



Novel *WDR62* and *MTR* Variants in a Patient With Autosomal Recessive Primary Microcephaly-2 With Polymicrogyria and Homocystinuria-Megaloblastic Anemia

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Abstract

Background: Autosomal recessive primary microcephaly-2 (MCPH2) is a rare genetic disorder with clinical and genetic heterogeneity. This study aimed to perform high-throughput whole-exome sequencing (WES) to facilitate the diagnosis of the genetic variants responsible for MCPH2 and the comorbidities.

Materials and Methods: The WES was performed for a 3-year-old boy with primary microcephaly-2 and homocystinuria-megaloblastic anemia in a consanguineous family. Sequencing and variant calling was conducted by standard bioinformatics tools. Filtering was performed to prioritize novel variants. Finally, the effect of variants on the protein structure and function was assessed using web prediction tools.

Results: Using WES, two novel homozygous variants and three novel homozygous variants were identified in the *WDR62* and *MTR* genes as the causes of MCPH2 and homocystinuria-megaloblastic anemia in the affected child, respectively. These frameshift insertion variants are classified as pathogenic and affect the structure and feature of the *WDR62* and *MTR* proteins by changing amino acid sequence and causing nonsense-mediated RNA decay (NMD).

Conclusion: Magnetic resonance imaging (MRI) supported polymicrogyria and impaired cerebral cortical development in the affected child. *WDR62* as a causative gene plays an essential role in cerebral cortical development, and its pathogenic disease-causing variants are considered as causing factors for MCPH2. Homocystinuria-megaloblastic anemia was a comorbidity associated with microcephaly in this patient, and its variants were confirmed by WES. Overall, performing WES is a necessary and accurate way to rapidly identify the exact causative genetic variants in MCPH2 and the homocystinuria-megaloblastic anemia and manage the disease.

Keywords: MCPH2, Whole Exome Sequencing, *WDR62* gene, *MTR* gene

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Introduction

Microcephaly is considered a neurodevelopmental disorder in which head circumference (HC) is more than two or three standard deviations (severe microcephaly) below the mean for age and gender-matched population (1-6). The Center for Disease Control and Prevention has estimated that the incidence of microcephaly is about 2-10/10000 live births in the United States (7).

Primary microcephaly (MCPH) is an autosomal recessive, heterogeneous subtype of microcephaly (8). In most patients, there are malformations on the

cortical, including simplified cerebral cortical gyral pattern, polymicrogyria, lissencephaly, pachygyria, and hypoplasia of the corpus callosum. The neurological and behavioral features include mental retardation, poor motor development, and seizure (9, 10).

Based on the Online Mendelian Inheritance in Man (OMIM) database, because of the heterogeneity of MCPH, several autosomal recessive loci are responsible for MCPH. Over 25 genes have so far been identified for MCPH, including the *WDR62* gene (9). Many of these genes encode proteins interacting with the centrosome

and mitotic spindle (3, 11-13).

Primary microcephaly-2 (MCPH2; OMIM 604317), which is a common form of primary microcephaly, is caused by homozygous or compound heterozygous mutations that occur in the WDR62 gene on chromosome 19q13, encoding a WD repeat-containing protein 62 (9, 14). So far, nearly 40 pathogenic mutations have been reported in the WDR62 gene that are responsible for microcephaly and for a wide range of cortical malformations (15-19). In addition, the WDR62 gene plays a prominent role in cerebral cortical development and neuronal proliferation, migration, and organization (9, 20, 21). Consequently, MCPH2 patients have delayed psychomotor development, different head circumferences, and a broad spectrum of cortical malformations (11, 21).

Considering that primary microcephaly is a genetically and phenotypically heterogeneous disorder and can present with various comorbidities, it is challenging to accurately identify patients with primary microcephaly and other accompanying disorders, and the exact genes and variants that are responsible for this disorder. Identifying MCPH using gene panel testing based on known and common genetic variants is not so specific due to the continuous identification of new genes and variants and different accompanying disorders (8). With advances in genetics and genomics, whole genome sequencing or whole-exome sequencing (WES) is one of the essential prerequisites for the specific diagnosis of primary microcephaly and various comorbidities (22). In the present study, high-throughput WES technology was applied to identify the exact causative variants in a consanguineous family with a child with MCPH2 and homocystinuria-megaloblastic anemia.

Materials and Methods

Subjects and Clinical Evaluations

One Iranian consanguineous family was referred for genetic testing due to having a child with developmental delay, mental retardation, seizures, low HC, diffused mild hypotonia, and anemia that resulted in a clinical diagnosis of microcephaly and megaloblastic anemia. Informed written consent was taken from the family. Figure 1 illustrates more information about the family pedigree. An autosomal recessive inheritance was expected according to the pedigree. The proband was further subjected to neurological, biochemical, and physical examinations. A magnetic resonance imaging (MRI) scan was performed on the proband.

Whole Exome Sequencing and Bioinformatic Analysis

WES was used to detect the variants in the genomic DNA sample in the proband. Genomic DNA was extracted from the peripheral blood by utilizing DNA Extraction Kit DNP (Sinacolon, Iran) according to the standard

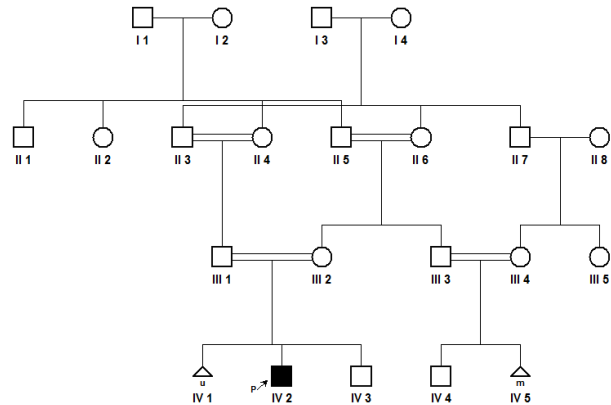


Figure 1. A Pedigree of a Consanguineous Family Representing MCPH2. Note. MCPH2: Autosomal recessive primary microcephaly-2.

protocols. The genomic DNA samples were quantified via the Thermo Scientific NanoDrop Spectrophotometer.

Whole-exome capture for DNA samples from pedigree's proband was then performed with SureSelect Target Enrichment System for Illumina Paired-End Sequencing Library (Agilent Technologies, Santa Clara, CA). DNA sequencing was performed on the Illumina platform (Illumina HiSeq, San Diego, CA). Raw data were converted to FASTQ files using bcl2fastq v1.8.4 (Illumina). Next, reads were mapped on the UCSC hg19 reference genome with BWA-MEM. ANNOVAR software was used to call and annotate single-nucleotide and indel variants. Among them, novel homozygous variants with a minor allele frequency of < 1% were checked on 1000 Genome Project, Exome Variant Server, Clinvar, Exome Aggregation Consortium (ExAC), dbSNP, and the HGMD database. To identify genetic variants with potential pathogenic effects on proteins, various computational predictors were used, including Alamut Visual Plus™ and Mutation Tester.

Results

Clinical Description

The affected child had clear phenotypical characteristics of primary microcephaly with cortical malformations and megaloblastic anemia. This 3-year-old boy was born at full term by normal vaginal delivery and had a small head size at birth. He had a significant developmental handicap, decreased muscle bulk, and diffuse mild hypotonia. Moreover, he suffered from moderate mental retardation and was unable to sit, walk, and speak. The brain MRI of the patient showed moderate to severe microcephaly with the malformations of cortical development (Figure 2). Biochemical tests confirmed homocystinuria-megaloblastic anemia. Both of his parents were intellectually normal with mild anemia. There were two miscarriages in this pedigree, including one previous miscarriage in this family and one in the maternal cousin. The maternal or environmental causes

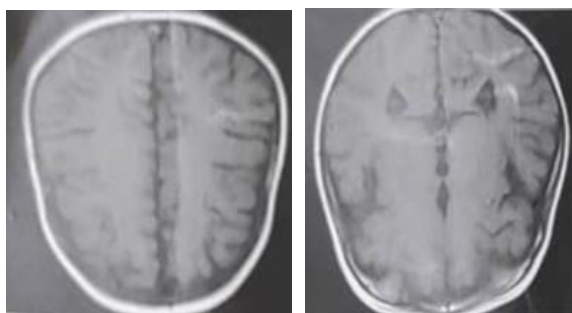


Figure 2. Brain Magnetic Resonance Imaging of the Patient, Showing Polymicrogyria and Simplified Gyrus of the Cerebral Cortex.

have not been identified in this family.

Molecular Investigations

The WES was performed on DNA samples related to the proband, and five novel homozygous frameshift variants were identified accordingly. Two of these frameshift insertion variants occurred in the WDR62 gene in exon 30. These frameshift mutations predict p. Q1310fs (c.3931dupC) and p.Q1305fs (c.3916dupC). Based on American College of Medical Genetics and Genomics guidelines and criteria, these variants were classified as pathogenic; they were neither found in ExAC nor 1000G and have not been reported before (23, 24). Various computational predictors have demonstrated that these are disease-causing variants and affect WDR62 protein structure and function by changing the amino acid sequence and causing nonsense-mediated RNA decay (NMD). These variants are deleterious and classified as having a splice site effect.

Three of these frameshift insertion variants occurred in the MTR gene in exon 27 and could predict p.R934fs (c.2801delG) and p.R578fs (c.1733delG), exon 28, and p.R985fs (c.2954delG). These variants were also predicted to be deleterious and pathogenic and affect the structure and function of the MTR protein by changing amino acid sequence and causing NMD. These variants were neither found in ExAC nor 1000G and have not been previously reported in public databases.

Discussion

MCPH2 is one kind of neurodevelopmental disorder characterized by moderate to severe mental retardation, microcephaly, and variable degree of cortical malformation, including microgyria, pachygyria, hypoplasia of the corpus callosum, and lissencephaly. The other clinical features are delayed psychomotor development in all patients, and seizures in some patients (4, 8, 25-27). Notably, the incidence of most rare genetic abnormalities such as MCPH is high in Iran and the Middle East because of the high rate of consanguineous marriages (28-30). The main purpose of this study was to find the exact genetic cause of MCPH2 using WES in Iranian consanguineous families,

particularly, in conditions where MCPH is accompanied by comorbidities.

Using WES, two novel frameshift variants (i.e., c.3931dupC:p.Q1310fs and c.3916dupC:p.Q1305fs) were identified in the exon 30 of the WDR62 gene, as well as three novel frameshift variants (i.e., c.2801delG:p.R934fs and c.1733delG:p.R578fs) in the exon 27 and 28 (c.2954delG:p.R985fs) of the MTR gene. Based on some bioinformatic tools (e.g., Alamut Visual Plus™ and Mutation Tester), these frameshift variants cause NMD and affect the structure and function of WDR62 and MTR proteins; moreover, these variants have deleterious and pathogenic effects.

The WDR62 gene is located on chromosome 19q13.12. This gene has 32 exons and 50230 base pairs and encodes 1,523 amino acids (20). It has previously been reported that recessive mutations in this gene are related to MCPH with additional abnormal phenotypes (20, 21, 30, 31). WDR62 expression is high in the developing brain in neural progenitors and postmitotic neurons, localized in the ventricular and subventricular zones and the cortical plates (21, 32).

Finlay et al and Faheem et al proved that WDR62 mutations lead to a decrease in the generation of cerebellar cortical neurons during the early stages of neurogenesis, confirming the phenotypic heterogeneity observed in MCPH2 (33, 34). Specifically, WDR62 expression is at the spindle poles in neural progenitor cells during prophase to metaphase transition and is not expressed during the interphase (21, 35, 36). Farag et al found that mutations in WDR62 cause defects in centrosome integrity and the spindle which is the main cause of MCPH2 microcephaly (37). For further confirmation, by examining the expression of this gene in Hella cells, it was proven that WDR62 accumulates in the centrosome (37). Furthermore, the siRNA knockdown of WDR62 decreases the proliferation of cortical progenitors and centrosome integrity, and disturbs the orientation of mitotic spindles (34, 37). The medical and MRI evaluation of the proband showed the brain and cerebellar atrophy and polymicrogyria in the proband, confirming that the MCPH2 in the proband can be due to the novel disruptive variants in the WDR62 gene. Other neurological symptoms in the proband, including delayed motor skills, seizures, developmental delay, mental retardation, low HC, and diffused mild hypotonia, further confirmed MCPH2. Additionally, based on the history of two miscarriages in this pedigree, it may be concluded that WDR62 variants or/and MTR variants may cause these miscarriages.

The MTR gene is located on chromosome 1q43. It encodes 5-methyltetrahydrofolate-homocysteine methyltransferase which catalyzes the final step in methionine biosynthesis. The mutation in this gene is responsible for methylcobalamin deficiency

complementation group G, which is characterized by homocystinuria-megaloblastic anemia and cblG complementation type (38-40). The cblG phenotype is inherited as an autosomal recessive and has clinical and biochemical heterogeneity (41, 42). As shown in the proband, it has previously been reported that the cblG phenotype can be present with neurological symptoms (43-47). Komulainen-Ebrahim et al and Kripps et al observed the neurological symptoms as a result of methionine synthase deficiency, along with the need for early treatment to reduce the neurological symptoms (48, 49).

Conclusion

In general, WES is an effective and accurate genetic diagnosis tool in genetically and phenotypically heterogeneous disorders such as MCPH2, especially when it is associated with different comorbidities. Further, the WES analysis of families with MCPH2 is required for early diagnosis and management of the patient when the disease is accompanied by methionine synthase deficiency. Our results expand the mutation spectrum of the WDR62 and MTR genes and will be beneficial in the genetic diagnosis of MCPH2 and homocystinuria-megaloblastic anemia patients, genetic counseling, and prenatal diagnosis and thus reduce the rate of this disease.

Authors' Contributions

Study conception and design: All authors; Data collection: TD and AK; Analysis and interpretation of the results: All authors; Writing, reviewing, editing, visualization, and supervision: TD and DMKT; Project administration and funding acquisition: DMKT.

Conflict of Interest Disclosures

The authors declared no conflict of interests.

Ethical Statement

The study was approved by the Institutional Review Board of Sabzevar University of Medical Sciences (with the ethics code of Medsab.REC.94.170).

Informed Consent

The informed consent form was obtained from all patients.

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References

- Hanzlik E, Gigante J. Microcephaly. *Children (Basel)*. 2017;4(6):47. doi: 10.3390/children4060047.
- Heide M, Huttner WB. Human-specific genes, cortical progenitor cells, and microcephaly. *Cells*. 2021;10(5):1209. doi: 10.3390/cells10051209.
- Alcantara D, O'Driscoll M. Congenital microcephaly. *Am J Med Genet C Semin Med Genet*. 2014;166C(2):124-39. doi: 10.1002/ajmg.c.31397.
- von der Hagen M, Pivarcsi M, Liebe J, von Bernuth H, Didonato N, Hennermann JB, et al. Diagnostic approach to microcephaly in childhood: a two-center study and review of the literature. *Dev Med Child Neurol*. 2014;56(8):732-41. doi: 10.1111/dmcn.12425.
- Baxter PS, Rigby AS, Rotsaert MH, Wright I. Acquired microcephaly: causes, patterns, motor and IQ effects, and associated growth changes. *Pediatrics*. 2009;124(2):590-5. doi: 10.1542/peds.2008-2784.
- Abuelo D. Microcephaly syndromes. *Semin Pediatr Neurol*. 2007;14(3):118-27. doi: 10.1016/j.spen.2007.07.003.
- Mai CT, Kucik JE, Isenburg J, Feldkamp ML, Marengo LK, Bugenske EM, et al. Selected birth defects data from population-based birth defects surveillance programs in the United States, 2006 to 2010: featuring trisomy conditions. *Birth Defects Res A Clin Mol Teratol*. 2013;97(11):709-25. doi: 10.1002/bdra.23198.
- Jean F, Stuart A, Tarailo-Graovac M. Dissecting the genetic and etiological causes of primary microcephaly. *Front Neurol*. 2020;11:570830. doi: 10.3389/fneur.2020.570830.
- Online Mendelian Inheritance in Man OMIM, Johns Hopkins University (Baltimore, MD). Available from: <https://omim.org/>.
- Guerrini R, Dobyns WB. Malformations of cortical development: clinical features and genetic causes. *Lancet Neurol*. 2014;13(7):710-26. doi: 10.1016/S1474-4422(14)70040-7.
- Sgourdou P, Mishra-Gorur K, Saotome I, Henagariu O, Tuysuz B, Campos C, et al. Disruptions in asymmetric centrosome inheritance and WDR62-Aurora kinase B interactions in primary microcephaly. *Sci Rep*. 2017;7:43708. doi: 10.1038/srep43708.
- Barbelanne M, Tsang WY. Molecular and cellular basis of autosomal recessive primary microcephaly. *Biomed Res Int*. 2014;2014:547986. doi: 10.1155/2014/547986.
- Zaqout S, Morris-Rosendahl D, Kaindl AM. Autosomal recessive primary microcephaly (MCPH): an update. *Neuropediatrics*. 2017;48(3):135-42. doi: 10.1055/s-0037-1601448.
- Kristofova M, Ori A, Wang ZQ. Multifaceted microcephaly-related gene MCPH1. *Cells*. 2022;11(2):275. doi: 10.3390/cells11020275.
- Wang R, Khan A, Han S, Zhang X. Molecular analysis of 23 Pakistani families with autosomal recessive primary microcephaly using targeted next-generation sequencing. *J Hum Genet*. 2017;62(2):299-304. doi: 10.1038/jhg.2016.128.
- Nardello R, Fontana A, Antona V, Beninati A, Mangano GD, Stallone MC, et al. A novel mutation of WDR62 gene associated with severe phenotype including infantile spasm, microcephaly, and intellectual disability. *Brain Dev*. 2018;40(1):58-64. doi: 10.1016/j.braindev.2017.07.003.
- Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini SS, Chen W, et al. Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature*. 2011;478(7367):57-63. doi: 10.1038/nature10423.
- McDonnell LM, Warman Chardon J, Schwartzentruber J, Foster D, Beaulieu CL, Majewski J, et al. The utility of exome sequencing for genetic diagnosis in a familial microcephaly epilepsy syndrome. *BMC Neurol*. 2014;14:22. doi: 10.1186/1471-2377-14-22.
- Miyamoto T, Akutsu SN, Fukumitsu A, Morino H, Masatsuna Y, Hosoba K, et al. PLK1-mediated phosphorylation of WDR62/MCPH2 ensures proper mitotic spindle orientation. *Hum Mol Genet*. 2017;26(22):4429-40. doi: 10.1093/hmg/ddx330.
- Bilgüvar K, Öztürk AK, Louvi A, Kwan KY, Choi M, Tatli B, et al. Whole-exome sequencing identifies recessive WDR62 mutations in severe brain malformations. *Nature*. 2010;467(7312):207-10. doi: 10.1038/nature09327.
- Yu TW, Mochida GH, Tischfield DJ, Sgaier SK, Flores-Sarnat L, Sergi CM, et al. Mutations in WDR62, encoding a centrosome-associated protein, cause microcephaly with

- simplified gyri and abnormal cortical architecture. *Nat Genet.* 2010;42(11):1015-20. doi: [10.1038/ng.683](https://doi.org/10.1038/ng.683).
22. Rump P, Jazayeri O, van Dijk-Bos KK, Johansson LF, van Essen AJ, Verheij JB, et al. Whole-exome sequencing is a powerful approach for establishing the etiological diagnosis in patients with intellectual disability and microcephaly. *BMC Med Genomics.* 2016;9:7. doi: [10.1186/s12920-016-0167-8](https://doi.org/10.1186/s12920-016-0167-8).
 23. Oza AM, DiStefano MT, Hemphill SE, Cushman BJ, Grant AR, Siegert RK, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. *Hum Mutat.* 2018;39(11):1593-613. doi: [10.1002/humu.23630](https://doi.org/10.1002/humu.23630).
 24. Biesecker LG, Harrison SM. The ACMG/AMP reputable source criteria for the interpretation of sequence variants. *Genet Med.* 2018;20(12):1687-8. doi: [10.1038/gim.2018.42](https://doi.org/10.1038/gim.2018.42).
 25. Zaqout S, Kaindl AM. Autosomal recessive primary microcephaly: not just a small brain. *Front Cell Dev Biol.* 2021;9:784700. doi: [10.3389/fcell.2021.784700](https://doi.org/10.3389/fcell.2021.784700).
 26. Naveed M, Kazmi SK, Amin M, Asif Z, Islam U, Shahid K, et al. Comprehensive review on the molecular genetics of autosomal recessive primary microcephaly (MCPH). *Genet Res (Camb).* 2018;100:e7. doi: [10.1017/s0016672318000046](https://doi.org/10.1017/s0016672318000046).
 27. 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(5):717-28. doi: [10.1086/429930](https://doi.org/10.1086/429930).
 28. Corry PC. Consanguinity and prevalence patterns of inherited disease in the UK Pakistani community. *Hum Hered.* 2014;77(1-4):207-16. doi: [10.1159/000362598](https://doi.org/10.1159/000362598).
 29. Saki-Malehi A, Seddigh-Rad G, Sayyahi A, Mousavi-Far F, Veysi M, Rahim F. The pattern of inherited microcephaly and role of the consanguineous marriage: a study from Southwestern Iran. *Ethiop J Health Dev.* 2017;31(2):119-23.
 30. Nicholas AK, Khurshid M, Désir J, Carvalho OP, Cox JJ, Thornton G, et al. WDR62 is associated with the spindle pole and is mutated in human microcephaly. *Nat Genet.* 2010;42(11):1010-4. doi: [10.1038/ng.682](https://doi.org/10.1038/ng.682).
 31. 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. *Eur J Hum Genet.* 1999;7(7):815-20. doi: [10.1038/sj.ejhg.5200385](https://doi.org/10.1038/sj.ejhg.5200385).
 32. 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. doi: [10.1186/1750-1172-6-39](https://doi.org/10.1186/1750-1172-6-39).
 33. Finlay BL, Darlington RB. Linked regularities in the development and evolution of mammalian brains. *Science.* 1995;268(5217):1578-84. doi: [10.1126/science.7777856](https://doi.org/10.1126/science.7777856).
 34. Faheem M, Naseer MI, Rasool M, Chaudhary AG, Kumosani TA, Ilyas AM, et al. Molecular genetics of human primary microcephaly: an overview. *BMC Med Genomics.* 2015;8(Suppl 1):S4. doi: [10.1186/1755-8794-8-s1-s4](https://doi.org/10.1186/1755-8794-8-s1-s4).
 35. Bogoyevitch MA, Yeap YY, Qu Z, Ngoei KR, Yip YY, Zhao TT, et al. WD40-repeat protein 62 is a JNK-phosphorylated spindle pole protein required for spindle maintenance and timely mitotic progression. *J Cell Sci.* 2012;125(Pt 21):5096-109. doi: [10.1242/jcs.107326](https://doi.org/10.1242/jcs.107326).
 36. Thornton GK, Woods CG. Primary microcephaly: do all roads lead to Rome? *Trends Genet.* 2009;25(11):501-10. doi: [10.1016/j.tig.2009.09.011](https://doi.org/10.1016/j.tig.2009.09.011).
 37. Farag HG, Froehler S, Oexle K, Ravindran E, Schindler D, Staab T, et al. Abnormal centrosome and spindle morphology in a patient with autosomal recessive primary microcephaly type 2 due to compound heterozygous WDR62 gene mutation. *Orphanet J Rare Dis.* 2013;8:178. doi: [10.1186/1750-1172-8-178](https://doi.org/10.1186/1750-1172-8-178).
 38. Leclerc D, Campeau E, Goyette P, Adjalla CE, Christensen B, Ross M, et al. Human methionine synthase: cDNA cloning and identification of mutations in patients of the cblG complementation group of folate/cobalamin disorders. *Hum Mol Genet.* 1996;5(12):1867-74. doi: [10.1093/hmg/5.12.1867](https://doi.org/10.1093/hmg/5.12.1867).
 39. Wilson A, Leclerc D, Saberi F, Campeau E, Hwang HY, Shane B, et al. Functionally null mutations in patients with the cblG-variant form of methionine synthase deficiency. *Am J Hum Genet.* 1998;63(2):409-14. doi: [10.1086/301976](https://doi.org/10.1086/301976).
 40. Watkins D, Ru M, Hwang HY, Kim CD, Murray A, Philip NS, et al. Hyperhomocysteinemia due to methionine synthase deficiency, cblG: structure of the MTR gene, genotype diversity, and recognition of a common mutation, P1173L. *Am J Hum Genet.* 2002;71(1):143-53. doi: [10.1086/341354](https://doi.org/10.1086/341354).
 41. Watkins D, Rosenblatt DS. Functional methionine synthase deficiency (cblE and cblG): clinical and biochemical heterogeneity. *Am J Med Genet.* 1989;34(3):427-34. doi: [10.1002/ajmg.1320340320](https://doi.org/10.1002/ajmg.1320340320).
 42. Rosenblatt DS, Thomas IT, Watkins D, Cooper BA, Erbe RW. Vitamin B12 responsive homocystinuria and megaloblastic anemia: heterogeneity in methylcobalamin deficiency. *Am J Med Genet.* 1987;26(2):377-83. doi: [10.1002/ajmg.1320260216](https://doi.org/10.1002/ajmg.1320260216).
 43. Outteryck O, de Sèze J, Stojkovic T, Cuisset JM, Dobbelaere D, Delalande S, et al. Methionine synthase deficiency: a rare cause of adult-onset leukoencephalopathy. *Neurology.* 2012;79(4):386-8. doi: [10.1212/WNL.0b013e318260451b](https://doi.org/10.1212/WNL.0b013e318260451b).
 44. Arakawa T, Narisawa K, Tanno K, Hirooka Y, Ono T. Megaloblastic anemia and mental retardation associated with hyperfolc-acidemia: probably due to N5 methyltetrahydrofolate transferase deficiency. *Tohoku J Exp Med.* 1967;93(1):1-22. doi: [10.1620/tjem.93.1](https://doi.org/10.1620/tjem.93.1).
 45. Carmel R, Watkins D, Goodman SI, Rosenblatt DS. Hereditary defect of cobalamin metabolism (cblG mutation) presenting as a neurologic disorder in adulthood. *N Engl J Med.* 1988;318(26):1738-41. doi: [10.1056/nejm198806303182607](https://doi.org/10.1056/nejm198806303182607).
 46. Pollini L, Tolve M, Nardecchia F, Galosi S, Carducci C, di Carlo E, et al. Multiple sclerosis and intracellular cobalamin defect (MMACHC/PRDX1) comorbidity in a young male. *Mol Genet Metab Rep.* 2020;22:100560. doi: [10.1016/j.ymgmr.2019.100560](https://doi.org/10.1016/j.ymgmr.2019.100560).
 47. Motte J, Kneiphof J, Straßburger-Krogias K, Pitarokouli K, Fisse AL, Kappos L, et al. Hereditary defect of cobalamin metabolism with adolescence onset resembling multiple sclerosis: 41-year follow up in two cases. *Ther Adv Neurol Disord.* 2019;12:1756286419872115. doi: [10.1177/1756286419872115](https://doi.org/10.1177/1756286419872115).
 48. Komulainen-Ebrahim J, Saastamoinen E, Rahikkala E, Helander H, Hinttala R, Risteli L, et al. Intractable epilepsy due to MTR deficiency: importance of homocysteine analysis. *Neuropediatrics.* 2017;48(6):467-72. doi: [10.1055/s-0037-1603976](https://doi.org/10.1055/s-0037-1603976).
 49. Kripps KA, Sremba L, Larson AA, Van Hove JLK, Nguyen H, Wright EL, et al. Methionine synthase deficiency: variable clinical presentation and benefit of early diagnosis and treatment. *J Inherit Metab Dis.* 2022;45(2):157-68. doi: [10.1002/jimd.12448](https://doi.org/10.1002/jimd.12448).