Acta Neuropathologica (2023) 146:833–835
https://doi.org/10.1007/s00401-023-02635-4

CORRESPONDENCE

Normal meninges harbor oncogenic somatic mutations in meningioma-driver genes

Julien Boetto[1,2] · Isabelle Plu[1,3] · Yohan Ducos[1] · Antoine Blouin[1] · Yu Teranishi[1] · The Brainbank Neuro-CEB Neuropathology Network · Sara Bizzotto[1] · Michel Kalamarides[1,4] · Matthieu Peyre[1,4]

Received: 1 July 2023 / Revised: 31 August 2023 / Accepted: 15 September 2023 / Published online: 26 September 2023

While somatic driver gene mutations are considered the hallmark of cancer, oncogenic mutations have recently been found in non-diseased proliferative tissues, such as endometrial [13], esophageal [10], or skin epithelium [11] but also in low proliferative tissues such as the brain [9]. Those discoveries lead to the hypothesis that the mutational processes giving rise to tumors preexist in normal tissue.

Meningioma oncogenesis is dominated by the occurrence of well-known driver gene mutations that form co-exclusive mutational groups, but 20% of meningiomas do not harbor any somatic mutation [4–6]. This assertion leads to the hypothesis of non-mutational initiating events in some cases, and questions us about the early events of meningioma formation in the normal meningeal layers. To date, no study focused on the presence of oncogenic driver mutations in the meninges of healthy individuals. To investigate this point, we studied the presence of low variant allele frequency (VAF) variants in the main driver genes with previously described oncogenic potential in meningiomas and decided to select meninges in the elderly, where there is a significant increased incidence of meningiomas [2].

Meningeal layers were obtained from individuals from the brain donation program of our institution. We analyzed a total of 90 post-mortem meningeal samples derived from 5 individuals with no history of intracranial tumors. For each of the 5 participants, we analyzed 15 dura mater samples (8 at the anterior skull base, 4 at the falx, and 3 at the convexity), 3 arachnoid samples, and one brain control sample (Fig. 1a, Supplementary Methods). Pathological analysis using HE sections (Supplementary Fig. 1) and Ki67 labeling (data not shown) confirmed the absence of meningioma or meningothelial hyperplasia at microscopic level and the absence of Ki67-positive cells. We generated deep-targeted sequencing data (average depth of 1760 × per sample across targeted regions, Supplementary Table 1) using a specific capture device covering intronic and exonic regions of 29 known meningioma-driver genes (Supplementary Table 2) and able to detect main chromosomal gains and losses (chromosome 1, 10, 18, and 22). We used Mutect2 and an in-house specific pipeline to call low VAF somatic variants (Fig. 1b and Supplementary Methods). For each individual, meningeal DNA was compared to brain DNA as control.

We obtained a total of 6493 variants, and conservatively filtered out variants to obtain high-confidence variants (Supplementary Methods). Among the 102 high-confidence variants, we kept only the 30 variants with functional impact (Supplementary Methods, Supplementary Table 3). Four occurred in meningioma major driver genes (one in *NF2* and three in *TRAF7*, Fig. 1c, d, Supplementary Table 3) in 4 separate patients (80% of the individuals). Median VAF for these variants was 0.86%. All four were predicted damaging and pathogenic by multiple algorithms (Fig. 1d and Supplementary Methods) and three of them were already described in meningiomas. Besides, all variants were uniquely found in one sample and none were seen in several samples within the same individual, even for neighbor samples separated only by few millimeters. Importantly, no variant was detected in the main hotspots of other oncogenic genes (*AKT1*, *SMO, PIK3CA*, Supplementary Table 4 and Supplementary Methods). To validate our variants, we performed droplet digital PCR (ddPCR) for three variants for

The members of The Brainbank Neuro-CEB Neuropathology Network are listed in Acknowledgements.

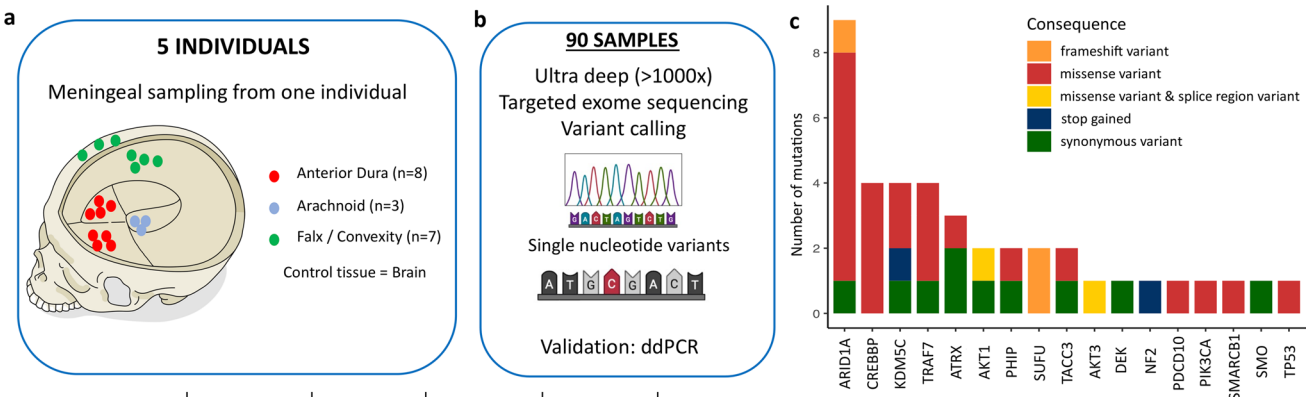
✉ Matthieu Peyre
matthieu.peyre@aphp.fr

[1] Sorbonne University, Paris Brain Institute, CRICM INSERM U1127 CNRS UMR 7225, APHP, 75013 Paris, France

[2] Department of Neurosurgery, Gui de Chauliac Hospital, CHU de Montpellier, 34090 Montpellier, France

[3] Department of Neuropathology, AP-HP, Hôpital Pitié Salpétrière, 75013 Paris, France

[4] Department of Neurosurgery, APHP, Hopital Pitié Salpêtrière, 47-91 Bvd de l'Hopital, 75013 Paris, France



d

Subject ID	P1	P2	P3	P4
Somatic mutation	*NF2* pTyr101*	*TRAF7* p.Lys615Glu	*TRAF7* p.Asn520Ser	*TRAF7* p.Asp585Ala
Tissue	Dura mater	Arachnoïd	Dura mater	Dura mater
Topography	ASB	Skull Base	ASB	ASB
VAF (ES-ddPCR), %	0.9 - 1.2	0.82 - 0.75	0.93 - 1.3	0.82 - NA
Predicted pathogenicity	Pathogenic	Pathogenic	Pathogenic	Pathogenic
Described in Meningioma	Yes	Yes	Yes	No

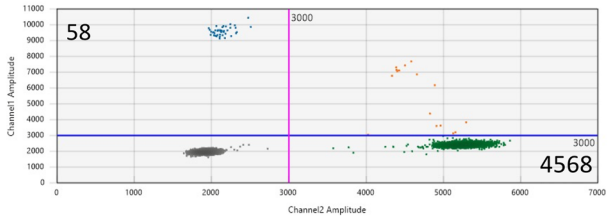


Fig. 1 **a** Illustration of the meningeal sampling for each individual. **b** Illustration of the methodology of the study. **c** Barplot illustrating the main genes and the somatic variants detected by ultra-deep-targeted sequencing. Variants are reported ordered by gene mutation frequency, and colored depending on the consequence of the mutation. **d** Summary of the four mutations present in the normal meninges in main driver genes for meningioma (*NF2* and *TRAF7*). VAFs are reported as obtained from exome sequencing (ES) and ddPCR validation. **e**. Two-dimensional ddPCR plot for the *NF2 p.Y101** mutation in P1_AD5 sample. The wild-type and mutant droplets are displayed, respectively, on the *x*- and *y*-axis. The number of mutant and wild-type droplets is displayed in the top left and bottom right, respectively

which assays were commercially available (*NF2 p.Tyr101**, *TRAF7 p.Lys615Glu* and *TRAF7 p. Asn520Ser*) (Fig. 1e, Supplementary Fig. 2), which confirmed the presence of the three variants and the respective VAFs.

Somatic mutations associated with cancer can be either driver mutations (able to promote clonal expansion), or passenger mutations that do not confer proliferative advantage. Here, we described the presence of passenger mutations in genes implicated in meningioma (*ARID1A, CREBBP*, *KDM5C*, or *TP53,* Fig. 1c), revealing the mutational profile of the normal meningeal layers. *ARID1A*, a gene involved in chromatin remodeling processes, was the top-mutant gene in our samples, and is mutated in anaplastic meningiomas [7]. Several studies in normal tissue (colon, skin, or esophagus) already described frequent mutations in this gene before tumor formation [16]. It is unknown whether *ARID1A* mutations in meningiomas are inherited from normal progenitors without playing a role in cancer progression, or if they could act as a first hit in aggressive meningioma clonal selection. No CNV event (e.g., loss of 22q) was detected in any of our samples (Supplementary Fig. 3).

All together, these results suggest that somatic mutations in driver or passenger genes associated with meningioma tumorigenesis are already present at low VAF in the normal meningeal layers of elderly individuals, without apparent meningeal pathology. The occurrence of the driver mutations may constitute an early event in a pathogenic progression through meningioma formation. The high frequency of somatic mutations in elderly dura mater is in line with the previous reports showing a high rate of karyotype abnormality and somatic mutational signatures suggestive of defective DNA damage repair in dura mater-derived cell lines compared to skin-derived cell lines from the same individual [3]. Our major finding concerns the presence of pathogenic *TRAF7* mutations in the anterior skull base dura or arachnoid, mirroring the location of *TRAF7*-mutant meningiomas and the high expression of *TRAF7* in neural crest-derived meninges during embryogenesis [12]. The occurrence of *TRAF7* mutations in the normal meninges also questions its intrinsic pathogenic value, since meningiomas frequently harbor a second co-mutation ($KLF4^{K409Q}$ or one of the main oncogenes of the PI3K pathway, *AKT1* and *PIK3CA*) [1, 5]. Our results are in line with the previous reports that suggest that *TRAF7* (as well as *NF2*) mutation is typically an early event acquired first in case of co-mutations [8].

Interestingly, driver mutations were present both in the arachnoid and dura mater samples. As arachnoid cells were present in dura mater samples in small amounts

(Supplementary Fig. 1f), it is not possible to determine which cell population harbored the oncogenic mutation. Additional studies are mandatory to determine the cell of origin of meningioma, that could be a quiescent meningeal stem cell physiologically present in both layers, able to proliferate and form different histological subtypes from a single cell of origin [15].

To conclude, our data are in favor of the spatially restricted local expansion of cellular clones that carry passenger and/or driver mutations that remain quiescent until secondary additional mechanisms still to discover trigger the tumoral proliferation. These mechanisms could depend on environmental factors (such as hormonal exposure) and rely on genetic (such as co-mutations) or epigenetic (such as methylation modifications) phenomenon [15]. Our targeted sequencing approach evaluated only genes known to be oncogenic in meningioma, and provides first evidence that oncogenic mutations exist in these genes. Future studies will address the total mutation load of normal meningeal samples and study potential variants in other genes. As a cross-sectional analysis in the elderly, this study does not address the question of mutations in young individuals and the evolution of the meningeal mutational burden with time. Detecting ultra-low mosaic clonal events remains technically challenging, and thus, additional mutations may also exist that bypassed our detection threshold [14]. Future studies addressing the accumulation of genetic variants or other molecular alterations in the normal meningeal layers will help to continue dissecting early mechanisms of meningioma oncogenesis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00401-023-02635-4.

Acknowledgements The authors would like to thank the following associations: ARSLA, CSC, France DFT, Fondation ARSEP, Fondation Vaincre Alzheimer, France Parkinson. The Neuro-CEB Neuropathology network includes: Dr. Franck Letournel (CHU Angers), Dr. Marie-Laure Martin-Négrier (CHU Bordeaux), Dr. Maxime Faisant (CHU Caen), Pr. Catherine Godfraind (CHU Clermont-Ferrand), Pr. Claude-Alain Maurage (CHU Lille), Dr. Vincent Deramecourt (CHU Lille), Dr. Mathilde Duchesne (CHU Limoges), Dr. David Meyronnet (CHU Lyon), Dr. Clémence Delteil (CHU Marseille), Pr. Valérie Rigau (CHU Montpellier), Dr. Fanny Vandenbos-Burel (Nice), Pr. Danielle Seilhean (CHU PS, Paris), Dr. Susana Boluda (CHU PS, Paris), Dr. Isabelle Plu (CHU PS, Paris), Dr. Dan Christian Chiforeanu (CHU Rennes), Dr. Florent Marguet (CHU Rouen), and Dr. Béatrice Lannes (CHU Strasbourg).

Funding This work was supported by a grant from the Fondation de l'Avenir to Matthieu Peyre (Project No. AP-RM-21015).

References

1. Abedalthagafi M, Bi WL, Aizer AA, Merrill PH, Brewster R, Agarwalla PK et al (2016) Oncogenic PI3K mutations are as common as AKT1 and SMO mutations in meningioma. Neuro Oncol 18:649–655. https://doi.org/10.1093/neuonc/nov316
2. Achey RL, Gittleman H, Schroer J, Khanna V, Kruchko C, Barnholtz-Sloan JS (2019) Nonmalignant and malignant meningioma incidence and survival in the elderly, 2005–2015, using the Central Brain Tumor Registry of the United States. Neuro Oncol 21:380–391. https://doi.org/10.1093/neuonc/noy162
3. Argouarch AR, Schultz N, Yang AC, Jang Y, Garcia K, Cosme CG et al (2022) Postmortem human dura mater cells exhibit phenotypic, transcriptomic and genetic abnormalities that impact their use for disease modeling. Stem Cell Rev Rep 18:3050–3065. https://doi.org/10.1007/s12015-022-10416-x
4. Brastianos PK, Horowitz PM, Santagata S, Jones RT, McKenna A, Getz G et al (2013) Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. Nat Genet 45:285–289. https://doi.org/10.1038/ng.2526
5. Clark VE, Erson-Omay EZ, Serin A, Yin J, Cotney J, Ozduman K et al (2013) Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. Science 339:1077–1080. https://doi.org/10.1126/science.1233009
6. Clark VE, Harmancı AS, Bai H, Youngblood MW, Lee TI, Baranoski JF et al (2016) Recurrent somatic mutations in POLR2A define a distinct subset of meningiomas. Nat Genet 48:1253–1259. https://doi.org/10.1038/ng.3651
7. Collord G, Tarpey P, Kurbatova N, Martincorena I, Moran S, Castro M et al (2018) An integrated genomic analysis of anaplastic meningioma identifies prognostic molecular signatures. Sci Rep 8:13537. https://doi.org/10.1038/s41598-018-31659-0
8. Dogan H, Blume C, Patel A, Jungwirth G, Sogerer L, Ratliff M et al (2022) Single-cell DNA sequencing reveals order of mutational acquisition in TRAF7/AKT1 and TRAF7/KLF4 mutant meningiomas. Acta Neuropathol 144:799–802. https://doi.org/10.1007/s00401-022-02485-6
9. Ganz J, Maury EA, Becerra B, Bizzotto S, Doan RN, Kenny CJ et al (2022) Rates and patterns of clonal oncogenic mutations in the normal human brain. Cancer Discov 12:172–185. https://doi.org/10.1158/2159-8290.CD-21-0245
10. Martincorena I, Fowler JC, Wabik A, Lawson ARJ, Abascal F, Hall MWJ et al (2018) Somatic mutant clones colonize the human esophagus with age. Science 362:911–917. https://doi.org/10.1126/science.aau3879
11. Martincorena I, Roshan A, Gerstung M, Ellis P, Van Loo P, McLaren S et al (2015) Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. Science 348:880–886. https://doi.org/10.1126/science.aaa6806
12. Mishra-Gorur K, Barak T, Kaulen LD, Henegariu O, Jin SC, Aguilera SM et al (2023) Pleiotropic role of TRAF7 in skull-base meningiomas and congenital heart disease. Proc Natl Acad Sci USA 120:e2214997120. https://doi.org/10.1073/pnas.2214997120
13. Moore L, Leongamornlert D, Coorens THH, Sanders MA, Ellis P, Dentro SC et al (2020) The mutational landscape of normal human endometrial epithelium. Nature 580:640–646. https://doi.org/10.1038/s41586-020-2214-z
14. Salk JJ, Schmitt MW, Loeb LA (2018) Enhancing the accuracy of next-generation sequencing for detecting rare and subclonal mutations. Nat Rev Genet 19:269–285. https://doi.org/10.1038/nrg.2017.117
15. Tomasetti C, Li L, Vogelstein B (2017) Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. Science 355:1330–1334. https://doi.org/10.1126/science.aaf9011
16. Wijewardhane N, Dressler L, Ciccarelli FD (2021) Normal somatic mutations in cancer transformation. Cancer Cell 39:125–129. https://doi.org/10.1016/j.ccell.2020.11.002