Activating mutations of the GNAQ gene: a frequent event in primary melanocytic neoplasms of the central nervous system

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Abstract Primary melanocytic neoplasms of the central nervous system (CNS) are uncommon neoplasms derived from melanocytes that normally can be found in the leptomeninges. They cover a spectrum of malignancy grades ranging from low-grade melanocytomas to lesions of intermediate malignancy and overtly malignant melanomas. Characteristic genetic alterations in this group of neoplasms have not yet been identified. Using direct sequencing, we investigated 19 primary melanocytic lesions of the CNS (12 melanocytomas, 3 intermediate-grade melanocytomas, and 4 melanomas) for hotspot oncogenic mutations commonly found in melanocytic tumors of the skin (BRAF, NRAS, and HRAS genes) and uvea (GNAQ gene). Somatic mutations in the GNAQ gene at codon 209, resulting in constitutive activation of GNAQ, were detected in 7/19 (37%) tumors, including 6/12 melanocytomas, 0/3 intermediate-grade melanocytomas, and 1/4 melanomas. These GNAQ-mutated tumors were predominantly located around the spinal cord (6/7). One melanoma carried a BRAF point mutation that is frequently found in cutaneous melanomas (c.1799 T>A, p.V600E), raising the question whether this is a metastatic rather than a primary tumor. No HRAS or NRAS mutations were detected. We conclude that somatic mutations in the GNAQ gene at codon 209 are a frequent event in primary melanocytic neoplasms of the CNS. This finding provides new insight in the pathogenesis of these lesions and suggests that GNAQ-dependent mitogen-activated kinase signaling is a promising therapeutic target in these tumors. The prognostic and predictive value of GNAQ mutations in primary melanocytic lesions of the CNS needs to be determined in future studies.

Keywords Primary melanocytic neoplasms · Melanocytoma · GNAQ · Central nervous system · MAP kinase pathway

Introduction

Primary melanocytic neoplasms of the central nervous system (CNS) are uncommon neoplasms occurring in diffuse or localized form [5, 8]. Diffuse lesions such as melanocytosis and melanomatosis generally occur in the setting of dermatologic syndromes (neurocutaneous melanosis, nevus of Ota) [1, 16, 18]. Localized lesions present as leptomeningeal masses and consist of a spectrum ranging from ‘well differentiated’ melanocytomas to lesions of intermediate malignancy and overtly malignant melanomas [6]. They are derived from scattered melanocytes that are normally present in the leptomeninges, especially at the base of the brain, in the posterior fossa and around the
upper cervical spinal cord. Melanocytomas are solitary, low-grade tumors that do not invade surrounding structures [5]. They are usually characterized by a benign clinical course, but local recurrence can occur [14, 25]. Intermediate grade lesions show histological features suggestive of aggressive behavior, such as invasion of the CNS but lack the overt cytological atypia of melanomas [6]. The biological behavior of intermediate-grade melanocytomas is unclear [5]. Primary melanomas of the CNS occur throughout the neuroaxis with a predilection for the spinal cord and posterior fossa. They are cytologically similar to melanomas arising in other sites and may metastasize to remote organs. Diffuse spreading of a primary meningeal melanoma through the subarachnoid space is referred to as meningeval melanomatosis [5, 27]. Discrimination between primary and metastatic melanocytic lesions of the CNS is important, because patients with metastatic disease carry a worse prognosis, with a life expectancy of less than 1 year in most studies [7, 23]. In addition, in some cases of primary melanomas of the CNS, long-term survival and even ‘cures’ have been documented after complete surgical excision [6, 29]. However, especially in cases where the melanocytic tumor presents as a solitary mass in the meninges and the patient is not known to have a melanocytic tumor of the skin, this differential diagnosis can be very difficult, both at the clinical and histological levels. While the molecular genetics of cutaneous melanomas has been investigated in numerous studies, the genetic alterations underlying primary CNS melanocytic lesions have not yet been addressed [5]. In melanocytic lesions of the skin—benign nevi as well as melanomas—oncogenic mutations in signaling components of the MAP kinase pathway are frequent [11, 22]. These mutations mostly involve exon 15 of the BRAF gene and exon 3 (codon 61) of the proto-oncogene NRAS. Mutations in HRAS are less frequent [13, 20]. Recently, in uveal melanomas and in some intradermal melanocytic lesions, such as blue nevi and nevi of Ota, somatic activating mutations of the GNAQ gene (or ‘G alpha q gene’) at codon 209 have been reported [17, 32]. The GNAQ gene maps on chromosome 9q21, and encodes a heterotrimeric GTP-binding protein α-subunit that couples G-protein coupled receptor signaling to the MAP kinase pathway [24]. GNAQ codon 209 mutations form an alternative route to MAP kinase activation [32]. In the present study, we investigated the mutation status of the GNAQ, BRAF, NRAS, and HRAS genes in a group of 19 primary melanocytic lesions of the CNS and found that somatic mutations in the GNAQ gene at codon 209 are relatively frequently present in these tumors. While the exact diagnostic, prognostic, and predictive value of GNAQ mutations in primary melanocytic lesions of the CNS is not yet clear, it is to be expected that a better knowledge of the genetic background of these lesions may not only facilitate adequate diagnosis but also identification of (novel) therapeutic targets, and thereby ultimately may have predictive value as well.

**Materials and methods**

**Patients and histopathology**

For this retrospective study, formalin-fixed and paraffin-embedded (FFPE) tissues of 19 primary melanocytic lesions of the CNS were retrieved from archives of various Departments of Pathology in The Netherlands and Germany. Cases from the Netherlands diagnosed between 1991 and 2009 were obtained through the Dutch nationwide histopathology and cytopathology data network and archive (PALGA) [9]. The study was performed in accordance with the ethical standards for this type of investigation in The Netherlands. Histology was revised by two pathologists (HK, BK). The diagnosis of ‘melanocytoma’, ‘intermediate-grade melanocytoma’ or ‘melanoma’ was based on histomorphological criteria, as described by Brat et al. [5, 6], and immunohistochemical stains (S100 positivity and at least one additional melanocytic marker (HMB45 or MelanA) positive in combination with lack of EMA staining). Scoring of histology included nuclear pleomorphism (mild, moderate or severe), mitotic activity, necrosis, melanin pigmentation, and CNS invasion.

**DNA extraction**

About three manually dissected sections of 10-µm FFPE tissue with an estimated tumor cell percentage of at least 60% were used for DNA extraction. After deparaffinization and rehydration, the tissues sections were incubated in proteinase K, followed by subsequent affinity-purification of the DNA (QIAGEN GmbH, Germany). DNA sample concentration was assessed spectrophotometrically (260/280 nm using a NanoDrop spectrophotometer, Peqlab Biotechnologies, Erlangen, Germany). DNA quality of the samples was tested using the BIOMED-2 gene control PCR, in which gene segments of house-keeping genes are amplified, yielding different fragment sizes (100, 200, 300, and 400 bp), depending on the extent of fragmentation of the DNA [31]. All extracted DNA samples allowed amplification of at least the 200-bp amplicon of the BIOMED-2 gene control PCR.

**Mutation analyses**

Direct sequence analysis of the GNAQ, BRAF, NRAS, and HRAS genes was performed on 19 primary melanocytic lesions of the CNS. Exon 5 of GNAQ, harboring codon 209
which is essential for GTP hydrolysis, was sequenced [19]. Furthermore, we performed sequence analysis of exon 15 of \(BRAF\) and exon 3 of \(NRAS\) and \(HRAS\), since these are well known hotspot regions for oncogenic mutations in melanocytic lesions of the skin [2, 13, 20]. Primer sequences used are listed in Table 1. All primers, except for \(GNAQ\), contained a M13 forward or reverse consensus sequence for sequencing the different exons. PCR amplification of exon 5 of \(GNAQ\) was performed in a total volume of 25 \(\mu\)L, containing 50 ng DNA, PCR-buffer IV (Integro), 37 mM MgCl\(_2\), 250 \(\mu\)M of each deoxynucleotide triphosphate, 37.5 \(\mu\)g bovine serum albumin (Sigma), 10 pmol of each primer, and 0.05 units of thermostable DNA polymerase (Sigma). DNA amplification was performed in a PTC 200 Thermal Cycler (MJ Research). The PCR was started with 5 min at 92°C and followed with 35 cycles of denaturation 45 s at 94°C, annealing at 62°C for 45 s and extension at 72°C for 45 s, followed by a final extension at 72°C for 20 min and cooling down for 5 min at 20°C. PCR amplification of exon 15 of \(BRAF\) and exon 3 of \(NRAS\) and \(HRAS\) were performed in a total volume of 20 \(\mu\)L. The PCR mix contained 50 ng DNA, buffer IV (Integro), 3 mM MgCl\(_2\), 200 \(\mu\)M of each deoxynucleotide triphosphate, 30 \(\mu\)g bovine serum albumin (Sigma), 10 pmol of each primer, and 0.25 units of thermostable DNA polymerase (Sigma). DNA amplification was performed in a PTC 200 Thermal Cycler (MJ Research). The PCR was started with 5 min at 94°C and followed with 30 cycles of denaturation 45 s at 94°C, annealing at 60°C for 45 s and extension at 72°C for 45 s, with a final extension at 72°C for 5 min. All PCR products were purified with MinElute plates (Qiagen). One microliter of the PCR product was used for the sequence reaction on an ABI PRISM 3700 DNA analyser (Applied Biosystems). Both strands were sequenced using the M13 primers. For all mutations detected, normal tissue was tested to exclude germline mutations (archival FFPE skin tissue).

### Table 1: Primers used for mutation analyses

| Gene | Exon | Forward (Fw)       | Primer sequence 5'-3'                  |
|------|------|-------------------|---------------------------------------|
| GNAQ | 5    | Fw                | TTTCCCTAAGTTTGTAAAGTGTTGCG             |
|      |      | Rw                | ATCCCATTTTCTCTCTCTGACC                 |
|      | 15   | Fw                | CTTTACCTACTACCTACCTAC                 |
|      |      | Rw                | AAAAATAGCCTCAATTCCTTAC                |
| NRAS | 3    | Fw                | GATTCCTACAGAAAACCAAGTGG               |
|      |      | Rw                | TAATGCTCCTAGTACCTGTCAG                |
| HRAS | 3    | Fw                | CTGCAAGGATCTCTACCGGA                  |
|      |      | Rw                | ACT TGGTGTGTGTGATGGCA                  |

**Results**

Patient and histopathological characteristics

Our study group consisted of 12 melanocytomas, 3 intermediate-grade melanocytomas and 4 primary melanomas of the CNS. Table 2 summarizes the respective patient and histopathological characteristics. In each patient, no primary melanoma localizations elsewhere in the body were known to be present. Histology revealed melanocytomas as being often heavily pigmented lesions consisting of spindle and/or epithelioid cells arranged in fascicles, sheets and/or compact nests (Fig. 1a, b). Nucleoli were inconspicuous. Mitotic activity was low (0–1 per 10 HPFs). In some cases focal necrosis was present. Nuclear pleomorphism was mostly mild. As summarized in Table 2 the melanocytomas often recurred. Three tumors were classified as intermediate-grade melanocytomas based on increased mitotic activity (2–5 per 10 HPF) and CNS invasion (patients 1, 6, and 17) (Fig. 1b, c). In the melanomas, nuclear pleomorphism was prominent, together with conspicuous nucleoli, higher mitotic activity (>7 per 10 HPF) and often extensive necrosis (Fig. 1d). All lesions were positive for S100, HMB-45 and/or MelanA, and lacked staining for EMA, the latter to exclude melanotic meningioma.

#### Mutation analyses

In this group of 19 primary melanocytic neoplasms of the CNS, we detected 7 mutations in the \(GNAQ\) gene (37%) (Table 3). All mutations were present in codon 209 (p.Gln209Pro and p.Gln209Leu) and were somatic mutations (Fig. 2). Of these seven \(GNAQ\) mutant lesions, six were melanocytomas (50%) and one was a melanoma (1/4, 25%). The intermediate-grade melanocytomas (\(n = 3\)) contained no mutations in the \(GNAQ\) gene. Of the \(GNAQ\)-mutated melanocytomas, five were located in the leptomeninges of the spinal cord and one attached to the tentorium cerebelli. All but one \(GNAQ\)-mutated melanocytomas were strongly pigmented. The one melanoma containing a \(GNAQ\) mutation was located in the spinal cord (sacral) and was mildly pigmented. Mutation analysis of the \(BRAF\) gene revealed one \(BRAF\) mutation (c.1799 T>A, p.V600E), in a melanoma. No \(HRAS\) or \(NRAS\) mutations were detected in any of the samples.

**Discussion**

Primary melanocytic tumors of the CNS consist of a spectrum of rare neoplasms derived from scattered melanocytes located in the leptomeninges. These melanocytes
are derived from the neural crest during early embryonic development and are frequently encountered in the recesses of the sulci at the base of the brain and around the brain stem and upper part of the cervical spinal cord [12]. Up to now, the genetic alterations associated with these neoplasms are unknown. There is an increasing evidence that melanocytic neoplasms in general are a heterogeneous group of tumors with different molecular changes in melanocytic lesions from different body sites. Most melanocytic nevi and melanomas of the skin show oncogenic mutations in signaling components of the MAP kinase pathway, in particular \textit{BRAF} and \textit{NRAS} [11, 22], although in uveal melanoma, Spitz nevi and blue nevi, these mutations are infrequent [26]. Very recently, mutations in the \textit{GNAQ} gene at codon 209 were described as an alternative route to MAP kinase activation in a particular subgroup of melanocytic neoplasms, namely uveal melanomas and specific intradermal melanocytic lesions such as blue nevi and nevi of Ota [17, 32]. We analyzed a group of 19 primary melanocytic lesions of the CNS for hotspot oncogenic mutations as described in melanocytic tumors of the skin (exon 15 of \textit{BRAF} gene, exon 3 of \textit{NRAS}, and exon 3 of \textit{HRAS}) and uvea (exon 5 of \textit{GNAQ}). In 7 out of these 19 CNS melanocytic tumors a somatic \textit{GNAQ} mutation was present at codon 209 (37%). This gene is located on chromosome 9q21 and encodes GTP-binding proteins, a family of heterotrimeric proteins that couple cell surface receptors to intracellular signaling pathways, such as the MAP kinase pathway. Codon 209 encodes the catalytic domain of \textit{GNAQ}. Mutations in this catalytic domain prevent hydrolysis of GTP and turns \textit{GNAQ} into its active, GTP-bound state. In uveal melanomas, identical somatic

### Table 2: Patient and histopathological characteristics

| Patient | Sex | Age | Diagnosis\(^a\) | Location\(^b\) | Cell type\(^c\) | Nuclear pleomorphism\(^d\) | Mitoses\(^e\) | Necrosis\(^f\) | CNS invasion | Pigmentation\(^g\) | Available follow-up |
|---------|-----|-----|-----------------|---------------|----------------|-----------------------------|---------------|---------------|---------------|-------------------|-------------------|
| 1       | F   | 50  | IM Th11-12      |               | S              | ++                          | 2             | –             | Yes           | +                 | na                |
| 2       | F   | 27  | MC Right cerebello-pontine angle | E              | + (+)          | 0              | +             | na           | No            | +                 | na                |
| 3       | M   | 41  | MC C0-C3        |               | E              | + (++)                     | 0             | +             | na            | +++               | na                |
| 4       | na  | na  | MM LM\(^h\)     |               | E              | ++                         | >15           | –             | na            | No                | R                 |
| 5       | na  | na  | MC LM           |               | Mx             | + (++)                     | 1             | +             | na            | +                 | R                 |
| 6       | F   | 68  | IM Cerebellar tentorium | E              | +               | 5             | No           | ++           | na            | No                | R                 |
| 7       | M   | 27  | MC Cerebellar tentorium | Mx            | +               | 1             | No           | ++           | na            | No                | R                 |
| 8       | F   | 44  | MC Pineal region | S              | +               | 1             | No           | +             | +              | R                 |
| 9       | M   | 55  | MC C3-6         |               | Mx             | +                          | 0             | –             | na            | ++                | R                 |
| 10      | F   | 59  | MM S2           |               | Mx             | +++                        | 8             | +             | Yes           | +                 | na                |
| 11      | na  | na  | MC C5-6         |               | E              | +                         | 0             | –             | na            | +++               | R                 |
| 12      | M   | 41  | MC Th6          |               | S              | +                         | 0             | +             | No            | +++               | R                 |
| 13      | F   | 45  | MC L3-4         |               | Mx             | + (++)                     | 0             | –             | na            | +++               | R                 |
| 14      | na  | na  | MC Th11         |               | S              | +                         | 1             | –             | na            | +                 | R                 |
| 15      | M   | 49  | MM Frontal lobe left | E              | ++                        | 7             | +++                   | na            | No                | na                |
| 16      | na  | na  | MC Cerebellar   |               | S              | +                         | 0             | –             | No            | ++                | na                |
| 17      | na  | na  | IM LM           |               | E              | +                         | 2             | –             | Yes           | +++               | na                |
| 18      | na  | na  | MC na           |               | S              | +                         | 0             | –             | na            | +++               | na                |
| 19      | M   | 7   | MM Temporal lobe right | E              | +++                        | >10           | –             | Yes           | +                 | Cong. nevus\(^i\) |

\(F\) female; \(M\) male; \(na\) not analyzed/data not available; \(R\) recurred

\(^a\) Diagnosis: \textit{MC} melanocytoma; \textit{MM} melanoma; \textit{IM} intermediate grade melanocytoma

\(^b\) Location: \textit{LM} leptomeningeal (more specific information about location could not be retrieved)

\(^c\) Cell type: \(S\) spindle; \(E\) epithelioid; \(Mx\) mixed

\(^d\) Scoring of nuclear pleomorphism: \(+\) mild; \(+ +\) moderate; \(+ ++\) severe

\(^e\) Number of mitotic figures per 10 HPF

\(^f\) Scoring of necrosis: \(+\) focal; \(+ +\) moderate; \(+ ++\) extensive

\(^g\) Scoring of pigmentation: \(+\) mild; \(+ +\) moderate; \(+ ++\) severe

\(^h\) \(na\) no CNS tissue present in slide for analysis

\(^i\) Patient known with a giant congenital melanocytic nevus
mutations of GNAQ at codon 209 have been described [21, 32]. GNAQ is important in melanocyte homeostasis and survival of melanocytes early in neural crest development [28].

The presence of GNAQ mutations in primary melanocytic neoplasms of the CNS as well as in uveal melanomas and intradermal melanocytic proliferations such as nevi of Ota and blue nevi [17, 32] is interesting as these lesions share some other features. First of all, these melanocytic tumors are non-epithelium-related neoplasms. GNAQ mutations might, thus, preferentially occur in melanocytes already present in extra-epithelial structures such as dermis

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**Table 3** Mutation analysis of the GNAQ, BRAF, NRAS, and HRAS genes

| Patient | Diagnosis | GNAQ | BRAF | NRAS | HRAS |
|---------|-----------|------|------|------|------|
| 1       | IM        | wt   | wt   | na   | na   |
| 2       | MC        | wt   | wt   | wt   | na   |
| 3       | MC        | c.626 A>C (p.Gln209Pro) | wt | wt | wt |
| 4       | MM        | wt   | wt   | wt   | wt   |
| 5       | MC        | wt   | wt   | wt   | wt   |
| 6       | IM        | wt   | wt   | wt   | wt   |
| 7       | MC        | c.626 A>C (p.Gln209Pro) | wt | wt | wt |
| 8       | MC        | wt   | wt   | wt   | wt   |
| 9       | MC        | c.626 A>C (p.Gln209Pro) | wt | na | wt |
| 10      | MM        | c.626 A>T (p.Gln209Leu) | wt | wt | na |
| 11      | MC        | wt   | wt   | wt   | wt   |
| 12      | MC        | c.626 A>T (p.Gln209Leu) | wt | na | na |
| 13      | MC        | c.626 A>T (p.Gln209Leu) | wt | wt | na |
| 14      | MC        | c.626 A>T (p.Gln209Leu) | wt | wt | wt |
| 15      | MM        | wt   | c.1799 T>A (p.V600E) | na | wt |
| 16      | MC        | wt   | wt   | wt   | wt   |
| 17      | IM        | wt   | wt   | na   | na   |
| 18      | MC        | wt   | wt   | wt   | wt   |
| 19      | MM        | wt   | wt   | wt   | wt   |
and leptomeninges. Second, they often share strong melanin pigmentation. Third, in this context, the nevus of Ota is interesting because this is a ‘dermal melanocytosis’, mostly congenital, involving the skin innervated by the first and the second branch of the trigeminal nerve. The nevus of Ota is often associated with ‘ocular melanocytosis’, involving the sclera, conjunctiva, and uveal tract. The involvement of different anatomical structures in the nevus of Ota might indicate that the \(GNAQ\) gene product plays a role in migration of melanocytes early during embryonic development. Histology of this nevus of Ota ranges from scattered dendritic melanocytes to a morphology strongly resembling blue nevi [4]. These nevi of Ota are not only associated with the development of uveal melanoma but are also associated with the presence of CNS melanocytoma [25]. Thus, it appears that \(GNAQ\) mutations are preferentially present in a group of non-epithelium-related melanocytic lesions, sharing histological features and occurring in an anatomical distribution indicating a possible role of \(GNAQ\) in migration of melanocytes early during embryonic development. Interestingly, tumorigenicity studies in nude mice with injection of human \(GNAQ^{Q209L}\) resulted in heavily pigmented melanocytic tumors at the injection site [32]. Furthermore, dominant dark skin (Dsk) mutations that are found in mutant mice with increased dermal melanin, are mutations of the mouse \(GNAQ\) gene, and the hyperpigmentation in these mutant mice is due to an increase of intradermal, but not epidermal melanocytes. It is important to note here, however, that these Dsk mutations are different from the oncogenic human \(GNAQ\) mutation at codon position 209 [33]. Other studies in mice have shown that activating mutations in \(GNAQ\) or \(Galpha11\), another gene encoding G-protein subunits, result in an aberrant accumulation of melanin-producing melanocytes in the dermal layer of the skin [15].

The finding that \(GNAQ\)-mutated melanocytic lesions (uveal melanoma, blue nevi [10, 26, 34] and our series of melanocytic lesions of the CNS) only infrequently carry \(BRAF\), and \(NRAS\) mutations might be helpful for differential diagnostic purposes. For instance, in our series, one melanoma contained a \(GNAQ\) mutation, which, in the differential diagnosis with a metastasis of a primary cutaneous melanoma—often harboring \(BRAF\) or \(NRAS\) mutations—might favor a primary location in the CNS. So, the presence of \(GNAQ\) mutations and lack of \(BRAF\) or \(NRAS\) mutations in melanocytic neoplasms of the CNS seems to strongly indicate a primary CNS tumor, a diagnosis that has obvious prognostic implications. Vice versa, as \(BRAF\) point mutations are a frequent event in cutaneous melanomas [30], the one melanoma in our series with a \(BRAF\) point mutation (case 15; c.1799 T\[A, p.V600E\]) might be a metastasis rather than a primary tumor. The fact that in this patient the tumor was located in the frontal lobe (rather than in the posterior fossa or around the spinal cord) might support this notion. However, according to the available data, a melanocytic tumor outside the CNS was absent in this patient. In our study, \(GNAQ\) mutations were preferentially present in the melanocytomas, while the intermediate melanocytomas and melanomas were only infrequently mutated. This might suggest that the presence of a \(GNAQ\) mutation favors a benign or low-grade course. On the other hand, activating \(GNAQ\) mutations are also reported in uveal melanomas, and, in addition, are shown to have no effect on disease-free survival in these neoplasms [3]. In conclusion, mutations in the \(GNAQ\) gene are a frequent event in primary melanocytic neoplasms of the CNS. This finding provides an important new insight in the pathogenesis of melanocytic CNS lesions, and suggests that \(GNAQ\)-dependent mitogen-activated kinase signaling is a promising therapeutic target in these tumors.

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Conflict of interest statement The authors declare that they have no conflict of interest.
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