

## Original Article

# Hereditary spinal schwannomas disease: a report for pedigree cases

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Received September 7, 2015; Accepted November 23, 2015; Epub March 15, 2016; Published March 30, 2016

**Abstract:** A 33-year-old male patient was found in clinic, which was hospitalized for surgical treatments due to the multiple tumors in vertebral canal. The earliest onset of the disease for him occurred when he was 19 years old. Up to now, he has been performed 7 times of surgical treatment. Tracing his family history, the pedigree contains 48 members. According to the diagnostic criteria proposed by Macollin and the International Schwannomatosis Workshop in 2011, nine patients were diagnosed (five died), and two were suspected patients. Meanwhile, neither neurofibromatosis type I with café au lait spots on the skin, nor multiple neurofibromatosis type II history with vestibular nerve function deficiency or hearing impairment was found in the pedigree. The vestibular nerve space occupying lesion was also not found in cranial MRI examination. The excisional tumor was confirmed to be schwannomas by pathological examination. The preliminary gene sequencing for the *NF2* and *SMARCB1* gene demonstrated that there was a missense mutation (C.593G→A) in exon 6 codon 198 in blood of the proband (III:1), a nonsense mutation (AAG→TAA) in exon 4 codon 149 in blood of II:5 and II:2, a missense mutation (C.593G→A) in exon 6 codon 198 in tumor tissue of III:1, and a missense mutation (C.119T→G) in exon 2 codon 40 in tumor of III:2. The synonymous mutation (C.93G→A) in exon 1 codon 31 of the *SMARCB1* gene was detected in the tumor and blood samples from many members of this pedigree. Hence, this family could be confirmed to be a certain hereditary spinal schwannoma pedigree and the synonymous mutation (C.93G→A) in exon 1 codon 31 of the *SMARCB1* gene may be the hereditary disease causing mutation.

**Keywords:** Schwannomas, schwannomas disease, diagnostic criteria, Non vestibular schwannoma, *SMARCB1*, *NF2*, genic mutation

## Introduction

Schwannoma is a rare kind of Schwann cell derived benign tumor, with specific clinical manifestations of multiple nerve sheath tumors on spinal cord or peripheral nerve fibers, neither intradermal and nor vestibular schwannomas (VS), as well as café au lait spots on the skin, neurofibromatosis type 1/2 (NF1/2) fundus Lisch nodules, which was also called as NF3 [1]. In this paper, a certain hereditary spinal schwannoma pedigree was reported.

## Materials and methods

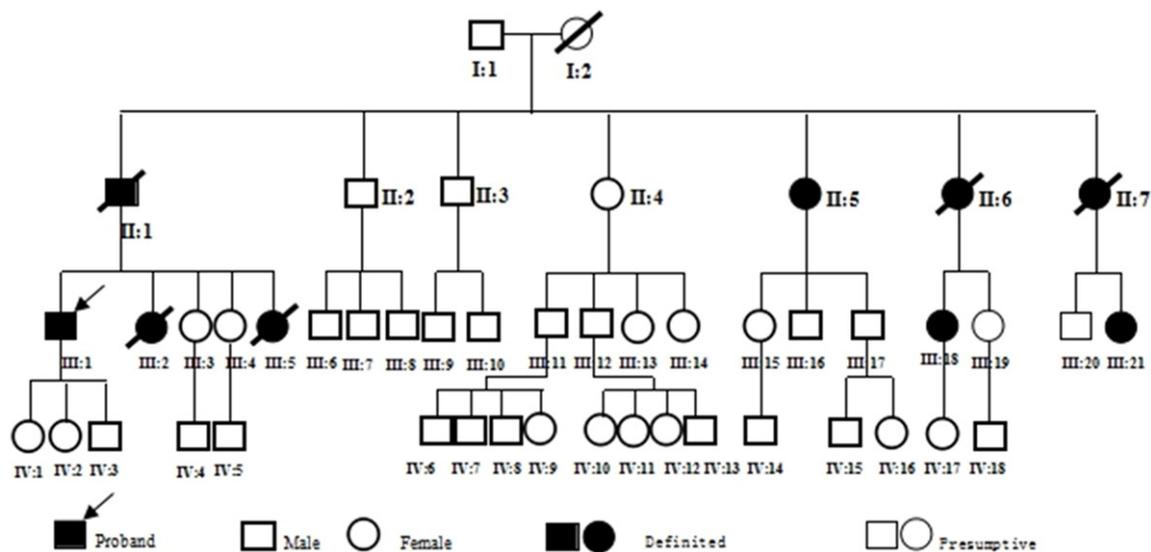
### Family members and clinical investigations

This pedigree containing 48 members came from Guangdong Province of China (**Figure 1**). In accordance with previous diagnostic criteria

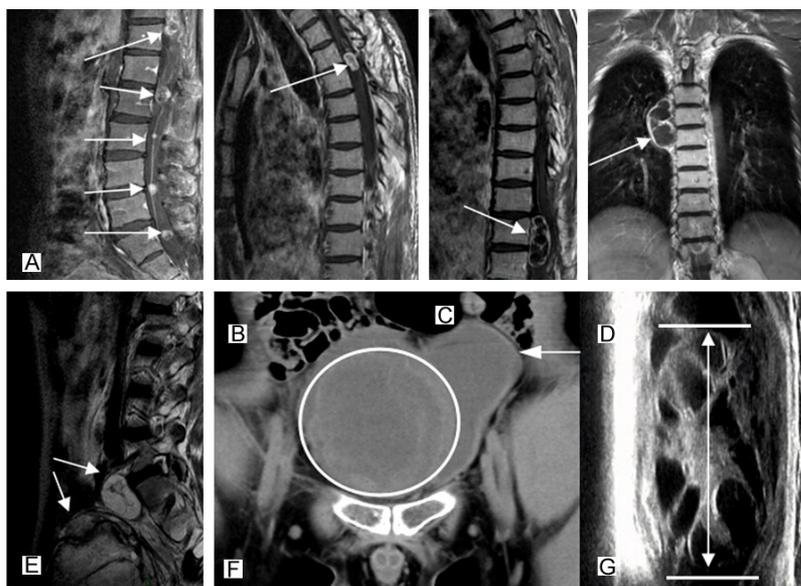
proposed by Macollin [2] and the International Schwannomatosis Workshop in 2011 [3], nine patients were diagnosed (five died), and two were suspected patients. After the family members signed informed consent forms, the detailed histories of their disease were collected. All family members received physical examination and imaging tests (spinal canal myelography or lumbar and cranial MRI). Blood samples from all members were collected. Individuals in whom a tumor was detected by MRI underwent surgical treatment, and tumor samples were collected.

Proband III:1, 33-year-old male, had received four surgical treatments since the onset of disease at the age of 19 years. In September 2006, the patient was first hospitalized because of neck, chest and back pain for 5 years and

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**Figure 1.** Pedigree chart of the familial spinal schwannomas.



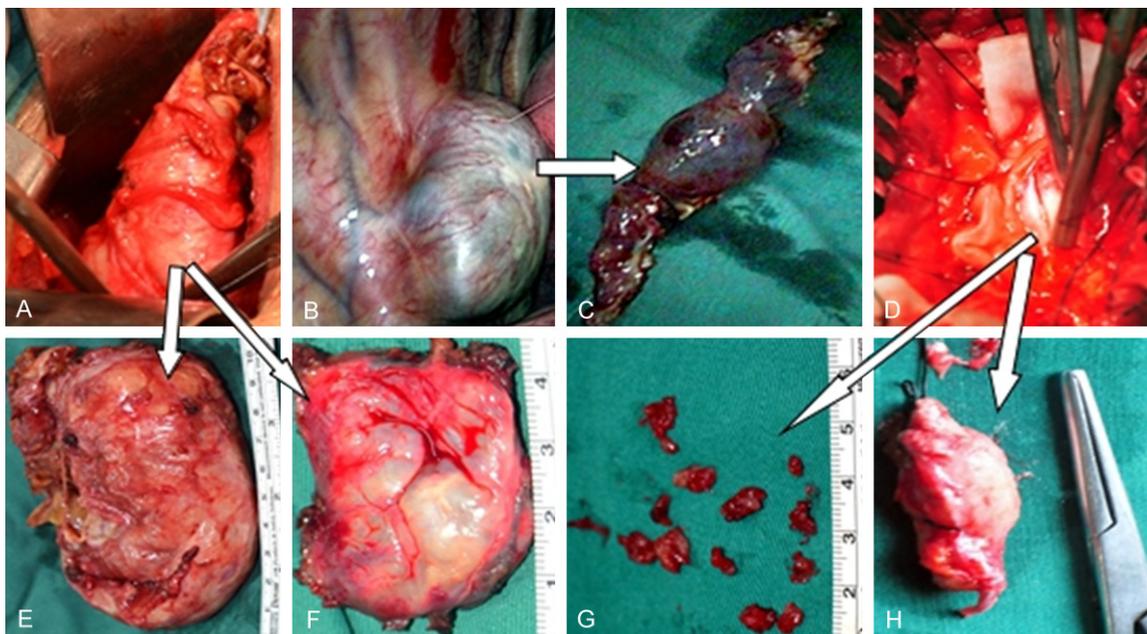
**Figure 2.** The MRI images for the hospitalized proband in 2013: (A) and (C) lumbar vertebra; (B) thoracic vertebra; Multiple occupying lesions (D) on the side of the thoracic vertebra and (E) in pelvic cavity; (F) Pelvic CT image, displaying huge occupying lesion (10\*9.5\*11 cm); (G) B-type ultrasonography image on right lower limb, displaying cystic tumor with size of 5.8\*4.7 cm on right sciatic nerve, not connected with canalis spinalis, pressing the bladder to left.

walking dysfunction for 1 month. MRI revealed multiple tumors on the neck and thoracic vertebra. Excision of neck and thoracic duct tumors was conducted. Yellowish oval circumscribed tumors (size: 2×0.8, 1×0.6, 2.5×0.8, 0.4×0.3 cm) were visible in the dorsal region of T<sub>3</sub>, the dorsal region of T<sub>1</sub>, the ventral region of C5 and the dorsal region of C6, respectively, adhesive with nerve branches. The Symptom was reli-

eved after surgical removal of the tumors. In January 2011, this patient was hospitalized again because of later back pain with numbness in the lower limbs for 1 month. MRI revealed multiple space-occupying lesions in the cervical, thoracic and lumbar spinal cords. Posterior neck and thoracic tumor excision was conducted. A 2.0×1.5 cm tumor was observed in the dorsal region of the spinal segment at T<sub>1</sub>. Four tumors (1.8×1.6 cm, 2.3×1.4 cm, 2.0×1.0 cm, 1.8×1.6 cm) were observed in the dorsal region of spinal segments at T<sub>10</sub>-L<sub>2</sub>, which were well circumscribed and adhesive nerve roots. The Symptom was relieved after completely removal

of the tumors. In March 2013, this patient was hospitalized for the third time because of later back pain with numbness in the lower limbs for 3 month. MRI revealed multiple tumors in the lumbosacral spinal canal, an extramedullary subdural stripped tumor at T<sub>11</sub> and T<sub>12</sub>, a tumor to the right of the vertebral body at T<sub>6</sub> and T<sub>7</sub>, and extramedullary subdural nodular lesions at T<sub>1</sub> and T<sub>3</sub>. Thoracolumbar spinal tumor excision

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**Figure 3.** Posterior thoracic and lumbar tumor excision, anterior pelvic tumor excision, as well as intrathoracic tumor resection by thoracoscopy were performed on the proband in 2013. (E and F) The large excisional tumor from (A) pelvic cavity; (C) The big excisional tumor from thoracic vertebra (B) under laparoscope; (G and H) The excisional multiple space-occupying tumor. All tumors were confirmed to be schwannomas by pathological examination.

was performed. Tumors (2.5×4.5 cm and 2.0×4.0 cm) with levels of T<sub>10-12</sub> and L<sub>2-L4</sub> were detected in intradural extramedullary, which were completely removed. In August 2013, he was hospitalized for the fourth time because of Chest back pain associated with double lower limbs paraplegia for 2 month. MRI revealed T<sub>3</sub> and T<sub>11/12</sub> tumors. With enhanced scanning ring enhancement, A T6/7 tumor with size of 2×2×3 cm was found on the right side of the vertebral body. Pelvic CT displayed multiple cystic lesions (biggest size: 10×9.5×11 cm). B-type ultrasonography on right lower limb showed two cystic tumors (5.3×2.2 cm, 5.8×4.7 cm) on the sciatic nerve of the right thigh, and a tumor (5.5×2.2 cm) on the tibial nerve of right leg (**Figure 2**). Posterior thoracic and lumbar tumor excision, anterior pelvic tumor excision, as well as intrathoracic tumor resection by thoracoscopy were performed (**Figure 3**). A solid-cystic tumor (10×9.5×11 cm) was detectable in the right lower quadrant and pelvic cavity. The dark red liquid outflowed after tumor incision, the necrosis tissues were found in the tumor. A T6/7 cystic mass with size of 2×2×3 cm and at right side of the spine were found in Right thoracic cavity, sharpness of border. Multiple tumors were found in the thoracic and lumbar spinal canal. The largest one with size of 5.6×3.2×2.3 cm is pale white and in level of T10/11.

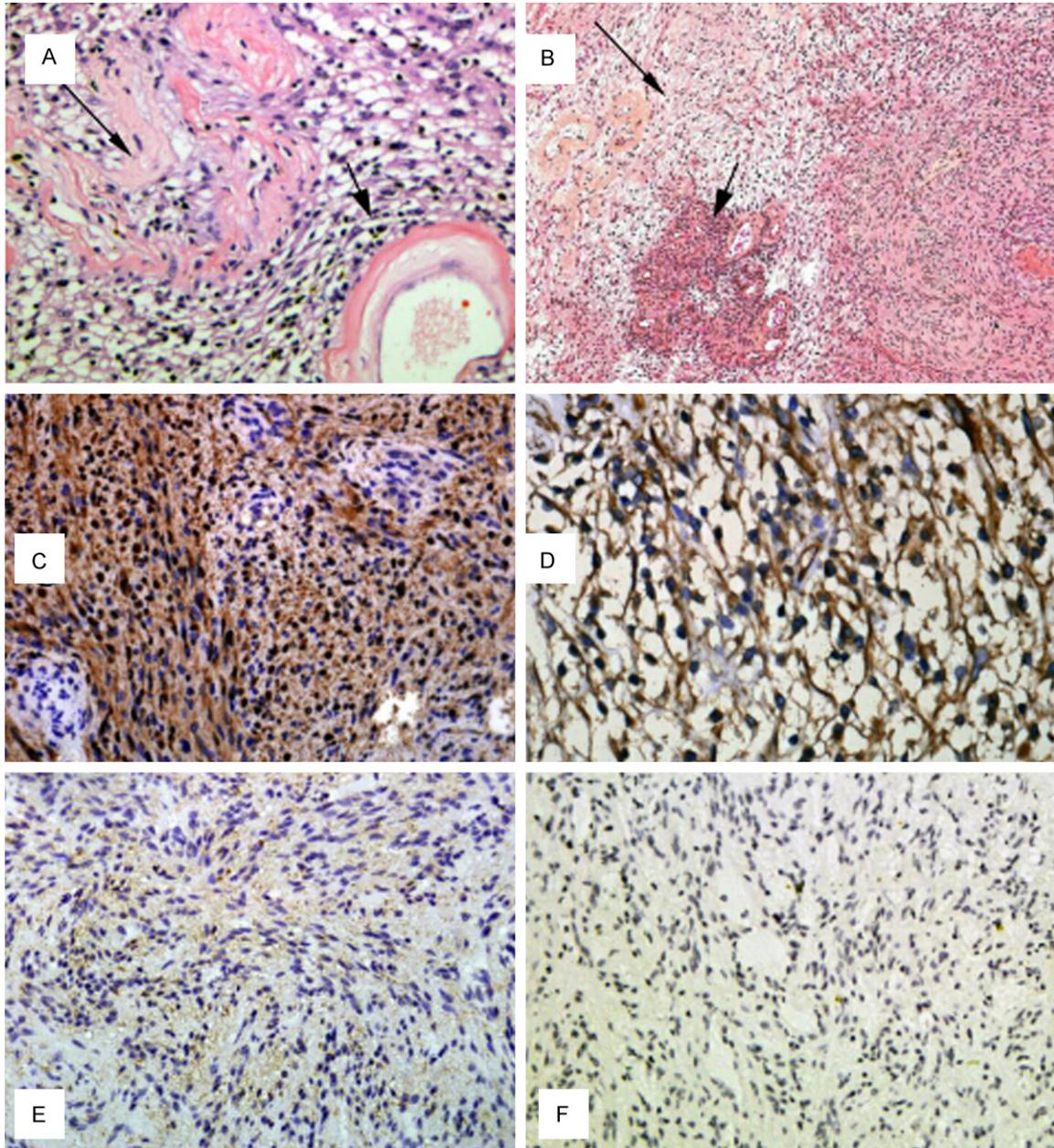
II:1 is father of proband III:1, who suffered from lower limb weakness, had experienced gradually progressive paraplegia and urinary and fecal incontinence since 1980, lower extremity panplegia in 1995, and died in 1999. Thoracic lumbar spinal canal tumors were found during surgery. III:2 is the first young sister of proband III:1, who had received 3 times surgical treatments because of tumors suffering. She suffered a sciatic nerve tumor in 2002, multiple tumors at C<sub>12</sub> and T<sub>12-S1</sub> in 2004, and extramedullary subdural multiple tumors with levels of T<sub>1/2-L1/2</sub> in 2007, showing progressive lower limb paralysis and dysuria, gradually leading to limb paralysis and headache. MRI revealed a brain tumor. She abandoned the treatment and died in 2010. III:5 is the latest young sister of proband III:1, who suffered chest and back pain, headache and vomiting in 2007. MRI demonstrated a tumor with short T1, T2 and high signal intensity in the subarachnoid space at T<sub>6-8</sub>. CT scan revealed a space-occupying lesion in the left cerebellar hemisphere. One month after tumor resection, she died of pulmonary infection. II:5 is the fifth aunt of proband III:1, who experienced pain in the abdomen and right lower limb in 1995. Two tumors with levels of T11 and T12 were found during the surgery. She keeps healthy after the surgery up to now. II:6 is the sixth aunt of proband

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**Table 1.** Data on family members with rare hereditary spinal schwannomatosis

No.	III:1	II:1	III:2	III:5	II:5	II:6	II:7	III:18	III:21
Gender	Male	Male	Female	Female	Female	Female	Female	Female	Female
Age of onset	19	24	13	16	37	17	26	24	20
Age of death	-	43	27	17	-	24	32	-	-
Pain	+++	++	++	++++	++	+	++++	++++	+++
Weakness/paraplegia	+++	++++	++++	++++	+	++	++++	-	-
Bowel and bladder disorders	x	√	√	x	x	√	√	x	x
Vestibular schwannomatosis	x	x	x	x	x	x	x	x	x
Milk coffee spot/Lisch nodules	x	x	x	x	x	x	x	x	x
Times of surgery	6	> 5	3	1	1	3	2	2	1
Number of tumor	17	> 5	5	2	2	> 4	5	2	1
Position of tumor	Spinal cord, thoracic cord, caudaequina, pelvic cavity, thigh	Thoracic vertebra, caudaequina, right shoulder	Thigh, neck, thoracic cord, caudaequina	Thoracic vertebra, left cerebellum	Thoracic cord	Thoracic cord, caudaequina	Thoracic cord, caudaequina	Cervical cord	Lumbar vertebra, caudaequina
Complication	-	-	-	Pneumonia	Lumber disc herniation	Urinary tract infection	-	-	-

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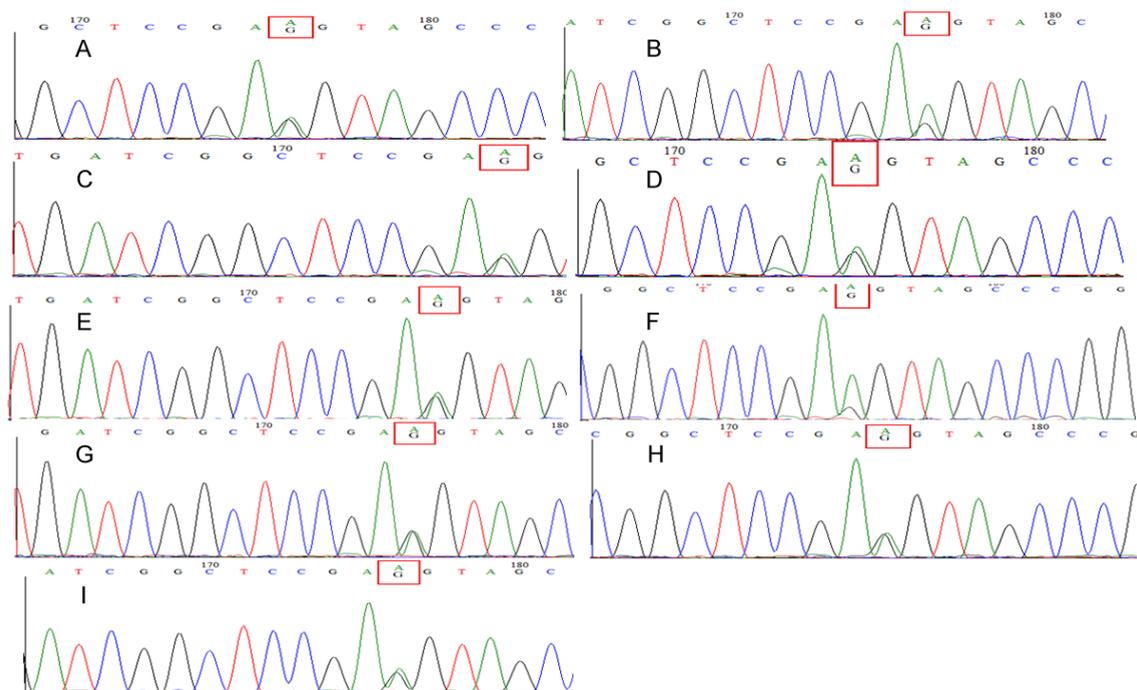


**Figure 4.** Immunohistochemistry results of the tumors from the Pedigree: A and B. H&E staining images, Antoni A area (Coarse short arrow) and Antoni B area (Slender arrow) could be observed, verocay globules could be found in Antoni A area; C. Strong positive staining of S-100; D. Positive staining of Vimentin; E. Strong positive staining of merlin; F. INI1 protein staining image: mosaic-like pattern.

III:1, who was hospitalized three times from 1981 to 1987, presenting with progressive lower limb paraplegia and bowel and bladder disorders. The T<sub>11</sub>, L<sub>2</sub> and L<sub>3</sub> Tumors were observed. Symptoms were not lessened after the third surgery. She had a urinary tract infection and died in 1988. II:7 is the latest aunt of proband III:1, who presented with lower back pain and progressive lower limb paraplegia, and received

twice surgeries. Multiple tumors were observed in levels of T<sub>12</sub>-L<sub>4</sub>. Paraplegia was not relieved after the secondary surgery. Bowel and bladder disorders appeared. She died in 2001. III:18, III:19 and III:19 are daughters of II:6. III:18 received twice cervical cord tumor resection. III:21 received lumbar multiple tumor resection. III:19 suffered cervical cord space-occupying lesion (MRI), but refused surgical treatment.

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**Figure 5.** The synonymous mutation (C.93G→A) in exon 1 codon 31 of the *SMARCB1* gene detected in the tumor and blood samples from many members of this pedigree. Form A to I: *SMARCB1* gene sequence diagram of exon 1 from the Blood, cervical spinal tumor, thoracic spinal tumor, pelvic tumor of the proband, blood of the oldest daughter of the proband (IV:1), thoracic spinal tumor of the father of the proband (II:2), as well as blood of III:18, III:20, and III:21. All Mutations occurred in exon 1 codon 31, nucleic acid sequence from GAG to GAA.

III:20 is son of II:7, who received multiple tumor resection lateral to the inferior extremity of the right leg, but refused pathological examination. Pathological examination demonstrated that the obtained tumors from diagnosed patients during surgery were schwannomas.

### Blood and tumor samples

Blood samples of 43 family members were collected, except the five dead patients. The removed fresh tumor samples during the surgery included thoracic cord, thoracic cavity and pelvic cavity from III:1, and the cervical cord tumor from III:18. Fresh tumor samples were stored at -80°C. The following tissues were embedded in paraffin: cervical cord of III:1, thoracic cord of II:1, thoracic cord of III:2, and thoracic cord of III:5. Unfortunately, blood samples could only be obtained from II:5 and III:21. No blood or tumor samples were obtained from II:6 and II:7, who were dead. All tumor samples were stained with H&E, DAB visualized for immunohistochemical staining. The applied Antibodies were as follows: vimentin and S-100 (Dako Cytomation Denmark A/S), *SMARCB1* gene expression

product INI1 (BD Biosciences San Jose, CA, USA), and NF2 gene expression product Merlin (Abcam, Cambridge, Great Britain).

### DNA extraction, amplification and mutation analysis

DNA was extracted from 43 blood samples and eight tumor samples. Tumor samples included thoracic cord, thoracic cavity and pelvic cavity of III:1, and fresh cervical cord tumor of III:18, as well as paraffin-embedded tumor samples of cervical cord of III:1, thoracic cord of II:1, thoracic cord of III:2, and thoracic cord of III:5. After DNA extraction, primers were designed using 17 exons of the *NF2* gene and nine exons of the *SMARCB1* gene in accordance with previously described methods [1, 4]. After PCR amplification, nine exons from each of the *NF2* and *SMARCB1* genes were directly sequenced by the Sanger method. The copy number of *NF2* and *SMARCB1* genes was analyzed using multiple ligation-dependent probe amplification (MLPA). The study was approved by the Ethics Committee of Affiliated Hospital of Guangdong Medical College in China.

### Results

This pedigree is a rare family with hereditary spinal schwannomatosis. Multiple schwannomas were found during surgery and observed by MRI. There were no café au lait spots on the skin, Lisch nodules or vestibular schwannomas. Clinical manifestations present limb pain, weakness and numbness. Symptoms could not be lessened by oral non-steroidal analgesics. Progressive lower limb paralysis occurred. Some patients suffered bowel and bladder disorders. These diseases were easy to relapse after surgery and in poor prognosis. III:5 had severe pneumonia, and died within 1 month after discharge from hospital, while II:6 had aurinary tract infection (see **Table 1**). The hematoxylin and eosin staining results demonstrated that all tumor samples were schwannomas. Immunohistochemistry showed positive expression of S-100 and vimentin. INI1 protein staining results revealed a mosaic-like pattern, indicating schwannomatosis. Positive staining for merlin protein indicated normal expression of the *NF2* gene (**Figure 4**).

The diverse mutations were shown in *SMARCB1* gene analysis. No abnormal copy number for the *NF2* or *SMARCB1* genes were observed in MLPA analysis. Direct gene sequencing for the *NF2* gene demonstrated that there was a missense mutation (C.593G→A) in exon 6 codon 198 in blood of the proband (III:1), a nonsense mutation (AAG→TAA) in exon 4 codon 149 in blood of II:5 and II:2, a missense mutation (C.593G→A) in exon 6 codon 198 in tumor of III:1, and a missense mutation (C.119T→G) in exon 2 codon 40 in tumor of III:2. *SMARCB1* gene exon sequencing suggested various mutations, as shown in **Figure 5**.

In blood samples from the proband, his oldest daughter (IV:1), father (II:2), two younger female cousins (II:18, 11:21) and a younger male cousin (II:20), and three tumor samples from the proband, a synonymous mutation (C.93G→A) was detected in exon 1 codon 31 of the *SMARCB1* gene. In a blood sample from the son (IV:3) of the proband, a synonymous mutation (C.897G→A) was detectable in exon 7 codon 299 of the *SMARCB1* gene. These synonymous mutations were firstly detected in this family with schwannomatosis, but the pathogenic mechanism remains unclear.

A missense mutation was detected in exon 9 codon 383 of the *SMARCB1* gene in blood samples of IV:1 and IV:3, which would induce an amino acid substitutions (P.Pro383Leu). A missense mutation was detected in exon 1 codon 18 of the *SMARCR1* gene in a tumor sample from the thoracic cord of III:2, which would lead to an amino acid substitution (P.Gln18Leu). A missense mutation was found in exon 1 codon 7 in the tumor sample of lumbar vertebra of III:5, which would also cause an amino acid substitution (P.Ser7Ile).

A nonsense mutation (C.91G→T) was found in exon 1 codon 31 in the tumor sample from thoracic cord of III:2. This mutation would induce premature termination of peptide chain synthesis (P. Glu31X).

### Discussion

Schwannomatosis is the third independent type for multiple nerve sheath tumors, with the characterizations of neither intradermal nor vestibular schwannomas. It is significantly different with NF1 and NF2 both in genetics and clinical manifestations. However, the neurinomatosis in clinical is easily confused with NF2, especially the chimeric NF2. In the literature about NF2 published from 1990 to 2003, there were no distinguished schwannomatosis, as they are very similar in clinical. In 2003, the results of the genetics research showed that no direct connection of NF2 gene and familial schwannomas disease. In 2007, Hulsebos et al. reported a pedigree of schwannomas and genetic studies suggest that the pathogenesis may be related to *SMARCB1* germline mutations. Then, the *SMARCB1* gene became the focus of the schwannomas disease pathogenesis. However, many studies confirmed the *SMARCB1* gene mutation was only found in 40%-50% of familial schwannomatosis. Moreover, the mutation were only found in 10% cases of sporadic schwannomatosis [1, 5, 6]. According to the previous diagnostic criteria proposed by Macollin [2] and the International Schwannomatosis Workshop in 2011 [3], nine patients were diagnosed (five died), and two were suspected patients in this report. *SMARCB1* gene mutations were found in 6 patients of them, occupying 54% of family schwannomatosis cases. Considering that not all family members were taken spinal MRI exami-

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nation, there may be some missed diagnosis, resulting in inaccurate data. The synonymous mutation (C.176G→A) in exon 1 codon 31 of the *SMARCB1* gene was detected in the blood samples from the proband, his oldest daughter (IV:1), father (II:2), two younger female cousins (II:18, 11:21) and a younger male cousin (II:20), as well as three tumor samples from the proband. These synonymous mutations were firstly detected in pedigree with schwannomatosis. According to the degeneration rule for amino acid codon, the synonymous mutation does not cause the amino acid encoding error. However, the above members (except the children of proband) are all in this disease, indicating that synonymous mutation may also lead to a mutation effect. Despite few literature for the synonymous mutation, it could nowadays be confirmed that the mutation effect is usually harmful, which is more prone to promoting cancer gene rather than restraining cancer gene [7], leading to skipping of exon [8], variation of splicing donor site [9], etc. Up to now, the mechanism of mutation effect caused by the synonymous replacement of the codon is not clear. Studies have indicated that the synonymous substitution of codon may affect the accuracy and speed of translation, especially through the enhancement the C-ending content of the four-fold codons [10]. More research works are required for the exploration of the mechanism that schwannomas disease caused by synonymous mutations. For the schwannomas diseases caused by germline mutations in the *SMARCB1* gene, it could be speculated that mutation may be inherited to offspring through the "two-hit" mode. In the "two-hit" procedure, the first hit occurred in the gametids, leading to germ line mutation without clinical symptoms. When the mutation the gametids were inherited to the next generation, germline mutation and somatic mutation simultaneously occurred ("two-hit"), presenting clinical symptoms. According to the "two-hit" theory, the first hit in this pedigree occurred in the grandfather or grandmother of the proband, neither clinical symptoms, nor *SMARCB1* gene mutations detected from the blood (somatic cells) samples. After the combination of their gametes, mutation was inherited to the father and the aunts of the proband by the "two-hit", leading to spinal schwannomas diseases. The mutations were found in both blood and tumor samples by genetic testing. Then, the mutations continue to be inherited to third and fourth generations,

leading to the diseases on the proband, his sister, and cousins. Although the oldest daughter of the proband has not yet clinical symptoms, the *SMARCB1* gene mutation in the same site has been found in her blood testing. It is reported [11, 12] that the young children may be risk to the malignant rhabdomyosarcoma in the cases of *SMARCB1* gene mutation and the risk also increases with age. The Heterozygous mutations in the survivors may promote the susceptibility of schwannomas disease. Despite some research works found that the somatic mutations for the *NF2* gene appears in the cases of schwannomas disease [5, 6, 13], the *NF2* gene mutations in different sites were detected in blood and tumor tissues from only few patients of the pedigree, indicating that *NF2* gene mutation is not the necessary conditions for schwannomas disease [1].

As the *SMARCB1* gene mutations were detected only in half of the members in the pedigree suffered the schwannomas disease, it is obvious that the *SMARCB1* gene germline mutations have been unable to fully explain the genetic basis of pedigree of schwannomas disease. With the deepening of the research, some scholars have explore the genetic mechanism of schwannomas disease from the fields outside of the *smarcb1* gene and even 22q. Piotrowski et al. [4] found a new *LZTR1* gene located at the long arm of chromosome 22, which have confirmed to be a cancer promoting gene and could induce schwannomas disease in the patients without *SMARCB1* gene mutation. These two pathogenic genes *LZTR1* and *SMARCB1* show that there is a potential functional association with chromatin remodeling mechanism, which plays a vital role in the adaptation of cellular differentiation and adaptation to environmental stimuli. Zhang et al. [14] found the relationship between missense mutation of *COQ6* gene and the susceptibility of familial schwannomatosis through full genome sequencing method, suggesting that the familial schwannomatosis may be not a single-gene disease. It is worth to mention that, although more and more research works have been focused on the genetics mechanism of schwannomas disease caused by *SMARCB1* gene mutation, there is still rare contributions devoted to the influence of the *SMARCB1* gene mutation on the function of the expressed *INI1* protein, as well as the downstream molecular mechanisms.

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We report a pedigree of hereditary spinal schwannomas. The family affected members suffered schwannomas disease meet diagnostic criteria proposed by MacCollin, which will help to increase the understanding of schwannomas. As the genetic schwannomas pedigree in China are rarely reported, the preliminary gene sequencing indicates that the synonymous mutation (C.93G→A) in exon 1 codon 31 of the SMARCB1 gene may be the hereditary disease causing mutation, and this is direction for our future works.

### Acknowledgements

This project was supported by the Natural Science Foundation of Guangdong Province in China (Grant No. S2013010012473), and supported by Zhengzhou technology research and development funds support project No. 153-PKJGG063.

### Disclosure of conflict of interest

None.

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