Spinal neurofibromatosis with NF1 mutation in a classic neurofibromatosis type 1 family: A case report and literature review

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Abstract
Background: Spinal neurofibromatosis (SNF) is a related form of Neurofibromatosis type 1 (NF1) with a low incidence. Here, we report a SNF patient with NF1 (OMIM *613113) mutation in a classic NF1 family to enrich the case data.

Methods: We presented the clinical data of a 27-year-old female suffered from SNF. Two NF1 individuals (the mother and the brother) in the patient’s family were also described. In the SNF patient, tumors in cervical were removed by surgical operation after the spinal MRI evaluation. Hematoxylin-eosin staining and immunohistochemistry were performed to better characterize the excised tumors. NF1 exons of the patient and her NF1 families were further sequenced by the next-generation sequencing technology.

Results: The patient developed irregular café-au-lait macules, multi-subcutaneous nodules, recurrent numbness, and weakness of both lower extremities. Multiple neurofibromas were found in the whole spine by spinal MRI. Tumor-like cells and hyperplasia of ganglion cells were found in the excised tissue by H&E staining and immunohistochemistry, respectively. One-year follow-up on the SNF patient showed that after the surgery lower limb pain, numbness and convulsion were completely relieved. A common germ-line pathogenic mutation (NM_000267.3:c.1721 +3A>G) was found in both the SNF patient and her classic NF1 families.

Conclusion: A case of SNF with classic NF1 mutation in a classic NF1 family was identified for the first time, indicating that SNF may share the same gene mutation with NF1, while the different manifestation of NF1 and SNF may be related to gene modification.

Keywords
classic neurofibromatosis type 1, NF1 gene mutation, spinal neurofibromatosis

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INTRODUCTION

Neurofibromatosis type 1 (NF1) is a complex multi-system human disorder caused by NF1 (OMIM *613,113) mutation which is located on the 11.2 locus of chromosome 17. NF1 is a large gene with a length of about 350 kb and a high rate of mutation that may be related to the excessive length of the gene (McClatchey, 2007). About 50% of NF1 patients carried NF1 de novo mutation with a mutation rate of 1/10,000 which is the highest among all known genes in human (Tadini et al., 2014). The reported incidence of NF1 varies from 1/2000 to 1/5000, regardless of ethnic and racial background (Rasmussen & Friedman, 2000). However, spinal cord-related neurofibromatosis with clinical implications is relatively rare, occupying about 5% of NF1 patients (Huson, Harper, & Compston, 1988).

Neurofibroma, the typical clinical manifestation of NF1, can occur in skin, subcutaneous, soft tissue, and even inside and outside the spinal canal. Spinal neurofibromatosis (SNF) is an alternative form of NF1, in which spinal cord neurofibromas are the main clinical characteristic. The concept of SNF was first cited and presented by Noman and Harold in 1976 (Leeds & Jacobson, 1976). During the next two decades, SNF was successively reported in different families and has been called "familial spinal neurofibromatosis" and "hereditary neurofibromatosis" (Pulst, Riccardi, Fain, & Korenberg, 1991; Ruggieri et al., 2015).

The clinical manifestations of NF1 can vary enormously. Patients from the same family may have different clinical manifestations (Upadhyaya et al., 2009). The clinical features of NF1 include café-au-lait macules, neurofibromas, axillary groin freckles, Lisch nodules, choroidal freckles, learning disabilities, skeletal dysplasia, and so on. Sbidian et al. (2010) proposed a NF1 score for predicting neurofibromatosis in adults and similar atypical manifestations of SNF including subcutaneous neurofibromas (>2), age (<30 years old), no cutaneous neurofibromas, and café-au-lait macules (<6 spots) were found to increase NF1 score in vivo.

Due to low incidence and lacking of reported cases, few therapeutic strategies have been developed toward SNF. The therapeutic goal of SNF is mainly focused on relieving the symptoms, increasing the survival rate and improving the quality of patients’ life. Till now, surgical excision is one of the most used clinical management. In SNF, tumors may disturb the vital center such as spine, spinal cord and nerve roots, surgical excision of the tumors requires adequate clinical experience. Timing of operation is another challenge to overcome. Uncertain genotype-phenotype relationship and lack of typical clinical symptoms make it difficult for SNF patients to be diagnosed, asymptomatic children are more likely to miss the early diagnosis (Korf, 2015). Nerve compression, the most obvious symptoms in SNF, that greatly affect the patients’ quality of life is introduced as one of the surgical indications for SNF (Ferner & Gutmann, 2013).

In recent years, few studies of multiple neurofibromas in spinal canal have been reported. In this study, a SNF patient with NF1 mutation in a classic NF1 family was reported. The clinical manifestation and gene mutation of SNF were also discussed to illustrate the pathogenesis, diagnosis, and prognosis of SNF, as well as, the timing of operation.
thoracic 11-sacral 5 spinal canals. Some nerve roots were compressed by tumor occupancy. Constriction and absorption were also found in some bones.

In this case, no neurofibroma-related symptoms were found in patient’s infancy and childhood. Scattered subcutaneous nodules throughout the body appeared in adolescence. The latest attack was happened in 28th week pregnancy, and the delivery was carried out by cesarean section due to this attack. The patient underwent a surgical intervention to remove the tumors in cervical 1–6 which constrict the nerves (Figure 3c). Tumor-like cells were found in the excised tissue by hematoxylin-eosin (H&E) staining (Figure 4 Left). Hyperplasia of ganglion cells was also found in the tumor by immunohistochemistry (Figure 4 Right). During the year of following up after the surgery, lower limbs pain, numbness, and occasional convulsion were completely relieved in the patient.

The mother and the brother in the family presented classic NF1 features and related medical history. Characteristic symptoms, like multiple neurofibromas nodules protruding and typical café-au-lait macules (> 6 spots) were emerged (54 years old) after the mother's marriage. Since the life

**FIGURE 1** (Upper) Pedigree of the studied family with classic NF1 and (Lower) diagram showing the mutation (NM_000267.3:c.1721 + 3A>G) in exon 11 on the NF1 (NCBI Reference Sequence: NG_009018.1) were showed
quality was not affected, no effective treatment was applied. The brother (28 years old) presented with classical features of NF1 including café-au-lait macules (>6 spots), multiple subcutaneous neurofibromas, one of these affecting the corner of the right eye that impaired the visual field with age and still growing after three operations. However, no axillary groin freckles, Lisch nodules, choroidal freckles, and scoliosis were found in the brother.

### 3.2 Gene sequencing

The exon sequencing of NF1 showed three common heterozygous mutations in all the family members with SNF or classic NF1. Two common mutations were found in the intron region and suspected to be benign. A common germ-line pathogenic mutation, NM_000267.3:c.1721 + 3A>G, located in exon15 of NF1, was identified and confirmed by Sanger sequencing.

**FIGURE 2** (Left) Irregular café-au-lait macules on the skin and (Right) subcutaneous nodules scattered on the right neck were shown.

**FIGURE 3** (a) Sagittal T2-weighted MRI of the whole spine showed neurofibromas invaded every paravertebral area with numerous small nodules infiltrating the sacrococcygeal soft tissue. Preoperative (b) and postoperative (c) images of spinal canal cervical 1–6.
DISCUSSION

SNF is characterized by bilateral, histologically proven neurofibromas of all spinal roots leaving no intact segments, with or without other manifestations of the NF1 diagnostic criteria. SNF can occur in an early age, but generally be noticed only when the spinal cord is severely compressed by tumors (Ruggieri et al., 2015). Symptomatic SNF accounted for only 5% of NF1 (von Deimling, Krone, & Menon, 1995; Huson et al., 1988), however, Thakkar, Feigen, and Mautner (1999) examined 1,400 NF1 patients by MRI and found that only 23 patients had spinal neurofibromas with obvious symptoms, accounting for about 1.6%. Compare with classic NF1 patients, the incidence of café-au-lait macules, axillary groin freckles, and cutaneous neurofibromas in SNF patients was much lower (Ruggieri et al., 2015). Owing to the lack of typical clinical manifestations, many SNF was diagnosed unexpectedly by spinal MRI for other reasons (Sbidian et al., 2010). For adolescents or fertile women that have NF1 family story, spinal CT or MRI should be reviewed regularly. (Ruggieri et al. (2015) found that café-au-lait macules were larger, lighter, and more irregular in SNF when compared with classic NF1. For children, multiple (sometimes less than six spots, but also a larger number), large, light brown, irregular margins of café-au-lait macules indicate a risk of SNF (Ruggieri et al., 2015). Although some SNF does not have typical clinical manifestations of NF1, their symptoms are all related to changes in the NF1 gene (Ruggieri et al., 2015). In this study, the same NF1 mutation (NM_000267.3:c.1721 + 3A>G) was detected in both classic NF1 patients and SNF patient, indicating that SNF is belonging to the NF1 catalog. This point of view is also supported by a Spanish research group (Ares et al., 1998) that they found a coding shift mutation of NF1 gene in SNF in 1998.

NF1 lacks mutation hotspots (Gutmann et al., 2017) and about 90% of NF1 mutations are point mutations. Because of the wide spectrum of mutations in NF1, clinical studies have shown no clear genotype-phenotype correlation (Abramowicz & Gos, 2014). Nevertheless, three genotype-phenotype correlations have been found as follows: loss of whole-gene base pairs leads to facial deformity, mental decline, and increased cancer incidence; codon 1809 mutation leads to café-au-lait macules (Rojnueangnit et al., 2015), short stature, pulmonary artery stenosis, and lack of gross plex or epidermal neurofibromas; base pair deletion in exon 17 causes no neurofibroma symptoms (Upadhyaya et al., 2007). The knowledge about the genotype-phenotype correlation of SNF is also limited due to a lack of related study. Some researchers have found that the incidence of missense mutation and splice-site mutation in SNF is higher than that in NF1, which may throw a light on future genotype-phenotype correlation studies of SNF (Upadhyaya et al., 2009). In this study, we reported a variable clipping mutation of NF1 (NM_000267.3:c.1721 + 3A>G), which occurred at the junction of intron and exon 15. This mutation can result in partial deletion of neurofibroma protein and limited cell proliferation inhibition (De Luca et al., 2005). It was first reported by Purandare (Purandare, Lanyon, & Connor, 1994) in 1994, but case-related information was absent. Up to now, the pathogenesis of SNF is still unclear. The possibility of gene modification has been proposed many a time (Nicita et al., 2014; Tadini et al., 2014). In this study, classic NF1 and SNF were found in a family with the same NF1 mutation, suggesting that SNF is an NF1 related disease and may be caused by not only gene mutation but also gene modification. Moreover, in this case only the patient had SNF, her mother and brother suffered from classic NF1, and the other members of the family did not show NF1. It is questionable that SNF is "hereditary" or "familial".

The importance of the tumor microenvironment in NF1 has been demonstrated in small animal models (Yang et al., 2008). Although neurofibromas occur equally in men and women with NF1, hormone level changes during pregnancy in NF1 patients can directly affect neurofibroma-deficient cells, increasing the size and number of neurofibromas and worsening the condition (Roth, Petty, & Barald, 2008). In this study, the patient also developed spinal cord compression and diagnosed with SNF during pregnancy. However, the underlined mechanism of gonadal sex hormones in the
development of neurofibromas is still far from clear, further related study is necessary.

Aggravated nerve compression symptom is considered as one of the surgical indications for SNF patients (Ferner & Gutmann, 2013). In this case, before the surgery, nerve compression symptoms like limbs numbness and weakness were emerged in the patient, while after the surgery these compression-related symptoms were greatly alleviated. The tumors responsible for nerve compression should be excised as much as possible, but excising tumors that close to the important nerves and blood vessels may cause secondary injury and massive hemorrhage (Lin & Gutmann, 2013). Therefore, the main objective of the operation is to relieve the obvious clinical symptoms and improve the life quality of the patients. The prognosis of surgical resection of tumors in NF1 is generally good to alleviate the clinical symptoms of patients. In this case, lower limbs numbness and weakness were totally disappeared in the follow-up year after the operation. Further study on the molecular and genetic levels of SNF could benefit the timing of surgery.

Recurrence and prognosis of SNF are rarely reported. A review article reported an 8.4% to 10.7% 5-year recurrence rate of patients with most resection of spinal neurofibromas (Ito, Aoyama, Miyaoka, Horiuchi, & Hongo, 2015). Total resection of tumors can reduce the recurrence rate, but the incidence of surgical complications such as infection, hematoma, cerebrospinal fluid leakage, and meningitis will increase accordingly (Ito et al., 2015).

This study identified a new case of SNF in a family with classic NF1 mutation (NM_000267.3:c.1721+3A>G) for the first time. It is indicated that SNF belongs to the NF1 catalog and may share the same gene mutation with NF1, while the different manifestation of NF1 and SNF may be related to gene modification. Surgical excision could relieve the nerve compression symptoms and greatly improve the quality of life, is an effective treatment in SNF. No recurrence of tumor was found in this patient and further follow-up would be continued. Conclusively, this study enriched the case data of NF1 and SNF, further benefited the diagnostic, pathologic, and therapeutic study of SNF.

CONFLICT OF INTEREST

All authors state that no financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

AUTHORS’ CONTRIBUTIONS

W Z designed the work. Z N investigated the family history and collected the clinical data of the patient. Z Y analyzed the data, made the graphs, and was a major contributor in writing the manuscript. G C, W W and L H helped with the gene sequencing. Y S and D C were involved in clinical data collecting. All authors read and approved the final manuscript.

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