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ORIGINAL PAPER

A mosaic pattern of INI1/SMARCB1 protein expression distinguishes Schwannomatosis and NF2-associated peripheral schwannomas from solitary peripheral schwannomas and NF2-associated vestibular schwannomas

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Abstract
Background The *INI1/SMARCB1* gene protein product has been implicated in the direct pathogenesis of *schwannomas* from patients with one form of schwannomatosis [*SWNTS1*; MIM # 162091] showing a mosaic pattern of loss of protein expression by immunohistochemistry [93% in familial vs. 55% in sporadic cases].
Aim of study To verify whether such INI1/SMARCB1 mosaic pattern could be extended to all schwannomas arising in the sporadic and familial schwannomatoses [i.e. to SMARCB1-related (SWNTS1) or LZTR1-related (SWNTS2) schwannomatosis or to SMARCB1/LZTR1-negative schwannomatosis] and whether it could be involved in classical NF2 or solitary peripheral schwannomas
Methods We blindly analysed schwannoma samples obtained from a total of 22 patients including (**a**) 2 patients (2 males; aged 38 and 55 years) affected by non-familial SMARCB1-associated schwannomatosis (SWTNS1); (**b**) 1 patient (1 female; aged 33 years) affected by familial schwannomatosis (SWTNS1/ SMARCB1 germ line mutations); (**c**) 5 patients (3 males, 2 females; aged 33 to 35 years) affected by non-familial (sporadic) LZTR1-associated schwannomatosis (SWNTS2); (**d**) 3 patients (3 males; aged 35 to 47 years) affected by familial schwannomatosis (SWTNS2/ LZTR1 germ line mutations); (**e**)

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2 patients (1 male, 1 female; aged 63 and 49 years, respectively) affected by non-familial schwannomatosis (SWTNS, negative for SMARCB1, LZTR1 and NF2 gene mutations); (**f**) 4 patients (3 males, 1 females; aged 15 to 24 years) affected by classical NF2 (NF2: harbouring NF2 germ line mutations; and (**g**) 5 patients (3 males, 2 females; aged 33 to 68 years) who had solitary schwannomas. [follow-up = 15–30 years; negative for constitutional/somatic mutation analysis for the *SMARCB1*, *LZTR1* and *NF2* genes] were (blindly) analyzed. The INI1/SMARCB1 immunostaining pattern was regarded as (1) *diffuse* positive nuclear staining [= retained expression] or (2) *mosaic* pattern [mixed positive/negative nuclei = loss of expression in a subset of tumour cells].

Results All solitary peripheral schwannomas and NF2-associated vestibular schwannomas showed diffuse nuclear INI1/SMARCB1 staining in 97–100% of neoplastic cells; schwannomas obtained from all cases of non-familial and familial schwannomatosis and NF2-associated non-vestibular schwannomas showed a mosaic pattern ranging from 10 to 70% of INI1/SMARCB1-positive expression. We did not record a complete lack of nuclear staining.

Conclusions The present data suggests that (a) mosaic loss of immunohistochemical INI1/SMARCB1 expression, despite the interlesional variability, is a reliable marker of schwannomatosis regardless of the involved gene and it might help in the differential diagnosis of schwannomatosis vs. solitary schwannomas and (b) INI1/SMARCB1 expression is not useful in the differential with mosaic NF2, since NF2-associated peripheral schwannomas show the same immunohistochemical pattern.

Keywords Schwannomatosis · SWNTS · Neurofibromatosis type 2 · NF2 · SMARCB1 · LZTR1 · INI1 · Mosaicism · Neurofibromatosis · Histology · Immunohistochemistry

Introduction

The *neurofibromatoses*, including *neurofibromatosis type 1* (NF1), *neurofibromatosis type 2* (NF2) and *schwannomatosis* (SWNTS), comprise a group of genetically distinct disorders of the nervous system that are unified by the predisposition to develop nerve sheath tumours [1–3]. All three types of neurofibromatosis have tumour manifestations (consistent with tumour-suppressor status) and non-tumour manifestations [4, 5]. The occurrence of bilateral vestibular schwannomas and schwannomas of other cranial and peripheral nerves (including intradermal nerves), central nervous system meningiomas, ependymomas, and gliomas, cataracts, epiretinal membrane, and optic nerve sheath meningiomas characterize classical NF2 [6, 7].

SWNTS, the most recently identified form of neurofibromatosis, is characterized by exclusive [8–10] (or almost exclusive) [11–13] peripheral nervous system involvement including multiple non-vestibular, non-intradermal schwannomas and chronic pain in the absence of other signs of NF2 [4, 8–13]. The presence of (unilateral) vestibular schwannoma, as exclusion criteria for the diagnosis of schwannomatosis, has been recently questioned [12, 13], and mosaic forms of NF2 [*mosaic NF2*, characterized by unilateral vestibular schwannomas and ipsilateral cranial and/or peripheral nervous system schwannomas or by meningiomas] [14–17] as well as the observation of *LZTR1*-associated (and possibly other schwannomatosis-associated genes) [18, 19] unilateral vestibular schwannoma [17] make the original [8, 9] and revised criteria for schwannomatosis [10] a challenge [12, 13, 19–21].

Two major clinical/molecular forms of schwannomatosis have been described so far [4, 5, 7, 19, 21–27]. SWNTS1 [MIM # 162091] [22, 24] has been described as a distinct form of neurofibromatosis in 2007 [22] and it is caused by constitutional inactivating mutations of the SMARCB1 gene [SWI/SBF-related matrix-associated actin-dependent regulator of chromatin, subfamily B, member 1; MIM # 601607]. SMARCB1 is located 6 Mb centromeric to the NF2 gene at 22q11.23 and encodes a protein belonging to the SWI/SNF ATP-dependent nuclear chromatin remodelling complex, which is involved in two distinct functions: (a) releasing repressive chromatin structures, allowing the transcriptional machinery to access its targets more effectively and (b) binding to and enhancing the DNA joining activity of HIV-1 integrase. The SWNTS1 phenotype includes families with multiple schwannomas and multiple extra-axial/extra-medullary meningiomas and, rarely, unilateral vestibular schwannoma [13]. SWI/SNF is a tumour suppressor implicated also in the genesis of malignant rhabdoid tumours.

The most recently characterized form of schwannomatosis is SWNTS2 [MIM # 615670] [25–27], caused by constitutional/germ line inactivating mutations of the LZTR1 gene [leucine zipper-like transcriptional regulator 1; MIM # 600574]. LZTR1, localized in centromeric position compared to SMARCB1, encodes a member of the BTB-kelch superfamily, confined exclusively in the Golgi network where it may help to stabilize the Golgi complex. The SWNTS2 phenotype is characterized by a later onset of the disease (i.e. 20–60 years) and by the development of schwannomas affecting various body regions including the extremities, spinal cord, chest wall and subcutaneous regions. To complicate matters, individuals carrying LZTR1 mutations may, at a low frequency, develop vestibular schwannomas [27] or, even, not show any clinical signs of the disease [19].

NI1/SMARCB1 protein is ubiquitously expressed in all cell types and it is known to regulate cell cycle, growth and differentiation [4, 5, 22–24]. Mutations in SMARCB1 are also involved in the development of atypical teratoid/rhabdoid tumours (AT/RT) and malignant rhabdoid tumours (which are aggressive paediatric malignant tumours of the central nervous system and kidneys, respectively) [28–30].

AT/RT, occurring either sporadically and in the context of a tumour-suppressor gene syndrome, show diffuse loss of INI/SMARCB1 immunohistochemical expression, a feature often used in the pathological diagnosis of these tumours. Schwannomatosis-associated schwannomas have both constitutional and somatic mutations of the SMARCB1 gene and show a mosaic pattern of loss of INI1/SMARCB1 expression by immunohistochemistry; the mosaic pattern was seen in 93% of tumours from familial schwannomatosis vs. 55% of sporadic schwannomatosis vs. 83% of NF2-associated tumours and 5% of solitary, sporadic schwannomas, suggesting a tumour composition of mixed null and haploinsufficient cells and a different pathway of tumorigenesis occurring in solitary, sporadic tumours [31, 32].

In order to verify whether these mosaic patterns could be extended to all schwannomas arising in schwannomatosis comparing to classical NF2 and sporadic, solitary peripheral schwannomas, we evaluated the immunohistochemical profile of INI1/SMARCB1 in a group of schwannomas from 13 patients with molecular diagnosis of schwannomatosis (harbouring either constitutional SMARCB1 or LZTR1 mutations), from 4 patients affected by NF2 (carrying NF2 constitutional mutations) and from 5 patients affected by solitary ones.

Subjects, materials and methods

Subjects

Patients enrolled in the immunopathology/immunochemistry study have been referred to our institutions in Catania, Rome and Florence and are divided as follows: (a) 2 patients (2 males; aged 38 and 55 years) fulfilling the diagnostic criteria for non-familial SWNTS1 (i.e. harbouring SMARCB1 germ line mutations) [8–10]; (b) 1 patient (1 female; aged 33 years) affected by familial SWTNS1 schwannomatosis (i.e. harbouring SMARCB1 germ line mutations); (c) 5 patients (3 males, 2 females; aged 33 to 53 years) affected by non-familial SWTNS2 (i.e. harbouring LZTR1 germ line mutations); (d) 3 patients (3 males; aged 35 to 47 years) affected by familial SWTNS2 (i.e. harbouring LZTR1 germ line mutations); (e) 2 patients (1 male, 1 female; aged 63 and 49 years, respectively) affected by non-familial SWTNS SMARCB1-, LZTR1-negative; (f) 4 patients (3 males, 1 female; aged 15 to 24 years; mode 19 years) affected by classical NF2 [6, 7]; and (g) 5 patients [3 males vs. 2 females; aged 33 to 68 years; median 52 years; mode 54 years] with solitary schwannomas (non-NF2, non-SMARCB1, non-LZTR1) after long-term follow-up [i.e. 15 to 30 years].

Diagnostic work-up

All patients underwent [6, 7, 33–35] (a) magnetic resonance imaging (MRI) study of the brain and spinal cord and ultrasound and full body MRI studies; (b) full ophthalmologic study including fundoscopy; (c) brainstem and auditory evoked potentials (BAEP) coupled with otolaryngology examination. Written informed consent was obtained from all recruited individuals or from their parents/guardians. The study was approved by the Ethical Committee [*Catania 1*], located at the University Hospital (AOU) "*Policlinico-Vittorio Emanuele*" in Catania, Italy.

Surgery

Tumours in the two patients with non-familial SWNTS1 were excised from the the tibial and ulnar nerves. Tumours in the patient with familial SWNTS1 were excised from the thigh. Tumours were removed from the hand, arm, leg, thigh and foot, respectively, in the 5 patients with non-familial SWNTS2. Tumours were excised from the forearm, the trigeminal nerve and the hand in the two patients with non-familial SWTNS2 (*SMARCB1/LZTR1/NF2* negative).The 4 NF2 patients had their tumours removed from the vestibular nerves (n = 2), the temporal region (n = 1) and the spine (n = 1). Five patients had isolated, solitary schwannomas excised from the hands (n = 3) and feet (n = 2). We obtained multiple tumours from the non-familial and familial SWTNS1 and SWTNS2 patients and from the non-familial SWTNS patients; single tumours from the remaining NF2 patients and from the 5 patients with isolated schwannomas (see also Tables 1 and 2).

Molecular analysis

DNA was extracted from blood leucocytes using standard procedures with phenol/chloroform extraction and ammonium acetate/ethanol precipitation. DNA from formalin-fixed paraffin-embedded tissues was isolated according to manufacturers' manual of QIAamp DNA FFPE Tissue Kit (Qiagen, Helden, Germany). The entire coding sequences of SMARCB1, LZTR1 and NF2 were sequenced using capillary sequencing. All primers were designed using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/) and ordered from MWG-Biotech AG (Ebersberg, Germany). Primer sequences are available on request. Capillary sequencing was performed on 310 Capillary DNA Analyzer (Life Technologies, Carlsbad, CA, USA). Raw and analyzed sequence results were visualized on Sequence Scanner v1.0 (Life Technologies, Carlsbad, CA, USA).

Immunohistochemical analysis

Immunohistochemical analysis was performed (blindly) on 5-μ-thick formalin-fixed, paraffin-embedded, tissue sections using BAF47, a monoclonal antibody directed against the

Table 1 Clinical and molecular features of patients with schwannomatosis

Patient	Sex/age	Disease	Inheritance	Schwannomas [onset] [histology/surgery]	Schwannomas [other localizations]	Germline mutation	Somatic mutation	INI % loss [pattern]
92/3602	M/38	SWNTS1	Sporadic	Multiple [age 26] [+ R posterior tibial]	R arm, R leg	*SMARCB1*: c.41C>A, p.(Pro14His)	Absent	50% [mosaic]
73030	M/55	SWNTS1	Sporadic	Multiple [age 44] [+ R ulnar nerve]	R upper limb/thorax	*SMARCB1*: c.*82C>T	Absent	70% [mosaic]
T691	F/33	SWNTS1	Familial	Multiple [age 23] [+ R thigh]	Back, R leg	*SMARCB1*: c.1118G>A, r.1118_1119ins1118+1_?, p.(Arg373Lysfs*49)	Absent	55% [mosaic]
T645	F/53	SWNTS2	Sporadic	Multiple [age 34] [+ R leg]	R arm	*LZTR1*: c.560T>G, p.(Leu187Arg)	Absent	30% [mosaic]
T682	F/37	SWNTS2	Sporadic	Multiple [age 33] [+ L arm]	Neck, R hand	*LZTR1*: c.513delG, p.(Leu171Phefs*29)	*NF2*: c.1737+1G>T, r.spl?	75% [mosaic]
T700	M/44	SWNTS2	Sporadic	Multiple [age 29] [+ R thigh]	R foot/thorax	*LZTR1*: c.1394C>A, p.(Ala465Glu)	Absent	60% [mosaic]
T803	M/33	SWNTS2	Sporadic	Multiple [age 32] [+ R foot]	R hand/arm	*LZTR1*: c.855C>G, p.(Tyr285*)	NA	85% [mosaic]
74726	F/46	SWNTS2	Sporadic	Multiple [age 35] [+ R brachial plexus]	R upper limb, L leg	*LZTR1*: c.320+2T>C, r(spl?)	NA	70% [mosaic]
9950	M/63	SWNTS	Sporadic	Multiple [age 53] [+ L forearm, R foot]	L upper limb, R foot	Absent	Absent	65% [mosaic]
698/9	F/49	SWNTS	Sporadic	Multiple [age 39] [+ L hand, 3rd finger]	L upper limb, R leg	Absent	Absent	50% [mosaic]
T690	M/47	SWNTS2	Familial	Multiple [age 24] [+ R forearm]	R foot, R leg	*LZTR1*: c.1373dupG, p.(His459Profs*210)	Absent	85% [mosaic]
T730	M/41	SWNTS2	Familial	Multiple [age 31] [+ R trigeminal nerve]	R foot/thorax	*LZTR1*: c.1602delA, p.(Lys53Asnfs*22)	*NF2*: c.606delA, p.(Ala203Leufs*6)	40% [mosaic]
T761	M/35	SWNTS2	Familial	Multiple [age 23] [+ R hand]	Thoracic, R thigh	*LZTR1*: c.2278T>C, p.(Cys760Arg)	Absent	60% [mosaic]

M male, *F* female, *R* right, *L* left, + performed/present, – not performed/absent, *VS* vestibular schwannoma, *SS* spinal schwannoma, *C* cerebral, *S* spinal, *T* thoracic, *TS* thoracic schwannoma, *CNS* cranial nerve schwannoma, *ONM* optic nerve schwannoma

[a] After full ophthalmology; ear, nose and throat examination; ultrasound examination of the four limbs; audiometry; brainstem and auditory evoked potentials; complete brain and spinal MRI. All parents and (other) 1st-degree relatives were evaluated clinically and by the same investigations as above to rule out NF2 stigmata

Table 2 Clinical and molecular features of patients with NF2 and solitary peripheral schwannomas

Patient	Sex/age	Disease	Schwannomas [onset] [histology/surgery]	Germline mutation	INI % loss [pattern]
00/7877	M/15	NF2	VSs, SSs, S ependymoma, cataract, ONM [+ L VS]	*NF2*: c.984_985delGAinsTT p.(Lys329*)	2% [diffuse]
05/45871	F/21	NF2	VSs, SSs, C + S meningiomas [+ R P skull S]	*NF2*: c.586C>T, p.(Arg196*)	50% [mosaic]
11/86014	M/18	NF2	VSs, TS, SSs [+ R TS]	*NF2*: c.600-3C>T, r.(spl?)	30% [mosaic]
15/4371	M/24	NF2	VSs, CNSs, SSs, S ependymomas, ONM [+ L TS]	*NF2*: c.1090delA, p.(Glu366Lysfs*10)	25% [mosaic]
2760/90	M/37	Solitary peripheral schwannomas[a]	Isolated [age 22] [+ R hand: 2nd finger]	Absent	2% [diffuse]
1746/08	F/45	Solitary peripheral schwannomas[a]	Isolated [age 29] [+ L hand: 3rd finger]	Absent	5% [diffuse]
33768	M/54	Solitary peripheral schwannomas[a]	Isolated [age 31] [+ R foot: 2nd toe]	Absent	8% [diffuse]
72365	M/58	Solitary peripheral schwannomas[a]	Isolated [age 30] [+ L foot: plantar]	Absent	2% [diffuse]
3217	M/62	Solitary peripheral schwannomas[a]	Isolated [age 42] [+ R hand: 4th finger]	Absent	2% [diffuse]

M male, *F* female, *R* right, *L* left, + present, *VS* vestibular schwannoma, *SS* spinal schwannoma, *C* cerebral, *S* spinal, *T* thoracic, *TS* thoracic schwannoma, *CNS* cranial nerve schwannoma, *ONM* optic nerve schwannoma

[a] After full ophthalmologic examination; ear, nose and throat examination; ultrasound examination of the four limbs; audiometry; brainstem and auditory evoked potentials; complete brain and spinal MRI. All parents and (other) first-degree relatives were evaluated clinically and by the same investigations as above to rule out NF2 stigmata

INI1/*SMARCB1* gene product (1:100, BD Transduction Laboratories, San Diego, CA). Immunohistochemical studies were performed with the labelled streptavidin-biotin peroxidise detection system using the Ventana automated immunostainer (Ventana medical Systems, Tucson, AZ). Briefly, the sections were deparaffinized and hydrated in a series of "dewax" solutions and alcohol. Heat-induced antigen retrieval was performed with a high-pH antigen retrieval buffer (ER2). Incubation with primary antibody was followed by incubation with a secondary antibody and substrate. Finally, the sections were counterstained and cover slipped. Any nuclear staining was considered as indicative of INI1/SMARCB1 expression. The *INI1*/*SMARCB1* nuclear immunostaining was interpreted as "diffuse" if the expression was retained in more than 90% of neoplastic cells or as a "mosaic pattern" if mixed positive and negative nuclei were seen, consistent with loss of expression in a subset (at least 10% of cells) of tumour cells. Endothelial cells of normal blood vessels were used as internal positive controls. Sections from a paediatric renal rhabdoid tumour, typically negative for *INI1*/*SMARCB1* expression, were included in the study and served as negative control.

Results

Molecular analysis

DNA analysis for the SMARCB1, LZTR1 and NF2 genes revealed that 3 patients harboured constitutional SMARCB1 gene mutations and 8 patients harboured constitutional LZTR1 gene mutations; mutational analysis for SMARCB1, LZTR1 and NF2 genes yielded negative results in 2 patients with classical signs of SWTNS and in 5 cases of solitary (sporadic) peripheral schwannomas; all the 4 cases with classical NF2 harboured NF2 gene mutations (see Tables 1 and 2).

Immunohistochemistry

All *solitary peripheral schwannomas* showed diffuse nuclear positivity for INI1/SMARCB1 ranging from 97 to 100% of neoplastic cells [Fig. 1].

Conversely, peripheral schwannomas from non-familial [Fig. 2] and familial [Fig. 3] *schwannomatosis* (SWNTS1 and SWNTS2) and from non-familial SWNTS negative for mutational analysis of *SMARCB1* and *LZTR1* genes showed a mosaic pattern, alternating positive and negative nuclei, consistent with the loss of INI/SMARCB1 expression in a subset of tumour cells, ranging from 10 to 70% of the analyzed cases [28, 31]. None of the schwannomas analyzed [Figs. 1, 2 and 3] showed a complete negative immunostaining as typically observed in renal rhabdoid tumours [29, 30]. As previously reported [31, 32], in the mosaic

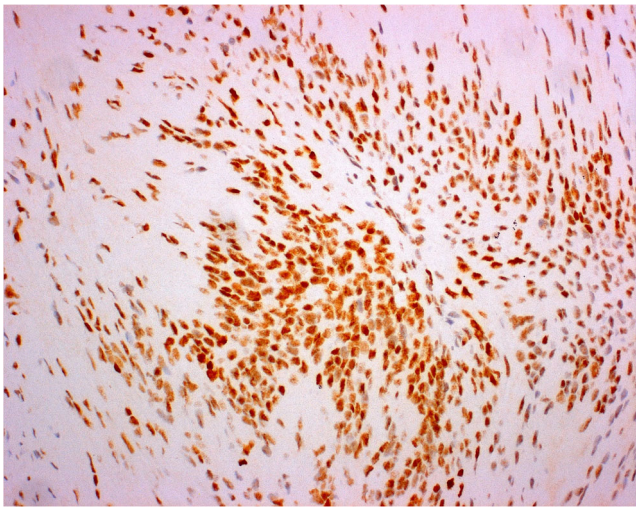

Fig. 1 Five-micro-thick formalin-fixed, paraffin-embedded, tissue section of a solitary peripheral schwannoma showing diffuse *INI1/SMARCB1* nuclear immunostaining positivity ranging from 97 to 100% of neoplastic cells

pattern [Figs. 2 and 3], the negative and positive cells were intimately intermixed. The *NF2-associated vestibular schwannomas* showed diffuse nuclear positivity while a mosaic pattern for INI1/SMARCB1 was seen in the *NF2-associated peripheral schwannomas* [Fig. 4]. The exact percentage of neoplastic cells with loss of INI1/SMARCB1 expression is reported in Tables 1 and 2.

Discussion

In the present series, 100% of patients who presented *solitary peripheral schwannomas,* i.e., a single schwannoma which remained solitary after a long-term observation (in the present series, 15 to 30 years) and 50% of NF2-associated vestibular schwannomas showed diffuse nuclear positivity ranging from 97 to 100% of neoplastic cells (Table 1, and Figs. 1 and 4). This is partially in line with previous studies that showed diffuse staining in 95% of solitary schwannomas [28, 31]. Conversely, 100% of schwannomatosis-associated schwannomas showed an INI1/SMARCB1 mosaic expression pattern alternating positive and negative nuclei, consistent with the loss of INI1/SMARCB1 expression in a subset of tumour cells, ranging from 10 to 70% of the analysed cases (Table 2, and Figs. 2, 3 and 4). Interestingly, the presence of the INI1/SMARCB1 mosaic expression pattern was independent from the presence of a constitutional SMARCB1 mutation as it could be found in schwannomas from patients affected by either familial and non-familial SWNTS1 and SWNTS2 and non-familial SWTNS not related to SMARCB1 or LZTR1 genes. This is in contrast with previous studies, which recorded mosaic patterns only in 55% of sporadic schwannomatosis (vs. 95% of tumours from familial schwannomatosis) [32].

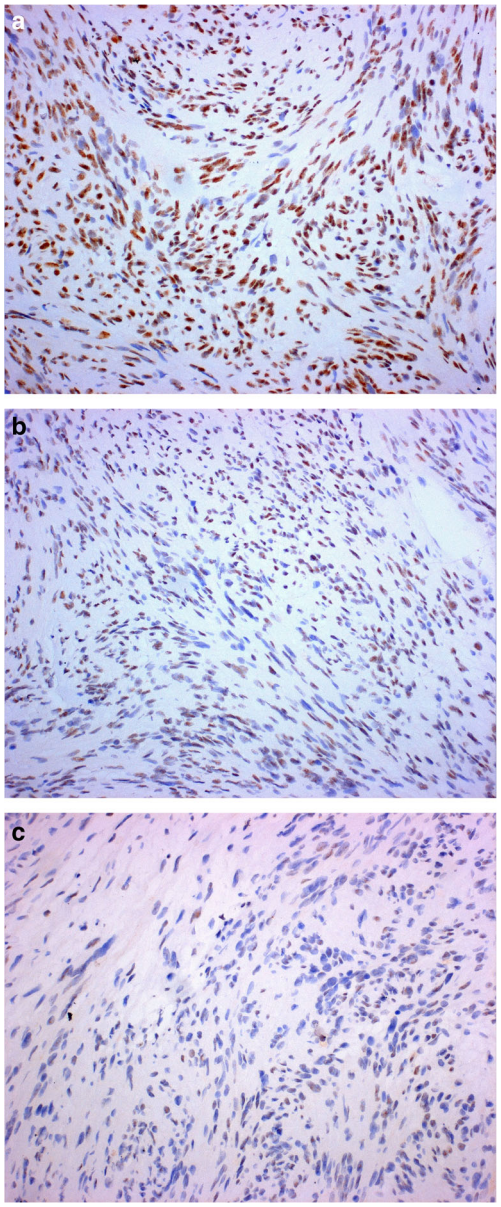

Fig. 2 Five-micro-thick formalin-fixed, paraffin-embedded, tissue sections of schwannomas taken from patients affected by (*non-familial*) schwannomatosis SWTNS1 reveal a mosaic pattern (alternating positive and negative nuclei), consistent with the loss of INI1/SMARCB1 expression in a subset of tumour cells, ranging from 10% (**a**) to 50% (**b**) or 70% (**c**) in the different cases

Besides, a mosaic expression pattern characterized also the INI1/SMARCB1 immunostaining of the NF2-associated non-vestibular peripheral schwannomas (Fig. 4b).

Thus, we demonstrated that *solitary peripheral schwannomas* retained the immunohistochemical expression of INI1/SMARCB1 in 97% to 100% of neoplastic cells, suggesting a different pathway of tumorigenesis and confirming the differences between the two clinical phenotypes, i.e. solitary peripheral vs. schwannomatosis, irrespective of non-familial vs. familial patterns of inheritance , *SMARCB1* vs. *LZTR1* mutations or lack of these mutations.

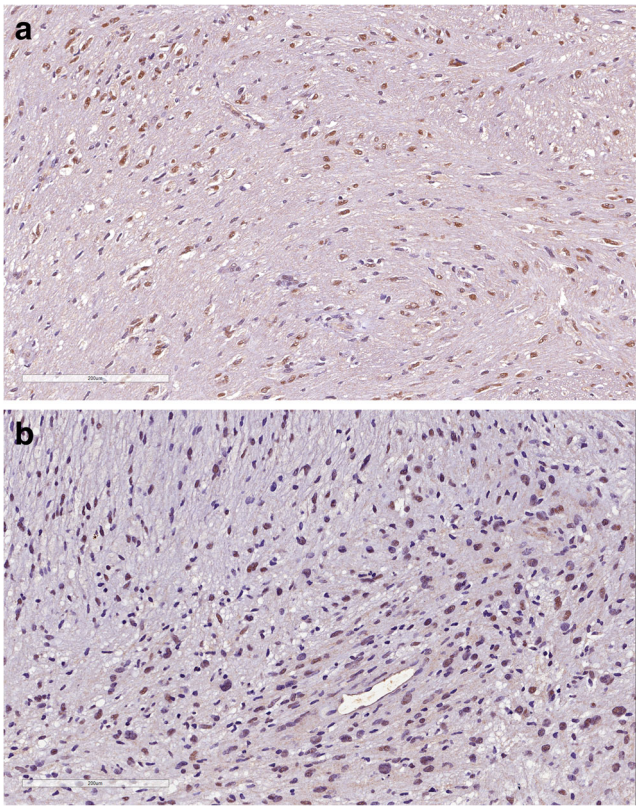

Fig. 3 Five-micro-thick formalin-fixed, paraffin-embedded, tissue sections of schwannomas taken from patients affected by (*familial*) schwannomatosis SWTNS2 reveal a mosaic pattern (alternating positive and negative nuclei), consistent with the loss of INI1/SMARCB1 expression in a subset of tumour cells, ranging from 40% (**a**) to 80% (**b**)

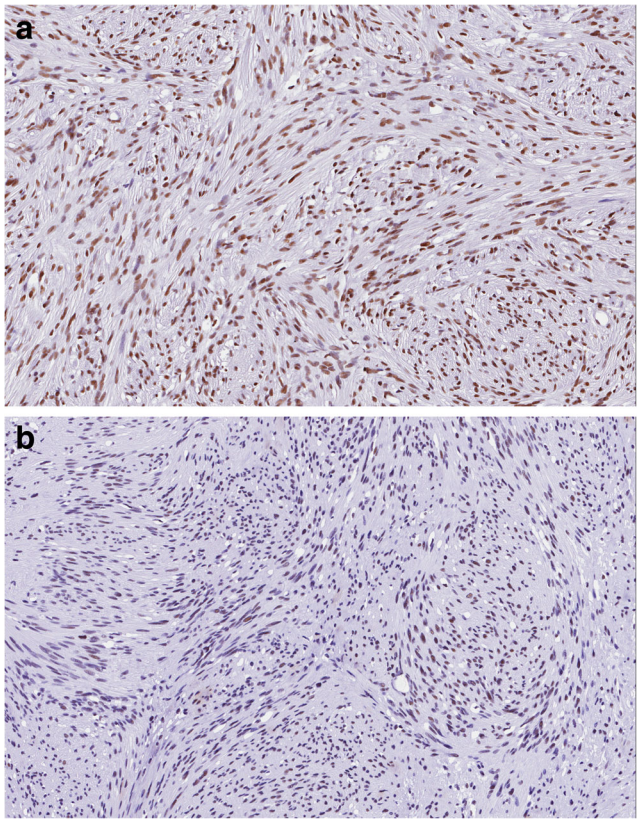

Fig. 4 Five-micro-thick formalin-fixed, paraffin-embedded, tissue sections of **a** *NF2*-associated vestibular schwannomas reveal a diffuse *INI1/SMARCB1* nuclear immunostaining vs. a mosaic pattern of *INI1/SMARCB1* expression in **b** *NF2*-associated non-vestibular peripheral schwannomas

In addition , our data confirm the involvement of *INI1/SMARCB1* in the development of all cases of non-familial schwannomatosis, the term non-familial, as opposed to familial schwannomatosis, should indicate only the onset of a de novo mutation in SMARCB1 or LZTR1 or in another, not yet identified, gene. The differences of mosaic expression pattern between non-familial vs. familial cases of schwannomatosis so far recorded in the literature could simply reflect the different diagnostic criteria used by our group: we were very restrictive and included cases in which two different somatic mutations of NF2 in multiple tumours of the patient were identified. [5, 35, 36].

Interestingly, we recorded a diffuse nuclear positivity of INI1/SMARCB1 in one case of NF2-associated vestibular schwannoma (Table 2 and Fig. 4a). These findings could suggest a different pathway of tumorigenesis between NF2-associated vestibular and non-vestibular (peripheral) schwannomas even though that needs confirmation by studies in larger series.

In conclusion, we found that mosaic loss of immunohistochemical INI1/SMARCB1 expression, despite the interlesional variability, is a reliable marker of schwannomatosis regardless of the involved gene and it might help in the differential diagnosis of schwannomatosis vs. solitary schwannomas. However, it cannot be useful in the differential with mosaic NF2, since NF2-associated peripheral schwannomas show the same immunohistochemical pattern [37].

Compliance with ethical standards Written informed consent was obtained from parents or guardians of all recruited individuals. The study was approved by the Ethical Committee [*Catania 1*], located at the University Hospital (AOU) "*Policlinico-Vittorio Emanuele*" in Catania, Italy.

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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