



The Study on the Clinical Phenotype and Function of HPRT1 Gene

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Abstract

Background: Lesch-Nyhan disease (LND) is a rare x-linked purine metabolic neurogenetic disease caused by enzyme hypoxanthine-phosphoribosyltransferase (HGprt) deficiency, also known as self-destructive appearance syndrome. A series of manifestations are caused by abnormal purine metabolism. The typical clinical manifestations are hyperuricemia, growth retardation, mental retardation, short stature, dance-like athetosis, aggressive behavior, and compulsive self-harm. **Methods:** We identified a point mutation c.151C > T (p. Arg51*) in a pedigree. We analyzed the clinical characteristics of children in a family, and obtained the blood of their parents and siblings for second-generation sequencing. At the same time, we also analyzed and compared the expression of HPRT1 gene and predicted the three-dimensional structure of the protein. And we analyzed the clinical manifestations caused by the defect of the HPRT1 gene. **Results:** The mutation led to the termination of transcription at the 51st arginine, resulting in the production of truncated protein, and the relative expression of HPRT1 gene in patients was significantly lower than other family members and 10 normal individuals. **Conclusion:** This mutation leads to the early termination of protein translation and the formation of a truncated HPRT protein, which affects the function of the protein and generates corresponding clinical manifestations.

Keywords

Lesch-Nyhan disease, HPRT1 gene

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Introduction

The human *HPRT1* gene is located on the X-chromosome (Xq26.3). This gene has only one functional mRNA transcript that encodes the enzyme hypoxanthine-guanine phosphoribosyltransferase.¹ It is one of the key enzymes in the salvage pathway of purine synthesis. Mutation in the *HPRT1* gene is the most common reason underlying Lesch-Nyhan syndrome (LNS). The main manifestations of this syndrome include hyperuricemia, growth retardation, mental retardation, short stature, dance-like athetosis, aggressive behavior, and compulsive self-harm. Here, we report a point mutation of *HGprt*-related neurological dysfunction (HND), caused by a nonsense mutation in exon 3 of *HPRT1*, c.151C > T (p. Arg51*). This mutation leads to an early termination codon, which may cause protein truncation or activate nonsense-mediated mRNA degradation, thus affecting the function of the protein products encoded by *HPRT1*.

Material and Methods

We collected the venous blood of children, siblings and parents for second generation sequencing analysis. Next, we used Covaris ultrasound to cut the genomic DNA, and hybridize with the array captured in the NimbleGen 2.0 probe sequence to obtain the exon DNA library. First of all, we used the Illumina TruSeq DNA sample preparation kit (Illumina, Inc., CA, USA) to obtain the pre-capture library and amplified it by laser capture microdissection. We carried out Agilent DNA 1000 chip analysis to determine the concentration, size

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distribution and quality of the capture library, and further carried out the enrichment and quality of the quantitative real-time (qPCR) analysis library. Total RNA from the patients, siblings and parents her were extracted using a QIAGEN RNA Preparation Kit (QIAGEN Inc., CO, Germany). cDNA from these samples was subjected to reverse transcription and synthesized using a PrimeScript™ Strand cDNA Synthesis Kit/RT Master Mix (Takara Shuzo Co, Ltd). In this study, we choosed glyceraldehyde phosphate dehydrogenase (GAPDH) as an internal reference gene and used the following primers and double labeled probes to detect HPRT1 gene expression: Forward primer TTA TGGACAGGACTGAACGTCT; Reverse primer TGTA ATCCAGCAGGTCAGCAA. After initial denaturation at 95.0 °C for 3 min, the reaction was cycled 35 times at 95.0 °C for 30 s and 60.0 °C for 45 s. The transcriptional level was determined by three repetitive samples and expressed as the standard error of mean \pm mean. The samples for statistical analysis of qPCR data were compared using the 2- $\Delta\Delta$ Ct method.

Protein Structure Prediction Using the I-TASSER Server for Nonsense Mutations

The Iterative Threading ASSEMBLY Refinement (I-TASSER) suite pipeline consists of four general steps: thread template identification, iterative structure assembly simulation, model selection and optimization, and structure-based function annotation. The server is available from <http://zhanglab.ccmb.med.umich.edu/ITASSER>.

Ethical Considerations

This study was approved by the Ethics Committee of Shanghai Children's Hospital. The informed consent of the patient's parents has been obtained when using blood samples for genetic analysis. The study is in line with the Chinese Bioethics laws and the Helsinki declaration.

Harmful Prediction of Protein

We used mutation taster to predict the effect of mutation on protein, and the results show that the effect of mutation on protein is harmful, the predicted results are as follows: phyloP:0.573;1.487;3.321 phastcons:1;1;1. The results showed that the mutation point and its pre-and post-sequence are highly conservative.

Results

Clinical Data Case 1 :

A male patient (currently 10 months old), G4P3, with birth weight 3510 g, was delivered by cesarean section because of a "scarred uterus;" he had no asphyxia after birth, and had no history of rescue. His mother's antenatal examination was normal and she did not take any drugs during pregnancy. His

parents were in good health, with no infectious or chronic diseases, and no record of familial genetic disease. The child visited our hospital because of lag in his motor and language development compared to normal children of the same age. He was first admitted to our hospital at the age of 5 months and 13 days. A detailed inquiry of his medical history revealed the following: the child could raise his head for only 1–2 s at the age of 3 months, and when admitted to the hospital, this time was a few seconds to 1 min; he could make sounds such as "babbling;" traceable, he was responsive to teasing, and cried frequently ; he had ungrasping, soft limbs, and his limb muscle tension was lower than that in normal children of the same age. When he was 4 months old, developmental screening suggested that his major motor development, fine motor adaptability, personal-social development level, and language ability was equivalent to that of 2.8-month old, 2.2-month old, 2.6-month old, and 2.8-month old children, respectively. No obvious abnormality was found in the head nuclear magnetic resonance (NMRI), electroencephalogram (EEG), brain topography, and blood tandem mass spectrometry; lactic acid, blood ammonia, and 25-hydroxyvitamin D levels as well as thyroid function were normal. After admission to our hospital for completing the relevant examination, blood biochemistry indicated high levels of uric acid at 673 μ mol/l (normal value 90–420 μ mol/l) and the video-EEG analysis was normal. The child is currently 10 months old; he can sit independently but cannot climb; he can stand by supporting the bench independently; he can grab things, but still can only make "babble" sounds; he can laugh, but is shy with strangers, and cannot express "goodbye" by waving his hand. His elder brother is 10 years old and is described in this report as Case 2. His elder sister, who is 6 years old, is normal and healthy. *Case 2 :*

This male patient, 10 years old, G1P1, is the elder brother of Case 1. The family members reported that this child had a history of hypoxia at birth; he had clenched his fists after birth, arched his hands backward and outward, twisted his feet around each other, and tilted his head back; he could pronounce "Dad, Mom, Grandpa, Grandma" when he was 1 year old, but still could not speak in long sentences. Currently, he is unable to sit and stand independently; he shows limb movement disorders, mental retardation, and dance-like gestures of hands and feet. The child has undergone rehabilitation training in other hospitals in the past; however, his condition has not improved, and his specific diagnosis and treatment in the past is unclear.

Real-Time Quantitative PCR (RT-qPCR) Analysis of the Expression of the HPRT1 Gene

The expression of HPRT1 gene in this family was analyzed by RT-qPCR. In order to evaluate the expression level of DNA, we analyzed the expression level of HPRT1 gene and housekeeper gene GAPDH gene respectively. The results showed that the relative expression of HPRT1 gene in patients was significantly

lower than that in other family members and 10 normal individuals (Figure 1).

the Nonsense Mutation Result in Changes in Protein Function and Structure

Due to the point mutation of *HPRT1* gene, the 51st arginine becomes a stop codon and the translation ends. These changes lead to the formation of a truncated protein with 51 amino acids and a lack of 167 amino acids at the c-terminal of the wild-type protein. We compared the three-dimensional structure of the wild type and mutated the *HPRT1* protein, and found that the conserved region of the mutated *HPRT1* protein was deleted, which may affect the inherent stability of the protein and affect the protein-protein interaction. (Figures 2 and 3)

Distribution and Conservation of the Mutation in the HPRT1 Gene

We analyzed the pathogenic mutations reported on National Centre for Biotechnology Information (NCBI) and found that these mutations were all located in the conserved domain (Figures 4 and 5).

Discussion

The human *HPRT1* gene is located on the X-chromosome (X q26.3). This gene has only one functional mRNA transcript that encodes the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPrt),¹ which is one of the key enzymes involved in the salvage synthesis of purine nucleotides. The function of the HGPRT enzyme is to catalyze the conversion of hypoxanthine nucleotide (IMP), and also to convert guanine to guanosine monophosphate (GMP).² There is no de novo pathway to synthesize purine nucleotides from 5-phosphoribose to uric acid in brain tissue, purine nucleotides can only be synthesized through the remedial pathway of HGPrt action, so the deficiency of HGPRT enzyme affects the growth and development of nervous system.³ The activity of HPRT enzyme is also affected when the gene is abnormal, depending on the degree of the influence. Clinically, there may be the following manifestations: 1. HPRT-related hyperuricemia (HRH): the enzyme is partially affected, only hyperuricemia is present in clinic, and there is no related manifestation of nervous system. 2. HPRT-related nervous system dysfunction (HRND): the enzyme activity was partially affected, with clinical manifestations of hyperuricemia, partial nervous system dysfunction, but no self-injury behavior. 3. Lesch-Nyhan syndrome (LNS): also known as self-destructive appearance syndrome, the enzyme activity is completely lost, is a group of growth retardation, mental retardation, short stature, dance-like athetosis, aggressive behavior, obsessive-compulsive self-harm, hyperuricemia and other symptoms of syndrome.⁴

In this study, a mutation (c.151C > T (p. Arg51*)) was found in the *HPRT1* gene in these cases. Whole-exon sequencing results indicated that the two boys harbored hemizygous mutations, whereas their mother and sister were heterozygous for the mutation. The results also confirmed that the disease is X-linked recessive. This variation has not yet been included in the normal population database, gnomAD. According to the American College of Medical Genetics and Genomics mutation classification guidelines, the laboratory classified this variation as a pathogenic variation. This mutation leads to early termination of protein translation and may form a truncated HGPRT protein, which affects the protein function and produces the corresponding clinical manifestations. Presently, at the age of 10 months, the younger child (Case 1) is still unable to climb, cannot change hands, has low muscle tone, and high levels of blood uric acid, with normal video-EEG and head NMRI results. These clinical manifestations suggest *HGPrt*-related nervous system dysfunction (HND), in which the patient often shows self-injuring behavior; the median age of onset of general self-injury behavior is 2 years old.⁵ At present, since this child is young, self-injury behavior is difficult to detect, and we are still unable to test for the HGPRT enzyme; therefore, diagnosis mainly depends on clinical manifestations and other related laboratory examinations and it is necessary to monitor the changes in their condition. During whole-exon sequencing, the elder brother was also found to harbor the hemizygous mutations, variation and was diagnosed with cerebral palsy after birth. The mother and sister of the patient were heterozygous with no corresponding clinical manifestations.

The full length of the *HPRT1* gene is 657 nucleotides. In this case, the mutation was found in c.151C > T (p. Arg51*) and occurred in exon 3. The mutation led to transcription termination at the 51st arginine residue, resulting in the production of a truncated protein. The relative expression of *HPRT1* in the patients was lower than that in normal individuals and their family members. Although the mother and daughter showed no abnormal manifestations, the *HPRT1* expression was lower than that in a normal random control group of 10 individuals. HGPRT is a tetramer composed of four identical subunits, and there are active sites of the enzyme on the β - α - β structure of each monomer. Gene mutations may damage the secondary structure of the monomer and affect the binding of the active site to the enzyme action, or affect the binding of the tetramer, resulting in enzyme inactivation.⁶ After analyzing the protein structure in the patients we found that the mutation was located in the only functional domain. As a result of this mutation, the protein with a total length of 218 amino acids was truncated into a short chain containing only 51 amino acids, and the important sites were lost, resulting in a non-functional enzyme. The mutation led to transcription termination at the 51st arginine residue, resulting in the production of a truncated protein. We speculated that the mutation caused self-inactivation of mRNA, resulting in a nonsense mutation at the termination codon, thus affecting the normal function of HGPrt proteins and causing abnormalities in purine metabolism. Related studies have also shown that such nonsense mutations

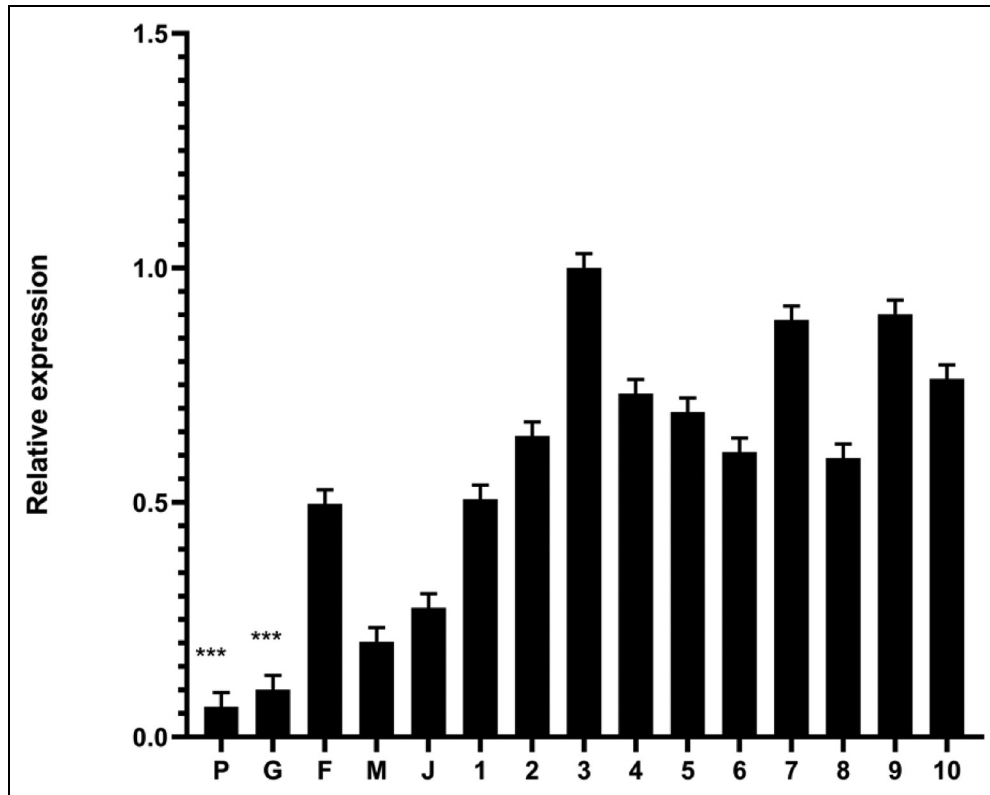


Figure 1. The relative expression level of HPRT1 gene in patients, family members and 10 random controls. (P:case1; G:case2; F:father; M: mother; J:sister).

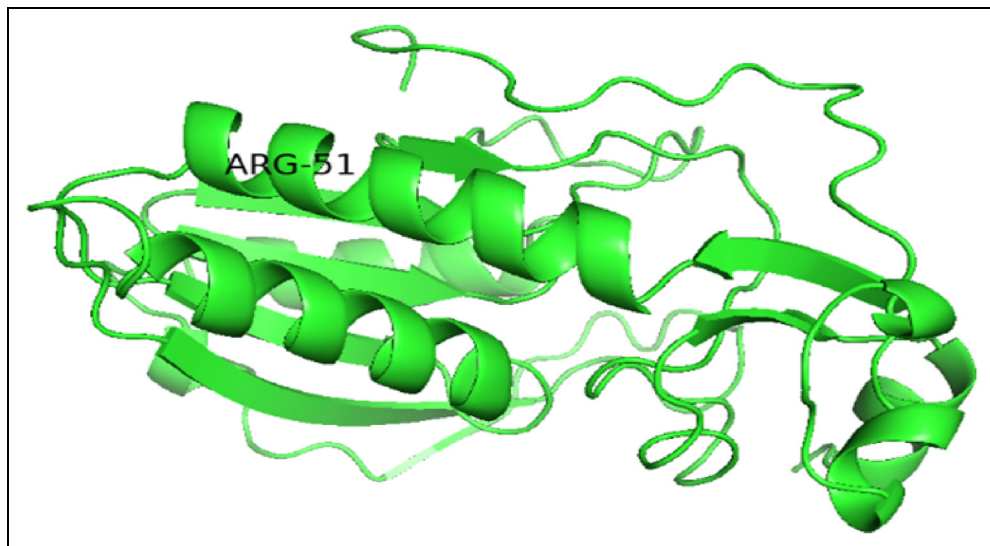


Figure 2. The three-dimensional structure of normal HPRT1 protein.

produce premature termination codons, which may lead to related molecular defects such as degradation of transcripts containing termination codons through the nonsense-mediated mRNA degradation pathway, an important post-transcriptional monitoring mechanism developed in eukaryotic cells during evolution. It recognizes and degrades abnormal mRNAs

containing termination codons through a variety of cellular components to effectively avoid the accumulation of truncated proteins, and thus exerting a protective effect on the body.⁷

Upon reviewing the related literature, we found that among the more than 400 *HPRT* mutation sites reported, the incidence of LNS in the Chinese population is particularly low^{8,9} Exon 3



Figure 3. The three-dimensional structure of abnormal HPRT1 protein.

	41	45	48	49	51	57	58	65	70	71	74	78	80	104	109	110	123	130	132	140	143	152	153	161	168	170	176	194	199	201																			
H. sapiens:	...GL	FTER	IAR	M	C	L	G	GY	FFAD	L	L	D	S	Q	ST	...L	ST	NV	LIV	...T	G	T	M	Q	T	R	Q	Y	N	A	S	...E	T	P	R	S	...P	D	...L	D	Y	...Y	F	R	D	...			
P. troglodytes:	...GL	FTER	IAR	M	C	L	G	GY	FFAD	L	L	D	S	Q	ST	...L	ST	NV	LIV	...T	G	T	M	Q	T	R	Q	Y	N	A	S	...E	T	P	R	S	...P	D	...L	D	Y	...Y	F	R	D	...			
M. mulatta:	...GL	FTER	IAR	M	C	L	G	GY	FFAD	L	L	D	S	Q	ST	...L	ST	NV	LIV	...T	G	T	M	Q	T	R	Q	Y	N	A	S	...E	T	P	R	S	...P	D	...L	D	Y	...Y	F	R	D	...			
C. lupus:	...GL	FTER	IAR	M	C	L	G	GY	FFAD	L	L	D	S	Q	ST	...L	ST	NV	LIV	...T	G	T	M	Q	T	R	Q	Y	N	A	S	...E	T	P	R	S	...P	D	...L	D	Y	...Y	F	R	D	...			
B. taurus:	...GL	FTER	IAR	M	C	L	G	GY	FFAD	L	L	D	S	Q	ST	...L	ST	NV	LIV	...T	G	T	M	Q	T	R	Q	Y	N	A	S	...E	T	P	R	S	...P	D	...L	D	Y	...Y	F	R	D	...			
M. musculus:	...GL	HTER	IAR	M	C	L	G	GY	K	FAD	L	L	D	S	Q	ST	...L	ST	NV	LIV	...T	G	T	M	Q	T	R	Q	Y	S	...A	S	...R	T	S	R	S	...P	D	...L	D	Y	...Y	F	R	D	...		
R. norvegicus:	...GL	HTER	IAR	M	C	L	G	GY	K	FAD	L	L	D	S	Q	ST	...L	ST	NV	LIV	...T	G	T	M	Q	T	R	Q	Y	S	...A	S	...R	T	S	R	S	...P	D	...L	D	Y	...Y	F	R	D	...		
G. gallus:	...GL	FTER	IAR	M	C	L	G	GY	FFAD	L	L	D	S	Q	ST	...L	ST	NV	LIV	...T	G	T	M	Q	T	R	Q	Y	N	A	S	...E	T	P	R	S	...P	D	...L	D	Y	...Y	F	R	D	...			
D. rerio:	...GL	FTER	IAR	M	C	L	G	GY	FFAD	L	L	D	S	Q	ST	...L	ST	NV	LIV	...T	G	T	M	Q	T	R	Q	Y	N	A	S	...E	T	P	R	S	...P	D	...L	D	Y	...Y	F	R	D	...			
C. elegans:	...GL	FVVR	IAR	I	C	L	G	SY	FF	E	T	A	I	V	E	S	M	ST	...L	D	E	...S	V	I	V	...T	G	R	L	A	K	...H	E	...T	G	...A	L	...R	V	...R	V	...E	...L	D	Y	...K	R	D	...
	p. Leu41Pro	p. Arg45Lys	p. Arg48His	p. Leu49Phe	p. Arg51*	p. Met57Thr	p. Gly58Arg	p. Leu65Phe	p. Gly70Glu	p. Gly71Val	p. Phe74Cys	p. Leu78Val	p. Asp80Val	p. Ser104Arg	p. Gln109Ter	p. Ser110Leu	p. Ser123Ter	p. Val130Asp	p. Ile132Met	p. Gly140Asp	p. Met143Lys	p. Gln152Ter	p. Tyr153Ter	p. Ala161Ser	p. Thr168Ile	p. Arg170Ter	p. Pro176Leu	p. Asp194Asn	p. Phe199Val	p. Asp201Gly																			

Figure 4. The conserved analysis of HPRT1 gene. HPRT1 mutation spectrum. Variant listed as red was discovered in this paper.

may be the mutation hotspot of the gene.¹⁰ In this case, the mutation was also located in exon 3, similar to most of the pathogenic mutation sites reported on NCBI. The amino acids encoded by exon 3 belong to the CpG island region of *HPRT*, which is an important regulatory region in some genes.¹¹ Although mutations in the *HPRT1* gene occur commonly, there are also some isolated hot spots, and the same mutations occur many times in unrelated patients. The two most common hotspots are c. 151C > T and c. 508 C > T, wherein both these mutations result in the conversion of arginine-encoding codons to stop codons (p. R51 * and p. R170 *, respectively), possibly through similar mechanisms

including methylation of the CpG sequence cytosine, and the resulting 5-methylcytosine that produces thymine through deamination.¹²

The *HPRT1* gene participates in the metabolism of purine, and defects in its protein products can lead to the accumulation of uric acid, the final product of purine metabolism. The crystallization of uric acid in different tissues and organs can lead to growth retardation, gout, musculoskeletal changes, and uric acid nephropathy^{13,14} Although it is speculated that this mutation may affect brain development, its effect on the nervous system has not been specifically verified. Schretlen and others

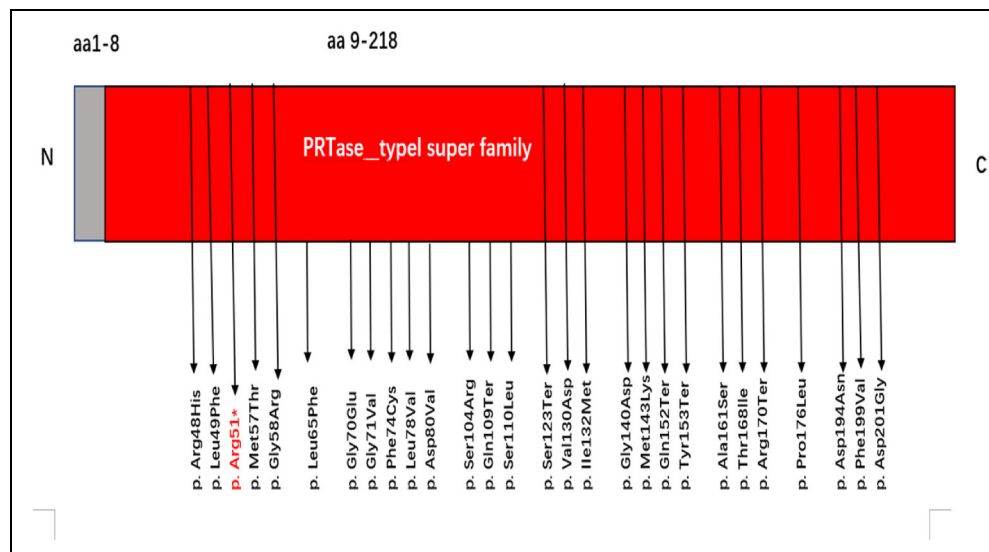


Figure 5. The distribution of mutant sites in domains (The sequence of proteins corresponding to missense mutation sites in different species).

have shown that the areas exhibiting reduced white matter volume in LNS and its variants are consistent with areas that affect neurobehavior. It is also speculated that these behavioral abnormalities may be related to a decrease in the white matter volume; however, its specific pathogenesis is not clear,¹⁵ and therefore needs to be studied further. At present, there is no specific treatment for the disease, the main treatment drug is allopurinol, allopurinol is an isomer of hypoxanthine, has the effect of xanthine oxidase inhibitor, can block the conversion of xanthine and hypoxanthine to uric acid. Although treatment cannot improve the outcome of the nervous system,¹⁶ it is still the drug which most widely used to control uric acid in patients with HGprt deficiency.¹⁷ It can also control the amount of purine in the diet to alleviate this disease.

To sum up, although there is no specific treatment for HPRT1-related diseases, we can diagnose the disease through early genetic detection, provide fertility guidance, and carry out corresponding intervention as soon as possible to improve the prognosis of the disease.

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Author Contributions

YC and MG determined the study design and performed the study. MG drafted the manuscript. LL, YW, AW, CW, SW, FY and YZ were responsible for data collection and analyzed the data. All authors read, critically revised, and approved the final manuscript.

Availability of Data and Material

All datasets for this study are included in the manuscript/supplementary files.

Consent for Publication

Signed informed consent was obtained from the patient's guardian for publication of any potentially identifiable images or data included in this article. Consent for publication is obtained from all participants.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Shanghai Children's Hospital. Informed consent to use blood samples for genetic analysis was obtained from the patient's parents. The study complied with Chinese bioethics laws and the Helsinki declaration.

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Informed Consent

Not applicable, because this article does not contain any studies with human or animal subjects.

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Trial Registration

Not applicable, because this article does not contain any clinical trials.

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