Case report

A Novel Heterozygous \textit{NF1} Variant in a Neurofibromatosis-Noonan Syndrome Patient with Growth Hormone Deficiency: A Case Report

Qin S et al. Short Stature due to a Novel \textit{NF1} Variant

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What is already known on this topic?
Neurofibromatosis-Noonan syndrome (NFNS) is a rare autosomal-dominant hereditary disease characterized by clinical manifestations of both neurofibromatosis type 1 (NF1) and Noonan syndrome. Several \textit{NF1} gene mutations have been reported to be associated with NFNS, such as a de novo heterozygous deletion of exons 1–58 and a heterozygous mutation c.7549 C > T in exon 51.

What this study adds?
In this work, a novel heterozygous mutation in the \textit{NF1} gene was identified in an NFNS patient, who showed phenotypic features of both NF1 and Noonan syndrome. Short stature, a common feature of NFNS, can be caused by growth hormone deficiency. There is no consensus as to whether NFNS patients should be treated with recombinant growth hormone.

Abstract
Neurofibromatosis-Noonan syndrome (NFNS), a rare autosomal-dominant hereditary disease, shows the manifestations of both neurofibromatosis type 1 (NF1) and Noonan syndrome. We present a case of NFNS with short stature due to the heterozygous nonsense variant of the \textit{NF1} gene. A 12-year-old boy was admitted for short stature, numerous café-au-lait spots, low-set and posteriorly rotated ears, sparse eyebrows, broad forehead and inverted triangular face, and nodular abnormal lesions revealed by cranial and spine magnetic resonance imaging. A novel heterozygous c.6189C > G (p.Tyr2063*) variant in \textit{NF1} gene was identified by the molecular analysis. Because exogenous growth hormone (GH) may enlarge nodular abnormal lesions, the patient did not receive GH treatment. During the follow-up, Lisch nodules were found in ophthalmologic examination. NFNS, a variant form of NF1, is caused by heterozygous mutations in the \textit{NF1} gene. The mechanism of GH deficiency caused by NF1 is still unclear. Whether NFNS patients should be treated with exogenous GH is still controversial.

Keywords: Neurofibromatosis Noonan syndrome, growth hormone deficiency, \textit{NF1} gene

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Introduction
Neurofibromatosis-Noonan syndrome (NFNS) (OMIM # 601321), a rare autosomal-dominant hereditary disease, was first reported in 1985 by Allanson and colleagues. Patients with NFNS have clinical manifestations of both neurofibromatosis type 1 (NF1) (OMIM # 162200) and Noonan syndrome (NS) (OMIM # 163950)\(^1\)\(^2\). NFNS, NF1, and NS belong to the RASopathies caused by dysregulation of the RAS-mitogen-activated protein kinase (MAPK) signaling pathway\(^3\). Several NF1 gene mutations have been reported to be associated with NFNS\(^3\)\(^-\)\(^7\), such as a de novo heterozygous deletion of exons 1–58\(^7\) and a heterozygous mutation c.7549 C > T in exon 51\(^6\). However, heterozygous nonsense variant c.6189 C > G (p.(Tyr2063*)) in the NF1 gene has not been reported. Here, we report a novel NF1 variant detected in a 12-year-old patient with NFNS who had short stature. The case report may help improve the understanding of the disease.

Case Report
A 12-year-old boy was referred for the evaluation of short stature. His parents were not consanguineous and there was no family history of genetic disease. At the time of birth, café-au-lait spots of different sizes were observed. He was born of full-term delivery, and his body length and birth weight were 52 cm and 3.8 kg, respectively.

On physical examination, the height and weight of the child were 136.5 cm [−2 standard deviation (SD) to −3SD] and 25.5 kg (−2SD to −3SD), according to the height- and weight-standardized growth charts for Chinese children and adolescents aged 0–18 years\(^8\). Upper/lower segment ratio was 0.94, and the arm span was 130 cm suggesting no skeletal deformity. The dysmorphic facial features including low-set and posteriorly rotated ears, sparse eyebrows, broad forehead, and inverted triangular face were suggestive of NS (The patient's parents declined to provide photos for publication). Relative macrocephaly, axillary freckling, and more than six café-au-lait spots (Figure 1A) with a maximal one of 2.5 cm × 4.2 cm (Figure 1B) suggested NF1. No abnormalities were detected on neurological and cardiovascular examinations. His developmental milestones were normal for his age and he had no cutaneous neurofibroma. No Lisch nodules were observed on ophthalmological examination during the first diagnosis. The pubertal stage was classified as Tanner stage 1. Serum insulin-like growth factor-1 (IGF-1) levels were approximately −1 SD for his age. Peak growth hormone (GH) response to pyridostigmine bromide and L-dopa was 4.38 ng/mL. Male tumor marker levels as well as thyroid and adrenal gland function tests were normal. The detailed auxological parameters and hormone levels of this case are shown in Table 1.

Chest radiography showed thoracolumbar scoliosis (Figure 2A), and the bone age lagged 2 years behind the actual age. Cranial magnetic resonance imaging (MRI) showed abnormal nodular signals in bilateral basal ganglia-thalamus region (Figure 2B). Spinal cord MRI revealed slight localized thickening and nodular appearance of C8 nerve originating from the left brachial plexus nerve (Figure 2C); thoracic spinal cord showed no obvious abnormality, while the anterior branch of the 5th lumbar nerve on the left was slightly thicker than the contralateral at L5/S1 level (Figure 2D).

Genetic analyses were conducted for the patient and his parents. Whole-exome sequencing of the peripheral blood was performed. Molecular analysis revealed a novel heterozygous c.6189 C > G (p.(Tyr2063*)) variant in the NF1 (NM_000267.3) gene (Figure 3). No mutation was found in the PTPN11 gene. This novel variant is predicted as likely pathogenic according to American College of Medical Genetics consensus recommendations (null variant, variant not found in public databases)\(^9\). However, his parents did not carry the above-mentioned gene mutation.

Although the boy had growth hormone deficiency (GHD), he was not treated with recombinant GH replacement therapy as the application of exogenous GH may enlarge nodular lesions of the brain and spinal cord.

One year after the diagnosis, his height and weight were still 2 to 3 SD below the Chinese reference standards. At the age of 14 years, his height and weight were 150 cm (−2SD to −3SD) and 33.2 kg (−2SD to −3SD), respectively.

Anthropometric follow-up data are shown in Table 1. Till date, the child has not been treated with recombinant GH. However, several Lisch nodules were observed on ophthalmologic examination at a one-year follow-up visit (Figure 1C).

Discussion
NF1 and NS are both related to abnormalities in the RAS-MAPK signaling pathway, but have distinct differences at the genetic level. In NF1 patients, neurofibromin, encoded by the NF1 gene and acting as a negative regulator in the Ras-MAPK pathway, can inactivate or deregulate Ras-GTPase. However, NS is genetically heterogeneous. PTEN11, RAF1, SOS1, KRAS, BRAF, SOS2 and 14 other genes are related to NS; in particular, the PTEN11 gene has been implicated in the etiology of more than 50% of NS cases. In NS patients, SHP2 protein, encoded by the PTEN11 gene and acting as a positive regulator of Ras-mediated signaling transduction, can activate Ras signaling pathway. Several NFNS patients have been reported till date. Among these, several cases showed the co-occurrence of NF1 and PTEN11 mutations; however, the majority of genetic studies only identified mutations in the NF1 gene. To determine whether NFNS represents a variable manifestation of either NS or NF1, or is an independent disease, studies have found that NFNS, a variant form of NF1, is caused by heterozygous mutations in the NF1 gene. NF1 was also the only pathogenic variant gene causing NFNS in our patient. Genetically, the variants related to NF1 and NFNS are associated with 17p11.2. Moreover, the combination of a mutation in the NF1 gene and an environmental epigenetic factor of muscle hypotonia may result in the NFNS phenotype.

Ekvall et al reviewed different NF1 mutations reported in patients with NF1 and NFNS, and identified peculiar characteristics of the variants associated with NFNS with respect to type and location. Firstly, compared to NF1, a higher prevalence of in-frame deletions and missense mutations were found in NFNS. Secondly, small insertions, splice-site mutations, and small indels were more common in NF1 compared to NFNS. Thirdly, NFNS showed a tendency for clustering of in-frame mutations in the GAP related domain (exon 20–27a). Finally, small deletions seemed to be clustered in the cysteine/serine-rich domain (exon 11–17). Subsequent searching literature related to NFNS found three novel mutations: a de novo heterozygous deletion including exons 1–58 of the NF1 gene, a novel heterozygous c.3052_3056delTTAGT (p.(L1018*)) variant, and a truncating mutation c.7846 C > T (p.(Arg2616*)) in a NS patient. In our case, the novel nonsense variant failed to conform to these characteristics. In NS patients, SHP2 protein, encoded by the PTEN11 gene, has been implicated in the etiology of more than 50% of NS cases. In NS patients, SHP2 protein, encoded by the PTEN11 gene, has been implicated in the etiology of more than 50% of NS cases.
GHD. Our patient showed clinical manifestations of NFNS with GHD and had a novel heterozygous mutation in the NF1 gene. However, due to the limited number of related trials, further research on the effectiveness and safety of recombinant GH or selumetinib in treating NFNS patients with GHD would be helpful.

**Informed Consent**: Written informed consent was obtained from the patient’s father.

**Author contributions**: Medical Practices: SQ, YXN, and JZ; Concept: SQ, YXN, and JZ; Design: SQ, YXN, and JZ; Data Collection or Processing: SQ, YDZ, and FDY; Analysis or Interpretation: SQ, YDZ, and FDY; Literature Search: SQ, YDZ, and FDY; Writing: SQ, YXN, and JZ.

**Conflicts of interest**: The authors declare no competing financial interests. Si Qin, Yindi Zhang, Fadong Yu, Yinxing Ni, and Jian Zhong declare that they have no conflict of interest.

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**References**


Figure 1. Phenotypic findings of the patient. A, B) café-au-lait spots on the skin. C) Ophthalmologic findings: Lisch nodules.
Figure 2. A) Chest radiograph showing thoracolumbar scoliosis. Magnetic resonance imaging of brain and spinal cord. B) Abnormal nodular signals in bilateral basal ganglia-thalamus region. C) Slight thickening of C8 (the left brachial plexus nerve). D) Slight thickening of the anterior branch of the 5th left lumbar nerve.
Figure 3. Results of NF1 Sanger sequencing.
NM_000267.3: c.6189 C > G, (p.(Tyr2063*)), Heterozygote, Nonsense

Table 1. Auxological parameters and hormone levels of the patient

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<tr>
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<tr>
<td>Age (years)</td>
<td>12</td>
<td>13</td>
<td>14</td>
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<tr>
<td>Bone age (years)</td>
<td>10</td>
<td>12.8</td>
<td>13.1</td>
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<tr>
<td>Height (cm)</td>
<td>136.5</td>
<td>143</td>
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<tr>
<td>Weight (kg)</td>
<td>25.5</td>
<td>27.8</td>
<td>33.2</td>
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<tr>
<td>BMI (kg/m^2)</td>
<td>13.7</td>
<td>13.6</td>
<td>14.8</td>
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<tr>
<td>Upper/lower segment ratio</td>
<td>0.94</td>
<td>0.93</td>
<td>0.92</td>
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<tr>
<td>Arm span (cm)</td>
<td>130</td>
<td>139</td>
<td>144</td>
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<td>Father’s height (cm)</td>
<td>172</td>
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<td>Mother’s height (cm)</td>
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<tr>
<td>Predicted adult height (cm)</td>
<td>170</td>
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<tr>
<td>FT4 (pmol/L)</td>
<td>7.31 (4.27 – 6.96)</td>
<td></td>
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<tr>
<td>FT3 (pmol/L)</td>
<td>10.18 (7.95 – 14.79)</td>
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<tr>
<td>TSH (μIU/mL)</td>
<td>0.95 (0.670 – 6.060)</td>
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<tr>
<td>ACTH (pg/mL)</td>
<td>17.20 (7.2 – 63.3)</td>
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<td>Blood plasma cortisol (μg/dL)</td>
<td>8.38 (AM: 6.71–22.54; PM: &lt; 10.00)</td>
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<tr>
<td>IGF-1 (μg/L)</td>
<td>202.00 (143 – 693)</td>
<td>330.00 (183 – 850)</td>
<td></td>
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<tr>
<td>Peak GH level in L-dopa test (ng/mL)</td>
<td>4.38 (≥ 10)</td>
<td></td>
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<tr>
<td>Peak GH level in pyridostigmine bromide test (ng/mL)</td>
<td>4.38 (≥ 10)</td>
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BMI: body mass index; FT4: free thyroxin; FT3: free triiodothyronine; TSH: thyroid-stimulating hormone; ACTH: adrenocorticotropic hormone; IGF-1: insulin-like growth factor-1; GH: growth hormone.