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GNAQ and *GNA11* mutant nonuveal melanoma: a subtype distinct from both cutaneous and uveal melanoma

E. Livingstone,[1] A. Zaremba,[1] S. Horn,[1,2] S. Ugurel,[1] B. Casalini,[3,4] M. Schlaak,[5] J.C. Hassel,[6] R. Herbst,[7] J.S. Utikal,[8,9] B. Weide,[10] R. Gutzmer,[11] F. Meier,[12] C. Koelsche,[13] E. Hadaschik,[1] A. Sucker,[1] H. Reis,[14] S. Merkelbach-Bruse,[15] M. Siewert,[1] F. Sahm,[3,4] A. von Deimling,[3,4] I. Cosgarea,[16] L. Zimmer,[1] D. Schadendorf,[1,17] B. Schilling[18] and K.G. Griewank[1,19]

[1]Department of Dermatology and [14]Institute of Pathology, University Hospital Essen, University of Duisburg-Essen, Hufelandstr. 55 45122, Essen, Germany
[2]Medical Faculty of the University Leipzig, Rudolf-Schönheimer-Institute of Biochemistry, Johannisallee 30 04103, Leipzig, Germany
[3]Department of Neuropathology and [13]Department of General Pathology, University Hospital Heidelberg, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany
[4]Clinical Cooperation Unit Neuropathology and DKTK, DKFZ, Heidelberg, Germany
[5]Department of Dermatology, LMU München, Frauenlobstraße 9–11 80337, Munich, Germany
[6]Department of Dermatology and National Center for Tumor Diseases, University Hospital Heidelberg, Im Neuenheimer Feld 460 69120, Heidelberg, Germany
[7]Department of Dermatology, Helios Klinikum Erfurt, Nordhäuserstr. 74, 99089, Erfurt, Germany
[8]Skin Cancer Unit, German Cancer Research Center (DKFZ), Heidelberg, Germany
[9]Department of Dermatology, Venereology and Allergology, University Medical Center Mannheim, Ruprecht-Karl University of Heidelberg, Mannheim, Germany
[10]Department of Dermatology, University of Tübingen, Liebermeisterstraße 25, 72076, Tübingen, Germany
[11]Department of Dermatology, Medizinische Hochschule Hannover, Carl-Neuberg-Straße 1, 30625, Hannover, Germany
[12]Department of Dermatology, Carl-Gustav-Carus University Hospital, Fetscherstr. 74, 01307, Dresden, Germany
[15]Institute of Pathology, University Hospital Cologne, Kerpener Str. 62, 50924, Cologne, Germany
[16]Dermatological Sciences, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK
[17]German Cancer Consortium (DKTK), Heidelberg, Germany
[18]Deptartment of Dermatology, University of Würzburg, Josef-Schneider-Str. 2, 97080, Würzburg, Germany
[19]Dermatopathologie bei Mainz, Bahnhofstr. 2B, 55268, Nieder-Olm, Germany

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Summary

Background *GNAQ* and *GNA11* mutant nonuveal melanoma represent a poorly characterized rare subgroup of melanoma with a gene mutation profile similar to uveal melanoma.

Objectives To characterize these tumours in terms of clinical behaviour and genetic characteristics.

Methods Patients with nonuveal *GNAQ/11* mutated melanoma were identified from the prospective multicentre tumour tissue registry ADOREG, Tissue Registry in Melanoma (TRIM) and additional cooperating skin cancer centres. Extensive data on patient, tumour and treatment characteristics were collected retrospectively. Targeted sequencing was used to determine tumour mutational burden. Immunohistochemistry staining was performed for programmed death-ligand 1 and BRCA1-associated protein (BAP)1. Existing whole-exome cutaneous and uveal melanoma data were analysed for mutation type and burden.

Results We identified 18 patients with metastatic *GNAQ/11* mutant nonuveal melanoma. Tumours had a lower tumour mutational burden and fewer ultraviolet signature mutations than cutaneous melanomas. In addition to *GNAQ* and *GNA11* mutations (nine each), six splicing factor 3b subunit 1 (*SF3B1*), three eukaryotic translation initiation factor 1A X-linked (*EIF1AX*) and four *BAP1* mutations were detected. In contrast to uveal melanoma, *GNAQ/11* mutant nonuveal melanomas frequently metastasized lymphatically and concurrent *EIF1AX*, *SF3B1* and *BAP1* mutations showed no apparent association with patient prognosis. Objective response to immunotherapy was poor with only one partial response observed in 10 treated patients (10%).

Correspondence
Elisabeth Livingstone.
Email: elisabeth.livingstone@uk-essen.de

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Conflicts of interest
See Appendix.

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Conclusions Our findings suggest that *GNAQ/11* mutant nonuveal melanomas are a subtype of melanoma that is both clinically and genetically distinct from cutaneous and uveal melanoma. As they respond poorly to available treatment regimens, novel effective therapeutic approaches for affected patients are urgently needed.

What is already known about this topic?

- The rare occurrence of *GNAQ/11* mutations in nonuveal melanoma has been documented.
- *GNAQ/11* mutant nonuveal melanomas also harbour genetic alterations in *EIF1AX*, *SF3B1* and *BAP1* that are of prognostic relevance in uveal melanoma.

What does this study add?

- *GNAQ/11* mutant nonuveal melanomas show metastatic spread reminiscent of cutaneous melanoma, but not uveal melanoma.
- *GNAQ/11* mutant nonuveal melanomas have a low tumour mutational burden that is higher than uveal melanoma, but lower than cutaneous melanoma.

What is the translational message?

- Primary *GNAQ/11* mutant nonuveal melanomas are a subtype of melanoma that is clinically and genetically distinct from both cutaneous and uveal melanoma.
- As metastatic *GNAQ/11* mutant nonuveal melanomas respond poorly to available systemic therapies, including immune checkpoint inhibition, novel therapeutic approaches for these tumours are urgently needed.

The discovery of somatic mutations in melanoma has led to a better understanding of melanoma pathogenesis and to the development of drugs targeting these mutations [i.e. v-raf murine sarcoma viral oncogene homolog B (BRAF) inhibitors], which constitute a major breakthrough in the treatment of patients with cutaneous melanoma. Most melanomas are derived from melanocytes located within the cutaneous epidermis, but they can also arise from pigmented cells of the cutaneous dermis, uvea and iris, mucosal membranes and leptomeninges.[1] Epidemiological and molecular genetic data suggest different mechanisms of melanoma genesis. Intermittent and chronic cumulative sun exposure is a major factor for many but not all melanoma subtypes.[2] While most sun-exposed cutaneous melanomas harbour oncogenic mutations in the mitogen-activated protein kinase (MAPK) signalling pathway [primarily activating mutations of *BRAF* (40%) and neuroblastoma rat sarcoma viral oncogene homolog (*NRAS*) (20%)],[3] these mutations are far less frequent in tumours arising in acral or mucosal melanoma and do not occur in uveal melanoma.[4]

In uveal melanoma, approximately 80% of somatic mutations affect *GNAQ* and *GNA11*.[5–7] *GNAQ* and *GNA11* code for members of the Gαq class of G-protein α subunits (Gαq and Gα11, respectively), which are involved in signalling via G-protein-coupled receptors. The mutations occur in the rat sarcoma viral oncogene (RAS)-like domain of the protein, leading to a constitutively activated GαQ/11 protein that essentially converts the GαQ/11 protein into an activated oncogene product.[8] Activated *GNAQ* and *GNA11* (*GNAQ/11*) mediates downstream stimulation of the MAPK pathway via phospholipase C (PLCβ) and protein kinase C (PKC) and of the phosphotidylinositol-3 kinase (PI3K)/protein kinase B (Akt)/mechanistic target of rapamycin and the yes-associated protein pathways.[9] All three signalling pathways are assumed to contribute to tumour growth and proliferation.

Apart from the mutational pattern, uveal melanoma also demonstrates a distinct biological behaviour regarding metastatic spread and treatment response. Despite excellent rates of local disease control, nearly 50% of patients will ultimately succumb to metastatic disease, with the most common metastatic site being the liver.[10] In contrast to cutaneous melanoma, treatment response to targeted therapy is poor with reported response rates of generally less than 10% for MAPK kinase (MEK), Akt and PKC inhibitors.[2,10,11] Immune checkpoint inhibition (ICI) using anti-programmed cell death protein (PD)-1 and/or anti-cytotoxic T-lymphocyte-associated protein (CTLA)4 antibodies has also been disappointing, with only individual patients responding to immunotherapy.[12–16] The low mutational burden of uveal melanoma, differences regarding expression of neoantigens between cutaneous and

uveal melanoma, an immunosuppressive tumour microenvironment, low immunogenicity and an immune-privileged site of uveal melanoma have been held responsible for the failure of immunotherapy.[17]

GNAQ/11 mutations can also occur in cutaneous melanocytic proliferations. They are frequent in dermal melanocytic tumours such as blue naevi (83%) and blue naevus-like melanoma (50%),[8] and less frequent in common cutaneous or mucosal melanoma.[18–20] According to the classic progression model, benign tumours (naevi) frequently harbour an initial activating mutation (e.g. a *BRAF* or *GNAQ/11* mutation). Acquisition of additional genetic alterations is required for progression to a malignant neoplasm (melanoma).[21] In uveal melanoma, additional mutations are typically found in splicing factor 3b subunit 1 (*SF3B1*), eukaryotic translation initiation factor 1A X-linked (*EIF1AX*) or BRCA1-associated protein (*BAP*)1. Interestingly, they mostly occur in a mutually exclusive manner and have prognostic relevance.[21] While inactivating *BAP1* mutations are associated with a poor prognosis,[22] *SF3B1* and *EIF1AX* mutated uveal melanomas show a more favourable course of disease.[23,24] Recently, we were able to demonstrate that *SF3B1* and *BAP1* alterations are present in 17% and 25% of blue naevus-like melanoma, respectively.[21] Johnson *et al.* additionally described concurrent *GNAQ/11* and *SF3B1* or *EIF1AX* mutations in nonuveal melanoma.[25]

It is uncertain to what extent the molecular linkage between *GNAQ/11* mutant uveal and nonuveal melanoma also translates into a biological relationship, in particular regarding the frequency and pattern of metastatic spread and the poor response to immunotherapy. We therefore identified patients with nonuveal melanoma who had metastatic disease and known *GNAQ/11* mutations, assessing course of disease, additional somatic mutations, tumour mutational burden (TMB) and mutation signature in order to investigate whether experiences with uveal melanoma can be transferred to nonuveal melanoma with *GNAQ/11* mutations.

Patients and methods

Patient selection

The database of the prospective multicentre translational study Tissue Registry in Melanoma (TRIM) was screened for nonuveal melanoma with known activating hotspot mutations of *GNAQ* and *GNA11*. The TRIM study has previously been described elsewhere.[26] In brief, formalin-fixed paraffin-embedded (FFPE) tumour tissues were prospectively collected and analysed within the framework of the skin cancer registry ADOREG (German Dermatologic Cooperative Oncology Group). The TRIM study was approved by the ethics committee of the University of Duisburg-Essen (15-6566-BO). Of 1060 patients registered in TRIM on 15 September 2018, 15 patients had *GNAQ/11* mutations.

Additional patients not captured in the registry (n = 9) were identified retrospectively from the dermatology departments of the university hospitals of Cologne (n = 3), Essen (n = 2), Heidelberg (n = 2), Tübingen (n = 1) and Würzburg (n = 1). For these patients, samples were collected during routine care and confirmatory *GNAQ/11* mutation analysis was performed using next-generation sequencing as described below. Histological evaluation was carried out by the local board-certified pathologists or dermatopathologists. Five cases (cases 2, 3, 4, 10 and 15) were described in a previous study.[21] Clinical data (including sex and age at diagnosis) in addition to tumour-specific information (localization, histological type, tumour thickness, ulceration, regression, sentinel node biopsy, stage at initial diagnosis, metastatic progress, type, duration and response to systemic treatment) was required for each patient and information was obtained from the local institution. The study was performed and approved in accordance with the guidelines of the ethics committee of the University of Duisburg-Essen (18-8110-BO).

DNA isolation

DNA was isolated from FFPE tumour tissue cut into 10-μm thick sections. After deparaffinization, sections were manually macrodissected according to standard procedures. Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Targeted sequencing and genetic analysis

All samples were sequenced applying a 10-gene custom amplicon-based panel as previously described,[21] covering known recurrent mutations in uveal and cutaneous melanoma [*GNAQ*, *GNA11*, phospholipase C-β4 (*PLCB4*), cysteinyl leukotriene receptor 2 (*CYSLTR2*), *EIF1AX*, *SF3B1*, *BAP1*, *BRAF*, *NRAS* and tyrosine-protein kinase (*KIT*)]. A 130-gene panel[27] was applied to determine TMB in eight samples where sufficient high-quality DNA for analysis was available (cases 2, 5, 7, 8, 10, 11, 12 and 18). Existing whole-exome data of cutaneous and uveal melanoma samples from The Cancer Genome Atlas (TCGA) were analysed for mutation type and mutation burden.[28,29] A detailed description of all methods applied for sequencing and genetic data in addition to the statistical analysis performed is listed in the supplemental material and methods (File S1; see Supporting Information).

BRCA1-associated protein 1 programmed death-ligand 1 immunohistochemistry

BAP1 immunohistochemistry (IHC) was performed, applying a rabbit polyclonal antibody recognizing amino acids 430–729 of the BAP1 protein (clone C-4, Santa Cruz Biotechnology, Dallas, TX, USA) as previously reported.[30] Nuclear staining was assessed for positivity. Programmed death-ligand 1 (PD-L1) expression was assessed in FFPE tumour specimens, with the use of a rabbit monoclonal antihuman PD-L1 antibody (clone 28-8) and an analytically validated automated immunohistochemical assay (PD-L1 IHC 28-8 pharmDx for Autostainer Link 48; Dako, Glostrup, Denmark).[31] PD-L1

positivity was defined as at least 5% of living tumour cells showing membranous staining of any intensity in a section containing at least 100 evaluable tumour cells.

Results

Sample cohort

Of the 24 patients in whom a *GNAQ/11* mutation was identified, six were excluded for further analysis as they either had ocular melanoma or nonmetastatic disease (two uveal melanoma, one ciliary body melanoma, one nonmetastatic disease) or because the *GNAQ/11* mutation could not be confirmed (two cases). In all other cases, a primary uveal melanoma was excluded by ophthalmological assessment and/or imaging by computed tomography/magnetic resonance imaging. The rate of *GNAQ/11* mutation in nonuveal melanoma in TRIM was 11 of 1039 patients (1·1%). The median age of the remaining 18 patients was 52 years (range 13–75) and 67% were male (Tables 1 and 2). In six patients, the tumour was diagnosed as melanoma of unknown primary (MUP). In patients with known primary, only five were clearly identified as melanoma on the first histopathology report. In two cases, the histopathologist could not initially differentiate between a primary melanoma and a benign melanocytic proliferation, and in four cases between a primary melanoma and a subcutaneous metastasis (Table 3). In eight patients, the primary was located in regions typical for blue naevus [scalp/head (n = 5) (Figure 1), buttocks/sacrococcygeal region (n = 2), scrotal (n = 1)]. In patients with known primary, median tumour thickness was 7·5 mm (range 0·85–8·9). Only two primaries were ulcerated.

Of the six patients diagnosed with MUP, three had macroscopic axillary lymph node metastases (patient 1, 10 and 14) and one had retroperitoneal lymph node metastases (patient 11) at initial diagnosis. In patients 12 and 17, it could not be determined whether the patients had primary cerebral melanoma or MUP with cerebral metastases.

Seven patients (39%) presented with a locoregional metastasis at initial diagnosis and five patients (28%) presented with distant metastasis. In patients without metastasis at initial diagnosis, median time to stage IV disease was 49 months (range 5–132). Four patients have not yet progressed to stage IV. At a median follow-up time of 48 months (range 3–201), 11 patients died as a result of melanoma.

Table 1 Summary of patient and tumour characteristics of the cohort of patients with nonuveal metastatic melanoma who had *GNAQ/11* mutation

Median age at diagnosis, years (range)	51·5 (13–75)
Median follow-up, months (range)	47·5 (3–201)
Sex	
Male	12 (67)
Female	6 (33)
Vital status	
Dead	11 (61)
Melanoma-specific death	11 (100)
Stage at diagnosis[a,b]	
Min. IA	1 (6)
IB	1 (6)
IIB	4 (22)
Min. IIIA	1 (6)
IIIB	2 (11)
IIIC	4 (22)
IV	5 (28)
Tumour thickness	
Not available	4 (22)
MUP	6 (33)
Median, mm (range)	7·5 (0·85–14)
Location of primary	
MUP	6 (33)
Head/neck	5 (28)
Upper extremity	1 (6)
Trunk[c]	5 (28)
Lower extremity	0
Vagina	1 (6)
Sentinel lymph node biopsy	
Done	3 (17)
Positive	3 (100·0)
Location of first metastasis	
Sentinel lymph node	3 (17)
Macroscopic lymph nodes	4 (22)
Cutaneous/subcutanoeus	3 (17)
Brain	2 (11)
Lung	2 (11)
Cardiac	1 (6)
Urethra	1 (6)
Multitopic	2 (11)

Data are presented as n (%) unless otherwise stated. MUP, melanoma of unknown primary. [a]Classified according to American Joint Committee on Cancer staging system, 8th edition. [b]Four patients progressed to a maximum stage III, five had stage IV without prior stage III disease, all other patients progressed to stage IV (one patient only had retroperitoneal lymph node metastases). [c]Two located on lower back/buttocks, one widespread on scrotum.

Distribution of mutations and immunohistochemistry for BRCA1-associated protein 1 and programmed death-ligand 1

In 13 patients, a metastasis was used for targeted amplicon sequencing and IHC, and the primary was assessed in five patients. *GNA11* and *GNAQ* were detected with equal frequency (Figure 2). Of the nine *GNA11* mutations, eight affected c.626A>T (pQ209L) and one affected c.627G>C (pQ209H). Of the nine *GNAQ* mutations, seven affected c.626A>T (pQ209L), one affected c.627A>T (pQ209H) and one affected c.626A>G (pQ209R). Additional mutations were found in 12 patients (67%). Six patients had *SF3B1* mutations (three c.1873C>T1 pR625C, three c.1874G>A pR625H), four patients had *BAP1* mutations (c.182_186delAGGTC pK61fs, c.757C>T pQ253*, c.1957G>T pE653*, c.1873C>T pP175F) and three patients had *EIF1AX* mutations (c.25G>C G9R, c.6delC pK3fs, c.10A>G pN4D). The patient with the vaginal melanoma harboured a *GNA11* c.627G>C pQ209H and a *KIT*

Table 2 Individual patient and tumour characteristics of patients with nonuveal metastatic melanoma who had *GNAQ/11* mutation

Patient no.	Sex	Age (years)[a]	Vital status	Follow-up time (months)	Stage at first diagnosis[b]	Tumour thickness (mm)	Fist-line systemic treatment	Response to first-line treatment	Seccond-line systemic treatment	Response to second-line treatment	Total treatment lines	Tissue used for analyses	Mutational status	BAP1 IHC	PD-L1 IHC
1	Male	56	Dead	73	IIIB	n.a.	Ipilimumab + nivolumab	Progressive disease	Pembrolizumab	Progressive disease	3	Metastasis	GNA11 Q209L EIF1AX K3fs SF3B1 R625C	n.d.	n.a.
2	Male	39	Alive	35	IIIC	Unknown	Nivolumab	Progressive disease	Ipilimumab + nivolumab + radiotherapy	PR	2	Primary	GNA11 Q209L SF3B1 R625H	+	pos
3	Male	64	Alive	201	IIB	7·5					0	Metastasis	GNA11 Q209L SF3B1 R625H	+	pos
4	Male	31	Alive	133	Min. IIIA	Unknown	Gemcitabine + treosulfan	Progressive disease	Ipilimumab	SD	6	Metastasis	GNA11 Q209L BAP1 Q253	neg	neg
5	Female	62	Dead	25	IIIB	Unknown	Pembrolizumab	Progressive disease	Ipilimumab	Progressive disease	2	Metastasis	GNA11 Q209L BAP1 E653*	neg	n.a.
6	Male	31	Dead	159	Min. I	Unknown	Ipilimumab + nivolumab	SD	Carboplatin + paclitaxel	SD	2	Primary	GNA11 Q209L	+	n.a.
7	Male	50	Dead	83	IIB	5·9	Pembrolizumab	Progressive disease	Ipilimumab + pembrolizumab	Progressive disease	7	Metastasis	GNA11 Q209L	n.d.	n.a.
8	Male	22	Dead	52	IV	14	Ipilimumab	Progressive disease	DTIC	SD	8	Metastasis	GNA11 Q209L	n.d.	pos
9	Female	56	Dead	147	IIB	8·0	Cisplatin, DTIC + vindesine	CR	Imatinib	SD	4	Primary	GNA11 Q209H KIT K642E	n.d.	n.a.
10	Female	39	Dead	158	IIIC	n.a.					0	Metastasis	GNAQ Q209L SF3B1 R625C	neg	neg
11	Male	32	Alive	35	IV	n.a.					0	Metastasis	GNAQ Q209L SF3B1 R625C EIF1AX N4D	neg	neg
12	Male	63	Dead	5	IV	n.a.	Pembrolizumab	Progressive disease			1	Metastasis	GNAQ Q209L SF3B1 R625H	+	neg
13	Female	59	Dead	82	IIB	6	Nintendanib + paclitaxel	SD	Pembrolizumab	PR	5	Metastasis	GNAQ Q209L BAP1 K61fs	neg	neg
14	Female	53	Dead	12	IV	n.a.	Ipilimumab	Progressive disease	Ipilimumab + nivolumab	Progressive disease	3	Metastasis	GNAQ Q209L EIF1AX GR9	+	n.a.
15	Male	35	Alive	43	IIIC	6					0	Primary	GNAQ Q209L	n.d.	n.a.
16	Female	13	Alive	29	IIIC	8·9					0	Metastasis	GNAQ Q209L	n.d.	neg
17	Male	75	Dead	3	IV	n.a.	Ipilimumab + nivolumab	Progressive disease			1	Metastasis	GNAQ Q209H	+	pos
18	Male	60	Alive	37	IB	0·85	Pembrolizumab +/− epacadostat	PR			1	Primary	GNAQ Q209R NRAS Q61K	+	n.e.

CR, complete response; n.d., not done; n.a., not applicable; n.e., not evaluable; SD, stable disease; PR, partial response; IHC immunohistochemistry; PD-L1, programmed death-ligand 1; BAP1, BRCA1-associated protein 1; DTIC, dacarbazine; pos, positive; neg, negative. [a]At diagnosis of melanoma. [b]Classified according to American Joint Committee on Cancer staging system, 8th edition.

Table 3 Histology and location of primary tumour and metastatic spread of individual patients

Patient no.	Original histological report primary	Location of primary tumour	SLNB performed	SLNB status	Location of first metastasis	All metastases
1	n.a.	Occult	n.a.		Lymph nodes left axilla	Pulmonal, bones, hepatic, lymph nodes, brain
2	Subcutaneous metastasis, DD blue naevus-like melanoma with neutropic growth	Scrotum	No		Cardiac	Lymph nodes, cardiac
3	Nodular melanoma	Head and neck	No		Cervical lymph nodes	In-transit metastasis, lymph nodes
4	Subcutaneous nodular melanoma infiltration	Head and neck	Yes, after local recurrence of primary tumour	Positive	SLN	Lymph nodes, hepatic, cutaneous, scrotal, soft tissue (kidney/urethra/pararectal)
5	Blue naevus DD clear cell sarcoma or MM	Right upper arm	No		Lung	Lymph nodes, hepatic, lung, soft tissue
6	Proliferating melanocytic nodus with uncertain dignity on congenital blue naevus	Right flank	n.a.		Thoracical wall, potentially also sentinel as pigmented naevus cells were detected within sentinel	Thoracic wall, retreoperitoneal, pleural, gastric, hepatic, pleural, mediastinal
7	Animal-type melanoma	Head and neck	No		Cutaneous	Cutaneous, subcutaneous, hepatic, bones
8	Compatible with metastasis of melanoma or subcutaneous malignant cellular blue naevus	Head and neck	No		Skin, liver	Lymph nodes, hepatic, cutaneous, lung, pleural, brain peritoneal, retroperitoneal, soft tissue
9	Vaginal melanoma	Vaginal	No		Urethra	Urethra, pelvis, lymph nodes, hepatic, adrenal gland, bones
10	n.a.	Occult	n.a.		Lymph nodes left axila	No additional metastases
11	n.a.	Occult	n.a.		Lymph nodes retriperitoneal	No additional metastases
12	MUP DD primary cerebral melanoma	Occult	n.a.		Brain	Brain
13	Not available	Head and neck	No		Lung	Lung, soft tissue (infrarenal), hepatic, cutaneous, muscular, peritoneal, retroperitoneal, pancreatic infiltration, adrenal gland, hilae lymph nodes, bones
14	n.a.	Occult	n.a.		Lymph nodes, pleura, lung, peritoneal, liver, kidney, paracardiac, spinal colum, cerebral	Lymph nodes, pleural, lung, peritoneal, hepatic kidney, paracardial, spinal column, brain
15	MM on the basis of a pre-existing blue naevus	Lower back/buttocks	Yes	Positive	SLN	No additional metastases
16	Subcutaneous metastasis vs. malignant blue naevus	Lower back/buttocks	Yes	Positive	SLN	No additional metastases
17	MUP DD primary cerebral melanoma	Occult	n.a.		Brain	Lung, brain
18	Nodular melanoma	Left scapula	No		Soft tissue/lymph nodes, cutaneous	Soft tissue, lung, lymph nodes, bone, hepatic, peritoneal, retroperitoneal, bones, cutaneous, paragastral, adrenal gland

DD, differential diagnosis; MM, malignant melanoma; MUP, melanoma with unknown primary; n.a., not applicable; SLN, sentinel lymph node; SLNB, SLN biopsy.

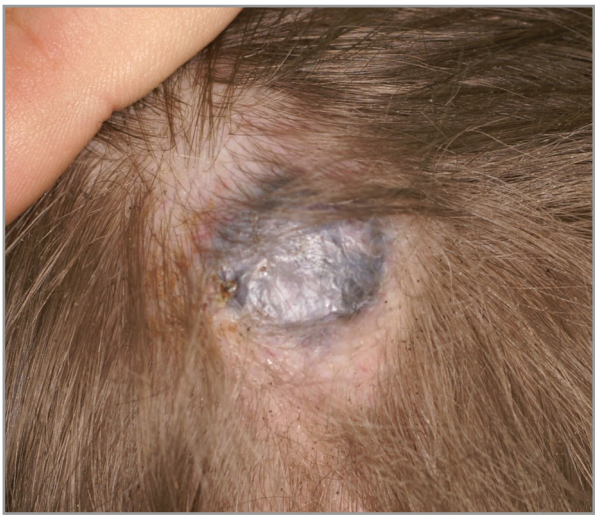

Figure 1 Blue naevus-like melanoma on the scalp of a 22-year old male patient (case 8). Breslow index 14 mm, no ulceration. The initial histology report described a lesion compatible with metastasis of melanoma or a subcutaneous malignant cellular blue naevus. At the time of initial diagnosis, the tumour had already metastasized to the skin and liver. The patient received ipilimumab and second-line treatment with dacarbazine and died approximately 2 years after the first melanoma diagnosis.

mutation (c.1924A>G pK642E). One patient with a *GNAQ* c.626A>G pQ209R mutation had a concurrent *NRAS* c.181C>A (pQ61K) mutation.

Loss of BAP1 protein expression could be confirmed by IHC in all four patients demonstrating a *BAP1* mutation. Of the 10 patients assessed for PD-L1 status, four showed positive staining and one was not evaluable.

Systemic therapy and response to treatment

A systemic first-line therapy for stage IV disease was given in 13 patients (Table 2). Most regimens (n = 10) were ICI-based; four of these were combination therapies (three anti-PD-1 antibody plus anti-CTLA4 antibody, one anti-PD-1 antibody plus epacadostat), four were anti-PD-1 antibody monotherapies and two were anti-CTLA4 antibody monotherapies. The best treatment response to ICI was progressive disease in seven patients, only one patient showed partial response (PR) (pembrolizumab plus epacadostat) and one exhibited stable disease (ipilimumab plus nivolumab). (Poly)chemotherapy treatments were administered in three patients, one in combination with nintedanib. One patient showed a complete response to cisplatin, dacarbazine and vindesine but progressed later. The other patients had stable disease and progressive disease as best responses. Second-line treatments were administered in 10 patients, seven of which were ICI treatments. Only two patients showed PR and a stable disease response to ICI therapy; however, one of these patients showed this response only in a concomitantly irradiated area. Three patients received chemotherapy or imatinib (patient with vaginal melanoma harbouring a KIT mutation), with stable disease as the best treatment response. Additional treatment lines were given in seven patients.

Tumour mutational burden and mutation distribution

To compare the mutational load and the amount of ultraviolet (UV) signature mutations, we assessed the TMB in eight samples, applying a larger sequencing panel covering 0·91 megabase of DNA. Our samples had lower mutation frequencies compared with other samples assessed using this panel (Figure 3a). Hypermutated samples were intentionally chosen for comparison. For each entity one case was used so that the comparison did not reflect a media of the single-nucleotide variant frequently mutated in each entity. To allow a comparison of *GNAQ/11* mutated tumours with a large cohort of other cutaneous melanomas, we assessed publicly available whole-exome datasets. *GNAQ/11* mutated tumours had lower mutational burden than *GNAQ/11* wildtype tumours ($P = 0{\cdot}02$, Wilcoxon test; Figure 3b). When we compared the substitution types of *GNAQ/11* mutated nonuveal melanomas (within TCGA) with *GNAQ/11* wildtype cutaneous and uveal melanomas, an intermediate profile was identified, demonstrating fewer C>T alterations (median 70·7%) than most cutaneous melanoma samples (85%); however, this was considerably more than in uveal melanoma samples (48%, Figure 3c).

Discussion

In summary, we analysed a cohort of metastatic *GNAQ/11* mutant melanomas that were not of uveal origin. These tumours are rare[32,33] and our data demonstrate that their biological behaviour is distinct from conventional cutaneous and uveal melanoma.

The histological evaluation of *GNAQ/11* mutant nonuveal melanomas seems to be challenging. In six tumours from our cohort the initial diagnosis was uncertain, as differentiation from benign melanocytic proliferations or subcutaneous metastasis was difficult. Cutaneous melanoma is mostly epidermally derived and histological detection of a dermal malignant melanocytic proliferation without epidermal involvement generally represents a cutaneous metastasis. However, in *GNAQ/11* mutant cutaneous melanomas the tumour arises in the dermis. Therefore, we believe that this data suggests that cases of nonbenign dermal melanocytic proliferations should be sequenced to exclude a primary *GNAQ/11* mutant cutaneous tumour. Whenever a *GNAQ/11* mutation is detected, clinical analysis and/or imaging should be performed to exclude uveal melanoma, as most *GNAQ/11* mutant melanomas arise in the uveal region.

Interestingly, one-third of patients reported in this study were diagnosed with MUP. This rate is remarkably higher than the < 20% rate of MUP diagnosed among all patients with melanoma who present with regional or distant metastasis.[34] In two of the cases diagnosed as MUP, patients were reported

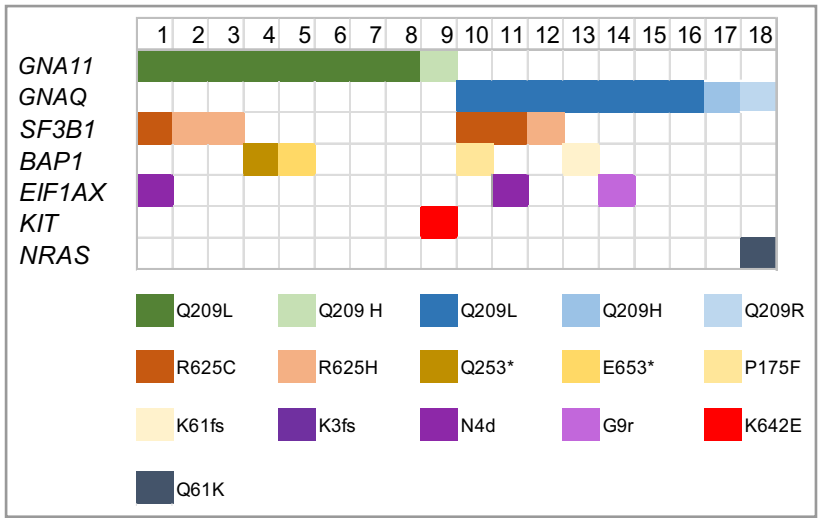

Figure 2 Activating mutations identified by targeted next-generation sequencing in 18 patients with metastatic melanoma and *GNAQ* or *GNA11* mutation. *SF3B1*, splicing factor 3b subunit 1; *BAP1*, BRCA1-associated protein; *EIF1AX*, eukaryotic translation initiation factor 1A X-linked; *KIT*, tyrosine-protein kinase; *NRAS*, neuroblastoma rat sarcoma viral oncogene homolog. Different protein alterations are depicted by assorted colour shades.

to have cerebral metastasis as the first tumour localization. Retrospectively, it is likely that both cases represented a cerebral primary rather than a metastasis (case 12 and case 17). Of the other four MUP cases, three patients had lymph node metastases and one patient had a polytopic metastasis at first diagnosis. Primary tumours in blue naevus-like melanoma can be located deep in the dermis and therefore they may never be detected, they may be mistaken for a subcutaneous metastasis (case 14) or before being recognized they may have gone into complete remission (cases 1, 10 and 11) and patients present with palpable lymph node metastases.

In this cohort of metastatic *GNAQ/11* nonuveal melanomas, most of the *GNAQ/11* mutations were the well-recognized activating Q209L hotspot mutations. Three rarer 209 mutations, a Q209R and two Q209H mutations were also identified. Interestingly, in two cases the tumours were found to harbour additional activating mutations in other genes. The *GNA11* Q209H samples also had a *KIT* K642E mutation and the *GNAQ* Q209R mutant sample had a concurrent *NRAS* Q61K mutation. This is unusual as mutations in *GNAQ/11* and activating *BRAF*, *RAS* or *KIT* mutations are generally mutually exclusive.[6,35] This could suggest that Q209R or Q209H mutations are less activating, with tumours requiring additional mutations to promote malignant behaviour. If this proves true, tumours should potentially be primarily grouped based on the other present activating mutation (i.e. *KIT* or *NRAS*). Larger cohorts and functional studies will be required to elucidate this assumption further.

Similar to uveal melanoma,[24,29,33] we found *GNAQ/11* mutations in cutaneous melanoma to co-occur frequently with *EIF1AX*, *SF3B1* or *BAP1* mutations. The numbers in this study are of course very low, but a clear association of different genetic events with prognosis, as observed in uveal melanoma, was not apparent. Patients with *BAP1* alteration tumours were not found to have an obvious poorer prognosis than patients with tumours harbouring *EIF1AX* or *SF3B1* mutations. These data will need to be assessed in larger cohorts (when available); however, our current data suggest that prognosis of patients with cutaneous *GNAQ/11* mutant melanoma cannot be reliably predicted based on *EIF1AX*, *SF3B1* or *BAP1* status.

The metastatic profile of our cohort draws attention to another interesting finding of our study. Uveal melanomas metastasize primarily haematologically to the liver[36] and initial lymph node metastasis, as is frequent in cutaneous melanoma, generally does not occur. The reason for this metastatic spread pattern is thought to be the lack of a lymphatic drainage system in the uvea. Another possibility is that uveal melanomas intrinsically metastasize haematologically. In our cohort of patients with cutaneous *GNAQ/11* mutant melanoma, almost all patients had lymph node metastases and some even had positive sentinel lymph nodes. This suggests that such genetic alterations are not associated with a primary haematological metastasis and despite having a similar genetic signature to uveal melanoma, *GNAQ/11* mutant nonuveal melanomas metastasize lymphatically and therefore routine clinical analysis of the draining lymphatic tissue should be performed.

TMB may be associated with poor response to immunotherapy.[37] High TMB has been associated with stronger response to immunotherapy in most cancers.[38] While TMB is high in cutaneous melanoma, it is notoriously low in uveal melanoma.[29] In both targeted sequencing of eight tumour samples from our cohort, and in an analysis of publicly available sequencing data from *GNAQ/11* mutant cutaneous melanoma in the TCGA cohort (Figure 3), a low TMB was detected. When examining the TCGA data, the difference in mutation number compared with conventional cutaneous melanoma samples was found to be statistically significant. The mutation profile of samples from our cohort showed fewer UV

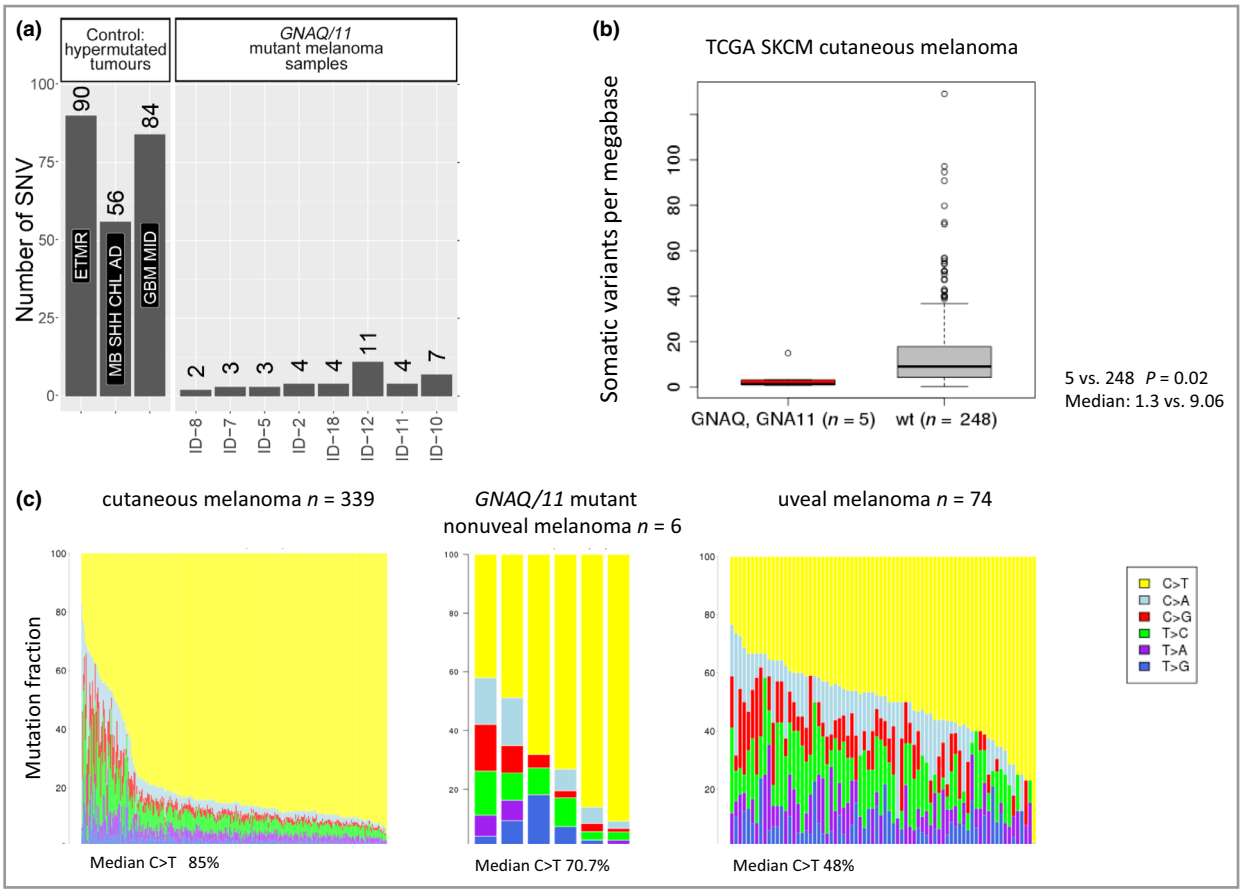

Figure 3 (a) Comparison of number of single-nucleotide variant (SNV) calling of eight *GNAQ/11* mutant melanoma samples vs. hypermutated tumours as controls. Tumour mutational burden of our cohort was low compared with other heavily mutated samples. (b) Mutational burden of The Cancer Genome Atlas (TCGA) melanoma tumours comparing *GNAQ/11* mutated vs. wildtype (wt) samples (Wilcoxon test). The sums of nonsynonymous somatic mutations are shown, which can be assumed to have the potential to create neoantigens. *GNAQ/11* mutated tumours have significantly lower mutational burden than *GNAQ/11* wildtype tumours. (c) Comparison of single-nucleotide substitutions for somatic variants in a cutaneous melanoma cohort (n = 339), *GNAQ/11* mutant nonuveal melanoma (n = 6) and a uveal melanoma cohort (n = 74) showed an intermediate profile of nonuveal *GNAQ/11* mutated melanomas demonstrating a lower ultraviolet gene signature (C>T alterations, yellow) than cutaneous melanoma and a higher ultraviolet gene signature than uveal melanoma. ETMR, embryonal tumours with multilayer rosettes; GBM MID, glioblastoma multiforme midline; MB SHH CHL AD, methylation class medulloblastoma, subclass sonic hedgehog A (children and adult); SKCM, skin cutaneous melanoma.

mutations compared with cutaneous melanoma. The explanation for this appears to be straightforward, as *GNAQ/11* mutant cutaneous tumours usually arise in the dermis and are only modestly exposed (if at all) to UVB irradiation, which is primarily absorbed in the epidermis. However, the TCGA data analysis did suggest that more mutations with a UV signature are found in *GNAQ/11* mutant cutaneous melanomas than uveal melanomas.

In recent years, therapeutic options for cutaneous melanoma have improved dramatically with the use of BRAF and MEK inhibitors, and impressive therapeutic responses have been demonstrated by ICI.[39–43] However, these therapeutic advances cannot be transferred to *GNAQ/11* mutant uveal melanomas. BRAF/MEK inhibition is not an option in *GNAQ/11* mutant tumours. Immunotherapy has demonstrated poor efficacy in uveal melanoma.[12,44] The findings of our current cohort suggest that *GNAQ/11* mutant nonuveal melanomas also respond poorly to immunotherapy. The efficacy of immunotherapy will need to be further assessed in larger studies. Considering a lack of other effective therapeutic approaches, immunotherapy certainly remains a valid therapeutic option, potentially even the best option that is currently available. When novel therapies for uveal melanoma become available, these might also prove to be valuable for nonuveal *GNAQ/11* mutant melanoma.

Our study has some limitations. The significance of our findings is limited by the small number of cases owing to the rarity of *GNAQ/11* mutant nonuveal tumours. Validation studies should be performed using larger cohorts. Furthermore, the studies were performed on heterogeneous samples, as both primary tumours and metastases were used, and tissue samples were derived at different timepoints during the

disease course. Moreover, there was also heterogeneity with regard to systemic treatments. While these factors could affect mutational status, they are not likely to have influenced the genes relevant for this study. PD-L1 expression can vary between primary and metastatic tissue and can change with systemic treatment. As it is not a clear marker for response to therapy in melanoma, we intentionally did not focus on PD-L1 expression in our study; however, we did report it for the sake of completeness. The TMB analysis could be performed in only eight patients owing to the lack of sufficient high-quality DNA in the remaining samples.

In summary, the clinical data and the mutation profile suggest that *GNAQ/11* mutant nonuveal melanomas differ significantly from conventional cutaneous melanoma. Despite a strong genetic relationship, they also differ from uveal melanoma by having more UV mutations and a different metastatic pattern. Similar to uveal melanoma, it appears that patients with these tumours do not benefit sufficiently from recent treatment advances in advanced melanoma and require novel therapeutic approaches.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

File S1 Supplemental material and methods.

Table S1 Genes covered in the applied sequencing panel.

Table S2 Summary of next-generation sequencing results of investigated genes in the *GNAQ/11* mutant cutaneous melanoma cohort with metastasis.

Appendix

Conflicts of interest: E.L. has served as a consultant and/or has received honoraria from Amgen, Actelion, Roche, Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Janssen, Medac, and has received travel support from Amgen, Merck Sharp & Dohme, Bristol-Myers Squibb, Amgen, Pierre Fabre, Sunpharma and Novartis (outside the submitted work). A.Z. has received travel support from Novartis and Bristol-Myers Squibb (outside the submitted work). S.U. declares research support from Bristol-Myers Squibb and Merck Serono, speaker and advisory board honoraria from Bristol-Myers Squibb, Merck Sharp & Dohme, Merck Serono, Novartis and Roche, and has received travel support from Bristol-Myers Squibb, Merck Sharp & Dohme (outside the submitted work). M.S. has served as a consultant and/or has received honoraria from Amgen, Roche, Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Kyowa Kirin and has received travel support from Amgen, Sunpharma and Novartis. J.C.H. has served as consultant and/or has received honoraria from Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Pierre Fabre, Pfizer, Roche and Sanofi Aventis, and has received travel support from Pierre Fabre (outside the submitted work). R.H. has served as consultant and/or has received speaker's honoraria from Amgen, Roche, Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Pierre Fabre and Sunpharma. J.S.U. is on the advisory board and/or has received honoraria and travel support from Amgen, Bristol-Myers Squibb, GSK, LeoPharma, Merck Sharp & Dohme, Novartis, Pierre Fabre and Roche (outside the submitted work). B.W. has served as consultant and/or has received honoraria from Amgen, Roche, Bristol-Myers Squibb, Merck Sharp & Dohme, Philogen, Curevac and Novartis. R.G. reports research support from Novartis, Pfizer, Johnson & Johnson, Amgen, MerckSerono in addition to speaker and advisory board honoraria from Roche Pharma, Bristol-Myers Squibb, GlaxoSmithKline, Novartis, Merck Serono, MSD, Almirall-Hermal, Amgen, Pierre Fabre, Sanofi, Leo Pharma, Amgen, Pfizer, Roche Posay, Incyte, Merck Serono, 4SC and Takeda. H.R. is on the advisory board of Bristol-Myers Squibb, has received honoraria from Roche and Bristol-Myers Squibb, travel support from Philips, Roche and Bristol-Myers Squibb, and grants from Bristol-Myers Squibb. S.M.-B. served as consultant and/or has received honoraria from Roche Pharma, Bristol-Myers Squibb, Novartis, Pfizer and AstraZeneca. F.S. reports travel support and/or speaker's honoraria and/or advisory board honoraria from Agilent, Medac, Illumina and AbbVie (outside the submitted work). A.v.D. reports travel support or speaker's honoraria or advisory board honoraria from Illumina and AbbVie (outside the submitted work). I.C. has received travel grants from Roche, GSK, Bristol-Myers Squibb, Teva and Novartis (outside the submitted work). L.Z. has served as a consultant and/or has received honoraria from Roche, Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Pierre Fabre, Sanofi and has received travel support from MSD, Bristol-Myers Squibb, Amgen, Pierre Fabre and Novartis (outside the submitted work). D.S. reports grants and other support from Bristol-Myers Squibb, personal fees from Bristol-Myers Squibb during the conduct of the study, personal fees from Amgen, personal fees from Boehringer Ingelheim, personal fees from InFlarX, personal fees and other support from Roche, grants, personal fees and other support from Novartis, personal fees from Incyte, personal fees and other support from Regeneron, personal fees from 4SC, personal fees from Sanofi, personal fees

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