A Novel Variant of SMARCB1 in Rare Familial Schwannomatosis Identified by Whole-Exome Sequencing and Genotype-Phenotype Correlation Analysis.

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Abstract

Background: Variants in the tumor suppressor gene *SMARCB1* could cause different conditions. In some cases, germline and somatic variants in *SMARCB1* are implemented in schwannomatosis. But the genotype and phenotype correlation for variants in *SMARCB1* has not been determined.

Methods: A Chinese schwannomatosis family with an autosomal dominant inheritance pattern was recruited. Whole-exome sequencing (WES) was performed to discover the causative variant, followed by Sanger sequencing. We evaluated the Human Gene Mutation Database (HGMD) regarding *SMARCB1* variants and validated associated phenotype records to assess phenotype-genotype relationships.

Results: A novel deletion variant c.885_896delGAAGCTGTGCTC p.(295_299del) in *SMARCB1* was identified in the affected family members and cosegregated with phenotypes in the pedigree. About 51.1% of variants in *SMARCB1* located in Snf5 subunit, 80.7% of variants were loss-of-function (LOF) variants, and more variants located in the Snf5 subunit of *SMARCB1* in Rhabdoid tumour (67.8%) than that in schwannomatosis (25.7%).

Conclusions: Our study expands the variant spectrum of *SMARCB1* and the genetic background of schwannomatosis, confirms the clinical indications for genetic screening of the *SMARCB1* gene, and has implications for genetic counseling in this disease.

Background

Schwannomatosis (MIM 162091) is a rare disorder characterized by the predisposition to develop benign multiple non-vestibular schwannomas and, in some cases, meningiomas and so on [1-3]. Examination and diagnosis of schwannomatosis has been hampered by the rarity of familial cases and the difficulty in separating schwannomatosis from NF2 cases [4]. Schwannomatosis is very rare, having an incidence of 0.58 cases/1 million persons every year [5]. Familial and sporadic schwannomatosis accounts for about 15% and 85% of reported cases, separately [6]. *SMARCB1*, a tumor suppressor gene, is involved in very low proportion of schwannomatosis patients: about 50% of the familial cases in autosomal dominant fashion with incomplete penetrance, but no more than 10% of the sporadic cases [7]. Schwannomatosis shares clinical symptoms and it can be challenging to distinguish with neurofibromatosis type 2 (NF2), particularly in cases of mosaic conditions [8]. *SMARCB1/INI1* is one of the core subunit of the ATP-dependent SWI/SNF chromatin remodeling complex, located at chromosomal position 22q11.2 [9]. So far, only 135 *SMARCB1* variants have been reported in the Human Gene Mutation Database (Professional 2021.2, <http://www.hgmd.org>). Variants in *SMARCB1* can cause different phenotypes, and the exact genotype-phenotype correlation for the variants in *SMARCB1* need to be further clarified [10]. In this study, we revealed a novel variant c.885_896delGAAGCTGTGCTC (p.(296_299del)) (NM_003073.5) in *SMARCB1* in a Chinese family with schwannomatosis.

Patient Data And Methods

Subjects and clinical evaluation

We observed a schwannomas family with autosomal dominant inheritance in our study. The diagnosis of schwannomas followed the consensus criteria of the National Institutes of Health, and the proband was confirmed with schwannomas by excisional biopsy and magnetic resonance imaging (MRI). Written informed consent was obtained from all participants, and this study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Zhengzhou University. All procedures were carried out in accordance with the Declaration of Helsinki.

Variant screening and analysis

DNA was extracted from blood samples according to standard methods using commercially available kits (QIAGEN, Venlo, The Netherlands). Whole-exome sequencing was based on the illumina sequencing platforms. The sequence reads were aligned to the human reference genome reference obtained from the UCSC database version hg19 (<http://genome.ucsc.edu>). The mean sequencing depth was 104.52×, and 98.23% of the targeted exome with >20x sequencing depth was obtained to accurately determine variants. Sequence variant interpretation was according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

Results

Clinical Manifestation

Three patients of the family were clinically diagnosed with schwannomas (**Fig. 1A**). The proband (III1), a 27-year-old woman, was born normally. Lumbar pain occurred during rope skipping at the age of 18 years. The symptoms were gradually aggravated after stopping activities, and the night pain was the most serious. The proband developed a mass the size of sour jujube in the right neck half a month before admission at the age of 20 years. Pain and numbness were manifested in the right upper limb after extrusion. The tumor in the superior trunk of the right brachial plexus was diagnosed after operation. The pathological results showed (right supraclavicular fossa) schwannoma. After a year (at the age of 21 years), because of the occupied spinal canal space, the proband was admitted to the hospital. Intraspinial schwannoma was diagnosed during the operation, and the pathological results showed the schwannoma in the thoracic 1, 2 spinal canal. At the age of 24 years, the mass was found on the posterior thigh, the occupied left sciatic nerve was diagnosed during the operation. and the pathological results suggested the schwannoma of the left lower limb. Two years later (at the age of 26 years), pains without obvious cause occurred in the left upper limb. She was admitted to hospital with multiple skin lesions with nodules (**Fig. 1B**) and cervical intraspinal space occupying lesions. After operation, the operative and pathological findings and MRI results demonstrated schwannomas (intracranial, cervical intraspinal, thoracic intraspinal space occupying) (**Fig. 2A-D**).

The proband (Ⅱ1), a 48-year-old man, was diagnosed with L4L5 intraspinal schwannoma and multiple schwannoma within both thighs (**Fig. 2E-F**).

The late proband (Ⅱ1), was dictated as schwannoma by clinical symptom descriptions without definitive diagnosis, and died at the age of 69.

Variant analysis of *SMARCB1*

Whole-exome sequencing and Sanger sequencing revealed the heterozygous variant of the affected family members c.885_896delGAAGCTGTGCTC (p.(296_299del)) in *SMARCB1* (**Fig. 1C**). The variant in *SMARCB1* was absent in the healthy family members, suggesting the variant cosegregated with the phenotype in this family (**Fig. 1A**). Multiple sequence alignment of 5 different species indicated the conservation around the 296_299 residues within the Snf 5 subunit of the SMARCB1 (**Fig. S1**). According to ACMG, the variant of *SMARCB1* was predicted to be likely pathogenic (PM1+PM2+PP1+PP3). Moreover, the variant was novel because it did not present in dbSNP, HGMD database, nor had been previously reported.

Discussion

In this Chinese pedigree, a novel and rare deletion variant (c.885_896delGAAGCTGTGCTC p.(296_299del)) in exon 7 was identified, which results in a truncated protein of 4 amino acid residues instead of full-length INI protein.

SMARCB1 mutated familial schwannomatosis transmitted in autosomal dominant fashion with incomplete penetrance accounts for about 7-8% of reported cases. In our study, the variant (c.885_896delGAAGCTGTGCTC p.(296_299del)) in *SMARCB1* has been shown to segregate with disease in the family members in 2 generations. Multiple sequence alignment of 5 different species reveals a high degree of conservation within the Snf5 subunit, implying its functional importance and the potential pathogenicity of the variant. The novel variant is a likely disease-causing loss of function variant according to the ACMG classification guidelines.

It is interesting that variants in *SMARCB1* can cause two very different phenotypes: schwannomatosis or malignant rhabdoid tumours (MRTs), and it may depends on the the variant types and location within the domain. Nontruncating (missense or splicing) variants are the major inherited variants found in familial schwannomatosis. Moreover, a higher proportion of truncating variants were found in sporadic schwannomatosis patients. The most common alteration identified so far is found in exon 1, c.41C>A (p.Pro14His) and within the 3'UTR at c.*82C>T, indicating the variant hotspots at either end of the gene in both familial and sporadic forms of schwannomatosis [11,12]. In contrast, the variants in the central exons or which delete all, or large parts, of the coding sequence often occurred in rhabdoid tumours. However, no exact genotype-phenotype correlation between variants in *SMARCB1* has been found so far. Intriguingly, the deletion variant (c.885_896delGAAGCTGTGCTC p.(296_299del)) in our study located in the central exon (exon 7), caused familial schwannomatosis. To assess the phenotype-genotype relationship of *SMARCB1*, We evaluated the Human Gene Mutation Database (HGMD) regarding SMARCB1 variants and validated associated phenotype records. Overall, we found that about 51.1% (69/135) of variants in *SMARCB1* were located in Snf5 domain, 80.7% (109/135) of variants were loss-of-

function (LOF) variants (Table 1). 44% (59/134) and 26.2%(35/134) of variants were related to rhabdoid tumour and schwannomatosis separately (Table 2). We further compared the variants in rhabdoid tumour and schwannomatosis, LOF variants are the main variant type in both diseases—Rhabdoid tumour VS schwannomatosis: 79.7% (47/59) VS 82.9% (29/35)) (Table 3), while more variants located in the Snf5 subunit of SMARCB1 in rhabdoid tumour (67.8%, 40/59) than that in schwannomatosis (25.7, 9/35) (Table 2). These results indicated that LOF variants within the Snf5 subunit of SMARCB1 inclined to cause rhabdoid tumour.

Malignant peripheral nerve sheath tumors arising in schwannomatosis patients were reported in some cases [13]. But the prognosis on the schwannomatosis caused by *SMARCB1* variant was uncertain. The oldest patient (1) in our study was up to 69 years old, and only showed multiple schwannomas with favorable outcome. Other patients (27 and 48 years-old, separately) in this pedigree has the similar symptoms, and need further follow-up.

Our study highlights the importance of genetic testing and counseling in patients with schwannomatosis, showed the consistent phenotype of the patients with the variant and expands the genetic background of schwannomatosis. Further and more studies of genotype and phenotype relationship are needed in order to better determine the cancer risk for carriers of *SMARCB1* germline variants.

Declarations

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Authors' contributions

CW contributed to the study conception and design. The manuscript was written by CW and YC. CW and XZ contributed to data retrieval and statistical analysis. Clinical data collection, genetic counseling and follow-up were performed by XK. WES analysis was performed by QL. CW performed Sanger sequencing. DNA was extracted by CC. CW, YC and CC modified and proofread the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from all participants.

Consent for publication

All patients in this study provided their consent for publication.

Competing interests

The authors declare that they have no conflict of interests.

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Tables

Table 1 Variant types of *SMARCB1*

(HGMD® professional 2021.2)

Variant Type	Total Number	Varants in Snf 5 domain	Resources
Missense/Nonsense	44	14	HGMD
Splicing	20	7	
Regulatory	3	0	
Small insertions	12	5	
Small indels	3	2	
Gross deletions	25	22	
Gross insertions	6	6	
Complex	2	2	
Repeats	0	0	
Small deletions+1	20+1	11+1	HGMD+This report
Total	135+1	69+1	

Table 2 Genotype-phenotype correlation of the variants in *SMARCB1*

(HGMD® professinal 2021.2)

Disease/phenotype	Varants in Snf 5 domain	Number of variants
Rhabdoid tumour	39	58
Rhabdoid tumour predisposition syndrome	1	1
Schwannomatosis, familial	5	22
Schwannomatosis	4	13
Rhabdoid tumour predisposition & schwannomatosis	1	1
Coffin-Siris syndrome	4	11
Atypical teratoid/rhabdoid tumours	2	6
Brain and Kidney tumours	2	3
CNS Atypical Teratoid/Rhabdoid tumours	1	3
Rhabdoid predisposition syndrome	2	2
Atrioventricular septum defects	1	1
Atypical teratoid/rhabdoid tumour	1	1
Cornelia de Lange syndrome	1	1
Developmental delay and hypotonia	0	1
Intellectual disability, severe syndromic	0	1
Kidney tumours	0	1
Kleefstra syndrome	0	1
Lung adenocarcinoma	0	1
Multiple congenital anomalies	1	1
Multiple meningiomas	0	1
Nicolaides-Baraitser syndrome	0	1
Ovarian serous cystadenocarcinoma	0	1
Posterior fossa brain tumours	1	1
Ventricular septal defect	0	1
TOTAL	66	134

Table 3 Variant types of *SMARCB1* in Rhabdoid tumour VS Schwannomatosis.

Rhabdoid tumour	Rhabdoid tumour predisposition syndrome	Schwannomatosis, familial	Schwannomatosis
1	0	5	2
0	0	1	1
12	0	3	1
4	1	8	3
0	0	1	1
6	0	2	1
0	0	1	1
21	0	0	0
3	0	0	1
1	0	0	0
0	0	0	0
10	0	1	2
58	1	22	13

Figures

Figure 1

Pedigree analysis of the familial schwannomatosis patients. A: The simplified pedigree spanning 3 generations. Squares and circles denote males and females respectively. Filled squares or circles indicate probands (marked with the arrow), and the unfilled squares or circles are unaffected family members. B: Skin symptoms of schwannomatosis (skin lumps) are noted. C: Sequencing results of SMARCB1 of III1, III2 and III1.

Figure 2

Pathological histology analysis and MRI tests of the familial schwannomatosis patients. A: Chest wall tumors of $\square 1$ were resected and stained with Haematoxylin and eosin (H&E). B-D: MRI tests demonstrating several benign intracranial, cervical intraspinal and thoracic intraspinal schwannomas (arrows). E: Tumors from the left leg of $\square 1$ were resected and stained with Haematoxylin and eosin (H&E). F: MRI tests demonstrating schwannomas within the left leg (arrows).

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