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RESEARCH

Mutations in G Protein Encoding Genes and Chromosomal Alterations in Primary Leptomeningeal Melanocytic Neoplasms

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Abstract Limited data is available on the genetic features of primary leptomeningeal melanocytic neoplasms (LMNs). Similarities with uveal melanoma were recently suggested as both entities harbor oncogenic mutations in *GNAQ* and *GNA11*. Whether primary LMNs share additional genetic alterations with uveal melanoma including copy number variations is unknown. Twenty primary LMNs ranging from benign and intermediate-grade melanocytomas to melanomas were tested by direct sequencing for hotspot mutations in the genes *GNA11, GNAQ, BRAF, NRAS* and *HRAS*. Furthermore, the lesions were tested for copy number variations of chromosomes frequently present in uveal melanoma (1p, 3, 6 and 8q) by multiplex ligation-dependent probe amplification (MLPA). Genome-wide analyses of copy number alterations of two leptomeningeal melanocytic neoplasms were performed using the OncoScan SNP-array. $GNAQ^{Q209}$ mutations were present in eleven LMNs, while two of 20 cases carried a $GNA11^{Q209}$ mutation. No *BRAF*, *HRAS* or *NRAS* hotspot mutations were detected. Monosomy 3 and gain of 8q were present in one leptomeningeal melanoma, and one intermediate-grade melanocytoma harbored a gain of chromosome 6. With MLPA, the melanocytomas did not show any further gross chromosomal variations. Our data shows that primary LMNs, like uveal melanoma, harbor oncogenic mutations in *GNAQ* and *GNA11* but lack mutations in *BRAF*, *NRAS* and *HRAS*. This finding may help in the differential diagnosis between a primary LMN and a metastasis from a cutaneous melanoma to the central nervous system. Copy number variations in some aggressive LMNs resemble those present in uveal melanoma but their prognostic significance is unclear.

Keywords Leptomeningeal melanocytic neoplasm · Melanocytoma · Melanoma · Central nervous system · *GNAQ* · *GNA11*

Abbreviations

LMN	leptomeningeal melanocytic neoplasms
MC	melanocytoma
IMC	intermediate-grade melanocytoma
LMM	leptomeningeal melanoma
UM	uveal melanoma
CM	cutaneous melanoma
CNS	central nervous system

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Introduction

Primary leptomeningeal melanocytic neoplasms (LMNs) comprise a spectrum of neoplasms ranging from benign melanocytoma to melanoma. [1] Melanocytomas (MCs) are slow-growing neoplasms generally characterized by a benign clinical course, although local recurrence and malignant transformation can occur. [2–5] Intermediate-grade melanocytomas (IMCs) show histological features that are suggestive of more aggressive behavior, and their clinical behavior is unpredictable. [1, 6] Primary leptomeningeal melanomas (LMMs) are histological similar to melanomas arising in other sites but rarely metastasize to remote organs. [7, 8]

Systematic study of LMNs is hampered by their very low incidence, and the genetic events underlying the oncogenesis of these tumors are largely unknown. Several recent studies, including our previous study, reported the presence of activating $GNAQ^{Q209}$ mutations in a substantial proportion of LMNs (37 %). [9–11] Identical $GNAQ^{Q209}$ mutations are present in uveal melanomas (UMs) and blue nevi, but they are very rare in cutaneous melanomas (CMs). [12–15] The *GNAQ* gene encodes the α-subunit of G-proteins ($G\alpha_q$) that are associated with G protein coupled receptors on the cell surface. [16] Substitution of the critical glutamine (Q at codon 209) or arginine (R at codon 183) of $G\alpha_q$ leads to decreased GTPase activity, preventing hydrolysis of GTP and locking *GNAQ* in its active, GTP-bound state. This results eventually in constitutive activation of the MAPK pathway. [12, 14, 16–18] Activating mutations in R183 of *GNAQ* are very rare in UMs and blue nevi, and little is known about its incidence in LMNs. [14, 19]

Activating mutations in *GNA11*, a homologue of *GNAQ* located on chromosome 19p13, are present in UMs and blue nevi, and were more recently demonstrated in some cases of LMNs as well. [10, 14, 19, 20]

The gross chromosomal alterations present in LMNs are yet to be investigated. In UMs, monosomy 3 is a frequent event and strongly associated with metastatic disease (50 %). [21, 22] Other frequent chromosomal aberrations in UMs include gain of chromosome 8q that is also associated with metastasis, and loss of chromosome 6p, that is associated with good prognosis. [21] Based on the presence of *GNAQ* and *GNA11* mutations in both LMNs and UMs, we hypothesized that LMNs might also share some gross chromosomal alterations with UMs as well.

In this study, we investigated a series of twenty LMNs for mutations in Q209 and R183 of both *GNA11* and *GNAQ*, as well as for other well-known "hotspot" mutations in *BRAF*, *NRAS* and *HRAS*. Additionally, lesions were tested for copy number variations of chromosomes frequently present in UMs (1p, 3, 6, 8q) using MLPA and two LMN cases were selected for in depth investigation of copy number alterations across the genome using the OncoScan SNP-array. Genetic changes were correlated with clinicopathological characteristics and patient follow-up data.

Materials and Methods

Patients and Histopathology

For this retrospective study, we collected formalin-fixed, paraffin-embedded tissues of 20 LMNs, including 14 cases from our previously published series. [9] The diagnosis of 'melanocytoma' (MC) ($n=11$), 'intermediate-grade melanocytoma' (IMC) ($n=5$) or 'leptomeningeal melanoma' (LMM) ($n=4$) was based on the criteria previously described. [1, 6, 9] None of these patients had clinical evidence of primary melanoma outside the central nervous system (CNS).

DNA Extraction and Mutational Analysis

DNA extraction and assessment of DNA quality by the BIOMED-2 control gene PCR were performed as previously described. [9] All but seven DNA samples allowed amplification of the 200 bp amplicon, minimally. [23] The remaining cases ($n=7$) allowed amplification of the 100 bp-amplicon only, and still could be evaluated by mutational analysis (Table 1).

Twenty LMNs were investigated for "hotspot" mutations: Q209 mutations in exon 5 and R183 mutations of exon 4 in *GNAQ* and *GNA11*; V600 mutations in *BRAF*; G12, G13, G15 mutations in exon 2 and Q61 mutations in exon 3 of the *NRAS* gene; and Q61 mutations in exon 3 of *HRAS*. Primer sequences are listed in Online Resource 1. PCR conditions as well as assessment on the ABI PRISM 3730 DNA analyzer were standardized and described previously. [9] PCR for direct sequence analysis of exon 4 of *GNAQ* and *GNA11* were performed using the Amplitaq Gold 360 Master Mix (Applied Biosystems) with standardized cycling conditions and duplicate PCRs were submitted for Sanger sequencing on an ABI PRISM 3730 DNA analyzer (Life Technologies, Foster City, USA).

Determination of Copy Number Variations

1. MLPA procedure We used the MLPA P027-B1 assay prepared by MRC-Holland (Amsterdam, the Netherlands) containing probes on chromosomes 1p, 3, 6, and 8q (as the MLPA assay contains only one probe for chromosome arm 8p we did not include 8p analysis in our study).

A further twelve probes were used as reference probes (details available at; http://www.mlpa.com). Immortalized cell lines of healthy controls were used as baseline control

Table 1 Patient characteristics, histopathology and results of mutational analysis and copy number changes detected by MLPA

Patient	Sex	Age	Diagnosis	Location	Cell type	Pigmentation	Follow-up	*GNA11* ex 5	*GNA11* ex 4	*GNAQ* ex 5	*GNAQ* ex 4	Chrom 1p	Chrom 3p	Chrom 3q	Chrom 6p	Chrom 6q	Chrom 8q
1*	F	27	MC	right CPA	E	no	Local recurrence. No distant metastases.	wt	wt	wt	wt	-	-	-	-	-	-
2 *, #	M	41	MC	C0-C3	E	3+	Local recurrence. Stable since. Alive.	wt	wt	c.626A>C (p.(Gln209Pro))	wt	-	-	-	-	-	-
3 *, #, +	M	27	MC	cerebellar tentorium	Mx	2+	LM seeding. Stable since. Alive.	wt	wt	c.626A>C (p.(Gln209Pro))	wt	na	na	na	na	na	na
4*	M	55	MC	C3-6	Mx	2+	Local recurrence. Stable since. Alive.	wt	wt	c.626A>C (p.(Gln209Pro))	wt	-	-	-	-	-	-
5 *, #, +	na	na	MC	C5-6	E	3+	Tumor spread in neck and vertebra. Deceased.	wt	wt	wt	wt	na	na	na	na	na	na
6 *, #, +	M	41	MC	Th6	S	3+	Local recurrence.	na	na	c.626A>T (p.(Gln209Leu))	na	na	na	na	na	na	na
7 *, #, +	F	45	MC	L3-4	Mx	3+	LM seeding. No distant metastases. Alive.	wt	wt	c.626A>T (p.(Gln209Leu))	wt	na	na	na	na	na	na
8 *	na	na	MC	Th11	S	1+	na	wt	wt	c.626A>T (p.(Gln209Leu))	wt	-	-	-	-	-	-
9 *, +	na	na	MC	cerebellar	S	2+	na	wt	wt	wt	wt	na	na	na	na	na	na
10 *, #, +	na	na	MC	na	S	3+	na	wt	wt	wt	wt	na	na	na	na	na	na
11	na	na	MC	EM, ID	E	3+	No recurrence. Stable. Alive.	wt	wt	c.626A>T (p.(Gln209Leu))	wt	-	-	-	-	-	-
12	na	na	IMC	IM	Mx	3+	No recurrence. Stable. Alive.	wt	wt	wt	wt	-	-	-	-	na	na
13	na	na	IMC	cauda	S	2+	No recurrence. Stable. Alive.	wt	wt	c.626A>T (p.(Gln209Leu))	wt	-	-	-	-	-	-
14 &	M	na	IMC	IM	E	1+	Local recurrence. LM seeding. Alive.	wt	wt	c.626A>T (p.(Gln209Leu))	wt	-	-	-	gain&	gain&	na
15 *, +	na	na	IMC	LM	E	3+	na	na	na	wt	na	na	na	na	na	na	na
16 *	F	68	IMC	cerebellar tentorium	E	1+	Deceased (not disease related)	c.626A>T (p.(Gln209Leu))	wt	wt	wt	-	-	-	-	-	-
17 *, #	na	na	LMM	LM	E	no	na	wt	na	wt	wt	-	na	na	na	na	-
18 *, &	F	59	LMM	S2	Mx	1+	Distant metastases (bone, lungs). Deceased.	wt	wt	c.626A>T (p.(Gln209Leu))	wt	-	loss&	loss&	-	-	gain&
19	F	32	LMM	Th10-11	Mx	1+	LM seeding. No extradural disease. Deceased.	c.626A>T (p.(Gln209Leu))	wt	wt	wt	-	-	-	gain	loss	-
20	M	55	LMM	L1-L2	Mx	1+	LM seeding. No distant metastases. Deceased.	wt	wt	c.626A>C (p.(Gln209Pro))	wt	na	na	na	na	na	na

F female, *M* male, *MC* melanocytoma, *IMC* intermediate-grade melanocytoma, *LMM* leptomeningeal melanoma

CPA cerebello-pontine angle, *EM* extramedullary, *ID* intradural, *LM* leptomeningeal, *S* spindle, *E* epithelioid, *Mx* mixed

* cases of which results of mutation analysis were in part described previously [9]

- no copy number changes with MLPA

na not available (sex, age, location, follow-up) or not assessable (MLPA and/or mutation analysis)

cases with 100 bp-amplicon in the BIOMED-2 gene control PCR (n=7). The remaining cases (n=13) allowed amplification of the 200 bp-amplicon, minimally

& copy number alterations of two cases confirmed by use of OncoScan analysis (Affymetrix)

Cases not assessable in the mutational analysis (data not further shown): case 6 for *GNA11* and *NRAS*, case 7 for *HRAS*, case 10 and 18 for *NRAS* ex 2, case 15 for *GNA11, NRAS* and *HRAS,* case 17 for *GNA11* exon 4

Nine cases not assessable with MLPA (*na*), possibly due to the combination of low quality DNA (100 bp-amplicon in the BIOMED-2 gene control PCR, marked with #) and/or heavily pigmentation, marked with +

samples. MLPA was performed according to the manufacturer's instructions, and as previously described. [21] Samples were analyzed on an ABI 3730 DNA analyzer using the Genescan software (Life Technologies, Foster City, USA). Details on data analysis can be found in Online Resource 2.

2. OncoScan analysis One LMM and one IMC (Table 1), selected due to the presence of copy number variations (CNVs) detected by MLPA, were further investigated for CNVs across the whole genome by OncoScan analysis (molecular inversion probe technology), as described by Wang et al. [24] This array consists of ~335,000 probes of which the majority (~283,000) are SNP based. DNA was processed by the Affymetrix Research Services Laboratory (ARSL, Santa Clara, California, USA). The normalized OncoScan data (^{2}Log(test/reference)-ratios and B-allele frequency plots) were analyzed with the Nexus copy number software version 5.1 (Biodiscovery, Inc, El Segundo, California, USA) for copy number calling.

Statistical Analysis

Fisher's exact test was used to test whether significant associations were present between mutational status and cell type; location of the lesion (spinal versus intracranial); disease-related death; and clinical behavior of the lesion (stable versus progressive lesions, the latter including local recurrence, leptomeningeal seeding and/or distant metastasis). Statistical analyses were performed using SPSS 16.0 for Windows (IBM Statistics 20, IBM Corporation, Armonk, New York, United States).

Results

Patients and Histopathological Characteristics

Our study group consisted of 11 MCs, five IMCs and four LMMs (Table 1). Most lesions were located around the spinal cord (13/20). MC recurred in four patients and aggressive behavior with leptomeningeal seeding or locoregional disease progression was present in three MC patients (#3, #5, #7). One IMC recurred with leptomeningeal seeding (patient #14), while two IMCs had stable disease (patients #12 and #13). Follow-up data was available for three of four LMM patients and all had succumbed to their disease. One LMM patient presented with a melanoma in the sacral region and developed distant metastases in the bone and lungs two years after initial diagnosis (patient #18). The late manifestation of other melanoma locations in this patient is supportive of the sacral lesion being the primary tumor. The other two LMM patients demonstrated subsequent leptomeningeal seeding but did not develop distant metastases (patients #19 and #20).

Mutational Analysis

A summary of the mutational analyses is provided in Table 1. Briefly, mutations in codon 209 of *GNAQ* in were detected in 11 neoplasms (55 %), while in two other LMNs a mutation in codon 209 of *GNA11* was observed (10 %). In this series, GNA11^{Q209} and GNAQ^{Q209} mutations were mutually exclusive. Both GNA11^{Q209} mutations resulted in substitution of glutamine at position 209 by leucine c.626A>T (p. (Gln209Leu)) alias Q209L (Fig. 1a), and were present in an IMC and LMM (patients #16 and #19) (Fig. 1b and c). GNAQ^{Q209} mutations were present in seven of 11 MCs (64 %), two of five IMCs (40 %) and two of four LMMs (50 %). These GNAQ^{Q209}

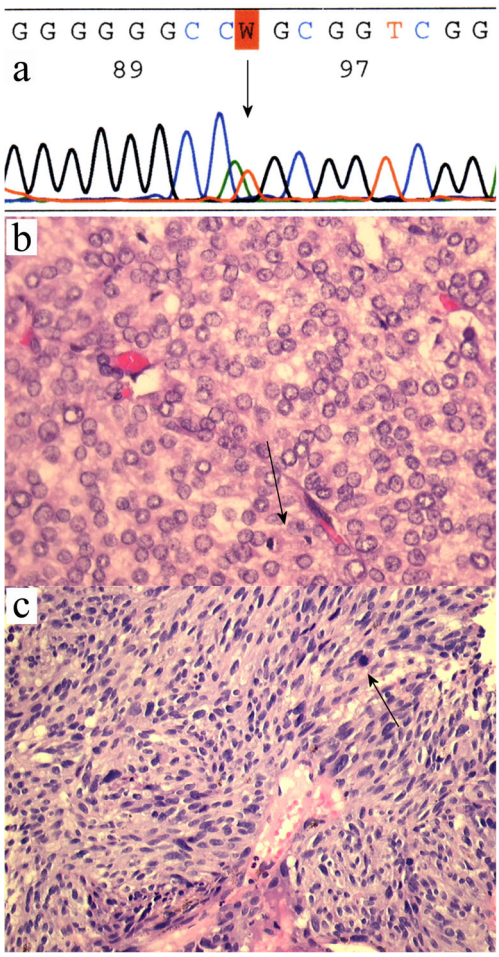

Fig 1 The GNA11^{Q209L} mutation was present in patient #16 (IMC) and patient #19 (LMM). **a.** Forward sequence tracing for *GNA11* surrounding codon 209 of exon 5, showing the mutation c.626 A>T (p. (Gln209Leu)) for patient #16 (IMC). **b.** *GNA11*-mutated IMC in the cerebellar tentorium (magnification 400x) showing an epithelioid cell type and increased mitotic activity (*arrow*) (patient #16). **c.** *GNA11*-mutated melanoma in a 32-year-old female (magnification 200x) located at the spinal region Th10-11 (patient #19), showing a mixed cell type, nuclear pleomorphism and mitotic activity (*arrow*)

mutations resulted in substitution of glutamine to leucine in 64 % (7/11) and to proline in 36 % of cases (4/11).

In all samples, "hotspot" mutations in exon 4 of *GNAQ* and *GNA11*, and in *BRAF*, *HRAS* or *NRAS* were absent.

There was no significant association between *GNAQ/GNA11* mutational status and cell type, location of the lesion, disease-related death, or clinical behavior (Fisher's exact, $P>0.05$).

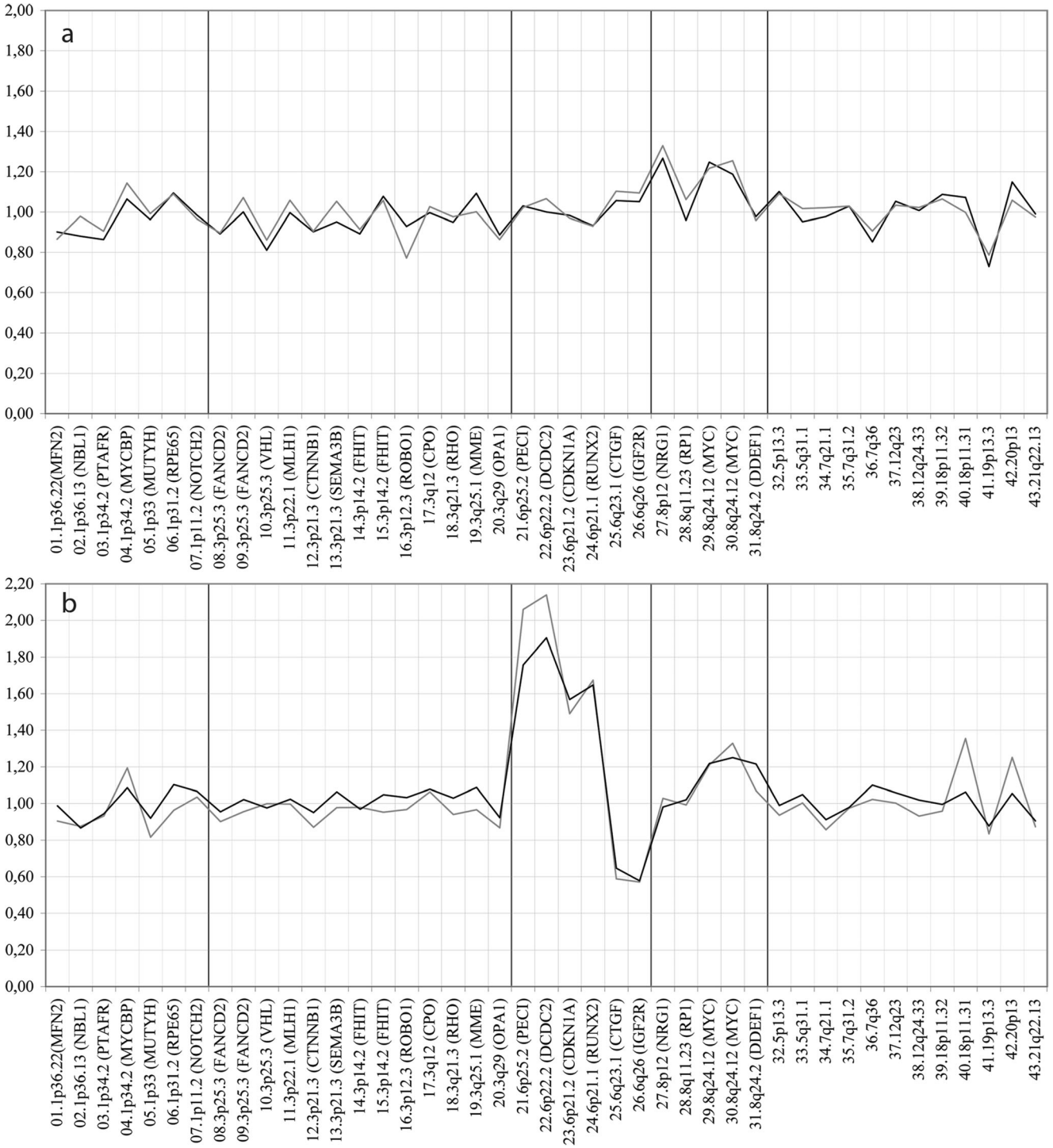

Fig 2 MLPA results for patient #8 (*GNAQ*-mutated MC) and patient #19 (*GNA11*-mutated LMM). **a.** MLPA results of a *GNAQ*-mutated MC located in the spinal canal at level of the 11th thoracic vertebrae (patient #8), without evident copy number changes. The ratio of 1.3 at 8p12 (probe 27) and the ratio of 1.2-1.3 at 8q24.12 (probes 29 and 30) were considered within the normal range. **b.** *GNA11*-mutated primary leptomeningeal melanoma showing gain of 6p and loss of 6q by MLPA (patient #19). The ratio of 1.3 at 8q24.12 (probe 30) was considered within the normal range

Copy Number Variations

MLPA results for eleven cases were of sufficient quality for further analysis (Table 1). Five MC cases did not harbor gross CNVs for the chromosome regions investigated (Fig. 2a, patients #1, #2, #4, #8 and #11). Three of four IMCs did not show chromosomal CNVs (patient #12, #13 and #16; patient #12 and #13 had stable disease and patient #16 died of non-disease related illness. Of note, patient #12 could only be evaluated for 1p, 3p, 3q and 6p). One IMC showed gain of chromosome 6 confirmed by OncoScan analysis (Fig 3a, Online Resource 3, patient #14). This was a *GNAQ*-mutated IMC showing local recurrence and leptomeningeal seeding at follow-up. With the OncoScan analysis, no other gross chromosomal CNVs were detected in this sample. Of the two LMMs that were evaluated using MLPA, one *GNA11*-mutated LMM showed gain of 6p, and loss of 6q (Fig. 2b, patient #19). The patient with this LMM suffered from leptomeningeal seeding at presentation but had no extradural melanoma dissemination. The second case was a *GNAQ*-mutated LMM that demonstrated monosomy 3 and gain of 8q, confirmed by OncoScan analysis (patient #18) (Fig. 3b). In addition, the OncoScan data revealed the following CNVs for this LMM: loss of chromosome 1p, loss of chromosome 2p, loss of chromosome 8p, loss of chromosomes 10, 11 and 18, and gains of chromosomes 4p, 17 and 22 (Fig. 3b, Online Resource 4).

Discussion

We previously demonstrated the presence of oncogenic mutations in Q209 of *GNAQ* in a series of LMNs (37 %). [9] In the present study, we broadened our sequencing analysis and

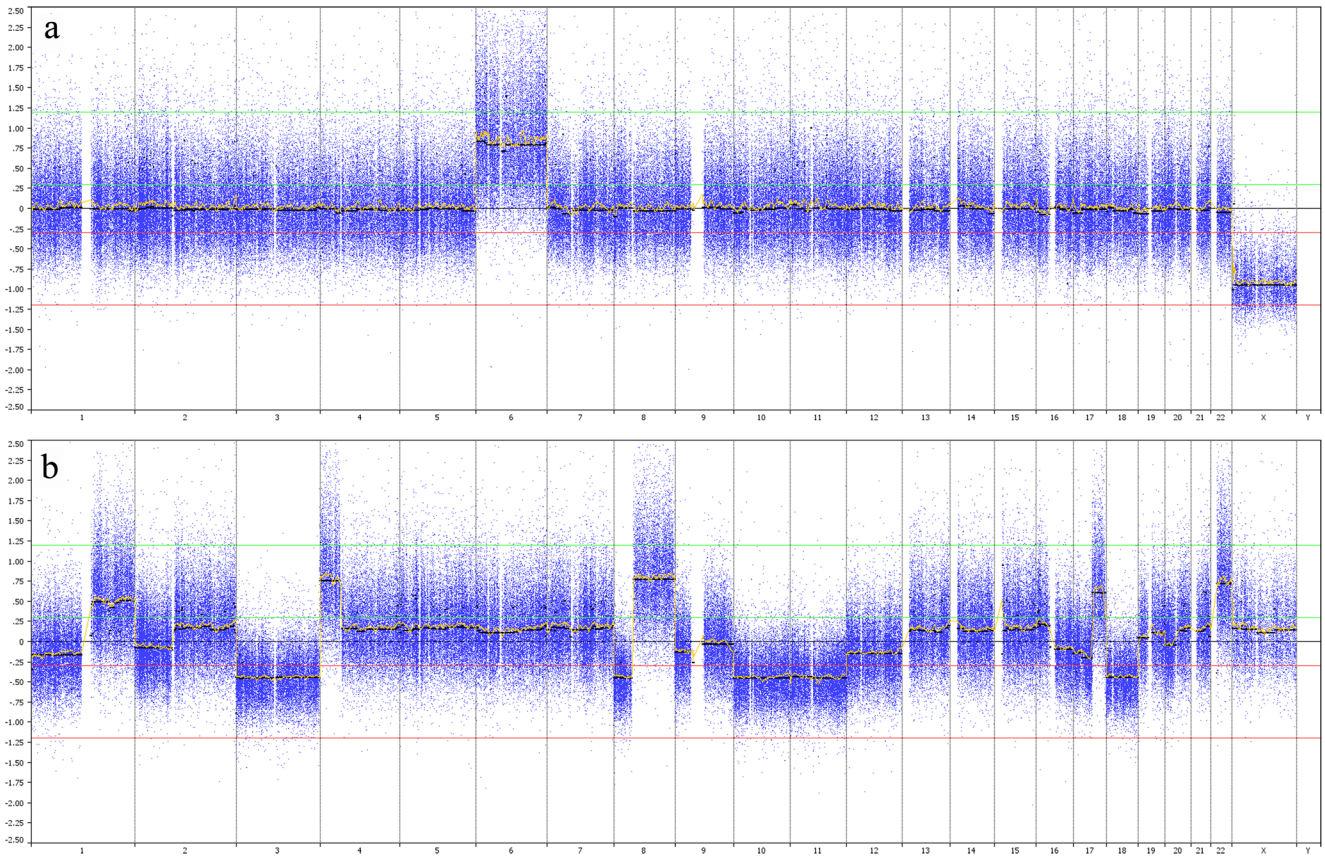

Fig. 3 Copy number variation plots obtained by OncoScan SNP-analysis for patient #14 (**a**) (IMC) and patient #18 (**b**) (LMM), using the NEXUS 5.1 software package. The normalized and log2-transformed patient-over-reference intensity ratios (^{2}Log(T/R)) for each array probe are displayed (blue dots) according to their chromosomal position (from 1pter – Yqter), with each chromosome separated with a dotted vertical line. The results of a statistical copy number algorithm are shown by the black horizontal lines, with a normal copy number (n=2) at the 0-line, a loss (deletion) going down (negative probe values) and a gain (duplication/amplification) going up (positive probe values). The corresponding allelic frequency plots, which are also obtained by the OncoScan SNP-analysis, are shown in the Supplementary Figures. The information regarding the CNVs and the corresponding allele frequency plots is needed for a correct interpretation of the copy number results (see Online Resources 3 and 4). The genome profile of the IMC (patient #14) is depicted in panel (**a**) and demonstrates gain of chromosome 6 and (normal) male hemizygosity for chromosome X. The genome profile of the LMM in patient #18 is depicted in panel (**b**), and demonstrates losses of chromosome 1p, 2p, 3, 8p, 10, 11 and 18. Clear gains are present of chromosome 4p, 8q, chromosome 17 (loss of 17q21.22q25.3) and 22

tested a series of 20 LMNs for the presence of "hotspot" mutations in Q209 and R183 of *GNA11* and *GNAQ*, as these "hotspots" were more recently shown to be mutated in cases of LMNs as well. [10, 19, 20] We identified two LMNs harboring a *GNA11* mutation at codon 209 (Q209L) (10 %). This result is very similar to the study of Gessi et al. who detected a $GNA11^{Q209L}$ mutation in two out of 16 LMNs (13 %). [10] Our study thus confirms that $GNA11^{Q209}$ mutations are a rare event in LMNs (~10 %). A similarly low $GNA11^{Q209}$ mutational frequency has been reported in blue nevi (7 %) while in UMs this frequency is higher (32 %). [14] Furthermore, the MC cases in our series did not show any $GNA11^{Q209}$ mutations (n=11) and this was also the case in the six MCs in the series of Gessi et al. [10] However, $GNA11^{Q209}$ mutations do occur in MCs, as two MC cases have been reported to harbor such mutation (Q209P and Q209L, respectively) [19, 20]. In 55 % of LMNs, we detected mutations in codon 209 of GNAQ, which is in the range of Gessi et al. (43 %) and similar to the $GNAQ^{Q209}$ mutational frequency in UMs (45 %) and blue nevi (55 %). [9, 10, 14]

In UMs and blue nevi, mutations at codon 183 of both *GNAQ* and *GNA11* are rare (1–3 % in either gene, tested in >200 cases). [14] In contrast, little is known about the $GNAQ^{R183}$ and $GNA11^{R183}$ mutational frequency in LMNs. Up to now, only one smaller case series searched for mutations in this codon of both genes and detected one activating $GNAQ^{R183}$ mutation in five LMN cases. [19] In our study, however, mutations in codon 183 of *GNAQ* and *GNA11* were absent, suggesting that these mutations are a rare event in LMNs.

At the moment, it is unclear whether *GNAQ* or *GNA11* mutations in LMNs have any association with histological characteristics or clinical behavior. In UM, such an association has not been found. [14, 25, 26] Furthermore, although inhibition of the *GNAQ/GNA11*-dependent MAPK signaling pathway is now under investigation for UMs, it remains to be determined whether a similar approach would be effective in LMNs. [27–33]

In our present series of adult LMN cases, we did not find any *BRAF*, *NRAS* or *HRAS* mutations. Similar results were obtained by Wang et al. [34] Of note, in contrast to the adult setting, we and others recently showed that mutations at Q61of *NRAS* rather than *GNAQ* or *GNA11* are involved in the development of LMNs in children. [10, 35, 36]

The incidence of CNS metastasis in patients with melanoma ranges from 10 % to 40 % in clinical studies. [37] This high frequency of CNS metastasis in melanoma patients is in contrast to the very low incidence of primary melanoma of the CNS. [1] Still, the discrimination between a primary versus metastatic melanocytic tumor in or around the CNS is relevant because of different patient work-up and the substantially better prognosis of patients with a primary neoplasm, even when the tumor is malignant. [38–41] In contrast to primary LMNs, mutations in *GNAQ* and *GNA11* are absent or very rare in cutaneous melanoma (CM) (1.4 %, tested in >150 cases), acral melanoma (0 %) and mucosal melanoma (0 %).

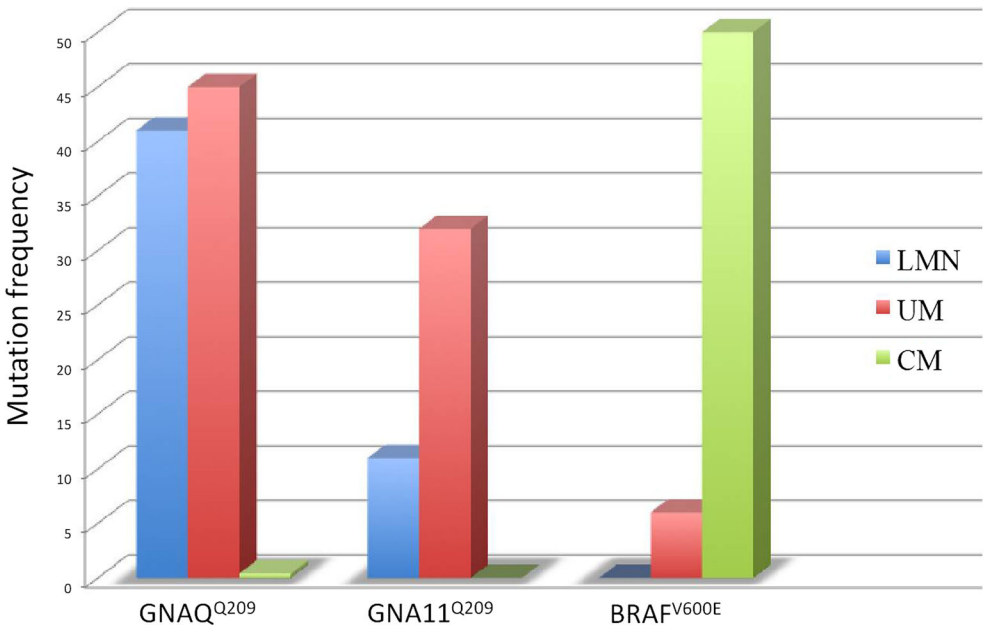

Fig 4 Frequency of *GNAQ*, *GNA11* and *BRAF* "hotspot" mutations for LMNs, UMs and CMs. Demonstration of a *GNAQ* or *GNA11* mutation in a CNS melanocytic tumor may be helpful in discriminating a primary LMN from a CM metastasis in/around the CNS, as CMs only very rarely harbor *GNAQ* or *GNA11* mutations. Vice versa, the demonstration of a *BRAF* mutation strongly favors a metastasis from a CM, as CMs show a high frequency of *BRAF* mutations. Note: the mutation frequencies include the hotspot mutations at codon 209 of *GNAQ* and *GNA11*, and codon 600 of *BRAF*, and were based on the following series, including the present study: [9, 10, 13, 14, 34, 46–49]

[14, 32] As a consequence, molecular testing of these genes could be helpful for elucidating the origin of a melanocytic tumor in or around the CNS. For instance, demonstration of a *GNAQ* or *GNA11* mutation in a CNS melanocytic tumor favors a primary LMN, although a metastasis from a UM or malignant blue nevus still needs to be excluded. Conversely, the demonstration of a *BRAF* mutation favors a metastasis from a CM as these melanomas show a high frequency of *BRAF* mutations (50 %). [42, 43] (Fig. 4)

Based on the presence of *GNAQ* and *GNA11* mutations in both LMNs and UMs, we postulated that UMs and the especially aggressive LMNs might share chromosomal copy number variations. The strong association of monosomy 3 and gain of 8q with poor UM patient prognosis is well documented. [21, 22, 44] Gain of 6p in UMs is often mutually exclusive with monosomy 3 and correlates with a better prognosis. [21, 22, 45] However, in our study, only two lesions carried the common gross chromosomal abnormalities seen in UM: one *GNAQ*-mutated LMM showed monosomy 3 and gain of 8q; and one *GNA11*-mutated LMM showed gain of 6p and loss of 6q. The former patient developed metastases in the bone and lungs two years after initial diagnosis, but no liver metastases were demonstrated. The latter patient showed leptomeningeal seeding but no distant metastases. In addition, one *GNAQ*-mutated IMC, presenting with local recurrence and leptomeningeal seeding, also showed gain of chromosome 6. Due to the limited number of patients that could be tested, however, it is still unclear if the gross chromosomal changes in LMNs have any prognostic implications.

We conclude that LMNs share some genetic aberrations with UMs, especially $GNAQ^{Q209}$ and $GNA11^{Q209}$ mutations, although the latter are, in contrast to UMs, an infrequent event in LMNs. Mutations in $GNAQ^{R183}$ and $GNA11^{R183}$ were absent in our series, suggesting that these mutations are rare in LMNs, as in UMs. The demonstration of a *GNAQ* or *GNA11* mutation in a LMN may have diagnostic value, allowing the discrimination of a primary CNS melanocytic tumor from a cutaneous melanoma metastasis. Copy number variations in some aggressive LMNs resemble those present in UMs including monosomy 3 and gain of 8q but their exact prognostic significance is so far unclear.

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