Comprehensive genomic analysis of sporadic multiple meningiomas reveals clonal origin and histotype-specific evolution: a case report

Maki Sakaguchi
maki1101@kuhp.kyoto-u.ac.jp

Kanazawa University Graduate School of Medical Sciences: Kanazawa Daigaku Daigakuin Iyaku Hokengaku Sogo Kenkyuka Iyaku Hoken Gakuiki Igakurui  https://orcid.org/0009-0007-0321-3094

Masafumi Horie
Kanazawa University Graduate School of Medical Sciences: Kanazawa Daigaku Daigakuin Iyaku Hokengaku Sogo Kenkyuka Iyaku Hoken Gakuiki Igakurui

Yukinobu Ito
Kanazawa University Graduate School of Medical Sciences: Kanazawa Daigaku Daigakuin Iyaku Hokengaku Sogo Kenkyuka Iyaku Hoken Gakuiki Igakurui

Shingo Tanaka
Kanazawa University Graduate School of Medical Sciences: Kanazawa Daigaku Daigakuin Iyaku Hokengaku Sogo Kenkyuka Iyaku Hoken Gakuiki Igakurui

Keishi Mizuguchi
Kanazawa University Hospital: Kanazawa Daigaku Fuzoku Byoin

Hiroko Ikeda
Kanazawa University Hospital: Kanazawa Daigaku Fuzoku Byoin

Etsuko Kiyokawa
Kanazawa Medical University: Kanazawa Ika Daigaku

Mitsutoshi Nakada
Kanazawa University Graduate School of Medical Sciences: Kanazawa Daigaku Daigakuin Iyaku Hokengaku Sogo Kenkyuka Iyaku Hoken Gakuiki Igakurui

Daichi Maeda
Kanazawa University Graduate School of Medical Sciences: Kanazawa Daigaku Daigakuin Iyaku Hokengaku Sogo Kenkyuka Iyaku Hoken Gakuiki Igakurui

Research Article

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Abstract

Meningioma is the most common primary intracranial tumor in adults, with up to 10% manifesting as multiple tumors. Data on the genomic and molecular changes in sporadic multiple meningiomas are scarce, leading to ongoing debates regarding their evolutionary processes. A comprehensive genetic analysis of a large number of lesions, including minute occult meningiomas (MOMs), is necessary to explore these two possible origins: clonal and independent. In the present study, we performed whole-exome sequencing and analyzed somatic single-nucleotide variants (SNVs), insertions/deletions (INDELs), and copy number alterations (CNAs) in a patient with sporadic multiple meningiomas. These meningiomas included two mass-forming lesions of different histological subtypes (transitional and chordoid) and three MOMs. Genetic analysis revealed CNAs on chromosomes 22q and Y as common abnormalities in the two largest tumors. Furthermore, we identified SNV/INDELs unique to each focus, with NF2 mutation prevalent in the transitional meningioma and CREBBP mutation in the chordoid meningioma. Loss of chromosome 22 was detected in all three MOMs, whereas an NF2 somatic mutation was found only in the largest MOM. Overall, we elucidated the clonal origin and histotype-specific evolution of multiple meningiomas in this case. CNAs may serve as the initial driving event in meningioma development.

Introduction

Meningioma is the most common primary intracranial tumor in adults. Its incidence is higher in women than in men but the reason for this difference is unclear [1]. Most meningiomas are solitary; however, up to 10% of cases manifest as multiple tumors [2]. Recent evidence suggests that the incidence of multiple meningiomas may be even higher than 10% [3]. Multiple meningiomas are either sporadic or familial, with some sporadic cases being radiation-induced. The standard treatment for meningioma is surgical resection. In patients with multiple meningiomas, clinical decisions regarding the lesions that should be surgically resected and the order of their resection are often difficult. It is a chronic disease that requires repeated interventions and lifelong surveillance to achieve disease control [4]. In terms of prognosis, patients with multiple meningiomas exhibit shorter overall survival, progression-free survival, and time to second intervention than patients with a solitary meningioma [3, 5]. Notably, a study that involved a large cohort of patients with multiple meningiomas revealed that a greater number of lesions, older age at diagnosis, and male sex were significantly negatively associated with overall survival [3].

Recent advances in the genomic analysis of solitary meningiomas have shed light on the relationships among the histological type, site of origin, malignancy, and prognosis [6, 7]. Approximately 50% of meningiomas exhibit NF2 mutations and/or loss of chromosome 22, where NF2 is located. These genomic changes have been proven to be associated with atypical clinical and histological presentations due to genomic instability. Specifically, they show a predilection for meningiomas of the cerebrum, cerebellar hemispheres, posterior skull base, and spinal regions as well as transitional and fibrous subtypes [8, 9]. Meningiomas without NF2 alterations are clinically benign and typically localized to the medial skull base. Their characteristic genomic changes include mutations of TRAF7, KLF4, AKT1, and
SMO. These mutations occur in a histotype-specific manner. Meningothelial and transitional meningiomas frequently harbor TRAF7 and either AKT1 or SMO mutations [9]. Mutations in SMARCE1, BAP1, or a combination of TRAF7 and KLF4 are associated with clear cell, rhabdoid, or secretory meningioma variants, respectively [9, 10].

Multiple meningiomas are associated with familial tumor syndromes such as neurofibromatosis type 2 and schwannomatosis, which are genetically characterized by germline mutations of NF2 and SMARCB1, respectively [11]. However, data on the genomic and molecular changes in patients with sporadic multiple meningiomas are scarce, leading to ongoing debates regarding their evolutionary processes [12, 13]. Two hypotheses have been proposed to explain the pathogenesis of sporadic multiple meningiomas: a clonal origin and an independent origin. Studies supporting the clonality hypothesis suggest that multiple meningiomas arise from a specific neoplastic clone that proliferates along the meninges to form multifocal lesions [12]. This hypothesis is supported by observations that most sporadic multiple meningiomas exhibit identical histological features. By contrast, some researchers consider multiple meningiomas as independent lesions because some of these tumors exhibit various histological subtypes or grades [14, 15]. A comprehensive genetic analysis of a large number of lesions, including minute occult meningiomas (MOMs), is necessary to resolve this issue. Although gaining a thorough understanding of meningiomas development requires the examination of MOMs, a putative precursor of mass-forming meningioma, no reports to date have discussed the genomic changes in MOMs.

In the present study, we performed whole-exome sequencing (WES) and analyzed somatic single-nucleotide variants (SNVs), insertions/deletions (INDELs), and copy number alterations (CNAs) in a patient with sporadic multiple meningiomas. The meningiomas comprised two mass-forming lesions of different histological subtypes (transitional and chordoid) and three MOMs. The clonality and evolutionary processes of these lesions were analyzed to elucidate the pathogenesis of sporadic multiple meningiomas.

Clinical summary

An 83-year-old man was incidentally discovered to have bilateral frontal convexity tumors 9 years prior to presentation. These asymptomatic tumors were monitored over time and identified as meningiomas through imaging. The patient had no familial history of the disease and no evidence of neurofibromatosis. He subsequently developed cognitive dysfunction coinciding with the identification of a new lesion on the left sphenoidal ridge. Preoperative magnetic resonance (MR) imaging revealed that this was the largest tumor, measuring 50 mm, and it exhibited strong homogenous enhancement. In addition, multiple smaller tumors up to 24 mm in size were observed in the bilateral convexity regions (Fig. 1a).

Pathological and genetic findings
We simultaneously resected two largest mass-forming meningiomas, one in the left convexity (T1) and one on the left sphenoidal ridge (T2), along with the left convexity dura, which contained three MOMs (MOM1–MOM3). T1 was a transitional meningioma composed of proliferative meningothelial cells arranged in bundles or whorls (Fig. 1b). T2 was a chordoid meningioma consisting of cord-like arrays of epithelioid cells within an abundant basophilic myxoid matrix (Fig. 1c). T1 and T2 were grade 1 and 2 tumors, respectively, according to the 2016 World Health Organization (WHO) classification. In the dura surrounding the left convexity tumor, scattered MOMs composed of oval or spindle-shaped meningothelial cells with or without psammoma bodies were observed (Fig. 1d–g).

Immunohistochemistry showed that T1 and T2 were focally positive for epithelial membrane antigen (EMA) and progesterone receptor (PgR) and that T2 was negative for brachyury. The primary antibodies used in the immunohistochemical analysis are listed in Supplementary Table 1.

WES was conducted to assess the clonality of the two resected meningiomas. Genomic DNA was extracted from frozen specimens of T1, T2 and background normal brain tissue. Libraries were prepared for each sample using a SureSelect Human All Exon V6 kit (Agilent Technologies) in accordance with the manufacturer’s recommendations. CNA analysis indicated loss of chromosomes 22q and Y in T1 and loss of chromosomes 1p, 10q, 22q, and Y in T2 (Fig. 2a). Thus, loss of chromosomes 22q and Y were common events in both T1 and T2. Mutational analysis revealed 52 and 66 SNVs/INDELs in T1 and T2, respectively. After the application of strict filtering criteria, 14 mutations were retained in each tumor, with no overlapping mutations between T1 and T2 (Table 1). Among these genes, NF2 frameshift mutation (c.503delC:p.K170Rfs*43) in T1 and CREBBP frameshift mutation (c.3923delT:p.L1308Cfs*30) in T2 were highlighted upon comparison with previously published data on genes mutated in at least two cases of meningiomas (Fig. 2b) [16]. Sanger sequencing was performed to validate the identified gene mutations, confirming the mutations of NF2 in T1 and CREBBP in T2 (Fig. 3a). Homozygous deletions of CDKN2A and CDKN2B, as well as TERT promoter hotspot mutations that are indicative of WHO grade 3 tumors, were not detected in either T1 or T2 by WES and Sanger sequencing (data not shown).
Table 1
Nonsynonymous gene mutations from the transitional meningioma (T1) and chordoid meningioma (T2) detected by whole-exome sequencing*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation type</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transitional meningioma (T1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REPS2</td>
<td>Missense</td>
<td>c.G650A</td>
<td>p.S217N</td>
<td>74.3%</td>
</tr>
<tr>
<td>NF2</td>
<td>Frameshift</td>
<td>c.503delC</td>
<td>p.K170Rfs*43</td>
<td>44.8%</td>
</tr>
<tr>
<td>OXCT1</td>
<td>Missense</td>
<td>c.G296A</td>
<td>p.R99Q</td>
<td>43.8%</td>
</tr>
<tr>
<td>ZNF469</td>
<td>Missense</td>
<td>c.C11599G</td>
<td>p.Q3867E</td>
<td>40.7%</td>
</tr>
<tr>
<td>COL4A2</td>
<td>Missense</td>
<td>c.C3076T</td>
<td>p.P1026S</td>
<td>38.5%</td>
</tr>
<tr>
<td>GLI4</td>
<td>Missense</td>
<td>c.G283A</td>
<td>p.G95R</td>
<td>38.4%</td>
</tr>
<tr>
<td>TRPA1</td>
<td>Missense</td>
<td>c.T1448C</td>
<td>p.M483T</td>
<td>38.1%</td>
</tr>
<tr>
<td>SOS1</td>
<td>Missense</td>
<td>c.C3589G</td>
<td>p.P1197A</td>
<td>34.7%</td>
</tr>
<tr>
<td>PPP1R7</td>
<td>Missense</td>
<td>c.G907C</td>
<td>p.A303P</td>
<td>34.5%</td>
</tr>
<tr>
<td>CLCN2</td>
<td>Missense</td>
<td>c.C2096T</td>
<td>p.S699F</td>
<td>34.5%</td>
</tr>
<tr>
<td>DNAH5</td>
<td>Stop-gain</td>
<td>c.C6763T</td>
<td>p.R2255X</td>
<td>34.2%</td>
</tr>
<tr>
<td>COL16A1</td>
<td>Missense</td>
<td>c.C162G</td>
<td>p.154M</td>
<td>33.1%</td>
</tr>
<tr>
<td>CYB5R4</td>
<td>Missense</td>
<td>c.G923T</td>
<td>p.G308V</td>
<td>24.4%</td>
</tr>
<tr>
<td>DNAH12</td>
<td>Missense</td>
<td>c.A2274C</td>
<td>p.K758N</td>
<td>20.8%</td>
</tr>
<tr>
<td><strong>Chordoid meningioma (T2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHDC1</td>
<td>Missense</td>
<td>c.G1760A</td>
<td>p.R587Q</td>
<td>57.1%</td>
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<tr>
<td>TYK2</td>
<td>Missense</td>
<td>c.C2315T</td>
<td>p.P1985fs</td>
<td>50.5%</td>
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<tr>
<td>TNXB</td>
<td>Frameshift</td>
<td>c.5955delC</td>
<td>p.P1985fs</td>
<td>48.0%</td>
</tr>
<tr>
<td>SUCO</td>
<td>Missense</td>
<td>c.G530A</td>
<td>p.S177N</td>
<td>44.5%</td>
</tr>
<tr>
<td>DPH7</td>
<td>Missense</td>
<td>c.C349T</td>
<td>p.R117W</td>
<td>44.3%</td>
</tr>
<tr>
<td>SUN1</td>
<td>Stop-gain</td>
<td>c.G1638A</td>
<td>p.W546X</td>
<td>44.2%</td>
</tr>
<tr>
<td>NLRC3</td>
<td>Missense</td>
<td>c.C1340T</td>
<td>p.S447L</td>
<td>43.5%</td>
</tr>
<tr>
<td>ZNF280D</td>
<td>Missense</td>
<td>c.C359T</td>
<td>p.S120L</td>
<td>42.7%</td>
</tr>
</tbody>
</table>

* The filtering steps were performed according to the following four parameters: gnomad_AF_popmax < 0.01, normal VAF (NVAF) < 0.05, tumor VAF (TVAF) > 0.2, and TVAF/NVAF > 5.0.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation type</th>
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<th>Amino acid change</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLMAP</td>
<td>Stop-gain</td>
<td>c.C61T</td>
<td>p.Q21X</td>
<td>41.9%</td>
</tr>
<tr>
<td>NCOA6</td>
<td>Missense</td>
<td>c.T3095C</td>
<td>p.1032A</td>
<td>40.4%</td>
</tr>
<tr>
<td>MYH7</td>
<td>Missense</td>
<td>c.A3536G</td>
<td>p.E1179G</td>
<td>39.8%</td>
</tr>
<tr>
<td>CREBBP</td>
<td>Frameshift</td>
<td>c.3923delT</td>
<td>p.L1308Cfc*30</td>
<td>37.3%</td>
</tr>
<tr>
<td>TLR9</td>
<td>Missense</td>
<td>c.A2432G</td>
<td>p.D811G</td>
<td>30.6%</td>
</tr>
<tr>
<td>RNF208</td>
<td>Stop-gain</td>
<td>c.G198A</td>
<td>p.V222M</td>
<td>29.5%</td>
</tr>
</tbody>
</table>

* The filtering steps were performed according to the following four parameters: gnomad_AF_popmax < 0.01, normal VAF (NVAF) < 0.05, tumor VAF (TVAF) > 0.2, and TVAF/NVAF > 5.0.

Finally, to elucidate the clonal origin and histotype-specific evolution of multiple meningiomas, Sanger sequencing and Fluorescence in situ hybridization (FISH) of the three MOMs were performed, and the gene mutations and CNAs were compared to those of T1 and T2. The three MOMs of the dura mater were microdissected from sections stained with HE for DNA extraction. Sanger sequencing was performed to evaluate the gene mutations. Notably, sequencing of the three MOMs showed the same NF2 mutation in the largest MOM3 (Fig. 3a), whereas the CREBBP mutation was not found in any of the specimens. The primers used in the Sanger sequencing are listed in Supplementary Table 2.

Furthermore, FISH was performed to evaluate the copy number of chromosome 22 in the three MOMs using an NF2 (22q12) deletion probe (Guang Zhou LBP Medicine Science & Technology, Guangzhou, China). Each lesion was compared to a section stained with hematoxylin and eosin (HE) to aid the identification of proliferative meningeal cells. After identifying an internal control, such as endothelial cells or lymphocytes, the number of nuclei was counted in at least 30 cells. The criteria for determining the presence of loss of chromosome 22 was defined as the presence of ≥ 4.2% deleted cells based on a previous study using age- and sex-matched normal control bone marrow samples [17]. FISH of chromosome 22 in the three MOMs revealed scattered cells exhibiting monosomy of chromosome 22q with one red and one green signal (Fig. 3b). The percentages of the meningeal cells displaying deletions in each lesion were as follows: 67.1% in MOM1, 40.9% in MOM2, and 50.0% in MOM3. All of these lesions were determined to have a loss of chromosome 22q. Collectively, they were considered to represent the clonal origin with loss of chromosome 22q, which then underwent histotype-specific evolution and acquired NF2 and CREBBP mutations (Fig. 3c, d).

Discussion

We performed WES of solitary multiple meningiomas of different histological subtypes and identified CNAs on chromosomes 22q and Y as common genetic abnormalities. SNVs/INDELs unique to each focus were also detected. All of the lesions, including MOMs, were considered to be associated with NF2 based on the loss of chromosome 22q. Interestingly, the transitional meningioma (T1) had a second hit
of NF2 and the chordoid meningioma (T2) had a CREBBP mutation. CREBBP is a chromatin-remodeling gene that is more enriched in chordoid than non-chordoid meningiomas [18]. This difference indicates that an epigenetic abnormality caused by CREBBP mutation may induce chordoid change against a background of chromosome 22 loss. The multiple meningiomas in this study were thought to be of clonal origin with histotype-specific evolution. A WES analysis of multiple meningiomas demonstrated clonal origin in five of six cases and subsequent branched evolution that resulted in inter-tumoral heterogeneity represented by different histologic types and grades [13]. Other studies of multiple meningiomas of different histological types have suggested that each lesion develops independently, but these studies did not evaluate CNAs and may not have accurately determined clonality [15, 19]. Accurate assessment of the clonality of multiple meningiomas requires not only mutational analysis but also CNA analysis. Because multiple meningiomas may be both clonal and independent in origin, a large study is necessary.

Our study is the first to analyze genetic changes in MOMs. Loss of chromosome 22 was detected in all three MOMs, whereas NF2 somatic mutation was found only in the largest MOM. It is often difficult to determine whether microscopic proliferative lesions consisting of meningothelial cells are reactive or neoplastic simply by observing their morphology. The presence of the same CNA in our patient indicated that the MOMs were neoplastic. The presence of CNAs has been reported in precancerous lesions such as intestinal metaplasia (which is a risk factor for gastric cancer) and clonal hematopoiesis (which is implicated in the development of hematological malignancies) [20, 21]. Our findings indicate that CNAs are linked to the development of meningioma and that they precede SNVs/INDELs as genetic abnormalities, in line with a previous study of multiple meningiomas [13].

Chromosome 22q deletion is identified in approximately 50% of meningiomas, marking this as a critical early event in the onset of NF2-related meningiomas [22, 23]. Our study suggests that loss of chromosome Y (LOY) is also an initial event in the development of meningioma, concurrent with the loss of chromosome 22. However, the clinical and biological significance of the Y chromosome in meningioma remains largely unexplored. To date, only a few studies that have used FISH analysis of meningiomas have shown that LOY in men represents the second most frequent aberration, accounting for 28–46% of cases, following loss of chromosome 22 [17, 24]. Qi et al. compiled an extensive catalog of LOY across more than 5,000 primary tumors from men in The Cancer Genome Atlas, demonstrating that LOY is exceedingly prevalent in numerous tumor types and suggesting its potential driving role in uveal melanoma [25]. Furthermore, LOY is associated with adverse outcomes in patients with bladder cancer, and cancer cells exhibiting LOY have been shown to modify T-cell functionality, leading to exhaustion of CD8+ T cells in the tumor microenvironment and increasing their susceptibility to PD-1-targeted immunotherapy [26]. In the context of meningioma, LOY may play a role in the tumorigenesis of meningiomas in men, potentially contributing to the poorer prognosis in men than in women.

**Conclusion**
Genomic analysis of solitary multiple meningiomas of different histological subtypes revealed the clonal origin and histotype-specific evolution. CNAs may serve as the initial driving event in meningioma development. Further investigation involving a larger cohort is warranted.

Declarations

Acknowledgement

We thank Ms. Yuki Mitani for her excellent technical assistance. This work was supported by JSPS KAKENHI (Grant Number JP23H05340, MS).

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This study was approved by the Medical Ethics Committee of Kanazawa University (No.12644).

Author Contributions

Sakaguchi, Horie, Maeda contributed to the study conception and design. Material preparation was performed by Tanaka, Nakada. Data collection and analysis were performed by Sakaguchi, Horie, Ito, Mizuguchi, Ikeda, Kiyokawa, Maeda. The first draft of the manuscript was written by Sakaguchi, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Consent to participate

Written informed consent was obtained from the patient.

References


Figures
Figure 1

(a) Gadolinium enhanced T1-weighted magnetic resonance image and (b–g) histological images of the patient in the present case

In addition to the multiple lesions in the bilateral convexities, (a) a large tumor with strong homogenous gadolinium enhancement was present in the left sphenoidal ridge. The two large tumors located in the
left convexity (T1) and on the left sphenoidal ridge (T2) were resected. Arrowhead: non-resected multiple lesions in the convexity; arrow: resected tumors. Histologically, (b) T1 was composed of proliferative meningothelial cells arranged in bundles or whorls, whereas (c) T2 exhibited cord-like arrays of epithelioid tumor cells within an abundant basophilic myxoid matrix. (d) Macro-image of the three microdissected areas of the dura surrounding the left convexity tumor. (e–g) Microscopically, three scattered minute occult meningiomas (MOMs) composed of oval or spindle-shaped meningothelial cells with or without psammoma bodies were identified.

![Figure 2](image)

**Figure 2**

(a) Copy number alterations and (b) deletion mutations of T1 and T2 detected by whole-exome sequencing

(a) T1 showed loss of chromosomes 22q and Y, whereas T2 exhibited loss of chromosomes 1p, 10q, 22q, and Y. Homozygous deletions of CDKN2A and CDKN2B were not detected in either T1 or T2. (b) Information and coordinates of genetic mutations observed in T1 and T2. The NF2 frameshift mutation (c.503delC:p.K170Rfs*43) in T1 and CREBBP frameshift mutation (c.3923delT:p.L1308Cfs*30) in T2 were considered significant.
Figure 3

Mutation and copy number analyses of minute occults meningiomas (MOMs) by Sanger sequencing and fluorescence in situ hybridization of chromosome 22

(a) The NF2 frameshift mutation in T1 and CREBBP frameshift mutation in T2 were confirmed by Sanger sequencing. In addition, the same NF2 mutation was detected in the largest MOM (MOM3). (b) All three
MOMs (MOM1–MOM3) contained scattered cells exhibiting monosomy of chromosome 22q with one red and one green signal (arrowhead: heterozygous deletion; arrow: non-deleted). (c) Summary of CNA of chromosome 22 and mutations of NF2 and CREBBP in three MOMs and two tumors. (d) Phylogeny inferred from the somatic CNA and SNV/INDEL.

**Supplementary Files**

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