CASE REPORT

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A Novel Heterozygous *NF1* Variant in a Neurofibromatosis-Noonan Syndrome Patient with Growth Hormone Deficiency: A Case Report

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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 NS. Several *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?

A novel heterozygous mutation in the *NF1* gene was identified in an NFNS patient, who showed phenotypic features of both NF1 and NS. Short stature, a common feature of NFNS, can be caused by growth hormone (GH) deficiency. There is no consensus as to whether NFNS patients should be treated with recombinant GH.

Abstract

Neurofibromatosis-Noonan syndrome (NFNS), a rare autosomal-dominant hereditary disease, is characterized by clinical manifestations of both neurofibromatosis type 1 (NF1) and NS. We present a case of NFNS with short stature caused by a heterozygous nonsense variant of the *NF1* gene. A 12-year-old boy was admitted because of short stature, numerous café-au-lait spots, low-set and posteriorly rotated ears, sparse eyebrows, broad forehead, and inverted triangular face. Cranial and spinal magnetic resonance imaging showed abnormal nodular lesions. Molecular analysis revealed a novel heterozygous c.6189 C > G (p.(Tyr2063*)) variant in the *NF1* gene. The patient was not prescribed recombinant growth hormone (GH) therapy because exogenous GH may have enlarged the abnormal skeletal lesions. During follow-up, Lisch nodules were found in the ophthalmologic examination. NFNS, a variant form of NF1, is caused by heterozygous mutations in the *NF1* gene. The mechanism of GH deficiency caused by NF1 is still unclear. Whether NFNS patients should be treated with exogenous GH remains controversial.

Keywords: Neurofibromatosis-Noonan syndrome, growth hormone deficiency, *NF1* gene

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 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 (3,4,5,6,7), 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, the heterozygous nonsense variant c.6189 C > G, (p.(Tyr2063*)) in the *NF1* gene has not previously been reported. Here, we report a novel *NF1* variant detected in a 12-year-old patient with NFNS who had short stature.

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Case Report

A 12-year-old boy was referred for evaluation of short stature. His parents were not consanguineous and there was no family history of genetic disease. At birth, café-au-lait spots of different sizes had been observed. He was born of full-term, vaginal birth and his birth length and 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 -3 SD] and 25.5 kg (-2 SD to -3 SD), 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. Unfortunately, the patient's parents declined to provide clinical photographs for publication. Relative macrocephaly, axillary freckling, and more than six café-au-lait spots (Figure 1A) with the largest measuring 2.5 cm × 4.2 cm (Figure 1B) suggested NF1. No abnormalities were detected on neurological and cardiovascular examination. His developmental milestones were normal for his age and he had no cutaneous neurofibroma. No Lisch nodules were observed on ophthalmological examination during the initial 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 are shown in Table 1.

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

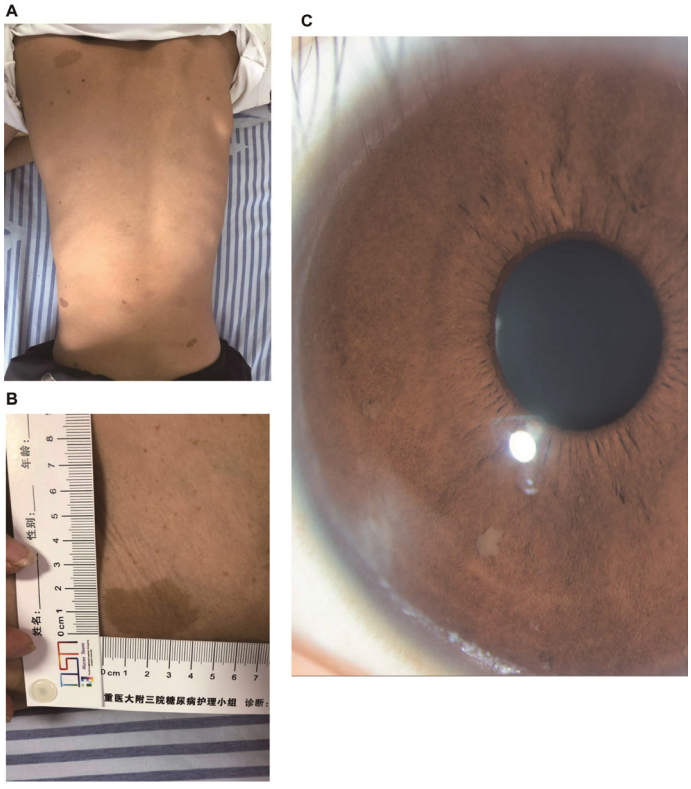

Figure 1. Phenotypic findings of the patient. A, B) Café-au-lait spots on the skin. C) Ophthalmologic findings: Lisch nodules

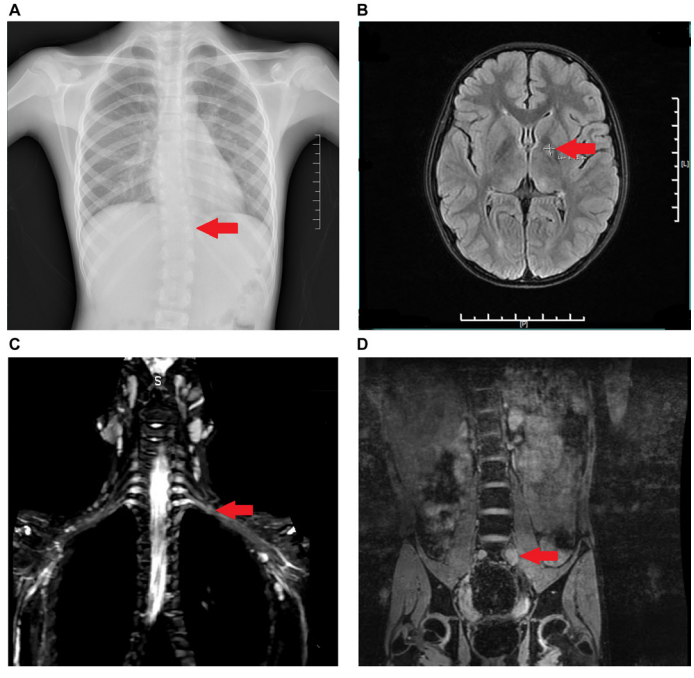

Figure 2. A) Chest radiograph showing thoracolumbar scoliosis. Magnetic resonance imaging of brain and spinal cord. B) Abnormal nodular signals in bilateral basal ganglia-thalamus region. C) Slight thickening of C8 (the left brachial plexus nerve). D) Slight thickening of the anterior branch of the fifth left lumbar nerve

Genetic analyses were conducted for the patient and his parents. Whole-exome sequencing of the peripheral blood DNA 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 same gene mutation.

Although the boy had GH deficiency (GHD), he was not treated with recombinant GH replacement therapy as the

Table 1. Auxological parameters and hormone levels of the patient

Age (years)	12	13	14
Bone age (years)	10	12.8	13.1
Height (cm)	136.5	143	150
Weight (kg)	25.5	27.8	33.2
BMI (kg/m^2)	13.7	13.6	14.8
Upper/lower segment ratio	0.94	0.93	0.92
Arm span (cm)	130	139	144
Father's height (cm)	172		
Mother's height (cm)	155		
Predicted adult height (cm)	170		
fT4 (pmol/L)	7.31 (4.27-6.96)		
fT3 (pmol/L)	10.18 (7.95-14.79)		
TSH (µIU/mL)	0.95 (0.670-6.060)		
ACTH (pg/mL)	17.20 (7.2-63.3)		
Blood plasma cortisol (µg/dL) (8:00 am)	8.38 (am: 6.71-22.54; pm: < 10.00)		
IGF-1 (µg/L)	202.00 (143-693)	330.00 (183-850)	
Peak GH level in L-dopa test (ng/mL)	4.38 (≥10)		
Peak GH level in pyridostigmine bromide test (ng/mL)	4.38 (≥10)		

BMI: body mass index, fT4: free thyroxine, fT3: free tri-iodothyronine, TSH: thyroid-stimulating hormone, ACTH: adrenocorticotropic hormone, IGF-1: insulin-like growth factor-1, GH: growth hormone

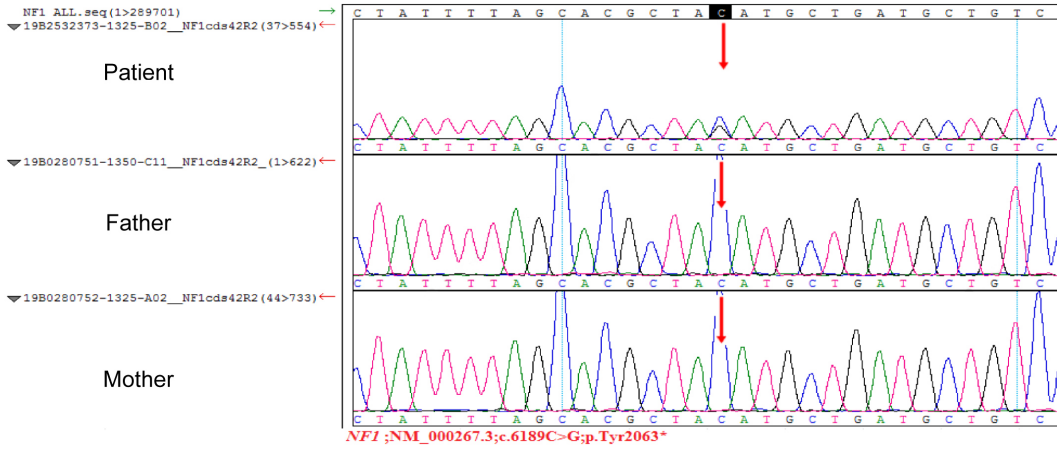

Figure 3. Results of *NF1* Sanger sequencing. NM_000267.3: c.6189 C > G, (p.(Tyr2063*)), heterozygote, nonsense

use of exogenous GH may enlarge the nodular lesions in 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 (-2 SD to -3 SD) and 33.2 kg (-2 SD to -3 SD), respectively. Anthropometric follow-up data are shown in Table 1. At the time of writing the child has still 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 patients with NF1, neurofibromin, encoded by the *NF1* gene and acting as a negative regulator in the Ras-MAPK pathway (6), can inactivate or deregulate Ras-GTPase. However, NS is genetically heterogeneous. *PTPN11*, *RAF1, SOS1, KRAS, BRAF, SOS2* and 14 other genes are related to NS; in particular, the *PTPN11* gene has been implicated in the etiology of more than 50% of NS cases (10). In NS patients, SHP2 protein, encoded by the *PTPN11* gene and acting as a positive regulator of Ras-mediated signaling transduction, can activate Ras signaling pathway (6).

Several NFNS patients have been reported to date. Among these, several cases showed the co-occurrence of *NF1* and *PTPN11* mutations (4,11); however, the majority of genetic studies only identified mutations in the *NF1* gene (7,12). Investigations have been performed to determine whether NFNS represents a variable manifestation of either NS or NF1, or is an independent disease. These investigations (3,13) 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 the presented case.

Genetically, the variants related to NF1 and NFNS are associated with 17p11.2 (5,10,13,14,15,16,17). Moreover, the combination of a mutation in the *NF1* gene and an environmental epigenetic factor resulting in muscle hypotonia may cause the NFNS phenotype (18,19). Ekvall et al. (3) 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, splicing 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). A subsequent literature search related to NFNS found three novel mutations: a *de novo* heterozygous deletion including exons 1-58 of the *NF1* gene (7), a novel heterozygous c.3052_3056delTTAGT (p.(L1018*)) variant (12), and a truncating mutation c.7846 C > T (p.(Arg2616*)) (20). In our case, the novel nonsense variant failed to conform to these characteristics. Although *de novo* occurrence is the likely explanation for this patient's illness, gonadal mosaicism in one of his parents could not be excluded.

Moreover, the pathogenicity of this nonsense variant was predicted by PolyPhen, Sorting Intolerant From Tolerant (SIFT), the loss of function (LoF) tool (21), and Combined Annotation Dependant Depletion (CADD) phred scores (22). On *in silico* analysis, the LoF tool score was 0.116, and CADD phred score for the same variant was 36.0; however, PolyPhen and SIFT failed to predict this variant. A low LoF tool score implies a damaging effect and a CADD score above 10 indicates a deleterious effect of the variant on the gene (23). Therefore, this novel variant may impair both the gene structure and protein function.

GHD is common in patients with NF1, NS, and NFNS. There is a plausible explanation for the relationship between GHD and NF1. First, the *NF1* gene may affect pituitary development. Hegedus et al. (24) showed a reduced size of the anterior pituitary gland and decreased body weight in mice with deactivated *NF1* gene; in addition, the reduced anterior pituitary gland size could present decreased expression of neurofibromin in the hypothalamus, resulting in diminishing production of GH-releasing hormone, GH, and IGF-1. Therefore, a common cause of short stature in these diseases is suprasellar lesions, but GHD can be found in some patients lacking a fundamental suprasellar lesion (25). Second, neurofibromin may regulate body growth by acting on the hypothalamic-pituitary axis (24) but the pathophysiological mechanisms and related signaling pathways are still unclear. This may explain why our patient had GHD in the absence of pituitary lesions. Further high-quality studies are required to uncover the underlying signaling pathways.

Recombinant GH replacement is the canonical treatment for GHD (26) but there is no consensus as to whether NFNS patients should be treated with exogenous GH. To date, only two case reports (20,27) have described GH treatment in NFNS with GHD. Use of recombinant GH may lead to the enlargement of nodular abnormal lesions and so recombinant GH replacement therapy was not used in the

presented case because of these probable contraindications. Selumetinib, an oral, specific inhibitor of MAPK kinase 1 and 2, has been used in NF1 children with inoperable plexiform neurofibromas (28). However, this drug was not available for our case in China.

Conclusion

Owing to the rarity of NFNS and difficulty in clinical diagnosis, there is limited experience in managing NFNS patients with GHD. The presented case 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.

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Ethics

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Si Qin, Yinxing Ni, Jian Zhong, Concept: Si Qin, Yinxing Ni, Jian Zhong, Design: Si Qin, Yinxing Ni, Jian Zhong, Data Collection or Processing: Si Qin, Yindi Zhang, Fadong Yu, Analysis or Interpretation: Si Qin, Yindi Zhang, Fadong Yu, Literature Search: Si Qin, Yindi Zhang, Fadong Yu, Writing: Si Qin, Yinxing Ni, Jian Zhong.

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