly, polymicrogyria, schizencephaly, pachygyria, and cortical dysplasia. Genetic counseling in 2 families whose genetic diagnosis was determined prevented them from having another child with MCPH. ***Discussion/Conclusion:*** Detection and reporting of novel variants is an important step in eliminating this disorder by providing families with appropriate genetic counseling.

Introduction

Primary microcephaly (PM) is characterized by congenital microcephaly (occipitofrontal head circumference below −2 SD at birth and below −3 SD following 6 months of age) [Thornton and Woods, 2009; Kaindl et al., 2010]. The etiology of microcephaly can be attributed to genetic or environmental causes (such as in utero alcohol exposure, infections, perinatal hypoxia, and hypoglyce-

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mia) [Deurinckx et al., 2021]. Autosomal recessive primary microcephaly (MCPH) is characterized by decreased brain growth in the prenatal period, congenital microcephaly, intellectual disability (ID), and developmental delay without extra-central nervous system malformations in the postnatal period [Létard et al., 2018]. MCPH occurs in 1:30,000–250,000 live births, with rates varying by geographic region [Zaqout et al., 2017].

MCPH is a group of genetically heterogeneous diseases that, according to the OMIM database (http://omim.org/), have been linked to 25 different genes. The majority of these genes, which are associated with the etiology of MCPH, have been identified in the last 10 years by technological developments in genetics [Jean et al., 2020]. In particular, whole-exome sequencing (WES) has increased our knowledge to detect the etiology in genetic diseases such as MCPH. Most of the proteins encoded by these genes affect cell division by acting on centrosomes, and variants of these genes cause a decrease in neurogenesis and cerebral cortex volume [Rasool et al., 2020].

It is difficult to recognize PM in the prenatal period without a genetic diagnosis. While PM was diagnosed at a rate of 20% before the 26th gestational week, it was shown that most of the cases were diagnosed at the 32nd gestational week [Woods, 2004]. Increased identification of the molecular genetic diagnosis will provide prenatal diagnosis and appropriate genetic counseling. In addition, MCPH is a disease with heterogeneity in genotype and phenotype. For this reason, it is important to determine the genetic causes and genotype-phenotype relationship in MCPH, which causes lifelong impairment. More molecular genetic studies including more cases with MCPH may result in better genotype-phenotype correlation. In addition, detection of carriers would be beneficial to reduce the incidence of MCPH.

In this study, we aimed to evaluate the clinical, genetic, and brain imaging findings of cases diagnosed with MCPH.

Material and Methods

We included cases who presented at the Department of Child Neurology and were diagnosed with MCPH through clinical interviews and examinations. All cases were evaluated by a pediatric neurologist and a medical geneticist. We included cases who had occipitofrontal head circumferences below −2 SD at birth and below −3 SD at last visit without extra-central nervous system malformations. Electroencephalogram (EEG), brain magnetic resonance imaging (MRI), and genetic analysis were performed for all cases.

Genetic Analysis

To study the molecular etiology of PM, genomic DNA has been isolated from the peripheral blood of patients using the QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany) with the manufacturer's protocols. All coding regions of the patient's human genome were sequenced using WES analysis through the Illumina NovaSeq Platform using the Agilent SureSelect V5 kit (Agilent, Santa Clara, CA, USA). We evaluated the Raw data via the Sophia DDM® data analysis platform. We used filtering steps to identify pathogenic variants associated with clinical characteristics as follows: (1) all missense, nonsense, frameshift, splice-site, indel, in-frame and synonymous variants, (2) variants with minor allele frequency <1.0% in population studies (1000 Genome [1000 G], ESP, ExAC, and Genome Aggregation Database [gnomAD]). The Genome Integrative Viewer was used to display sequence data. We controlled the novel variants in databases of HGMD® and ClinVar (http://ncbi.nlm.nih.gov/clinvar). Pathogenicity of new variants has been interpreted using in silico analysis tools (Mutation Taster, CADD [Combined Annotation Dependent Depletion]), probability of being loss of function intolerant (pLI) score. ACMG guidelines were followed for variant pathogenicity classification and American College of Medical Genetics and Genomics (ACMG) criteria [Richards et al., 2015]. Familial segregation was checked using Sanger sequencing.

Results

In this study, we evaluated the genetic results of the 41 cases with suspected MCPH diagnosis from 39 families. We evaluated WES findings of the 38 families including cases with suspected MCPH diagnosis. In addition, we included one family identified by a single-gene analysis (*WDR62*). Genetic diagnosis related to MCPH was provided in 11/39 (28.2%) of these families including 13/41 cases (31.7%). The genetic diagnoses we determined were as follows: a variant of the *ASPM* gene was found in 5/41 (12.2%) cases from 5 families, and variants of the *WDR62* gene were found in 8/41 (19.5%) cases from 6 families. We did not detect a clinically relevant variant in any of the MCPH genes other than *ASPM* and *WDR62*. In the *ASPM* gene, we detected 5 different variants including 3 frameshift (c.8862dupA, c.4162dupA, c.5219_5225delGAGG) and 2 nonsense (c.646G>T, c.7792C>T) variants. In the *WDR62* gene, we detected 5 different variants including 3 frameshift (c.3936dupC, c.2319delC, and c.384_385delAG) and 2 nonsense (c.1605dupT, c.2956C>T) variants. In addition, we detected the same variant (c.1605dupT) in 3 different unrelated families. We found a compound heterozygous genotype in 2 siblings from 1 family (c.3936dupC, c.2319delC) in the *WDR62* gene. When the distribution of the variants in the genes was investigated, variants of the *WDR62* gene were the most common cause (61.5%), and variants of the *ASPM* gene were the second

Table 1. Genetic findings in cases diagnosed with autosomal recessive primary microcephaly

Gene	Family	Case	Exon	Zygosity	Nucleotide variation	Amino acid variation	Mutation type	ACMG interpretation	Previous report
WDR62 (NM_001083961.2)	F1	1	30	Hetero	c.3936dupC	p.V1313Rfs*18	Frameshift	Pathogenic	[Nicholas et al., 2010; Rasool et al., 2020]
			19	Hetero	c.2319delG	p.S774Vfs*19	Frameshift	Likely pathogenic	Novel
		2	30	Hetero	c.3936dupC	p.V1313Rfs*18	Frameshift	Pathogenic	[Nicholas et al., 2010; Rasool et al., 2020]
			19	Hetero	c.2319delG	p.S774Vfs*19	Frameshift	Likely pathogenic	Novel
	F2	3	12	Homo	c.1605dupT	p.E536*	Nonsense	Pathogenic	[Poulton et al., 2014]
		4	12	Homo	c.1605dupT	p.E536*	Nonsense	Pathogenic	[Poulton et al., 2014]
	F3	5	12	Homo	c.1605dupT	p.E536*	Nonsense	Pathogenic	[Poulton et al., 2014]
	F4	6	12	Homo	c.1605dupT	p.E536*	Nonsense	Pathogenic	[Poulton et al., 2014]
	F5	7	24	Homo	c.2956C>T	p.Q986*	Nonsense	Pathogenic	Novel
	F6	8	4	Homo	c.384_385delAG	p.N131Wfs*3	Frameshift	Pathogenic	Novel
ASPM (NM_018136.5)	F7	9	19	Homo	c.8862dupA	p.V2955Sfs*12	Frameshift	Likely pathogenic	Novel
	F8	10	18	Homo	c.4162dupA	p.1388 fs*4	Frameshift	Likely pathogenic	Novel
	F9	11	3	Homo	c.646G>T	p.E216*	Nonsense	Pathogenic	Novel
	F10	12	18	Homo	c.5219_5225delGAGG	p.Arg1740Thrfs*7	Frameshift	Pathogenic	[Türkyılmaz and Sager, 2021]
	F11	13	18	Homo	c.7792C>T	p.Gln2598*	Nonsense	Pathogenic	[Türkyılmaz and Sager, 2021]

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Table 2. Clinical findings, electroencephalogram (EEG), and brain imaging findings in cases with genetic diagnosis of autosomal recessive primary microcephaly

Case	Age, years	Sex	Head circumference		Developmental delay		Seizures/ onset age	EEG	Brain MRI findings in addition to microcephaly	Dysmorphologic features
			at birth, cm (SD)	last visit, cm (SD)	speech	motor skills				
1	17	F	31 (−2.54)	46 (−7.71)	No speech	Walking at 5 years (ataxic and spastic gait)	+ 5 years	Nonconvulsive status epilepticus	No MRI findings	No
2	10	F	30 (−3.20)	45 (−5.11)	Speech impairment	Walking at 2 years 6 months (ataxic and spastic gait)	No	No EEG findings	No MRI findings	Sloping forehead
3	8	M	31.5 (−2.17)	44 (−5.4)	Speech impairment	Walking at 18 months	+ 6 years	Focal discharges from frontal region	Simplified gyral pattern, corpus callosum hypoplasia	Craniosynostosis, sloping forehead
4	Prenatal									
5	2	F	29.5 (−3,62)	42 (−3.96)	No speech	Toe walking	+ Neonatal period	No EEG findings	Simplified gyral pattern, polymicrogyria in the left frontal lobe, closed lip schizencephaly	No

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Table 2 (continued)

Case	Age, years	Sex	Head circumference		Developmental delay		Seizures/ onset age	EEG	Brain MRI findings in addition to microcephaly	Dysmorphologic features
			at birth, cm (SD)	last visit, cm (SD)	speech	motor skills				
6	16	M	31 (−2.54)	48 (−6.26)	Speech impairment	Walking at 4 years	No	Diffuse cerebral dysfunction	Retardation in myelination, pachygyria, lissencephaly	Prominent forehead
7	5	F	30 (−3.20)	42 (−5)	Speech impairment	Walking at 2 years (ataxic gait)	+ 4 years	Bilateral central discharges focal epilepsy	Lissencephaly	No
8	10	F	31 (−2.54)	44.5 (−5.57)	Speech impairment	Walking at 4 years 6 months	+ 2 years 6 months	Left cerebral hemisphere discharge	Cortical dysplasia Schizencephaly in the right parietal lobe, right frontotemporal pachygyria	No
9	7	F	31.5 (−2.17)	41 (−7.57)	Speech impairment	Walking at 18 months (paroxysmal dyskinesia)	No	No EEG findings	Lissencephaly	Prominent forehead
10	1.5	M	32 (−2.08)	41 (−5.05)	No speech	Unsupported sitting at 12 months	+ 9 months	Focal epileptic discharge	Simplified gyral pattern	Prominent forehead
11	6	M	N/A	39.5 (−3.5)	No speech	Broad-based gait	+ 8 months	Focal epileptic discharge	Thickening of the cerebral cortex, pachygyria	No
12	9	F	32 (−2.08)	43 (−6.07)	Speech impairment	Walking at 18 months	+ 7 years	Focal epileptic discharge	Symmetrical ventriculo-megaly, thin of the corpus callosum, simplified gyral pattern, polymicrogyria	Narrow and sloping, forehead
13	10	M	31 (−2.54)	45 (−5.88)	Speech impairment	Walking at 20 months	+ 5 years	Focal epileptic discharge	Symmetrical ventriculo-megaly, simplified gyral pattern, pachygyria	Synophrys, narrow and sloping forehead

most common cause (38.5%). We have found 6 novel variants and 4 previously reported variants in *ASPM* and *WDR62* genes (Table 1). Among these results, we reported the association between 2 variants of the *ASPM* gene and polymicrogyria in the literature [Türkyılmaz and Sager, 2021].

The occipitofrontal head circumference at birth ranged from −2.08 SD and −3.62 SD in our cases (Table 1). All cases displayed developmental delay (Table 1). Main brain imaging findings in our cases were lissencephaly, polymicrogyria, schizencephaly, pachygyria, and cortical dysplasia (Table 2). Brain imaging findings were associated with variants in both *ASPM* and *WDR62* genes. Epilepsy and seizures were present in 9/12 cases (75%), with almost all accompanying EEG and brain imaging findings (there is 1 case without EEG findings and 1 case without brain imaging findings) (Table 1).

Discussion and Conclusion

In this study, we identified a genetic cause related to MCPH in 13/41 cases (31.7%). In the previous literature, studies evaluating cases with MCPH have reported that no genetic cause could be found in 50–75% of Western European or North American and 20–30% of the Indian or Pakistani population [Zaqout et al., 2017]. Therefore, it has been emphasized that there may be more genetic causes that have not yet been associated with MCPH. Our findings are in line with previous literature because we could not find the genetic cause in 68.3% of the cases.

We detected variants of the *WDR62* gene in 6 families at a rate of 61.5%, and variants of the *ASPM* gene in 5 families at a rate of 38.5%. When the distribution of the variants in the genes related to MCPH was investigated, the *ASPM* gene (68.6%) is the most common and the *WDR62* gene (14.1%) is the second most common genetic contributor of MCPH [Zaqout et al., 2017]. On the other hand, we evaluated previous studies investigating the geographical distribution of cases with *ASPM* and *WDR62* variants. For variants of the *WDR62* gene, the Turkish population constitutes the most common number of families per country after the Pakistani community [Slezak et al., 2021], while the Turkish population exhibits a lower number of families per country for variants of the *ASPM* gene [Létard et al., 2018]. As a result of these findings, we suggest that the *WDR62* gene might be more prominent in the Turkish population. However, more studies are needed to test this finding.

Main brain imaging findings in our cases were lissencephaly, polymicrogyria, schizencephaly, pachygyria, and cortical dysplasia. Brain imaging findings were associated with variants in both *ASPM* and *WDR62* genes. In the basic definition of MCPH, there were no structural brain abnormalities other than the simplified gyral pattern and decreased brain volume associated with microcephaly. However, the following studies and case reports showed that MCPH especially associated with variants of the *WDR62* gene presented structural brain abnormalities [Zaqout et al., 2017]. The variants of the *WDR62* gene were associated with severe structural brain abnormalities such as lissencephaly, schizencephaly, and polymicrogyria [Bilgüvar et al., 2010]. And the authors stated that the *WDR62* gene has a different mechanism from other microcephaly genes that does not act on the centrosomes. Most of the reported brain malformations associated with variants of the *ASPM* gene were simplified gyral pattern (67%) and corpus callosum defects (31%) [Létard et al., 2018]. Atypical brain imaging findings such as polymicrogyria and syringomyelia were rarely reported in variants of the *ASPM* gene [Türkyılmaz and Sager, 2021]. However, we determined lissencephaly and pachygyria in cases carrying variants of the *ASPM* gene. We detected structural brain abnormalities in most of our cases. Differently, we did not obtain any brain imaging findings in 2 siblings carrying the same compound heterozygous variant of the *WDR62* gene. In the previous literature, few cases carrying compound heterozygous variants for the *WDR62* gene have been reported. Some of these studies determined polymicrogyria related to compound heterozygous variants of the *WDR62* gene [Murdock et al., 2011; Slezak et al., 2021].

We detected that 4 out of 5 patients with *ASPM* pathogenic variants displayed focal epileptic discharge. Epilepsy is not a common feature in MCPH [Mochida et al., 2001]. The incidence of epilepsy was found to be 3–8% in MCPH cases associated with the *ASPM* gene without cortical migration defect [Türkyılmaz and Sager, 2021]. However, we suggest that the reason for the detection of higher rates of epileptic discharges in cases with the *ASPM* variant in our study is the high rate of structural malformations in brain imaging findings. In the previous literature, the phenotype of epilepsy was commonly related to brain malformations in MCPH cases [Bhat et al., 2011].

As a result of the genetic diagnosis in this study, one of the families affected by MCPH related to variants in the *WDR62* gene was detected in the early period with a prenatal invasive genetic diagnosis. One family also applied to pre-implantation genetic diagnosis, as their first child

was genetically diagnosed by the *WDR62* variant. These parents, who are carriers of the *WDR62* variant, had their second child without MCPH. Tran et al. [2021] reported a prenatal diagnosis of *ASPM* variant in a Vietnamese family including 2 previous cases with microcephaly.

As a conclusion, MCPH is a group of genetically heterogeneous diseases that have been linked to 25 different genes. In addition, this number is increasing with the addition of new candidate genes. For this reason, we used WES in our cases. In our study, we did not detect a clinically relevant variant in any of the MCPH genes other than *ASPM* and *WDR62*. National differences may also be the reason why MCPH-related genes were not detected in our study other than these 2 genes. We suggest that it would be more appropriate to use targeted next-generation sequencing panels that are cheaper, faster, and easier to interpret than WES in the Turkish population. However, WES provides an opportunity to re-analyze cases for which the genetic cause of MCPH cannot be found. We have determined 6 novel and 4 previously reported variants in the *ASPM* and *WDR62* genes. Detection and reporting of novel variants is an important step in eliminating this disorder by providing families with appropriate genetic counseling [Batool et al., 2021].

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Statement of Ethics

The protocols used in this study were in compliance with the Declaration of Helsinki and were approved by the Ethics Committee of the Kartal Training and Research Hospital, University of Health Sciences (Protocol 2021/514/214/16, November 30, 2021). Written informed consent was obtained from the parent/legal guardian of the patient for publication of the details of their medical case.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

All authors designed the study. H.B., A.T., A.H.Ç., F.Ö., and H.O. worked on genetic part of study. S.G.S. and Y.A. provided clinical evaluation. H.B. and G.Ü.B. wrote the manuscript. All authors reviewed and approved the final manuscript.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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