



A novel *NF1* frame-shift mutation (c.702_703delGT) in a Chinese family with neurofibromatosis type 1

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ABSTRACT. This study aimed to characterize the clinical features of a Chinese pedigree with neurofibromatosis type 1 (NF1) and to identify mutations in the *NF1* gene. In this three-generation family containing 8 members, 5 had been diagnosed with NF1 and the others were asymptomatic. All members of the family underwent complete medical examinations. Molecular genetic analyses were performed on all subjects included in the study. All exons of *NF1* were amplified by polymerase chain reaction, sequenced, and compared with a reference database. Possible changes in function of the protein induced by amino acid variants were predicted by bioinformatic analysis. In this family, the 5 patients presented different clinical phenotypes, but all manifested typical café-au-lait macules. One novel frame-shift

mutation, c.702_703delGT, in exon 7 of *NF1* was identified in all affected family members, but not in the unaffected family members or in 102 normal controls. This mutation generates a premature stop codon at amino acid position 720. Additionally, a synonymous mutation c.702 G>A was found in 3 family members, including 2 affected and 1 normal individuals. In conclusion, our study suggests that a novel c.702_703delGT frame-shift mutation in *NF1* is likely to be responsible for the pathogenesis of NF1 in this family. To the best of our knowledge, it is the first time that a c.702_703delGT mutation has been identified in a family with neurofibromatosis type 1.

Key words: Neurofibromatosis type 1; *NF1* gene; Mutation

INTRODUCTION

Neurofibromatosis type 1 (NF1; OMIM#162200), also known as von Recklinghausen disease, is one of the most common autosomal dominant disorders in humans (Huson et al., 1989). It occurs in about 1:3500 individuals (Riccardi, 1992; Rasmussen and Friedman, 2000). Neurofibromas, which arise from peripheral nerve sheaths, are the key characteristics of NF1 (Von Deimling et al., 2000), and café-au-lait macules, axillary freckling, and Lisch nodules of the iris are also common (Huson and Hughes, 1994; Gutmann et al., 1997; Szudek et al., 2000). Café-au-lait macules and neurofibromas are frequently present from birth and increase in number with age. Skinfold freckling often appears next. Lisch nodules usually only occur in adults. The *NF1* gene was first associated with NF1 in 1990 and is located on chromosome 17q11.2, spanning 350 kbp of genomic DNA and containing 60 exons that encode the 2818 amino acid protein neurofibromin (Cawthon et al., 1990; Viskochil et al., 1990; Wallace et al., 1990). The *NF1* mutation rate is among the highest observed in human genes, with estimates ranging from 1/7800 to 1/23,000 gametes (Huson et al., 1989; Clementi et al., 1990; Littler and Morton, 1990; Riccardi, 1992; Takano et al., 1992; Li et al., 1995). To date, more than 800 independent mutations in the *NF1* gene among different ethnic groups have been described to be associated with neurofibromatosis type 1 (Heim et al., 1995; Origone et al., 2002; Cai et al., 2005; Bausch et al., 2007; Upadhyaya et al., 1997, 2008; Bottillo et al., 2009; Messiaen et al., 2011). About half of NF1 individuals have a positive family history (Clementi et al., 1990; Takano et al., 1992; North, 1993). In this study, alterations in the *NF1* gene were analyzed in a three-generation family containing 8 members, and a novel frame-shift mutation was found.

MATERIAL AND METHODS

Family recruitment and clinical examination

A three-generation pedigree with NF1 (Figure 1) was recruited from Chengdu, Sichuan Province in China. No consanguineous marriages were noticed in the family. All members of this family enrolled in the study underwent complete neurological and ophthalmologic examinations, including magnetic resonance imaging (MRI) of the central nervous system (3.0T; SIEMENS, Berlin, Germany) and slit-lamp microscopy.

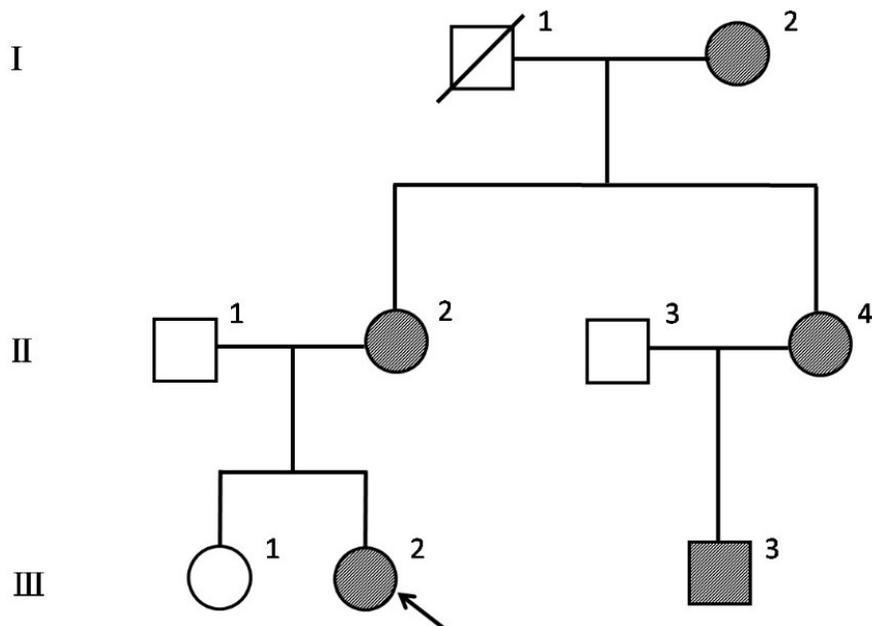


Figure 1. Pedigree of the Chinese family with neurofibromatosis type 1. Filled squares and circles are affected males and females, respectively. Arrowhead indicates proband (III-2.).

The study was prospectively reviewed and approved by the Medical Ethics Committees of both West China Hospital of Sichuan University and Shenzhen Eye Hospital of Jinan University. Written informed consent was obtained in accordance with the Declaration of Helsinki before blood samples were taken for analysis (see attachment for details). Three minors were enrolled in this study for whom written informed consent was obtained from a guardians on their behalf (see attachment for details). Neurofibromatosis type 1 was diagnosed according to criteria created by the National Institute of Health (NIH) (Anonymous, 1988; Gutmann et al., 1997; Huson and Hughes, 1994). Two or more of the following criteria are required for positive diagnosis: 1) 6 or more café-au-lait macules >5 mm in maximal diameter in prepubescent individuals or >15 mm in maximal diameter after puberty; 2) 2+ neurofibromas of any type or 1 plexiform neurofibroma; 3) freckling in the axillary or inguinal regions; 4) an optic pathway tumor; 5) 2+ Lisch nodules (iris hamartomas); 6) a distinctive osseous lesion, such as sphenoid wing dysplasia or thinning of the cortex of long bones (with or without pseudarthrosis); or 7) a first-degree relative (parent, sibling, or child) with NF1 diagnosed by the above criteria.

Mutation screening and sequence analysis of the *NF1* gene

Informed consent was obtained from all family members before peripheral blood samples were collected (see above). Genomic DNA was extracted from 0.2-mL blood samples with a Qiam Blood Kit (Qiagen, Hilden, Germany) following manufacturer instructions. DNA integrity was evaluated using 1% agarose gel electrophoresis. All exons of *NF1*

were amplified from genomic DNA of each participant by polymerase chain reaction (PCR) using the forward and reverse primers (Table 1). PCR was performed using 30- μ L reaction mixtures, each containing 1 μ g genomic DNA, 1X PCR buffer, 1.5 mM MgCl₂, 0.3 mM per dNTP component, 0.05 U Taq DNA polymerase, 0.3 μ M forward primer, and 0.3 μ M reverse primer. Cycling conditions included an initial denaturation step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 20 s, annealing at 48°-54°C for 30 s, and extension at 72°C for 2 min, followed by a final extension step at 72°C for 5 min. The amplified products were purified using a cycle-pure kit (OMEGA; Bio-Tek, Doraville, GA, USA) and sequenced on an ABI 3730XL automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences were compared pairwise with the published NF1 sequences. Mutations were named according to the nomenclature recommended by the Human Genomic Variation Society (HGVS, <http://www.hgvs.org/>).

Bioinformatic analysis

The ClustalW tool (<http://clustalw.ddbj.nig.ac.jp/top-e.html>) was used to align neurofibromin protein sequences among 8 different species.

RESULTS

Clinical findings of the pedigree

The proband (III-2) presented neurofibromas of the right orbit and abdomen, dozens of café-au-lait macules and axillary freckling, and was diagnosed with NF1 at the age of 3 years (Figure 2). Orbital CT revealed a subcutaneous soft tissue mass of the right eyelid and sphenoid wing dysplasia. Even though MRI demonstrated many high-T2-signal-intensity brain lesions, the patient did not have dyskinesia or sensory disturbance. This patient underwent surgical excision of orbital neurofibroma, and biopsy demonstrated plexiform neurofibroma. The proband's grandmother (I-2) was diagnosed with NF1 at the age of 35 years. During examination, nearly 100 subcutaneous neurofibromas were found scattered over her whole body, with a diameter that varied widely from 2 to 6 cm. This patient also presented sphenoid wing dysplasia and scattered café-au-lait macules. The mother of the proband (II-2) had hundreds of café-au-lait macules, iris hamartomas, and freckling in the axillary and inguinal regions. Patient II-4's clinic manifestation was similar to her mother's (I-2), but the size of subcutaneous neurofibromas was much smaller. III-3 was an 8-year-old boy, on whom only dozens of café-au-lait macules were found on his trunk and four limbs. No abnormalities were found in the rest of the family numbers.

NF1 mutation identification and analysis

Sequence analysis of *NF1* revealed a novel frame-shift mutation, c.702_703delGT, in exon 7 of all 5 patients but not in any of the asymptomatic members of this family or the 102 normal controls. The c.702_703delGT mutation cosegregated with disease within this family (Figure 3). One synonymous mutation, c.702 G>A, in exon 7 of *NF1* was also identified in 3 family members, including 2 affected family members (I-2, III-2) and 1 normal individual (III-1) (data not shown).

Table 1. Primers used in polymerase chain reaction for amplification of *NFI*.

Exon	Primer direction	Primer sequence (5'→3')	Product size (bp)
1	Forward	CAGGCCGCCTTCCCTCT	560
	Reverse	CTCCCTCACCTACTCTGT	
2	Forward	GTGGTTGATGCAGTTTCCCT	351
	Reverse	AATCACTTCCATACTTGAA	
3	Forward	GTAAAATGGAAGACTATTGT	294
	Reverse	CATAGGACTGCTCTTTGGT	
4	Forward	CTATCTATAGACAGATGTAG	404
	Reverse	ATTACTTCAGTAGTCCCAT	
5	Forward	CCAGGCTGGTCTTGAAC	349
	Reverse	CAGTTGGTGTCTAGTTCAG	
6-7	Forward	CCTTTCATTGCTTACAGAT	547
	Reverse	TATGTCACAAGTAGGCATT	
8	Forward	CCTTGGGTTTTACATAGT	638
	Reverse	CAGTAGCACTATATGGACAC	
9-11	Forward	GCTACATCTGGAATAGAAG	1217
	Reverse	GAAAACCAAGAGTGCATTCT	
12	Forward	GAAAGTTCCCGACAAAAGGAT	350
	Reverse	GAAGACCCATTCAATTCTCT	
13	Forward	GTTGGATAGCTATTATCCT	260
	Reverse	GCGTTTCAGCTAAACCCAA	
14	Forward	CCTTAGCAGTCACTGTCT	543
	Reverse	CAAATTGAGGGTATGTGAAT	
15	Forward	CTCCAGTGTATGTTTACCA	1056
	Reverse	CGTGGCTATTGAGCACTT	
16	Forward	CTAAGCTTCTTAAACTTGT	292
	Reverse	CTCACCATTACCATTCCAA	
17	Forward	CTCAAAACAGGAAGACAAC	297
	Reverse	CAGAAAACAAAACAGAGCACAT	
18	Forward	CTCTGTGAGTTATTGTATG	475
	Reverse	GTAGTGACCTTAAATACATT	
19-20	Forward	CTCTGCTCTTCTACTCCTT	575
	Reverse	CTGAGCGACTCTTGAAAGAT	
21-22	Forward	GTCATGGAAGAAATGTTGGAT	1067
	Reverse	CTGCTACATTTCACTAGAAATG	
23-24	Forward	CCAGAAGTTGTGTACGTTCT	847
	Reverse	CCACTAATACTTGAAGGTCAT	
25	Forward	AGGTAGTTCCTAAGGTTTAT	500
	Reverse	CCTGGTAAATGTGACTCTT	
26-27	Forward	CATTACACCATGCACAT	533
	Reverse	CCGCTTACTCTAATCACTT	
28-29	Forward	CAAGTGGTTGTCAACTTTG	599
	Reverse	CTTGCTTCATGCAGTGTAGT	
30	Forward	CCCTGTCAAAGTTGTCTTT	328
	Reverse	GGTAATCTCAACTGTAAAG	
31	Forward	GATAACCTTCTTCCCAAAT	743
	Reverse	CAGCTAATAAAAAAGTTCTCCA	
32-33	Forward	CTCAATTCCTCAACTCCTTGT	887
	Reverse	CAGGTACATGGATTTATGTG	
34	Forward	GTCTAATGTCAAGTCACATTG	324
	Reverse	GTTCACTATCCCATGACT	
35	Forward	CATGGTCCTGAGGTCTTT	347
	Reverse	GTGGCAAACCTCCTTCT	
36	Forward	TTGGTGCATGTTGCCAAAT	297
	Reverse	CTGTAAAGAGACCCAAAACAT	
37	Forward	ATTGATTAGTGGCATCTGT	616
	Reverse	GTGACATTTATACACCACT	
38	Forward	GGCATAGTGTGTTGTTGGT	485
	Reverse	CAAAACCCAAATCAAACCTG	
39	Forward	TCTGTCATAGGAGCCTCA	551
	Reverse	GTAAGTGAAAGTCTTCACTGG	
40	Forward	GCTTTCCTTTTACCAAACCTT	587
	Reverse	CAATGTGGCACCAGATAAAT	
41-42	Forward	GATTAGGCTGTTCCAATGAAT	768
	Reverse	GGTGTAGAGCACAAAACAT	
43-45	Forward	CAAAATGAAACATGGAAC	901
	Reverse	CTTACAACCTTGAGAACCAT	

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Table 1. Continued.

Exon	Primer direction	Primer sequence (5'→3')	Product size (bp)
46	Forward	CAGCTATTACTGTATGATCA	911
	Reverse	CCCAAATGAATGCACTCAT	
47	Forward	GGAAGATAAGCTGCTTTAT	264
	Reverse	TAGGAACCTCAAGGCAAAGT	
48	Forward	GGATTACTTATCTTGTCAT	290
	Reverse	CAAATTACTTCTGGTTTCT	
49	Forward	CCTTTCCTTGCAGAGTTGT	302
	Reverse	GTACTTTTGGGTGATCCTT	
50	Forward	AACAGGTACTATGCTCTTT	312
	Reverse	CTGCTTGCTCCATTAGTT	
51	Forward	GTTTGTATCCTAAAGCCCTT	424
	Reverse	CTTTGTACACTGACATGGA	
52-54	Forward	CACTTTATGTCCAACATT	1043
	Reverse	GACAGGCACGAAGGTGAAT	
55-56	Forward	CACATTATCTGGGGAAT	676
	Reverse	ACCTTCATTTTGCCAAAAGT	
57	Forward	GTTGTAAGTCTATGGTAGT	664
	Reverse	AAGTTCCTCTTCTGCCCAA	
58	Forward	CAGACAAAATCGCCTAATGAT	683
	Reverse	GGACTAACACTTTACTCTGT	



Figure 2. Clinical manifestations of patients in the Chinese family. **A.** Right orbital neurofibroma of proband. **B.** Biopsy of proband's right orbital neurofibroma. HE staining reveals that the main entity of neurofibroma is composed of spindle and oval Schwann cells; scattered Wagner-Meissner-like bodies, fibroblasts and mast cells are also noticed. **C.** Orbital axial CT of the proband shows sphenoid wing dysplasia on the right side and subcutaneous neurofibroma of right eyelid. **D.** Head axial MRI shows many high signal intensity lesions in T2-weight imaging (frontal lobe, temporal lobe and medulla oblongata). **E.** and **F.** Lots of subcutaneous neurofibromas and multiple café-au-lait macules scattered in patients II-4 and I-2, respectively. Patient II-2 (**G**) and patient III-3 (**H**) manifest café-au-lait macules in different size on their trunks.

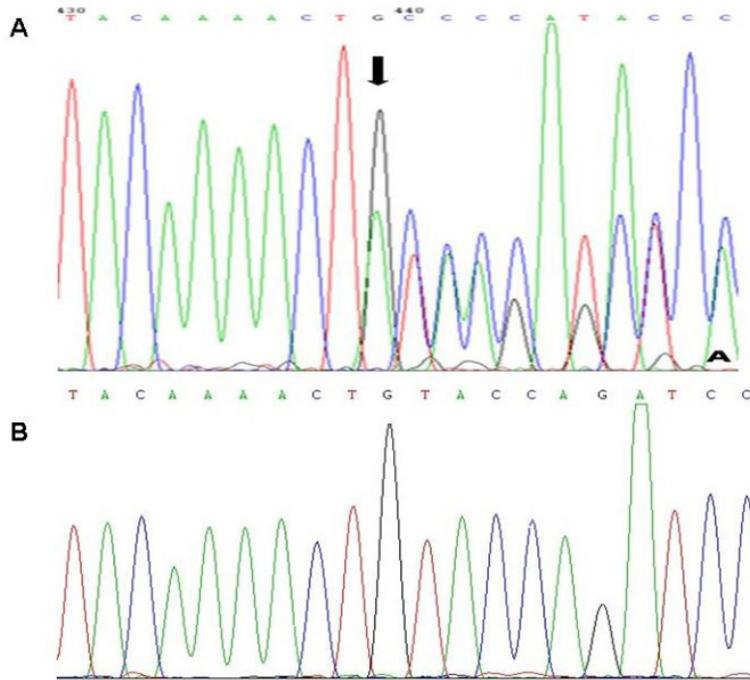


Figure 3. Sequencing results of the *NF1* gene. **A.** Frame-shift mutation c.702_703delGT, in exon 7 of the *NF1* gene (arrow) found in all patients. **B.** Wild-type sequence from an unaffected member.

Bioinformatic analysis

The deletion of a guanine and thymine in codon 702-703 of *NF1* exon 7 caused frame shift and introduced a premature stop signal at codon 720, which resulted in the termination of the amino acid sequence starting at position 234 of neurofibromin. The amino acid sequence around leucine in 234 was highly conserved for NF1, based on an analysis of orthologs from 8 different species by using an internet ClustalW tool (Figure 4).

Homo sapiens	.AVINSLEKAFWNWVENYPDEFKLYQIPQTDMAECAEKLFDLVDGFAESTKRKAAVWPLQII
Pan paniscus	.AVINSLEKAFWNWVENYPDEFKLYQIPQTDMAECAEKLFDLVDGFAESTKRKAAVWPLQII
Equus caballus	.AVINSLEKAFWNWVENYPDEFKLYQIPQTDMAECAEKLFDLVDGFAESTKRKAAVWPLQII
Ailuropoda melanoleuca	.AVINSLEKAFWNWVENYPDEFKLYQIPQTDMAECAEKLFDLVDGFAESTKRKAAVWPLQII
Macaca mulatta	.AVINSLEKAFWNWVENYPDEFKLYQIPQTDMAECAEKLFDLVDGFAESTKRKAAVWPLQII
Oryctolagus cuniculus	.AVINSLEKAFWNWVENYPDEFKLYQIPQTDMAECAEKLFDLVDGFAESTKRKAAVWPLQII
Mus musculus	.AVINSLEKAFWNWVENYPDEFKLYQIPQTDMAECAEKLFDLVDGFAESTKRKAAVWPLQII
Drosophila melanogaster	.AVINSLEKAFWNWVENYPDEFKLYQIPQTDMAECAGKLFDLVDGFAESTKRKAAVWPLQII

Figure 4. Mutation involving a highly conserved region. The frameshift mutation disrupt amino acid sequence starts at position 234; this region is highly conserved for NF1, which is demonstrated by analysis of orthologs from eight different species.

DISCUSSION

Neurofibromatosis type 1 is the most common autosomal dominant disorder with equal gender incidence (Huson et al., 1989). The presentation of most disease manifestations is age-dependent and its penetrance is thought to approach 100% by age 20 (Boyd et al., 2009). Significant advances in the understanding of NF1 stemmed from the discovery of the *NF1* gene (Cawthon et al., 1990; Viskochil et al., 1990; Wallace et al., 1990). Neurofibromin, the *NF1* gene protein product, has an RAS-GTPase activating protein-related domain that can inactivate p21-RAS by activating the intrinsic GTPase of p21-RAS-GTP to hydrolyze GTP to GDP (Xu et al., 1990; Golubic et al., 1992). RAS is a key component of many growth factor signaling pathways, indicating that neurofibromin serves as a regulator of signals for cell proliferation and differentiation, and in the absence of neurofibromin, it will drive uncontrolled cell proliferation (Viskochil, 1999). Therefore, *NF1* is thought to act as a tumor suppressor gene. To date, many types of mutations have been identified, such as chromosome abnormalities, insertions, deletions, point mutations, 3'-untranslated region mutations, stop mutations, and splicing mutations (Boyd et al., 2009; Rasmussen and Friedman, 2000). However, no true "hotspots" have been found in *NF1*. In this Chinese pedigree, a frame-shift mutation (c.702_703delGT) in exon 7 was identified in all affected patients, but not in any normal family member or healthy control. This c.702_703delGT *NF1* mutation produced a premature stop codon at position 720, which resulted in a truncated protein of 240-amino acid residues instead of full-length neurofibromin. The frame-shift mutation occurred at the first half of the gene in a region that is highly conserved for *NF1* based on analysis of orthologs from 8 different species, including *Homo sapiens*, *Pan paniscus*, *Equus caballus*, *Ailuropoda melanoleuca*, *Macaca mulatta*, *Oryctolagus cuniculus*, *Mus musculus*, and *Drosophila melanogaster* (Figure 4). Such a high degree of conservation argues for a functional importance of the relevant amino acid sequence. However, the NF1 protein may not exist at all in these affected patients based on nonsense-mediated mRNA decay (Muhlrad and Parker, 1994; Hentze and Kulozik, 1999; Wagner and Lykke-Andersen, 2002). Nonsense-mediated mRNA decay is a surveillance mechanism by which cells recognize and degrade mRNAs containing premature translation termination codons. Therefore, this genetic variant appears to be the causative mutation for the disease in this family.

To date, no obvious corresponding relationships between particular *NF1* mutations and resulting clinical manifestations have been identified. Our study also showed this absence of a close genotype-phenotype relationship, as patients in this pedigree presented different sizes of subcutaneous neurofibromas and other clinical manifestations. This feature suggests that other factors, including modifying genes, environmental influences, and stochastic factors, are likely involved in determining clinical manifestations (Rasmussen and Friedman, 2000).

In conclusion, the c.702_703delGT mutation in *NF1* in this pedigree appears to be the cause of NF1 in this family. This is the first time that this frame-shift mutation was found and this study adds a novel mutation to the existing spectrum of *NF1* mutations in patients with NF1 and provides further evidence that the loss or diminished function of the neurofibromin leads to neurofibromatosis type 1.

Conflicts of interest

The authors declare no conflict of interest.

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