



Original Article

A Missense Mutation in the HNMT Gene Responsible for Autosomal Recessive Intellectual Disability in an Iranian Family with Consanguineous Marriage

Seyed Hamidreza Mirabutalebi^{1,5} Ph.D., Mohammadreza Dehghani² MD., Ph.D., Nasrin Ghasemi³ MD., Ph.D., Mohammad yahya Vahidi Mehjardi² Ph.D., Mojtaba Movahedinia⁴ MD., Seyed Mehdi Kalantar^{1*} Ph.D.

¹ Department of Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

² Medical Genetics Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

³ Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

⁴ Department of Pediatrics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

⁵ Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

ABSTRACT

Article history

Received: 22 Feb 2022

Accepted: 10 Apr 2022

Available online: 15 Oct 2022

Keywords

Consanguineous family

HNMT gene

Intellectual disability

Whole exome sequencing

Background and Aims: One of the neurotransmitters in the brain is Histamine which acts as several biological mechanism regulators like inflammation, gastric acid secretion, and neuromodulation. Inactivation of Histamine occurs by histamine N-methyltransferase (HNMT) enzyme. The HNMT transfers a methyl group from S-adenosyl-L-methionine to Histamine and is the main process for the termination of neurotransmission actions of Histamine in the mammalian central nervous system.

Materials and Methods: In this case, a family was referred to the genetic clinic to diagnose the cause of their disorder. The clinical form, pedigree, and questionnaire were completed for the family, and the parents gave their written consent for all tests and photographs publication. Both siblings have moderate learning and intellectual disability. Whole exome sequencing was performed and Sanger sequencing for co-segregation was used.

Results: Bioinformatics analysis revealed a homozygous missense variant in *HNMT* (c.623T>C p.Leu208Pro) which causes non-syndromic autosomal recessive intellectual disability in this consanguineous family. Analysis of segregation confirmed this mutation. P.Leu208Pro mutation reduces the stability of the protein, which reduces the inactivation of Histamine.

Conclusion: *HNMT* should be considered an important gene in the genetic evaluation of consanguineous families with intellectual disability.

Introduction

Intellectual disability (ID) is a neurodevelopmental disease manifested by intellectual and adaptive behavior disturbance, and this disorder developed before the age of 18 during the neurodevelopment stage [1]. It is one of the most crucial concerns in health care. According to genetics, it is a heterogeneous disorder that occurs by different causative factors, including chromosomal aberrations and gene mutations. Noticeable numbers of people are affected by ID all around the world. It is estimated at approximately 1%, with a 1 to 3% prevalence in developed countries, which is twice as high as in undeveloped countries. Non-syndromic and syndromic are two groups of ID. In Non-syndromic ID, the clinical signs are mental retardation and learning disability, whereas in syndromic ID, in addition to mental retardation, other clinical features may also exist [2]. Genetic research has focused on the use of broad genomic techniques due to the heterogeneity of ID. These include genomic microarrays and, more recently, next-generation sequencing (NGS), including gene panels, whole exome, or genome sequencing (WES or WGS). Diagnosis of neurodevelopmental disorders has revolutionized by applying NGS methods, especially in ID disorder [3].

Histamine, a biogenic amino, regulates the secretion of gastric acid [4], acts as a neurotransmitter in the central nervous system, involved in inflammation and immune process, which is stored in basophils, mast cells of the airway and enterochromaffin cells [5]. Two

enzymes regulate the metabolism of Histamine; one of them is Histamine N-methyltransferase (HNMT), a cytoplasmic protein of the methyltransferase superfamily. In the mammalian brain, neurotransmitter action of histamine terminates by HNMT [6, 7]. The activity of this enzyme is observed in different human organs such as the kidney, liver, spleen, prostate, ovary, intestine, spinal cord, heart, brain, placenta, lung, stomach, and thyroid gland [8]. The length of this gene is about 34 kb and contains six exons placed on chromosome 2q22.1 [9, 10]. A decrease in *HNMT* transcription impairs histamine metabolism, which causes various disorders, namely asthma, bronchial hyper-responsiveness, and neurological disease [11, 12]. For instance, the expression level of *HNMT* alters in various neurological diseases; in depression, *HNMT* expression decreases as well as in Down syndrome. Also, the expression of HNMT mRNA increases in women with Alzheimer's disease [13]. In the present study, we found a mutation in *the HNMT* gene (c.623T>C p.Leu208Pro) in an Iranian family with consanguineous marriage affected by ID.

Materials and Methods

The study was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.MEDICINE.REC.1399.307). Families completed the clinical form, pedigree, and questionnaire. Besides, parents gave their written consent for all tests and photographs publication. Peripheral blood

samples of affected cases, their family, and healthy child of the family were collected in complete blood count tubes containing the anticoagulant Ethylenediaminetetraacetic acid. DNA was extracted from blood samples by the salting out method. Then, WES was performed on an Illumina platform HiSeq4000 machine (Illumina Inc., San Diego, CA, USA). The method coverage was 100-300X with a sensitivity of more than 99%. To control sequence read quality in the FASTQ file format FASTQC was used (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). The aligned file (BAM file) was produced using the Burrows-Wheeler Aligner (BWA) algorithm, and the hg19 was the reference genome. Picard Tools was utilized to mark and remove the duplicates and for base quality score recalibration (BQSR). The variant calling format (VCF) file was generated via the GATK HaplotypeCaller. ANNOVAR software and databases such as dbSNP, ExAC Browser or GnomAD, ClinVar, and InterVar were used to annotate the SNVs and indels in the raw VCF file. The causative variant was found by excluding the first intronic, synonymous, non-frameshift, and the variants out of the splice site regions. Then, the minor allele frequency variants higher than 1% in the population were excluded according to dbSNP databases and ExAC Browser. Causative pathogenic variants were investigated in the remaining variants by using the ClinVar database. Eventually, in silico tools containing SIFT, PROVEAN, MutationTaster, and LRT were used to determine variant effects on the protein and possible pathogenic changes in

affected cases. Sanger sequencing method and Co-segregation was performed to confirm the variant. Primer sequence (F: ACAGCAGCCCATCAAAGTCT, R: ACCTTCCCCTCTTTCTTAGCAC) was designed by primer3 plus software. Sanger sequencing was performed by BigDye™ Terminator v3.1 Cycle Sequencing Kit and ABI-3700 DNA analyzer (Thermo Fisher).

Clinical presentation

A consanguineous family from the Yazd province in Iran was chosen, in which the first-cousin parents had seven children; five of them were healthy (IV-3 _ IV-7), and four died in an accident (IV-4 _ IV-7). Two of their girls were affected with non-syndromic ID. The individual IV-1 in the pedigree (Figure 1) is proband with forty-year-old who was born with preterm delivery. They did not have developmental delays. Development of speech, motor function, and sociality was normal. The patient could independently do her tasks, such as eating and using the toilet.

However, after a few years, her parents suspected mental disorders due to her different behaviors compared to individuals of the same age. After neurology evaluation, moderate intellectual disability and severe learning disorder were diagnosed. In pedigree, an IV-2 individual is an IV-1 sibling (Figure 1) at thirty-eight years old, born after two years of her sibling's birth. Like her sister, she has all the signs of a milder phenotype. Essential laboratory tests such as hematological and biochemical parameters were normal in both individuals. Magnetic resonance imaging scanning, as well as chromosomal,

neurological, metabolic, ophthalmological, and dermatological examinations of them, were reported to be normal. These two affected cases did not show aggressive behavior, sleep disturbance, and urine incontinence. The siblings did not have neurological disorders, autistic features, congenital malformations, or facial dysmorphisms. In these two patients, body weight and height were both normal.

Results

We performed the WES technique. After filtering the variants, a single homozygous variant in *HNMT*, rs745756308, (NM_006895.3): c.623T>C, (p.Leu208Pro) in IV-1 individual was ranked as potentially pathogenic. Moreover, Sanger sequencing analysis for family individuals was performed

and indicated co-segregation with a recessive inheritance (IV-1: homo affected, IV-2: homo affected, IV-3: healthy Hetero, III-3: healthy Hetero, IV-4: healthy Hetero) (Figure 2). Analysis of the missense variant in the *HNMT* gene was presented in Table 1. According to this table, the variant (c.623T>C) is located on chromosome 2q22.1. According to ACMG guidelines, this mutation is a likely pathogenic variant. This mutation caused autosomal recessive non-syndromic ID (Table 1). The crystal structure of HNMT protein is available in databases, based on this structure, PyMol software (4-5-0 version) was used to check possible effects of the new variant on wild type protein model (Figure 3). A significant difference was not observed in the 3D structure of HNMT protein compared to wild-type.

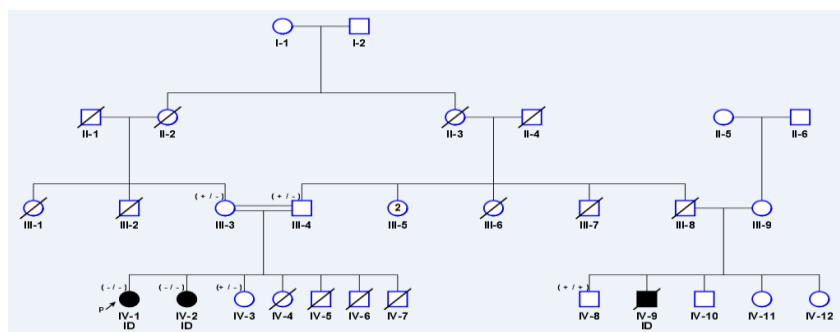


Fig. 1. Pedigree. Black symbols indicate affected individuals

Table 1. Analysis of variants identified by Whole Exome Sequencing in an Iranian family

Gene	Position		Variant type			Disease
	Cytoband	Exon	C	p	Zygoty	
HNMT	2q22.1	6	c.623 T>C	(p.Leu208Pro)	Homo T>C	Autosomal Recessive Nonsyndromic Intellectual Disability
In silico parameter						Variant classification
Inheritance	Mutation Taster	PROVEN	SIFT	LRT		ACMG guideline
Autosomal recessive	Disease-causing	Damaging	Damaging	Deleterious		Likely Pathogenic (PM2, PS3, PP3)

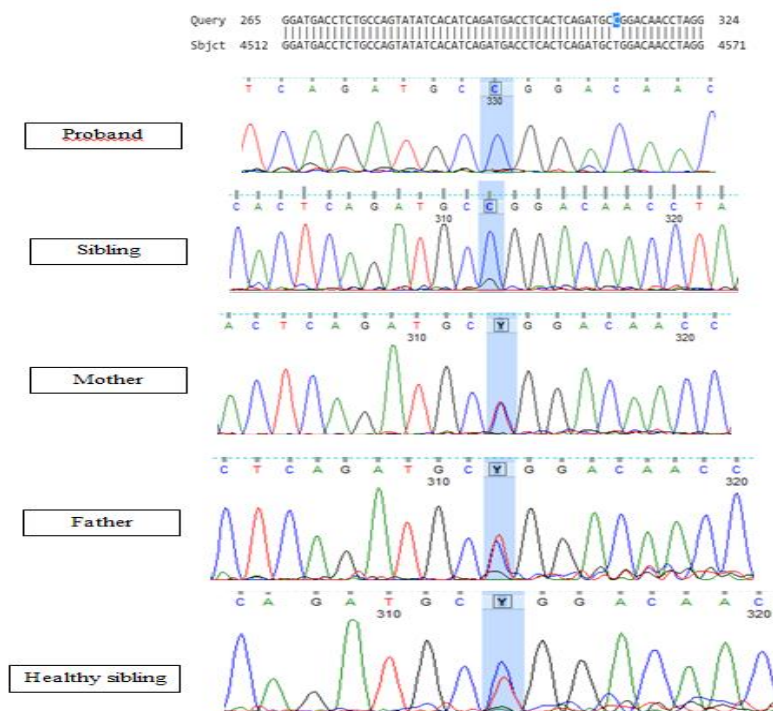


Fig. 2. Electropherogram of Sanger sequencing. Affected patients with ID, their parents, and healthy sister showing in the blue field the homozygous mutations of the index patient (T replaced by C).

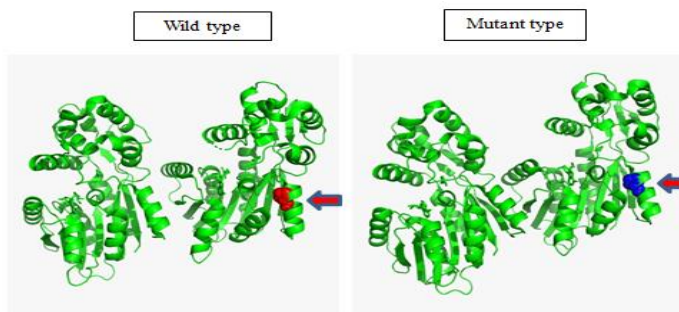


Fig. 3. p.Leu208Pro modeling within HNMT. PyMol software was used for modeling. The red arrow shows the place of residue 208 within the protein.

Discussion

Histamine regulates and controls many biological mechanisms in the adult human brain through its neurotransmitter actions. Moreover, in the development process of a rat fetus's brain, a high concentration of Histamine detects in different brain areas where differentiation of neurons occurs. It shows that Histamine as a neurogenic

element has a main role in brain development [14, 15]. Apoptosis induced by Histamine mediates via caspase activation and stimulation of the PKC- γ signaling pathway [16]. To prevent unpleasant reactions of Histamine, synthesis, and release should be regulated precisely [17]. The Histamine level is lower than the other amines in

the brain. Inactivation of Histamine occurs within a few minutes by the action of HNMT [18]. The WES method was carried out in the current study, and a homozygous missense mutation in the *HNMT* gene (c.623T>C) was detected in a consanguineous Iranian family. This study demonstrated two sisters with moderate intellectual and learning disabilities without dysmorphic features. Verhoeven et al. reported a male born from second cousins with a homozygous mutation in *HNMT*. This patient showed different phenotypes, such as autism, delay in speech, sleep disorders, and gastrointestinal diseases with moderate intellectual disability, which confirmed different actions of Histamine in males and females [19]. Heidari et al. (2015) explained this mutation in two Kurdish and Turkish families in another part of Iran with moderate ID, which is the same as the present report. Our cases did not report sleep disorders similar to Heidari et al.'s study [20].

In Verhoeven's study, the affected individual did not show any dysmorphic features, as in our study. Brain Histamine has a physiological role in sleep and stress regulation, and in ID cases who suffered from sleep disturbance and aggressive behavior, antihistaminic compounds which pass the blood-brain barrier are utilized to reduce these conditions. Aggressive behaviors and mental retardation in these cases are related to histamine N-methyltransferase deficiency [19]. MTase domain and the S domain are the two-domain structure of HNMT. The cofactor binding is performed by the MTase domain, and Histamine binding is carried out by both domains. The hydrophobic pocket contains Leu208 which has many

contacts with the residues in the same α - helix [21, 22]. Previous studies indicated that this residue is located in helix E of the MTase domain of HNMT protein and forms various hydrophobic interactions with other neighboring residues [20]. It is noticeable that L208 is located at a substantial distance from the protein's active site (ca. 18 Å from the substrate binding pocket). They suggest that mutation and amino acid replacement caused protein structure perturbations leading to compromised enzyme function. The recent analyses confirmed that side chain packing in this hydrophobic pocket is critical for the protein's structural stability. Thus, the L208P replacement can lead to loss of protein solubility, increased cytoplasm aggregation, and loss of function [23]. This confirmed characterization of the generated variants and proteins clearly suggested that position 208 in HNMT protein is susceptible to amino acid replacements resulting in a deterioration of stability and enzyme activity, which strongly depends on the properties of the amino acid side chain. Changes in *HNMT* gene stability and affinity and lack of stabilization and solubilization of Leu208Pro confirmed this replacement of function of HNMT in ID affected family. HNMT short isoform contains 126 amino acids in N-terminal (Genbank ID: NM_001024075.1; NP_001019246.1 (Q8IU56). Leu208Pro mutation, which was detected in the family, influenced the longer isoform of HNMT. Changes in *HNMT* gene stability and affinity and lack of stabilization and solubilization of Leu208Pro confirmed this replacement of function of HNMT in ID affected family.

HNMT short isoform contains 126 amino acids in N-terminal (Genbank ID: NM_001024075.1; NP_001019246.1 (Q8IU56). Leu208Pro mutation, which was detected in the family, influenced the longer isoform of HNMT. Various mutations affect stability, function, and isoforms, leading to several family clinical features influenced by ID [20]. This mutation has destructive effects on neurodevelopment and may alter the level of Histamine in the central nervous system. The *HNMT* product and catabolite levels decrease, and in addition, in the etiology of disorder in this family, N-tele-methylhistamine has the main role.

Conclusion

In this study, we demonstrated that HNMT mutation is related to the non-syndromic form of

autosomal recessive intellectual disability. The mutation of p.Leu208Pro does not depend on the protein catalytic region. The proline residue rigidity would most likely change the helix conformation, destabilizing the protein and therefore affecting protein stability and the substrate-binding site. This study's findings demonstrated that *HNMT* plays the main role in human neurodevelopment. *HNMT* should be considered as one of the important genes in the genetic evaluation of consanguineous families with ID.

Conflict of Interest

The authors have no conflict of interest to declare.

Acknowledgment

This study was supported by Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

References

- [1]. Maulik PK, Mascarenhas MN, Mathers CD, Dua T, Saxena S. Prevalence of intellectual disability: A meta-analysis of population-based studies. *Res dev disabil*. 2011; 32(2): 419-36.
- [2]. Musante L, Ropers HH. Genetics of recessive cognitive disorders. *Trends in Genetics* 2014; 30(1): 32-9.
- [3]. Anazi S, Maddirevula S, Salpietro V, Asi YT, Alsahli S, Alhashem A, et al. Expanding the genetic heterogeneity of intellectual disability. *Human genetics*. 2017; 136(11): 1419-429.
- [4]. Loiselle J, Wollin A. Mucosal histamine elimination and its effect on acid secretion in rabbit gastric mucosa. *Gastroenterology* 1993; 104(4): 1013-1020.
- [5]. Schwartz JC, Arrang JM, Garbarg M, Pollard H, Ruat M. Histaminergic transmission in the mammalian brain. *Physiol Rev*. 1991; 71(1): 1-51.
- [6]. Maśliński C. Histamine and its metabolism in mammals part II: Catabolism of histamine and histamine liberation. *Agents and Actions* 1975; 5(3): 183-225.
- [7]. Hough LB, Green JP. Histamine and its receptors in the nervous system. *Handbook of neurochemistry*: Springer, 1984; p. 145-211.
- [8]. Yan L, Galinsky RE, Bernstein JA, Liggett SB, Weinshilboum RM. Histamine N-methyltransferase pharmacogenetics: association of a common functional polymorphism with asthma. *Pharmacogenetics and Genomics* 2000; 10(3): 261-66.
- [9]. Girard B, Otterness DM, Wood TC, Honchel R, Wieben ED, Weinshilboum RM. Human histamine N-methyltransferase pharmacogenetics: cloning and expression of kidney cDNA. *Molecular Pharmacology* 1994; 45(3): 461-68.
- [10]. Aksoy S, Raftogianis R, Weinshilboum R. Human histamine N-methyltransferase gene: structural characterization and chromosomal localization. *Biochem Biophys Res Commun*. 1996; 219(2): 548-54.
- [11]. Lindahl KM. The histamine methylating enzyme system in liver. *Acta Physiologica Scandinavica* 1960; 49(2-3): 114-38.
- [12]. Brown DD, Tomchick R, Axelrod J. The distribution and properties of a histamine-methylating enzyme. *J Biologic Chem*. 1959; 234(11): 2948-950.

- [13]. Yoshikawa T, Nakamura T, Yanai K. Histamine N-methyltransferase in the brain. *International Journal of Molecular Sciences* 2019; 20(3): 737.
- [14]. Panula P, Lintunen M, Karlstedt K. Histamine in brain development and tumors. *Seminars in cancer biology*. Elsevier; 2000.
- [15]. Molina-Hernández A, Díaz NF, Arias-Montaño JA. Histamine in brain development. *J Neurochem*. 2012; 122(5): 872-82.
- [16]. Hur J, Kang MK, Park JY, Lee SY, Bae YS, Lee SH, et al. Pro-apoptotic effect of high concentrations of histamine on human neutrophils. *Int Immuno-pharmacol*. 2003; 3(10-11): 1491-502.
- [17]. Darvas Z, Falus A. Histidine decarboxylase (HDC) enzyme and gene. *Histamine: biology and medical aspects* Budapest: Spring Med. 2004: 31-42.
- [18]. Jin C, Kalimo H, Panula P. The histaminergic system in human thalamus: correlation of innervation to receptor expression. *European Journal of Neuroscience* 2002; 15(7): 1125-138.
- [19]. Verhoeven WM, Egger JI, Janssen PK, van Haeringen A. Adult male patient with severe intellectual disability caused by a homozygous mutation in the HNMT gene. *BMJ Case Reports* 2020; 13(12): 235972.
- [20]. Heidari A, Tongsook C, Najafipour R, Musante L, Vasli N, Garshasbi M, et al. Mutations in the histamine N-methyltransferase gene, HNMT, are associated with non-syndromic autosomal recessive intellectual disability. *Human Molecular Genetics* 2015; 24(20): 5697-710.
- [21]. Pang YP, Zheng XE, Weinsilboum RM. Theoretical 3D model of histamine N-methyltransferase: insights into the effects of a genetic polymorphism on enzymatic activity and thermal stability. *Biochemical and Biophysical Research Communications* 2001; 287(1): 204-208.
- [22]. Horton JR, Sawada K, Nishibori M, Zhang X, Cheng X. Two polymorphic forms of human histamine methyltransferase: structural, thermal, and kinetic comparisons. *Structure* 2001; 9(9): 837-49.
- [23]. Tongsook C, Niederhauser J, Kronegger E, Straganz G, Macheroux P. Leucine 208 in human histamine N-methyltransferase emerges as a hotspot for protein stability rationalizing the role of the L208P variant in intellectual disability. *Mol Basis Dis*. 2017; 1863(1): 188-99.