



# NF1 frameshift mutation (c.6520\_6523delGAGA) association with nervous system tumors and bone abnormalities in a Chinese patient with neurofibromatosis type 1

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Genet. Mol. Res. 15 (2): gmr.15027572

Received September 1, 2015

Accepted December 8, 2015

Published April 7, 2016

DOI <http://dx.doi.org/10.4238/gmr.15027572>

**ABSTRACT.** Neurofibromatosis type 1, also known as NF1 or von Recklinghausen's disease, is a common neurocutaneous syndrome that presents with multiple café-au-lait patches, skinfold freckling, dermatofibromas, neurofibromas, and Lisch nodules. The mutations of the gene *NF1*, encoding the protein neurofibromin, have been identified as the cause of this disease. Here, we report a clinical and molecular study of a Chinese patient with multiple café-au-lait skin freckles, dermatofibroma, central and peripheral nervous system tumors, and bone abnormalities attributed to *NF1*. The patient showed >6 café-au-lait spots on the body and multiple dermatofibromas. A brain glioma and multiple nerve sheath tumors inside and outside the vertebral canal were identified by magnetic resonance imaging, which also showed

multiple intercostal nerve schwannomas and hydrocephalies above the cerebellar tentorium. Talipes equinus was also apparent. A mutation analysis of the *NF1* gene revealed a novel frameshift mutation in exon 43, consisting of a heterozygous deletion of four nucleotides (GAGA) between positions 6520 and 6523. No *NF1* mutations were detected in the patient's parents or younger brother. These results extend the list of known mutations in this gene. The absence of the *NF1* mutation in the healthy family members suggests that it is responsible for the NF1 phenotype. To our knowledge, this frameshift mutation represents a novel NF1 case, and may be associated with nervous system tumors and bone abnormalities.

**Key words:** Mutation analysis; *NF1* gene; Neurofibromatosis type 1; Multiple café-au-lait spots; Tumor; Bone abnormality

## INTRODUCTION

Neurofibromatosis type 1 (NF1) is a common autosomal dominant genetic disease associated with mutations in the gene neurofibromin 1 (*NF1*), and shows multi-system and multi-organ involvement, particularly that of the central nervous and dermal systems. The *NF1* gene, located on chromosome 17q11.2, was one of the earliest to be implicated in human pathogenesis, being cloned for the first time in 1990 (Cawthon et al., 1990; Wallace et al., 1990). The *NF1* genomic sequence spans 350 kb and contains 60 exons encoding the 2818 amino acids of the protein neurofibromin (Lee and Stephenson, 2007). It exhibits a high mutation rate, with approximately 50% of NF1 patients carrying novel mutations (Shen et al., 1996). Moreover, such mutations have a high rate of penetrance, close to 100% by age 20 (Edwards et al., 1988). NF1 presents with different clinical manifestations in different age groups. A large number of café-au-lait (CAL) spots are considered to be the only symptom occurring in infancy, with skinfold freckling often appearing next. Approximately 75% of patients first present with symptoms between 5 and 10 years old. In general, cutaneous neurofibromas occur during adolescence, and Lisch nodules are usually only seen in adults. Further manifestations appearing less frequently among NF1 patients include epileptic seizures, malignancies, plexiform neurofibromas, optic gliomas, learning difficulties, and bone dysplasias. Around half of NF1 sufferers have no family history of the disease (Upadhyaya et al., 1998). In this study, using polymerase chain reaction (PCR) and DNA sequencing, we screened members of a Chinese family, finding that one carried an *NF1* gene mutation, while three were genetically normal and healthy.

## MATERIAL AND METHODS

### Patient

The patient was a 17-year-old Chinese boy who had been attending the First Affiliated Hospital of Guangxi Medical University for 4 months because of poor physical health. He felt pain and weakness in the limbs, with occasional paroxysmal uncontrollable spastic limb tremors. He could relieve himself, but could not turn over, get up, or hold heavy items, and

presented with general mental handicap and poor calculation ability. He had lost 3 kg in the preceding 4 months.

When he was born, several warts of different sizes were present on the skin of his back. By the age of 10, these warts had increased in number. The illness was diagnosed as fibroma at another hospital, although the details of this are unknown. None of his family members suffered from NF1.

The clinical manifestations of this patient were consistent with a diagnosis of NF1 (Anonymous, 1988), namely, CAL spots and subcutaneous neurofibromas. During physical examination, CAL spots (0.5-2.0 cm in diameter) were found all over his body, and dozens of subcutaneous neurofibromas were present (0.5-1.5 cm in diameter). He presented with bone malformation characterized by equinus deformities in his lower extremities and strephenopodia of the left foot. His muscle tone clasp-knife response was increased, and muscle strength was weakened. He demonstrated hyperalgesia in the right limbs without sensory loss, dyskinesia, or synesthesia. A tendon reflex was present (+++) and the Babinski sign (+) was positive. When examined by magnetic resonance imaging (MRI), tumors were observed in the central and peripheral nervous systems. This scan also showed nodular long T1/T2 signal intensity, which was distributed in the rear right of the medulla oblongata, the right side of the basal ganglia region and both sides of the thalamus. The fourth ventricle outflow tracts were narrowed and the supratentorial ventricle was widened. Cystic-solid lesions of approximately 1.8 x 1.8 cm were found in the splenium of the corpus callosum. Abnormal nodular MRI signals of many different sizes were evident in the cervical vertebrae, extramedullary lumbar spinal canal, subdural space, right and left sides of the spinal nerve roots, and right and left sides of the intercostal nerves. The cervical spine and conus medullaris were compressed by schwannomas, leading to irregular thinning of the former. There were also nodules on the scalp, the back of the neck, and the right axillary soft tissue, as described above. No tumor was found in the auditory nerve bundle at inspection.

### **Mutation analysis**

Informed consent was obtained from all family members before peripheral blood samples were collected. Genomic DNA was extracted from 0.2 mL blood samples with a QIAamp DNA Blood Mini Kit (QIAGEN, Germantown, MD, USA) following the manufacturer protocol. We designed primers flanking all 60 coding exons of the *NF1* gene using the web-based version of the Primer 5.0 program (<http://www.premierbiosoft.com/>). All exons were amplified from the genomic DNA of each participant by PCR using 2X PCR Master Mix (Tian Gen, BeiJing, China). The amplified products were sequenced on an ABI 3500 automated DNA sequencer (Life Technologies, Carlsbad, CA, USA). Nucleotide sequences were then compared pairwise with published *NF1* sequences (RefSeq accession Nos. NM\_000267.3 and NG\_009018.1). Mutations were named according to the nomenclature recommended by the Human Genomic Variation Society (<http://www.hgvs.org/>). Experiments were performed at the molecular genetics facility of the Guangzhou Golden Field Medical Institute. The genetic variant was checked against the Human Gene Mutation Database (HGMD; <http://www.hgmd.cf.ac.uk>) and published literature available on to determine its novelty.

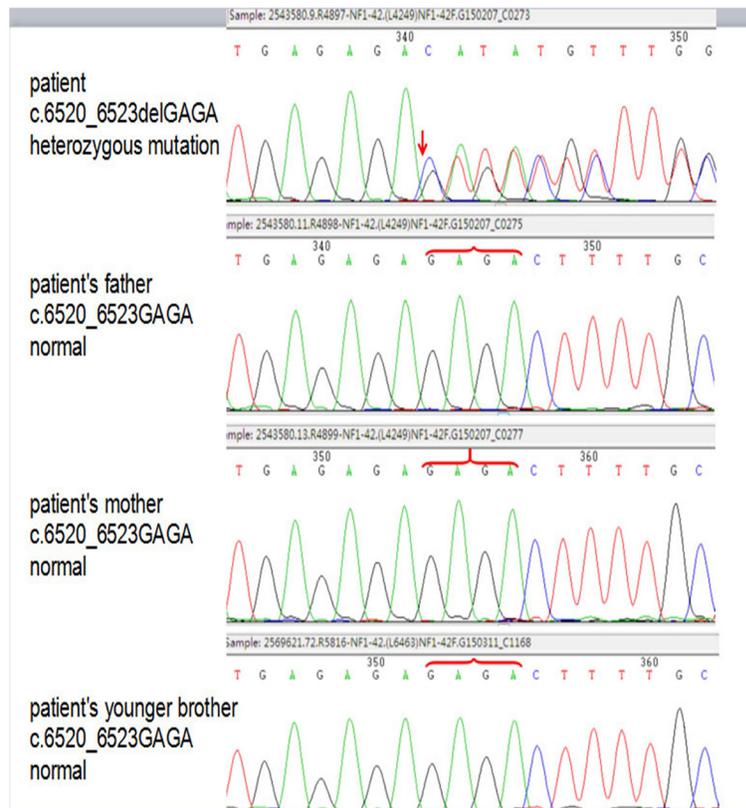
## RESULTS

### Clinical findings of the patient

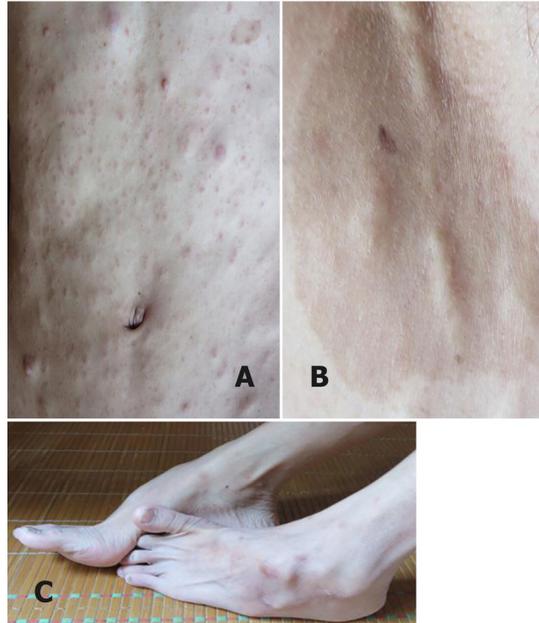
The patient presented dozens of subcutaneous neurofibromas of the abdomen and a faint café-au-lait macule on the inguinal and bone malformation characterized by equinus deformities in his lower extremities and strephenopodia of the left foot (Figure 1). During examination, dozens of café-au-lait macule were found scattered over his whole body, with a diameter that varied from 0.5 to 2 cm. MRI demonstrated tumors were observed in the central and peripheral nervous system (Figure 2).

### NF1 mutation identification and analysis

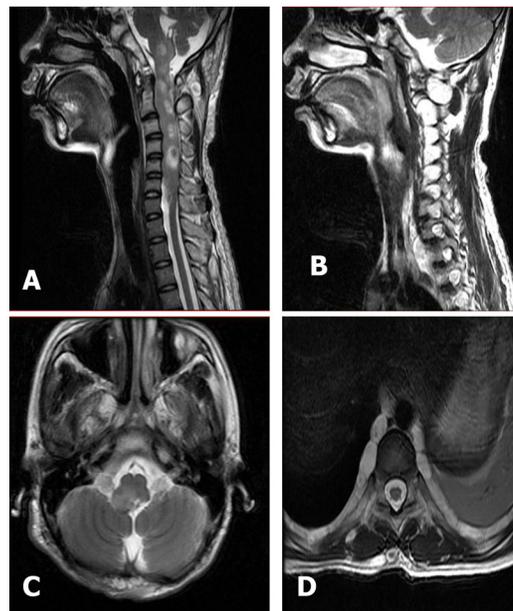
Direct DNA sequencing revealed a novel frameshift mutation (c.6520\_6523delGAGA) in exon 43 of the *NF1* gene. This aberration consisted of a deletion of four nucleotides (GAGA) at position 6520\_6523 (Figure 3). The mutation was not present in the parents or younger brother of the patient, and we believe that it represents a novel pathogenic sequence variation.



**Figure 1.** Sequencing results revealing the heterozygous mutation c. 6520\_6523de IGAGA identified from the patient's DNA sample. The red arrow indicates the mutation site.



**Figure 2.** A. Multiple subcutaneous neurofibromas on the abdomen. B. Giant café-au-lait, macule on the inguinal. C. Equines deformities of the lower extremities and strephenopodia of the left foot.



**Figure 3.** A. Glioma on the medulla and schwannomas on both sides of the cerebellopontine angle area (CPA). B. and C. Many schwannomas on the intervertebral foramina, and cervical spinal cord compression. D. Schwannomas appearing like a string of beads on the right and left sides of the intercostal nerves.

## DISCUSSION

NF1, also called von Recklinghausen's disease, is a common autosomal dominant disorder characterized by cutaneous or subcutaneous neurofibromas, CAL spots, Lisch nodules of the iris, freckling of axillary or inguinal regions, bone dysplasias, learning difficulties, neurogliomas, and malignant peripheral nerve sheath tumors. Approximately 15.0 to 19.87% of NF1 patients present with central nervous system tumors (Riccardi, 1993; Ruggieri, 1999), and it occurs in about 1 in 3500 individuals (Boyd et al., 2009). *NF1*, located on chromosome 17q11.2, is considered to be a classical tumor suppressor gene. When this sequence undergoes translocation or mutation, NF1 results from the alteration of its protein product, neurofibromin. A critical region of neurofibromin, encoded by exons 21-27a and known as the GAP-related domain (GRD), accelerates hydrolysis of the guanosine triphosphate bound to active RAS protein, leading to RAS inactivation (Abramowicz and Gos, 2014).

To date, approximately 1500 mutations have been detected in the *NF1* gene, including missense and nonsense mutations, shear mutation, insertions, deletions, and duplications (Abramowicz and Gos, 2014). Most of these changes (approximately 80%) result in a truncated form of the protein (Abramowicz and Gos, 2014).

In the present study, a new *NF1* mutation (c.6520\_6523delGAGA) was identified in our patient through direct sequencing of exons in the coding region of this gene. This novel sequence variation introduces a shift in the reading frame at codon 2174, and leads to the introduction of a premature stop at codon 2177, which truncates the encoded protein and causes loss of its normal functions. This mutation has not previously been recorded in the HGMD, dbSNP, or ESP6500 databases, nor has it been reported in the literature. As with other novel *NF1* mutations, this new variation is in exon 43.

The *NF1* gene has a high mutation rate of 1/10000, which is 100 times higher than that observed in most single-gene diseases (Hulsebos et al., 1996). Approximately half of all patients present with a new mutation (Lázaro et al., 1994) and no family history of the disease.

In a study of 500 German and Turkish neurofibromatosis patients, 278 unique mutations were detected across nearly every exon of *NF1* (Fahsold et al., 2000). This investigation showed no mutation hotspots, and most variations were revealed to be unique to individual patients. In addition, another study reported 75 previously described mutations and 22 new variants among 110 Italian NF1 cases. These mutations tend to be in coding regions, for instance, exons 4b, 7, 10a, 11, 15, 16, 23-1, 29, 31, 36, 37, and 45 are frequently involved (Losito et al., 2003). In our research, the patient, whose parents and younger brother demonstrated no *NF1* aberrations, presented with a novel mutation and no family history of neurofibromatosis. The new mutation was found on exon 43, thus although it exists outside the GRD, it still causes NF1 due to effects on the transcription and translation of the downstream sequence, resulting in changes to the structure and function of neurofibromin.

Most researchers maintain that different *NF1* mutations are not connected to specific clinical manifestations, despite their being widely reported as causing neurofibromatosis. There are seven key clinical manifestations of this disease. NF1, known as traditional neurofibromatosis, accounts for 85% of all cases, and manifests as massive neurofibromas, CAL spots, and Lisch nodules, with no central nervous system damage. NF2, the central or auditory type, presents as bilateral acoustic neuromas, and a small number of CAL spots and neurofibromas, without Lisch nodules. Our patient was 17 years old, with gliomas in the central nervous system being evident as central nervous system damage. His clinical

manifestations were similar to those expected with NF2, and fewer than those of NF1. We were unable to definitively diagnose our patient with either type given the gene sequencing results obtained. Thus, the sequence variation described here may be significant for *NF1* gene mutation classification. This mutation may be related to the malignant tumor observed in the central nervous system, such that the manifestations of the patient are similar to those of NF2. The patient also presented with developmental skeletal defects, such as strephenopodia and equinus deformities that differ from the types of skeletal damage cited in previous literature, namely scoliosis, lordosis and skull defects, including depressed skull. The bone deformities observed in this case have not been documented in prior related reports.

It is important that new functional NF1-causing gene variants are identified, and the findings described herein expand the spectrum of known *NF1* mutations. Moreover, our observations provide evidence encouraging the further study of connections between neurofibromatosis clinical manifestations and mutations. It seems likely that the c.6520\_6523delGAGA (p.Glu2174fs) aberration is linked to gliomas and equinus deformities. This mutation was first discovered and reported here. To our knowledge, there exist no works to date positing a definite connection between gene mutations and glioma or equinus deformities.

### Conflicts of interest

The authors declare no conflict of interest.

### ACKNOWLEDGMENTS

The authors are deeply grateful to all of the family members for their cooperation in this study. Research supported by grants from the National Natural Science Foundation of China (NNSF #81160168)

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