Observational Study

Pain correlates with germline mutation in schwannomatosis

Justin T. Jordan, MD, MPH[a,b,*], Miriam J. Smith, PhD[c], James A. Walker, PhD[a,d], Serkan Erdin, PhD[d],
Michael E. Talkowski, PhD[a,d], Vanessa L. Merker, BS[a], Vijaya Ramesh, PhD[a,d], Wenli Cai, PhD[e],
Gordon J. Harris, PhD[e], Miriam A. Bredella, MD[e], Marlon Seijo[b], Alessandra Suuberg, JD[d],
James F. Gusella, PhD[a,d,f], Scott R. Plotkin, MD, PhD[a,b]

Abstract

Schwannomatosis has been linked to germline mutations in the *SMARCB1* and *LZTR1* genes, and is frequently associated with pain.

In a cohort study, we assessed the mutation status of 37 patients with clinically diagnosed schwannomatosis and compared to clinical data, whole body MRI (WBMRI), visual analog pain scale, and Short Form 36 (SF-36) bodily pain subscale.

We identified a germline mutation in *LZTR1* in 5 patients (13.5%) and *SMARCB1* in 15 patients (40.5%), but found no germline mutation in 17 patients (45.9%). Peripheral schwannomas were detected in 3 *LZTR1*-mutant (60%) and 10 *SMARCB1*-mutant subjects (66.7%). Among those with peripheral tumors, the median tumor number was 4 in the *LZTR1* group (median total body tumor volume 30 cc) and 10 in the *SMARCB1* group (median volume 85cc), (*P*=.2915 for tumor number and *P* = .2289 for volume). mutation was associated with an increased prevalence of spinal schwannomas (100% vs 41%, *P* = .0197). The median pain score was 3.9/10 in the *LZTR1* group and 0.5/10 in the *SMARCB1* group (*P* = .0414), and SF-36 pain-associated quality of life was significantly worse in the *LZTR1* group (*P* = .0106). Pain scores correlated with total body tumor volume (rho = 0.32471, *P* = .0499), but not with number of tumors (rho = 0.23065, *P* = .1696).

We found no significant difference in quantitative tumor burden between mutational groups, but spinal schwannomas were more common in *LZTR1*-mutant patients. Pain was significantly higher in *LZTR1*-mutant than in *SMARCB1*-mutant patients, though spinal tumor location did not significantly correlate with pain. This suggests a possible genetic association with schwannomatosis-associated pain.

Abbreviations: 3′UTR = 3′ untranslated region, cRNA = coding ribonucleic acid, gDNA = genomic deoxyribonucleic acid, NF2 = neurofibromatosis 2, NGS = next generation sequencing, QOL = quality of life, SF-36 = Short Form 36, VAS = visual analog scale, WBMRI = whole-body magnetic resonance imaging.

Keywords: LZTR1, pain, schwannomatosis, SMARCB1, whole-body MRI

1. Introduction

Schwannomatosis is a hereditary syndrome that predisposes affected individuals to develop nonintradermal schwannomas and, less commonly, meningiomas.[1] While there is phenotypic overlap with neurofibromatosis 2 (NF2), early genetic studies of schwannomatosis ruled out mutations in the *NF2* locus as a cause.[2] To date, 2 genes have been associated with schwannomatosis, *SMARCB1* and *LZTR1*, making schwannomatosis the only form of neurofibromatosis with locus heterogeneity.[3,4] *SMARCB1* mutations are found more frequently in familial (45%) than sporadic cases (7%) of schwannomatosis,[5] while *LZTR1* mutations are found at similar frequency between the 2 (21% vs 20.5%).[6]

Schwannomatosis is notable for several unique characteristics when compared to NF2, including a much lower prevalence of vestibular schwannomas and meningiomas, a relatively higher prevalence of peripheral schwannomas, and frequent chronic, severe pain. The pain of schwannomatosis has been associated with tumor burden,[7] but not location, in previous studies, raising the question of a genetic contribution to an underlying pain syndrome.

The current theory of tumor development among individuals with either *SMARCB1*- or *LZTR1*-associated schwannomatosis involves a 3-step, 4-hit mechanism. A germline mutation in either *SMARCB1* or *LZTR1* is present on 1 allele, followed by a somatic

loss of heterozygosity event on chromosome 22q involving both the *NF2* gene and either the *SMARCB1* or *LZTR1* gene, and then a mutation in the *NF2* gene on the retained allele.[4,8] This series of mutational events demonstrates not only the importance of *NF2* loss in schwannoma development, but also the genetic heterogeneity among tumors even in the same individual. In addition, this model raises the possibility that the mechanism of pain generation may be distinct from that of tumorigenesis, with pain related to the germline *SMARCB1* or *LZTR1* mutation, while tumor formation is caused by biallelic loss of *NF2*.

The paucity of genotype–phenotype correlations identified to date for schwannomatosis patients prompted us to investigate with a novel combination of tools. We used a custom, high-efficiency targeted gene capture system for germline sequencing of relevant genes. To that data, we compared key features of schwannomatosis phenotype, including internal tumor number and size using whole body MRI (WBMRI) and regional MRI, as well as validated measures of patient-reported pain and pain-related quality of life (QOL).

2. Materials and methods

2.1. Cohort determination

The patient population for this study was derived from a pool of 282 individuals who participated in an IRB-approved study of WBMRI.[9] Analyzed patients had a clinical diagnosis of schwannomatosis as defined by prior publication, with 2 or more nonintradermal schwannomas, at least 1 with histological confirmation, and no evidence of vestibular tumor on high-quality MRI scan, no known constitutional *NF2* mutation, and no first-degree relative with NF2; alternatively, patients could meet clinical diagnostic criteria by having 1 pathologically confirmed nonvestibular schwannoma or intracranial meningioma plus a first-degree relative with schwannomatosis.[10] Participants with WBMRI, seen at Massachusetts General Hospital with clinically diagnosed schwannomatosis, with germline DNA available, and who had previously consented to germline genetic testing, were included. Among the 37 meeting these criteria, 7 individuals had known germline mutations determined previously by Sanger sequencing. The 30 remaining individuals underwent capture (described below), followed by targeted next generation sequencing (NGS) of the entire gene regions for *NF2*, *SMARCB1*, and *LZTR1*.

2.2. Whole-body MRI and clinical characterization

As a component of our previously published study, patients over the age of 18 years with clinically diagnosed schwannomatosis underwent WBMRI.[9] Phenotypic data collected at enrollment included neurologic symptoms and evidence of neuraxis tumors identified on previous regional imaging. All included patients consented to blood and tumor genetic testing, and all such data were linked to WBMRI and clinical data but otherwise rendered de-identified.

2.3. Tumor volumetrics

Tumor burden was determined as previously described.[9] A board-certified radiologist without knowledge of genotype reviewed each scan to identify tumors. Tumors were segmented using computerized volumetry, and whole-body tumor volume was assessed.[11]

2.4. Pain measurement

Pain phenotype was established using self-reported measures of pain intensity and pain-related QOL. Pain intensity was assessed using the visual analog scale (VAS), in which the patient places a vertical mark on a 10 cm horizontal line drawn between "no pain" on the left and "worst pain ever" on the right, to indicate their current level of pain. The distance of the marks from the left side of the scale were measured to the nearest tenth of a centimeter to obtain the final numerical pain score. Quality of life implications of pain were measured with the Short Form 36 (SF-36), version 1, bodily pain subscale. The SF-36 is a short, patient-completed questionnaire that explores various aspects of QOL over the past month, with higher scores reflecting better QOL and lower scores reflecting worse QOL. Norm-based scores were calculated for each patient using the QualityMetric Health Outcomes Scoring Software 2.0,[12] which transforms raw scores into a norm-based score with expected mean of 50 and SD of 10 using data from the general US population.

2.5. DNA extraction

As a component of our previously published work,[9] individuals consented to germline genetic analysis and voluntarily gave blood samples. Immortalized lymphoblast lines were established from peripheral blood samples as described previously.[13] Genomic DNA was extracted from cultured lymphoblast cells using a PureGene DNA isolation kit (Gentra Systems, Minneapolis, MN).

2.6. cRNA capture/NGS

Custom biotinylated 120-mer coding ribonucleic acid (cRNA) baits were created to capture the entire gene sequences of *SMARCB1*, *LZTR1*, and *NF2*. Using lymphoblast DNA from each patient, sequencing libraries were prepared using the SureSelect XT Target Enrichment System for Illumina Paired-End Multiplexed Sequencing (3 μg), by Agilent Technologies (G7530-90000). Namely, 3 μg of genomic DNA was sheared to approximately 175 bp fragments using the Covaris Focused-ultrasonicator. DNA fragments were end-repaired, adenylated, ligated to adapter oligos, and then amplified with 5 cycles of polymerase chain reaction (PCR) according to the protocol provided by the manufacturer. After quantification, 750 ng of each amplified DNA sample was hybridized overnight with the Capture Library. Following capture cleanup, each genomic deoxyribonucleic acid (gDNA) library was amplified with an additional 16 cycles of PCR, which also tagged each sample with an index-specific barcode. Final products were quantified using the TapeStation 2200 and pooled for rapid mode sequencing on the Illumina HiSeq 2500, which generated a median 4.09 million paired-end reads of 100 bp. Quality checking of raw sequence reads was assessed by FASTQC (version 0.10.1) (Andrew, S 2010, FASTQC, a quality control tool for high-throughput sequence data http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Further, sequence reads were aligned to human reference genome Ensembl GRCh37 (version 71) using bwa-mem (version 0.7.5a-r418) at its default parameter setting[14] (arXiv:1303.3997v2 [q-bio.GN]). Next, alignments were coordinate sorted duplicated reads filtered using Picard Tools's SortSam and MarkDuplicates algorithms, respectively (version 1.95; http://broadinstitute.github.io/picard/). Filtered alignments were further insertion–deletion (indel)-realigned and

Table 1

Mutational analysis of schwannomatosis patients.

Gene	Location	DNA mutation	Predicted protein change	Functional change
LZTR1	Exon 1	c.73delG	p.(Ala25Profs*17)	Frameshift deletion
LZTR1	Exon 10	c. 1084C>T	p.(Arg352*)	Stopgain
LZTR1	Exon 13	c. 1396C>T	p.(Arg466Trp)	Nonsynonymous SNV
LZTR1	Exon 20	c.2350_2360del	p.(Gln784Hisfs*63)	frameshift deletion
LZTR1	Exon 16	c.1786–1G>A	p.(?)	Splice-site
SMARCB1	Exon 4	c. 472C>T	p.(Arg158*), p.Arg121_Cys167delinsSer, p.Gln130_Cys167delinsPro, p.Leu266_Ser329delfs*31	Stopgain/Splice-site (Smith[19])
SMARCB1[B]	Exon 6	c.795+1G>T	p.Lys265_Leu266ins15, p.Glu210_Lys265delinsAlafs*14	Splice site (Smith[19])
SMARCB1[B]	Exon 6	c.795+1G>T	p.Lys265_Leu266ins15, p.Glu210_Lys265delinsAlafs*14	Splice site (Smith[19])
SMARCB1[A]	3′UTR	c.*82C>T	NA	Reduced expression (Smith[19])
SMARCB1[A]	3′UTR	c.*82C>T	NA	Reduced expression (Smith[19])
SMARCB1[A]	3′UTR	c.*82C>T	NA	Reduced expression (Smith[19])
SMARCB1[A]	3′UTR	c.*82C>T	NA	Reduced expression (Smith[19])
SMARCB1	3′UTR	c.*82C>T	NA	Reduced expression (Smith[19])

Superscript letters = members of the same family.

base-recalibrated using the Genome Analysis Toolkit (GATK, version 3.1.1).[15] GATK's HaplotypeCaller was used to call raw variants in each sample with the following argument set; –stand_call_conf 30 –stand_emit_conf 10 –minPruning 3. A high-confidence set of variants was selected from intragenic regions along with 30 kb upstream and downstream regions of captured genes with DP > 20 and GQ > 30 filters and they were further annotated by Annovar (version. 02-01-2016).[16] Candidate mutations were defined as exonic or splice site calls that were classified as either nonsense or deleterious missense (as predicted by *in silico* analysis or previous RNA studies), and were confirmed by Sanger sequencing analysis using an ABI Prism 3730 DNA analyzer. We used CNV-seq to call copy-number variations on intragenic regions of 5 target genes from capture-sequencing data [17]. We compared 30 subjects with schwannomatosis to an equal number of subjects with NF1 used as reference, with options—log2-threshold 0.2—*P*-value .05—genome human—global-normalization. Duplicated reads were marked by Picard Tool's MarkDuplicates algorithm (version 1.95) (https://broadinstitute.github.io/picard/) and further removed by SAMtools (version 0 1.18).[18]

2.7. Statistics

Summary statistics were compared between groups using a Wilcoxon test for 2-group comparisons and a Kruskal–Wallis test for 3-group comparisons of tumor number, total body tumor volume, pain scores, SF-36 bodily pain subscores, and other continuous variables. Chi-square statistics were used to compare binomial variables between groups, including presence of intracranial schwannomas, café-au-lait macules, and other clinical features. Spearman coefficients were used for comparisons of pain with either tumor number or total body tumor volume. Missing data were omitted from analyses due to a sample number too small for imputation. SAS version 9.4 was used for all statistics.

2.8. Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The studies were reviewed and approved by the Partners Healthcare Institutional Review Board.

3. Results

From our original 51 patient cohort of schwannomatosis patients who underwent WBMRI, 14 were excluded from this study due to having no lymphoblast DNA available for sequencing. Another 7 patients previously underwent Sanger sequencing analysis and exhibited a germline *SMARCB1* mutation; these were included here in the correlation analysis but not the targeted sequencing.[19,20] For the remaining 30 subjects, we used a custom biotinylated cRNA library to capture the entire gene regions of *SMARCB1*, *LZTR1*, and *NF2*, which were then analyzed by targeted NGS. The median library size was 4 million paired-end reads with 7.7 million uniquely mapping individual reads. Median coverage was 1060× with 72% efficiency of capture within the target regions. The results of genetic analysis are shown in Table 1.

No patients had deleterious *NF2* mutations or copy number variants on chromosome 22q in the germline. Of those sequenced by capture, 5/30 (16.7%) had a germline *LZTR1* variant, 8/30 (26.7%) had a germline *SMARCB1* variant, and 17/30 (56.6%) had no identified mutation. Mutations called by capture sequencing were verified by Sanger sequencing. Sanger confirmations of novel mutations are shown in Figure 1.

To the 30 capture-sequenced samples, we added the 7 samples for which mutations in SMARCB1 were already known by prior Sanger sequencing. Then, the 37 patients were divided into 3 groups based on their germline mutation: *LZTR1* (n=5), *SMARCB1* (n=15), or no identified alteration (n=17). Patient characteristics for each group are shown in Table 2. There was no significant difference between age at diagnosis, median tumor number, or median total body tumor volume between the 3 groups. No intracranial meningiomas were identified in any patient in this cohort. Patients with *LZTR1* mutations had a significantly higher
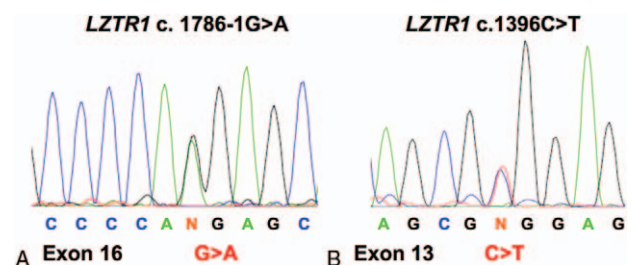

Figure 1. Sanger sequencing confirming novel mutational calls in *LZTR1*.

Table 2

Phenotype correlations by germline mutation.

	LZTR1 (n=5)	SMARCB1 (n=15)	Neither (n=17)	P values 3 groups	P value mutation groups only
Median age at diagnosis	46	48	39	.0944	.6056
Median pain score	3.9	0.5	3	.0992	.0414
Median bodily pain SF-36 subscore	41.8	53.8	41.8	.0115	.0106
Median number of tumors	1	3	2	.4777	.4316
Median total body tumor volume	6.96 cc	31.96 cc	11.49 cc	.5074	.3836
Meningiomas	0%	0%	0%	N/A	N/A
Spinal schwannomas	100%	40%	41.2%	.0472	.0195
Intracranial schwannoma	20%	0%	17.7%	.2144	.0756
Cutaneous schwannoma	40%	6.7%	11.8%	.1614	.0706
Subcutaneous schwannoma	60%	33.3%	23.5%	.3081	.2918
Female gender	40%	66.7%	41.2%	.3056	.2918
Café au lait macules	0%	6.7%	23.5%	.2415	.5536
Hearing loss	20%	26.7%	11.8%	.5605	.7656
Tinnitus	20%	0%	5.9%	.2291	.0756
Headaches	0%	20%	11.8%	.5052	.2781

prevalence of spinal schwannomas than patients with *SMARCB1* mutations (100% vs 40%, *P*=.0195). Further, patients with *LZTR1* germline mutations reported significantly higher pain intensity than *SMARCB1* patients (3.9 vs 0.5, respectively; *P*=.0414), and they reported significantly worse QOL, as assessed by SF-36 pain burden scores, than the *SMARCB1* group (*P*=.0106). Notably, inclusive of all mutation groups, neither pain intensity nor pain-related QOL was significantly different among patients with spinal schwannomas compared to patients without spinal schwannomas (*P*=.5071 and *P*=.3393, respectively). Additional phenotypic characteristics were compared between groups and were not significant (Table 2).

Pain scores were correlated with tumor number and total body tumor volume within groups. For all patients, VAS pain intensity scores correlated with total body tumor volume (rho=0.32471, *P*=.0499), though not with number of tumors (rho=0.23065, *P*=.1696) (Fig. 2). Within each gene grouping, no such correlations were found (data not shown).

4. Discussion and conclusions

Whereas 83% of surveyed schwannomatosis patients report chronic pain (≥3 months duration), and 75% take chronic pain medications (Plotkin, unpublished results), understanding the determinants of schwannomatosis-related pain is critical in order

to create accurate laboratory models that can be generated and studied. To that end, we used a combination of WBMRI data, a novel high-throughput method for genotyping schwannomatosis germline samples, and patient reported pain levels and pain-related QOL to investigate genotype–phenotype correlations in 37 individuals with schwannomatosis. While mutation groups had no significant difference in tumor burden, there was nonetheless a significant difference in pain intensity between groups, with self-reported pain being significantly greater in patients with a germline *LZTR1* mutation than those with a germline *SMARCB1* mutation. Similarly, pain-related QOL was significantly worse (nearly one standard deviation) among the former group. Although the incidence of spinal schwannomas was much greater in patients with *LZTR1* mutation than *SMARCB1* mutation, pain and pain-related QOL were not significantly different between patients with and without spinal schwannomas without regard to mutational status, indicating that tumor location is not the primary driver of pain. We also showed here that patient-reported pain scores correlate with total body tumor volume among patients with schwannomatosis, but not with number of tumors. This stands in agreement with our previous work.[7] Overall, our data indicate that schwannomatosis-associated pain may not only stem from total body tumor volume, but may also be related to the germline mutation that predisposes to schwannomatosis.
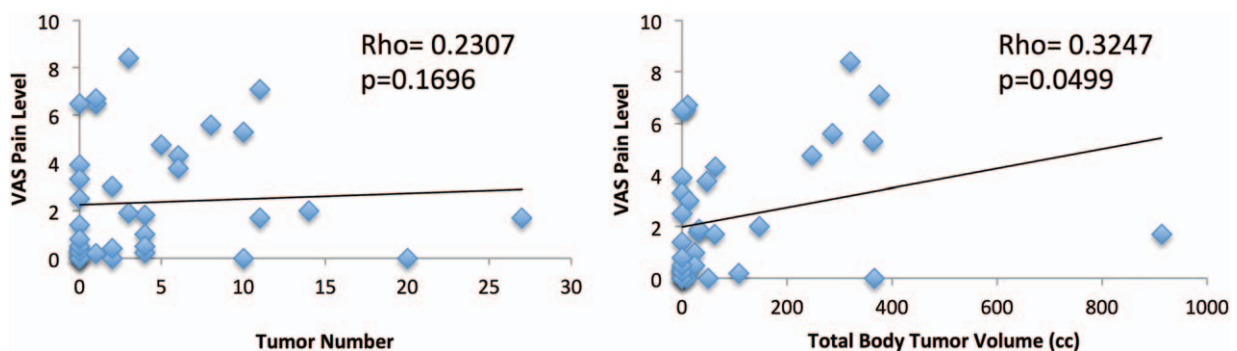

Figure 2. Correlations between pain and tumor burden. A significant correlation was found between patient-reported pain score and total body tumor volume, but not with tumor number.

The reason for differences in pain between mutational groups is unknown, but may be due to function of *SMARCB1* and *LZTR1* genes. *SMARCB1* is known to be involved in regulating expression of genes throughout the genome, due to its association with the SWI/SNF human chromatin remodeling complex. The function of *LZTR1* is currently less well established, but has been identified as a likely tumor suppressor gene and may have a functional link with *SMARCB1* through nuclear receptor corepressor (N-CoR) interactions.[4,21,22] Individuals with no identified mutation were not included in the primary analysis due to the presumed genetic heterogeneity of this population, a limitation of any genotype-phenotype study in this disease.

Patients with an *LZTR1* mutation were found to have spinal schwannomas significantly more frequently than the other schwannomatosis patients in this study. This may represent a mutation-specific tumor location predisposition, similar to sporadic olfactory groove meningiomas with *SMO* mutations.[23] Our understanding of this genotype–phenotype relationship will improve as more patients with schwannomatosis are characterized.

Our custom cRNA capture library is an efficient sequencing method for high-throughput genotyping, and provides high-quality genetic information for identification of single nucleotide variants or indels. Additionally, when pooled with individuals with non-22q disease, this method provides sufficiently high quality data for copy number variant analysis. While the present study only included analysis of exons, splice sites, and 3′ untranslated regions (3′ UTR), introns were also captured and sequenced and will be analyzed in future work for deleterious variants within known effector sequences.

Whole body MRI provides high-quality phenotypic information regarding tumor burden in schwannomatosis, and has revealed large differences in overall tumor burden between individuals within the same mutation groups. The WBMRI in our study included only large field-of-view coronal STIR images, which are not reliably able to identify intracranial or intraspinal tumors. Regional MRI data were only available where it had been clinically indicated. Also of note, WBMRI slice thickness was 5 mm in this study, thus small (yet potentially painful) tumors may have been present but beyond the resolution of WBMRI. These limitations will be addressed in future studies by the addition of dedicated brain and spine imaging and using smaller slice thickness.

Together, capture sequencing and WBMRI provide high quality data for identifying genotype–phenotype correlations in schwannomatosis. Our finding of increased pain among patients with *LZTR1* mutations is both important and timely, given that severe, chronic pain is common among patients with schwannomatosis, and that there are large-scale international efforts to create schwannomatosis-associated pain models and to find therapies for this unique population. While the small sample in our analysis is reflective of the rarity of this disease, further study of the identified genotype-phenotype correlation is warranted.

5. Author contributions

JTT: Study concept and design, acquisition of data, analysis and interpretation, critical revision of manuscript.

MJS: Analysis and interpretation, critical revision of manuscript

JAW: Acquisition of data, analysis and interpretation, critical revision of manuscript

SE: Acquisition of data, analysis and interpretation, critical revision of manuscript

MET: Study concept and design, acquisition of data

VM: Study concept and design, acquisition of data, analysis and interpretation, critical revision of manuscript

VR: Study concept and design, acquisition of data

WC: Study concept and design, acquisition of data, analysis and interpretation

GJH: Study concept and design, acquisition of data, analysis and interpretation

MAB: Study concept and design, acquisition of data, analysis and interpretation, critical revision of manuscript

MS: Analysis and interpretation, critical revision of manuscript

AS: Acquisition of data, analysis and interpretation

JFG: Study concept and design, analysis and interpretation, critical revision of manuscript, study supervision

SRP: Study concept and design, acquisition of data, analysis and interpretation, critical revision of manuscript, study supervision

References

[1] MacCollin M, Chiocca EA, Evans DG, et al. Diagnostic criteria for schwannomatosis. Neurology 2005;64:1838–45.

[2] MacCollin M, Willett C, Heinrich B, et al. Familial schwannomatosis: exclusion of the NF2 locus as the germline event. Neurology 2003;60:1968–74.

[3] Hulsebos TJ, Plomp AS, Wolterman RA, et al. Germline mutation of INI1/SMARCB1 in familial schwannomatosis. Am J Hum Genet 2007; 80:805–10.

[4] Piotrowski A, Xie J, Liu YF, et al. Germline loss-of-function mutations in LZTR1 predispose to an inherited disorder of multiple schwannomas. Nat Genet 2014;46:182–7.

[5] Smith MJ, Wallace AJ, Bowers NL, et al. Frequency of SMARCB1 mutations in familial and sporadic schwannomatosis. Neurogenetics 2012;13:141–5.

[6] Smith MJ, Isidor B, Beetz C, et al. Mutations in LZTR1 add to the complex heterogeneity of schwannomatosis. Neurology 2015;84:141–7.

[7] Merker VL, Bredella MA, Cai W, et al. Relationship between whole-body tumor burden, clinical phenotype, and quality of life in patients with neurofibromatosis. Am J Med Genet A 2014;164A:1431–7.

[8] Sestini R, Bacci C, Provenzano A, et al. Evidence of a four-hit mechanism involving SMARCB1 and NF2 in schwannomatosis-associated schwannomas. Hum Mut 2008;29:227–31.

[9] Plotkin SR, Bredella MA, Cai W, et al. Quantitative assessment of whole-body tumor burden in adult patients with neurofibromatosis. PLoS One 2012;7:e35711.

[10] Plotkin SR, Blakeley JO, Evans DG, et al. Update from the 2011 International Schwannomatosis Workshop: from genetics to diagnostic criteria. Am J Med Genet A 2013;161A:405–16.

[11] Cai W, Kassarjian A, Bredella MA, et al. Tumor burden in patients with neurofibromatosis types 1 and 2 and schwannomatosis: determination on whole-body MR images. Radiology 2009;250:665–73.

[12] Saris-Baglama RNDC, Chisholm GB, Plumb E, et al. QualityMetric Health Outcomes Scoring Software 2. 0: User's Guide, 2007.

[13] Anderson MA, Gusella JF. Use of cyclosporin A in establishing Epstein–Barr virus-transformed human lymphoblastoid cell lines. In Vitro 1984;20:856–8.

[14] Li H. Toward better understanding of artifacts in variant calling from high-coverage samples. Bioinformatics 2014;30:2843–51.

[15] McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010;20:1297–303.

[16] Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010;38:e164.

[17] Xie C, Tammi MT. CNV-seq, a new method to detect copy number variation using high-throughput sequencing. BMC Bioinformatics 2009;10:80.

[18] Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–9.

Medicine

[19] Boyd C, Smith MJ, Kluwe L, et al. Alterations in the SMARCB1 (INI1) tumor suppressor gene in familial schwannomatosis. Clin Genet 2008; 74:358–66.

[20] Smith MJ, Walker JA, Shen Y, et al. Expression of SMARCB1 (INI1) mutations in familial schwannomatosis. Hum Mol Genet 2012;21:5239–45.

[21] Underhill C, Qutob MS, Yee SP, et al. A novel nuclear receptor corepressor complex, N-CoR, contains components of the mammalian SWI/SNF complex and corepressor KAP-1 2000. J Biol Chem 2000; 275:40463–70.

[22] Frattini V, Trifonov V, Chan JM, et al. The integrated landscape of driver genomic alterations in glioblastoma. Nat Genet 2013;45:1141–9.

[23] Clark VE, Erson-Omay EZ, Serin A, et al. Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. Science 2013;339:1077–80.