A Chinese Family with Noonan Syndrome Associated with a Heterozygous LZTR1 Mutation

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Research

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Abstract

Background

Noonan syndrome (NS) is a multisystem disorder resulting from pathogenic mutations in more than 15 genes, among which *LZTR1* is newly discovered. However, its role in NS pathogenesis remains unclear, including its inheritance pattern.

Methods

We herein report a family with *LZTR*-related NS. We collected clinical data of the proband and her families. Four of them completed the whole exon sequencing.

Results

Two children with NS inherited the same heterozygous *LZTR1* variant, c.1149 + 1G > T, from their affected mother. Moreover, the proband was diagnosed as NS accompanied by growth hormone deficiency (GHD) without other associated gene mutations.

Conclusion

The variant c.1149 + 1G > T of *LZTR1* was previously reported in autosomal recessive NS, but this is the first report of an autosomal dominant form. Our reported cases provided evidence for a more complex inheritance pattern resulting from *LZTR1* c.1149 + 1G > T mutation. Occasionally, NS patients can have GHD.

Introduction

Noonan syndrome (NS, OMIM 163950) is a genetic multisystem heterogeneous disorder with a relatively high estimated incidence of every 1000–2500 live births ^[1] and can show autosomal dominant (AD) or autosomal recessive inheritance (AR) ^[2]. To date, more than fifteen genes associated with NS have been reported, including *PTPN11*, *SOS1*, *RAF1*, *BRAF*, *HRAS*, *KRAS*, *NRAS*, *SHOC2*, *MAP2K1*, *MAP2K2*, *CBL*, *RIT1*, *RASA2*, *A2ML1*, and *LZTR1* ^[3, 4]. Among them, variants of *LZTR1* have been newly associated with the etiology of NS since 2014 ^[5–8]. *LZTR1* (OMIM 600574) is the abbreviation for the leucine zipper-like transcriptional regulator 1 gene and is located on 22q11.2. At first, *LZTR1* gene loss-of-function mutations cause schwannomatosis ^[6, 9, 10]. Recently, no more than 50 cases of NS have been associated with pathogenic *LZTR1* mutations ^[3, 5, 7, 11–14], and NS can be inherited via AD or AR forms of *LTZR1* mutation. The reason of this variant hereditary pattern associated with *LTZR1* mutations in NS is not yet clear. To date, the factors underlying the hereditary differences among *LTZR1* mutations remain unclear,

but the c.1149 + 1G > T mutation of the *LZTR1* gene was reported in three cases of NS with an AR mode of inheritance^[11, 15]. Here, we report a family with AD NS associated with the c.1149 + 1G > T mutation of *LZTR1*.

Patients And Methods

Patient referral

Patient 1 was the proband (Figure 1, III-3) and a 6.6-year-old female. She was admitted to our hospital because of short stature and was the first child in her family. She was born at 41 weeks gestation to nonconsanguineous parents via vaginal delivery. Her birth weight and length were 1800 g (<P3rd) and 47 cm (<P3rd), respectively, and her psychomotor development was mildly delayed. From the newborn stage, the patient showed evident growth retardation. At the age of 6.6 years, her height, weight and body mass index (BMI) were 90.5cm (-6.3 SD), 11 kg (-4.9 SD) and 13.4 kg/m² (P10-25th), and her arm span/height and sitting height/height ratios were 0.92 (-2 SD) and 0.58 (+3 SD) according to the standard reference values^[16]. Except for her short stature and developmental delay, she exhibited the following features that are typical of NS (Figure 2): hypertelorism; downslanting palpebral fissures; epicanthal folds; low-set, oval-shaped, posteriorly rotated ears with a thick helix; a short broad nose with a depressed root and full tip; a deeply grooved and long philtrum; high and wide peaks of the vermilion; a highly arched palate; micrognathia; a short neck; cubitus valgus; scoliosis; café au lait spots; and mild hypertrichosis. She also presented with squinting, refractive errors and nystagmus. Her IGF-1 level was 61 µg/L (-1.9 SD), and the GH peak after the growth hormone stimulating test was 5.54 ng/ml. In addition, hormone levels of the adrenal gland, thyroid gland and gonad were normal. Tumor markers such as alpha-fetoprotein (AFP), human chorionic gonadotropin (HCG), and carcinoembryonic antigen (CEA) were negative. An echocardiogram revealed no congenital cardiac defect, while an electrocardiogram (ECG) showed frequent premature ventricular beats (the second and third rhythms) (Figure 3). Ultrasounds showed no abnormalities in the liver, kidneys, uterus, or ovaries, and X-rays showed hemivertebral deformity of the third thoracic vertebra and scoliosis (Cobb's angle = 28°). The patient's bone age was 5.5 years, and magnetic resonance imaging (MRI) of the craniocerebrum and spine showed the following: 1) pituitary height of 3.3 mm, and 2) no observation of schwannomatosis or occupying lesions (Figure 4). Her karyotype was 46, XY. According to the clinical manifestation and laboratory tests, she was diagnosed with NS^[17] with growth hormone deficiency (GHD). Whole exon sequencing "WES" study was completed, revealing the heterozygous mutation c.1149+1G>T of the *LZTR1* gene that she inherited from her mother (Figure 5).

Patient 2 (Figure 1, III-4) was the younger sister of the proband. She was 2.5 years old, and her birth weight and height were 3000 g and 49 cm, respectively, with a gestational age of 38 weeks. Her height and weight were 82.5 cm (-2.7 SD) and 10.7 kg (-1.8 SD). She had typical features of NS, including hypertelorism; downslanting palpebral fissures; epicanthal folds; low-set, oval-shaped, posteriorly rotated ears with a thick helix; a short broad nose with a depressed root and full tip; a deeply grooved philtrum; high and wide peaks of the vermilion; a highly arched palate; micrognathia; a short neck; and pectus

carinatum. Her development and cognitive ability were normal, and she had no cardiac or genitourinary defects. The patient was diagnosed with NS, and by WES she had the same heterozygous mutation, c.1149+1G>T, of the *LZTR1* gene (Figure 5).

Suspected patient 3 (Figure 1, II-6) was the mother of patients 1 and 2. She was 27 years old, and her height was 153 cm (-1.7 SD). She looked like a normal female except for presenting with hypertelorism; downslanting palpebral fissures; low-set, oval-shaped, posteriorly rotated ears with a thick helix; a highly arched palate; and prominent nasolabial folds. She had no cardiac, urogenital or skeletal defects and the same heterozygous mutation of the *LZTR1* gene (c.1149+1G>T) (Figure 5).

Suspected patient 4 (Figure 1, I-3) and **suspected patient 5** (Figure 1, II-7) were the mother and younger sister of patient 3, respectively. They had appearance features similar to those of patient 3, and their heights were 151 cm (-1.9 SD) and 153 cm (-1.7 SD), respectively.

Suspected patient 6 (Figure 1, III-5) was the daughter of patient 5 and was diagnosed with presumed NS. At the age of 3 years, she showed a short stature (84.3 cm, -3.2 SD); hypertelorism; downslanting palpebral fissures; low-set, oval-shaped, posteriorly rotated ears with a thick helix; a short broad nose with a depressed root and full tip; a deeply grooved philtrum; high and wide peaks of the vermilion; a highly arched palate; and micrognathia without congenital heart, urogenital, skeletal or cognitive defects.

Unfortunately, patients 4-6 rejected the genetic analysis, and patients 2-6 did not agree to share their photos.

Molecular genetic analysis

Written informed consents for gene testing were obtained from the patients' parents. Patients 1-3 and the proband's father underwent genetic testing. Five to ten milliliters of peripheral blood was collected in disposable vacuum tubes for genetic testing. Genomic DNA was isolated using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. After construction according to the standard protocol, whole exon sequencing (100X) of the libraries was performed with the SureSelect Human All Exon V6 array on the Illumina HiSeq X Ten Platform with the PE150 strategy. A standard bioinformatics pipeline was utilized for variant identification with the help of Genome Analysis Toolkit (GATK) ^[18] software following the best practice guidelines recommended by the GATK ^[19, 20]. Candidate variants were retained as follows: (1) rare variants with a minor allele frequency of < 1% in the ExAC, dbSNP, 1,000 Genomes, gnomAD and local databases, and (2) functional variants including frameshift, splice, nonsense, missense and synonymous variants that can affect splicing. Then, we utilized a hypothesis-free approach to analyze all phenotype-related genes. Eventually, the intersection of the above criteria led to the identification of a heterozygous mutation, c.1149+1G>T, under accession number NM_006767.3 in the *LZTR1* gene. The splicing mutation was ranked as "likely pathogenic" according to the American College of Medical Genetics and Genomics (ACMG) ^[21]. Sanger sequencing showed that the mutation was inherited from the proband's mother, who also had a mild phenotype related to NS (Figure 5). Notably, the proband's younger sister harbored the same heterozygous mutation

(Figure 5). The proband's father did not harbor the mutation and had no symptoms of NS (Figure 5). In previous literature, compound heterozygosity involving this variant (c.1149+1G>T) in *LZTR1* has been described in three patients with AR *LZTR1*-linked NS [11, 12, 15]. This mutation is classified as frameshift and can cause abnormalities at the splice site. As a result, the variant produces truncated proteins, which may affect the function of coded proteins. In the east Asian population database (ExAC_EAS), the frequency of this mutation is 0. The patients did not harbor other mutations that have been associated with NS, GHD, skeletal dysplasia and other genetic diseases.

Discussion

NS is a genetic multisystem condition, and pathogenic genes are associated with only 75% of NS patients, thereby making the clinical diagnosis of NS very important. The phenotype of NS is variable and includes a short stature, congenital heart defects and/or cardiomyopathy, a characteristic craniofacial dysmorphism and childhood benign or malignant tumors, including leukemia and solid tumors [22–24]. The diagnosis of NS depends mainly on the identification of typical clinical manifestations, such as a notable appearance, short stature, congenital heart disease and other dysplasia [1, 25, 26]. Our patients (III-3, III-4, III-5) were clinically diagnosed with NS based on their display of features typical of the disease. Patient 1 and patient 2 were subjected to WES, revealing the same variant, c.1149 + 1G > T, in *LZTR1* that was inherited from their mother (II-6). Their maternal grandmother (I-3) and maternal aunt (II-7) had a facial appearance similar to that of their mother (II-6). From previous reports, families with AD *LZTR1*-linked NS exhibited vertical transmission of the phenotype with differential penetrance [12]. Additionally, the phenotype of NS ranges greatly, from a normal appearance to features typical of NS. Therefore, our families displayed the AD mode of hereditary NS with incomplete penetrance.

To make accurate diagnoses quickly and effectively, we performed WES for molecular diagnoses. All the exons (including the 50 bp flanking piece on either side) were captured in a single reaction, and genes related to the RASopathies were thus considered. The average sequencing depth, average coverage and 10 × coverage (coverage of sites with a depth greater than 10) in the target region were 153.02×, 99.43% and 96.28%, respectively, indicating the high confidence level of variant calling. No other variants were detected in the *LZTR1* gene or in the other RASopathy genes in the proband. Nonetheless, exome sequencing is limited in detecting large deletions/duplications and deep intronic variants. Based on the fact that variant c.1149 + 1G > T in the *LZTR1* gene segregated with an NS-related phenotype in multiple affected family members, we speculated that the pedigree presented as dominant inheritance.

More than 15 gene mutations are known to be involved in the etiology of NS. Pathogenic variants in the genes encoding proteins implicated in the RAS-MAPK signaling pathway are responsible for NS. These gene mutations function upstream of the RAS/MAPK cascade or its regulation and dysregulate the RAS/MAPK pathway, leading to sustained or excessive activation of ERK (which defines RASopathies) [1]. *LZTR1*-related NS was recently described. *LZTR1* is a highly conserved gene and encodes a protein

characterized by six tandemly arranged Kelch motifs at the *N*-terminus and two BTB/POZ (broad complex, tramtrack and bric-a-brac/Pox virus and zinc finger) domains at the *C*-terminus. *LZTR1* is an important regulator of the normal cell cycle and acts as a tumor suppressor. Additionally, *LZTR1* is found to be a conserved regulator of RAS ubiquitination and signaling [27–30].

Previous studies have demonstrated that *LZTR1* mutations can be acquired via AR or AD inheritance [3, 5, 11, 12, 31]. In the current study, the variants of *LZTR1* associated with NS were located in both the Kelch and BTB domains, and AD NS has been attributed to the Kelch motifs, especially Kelch motifs 1–4 [14, 30, 31]. A new study showed that more than one RVxF motif is located between Kelch 5 and Kelch 6 in the *LZTR1* gene. RVxF is a binding location of the protein phosphatase-1 (PP1) [30, 32], and variant c.1149 + 1G > T is located in Kelch domains 5–6. This mutation can cause splice abnormalities and produce truncated proteins and thus may influence the binding function of the RVxF motif and PP1. To our knowledge, more than 50% of phosphoserine/threonine dephosphorylation reactions are catalyzed by PP1 in mammalian cells [33]. PP1 multifunctionally interacts with dozens of polypeptides that function as substrates, inhibitors, chaperones, anchoring/scaffolding proteins, and substrate-specifiers [32, 34] and even those associated with heart physiology [35]. The proband's arrhythmia may be associated with the dysfunction of PP1. Variant c.1149 + 1G > T has been reported in three NS patients with compound heterozygous mutation of *LZTR1* [11, 12, 15] (Table 1). Our patients had the NS phenotype and the heterozygous mutation inherited in the AD form, and variant c.1149 + 1G > T of *LZTR1* may thus result in NS of the AD or AR form.

The typical feature of NS is a short stature, but some NS patients have GHD, as previous studies reported. Three NS patients diagnosed with GHD [11, 12, 36]. In our study, the proband (III-3) was the fourth NS patient identifying as having GHD (Table 2). WES revealed no gene mutations associated with GHD in this patient, and the factors underlying GHD in these four NS patients were not clear.

In conclusion, the heterozygous variant c.1149 + 1G > T of *LZTR1* was transferred from maternal family members, and the manifestations of NS ranged widely. The variant c.1149 + 1G > T of *LZTR1* was previously reported in autosomal recessive NS, but this is the first report of an autosomal dominant form. Our reported cases provided evidence for a more complex inheritance pattern resulting from *LZTR1* c.1149 + 1G > T mutation. These results show that variant c.1149 + 1G > T of *LZTR1* may result in NS with an AD or AR form. Occasionally, NS patients can have GHD.

Abbreviations

AD: autosomal dominant; AR: autosomal recessive; GHD: growth hormone deficiency; LZTR1: leucine zipper-like transcriptional regulator 1; NS: Noonan syndrome; PP1: protein phosphatase-1.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Shenzhen Children's Hospital [No. 2020 (006)]. All subjects provided written informed consent in accordance with the Declaration of Helsinki.

Consent for publication

Written consent for publication was provided by the patients' parents.

Availability of data and materials

The dataset analyzed in the current study is available from the corresponding author upon reasonable request.

Conflict of Interest Statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled,

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Author contribution statement

X Z contributed to the data collection, data interpretation and writing of the manuscript. Z S contributed to the study design and reviewed the report. L W contributed to the clinical data collection and data interpretation. FF L contributed to the imaging data collection and data interpretation. ZZ L contributed to the gene mutation interpretation. WY Z contributed to the gene-phenotype analysis.

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Tables

Table 1. NS patients with variant c.1149+1G>T of *LZTR1* in the literature

Literature	Age (years)	Sex	Hereditary form	Variant location	Nucleotide change	Amino acid change	Origin of mutation	Phenotype				
								Facial and physical features	Short stature	Cardiac defect	ECG	Others
Perin, F, et al. ^[15]	4	M	AR	Kelch 6 and BTB might be lost	c.1084C>T	p.R362*	MC	Broad forehead; hypertelorism; downward-slanting palpebral fissures; posteriorly rotated ears with a thickened helix; broad thorax with a webbed neck	NA	Severe HCM; mild PVS	Broad QRS complexes; RBBB; left axis deviation	NA
				Kelch 6 and BTB might be lost	c.1149+1G>T	Disrupts splice site	FC					
Pagnamenta, A.T, et al. ^[11]	6.8	M	AR	BTB2	c.2062C>T	p.R688C	de novo	Blue irides; downslanting palpebral fissures; convergent squinting; ptosis; hypertelorism; low-set, posteriorly rotated ears; pectus carinatum; wide neck; joint hypermobility; square thumb	+	NA	NA	Mild developmental delay and delayed speech and language development; hypermetropia; hyperacusis; hypotonia
				Kelch 6 and BTB might be lost	c.1149+1G>T	Disrupts splice site						
Johnston, J.J, et al. ^[12]	2.1	F	AR	Kelch 1-6 and BTB might be lost	c.27delG	p.Q10fs	FC	Prenatal polyhydramnios; depressed, broad nasal bridge; relative macrocephaly; nevus flammeus on forehead; midface retrusion with marked frontal bossing; high anterior hairline; nevus flammeus on forehead; downslanted palpebral fissures; bilateral epicanthus with widely spaced eyes; long philtrum; full, sagging cheeks; short neck; broad chest; relatively short arms and legs	NA	Levocardia; small ASD; patent foramen ovale	Fetal bradycardia	Hypotonic; Intestinal malrotation
				Kelch 6 and BTB might be lost	c.1149+1G>A	Disrupts splice site	MC					
our study	6.6	F	AD	Kelch 6 and BTB might be lost	c.1149+1G>A	Disrupts splice site (donor)	MC	Hypertelorism; downslanting palpebral fissures; squinting; nystagmus; epicanthal folds; low-set, oval- shaped, posteriorly rotated ears, thick helix; short broad nose with a depressed root and full tip; deeply grooved and long philtrum; high and wide peaks of the vermilion; highly arched palate; micrognathia; short neck; cubitus valgus; scoliosis; pectus	+	-	Frequent premature ventricular beats	Delayed psychomotor development; hemivertebra deformity; refractive errors

								excavatum; café au lait spots; mild hypertrichosis				
	2.5	F	AD	Kelch 6 and BTB might be lost	c.1149+1G>A	Disrupts splice site (donor)	MC	hypertelorism; downslanting palpebral fissures; epicanthal folds; low-set, oval-shaped, posteriorly rotated ears with a thick helix; short broad nose with a depressed root and full tip; deeply grooved philtrum; high and wide peaks of the vermillion; highly arched palate; micrognathia; short neck; and pectus carinatum	+	-	-	-
	27	F	AD	Kelch 6 and BTB might be lost	c.1149+1G>A	Disrupts splice site (donor)	MC	hypertelorism; downslanting palpebral fissures; low-set, oval-shaped, posteriorly rotated ears with a thick helix; highly arched palate; and prominent nasolabial folds	-	-	-	-

NS: Noonan syndrome; F: female; M: male; AD: autosomal dominant; AR: autosomal recessive; -: feature absent; +: feature present; NA: not applicable; FC: farther carrier; MC: mother carrier; HCM: hypertrophic cardiomyopathy; PVS: pulmonary valve stenosis; ASD: atrial septal defect; RBBB: right bundle branch block.

Table 2. *LZTR1*-related NS patients with growth hormone deficiency in the literature

Literature	Age (years)	Sex	Hereditary form	Variant location	Nucleotide change	Amino acid change	Origin of mutation	Phenotype			
								Facial and physical features	Short stature	Cardiac defect	Others
Johnston, J.J, et al. ^[12]	3.2	F	AR	Within intron 16 of <i>LZTR1</i> affecting BTB 2	c.1943- 256C>T	*70G>A	MC, FC	Prenatal polyhydramnios; proptosis; ptosis; wide mouth; low- set ears; bulbous nasal tip; relative macrocephaly	+	HCM; small ASD	Delayed development; decreased muscle mass and motor coordination
Nakaguma, M. A, et al. ^[36]	12.5	M	AR	Kelch 5	c.881G>T	p.R294L	MC	Ptosis; triangular face; high-arched palate; low-set ears; micrognathia; pectus excavatum	+	Transposition of the great vessels; PVS; interventricular and interatrial communication	NA
				BTB 2	c.2212C>T	p.Q738*	FC				
Pagnamenta, A.T, et al. ^[11]	5	M	AD	Kelch 2	c.407A>G	p.Y136C	De novo	Congenital ptosis; depressed nasal bridge; low-set, posteriorly rotated ears; pointed chin; wide intermamillary distance; barrel- shaped chest; pectus excavatum; 2- 3 toe syndactyly; cryptorchidism	+	mild PVS	Delayed speech and language development; generalized hypotonia; delayed development
Our study	6.6	F	AD	Kelch 6 and BTB might be lost	c.1149+1G>A	Disrupts splice site (donor)	MC	Hypertelorism; downslanting palpebral fissures; epicanthal folds; squinting; nystagmus; low- set, oval-shaped, posteriorly rotated ears with a thick helix; short broad nose with a depressed root and full tip; deeply grooved and long philtrum; high and wide peaks of the vermilion; highly arched palate; micrognathia; short neck; cubitus valgus; scoliosis; pectus excavatum; café au lait spots; mild hypertrichosis	+	-	Delayed psychomotor development; hemivertebra deformity; refractive errors

NS: Noonan syndrome; F: female; M: male; AD: autosomal dominant; AR autosomal recessive; FC: farther carrier; MC: mother carrier; -: feature absent; +: feature present; NA: not applicable; HCM: hypertrophic cardiomyopathy; PVS: pulmonary valve stenosis; ASD: atrial septal defect.

Figures

Figure 1

Pedigrees of the Noonan syndrome (NS) families with variant c.1149+1G>T of the LZTR1 gene. The arrow indicates the proband. Black indicates that the patient exhibited variant c.1149+1G>T of LZTR1. Stripes indicate that the patient had a NS-like appearance to varying degrees. The number above the patients indicates their height.

Figure 2

Clinical images showing Noonan-like features in the proband, who provided consent. A) Café au lait spots, mild hypertrichosis. B) Low-set, oval-shaped, posteriorly rotated ears with a thick helix. C) Short broad nose with a depressed root and full tip, deeply grooved and long philtrum, high and wide peaks of the vermilion.

Figure 3

ECG of the proband. The ECG showed premature ventricular beats.

Figure 4

Imaging photos of the proband. A) The bone age was 5.5 years. B) X-ray imaging showing scoliosis (Cobb's angle = 28°). C) MRI showing hemivertebral deformity of the third thoracic vertebra, indicated by the yellow arrow. D) and E) Patient's craniocerebrum MRI showing no schwannomatosis or occupying lesions. F) Patient's spine MRI showing no schwannomatosis. The blue arrow indicates that the patient's pituitary height was 3.3 mm.

Figure 5

Sanger validation of mutation c.1149+1G>T of the LZTR1 gene in the proband's family. Variant c.1149+1G>T is shown in red. Absence of variant c.1149+1G>T is shown in green.