IN VIVO *39*: 1251-1261 (2025)
doi: 10.21873/invivo.13929

Pathogenic Variants and Allele Loss of the NF2 and LZTR1 Gene in Sporadic Vestibular Schwannoma

MARIA BREUN[1], TIM SCHULZ[1], CAMELIA M. MONORANU[2], RALF-INGO ERNESTUS[1], CORDULA MATTHIES[1], MARIO LÖHR[1] and LAN KLUWE[3]

[1]Department of Neurosurgery, University Hospital of Würzburg, Würzburg, Germany;
[2]Department of Neuropathology, Institute of Pathology, University Hospital of Würzburg, Würzburg, Germany;
[3]Department of Neurology, University Hospital Hamburg-Eppendorf, Hamburg-Eppendorf, Germany

Abstract

Background/Aim: Pathogenic variants and allele-loss of the *NF2* gene with Merlin loss as consequence is the driving genetic event for vestibular schwannoma development. Our knowledge about the pathogenic *NF2* variants in sporadic vestibular schwannoma is insufficient. Therefore, we analyzed a cohort of sporadic vestibular schwannomas by panel-sequencing.

Patients and Methods: Forty-one sporadic vestibular schwannomas from 26 male and 15 female patients were included. DNA from tumor tissues was sequenced with a custom panel for the *NF2* and *LZTR1* genes. Allele-loss of the *NF2* locus was also examined using multiplex-ligation-dependent probe-amplification. These genetic data were correlated with clinical parameters including hearing, tumor extension and growth.

Results: Among the 41 tumor samples, 34 had one pathogenic variant or an allele-loss of *NF2* gene and one tumor showed a pathogenic variant in the *LZTR1* gene. Allele frequencies of the total of 46 pathogenic variants varied from 0.05 to 0.82, and none of these variants was found in blood. For 6 tumors, no pathogenic variants were found while 4 of them had allele-loss of the *NF2* gene. When the tumors were divided into 3 groups according to the counts of inactivating events (pathogenic variants and allele loss), the clinical parameters including hearing, tumor structure in MRI, tumor growth, tumor size and postoperative facial function did not differ significantly.

Conclusion: There was no correlation between phenotype and genetic alterations of the *NF2 or LZTR1* gene in sporadic schwannomas. Genetic inactivating events are the precondition for the development of vestibular schwannomas but do not influence their growth and other features.

Keywords: Sporadic vestibular schwannoma, next generation sequencing (NGS), pathogenic variants, genotype, phenotype.

Maria Breun, Department of Neurosurgery, Section Experimental Neurosurgery, University of Würzburg, Josef-Schneider-Str. 11, D-97080 Würzburg, Germany. Tel: +49 93120124884, e-mail: breun_m@ukw.de

Received October 25, 2024 | Revised January 24, 2025 | Accepted January 31, 2025

Introduction

Vestibular schwannoma are benign tumors of the vestibular nerve in the cerebellopontine angle. Most of them arise sporadically and only about 5% grow in patients with neurofibromatosis type 2 (NF2), a genetic disorder with an inherited inactivating variant of the *NF2* gene, which codes for the tumor suppressor Merlin. Merlin's loss of function is the main known pathogenic factor in vestibular schwannoma pathogenesis. Our knowledge regarding genetic alterations in sporadic vestibular schwannoma is still insufficient, though Merlin inactivation is known as the main pathogenic factor in these tumors (1).

In patients of neurofibromatosis type 2 (NF2), mosaicism is frequent which means that the *de novo* pathogenic *NF2* variants are only in subgroups of the cells, depending on the time point in embryonic stage when the variant occurred. The hypothesis is, that sporadic vestibular schwannoma represents an extreme form of mosaic NF2 where the *de novo* pathogenic variants occur extremely late and, therefore, are only carried in an extremely small number of cells.

Target sequencing using custom panels enables effective detection of variants for hundreds of samples. Furthermore, it is far more sensitive than the conventional Sanger sequencing and can detect variants in low allele-frequencies. This is important for tumor tissues which may contain a large amount of non-tumor cells. In this study, we applied a custom panel covering the entire coding region of the *NF2* and leucine zipper-like transcriptional regulator 1 (*LZTR1)* genes for 41 sporadic vestibular schwannomas. In addition, we applied the multiplex ligation dependent probe amplification (MLPA) to assess copy number variations of all exons of *NF2* and two exons of the *LZTR1* gene.

Patients and Methods

Tissue samples and clinical data. The study was conducted according to the guidelines of the Declaration of Helsinki and approved on 17/03/2021 by the Institutional Review Board of the University Hospital Wuerzburg (#241/20). Written informed consent was obtained from all patients for the use of their tissue in this study. All patients were treated in the Neurosurgery Department of the University Hospital Wuerzburg between 2021 and 2022. Directly after surgical excision, the tissue was processed for DNA extraction. All samples were neuropathologically assessed according to EANO guidelines and WHO criteria (1, 2). Forty-one tumors were diagnosed as sporadic vestibular schwannoma and among them 6 were recurrences. For 32 out of the 41 patients, blood DNA was available and also analyzed by panel-sequencing as described below.

Patient clinical data was collected retrospectively (Table I, Table II). Hearing function and tumor extensions were categorized using the Hannover classification (3, 4) and tumor growth dynamics were classified by magnetic

Table I. *Summary of patients' clinical parameters.*

Patient characteristics	Sporadic vestibular schwannoma
Sex	Female: 26; Male: 15
Age (median, quartiles)	50.7±13 years
Tumor localization	Left nerve: 24 Right nerve: 17
Tumor extension	Purely intrameatal (T1): 0 Intra- and extrameatal (T2): 0 Filling the cerebellopontine cistern (T3): 7 T3A, 11 T3B Brainstem compression±dislocation of the fourth ventricle (T4): 8T4A, 15 T4B
Tumor progress per year	≤2 mm: 22; ≥2 mm: 13 Unknown: 6
Antoni classification	Antoni A: 14 Antoni B: 1 Antoni A/B: 23 Unknown: 3
Hannover classification for hearing	H1: 7 H2: 10 H3: 11 H4: 6 H5: 0 H6: 7
Recurrence	6
Previous surgery	5
Previous chemotherapy	1

resonance imaging during a "watch and wait" period before surgery if available (3, 5). Tumor growth of more than 2 mm in a year was categorized as rapid growing and less as slow growing. Vestibular schwannomas with a homogenous contrast enhancement were classified as homogenous, tumors with cystic components were categorized as cystic and with irregular contrast enhancement as inhomogeneous. Radiosurgery or bevacizumab treatment before surgery was defined as pretreatment.

DNA extraction and panel-sequencing. Total DNA was extracted from native tissue and from ethylendiamintetraacetat (EDTA)-blood from the patients utilizing the Gene Matrix Universal DNA Purification Kit (Roboklon, Berlin, Germany). Purified DNA samples were stored at –80˚C and subjected to targeted sequencing for the *NF2* and *LZTR1* genes using a custom panel of amplicons covering the entire coding and splicing sequences of the two genes. These amplicons were prepared into a library using an Illumina Ampliseq Plus kit (Illumina, Berlin, Germany). The libraries were sequenced on an Illumina iSeq100. The resulting reads were evaluated by an integrated "amplicon analysis module" (Illumina iSeq 100, v2.1.0) and the variants that deviated from the reference sequence were specified and further evaluated manually.

Multiplex ligation-dependent probe amplification (MLPA). In order to examine the copy number variations of exons and the entire gene an MLPA (multiplex ligation-dependent probe amplification) analysis was carried out for the *NF2* genes according Kluwe *et al*. (12). The data were evaluated using the analysis software Coffalyser (MRC-Holland, Version 240129.1959).

Statistical analysis. All statistical computations were performed with Graph Pad Version 9 (GraphPad Software, San Diego, CA, USA). Normality was tested by the Shapiro-Wilk test. Statistical significance was determined using the Mann-Whitney-*U*-test and the Kruskal Wallis test. $p<0.05$ was considered as statistically significant. Correlation was evaluated using the Pearson correlation coefficient.

Results

Basic clinical and genetic features of the tumors. A total of 41 vestibular schwannomas from 26 female and 15 male patients (mean age 50.5±13 years) were assessed for pathogenic variants of the *NF2* and *LZTR1* genes. All tumors were sporadic ones, meaning that none of the patients met the diagnostic criteria for *NF2*-related schwannomatosis. Among the 41 tumors, 35 were primary and 6 were recurrences. The clinical parameters of the patients are summarized in Table I. A total of 46 variants were found and none of them was detected in the available blood samples of 32 of the patients, ensuring that all these pathogenic variants are somatic. The allele frequencies of the somatic variants varied from 0.05 to 0.82 while 35 variants had allele frequencies below 50%. Especially, 12 (26%) variants had allele frequencies below 20%.-All pathogenic variants and allele loss of the *NF2* and *LZTR1* genes are summarized in Table III.

Clinical features versus counts of inactivating events in each tumor. Among the 41 tumors, 12 grew fast, 18 had a cystic or inhomogeneous appearance in magnet resonance imaging (MRI), 15 showed worse hearing or functional deafness and five patients had a pretreatment with a surgery before (Table II). One of these patients had, years before surgery, a chemotherapy and stem cell transplantation because of blood cancer as a child. In further analysis, the 41 tumors were divided into three groups, according to the counts of inactivating events in each of them (see Table IV): Group 0=tumors with no inactivating event (n=4); Group 1=tumors, each with one inactivating event (pathogenic variants or allele loss), including the tumor with unknown status of allele loss (n=8); Group 2=tumors with two or more inactivating events each (n=29). Regarding hearing (Figure 1B), a significant difference was found between Group 0, Group 1 and Group 2 with score 1.5 *versus* 2.25 and 3.5, respectively, and p=0.015.

Tumor size (Figure 1D) did not correlate with the counts of inactivating events. The mean size score was 4.2 (T3B-T4A) in Group 0, 4.2 (T3B-T4A) in Group 1 and 4.96

Table II. *Phenotype of the patients including symptoms, function, tumor development, tumor extension and pre-treatment.*

Sample No	Age	Sex	Side	Tumor extension.	KM enhancement	Tumor growth	Rapid *vs.* slow	Antoni type	Phenotype	Other tumors	Pre-treat.	Hearing	Fac. pre	Fac. post
1	56	1	1	6	One cyst	2 mm in 5 mo	2	1	Sudden hearing loss (1995/2000), hypoacusis	Non	2	4	I	V
2	35	1	1	6	Homogeneous	Upfront surgery	1	3	Sudden hearing loss 2020, Tinnitus	Non	2	4	I	I
3	59	2	1	5	Homogeneous	4 mm in 4 mo	1	3	Tinnitus, hypoacusis, headache	Non	2	3	I	I
4	54	1	2	4	Homogeneous	Upfront surgery	2	3	Hörsturz 2019, Tinnitus, Schwindel	Non	2	3	I	II
5	58	1	1	6	Homogeneous	2 mm in 2 mo	1	3	Hearing loss on the right, tinnitus	Neuroma N. abducens, Abdominal cyst	2	2	I	IV
6	23	1	1	5	Inhomogeneous, small cysts	15 mm in 3 y	1	1	Headache, tinnitus	Non	2	1	I	I
7	50	1	1	6	Homogeneous	Upfront surgery	3	3	Sudden hearing loss, hypoacusis	Non	2	2	I	I
8	49	1	2	4	Cystic	1.5 mm in 6 mo	1	2	Hypoacusis	Basal cell carcinoma	2	1	I	I
9	74	1	2	5	Cystic	Stable	2	3	Hypoacusis, tinnitus	Non	2	3	I	I
10	71	2	2	4	Homogeneous	Stable	2		Hypoacusis, balance disturbance	Prostate carcinoma with metastasis	2	1	I	I
11	51	2	2	4	Homogeneous	Stable	2	3	Hypoacusis, tinnitus	Non	2	1	I	I
12	44	1	2	3	Homogeneous	Stable	2	1	Dizziness	Pineal cyst	2	1	I	I
13	44	1	2	4	Homogeneous	Stable	2	1	Dizziness, hypoacusis	Non	2	3	I	I
14	63	1	2	4	Homogeneous	Stable	2	1	Hypoacusis, tinnitus	Non	2	2	I	I
15	41	1	1	5	Homogeneous	10 mm in 2 y	1	3	Sudden hearing loss, hypoacusis, headache	Non	2	2	I	I
16	59	1	2	4	Homogeneous small cysts	5 mm in 1.5 y	1	3	Dizziness, hypoacusis	Non	2	2	I	I
17	45	1	2	4	Homogeneous	Upfront surgery	3	3	Surditas	Non	2	6	I	I
18	58	1	2	4	Homogeneous	Upfront surgery	2	Nn	Hypoacusis	Non	2	3	I	III
19	61	2	2	4	Homogeneous	Stable	2	3	Hypoacusis	Non	2	3	I	I
20	61	1	2	3	Homogeneous	Stable	2	1	Hypoacusis	Non	2	1	I	I
21	41	2	2	3	Homogeneous	Stable	2	1	Hypoacusis, tinnitus	Non	2	3	I	II
22	55	1	2	3	Homogeneous	Stable	2	1	Hypoacusis, tinnitus, dizziness	Non	1	2	I	I
23	34	2	2	3	Homogeneous	Stable	2	1	Hypoacusis, tinnitus	Non	2	2	I	II
24	55	2	1	4	Homogeneous	Upfront surgery	3	3	Hypoacusis, tinnitus	Non	2	3	I	I

Table II. *Continued*

Table II. *Continued*

Sample No	Age	Sex	Side	Tumor extension	KM enhancement	Tumor growth	Rapid *vs.* slow	Antoni type	Phenotype	Other tumors	Pre-treat.	Hearing	Fac. pre	Fac. post
25	51	2	1	3	Homogeneous	Stable	2	3	Hypoacusis, tinnitus, sudden hearing loss	Non	2	3	I	I
26	49	1	2	5	Homogeneous	Stable	2	3	Surditas, headache	Non	1	6	II	II-III
27	68	1	1	6	Inhomogeneous, cystic	Stable	2	1	Hypoacusis, tinnitus, sudden hearing loss, dizziness	Non	2	3	I	I
28	56	1	1	6	Inhomogeneous, necrotic	2 mm in 3 mo	1	3	Dizziness, facial hypoesthesia, hypoacusis, sudden hearing loss	Non	2	4	I	I
29				6	Homogeneous	Rapid	1		Headache, vertigo, hypoacusis, facial hypoesthesia	Benign mammal tumor, abdominal cyst		1	I	I
30	63	1	1	6	Cystic	Upfront surgery	3	3	Hypoacusis, sudden hearing loss, vertigo, facial hypoesthesia, abducens paresis	Non	2	6	I	I
31	17	2	1	5	Homogeneous	6 mm in 1y	1	1	Surditas, facial paresis, cognitive deficits	Atypical teratoid/ rhabdoid tumor	1	6	I	I
32	41	1	2	5	Homogeneous	4 mm in 7 mo	1		Facial hypoesthesia	Non	2	6	I	III
33	62	1	1	5	Homogeneous	2 mm in 15 mo	2	3	Tinnitus	Non	2	4	I	I
34	41	1	1	6	Homogeneous	Stable	2	3	Hypoacusis, dizziness	Non	2	2	I	I
35	64	2	2	6	Homogeneous	Stable	2	3	Hypoacusis, vertigo	Non	2	4	I	II
36	53	2	1	6	Inhomogeneous, cystic	Upfront surgery	3	3	Hypoacusis, dizziness	Non	2	4	I	IV
37	27	1	2	3	Homogeneous	8 mm in 16 mo	1	1	Surditas	Melanoma	1	6	I	I
38	61	1	2	6	Homogeneous	Upfront surgery	3	3	Hypoesthesia of the tongue, taste disturbance, hypoacusis, facial hypoesthesia	Non	2	2	I	I
39	27	2	1	6	Homogeneous	10 mm in 1.5 y	1	3	Surditas	Non	1	6	I	II
40	44	2	2	6	Inhomogeneous, cystic	Stable	2	3	Hypoacusis, tinnitus, sudden hearing loss	Non	2	2	I	I
41	53	2	2	6	Inhomogeneous	Stable	2	1	Vertigo, hypoacusis	Non	2	3	I	III

Sex: 1: female, 2: male; side: 1: tumor on the right side; 2: tumor on the left side; Tumor extension according Hannover classification: 1: T1; 2: T2; 3: T3a; 4: T3b; 5: T4a; 6: T4b; Rapid *vs.* slow growing: 1: rapid; 2: slow; 3: no follow up available; Antoni Type: 1: Type A; 2: Type B; 3: Type A/B; Pretreatment regarding the vestibular schwannoma: 1: yes; 2: no; Hearing according to Hannover Classification: 1: H1; 2: H2; 3: H3; 4: H4; 5: H5; 6: H6; Facial function according to House Brackmann: I: HB1; II: HB2; III: HB3; IV: HB4; V: HB5.

Table III. *Type and depth of each pathogenic variant and its effect.*

Sample No	Mutation No	Position genomic	Exon	cDNA	Reference allele	Variant allele	Variant type	Sequence context	Consequence	% Alleles	Total alleles	Reference allele depth	Variant allele depth
1	1	30051658	6	592	C	T	SNV	Coding	Stop gained	38.9684814	349	213	136
2	2	30074239	14	1,501	AT	A	Deletion	Coding	Frameshift variant	29.2198582	1,410	998	412
	3	30070880	13	1,396	C	T	SNV	Coding	Stop gained	29.6296296	297	209	88
3	4	30069319	12	1,184	CA	C	Deletion	Coding	Frameshift variant	25.698324	179	133	46
	5	30069476	12	1,340+1	G	C	SNV	Intron	Splice donor variant	28.458498	253	181	72
4	6	30000060	1	73	AGGATCGTCACCATG GACGCCGAGATG	A	Deletion	Coding	Frameshift variant	61.3300493	406	157	249
5	7	30054229	7	651	C	A	SNV	Coding	Stop gained	14.619883	342	292	50
6	8	30050659	5	461	AC	A	Deletion	Coding	Frameshift variant	63.6948529	1,088	395	693
7	9	30067824	11	1,009	C	T	SNV	Coding	Stop gained	19.1911182	1,261	1,019	242
8	10	30000086	1	99	G	GA	Insertion	Coding	Frameshift variant	33.8435374	588	389	199
9	11								No pathogenic variant				
10	12								No pathogenic variant				
11	13	30000033	1	46	AG	A	Deletion	Coding	Frameshift variant	34.9593496	246	160	86
	14	30057328	8	810	G	T	SNV	Coding	Missense variant, Splice region variant	37.4	449	280	168
	15	30000035	1	48	GA	TT	MNV	Coding	Stop gained	35.4	246	159	87
12	16	30057329	8	810+1	G	T	SNV	Intron	Splice donor Variant	35.7	722	464	258
13	17								No pathogenic variant				
14	18	30067911	11	1,096	G	T	SNV	Coding	Stop gained	73.8	1,205	315	890
15	19	30035180	3	342	A	ACAACATTT	Insertion	Coding	Frameshift variant	10.7	1,020	911	109
16	20	30051666	6	599+1	G	A	SNV	Intron	Splice donor Variant	39.5	1,026	621	405
17	21	30051652	6	586	C	T	SNV	Coding	Stop gained	68.0	718	230	488
18	22	21351195	20	2,346	AACGC	A			Frameshift variant, Downstream gene variant	62.6			508
19	23								No pathogenic variant				
20	24								No pathogenic variant				
21	25	30032747	2	122	G	A			Stop gained	82.0			861
22	26	30000053	1	66	CA	C			Frameshift variant	63.6			723
23	27	30070931	13	1	G	T			Splice donor variant	72.6			610
24	28	30035108	3	270	AC	A			Frameshift variant	55.5			939

Table III. *Continued*

Table III. *Continued*

Sample No	Mutation No	Position genomic	Exon	cDNA	Reference allele	Variant allele	Variant type	Sequence context	Consequence	% Alleles	Total alleles	Reference allele depth	Variant allele depth
25	29	30032772	2	147	GGTGTGCCGGAC	G	Deletion	Coding	Frameshift variant	17	1,683	1,402	281
26	30	30050656	5	458	AC	A	Deletion	Coding	Frameshift variant	20	1,209	967	242
27	31	30077432	15	1,579	G	T	SNV	Coding	Stop gained	7	3,153	2,918	230
28	32	30051654	6	588	AG	A	Deletion	Coding	Frameshift variant	24	842	643	199
	33	30070823	13	-2	A	C	SNV	Intron	Splice acceptor variant	25	753	560	191
29	34								No pathogenic variant				
30	35	30050657	5	459	CG	C	Deletion	Coding	Frameshift variant	35	2,800	1,814	986
	36	30032866	2	1	G	T	SNV	Intron	Splice donor variant	34	1,705	1,127	578
31	37	30069295	12	1,160	AG	A	Deletion	Coding	Frameshift variant	65	1,488	522	966
32	38	30070921	13	1,437	CACGTA	C	Deletion	Coding	Frameshift variant	6	835	783	52
33	39	30054200	7	622	CTGAAGATAGCTCAGG ACCTGGAGATGTACGG	C	Deletion	Coding	Frameshift variant	11	1,793	1,602	191
	40	30069300	12	1,165	CAGATCACCGAGGA GGAGGCAAAACTT	C	Deletion	Coding	Frameshift variant	11	1,103	979	124
34	41	30050712	5	514	AG	A	Deletion	Coding	Frameshift variant, Splice region variant	15	1,922	1,643	279
	42	30050645	5	-1	G	A	SNV	Intron	Splice acceptor variant	16	1,971	1,652	319
35	43	30057285	8	767	TC	T	Deletion	Coding	Frameshift variant	5	4,217	4,003	214
36	44	30000029	1	42	C	CG	Insertion	Coding	Frameshift variant	29	2,147	1,518	629
37	45	30038257	4	430	T	TA	Insertion	Coding	Stop gained	41	1,020	599	421
38	46	30000021	1	34	AGCTCTCTCAAGAGG AAGCAACCCAAGACG TTCACCGT	A	Deletion	Coding	Frameshift variant	36	822	529	293
39	47	30064432	10	996	GC	G	Deletion	Coding	Frameshift variant, Splice region variant	24	2,025	1,547	478
	48	30064436	10	1	G	A	SNV	Intron	Splice donor variant	21	2,041	1,605	436
40	49	30000039	1	52	C	T	SNV	Coding	Stop gained	30	846	594	252
	50	30074183	14	-2	A	T	SNV	Intron	Splice acceptor variant	28	3,533		
41	51	30069295	12	1,160	AG	A	Deletion	Coding	Frameshift variant	44	999	557	442
	52	30077446	15	1,593	GA	G	Deletion	Coding	Frameshift variant	8	1,756		

Table IV. *Combination of genetic events in the 41 tumors analyzed.*

Gene	*NF2*							*LZTR1*
Number of pathogenic variants	0	0	1	1	1	2	2	1
Allele loss (0=no, 1=yes)	0	1	0	Not known	1	0	1	1
Total inactivating alterations	0	1			2		3	2
Groups	Group 0	Group 1			Group 2			
Number of tumors (41 in total)	4	8			27		1	1

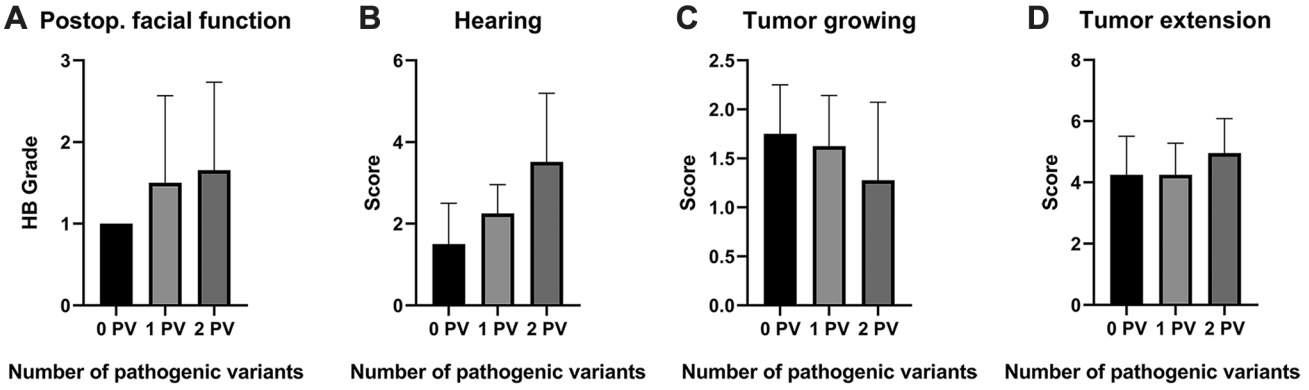

Figure 1. *Phenotype correlation with the number of pathogenetic variants (PV). A) Facial function according to House & Brackmann (HB) Grade; B) Auditory function according Hannover Classification; the difference between 1 PV and 2 PV was statistically significant (p=0.015). C) Tumor growth dynamic (1: rapid; 2: slow); D) Tumor extension according Hannover Classification.*

(T4A) in Group 2. These differences were not significant according to Kruskal Wallis test (p=0.18). When slow growing was coded as 2 and fast growing as 1, Group 0 had a score of 1.7 and was not significantly slower than the score of 1.6 of Group 1 or the score of 1.3 of Group 2 (Figure 1C). There were no differences in the preoperative facial function. By contrast, worse postoperative results were found more frequently in Groups 1 and 2, compared to Group 0, although the difference was not significant (p=0.37) (Figure 1A).

One tumor of Group 1 and three tumors of Group 2 were treated with radiation or medical before the surgery. Thus, in our small cohort the number of pathogenic variants increases with pretreatment. There were no differences regarding resection extension or postoperative regrowth. For all four tumors in group 0, MRI showed completely homogeneous signals. In contrast, inhomogeneous or cystic signals were found in 1/8 (13%) of group 1 and in 9/29 (31%) of group 2 tumors. Thus, the proportion of inhomogeneous tumors seems to increase with the counts of pathogenic variants.

Within group 2 tumors, some had two pathogenic variants, and some had one pathogenic variant plus an allele loss. However, clinical parameters including hearing, tumor structure in MRI, tumor growth and postoperative facial function were similar. Tumor size showed differences and there was a trend, but it did not reach significance (p=0.06).

Unexpected genetic findings and clinical presentation. In tumor 18 a frame-shifting variant was found in exon 20 of the *LZTR1* gene with an allele frequency was 66% (depth 508) and no pathogenic variant was found in the *NF2* gene. MLPA detected an allele-loss of the entire *NF2* locus. The two probes for two exons *LZTR1* also showed copy number reduction, indicating that the allele loss covers the *LZTR1* locus.

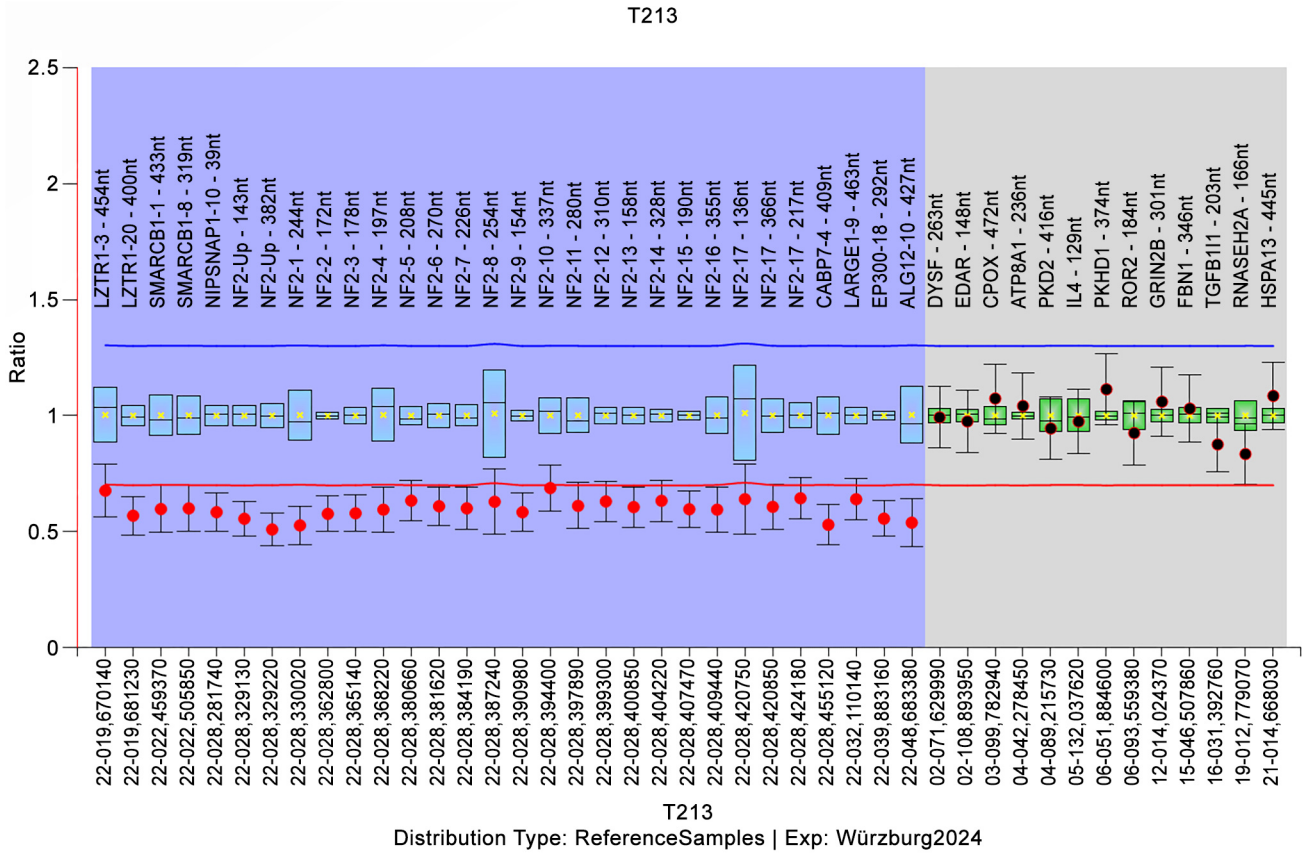

Figure 2. *MLPA of tumor #41 reveals an allele loss of the NF2 gene, which appeared heterozygotic.*

So, tumor #18 had a pathogenic frameshift variant of the *LZTR1* gene. The tumor had medium size, and the preoperative hearing function was also medium. MRI signals were homogeneous. The resection was complete and there has been no recurrence so far. Histology revealed a typical schwannoma.

Another unexpected finding was in tumor 41. Two pathogenic variants plus an allele loss of the *NF2* gene were found. The two frameshifting variants are in exons 12 and 15 with allele frequencies of 44% and 8%, respectively. The allele loss appeared heterozygotic in the MLPA data (Figure 2). So, tumor #41 had a high number of pathogenic variations. Although it was a large tumor it resulted in a medium hearing function preoperative. The tumor was inhomogeneous in MRI, the patient underwent surgery within three months after diagnosis. He received no pre-treatment and had no other tumors. Immediately after the operation, he had a facial palsy House Brackmann (HB)°III, but so far (after 1.5 years of follow up) no recurrence was observed.

Discussion

Using a panel covering the *NF2* and *LZTR1* gens, a total of 46 pathogenic variants were found in the 41 sporadic vestibular schwannomas. Panel sequencing has much higher sensitivity than the conventional Sanger sequencing and, therefore, can detect more variants in DNA from tumors, which often have non-tumor tissues of high proportion (6, 7). Indeed, 12 variants found in this study had allele frequencies below 0.2, which would likely not have been detected by Sanger sequencing. According to the

standard protocol for detecting somatic variants, only those in more than 5% of the total reads were recorded in the present study. However, it is still well possible that some specimens had extremely high proportion of non-tumor tissues and therefore pathogenic variants were in even lower allele frequencies which were not recorded. This may explain a part of missing pathogenic variants.

Missing pathogenic variants can also be explained by deep intronic variants, which are not covered by the applied panel. Deep intronic variants may cause alteration in the splicing and, therefore, can be pathogenic. A third explanation for not finding an inactivating event is, that the investigated piece of the tumor, contained a large portion of tumor-free tissue. This is due to the limited sensitivity of MLPA for detecting copy number variation. A fourth possible explanation of not finding an inactivating event is that the events may occur in other genes. If our panel did not include the *LZTR1* gene, we would miss this pathogenic variant. A recent genome-wide association study found that the 9p21.3 region is associated with risk of vestibular schwannomas (8). Thus, inactivating events may be in genes in those regions.

Though *LZTR1*-related schwannomatosis is more closely associated with non-cerebral tumors, vestibular schwannomas can also develop. Pathogenic *LZTR1*-variants have even been found in the blood as germline variants in 4 (3%) out of 161 patients with sporadic vestibular schwannomas (9). Therefore, our finding of a pathogenic *LZTR1*-variant in a sporadic vestibular schwannoma is in concordance with the previous findings.

There is no straightforward correlation between the type nor the counts of the inactivating events and clinical features in this cohort of sporadic vestibular schwannomas. Correlation of genotype with disease-severity in *NF2*-related schwannomatosis (10-12) does not apply to sporadic vestibular schwannomas. The type of genetic variant influences the patient age at which a tumor develops and the number of tumors a patient develops. However, growth and associated clinical features are more biological and may not be influenced by genetic but rather non-genetic factors.

Carlson *et al.* (13) found that only major chromosomal abnormalities correlated with an aggressive phenotype. Havik *et al.* (14) investigated the genetic landscape of sporadic vestibular schwannoma with whole genome sequencing and found a pathogenic *NF2*-variants in 35 out of 46 cases. In 16 cases, they found two mutational hits, but nothing is reported about the chronological sequence of the mutations and a higher number of pathogenic variants could not be linked to radiosurgery. Sporadic spinal schwannomas also show pathogenic variants in the NF2 gene and two mutations in one tumor have been reported by Carvalho *et al.* (15).

Conclusion

There was no correlation between clinical phenotype and genetic inactivating alterations of the *NF2* gene in sporadic schwannomas. Inactivation of the *LZTR1* gene is also involved in the development of sporadic vestibular schwannoma. Genetic inactivating events are the precondition for the development of vestibular schwannomas but do not influence their growth and other features.

Funding

The University of Würzburg supported this work in the funding program Open Access Publishing.

Conflicts of Interest

There are no conflicts of interest.

Authors' Contributions

All Authors contributed to the study conception and design. The study was supervised by MB and LK. Tumor tissue samples were provided by RE, CM, ML and CMM. Experiments were performed by LK, and data analyzed by TS, MB and LK. TS, MB and LK wrote the draft of the manuscript, which was subsequently revised by CMM, CM, ML and RE. All Authors read and approved the final manuscript. MB is the corresponding author.

References

1 Goldbrunner R, Weller M, Regis J, Lund-Johansen M, Stavrinou P, Reuss D, Evans DG, Lefranc F, Sallabanda K, Falini A, Axon P, Sterkers O, Fariselli L, Wick W, Tonn JC: EANO guideline on the diagnosis and treatment of vestibular schwannoma. Neuro Oncol 22(1): 31-45, 2020. DOI: 10.1093/neuonc/noz153

2 Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, Hawkins C, Ng HK, Pfister SM, Reifenberger G, Soffietti R, von Deimling A, Ellison DW: The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol 23(8): 1231-1251, 2021. DOI: 10.1093/neuonc/noab106

3 Samii M, Matthies C: Management of 1000 vestibular schwannomas (acoustic neuromas): hearing function in 1000 tumor resections. Neurosurgery 40(2): 248-262, 1997. DOI: 10.1097/00006123-199702000-00005

4 Hummel M, Perez J, Hagen R, Gelbrich G, Ernestus RI, Matthies C: When does hearing loss occur in vestibular schwannoma surgery? Importance of auditory brainstem response changes in early postoperative phase. World Neurosurg 95: 91-98, 2016. DOI: 10.1016/j.wneu.2016.07.085

5 Moffat DA, Kasbekar A, Axon PR, Lloyd SKW: Growth characteristics of vestibular schwannomas. Otol Neurotol 33(6): 1053-1058, 2012. DOI: 10.1097/MAO.0b013e3182595454

6 Behjati S, Tarpey PS: What is next generation sequencing? Arch Dis Child Educ Pract Ed 98(6): 236-238, 2013. DOI: 10.1136/archdischild-2013-304340

7 Contini E, Paganini I, Sestini R, Candita L, Capone GL, Barbetti L, Falconi S, Frusconi S, Giotti I, Giuliani C, Torricelli F, Benelli M, Papi L: A systematic assessment of accuracy in detecting somatic mosaic variants by deep amplicon sequencing: application to NF2 gene. PLoS One 10(6): e0129099, 2015. DOI: 10.1371/journal.pone.0129099

8 Sadler KV, Bowes J, Rowlands CF, Perez-Becerril C, van der Meer CM, King AT, Rutherford SA, Pathmanaban ON, Hammerbeck-Ward C, Lloyd SKW, Freeman SR, Williams R, Hannan CJ, Lewis D, Eyre S, Evans DG, Smith MJ: Genome-wide association analysis identifies a susceptibility locus for sporadic vestibular schwannoma at 9p21. Brain 146(7): 2861-2868, 2023. DOI: 10.1093/brain/awac478

9 Sadler KV, Bowers NL, Hartley C, Smith PT, Tobi S, Wallace AJ, King A, Lloyd SKW, Rutherford S, Pathmanaban ON, Hammerbeck-Ward C, Freeman S, Stapleton E, Taylor A, Shaw A, Halliday D, Smith MJ, Evans DG: Sporadic vestibular schwannoma: a molecular testing summary. J Med Genet 58(4): 227-233, 2021. DOI: 10.1136/jmedgenet-2020-107022

10 Evans DG: Neurofibromatosis type 2 (NF2): a clinical and molecular review. Orphanet J Rare Dis 4: 16, 2009. DOI: 10.1186/1750-1172-4-16

11 Ruttledge MH, Andermann AA, Phelan CM, Claudio JO, Han FY, Chretien N, Rangaratnam S, MacCollin M, Short P, Parry D, Michels V, Riccardi VM, Weksberg R, Kitamura K, Bradburn JM, Hall BD, Propping P, Rouleau GA: Type of mutation in the neurofibromatosis type 2 gene (NF2) frequently determines severity of disease. Am J Hum Genet 59(2): 331-342, 1996.

12 Kluwe L, Nygren AO, Errami A, Heinrich B, Matthies C, Tatagiba M, Mautner V: Screening for large mutations of the *NF2* gene. Genes Chromosomes Cancer 42(4): 384-391, 2005. DOI: 10.1002/gcc.20138

13 Carlson ML, Smadbeck JB, Link MJ, Klee EW, Vasmatzis G, Schimmenti LA: Next generation sequencing of sporadic vestibular schwannoma: necessity of biallelic NF2 inactivation and implications of accessory non-NF2 variants. Otol Neurotol 39(9): e860-e871, 2018. DOI: 10.1097/MAO.0000000000001932

14 Håvik AL, Bruland O, Myrseth E, Miletic H, Aarhus M, Knappskog PM, Lund-Johansen M: Genetic landscape of sporadic vestibular schwannoma. J Neurosurg 128(3): 911-922, 2018. DOI: 10.3171/2016.10.JNS161384

15 De Carvalho RM, DE Castro Sant' Anna C, Pinto GR, Paschoal EHA, Tuji FM, DO Nascimento Borges B, Soares PC, Júnior AGF, Rey JA, Chaves LCL, Burbano RR: Frequency of the loss of heterozygosity of the NF2 gene in sporadic spinal schwannomas. Anticancer Res 38(4): 2149-2154, 2018. DOI: 10.21873/anticanres.12455