

Identifying a novel frameshift pathogenic variant in a Chinese family with neurofibromatosis type 1 and review of literature

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Received: 2022-04-25 Accepted: 2022-11-3

Abstract

• **AIM:** To detect the pathogenic gene variant in a family with neurofibromatosis type 1 (NF1).

• **METHODS:** This patient with NF1 was sequenced using target sequence capture and high-throughput sequencing technology. After detecting the suspicious pathogenic variant type, the pathogenic variant sites of the patient and the patient's family members were verified by multiple ligation dependent probe amplification and Sanger sequencing. Sift, polyphen-2, Mutation Taster and GERP++ software were used to predict the pathogenicity of the unknown loci. The clinical data, diagnosis and treatment process of the patients were reviewed. Using the keyword "NF1; frameshift pathogenic variant", relevant literature was gathered for analysis from Chinese and international databases, with articles dating from the establishment of each database to April 2022.

• **RESULTS:** A heterozygous frameshift pathogenic variant of *NF1* in exon 33 was detected in the patient. The insertion of adenine in coding region 4486 resulted in the replacement of isoleucine with asparagine in protein 1497. Sanger sequencing validation and segregation analysis were performed, which demonstrated that the *NF1* gene was cosegregated with the disease phenotype in this family. This study identified a novel *NF1* heterozygous frameshift

mutation c.4486dupA (p.I1497Nfs*12). Relevant literature retrieval found 7 Chinese articles and 12 foreign articles. With *NF1* gene mutation, mutation types are diverse, including point mutation, frameshift mutation, splice site mutation, exon mutation, chimeric mutation and de novo mutation. Foreign reports are based on autosomal dominant inheritance.

• **CONCLUSION:** This study's results demonstrate that a novel deletion in exon 33 caused NF1 in this Chinese family, expanding the mutational spectrum of the *NF1* gene.

• **KEYWORDS:** neurofibromatosis type 1; frameshift pathogenic variant; monozygotic twins

DOI:10.18240/ijo.2023.01.07

Citation: Guo XH, Jin X, Wang B, Wang ZY. Identifying a novel frameshift pathogenic variant in a Chinese family with neurofibromatosis type 1 and review of literature. *Int J Ophthalmol* 2023;16(1):47-52

INTRODUCTION

Neurofibromatosis type 1 (NF1; Omim 16220) is one of the most common autosomal dominant diseases, affecting one in 2500-3000 people^[1]. Its characteristic manifestations include café-au-lait spots (CALs), Lisch nodules in the eye, fibromatous tumours of the skin and freckling of axillary and inguinal regions^[2]. Individuals with NF1 also have increased susceptibility to developing benign and malignant tumours, such as breast cancer, optic pathway gliomas, duodenal carcinoids, juvenile myelomonocytic leukaemia, pheochromocytomas and astrocytic neoplasms^[3]. NF1 is caused by a heterozygous pathogenic variant in the *NF1* tumour suppressor gene, located at chromosome 17q11.2^[4]. It distributes 60 exons, spans 350 kb of genomic DNA^[5] and has a 10-fold higher mutation rate than most other disease genes. This gene encodes neurofibromin, which harbours a functional GTPase-activating protein-related domain and downregulates p21-RAS, an essential regulator of cell growth. The mutational spectrum of the *NF1* gene is complex due to a large number of coding exons, and there is no strict relativity between genotype and phenotype^[6-9]. More than 3000 different inherited *NF1*

pathogenic variants have been reported in the Human Gene Mutation Database, including nonsense mutations, deletions, amino acid substitutions and insertions^[10]. Many types of mutations lead to the truncation or loss of neurofibromin, which results in uncontrolled cell proliferation^[7]. Almost half of NF1 patients have no family history of the disorder^[11]. The present study identified a novel *NF1* heterozygous frameshift pathogenic variant c.4486dupA (p.I1497Nfs*12) in a Chinese family with NF1. For analysis, the study used targeted next-generation sequencing to screen all genes associated with neurofibromatosis, identify pathogenic variants and analyse phenotypic heterogeneity. These data were combined with a literature review to improve clinical understanding of NF1 and reduce misdiagnosis and mistreatment.

SUBJECTS AND METHODS

Ethical Approval The Institutional Review Board of the General Hospital of the People's Liberation Army (PLA) approved the present study (Approval number: S2019-111-01). All participating family members provided informed written consent and were endorsed by their respective institutional review boards. All procedures used in the present study adhered to the tenets of the Declaration of Helsinki.

Clinical data Patient: male, 13 years old. The chief complaint was a subcutaneous mass in left upper eyelid with ptosis for 9 years (Figure 1A). Vitals: breathing 18 times/min, blood pressure 112/65 mm Hg, height 154 cm, weight 38 kg and body mass index (BMI) 16. Right eye visual acuity 1.0, left eye 0.4, -2.00 DS corrected 1.0. A 3×3 cm² soft mass was seen in the left upper eyelid, with unclear borders and no tenderness. The muscle strength of the levator muscle of the left eye was 2 mm, while the muscle strength of the levator muscle of the right eye was 10 mm. There were multiple CALS on the body, as well as axillary and inguinal freckles (Figure 1B). Both eyes of the patient had Lisch nodules with common exotropia and normal visual acuity (Figure 1D). There was an 8-cm café-au-lait macule on the right knee (Figure 1C). No neurological or skeletal abnormalities were observed. He had undergone tumour resection and was pathologically confirmed as plexiform neurofibroma (PN).

He has a sister and two triplet brothers, one of whom is his identical twin [monozygotic (MZ)]. His parents, sisters and dizygotic brother had no clinical features of NF1. His MZ brother also showed multiple, axillary and inguinal freckles and Lisch nodules (Figure 2).

Method and Process The Chinese family that participated in the study is from Henan Province. This family included two affected and four unaffected members who were analysed and followed clinically at the Chinese PLA General Hospital. Comprehensive ophthalmological examinations, including visual acuity, slit lamp and fundus examination, were



Figure 1 Clinical manifestations of patient with neurofibromatosis type 1 We can see subcutaneous mass in the left upper eyelid with ptosis (A), CALS on his face and back (B) and right knee with diameter of 8 cm (C) and Lisch nodules in both eyes (D). CALS: Café-au-lait spots.

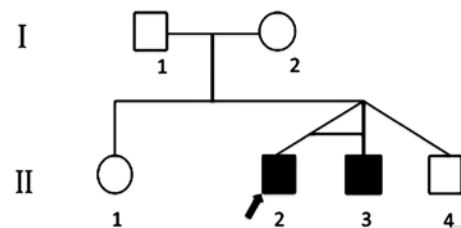


Figure 2 Pedigree of the Chinese family with NF1 In the pedigree, black squares are affected males, white squares and circles are healthy males and females respectively. Arrow denotes proband.

performed on both affected and unaffected family members. Computed tomography scans of the head were also performed on affected family members.

This study followed the methods as reported^[12]. Genomic DNA was prepared from peripheral blood lymphocytes of the pedigree members and normal controls using a QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany).

Genetic studies were performed using peripheral blood lymphocytes genomic DNA. The study used 1-3 µg of genomic DNA for targeted enrichment using the GenCap exome capture kit (MyGenostics) and the enrichment libraries were sequenced on Illumina HiSeq X Ten sequencer for a paired read at 150 bp. Variant filtration was used to filter variants. The filtering standard was as follows: 1) variants with mapping qualities <30; 2) the total mapping quality zero reads <4; 3) approximate read depth <5; 4) QUAL<50.0; 5) phred-scaled *P*-value using Fisher's exact test to detect strand bias >10.0.

After the above two steps, the data were transformed to VCF format. Variants were further annotated by ANNOVAR and associated with multiple databases, such as 1000 Genomes,

ESP6500, dbSNP, ExAC, Inhouse (MyGenostics) and the Human Gene Mutation Database; variants were predicted by SIFT, PolyPhen-2, MutationTaster and GERP++. The selection of potentially pathogenic mutations was based on the following process: 1) The mutation reading was greater than 5, and the mutation rate was not less than 30%. 2) Remove the mutation, and the mutation frequency in esp6500 and internal database exceeded 5% in 1000 g. 3) Delete if there was a mutation in the abnormal database (MyGenostics). 4) Delete synonyms. 5) If mutations were synonymous, they were retained. When the above jobs were finished, the pathogenic variants which were left were deemed to be the pathogenic variants. PCR amplification was used to do Sanger sequencing validation, and the primer sets were as follows: forward, 5'-CTGAAGCCGGGTATCAGAAA-3' and reverse, 5'-CAAGAAGATGCAAAGTAAAAAGCA-3'. The sequencing reaction was performed using the BigDye® v.1.1 Terminator cycle sequencing kit and the ABI Prism® 3130xl Genetic Analyzer (Life Technologies).

Document Retrieval Using “neurofibromatosis type 1, NF1, frameshift mutation” as keywords, a total of seven articles were retrieved from the China National Knowledge Internet database. Using “NF1; frameshift pathogenic variant” as the keyword, the biomedical literature (PubMed) database and ScienceDirect database were searched. Documents included were from the date of each database’s establishment to April 2022. A total of 12 foreign pieces of literature that met the requirements were retrieved.

RESULTS

After filtering the candidate variants of the proband in databases, a heterozygous frameshift pathogenic variant in *NF1* (c.4486dupA p.I1497Nfs*12) in exon 33 was detected (Figure 3A). The insertion of adenine in coding region 4486 resulted in a substitution of isoleucine to asparagine in the protein at position 1497. This can be seen in the comparison figure below (Figure 3B). The pathogenic variant was not reported in either of the aforementioned databases or the literature. Sanger sequencing validation and segregation analysis were performed, which demonstrated that the *NF1* (c.4486dupA p.I1497Nfs*12) gene was cosegregated with the disease phenotype in this family. The affected individuals, the proband and his MZ brother carried it, while the unaffected members did not. These results suggest that the *NF1* (c.4486dupA p.I1497Nfs*12) pathogenic variant is a novel causative mutation for *NF1*.

Document Retrieval A total of seven articles were retrieved from the Chinese database. A total of 12 foreign articles that met the requirements were retrieved from the foreign language databases. The studies included case analysis and multiple case analysis, mainly for *NF1* gene mutation, with various types of

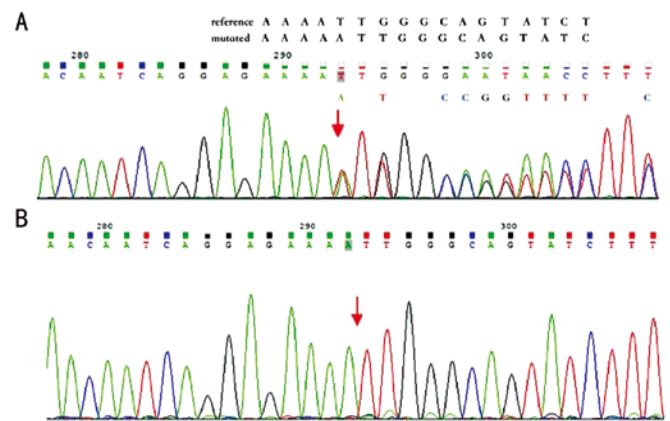


Figure 3 Sequencing results of the *NF1* gene A: The novel frameshift mutation c.4486dupA (p.I1497Nfs*12) in exon 33 of the *NF1* gene found in the proband and his MZ brother. B: Wild-type sequence from asymptomatic members. MZ: Monozygotic.

mutation, including point mutation, frameshift mutation, splice site mutation, exon mutation, chimeric mutation and de novo mutation. All reported autosomal dominant inheritance^[13-32].

DISCUSSION

Neurofibromatosis type 1 is a multisystem genetic disease with more than 3000 *NF1*-related pathogenic variants reported, but pathogenic variants in exon 33 are extremely underrepresented. The current study found a novel pathogenic variant c.4486dupA (p.I1497Nfs*12) in exon 33, located in the GTPase-activating protein-related domain. It is a frameshift pathogenic variant that generates a pre-terminating codon, resulting in the truncation of neurofibromin. Neurofibromin can inactivate p21-RAS by converting active p21-RAS-GTP to inactive p21-RAS-GDP and is a negative regulator of RAS signal pathways, which are responsible for cell survival and proliferation. Hence, truncated or absent neurofibromin leads to disorders of organism development and uncontrolled cell proliferation.

NF1 is a progressive neurocutaneous disease characterised by extreme clinical variability, from mild cutaneous findings to severe life-threatening complications. The disease can be observed among unrelated individuals, closely related family members and even within the individual patient at different times. In the present study, the affected individuals are MZ but have different clinical features. The proband in this study was 154 cm in height and 38 kg in weight at the age of 13, but his MZ brother was 162 cm in height and 49 kg in weight. A study by Yao *et al*^[33] also reported significant differences in the children’s height and weight. The height of *NF1* patients is often lower than the average level, and about 15% of *NF1* patients are lower than the third percentile^[34]. The proband is shorter than the average, but not lower than the third percentile. The pathogenic variant of the 5’ triad part of *NF1* gene may be related to short stature^[35].

Some studies of MZ twins with NF1^[36-37] have found that they were concordant in the number of CALS, axillary and inguinal freckling and Lisch nodules but were significantly discordant in tumours, especially PNs. The results of these studies are consistent with this study's findings. Because many NF1-related tumours require a second hit in the other *NF1* allele, this might explain the discordance in the twins' tumours. A PN is a severe subtype of NF1 and can involve the eyelid, orbital, periorbital and facial structures [termed orbital-periorbital PN (OPPN)]. Less than 10% of NF1 cases develop PN^[38]. Most OPPNs are distributed along the trigeminal nerve, and the most notable sign is blepharoptosis, which occurs in almost all cases, while congenital ptosis associated with NF1 has an incidence rate up to approximately 1%^[39] and is usually unilateral. Twins have been reported with concordance for unilateral ptosis. The ptosis in this report was derived from a plexiform tumour in the upper eyelid, which was also discordant in the twins. Other studies have shown that NF1 leads to impaired neuroretinal function in patients^[40]. The amplitude of the P1 wave with a central retinal degree of between 2° and 25° on multifocal electroretinogram (mfERG) decreased. The possible use of mfERG as a subclinical retinal damage indicator has a potential utility in clinical practice for the follow-up of NF1 patients.

Strabismus associated with OPPN has an incidence rate of 26%-75%. This is likely due to the mechanical restriction of the eye by the tumour's infiltration of orbital tissues and extraocular muscles; eye displacement can also occur due to orbital dysplasia^[41]. Severe vision loss may also result in sensory strabismus^[42]. However, the proband in this study had no intraorbital tumour infiltration or vision loss. Concomitant exotropia associated with the *NF1* gene is rare, while exotropia has an estimated prevalence of 1% in the general population and may result from certain hindrances to the development or maintenance of binocular vision. Avery *et al*^[43] found that the incidence of strabismus was higher among patients with ptosis, so the current study inferred that the concomitant exotropia of the proband might be secondary to visual occlusion and disruption of binocularity by a ptotic eyelid. Another study revealed that OPPN patients shared a common *NF1* pathogenic variant but had different symptoms in one family, and there were no mutational hot spots across different OPPN families^[44]. Furthermore, no apparent genotype-phenotype correlations were observed in the OPPN patients.

Sites *et al*^[37] reported on a pair of MZ twins who were discordant for NF1. The pathogenic NF1 pathogenic variant was found in all cell samples from the affected twin; for the unaffected twin, this was found in lymphoblastoid and buccal cells but not fibroblasts. Thus, mosaicism in NF1 pathogenic variants may be another cause of discordant clinical features in

MZ twins. Other potential non-hereditary mechanisms include distinct genetic modifiers^[36], environmental factors, epigenetic changes, copy number variants and post-zygotic mutations.

Genetic counselling is important because NF1 follows autosomal dominant inheritance. Although the parents reported herein had no signs of disease, they had the potential to produce another child with NF1 due to gonadal mosaicism^[10]. It is also necessary to follow the affected twins long-term because they are susceptible to tumours.

In conclusion, this study analysed a patient who was a twin in a Chinese family. A new deletion of exon 33 was found to lead to NF1. This discovery enriched the pathogenic variant spectrum of the *NF1* gene. It provides a theoretical basis for genetic counselling and prenatal diagnosis of NF1. Although genetic diseases cannot be cured, prompt genetic testing for suspicious patients can detect the existence of such disease as soon as possible, allowing clinicians to carry out reasonable comprehensive intervention early and delay the damage associated with the disease.

ACKNOWLEDGEMENTS

Authors' contributions: Conception and design: Guo XH and Jin X; Administrative support: Guo XH and Wang ZY; Provision of study materials or patients: Jin X and Wang B; Collection and assembly of data: Wang ZY and Wang B; Data analysis and interpretation: Wang ZY and Wang B; Manuscript writing: All authors; Final approval of manuscript: All authors.

Foundation: Supported by National High Level Hospital Clinical Research Funding (No.2022-PUMCH-A-031).

Conflicts of Interest: Guo XH, None; Jin X, None; Wang B, None; Wang ZY, None.

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