Core clinical symptoms and heterogeneous manifestations of NS / LAH: three case reports and review of the literature

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Case Report

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Abstract

Background Noonan-like syndrome with loose anagen hair-a rare autosomal dominant disease with very clinical heterogeneity, is difficult to make clinical diagnosis in the earlier period. We identified the cause of disease with severely short stature by WES on three pediatric cases. Meanwhile, we summarized the clinical manifestations of these three patients and reviewed the literature of the syndrome.

Case presentations We finally confirmed three cases of Noonan-like syndrome with loose anagen hair (NS / LAH) (OMIM # 607721) and hotspot mutation in SHOC2 gene (OMIM# 602775), which both involved in the RAS-MAPK signaling pathway. The mutation located in chr10: 112724120, NM_007373.3, c.4A>G (p.Ser2Gly) is de-novo heterozygous in all patients. NS / LAH show some core clinical symptoms, such as craniofacial anomalies, short stature, weight loss, abnormal hair, growth retardation and heterogeneous manifestations (macrocephaly, GH deficiency, congenital heart disease, skin pigmentation, mental retardation, etc.). In the study, we have also proved that it is safe and feasible to apply rhGH treatent on Noonan-like syndrome with loose anagen hair.

Conclusions Through our effort, we have identified a rare disease in China. The molecular characteristics, clinical manifestations, and therapeutic measure were summarized so that it can help to clinicians a better understanding of the disease and offer patients effective and timely assistance.

Introduction

Rasopathies is a type of syndrome with phenotypic overlap. Mutations in the relevant functional genes and downstream signaling genes in this pathway lead to RAS-related diseases(1). NS(Noonan syndromes)are the most common Rasopathy disease. It is currently believed that abnormal signal regulation of the RAS-MAPK pathway (Ras-mitogen-activated protein kinase signaling pathway) is the cause of NS(2). So far, a total of 20 genes have been reported to be associated with RASopathies, including A2ML1, BRAF, CBL, HRAS, KRAS, LZTR1, MAP2K1, MAP2K2, NF1, NRAS, PPP1CB, PTPN11, RAF1, RASA1, RASA2, RIT1, RRAS, SHOC2, SOS1, SOS2, and SPRED1(3). NS/LAH was first reported in 2003(4). Later, researchers conducted molecular biological examination on patients with NS/LAH, and confirmed the genetic cause of the disease (5). It can be observed that c.4A > G missense (p.Ser2Gly) is a hot spot mutation associated with NS/LAH. NS/LAH is rarely reported in the Chinese population, in this study, we confirm three cases of NS/LAH. Their clinical phenotype is consistent with the common manifestations of disease, but at the same time, they have their own specific characteristics. Our research expands the clinical phenotype of the disease and explores treatment options for this disease.

Case Presentation

Case 1

A 1.1-year-old boy was referred to our clinic for genetic evaluation, because of his growth retardation. The boy is the second-born of nonconsanguineous parents. He was born at 39+1 weeks of gestation and birth

weight 3.15kg, birth length 49cm, head circumference 34.5cm. There is no history of neonatal asphyxia, but Apgar values were not available. After birth, the boy was mixed feeding, and had no feeding difficulty. Physical development lagged compare with typically developing peers. His growth rate was about 9.5cm in his first year of life, and He cannot sit by himself at the age of 1.1y. His language development was within normal range. Hydrocephalus was found at the age of 6 months, but untreated. There was no history of similar diseases in the family. His father is 162cm, his mother is 153cm, both are healthy. And he has a healthy sister age 10y.

Physical examination: Ht :58cm (-7SD), Wt: 5.2kg (-5SD), head circumference: 50cm (+2SD), skin eczema, hair and eyebrow are thinning, left upper eyelid ptosis, Long eyelashes, high arched palate, prominent forehead, pectus carinatum(the prominent sternum), short limbs, deep palmar crease, joint hypermobility. No obvious abnormalities in spine.

The main laboratory examination: blood and urine routine tests, liver and kidney function, electrolytes, serum electrolyte, thyroid function, and blood tandem mass spectrometry test were normal. Growth hormone (GH) stimulate test: GH peak is 1.19 ng/ml(□10ng/ml). IGF-1: 51.9 ng/ml(55-327ng/ml). No pathogenic variation was observed in the FGFR3 gene sequencing analysis and micro-array test. Echocardiographic: ventricular septal hypertrophy□9.7mm(□ 8mm). Electrocardiogram indicated high left ventricular voltage. Cranial MRI: external hydrocephalus in the frontotemporal region. X-ray: osteoporosis, bone age was behind his peers, the spine was slightly scoliosis.

Follow-up: We keep intermittent following this patient for 6 years. And his ventricular septal was gradually increasing, the thickness of ventricular septal was 12mm at the age 3y. Cardiologist suggested Beta receptor blockers for oral medication at that time, but his parents did not adherence to the medication. Interesting, the thickness of his ventricular septal did not increase during the following examination at the age of 4y, even after the six-month GH treatment. He was under six month GH treatment with dosage of 0.1 IU/kg/d□and was benefit 4cm during the therapy. The growth chart of the patients was in Figure 1-A. At the age of 5y, he had bleeding caused by gastric ulcer after oral antipyretic, and then had partial gastrectomy.

Case 2

A 1.8 years old boy was referred to our clinic because of his growth retardation, mild high lactic acid and cryptorchidism. He was the first-born child of his family, his birth weight was 3.03kg, birth length was 49cm. He had no feeding difficulties in neonatal period. Physical development lagged compared with typical developing peers, but language development was similar to typical developing peers. Mild elevated blood lactic acid and hypoglycemia were found in his health examination. His father is 160cm and his mother is 155cm. They are physically healthy and has no history of the hereditary disease.

Physical examination: Ht :68.5cm (-5.6SD), Wt :7.6 kg (-4SD), head circumference 49.8cm (+2SD), skin eczema, the color of the skin was darker than his parents, fluffy hair, prominent forehead, sparse eyebrow, long lashes, joint laxity, cryptorchidism, normal penis, deep palmar crease.

The main laboratory examination : Blood glucose: 3.34mmol/L (3.9-6.2 mmol/L); lactic acid 1.8-2.8 mmol/L (0.5-1.6 mmol/L), fasting insulin: 12.2 (fasting 17.8-173 pmol/L). Blood gas analysis test, blood and urine routine tests, liver and kidney function, electrolytes, serum electrolyte, thyroid function, and blood tandem mass spectrometry test were normal. Growth hormone (GH) stimulate test: GH peak 2.35 ng/ml(≥10ng/ml). IGF-1 50 ng/ml (55-327ng/ml). The blood tandem mass spectrometry (amino acid and fatty acid analysis), urine organic acid test, microarray, and FGFR3 gene sequencing were normal. Cranial MRI: The brain volume was larger than the same age peer. Bone age was delayed. Scrotum ultrasonography showed both testicles were in the groin area. X-ray of spine, echocardiographic and electrocardiogram were normal.

Follow-up: We keep following this patient for 5 years. The blood lactic acid was within normal range several times. He started GH treatment (0.1IU/kg/d)from 3y old, and gained 6.2cm/7month, then stopped treatment by the parents without doctor's suggestion. When stop the GH treatment, his growth velocity was slower down. The growth chart of the patients was in Figure 1-B. There was no skeleton or cardiac anomality observerd during the follow-up period.

Case 3

A 2 months old girl was referred to our department because of her poor growth. The girl was the second child the family. During the pregnancy, there was complicated by polyhydramnios. She was born full-term, birth weight was 3.4kg, birth height was 50cm. The Apgar score were 10 - 8 - 9 -9. She had feeding difficulty and malnutrition, growth retardation, poor motor development, recurrent skin eczema and respiratory infections. The parents are healthy, and they are not consanguineous, without remarkable family history. She has a 4 years old brother, who is normal in growth and development. The girl was admitted to hospital because of pneumonia.

Physical examination: HT:50cm(-3SD), WT:3.98kg(-2SD), head circumference:41.5cm(+2SD), temperature:37.8℃, pulse:172times/min, respiratory rate:50 times/min, blood pressure:70/48mmHg, scaphocephaly, anterior fontanelle: 4*4cm flat and soft, prominent forehead, esotropia, micrognathia, high palate, muscular hypotonia, joint laxity, sparse scalp hair and eyebrow. A mass was touched about 4*4cm in the left epigastric.

The main laboratory examination: blood glucose: 3.7mmol/L, blood routine and hyper-sensitive C-reactive protein suggest she had an infection. Digital radiography (DR) of the thoracic chest indicates she had pneumonia and increased heart shadow. cranial ultrasound: subependymal hemorrhage, Echocardiography: patent foramen ovale, mild abnormality of the pulmonary valve. abdominal ultrasonography: mixed echogenic mass (4.0*3.6cm) in left abdomen, low echogenic mass (5.6*2.9*3.8cm) in the left lobe of the liver. That may be a hemangioma. Upper gastrointestinal opacification: descending colonic dilatation, upper-Abdomen routine, and enhancement scanning: multiple hemangiomas in the liver, cranial MRI: Abnormal structure of gyrus in the right frontal lobe. The result raises the possibility that pachygyria. Neonatal neurobehavioral score 31 points (not pass). Qualitative general movements assessment（GMS）: GMs (poor repertoire), term infant.

Follow-up: We keep follow-up this girl for 2.5 years. Now she is 2.5y, 76cm(-5SD),8.5kg(-4SD). She can stand and walk for while by herself. X-ray of spine and echocardiography were normal.

Methods

DNA extraction

Blood samples (2 mL of peripheral blood) were collected from the patient and his parents for the whole-exome sequencing. Genomic DNA was isolated using the Lab-Aid DNA kit (Zeesan Biotech Co., Ltd, China). And DNA was stored at -80 ℃.

Whole exome sequencing and Sanger sequencing

For whole-exome seq sequencing, genomic DNA samples were captured to create a sequencing library by Agilent SureSelect Human All Exon V5 Kit (Agilent Technologies, Santa Clara, CA) following the manufacturer's protocol. The prepared libraries were sequenced with a HiSeq2500 (Illumina, San Diego, CA). Sanger sequencing was used to verify the mutations and their origins. All procedures followed the manufacturer's instructions.

Sequencing data analysis

Genome Analysis Toolkit (GATK) was used for variant calling (GATK HaplotypeCaller) . The TGex software (LifeMap Sciences,USA) was used to annotate the selected SNVs and indels. “Rare deleterious” mutations were defined as those that met the following criteria: a) they led to a stop-gain, stop-loss, nonsynchronous, frame shift or splice-site mutation; (b) Reference Genome GRCh37 / hg19.

Interpretation of variation

Pathogenicity assessment is based on the ACMG/AMP guidelines□The American College of Medical Genetics and Genomics (ACMG)and the Association for Molecular Pathology(AMP)□Based on InterVar□□ he classification of variants into 'Benign', 'Likely benign', 'Uncertain significance', 'Likely pathogenic' and 'Pathogenic'.

Discussion And Conclusion

Three patients performed with severe growth retardation, macrocephaly. In order to clarify the genetic cause of the disease, we performed whole exon sequencing. Sequencing data were conjoint analyzed with clinical symptoms. And the same mutation of SHOC2 gene was confirmed in the three patients. SHOC2 gene is on the RAS/MAPK signaling pathway, c.4A>G (p. Ser2Gly) mutation was the hotspot mutation of the gene. Based on the findings, the final diagnosis of three children was NS / LAH (Noonan-like syndrome with loose anagen hair, OMIM: # 607721). We summarized the clinical symptoms of the three patients and compared with the literatures, as shown in Table 1, to find the core clinical phenotype of NS / LAH.

From Table 1, we found that mean birth weight was 3360±491.84g (reference levels: 2500-4000) and birth length was 47.76±2.89 cm (reference levels: 50cm). This would imply that newborns do not have low birth weight, but some children show symptoms of intrauterine growth retardation. At the same time, through the data, it is not hard to find that 59 cases of NS/LAH children showed short stature, light weight, craniofacial anomalies, sparse scalp hair and eyebrow, growth retardation. We take these symptoms as the core clinical manifestations of the syndrome. In addition, 96.43% of the patients will be associated with macrocephaly, 75.93% will be associated with congenital heart disease, 90.63% with growth hormone deficiency, 80.43% with mental retardation, 85.71% with Skin pigmentation, 84.21% with intracranial abnormality, 64.71% with feeding difficulties and 46.43% with abnormality of the skeletal system. The lowest clinical manifestations is abnormality of the genitourinary system (14.29%), 29.63% of the patients' mothers showed polyhydramnios during pregnancy. We think these clinical symptom are heterogeneous manifestations of NS / LAH. Multiple hemangioma in hepatic and gastric ulcer had not been described in these existing cases , and further analysis of the sample data is needed.

Since Tartaglia reported the first gene, PTPN11, which causes Noon's syndrome in 2001(6), significant progress has been made in elucidating the pathogenesis and genetics of this disease. It has been discovered that more genes in the RAS-MAPK pathway can lead to Noonan or Noonan-like syndrome. The SHOC2 gene is one of them. In 2003, Mazzanti first reported Noonan-like syndrome with loose anagen hair[4], this syndrome exhibits both Noonan features, including short stature, facial deformity, congenital heart disease, hypertrophic cardiomyopathy, skeletal abnormalities, and ectodermal abnormalities, such as easy to lose hair, hair growth is slow and sparse, lacking inner root sheath and outer root sheath. Until 2009, Cordeddu finally confirmed SHOC2c.4A>G, p.Ser2Gly missense mutation is the root of NS/LAH(7). Although the genetic background and living background of NS/LAH patients are different, the sites that cause disease are consistent. In theory, every base in the DNA molecule may be mutated, but in fact, the mutation site is not completely randomly distributed. Each part of the DNA molecule has a different mutation frequency and there is a mutation hotspot. At present, the reasons for the formation of mutation hot spots remain unclear. However, it is certain SHOC2c.4A>G, p.Ser2Gly is a mutation hotspot of NS/LAH. SHOC2 (leucine-rich repeat scaffold protein) is located at chr10:110,919,543-111,013,667 (10q25.2,GRCh38/hg38) containing 94,125 bases, consisting of 582 amino acids, molecular mass:64888 Da. Which acts as a positive modulator of the RAS-MAPK pathway. This specific mutation creates a recognition site for N-terminal myristoylation, in which a 14-carbon saturated fatty acid is covalently attached to the N-terminal and the result is constitutive membrane targeting, leading to increased RAS-MAPK signaling. The NS/LAH phenotype is complex, involving multiple organs and tissues. That causing difficulties in clinical diagnosis, especially in China where this syndrome is rarely reported. We found three cases of Noonan-like syndrome with loose anagen hair that benefited from the rapid development of second-generation sequencing technology in recent years, but It still should be noted that although genetic testing can aid or confirm the disease, That does not mean, however, we can exclude such diseases when known disease-associated gene variants are not detected.

Three patients showed the core clinical manifestations of the disease and also showed some clinical heterogeneity, such as severe digestive tract ulcers, multiple hemangiomas in the liver, etc. Among the

symptoms, short stature is the most common and most obvious symptom. The fundamental cause of short stature is caused by growth hormone deficiency. Growth hormone (GH) promotes human development and cell proliferation. The early studies showed that recombinant human GH (rhGH) improved the growth rate of NS patients(8). But the data is still limited and the protocol of GH therapy for NS/LAH is not well established. We attempted to apply a small dose of rhGH to these two short stature NS/LAHs (case1 and case2) and had a regular follow-up. The patient's symptoms improved and there were no adverse reactions. The lower dose of GH therapy remarkably improved the linear growth of the patient. The SHOC2 gene is a gene in the RAS-MAPK signaling pathway, while the RAS-MAPK signaling pathway is involved in growth factor-mediated cell proliferation, differentiation, and apoptosis. When extracellular stimulated signaling molecules such as growth factors and hormones bind to cell membrane surface receptors, growth factor receptor binding protein 2 (GRB2) recruits guanylate exchange factor (SOS1) and protein tyrosine phosphatase non-receptor type 11 (PTPN11) forms a complex that activates the MAPK signaling pathway cascade through a series of phosphorylation, and ultimately participates in cell proliferation and differentiation or transcription of related genes in the cytoplasm/nucleus (9).

In terms of adverse events, according to the literature, in addition to a small range of fluctuations in fasting insulin levels, rhGH has no significant effect on the structure, function of heart and glucose metabolism of NS patients (10). However, NS patients have a higher risk of hematologic and substantial tumors, and the standardized incidence ratio of childhood tumors is 8.1 (11). There have been reports of subcutaneous invasive granulose cell tumors, lymphoma, and mandibular giant cell tumor recurrence after treatment (12).Fortunately, by now, the three patients we treated have not shown the above symptoms.

After a period of rhGH treatment and regular cardiology and hematology, we believe that rhGH treatment on Noonan-like syndrome with loose anagen hair is safe and feasible. RhGH can significantly increase its growth rate but should be followed regularly during treatment, assess cardiac function, glucose metabolism levels and monitor the presence of tumors. However, it is necessary to clarify that Longterm correlation between genotype and rhGH therapy responsiveness needs to be addressed in a larger population.

Abbreviations

NS / LAH
Noonan-like syndrome with loose anagen hair
NS
Noonan syndromes
DR
Digital radiography

Ht
height
Wt
weight
RhGH
recombinant human growth hormone

Declarations

Availability of data and materials

Please contact authors for data requests.

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Contributions

Qiang Zhang, Xin Fan, Zailong Qin, and Shaoke Chen designed the manuscript and analyzed the literature. Qiang Zhang, Xin Fan wrote the manuscript and prepared the figures. All authors read and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

written informed consent were obtained from the parents for publication of this report and any accompanying images. The consent form was approved by the ethical committee of Guangxi Maternal and Child Health Hospital, China.

Consent for publication

All patient in this report give their consent for images (such as face and clinical feature) or other clinical information relating to be reported for academic purpose.

Competing interests

The authors declare that they have no competing interests.

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Table

Due to technical limitations, table 1 is only available as a download in the supplemental files section.

Figures

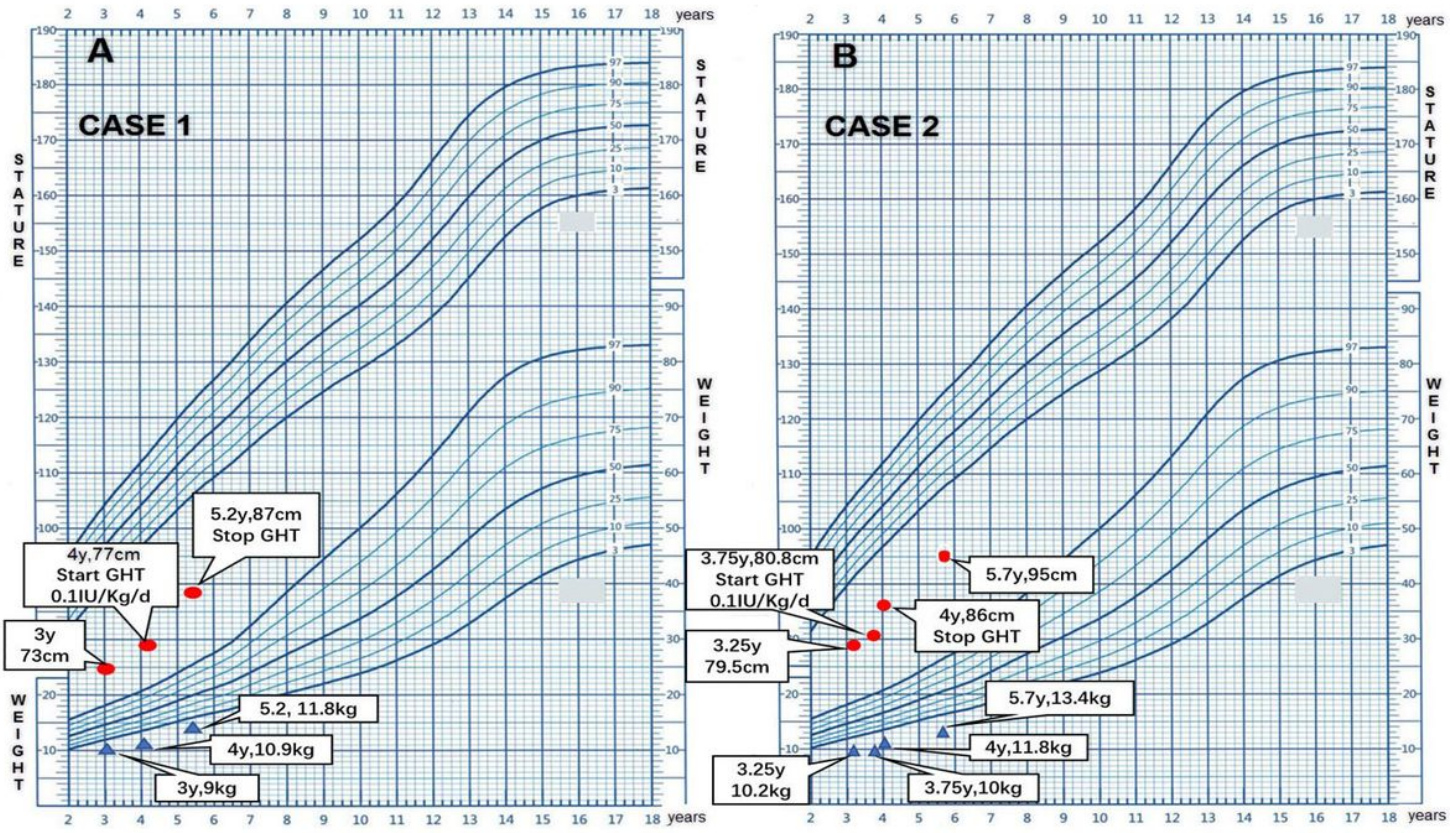

Figure 2

Height and Weight curves of the 2 patients. The red dot represents the height curve and the blue triangle represents the weight curve.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- Table1.pdf