J Human Gen Genom. 2018 July; 2(1):e81239. doi: 10.5812/jhgg.81239.

Published online 2018 July 15. **Research Article**

Novel Homozygous Nonsense Mutation of PMS1 in a Patient with Multifocal Schwanomatosis Schwanomatosis Resembling Neurofibromatosis Type 2

Mohammad Taghi Akbari,[1,2] and Mojgan Ataei Kachui[2,*]

[1]Medical Genetics Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, IR Iran
[2]Tehran Medical Genetics Laboratory, Tehran, IR Iran

[*]*Corresponding author*: Mojgan Ataei Kachui, Tehran Medical Genetics Laboratory, Taleghani Ave, Tehran, IR Iran. Tel: +98-2188896868, Fax: +98-2188896868, E-mail: mojganataei@gmail.com

Received 2018 July 01; **Accepted** 2018 July 10.

Abstract

Background: The type II form of neurofibromatosis (NF2) is an autosomal dominant multiple neoplasia syndrome characterized by tumors of the eighth cranial nerve (usually bilateral), meningiomas of the brain, and schwannomas of the dorsal roots of the spinal cord. The incidence of neurofibromatosistype II is 1 in 25,000 live births.
Methods: To further understand the genetic spectrum of NF2, we analyzed an individual affected with multifocal schwanomatosis by whole exome sequencing. Potential candidate mutations were checked in additional family members to determine if the putative mutation segregated with disease status.
Results: No pathogenic variant was identified in NF1 and NF2 genes however, a novel nonsense homozygous mutation p.Q675X in PMS1 gene was identified. Direct sequencing confirmed that the patient is homozygous and her parents are heterozygous for the identified variant.
Conclusions: To the best of our knowledge it is the first report of involvement of PMS1 mutations in NF2. However, genetic testing of NF1 and NF2 genes in affected tissues to rule out somatic mutations as well as functional study for the identified variant are required to clarify under what circumstances mutations of this gene cause tumorigenesis.

Keywords: Neurofibromatosis Type 2, PMS1, Schwanomatosis

1. Background

Neurofibromatosis type 2 is a rare multiple neoplasia syndrome caused by mutations in the NF2 tumour suppressor gene on chromosome 22q. Around one in 25000 livebirths are born with a mutation in this gene with approximately 100% penetrance by 60 years of age. Half of patients have hereditary form of the disease while the remainder have a de novo somatic mutation in NF2 gene. Patients are characterized by schwannomas, multiple meningiomas, spinal tumours, peripheral neuropathy, eye and skin lesions (1).

Mismatch repair (MMR) is a conserved pathway to preserve genome stability. The main role of the MMR system is the rectification of single nucleotide substitutions and insertion/deletion loops (IDLs) which occur during DNA replication (2).

The human MMR proteins homologues of the bacterial MutS consist of hMSH2, hMSH3 and hMSH6. Additionally, four human homologues of the bacterial MutL gene comprise hMLH1, hPMS1, hPMS2 and hMLH3 (3).

Several heterodimers of MLH1 have been recognized; which include complex of MLH1 and PMS2, complex of MLH1 and PMS1 and complex of MLH1 and MLH3 (4).

Mismatch repair cancer syndrome (MMRCS OMIM#276300) is an autosomal recessive rare childhood predisposition cancer syndrome which results from germ line mutations in one of the four MMR genes; MLH1, MSH2, MSH6 or PMS2 (5).

The tumour spectrum in patients carrying MMR gene mutation is very broad, including (6): hematological malignancies (7); brain tumors (8); multiple intestinal tract tumors (9, 10); and other malignancies. Moreover, some MMRCS patients present phenotypic features similar to neurofibromatosis type 1 (NF1) (2).

Here we report a novel homozygous nonsense mutation in PMS1 gene associated with multifocal schwanomatosis.

2. Methods

2.1. Subject Description

Index patient was a 30 years old female affected with unilateral acoustic schwanomatosis which had led to unilateral deafness. Signs of hearing impairment began from the age of 23 and gradually progressed to deafness. Moreover, schwannoma tumors in cervical and mandibular regions were removed by surgery at the age of 26. The patient and her two apparently healthy siblings are from consanguineous parents. The pedigree, including all family members is depicted in Figure 1.

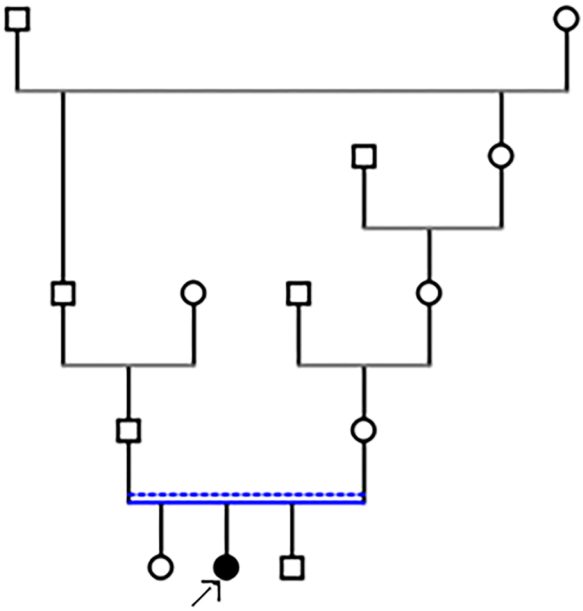

Figure 1. Family pedigree

2.2. Sample Preparation

Informed consent was taken from all participants involved in this study. Peripheral blood sampling was performed from affected individual and her parents. DNA was extracted using the conventional salting-out method. High purity DNA (OD260/OD280 > 1.8) was used for whole exome sequencing.

2.3. Whole Exome Sequencing

Whole exome sequencing of the patient was performed at BGI China. Complete genomics sequencing, known as combinatorial probe-anchor ligation (cPALTM) was employed to identify the bases of DNA Nano-Ball (DNB) populated in high-density DNA nanoarray.

2.4. Bioinformatics Analysis

Whole exome sequencing data was analyzed using Complete Genomics Analysis tools (CGA tools version 1.3.0 build 9; http://www.completegenomics.com/sequence-data/cgatools/). All high-quality trimmed reads were aligned to hg19 reference genome using the teramap. To remove common variants and alignment artifacts, variants with an allele frequency > 1% in the 1000 genomes database (http://www.1000genomes.org), higher than 1% in exome aggregation consortium (ExAC) were filtered out. We used a recessive genetic model which required the variant to be present in tested individual in homozygous form. To predict the potential impact of sequence variants on protein function, PolyPhen-2, SIFT and MutationTaster scores were assigned to each variant. We also obtained conservation scores using GERP and PhastCons to predict mutation impact based on evolutionary constraint analyses.

2.5. Validation of Mutations and Segregation Analysis

To validate the causal mutation(s) and mutation-disease segregation analysis in the pedigree, Sanger sequencing was carried out in affected and unaffected family members from whom DNA was available. Primers were designed using GENERUNR v3.4.0.0 and amplicons were sequenced by standard Sanger's sequencing technique using BigDye® terminator (Invitrogen, ABI, Foster City, CA). Primer sequences are available upon request.

3. Results

No pathogenic variant was identified in NF1 and NF2 genes however, however, filtering of variants identified a novel nonsense homozygous mutation p.Q675X in PMS1 gene in accordance with mode of inheritance in the pedigree. Direct sequencing confirmed that the patient is homozygous and her parents are heterozygous for the identified variant (Figure 2).

4. Discussion

Sequence integrity of the human genome is guaranteed by DNA repair systems such as MMR pathway. This system ensures the repairing of mismatched nucleotides and insertion-deletion loops that arise during DNA replication and recombination. DNA MMR proteins are encoded by evolutionary highly conserved genes (11).

Post meiotic segregation increased 1 (PMS1) gene is located on chr 2q31.1 contains 12 coding exons and encodes a 932 amino acid protein.

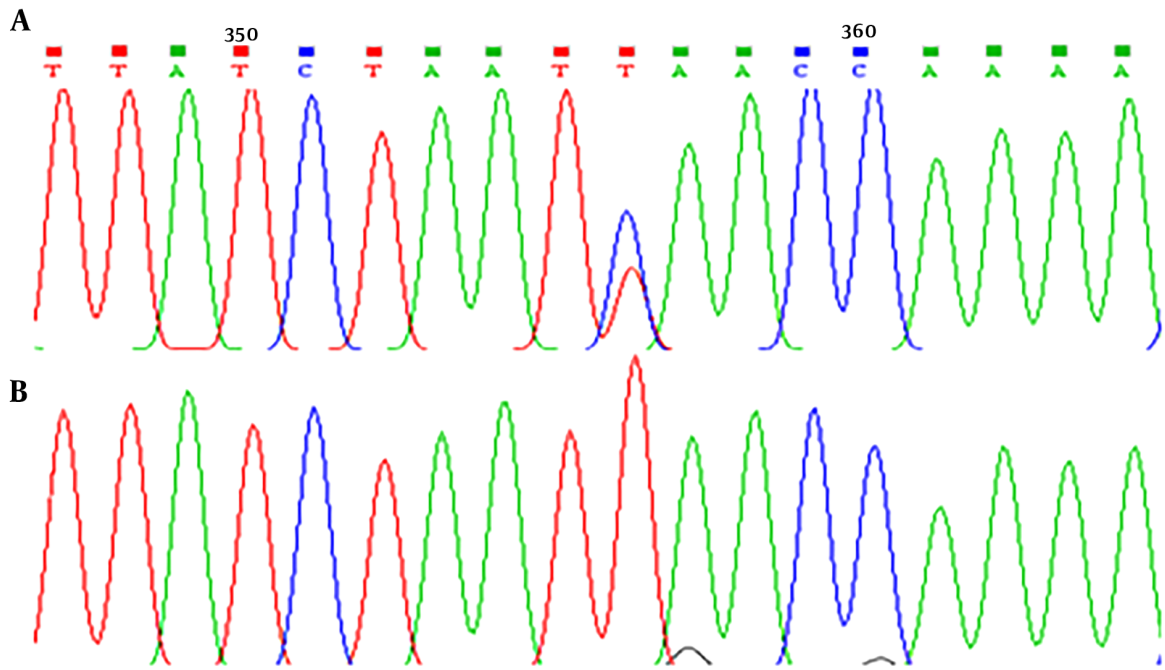

Figure 2. A, Sanger sequencing of healthy heterozygous parents for PMS1 mutation; B, Sanger sequencing of homozygous affected proband for PMS1 mutation.

In humans, hMLH1 and hPMS2 work as a heterodimer (12) also hPMS1 with hMLH1 forms another heterodimer and function together (13). A tract of 36 amino acid residues in hPMS1 and hPMS2 interact strongly with hMLH1 (14).

Although hPMS1 knockout mice showed mutation frequency higher than normal level (15) mutations in this gene are not frequent (16).

Nevertheless, Leung et al. (11), have shown the expression of hPMS1 gene in different cancer cells such as colorectal, gastric and cervical cancers and that this protein associates with hMLH1. They explained that the redundancy of hPMS1 and hPMS2 may describe the cause of low mutation frequency of these genes in cancers. Moreover, hPMS1 germline mutation was found in one HNPCC family (16).

Kondo et al. (14), showed that MutL homologous act competitively for the interacting domain in hMLH1. Late onset disease in our patient may indicate reparative role of other counterparts in MMR process in the absence of PMS1.

To the best of our knowledge it is the first report of involvement of PMS1 mutations in NF2. However, genetic testing of NF1 and NF2 genes in affected tissues to rule out somatic mutations as well as functional study for the identified variant are required to clarify under what circumstances mutations of this gene cause tumorigenesis.

Acknowledgments

We are grateful to the patient and her family for participation.

References

1. Asthagiri AR, Parry DM, Butman JA, Kim HJ, Tsilou ET, Zhuang Z, et al. Neurofibromatosis type 2. *Lancet*. 2009;**373**(9679):1974–86. doi: 10.1016/S0140-6736(09)60259-2. [PubMed: 19476995]. [PubMed Central: PMC4748851].
2. Baas AF, Gabbett M, Rimac M, Kansikas M, Raphael M, Nievelstein RA, et al. Agenesis of the corpus callosum and gray matter heterotopia in three patients with constitutional mismatch repair deficiency syndrome. *Eur J Hum Genet*. 2013;**21**(1):55–61. doi: 10.1038/ejhg.2012.117. [PubMed: 22692065]. [PubMed Central: PMC3522206].
3. Nunn J, Nagini S, Risk JM, Prime W, Maloney P, Liloglou T, et al. Allelic imbalance at the DNA mismatch repair loci, hMSH2, hMLH1, hPMS1, hPMS2 and hMSH3, in squamous cell carcinoma of the head and neck. *Oral Oncol*. 2003;**39**(2):115–29. [PubMed: 12509964].
4. Bridge G, Rashid S, Martin SA. DNA mismatch repair and oxidative DNA damage: implications for cancer biology and treatment. *Cancers (Basel)*. 2014;**6**(3):1597–614. doi: 10.3390/cancers6031597. [PubMed: 25099886]. [PubMed Central: PMC4190558].
5. Wimmer K, Etzler J. Constitutional mismatch repair-deficiency syndrome: have we so far seen only the tip of an iceberg? *Hum Genet*. 2008;**124**(2):105–22. doi: 10.1007/s00439-008-0542-4. [PubMed: 18709565].
6. Wimmer K, Kratz CP. Constitutional mismatch repair-deficiency syndrome. *Haematologica*. 2010;**95**(5):699–701. doi: 10.3324/haematol.2009.021626. [PubMed: 20442441]. [PubMed Central: PMC2864372].

7. Ripperger T, Beger C, Rahner N, Sykora KW, Bockmeyer CL, Lehmann U, et al. Constitutional mismatch repair deficiency and childhood leukemia/lymphoma–report on a novel biallelic MSH6 mutation. *Haematologica*. 2010;**95**(5):841–4. doi: 10.3324/haematol.2009.015503. [PubMed: 20015892]. [PubMed Central: PMC2864393].
8. Johannesma PC, van der Klift HM, van Grieken NC, Troost D, Te Riele H, Jacobs MA, et al. Childhood brain tumours due to germline bi-allelic mismatch repair gene mutations. *Clin Genet*. 2011;**80**(3):243–55. doi: 10.1111/j.1399-0004.2011.01635.x. [PubMed: 21261604].
9. Durno CA, Holter S, Sherman PM, Gallinger S. The gastrointestinal phenotype of germline biallelic mismatch repair gene mutations. *Am J Gastroenterol*. 2010;**105**(11):2449–56. doi: 10.1038/ajg.2010.215. [PubMed: 20531397].
10. Herkert JC, Niessen RC, Olderode-Berends MJ, Veenstra-Knol HE, Vos YJ, van der Klift HM, et al. Paediatric intestinal cancer and polyposis due to bi-allelic PMS2 mutations: case series, review and follow-up guidelines. *Eur J Cancer*. 2011;**47**(7):965–82. doi: 10.1016/j.ejca.2011.01.013. [PubMed: 21376568].
11. Leung WK, Kim JJ, Wu L, Sepulveda JL, Sepulveda AR. Identification of a second MutL DNA mismatch repair complex (hPMS1 and hMLH1) in human epithelial cells. *J Biol Chem*. 2000;**275**(21):15728–32. doi: 10.1074/jbc.M908768199. [PubMed: 10748105].
12. Kolodner RD, Marsischky GT. Eukaryotic DNA mismatch repair. *Curr Opin Genet Dev*. 1999;**9**(1):89–96. [PubMed: 10072354].
13. Raschle M, Marra G, Nystrom-Lahti M, Schar P, Jiricny J. Identification of hMutLbeta, a heterodimer of hMLH1 and hPMS1. *J Biol Chem*. 1999;**274**(45):32368–75. [PubMed: 10542278].
14. Kondo E, Horii A, Fukushige S. The interacting domains of three MutL heterodimers in man: hMLH1 interacts with 36 homologous amino acid residues within hMLH3, hPMS1 and hPMS2. *Nucleic Acids Res*. 2001;**29**(8):1695–702. [PubMed: 11292842]. [PubMed Central: PMC31313].
15. Prolla TA, Baker SM, Harris AC, Tsao JL, Yao X, Bronner CE, et al. Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair. *Nat Genet*. 1998;**18**(3):276–9. doi: 10.1038/ng0398-276. [PubMed: 9500552].
16. Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, et al. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature*. 1994;**371**(6492):75–80. doi: 10.1038/371075a0. [PubMed: 8072530].