

Original Article

A novel mutation of the *NF1* gene in a Chinese family with neurofibromatosis type 1

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Abstract: Background: Mutations in the neurofibromin 1 (*NF1*) gene are associated with clinical manifestations of neurofibromatosis type 1 (NF1). Objective: To clarify the relationship between *NF1* variants and disease phenotype. Methods: Peripheral blood samples were collected from a patient and her relatives and genomic DNA was extracted for next-generation sequencing (NGS) to detect potential variants; the results were validated by Sanger sequencing. Results: A novel frameshift variant c.4508_c.4509delAT (p.Asn1503fsTer26) was detected in exon 34 of the *NF1* gene in the patient and her daughter, but not in any other (healthy) family member. This c.4508_c.4509delAT (p.Asn1503fsTer26) frameshift variant of *NF1* may underlie NF1 in this family. Conclusions: This finding expands the spectrum of pathogenic mutations of the *NF1* gene, which could aid genetic counseling and prenatal diagnosis.

Keywords: Neurofibromatosis type 1, *NF1* gene, mutation, whole-exome sequencing, Sanger sequencing

Introduction

Neurofibromatosis is an autosomal dominant genetic disease characterized by abnormal development of the nervous system, skin, bones, and eyes. Neurofibromatosis is divided into Neurofibromatosis type I (NF1) and NF2, of which NF1 is the most common (90%), with a worldwide incidence of 1/3,000-1/2,000, regardless of race and gender [1]. NF1 is the result of an autosomal dominant heterozygous germline mutation in the neurofibromin 1 (*NF1*) gene. Patients with NF1 usually show an extreme degree of clinical heterogeneity within families, even in any given individual. The typical clinical manifestations are skin neurofibromas, café-au-lait macules (CALMs), freckling, and Lisch nodules. Some patients develop seizures, learning and cognitive disabilities, autism spectrum disorder, vascular disease, cardiac malformations, central nervous system tumors, skeletal abnormalities, and other syndromes [2]. Moreover, it has also been reported that patients may present with other syndromes that overlap with the clinical symptoms of NF1, such as Jaffe-Campanacci syndrome, Noonan syndrome, LEOPARD syndrome, Costello syn-

drome, and cardio-facio-cutaneous syndrome [3, 4]. Therefore, an early and definitive diagnosis can have a positive impact on the proactive management and interventions in complications to patients.

Mutations in the *NF1* gene are the direct genetic cause of NF1, which is located on chromosome 17q11.2, with a span of 350 kb and 60 exons, and encodes for neurofibromin of 2818 amino acids. It is expressed in neuronal cells, Schwann cells, smooth muscle cells, vascular endothelial cells, oligodendrocytes, astrocytes, and leukocytes [5]. *NF1* is a tumor suppressor gene encoding a neurofibromin involved in multiple signal transduction cascades such as Ras/RAF/MEK/ERK, Akt/mTOR, and AC/cAMP, which are relevant to cell proliferation and differentiation [6]. Neurofibromin also interacts with many other proteins involved in actin cytoskeleton rearrangements, neuronal cell formation, or intracellular transport [7]. To date, over 3000 pathogenic variants in the *NF1* gene without identified hot-spot mutation have been reported in the Human Genetic Mutation Database (<http://www.hgmd.org>). Approximately 50% of patients suffer from spontaneous

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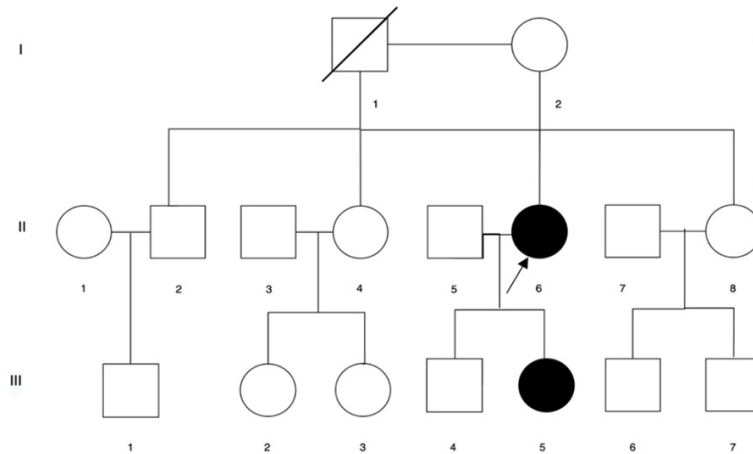


Figure 1. Pedigree analysis of the patient. There were 3 generations of this family. Filled black symbols indicate family members carrying c.4508_c.4509delAT. The proband is indicated by an arrow. Open symbols indicate family members without this mutation or with a typical clinical profile. A diagonal line through the square indicates that the individual is deceased.

mutations in the *NF1* gene that cause the disease, and the rest are inherited [8]. Pathogenic mutations are identified with *NF1* mainly resulting in truncated forms of neurofibromin. The majority of mutations (93%) are small mutations (including missense, nonsense, frameshift, or splicing mutations) [9]. Large deletions containing the entire *NF1* gene were found in approximately 5-11% of patients [10]. In the human genome, *NF1* is one of the largest genes with the highest rates of spontaneous mutations (estimated to be 1 in 10,000 alleles per generation), a high number of pseudogenes, and a wide mutation spectrum [11]. Clarifying the genotype-phenotype correlation by mutation analysis to further guide disease treatment remains a major challenge. Therefore, mutational analysis is recommended to identify causative mutations, perform prenatal genetic diagnosis, and provide additional evidence for genotype-phenotype correlation. In this study, we report a novel mutation in the *NF1* gene in a Chinese family. We establish a clinical diagnosis in the carriers of this mutation as well as genetic etiology through genetic testing.

Materials and methods

Subjects and clinical evaluation

A Han Chinese pedigree with *NF1* participated in the study (**Figure 1**). The study was carried out in accordance with the World Medical

Association Declaration of Helsinki. The patient and her family members voluntarily signed an informed consent form and peripheral blood samples were collected. The study was approved by the ethics committee of North Sichuan Medical College Hospital. Subjects with *NF1* were diagnosed according to International Consensus Group for Neurofibromatosis diagnostic criteria [12].

Genetic testing

Genomic DNA was obtained from each subject using the Blood Genome Column Medium Extraction Kit (Kangweishi, Beijing, China). A whole-exome library was constructed and the quality was verified. Enriched protein-coding exome fragments were sequenced with an MGISEQ-T7 series sequencer (Complete Genomics, San Jose, CA, USA); coverage of the target sequence was $\geq 99\%$. High-quality and reliable mutations were obtained through data filtering and screening. The detected pathogenic variants were evaluated with reference to major databases (such as Single Nucleotide Polymorphism Database, Exome Aggregation Consortium, Exome Sequencing Project, Online Mendelian Inheritance in Man, Human Gene Mutation Database [HGMD], ClinVar) using variant annotation software, and the function of the protein was predicted using various tools (such as Protein Variation Effect Analyzer, Sorting Intolerant From Tolerant, Polymorphism Phenotyping v2, Mendelian Clinically Applicable Pathogenicity, Rare Exome Variant Ensemble Learner, Mutationtaster) to select variants that could have deleterious effects on protein structure. After whole-exome sequencing, primers were designed to amplify the mutation site in the target gene and PCR amplification was performed. The primer sequences were as follows: AGAGCCTACTTTGAAGCTGAAG (forward) and ATGCCAACGCTAATCTGAACTT (reverse). PCR was conducted under the conditions: initial denaturation at 94°C for 5 minutes, and 30 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 35 seconds and a final

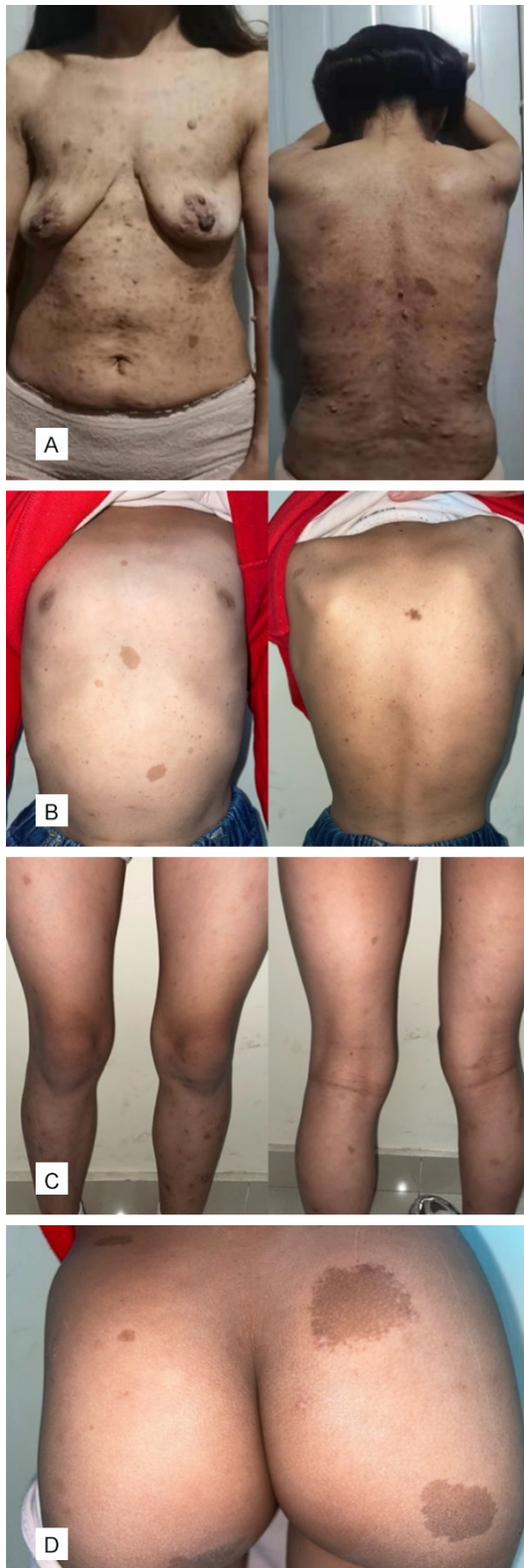


Figure 2. Clinical presentation of the patient. A. Scattered CALMs and neurofibroma on the trunk (II-6). B. CALMs and freckle-like depigmentation spots on the trunk (III-6). C. CALMs and freckle-like depigmentation spots on the extremities (III-6). D. CALMs on the buttocks (III-6).

hold at 72°C for 10 minutes. PCR products were electrophoretically separated on a 1% agar gel, purified, and subjected to Sanger sequencing (ABI 3730 system; Applied Biosystems, Foster City, CA, USA). DNASTAR software (DNASTAR, Madison, WI, USA) was used for sequence analysis and comparison.

Results

Clinical features of the study subjects

In this pedigree, 2 patients were clinically diagnosed with *NF1*. The proband (II-6) was a 46-year-old female from Sichuan Province who was born to nonconsanguineous parents with café-au-lait macules (CALMs) on her torso. As she grew older she developed a variety of subcutaneous neurofibromas all over her torso. The number and size of CALMs and subcutaneous neurofibromas increased with age. Physical examination revealed scattered oval CALMs of varying size-light brown in color with clear borders, and not protruding from the skin surface on the trunk and extremities, as well as scattered soft, solid, palpable nodules of varying size (**Figure 2A**). The proband's daughter, a 9-year-old patient, presented at birth with CALMs all over her body, the number and size of which increased with age; however, she did not have subcutaneous neurofibromas. Physical examination revealed large brown pigmentations and freckle-like depigmented spots on the trunk and extremities (**Figure 2B**). There were no abnormalities in ophthalmologic, orthopedic, and nervous system examinations in either patient. Other family members did not exhibit these clinical manifestations.

Genetic findings

A novel frameshift mutation c.4508_c.4509-delAT (p.Asn1503fsTer26) was found in exon 34 of the *NF1* gene that introduced a premature stop codon at position 1503 (**Figure 3A**). The same mutation was found in the daughter of the proband but not in any of the other (healthy) family members (**Figure 3B-E**). The mutation was classified as likely pathogenic (PVS1+PM2) according to American College of Medical Genetics and Genomics guidelines [13]. This novel *NF1* gene variant can be considered deleterious as it was associated with the *NF1* phenotype. The mutation has not been reported in HGMD, Leiden Open Variation Database, or in the latest literature on the *NF1* gene.

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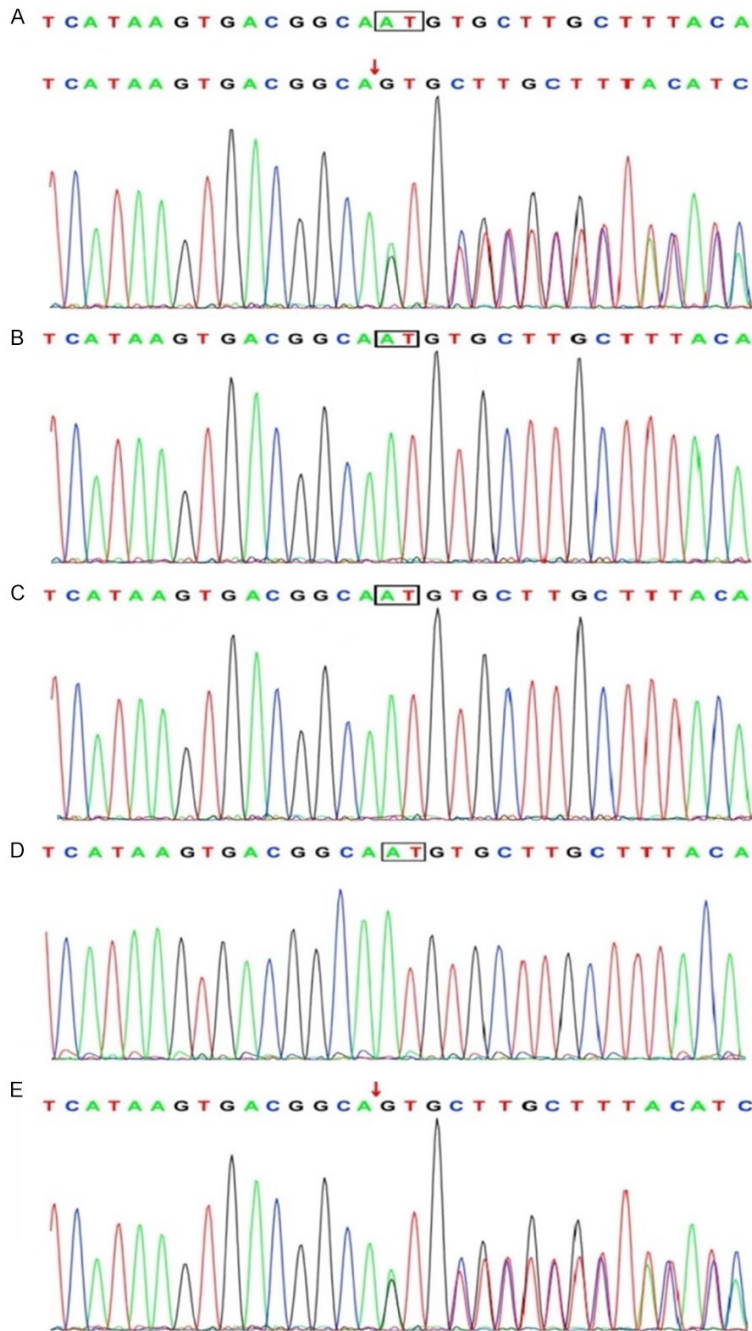


Figure 3. Genetic analysis of the patient and her family. A. Gene sequence of the patient (II-6). The c.4508_c.4509delAT mutation, a frameshift, was present in exon 34 of the *NF1* gene. B. Gene sequence of the patient's mother (I-2); the genotype of this locus was normal. C. Gene sequence of the patient's husband (II-5); the genotype of this locus was normal. D. Gene sequence of the patient's son (III-4); the genotype of this locus was normal. E. Gene sequence of the patient's daughter (III-5). Exon 34 of the *NF1* gene harbored the c.4508_c.4509delAT heterozygous mutation.

Discussion

NF1 is a classic neurofibromatosis with CALMs, neurofibroma, plexiform neurofibroma, freck-

les, optic glioma, Lisch nodes, and bone lesions as the main clinical manifestations; it may also be associated with learning and cognitive impairment [14]. In this study we identified a novel mutation in the *NF1* gene in a Chinese *NF1* family. Both the proband and her daughter showed typical skin presentation and were diagnosed with *NF1* according to international consensus diagnostic criteria. As the clinical manifestations of *NF1* can be heterogeneous, genetic testing plays an important role in *NF1* diagnosis and differential diagnosis from other diseases, and the latest diagnostic criteria for *NF1* have incorporated genetic testing [12]. For children with isolated CALMs, early diagnosis through genetic testing can provide parents with the opportunity for genetic counseling and intervention for learning disabilities and growth complications. Genetic testing is also important for distinguishing *NF1* from constitutive mismatch repair deficiency, Legius syndrome, or other conditions with similar clinical features.

NF1 is a neurocutaneous syndrome caused by mutations in the *NF1* gene, which is located on chromosome 17q11.2 and spans >280 kb of genomic DNA [15]. The *NF1* protein is a GTPase-activated protein (GAP) that negatively regulates Ras GAP. Mutations in the *NF1* gene result in disease due to loss of protein function. Common types of mutation in *NF1* include nonsense, missense, splice-site, and frameshift mutations due to inser-

tions or deletions, but there are no mutational hotspots [9]. Signaling molecules downstream of Ras including receptor tyrosine kinases, integrators, and ion channels are activated under

pathologic conditions, leading to uncontrolled cell growth and tumor formation [16]. In this study, we identified a frameshift mutation (c.4508_c.4509delAT) in exon 34 of the *NF1* gene that introduced a premature stop codon at position 1503 (p.Asn1503fsTer26), yielding a truncated protein that caused aberrant cell proliferation and tumor formation. The mutation was not present in the proband's mother, and her father had no similar clinical manifestations while he was alive, leading us to conclude that this mutation is a new pathogenic frameshift variant of *NF1*. Nonsense-mediated mRNA decay is a quality control mechanism that is activated when protein translation is prematurely terminated [17]. All these factors may contribute to the development of *NF1* in carriers of the *NF1* (c.4508_c.4509delAT) variant in this family.

The highly variable clinical features of *NF1* may result from *NF1* gene heterogeneity, modifier genes, epigenetic regulators, mosaicism, and environmental factors [18, 19]. Several studies have suggested that *NF1* mutations are closely related to the clinical phenotype of patients and may provide insight into the pathogenesis of *NF1* that can guide treatment. For example, the phenotype associated with p.Met992 deletion, p.Arg1809 substitution, and p.Arg1038-Gly substitution does not include plexiform neurofibromas and neurofibromas, making it difficult to diagnose *NF1* in these patients based on clinical presentation alone; however, testing for these mutations in children could lead to early diagnosis [20-22]. Lisch nodules are a typical pigmentation feature of *NF1* that is more common in patients with frameshift mutations [23]. Microdeletions occur frequently and have been linked to severe clinical manifestations of *NF1*, including an increased risk of malignant peripheral nerve sheath tumors and plexiform neurofibromas [24]. A large 1.4-/1.2-Mb microdeletion in 17q11.2 was found to cause overgrowth syndrome with bone age acceleration in patients, while whole-gene deletions and frameshift variants of *NF1* are associated with bone lesions [25, 26]. In the present study, the same *NF1* gene mutation was present in 2 generations of a Chinese family and the carriers showed characteristics of *NF1*. We believe that the proband's phenotype was caused by the detrimental effects of the *NF1* gene variant. However, since her daughter was

still young and had no typical symptoms or complications other than skin manifestations, she should be monitored for phenotypic changes as she grows older. To clarify the genotype-phenotype correlation in *NF1*, it is necessary to examine a broader spectrum of genetic variations in patients.

The main focus of *NF1* management has been targeted therapies based on defective genes, vaccines based on genetically modified viruses, and inhibitors targeting cell growth pathways [27, 28]. However, there are no specific treatments available as the association between *NF1* mutations and phenotype remains unclear. The results of our study provide a basis for studying genotype-phenotype correlations in *NF1*, which can inform clinical management.

In conclusion, *NF1* variants can be accurately and rapidly identified by combining whole-exome sequencing and Sanger sequencing. We identified a new pathogenic variant of *NF1* (c.4508_c.4509delAT) in a Chinese family affected by *NF1*. The identification of this variant expands the mutation spectrum of *NF1*, and can aid clinical diagnosis and genetic counseling.

Disclosure of conflict of interest

None.

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