

YAP1-fusions in pediatric NF2-wildtype meningioma

Philipp Sievers^{1,2} · Jason Chiang³ · Daniel Schrimpf^{1,2} · Damian Stichel^{1,2} · Nagarajan Paramasivam⁴ · Martin Sill^{5,6} · Tenzin Gayden^{7,8,9} · Belen Casalini^{1,2} · David E. Reuss^{1,2} · James Dalton³ · Kristian W. Pajtler^{5,6,10} · Daniel Hänggi¹¹ · Christel Herold-Mende¹² · Elisabeth Rushing¹³ · Andrey Korshunov^{1,2,5} · Christian Mawrin¹⁴ · Michael Weller¹⁵ · Matthias Schlesner⁴ · Wolfgang Wick^{16,17} · Nada Jabado^{7,8,9} · David T. W. Jones^{5,18} · Stefan M. Pfister^{5,6,10} · Andreas von Deimling^{1,2} · David W. Ellison³ · Felix Sahn^{1,2,5} 

Meningioma is the most common primary central nervous system (CNS) tumor [8]. In contrast to adulthood, meningiomas are rare among children and adolescents and frequently (about 38%) occur in the context of tumor predisposition syndromes [12]. In line with the frequent inactivation of *NF2* in adult meningiomas, neurofibromatosis type 2 is the most common inherited syndrome predisposing to the early

development of meningiomas, which are often multiple. Other germline alterations predisposing to meningioma development are *SMARCE1* [14] and *SUFU* mutations [1]. More recently identified drivers of meningiomas include *AKT1/TRAF7*, *SMO*, *KLF4/TRAF7*, and *PIK3CA* mutations [3, 5].

Felix Sahn
felix.sahn@med.uni-heidelberg.de

¹ Department of Neuropathology, Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany

² Clinical Cooperation Unit Neuropathology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany

³ Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN, USA

⁴ Bioinformatics and Omics Data Analytics, German Cancer Research Center (DKFZ), Heidelberg, Germany

⁵ Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany

⁶ Division of Pediatric Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany

⁷ Department of Human Genetics, McGill University, Montreal, QC H3A 1B1, Canada

⁸ Department of Pediatrics, McGill University, Montreal, QC H4A 3J1, Canada

⁹ The Research Institute of the McGill University Health Center, Montreal, QC H4A 3J1, Canada

¹⁰ Department of Pediatric Oncology, Hematology, Immunology and Pulmonology, University Hospital Heidelberg, Heidelberg, Germany

¹¹ Department of Neurosurgery, University Medical Centre Mannheim, University of Heidelberg, Mannheim, Germany

¹² Division of Experimental Neurosurgery, Department of Neurosurgery, University Hospital Heidelberg, Heidelberg, Germany

¹³ Institute of Neuropathology, University Hospital and University of Zurich, Zurich, Switzerland

¹⁴ Institute of Neuropathology, Otto-von-Guericke University, Magdeburg, Germany

¹⁵ Department of Neurology, University Hospital and University of Zurich, Zurich, Switzerland

¹⁶ Clinical Cooperation Unit Neurooncology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany

¹⁷ Department of Neurology and Neurooncology Program, National Center for Tumor Diseases, Heidelberg University Hospital, Heidelberg, Germany

¹⁸ Pediatric Glioma Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany

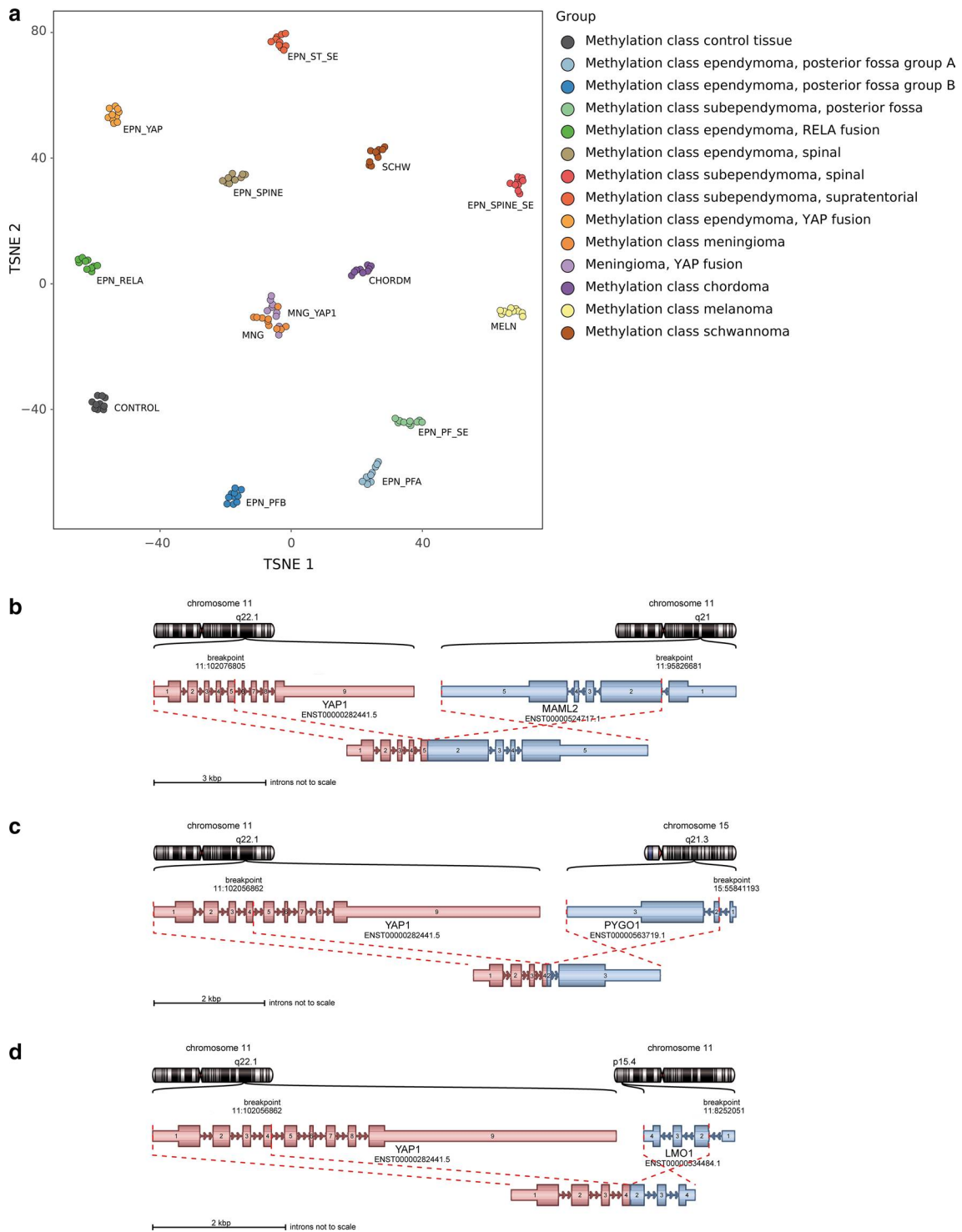


Fig. 1 a Unsupervised hierarchical clustering of DNA methylation profiles in nine *YAP1*-fused meningiomas (MNG_YAP1) alongside 128 well-characterized CNS neoplasms and control tissue shown in a two-dimensional representation of pairwise sample correlations using the 15,000 most variant probes by t-distributed stochastic neighbor embedding (t-SNE) dimensionality reduction. Reference methylation classes: ependymoma, posterior fossa group A (EPN_PFA), ependymoma, posterior fossa group B (EPN_PFB), ependymoma, RELA fusion (EPN_RELA), ependymoma, YAP fusion (EPN_YAP), sub-

ependymoma, posterior fossa (EPN_PF_SE), ependymoma, spinal (EPN_SPINE), subependymoma, spinal (EPN_SPINE_SE), subependymoma, supratentorial (EPN_ST_SE), meningioma (MNG), chordoma (CHORDM), melanoma (MELN), schwannoma (SCHW) and control tissue white matter (CONTROL). Schematic of the *YAP1-MAML2* fusion involving exons 1–5 of *YAP1* and exons 2–5 of *MAML2* (**b**), the *YAP1-PYGO1* fusion involving exons 1–4 of *YAP1* and exons 2–3 of *PYGO1* (**c**) and the *YAP1-LMO1* fusion involving exons 1–4 of *YAP1* and exons 2–4 of *LMO1* (**d**)

Table 1 Clinicopathological characteristics of the *YAPI*-fused meningioma cohort

Case #	Age (years)	Sex	Tumor location	Initial diagnosis	Genetic alteration
1	4	F	Lateral ventricles, third ventricle	MNG	<i>YAPI:MAML2</i>
2	3	M	Temporal	PXA	<i>YAPI:PYGO1</i>
3	1	M	Third ventricle, lateral ventricle	pHGG	<i>YAPI:MAML2</i>
4	2	M	Skull base	MNG	<i>YAPI:MAML2</i>
5	36	M	Optic nerve	MNG	<i>YAPI:MAML2</i>
6	8	F	Skull base (supra-/infratentorial)	MNG	<i>YAPI:LMO1</i>
7	17	M	Cavernous sinus	MNG	<i>YAPI:MAML2</i>
8	7	F	Parietal	MNG	<i>YAPI:MAML2</i>
9	7	F	Frontal	MNG	<i>YAPI:MAML2</i>

Histological meningioma subtypes meningiomas and WHO grade are provided in Supplementary Table 2. *MNG* meningioma, *PXA* pleomorphic xanthoastrocytoma, *pHGG* pediatric high grade glioma, *F* female, *M* male

The mutational underpinnings of sporadic pediatric meningioma have remained elusive to date. We report in-frame gene rearrangements predicted to result in fusions involving *YAPI* in nine meningiomas. We initially identified a *YAPI-MAML2* fusion by clinical RNA sequencing in a 4-year-old female patient with an intraventricular mass, histologically compatible with meningioma. Subsequently, based on our database of > 30,000 DNA methylation profiles of brain tumors [4], including > 1000 meningiomas (among them about 102 pediatric meningiomas, defined as age of diagnosis equal or below 18 years) [13], and corresponding copy number information, we additionally identified eight meningiomas with structural alterations affecting chromosome 11q around the *YAPI* locus (Online Resources Supplementary Figs. 2 and 3). All clustered with reference meningioma cases in t-SNE analysis of DNA methylation data (Fig. 1a) and showed histological and immunohistochemical features of meningioma (Online Resource Supplementary Fig. 1), despite two being initially diagnosed as glioma. Interestingly, of the nine cases two were classified as transitional and two as atypical meningiomas—both subtypes which often carry *NF2* mutations. With the presented case number and incomplete information on few samples, robust conclusions are, however, not derivable on subtype distribution. This limitation also applies to localizations. The clinicopathological characteristics are summarized in Table 1. RNA (seven samples) or exome (one sample) sequencing [15] revealed the presence of *YAPI* fusions in all eight additional tumors (Fig. 1b–d and Online Resource Supplementary Table 1).

Seven tumors harbored a rearrangement of *YAPI-MAML2* involving exons 1–5 ($n=5$) or only in exon 1 ($n=2$) of *YAPI* (NM_001130145) and exon 2–5 of *MAML2* (NM_032427). *YAPI-MAML2* fusions were verified by fluorescence in situ hybridization (FISH) performed in two cases (Online Resource Supplementary Fig. 4). A *YAPI-PYGO1* fusion was seen in a single case, containing exons 1–4 of *YAPI* and exons 2–3 of *PYGO1* (NM_015617). Additionally, a

YAPI-LMO1 fusion was detected in another case involving exons 1–4 of *YAPI* and exons 2–4 of *LMO1*. Of note, seven of the additional eight patients were children or adolescents, whereas one patient was an adult.

YAPI is a transcriptional co-activator and downstream effector of the HIPPO pathway that acts mainly through TEAD family transcription factors and regulates expression of genes involved in cell proliferation and apoptosis [6, 7, 19, 20]. Deregulation of the HIPPO pathway via overexpression of *YAPI*, leading to tumorigenesis is a frequent event in human malignancies including meningiomas [2, 9]. Rearrangements involving the *YAPI* gene have also recently been implicated as a driver in different types of cancer. Valouev et al. reported an in-frame gene fusion between *YAPI* and *MAML2* in nasopharyngeal carcinomas [16]. A similar fusion between *YAPI* and *MAMLD1* has been described in ependymoma [10, 11]. Both *MAML2* and *MAMLD1* are members of the Mastermind gene family and act as transcriptional co-activators of NOTCH signaling [17]. The *YAPI-MAML2* rearrangement combines the transcriptional activation domain of *MAML2* with the TEAD-binding domain of *YAPI*, which likely results in NOTCH-independent co-activation of TEAD-mediated HIPPO signaling [17]. While *PYGO1* has been associated with different types of cancer, structural rearrangements including *YAPI* and *PYGO1* have not been reported to the best of our knowledge. Notably, *PYGO1* also functions as a transcriptional co-activator in the Wnt pathway. *LMO1* acts as a transcriptional regulator with a tumor-promoting activity, but its role in tumors has not been well studied.

These alterations seem to act as an alternative to *NF2* inactivation, since no *NF2* alterations were detected in the present cohort. The *NF2* gene product, the tumor suppressor merlin, functions upstream of the HIPPO pathway and there is growing evidence suggesting a functional link between *NF2*, *YAPI* and activation of the HIPPO pathway [2, 18]. In line, *YAPI* fusion positive meningiomas clustered closer to

NF2 mutant cases than other pediatric meningiomas (Online Resource Supplementary Fig. 5). This observation parallels the virtually mutually exclusive alterations of *NF2* and *YAP1* in ependymoma. Since DNA methylation correlates with cell-of-origin, the proximity of *YAP1*- and *NF2*-altered cases in the clustering might indicate more similarities of their precursor cells compared to *SMARCE1* mutant meningiomas. However, the lack of *AKT1*, *SMO*, or *KLF4/TRAF7* mutant pediatric meningiomas and the few *SMARCE1* cases are limitations of this analysis.

Our findings identify *YAP1* fusions as a potential oncogenic driver in the development of meningiomas, predominantly in pediatric patients, and strengthen the hypothesis that deregulation of the HIPPO pathway is a central mechanism in meningioma tumorigenesis. Further studies in larger cohorts are needed to determine additional downstream functional consequences and a possible prognostic role of *YAP1* alterations in meningiomas.

Acknowledgements We thank L. Dörner and H. Y. Nguyen for skillful technical assistance and the microarray unit of the DKFZ Genomics and Proteomics Core Facility for providing Illumina DNA methylation array-related services. This study was supported by the German Cancer Aid (70112956) and Else Kröner-Fresenius Stiftung (EKFS 2015_A60). FS is a fellow of the Else Kröner Excellence Program of the Else Kröner-Fresenius Stiftung (EKFS 2017_EKES.24).

References

1. Aavikko M, Li SP, Saarinen S, Alhopuro P, Kaasinen E, Morgunova E et al (2012) Loss of SUFU function in familial multiple meningioma. *Am J Hum Genet* 91:520–526. <https://doi.org/10.1016/j.ajhg.2012.07.015>
2. Baia GS, Caballero OL, Orr BA, Lal A, Ho JS, Cowdrey C et al (2012) Yes-associated protein 1 is activated and functions as an oncogene in meningiomas. *Mol Cancer Res* 10:904–913. <https://doi.org/10.1158/1541-7786.MCR-12-0116>
3. 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>
4. Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D et al (2018) DNA methylation-based classification of central nervous system tumours. *Nature* 555:469–474. <https://doi.org/10.1038/nature26000>
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. Levy D, Adamovich Y, Reuven N, Shaul Y (2008) Yap1 phosphorylation by c-Abl is a critical step in selective activation of proapoptotic genes in response to DNA damage. *Mol Cell* 29:350–361. <https://doi.org/10.1016/j.molcel.2007.12.022>
7. Liu-Chittenden Y, Huang B, Shim JS, Chen Q, Lee SJ, Anders RA et al (2012) Genetic and pharmacological disruption of the TEAD–YAP complex suppresses the oncogenic activity of YAP. *Genes Dev* 26:1300–1305. <https://doi.org/10.1101/gad.192856.112>
8. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (2016) WHO classification of tumours of the central nervous system. IARC, Lyon
9. Overholtzer M, Zhang J, Smolen GA, Muir B, Li W, Sgroi DC et al (2006) Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. *Proc Natl Acad Sci USA* 103:12405–12410. <https://doi.org/10.1073/pnas.0605579103>
10. Pajtler KW, Witt H, Sill M, Jones DT, Hovestadt V, Kratochwil F et al (2015) Molecular classification of ependymal tumors across all CNS compartments, histopathological grades, and age groups. *Cancer Cell* 27:728–743. <https://doi.org/10.1016/j.ccell.2015.04.002>
11. Parker M, Mohankumar KM, Punchihewa C, Weinlich R, Dalton JD, Li Y et al (2014) C11orf95-RELA fusions drive oncogenic NF- κ B signalling in ependymoma. *Nature* 506:451–455. <https://doi.org/10.1038/nature13109>
12. Pathmanaban ON, Sadler KV, Kamaly-Asl ID, King AT, Rutherford SA, Hammerbeck-Ward C et al (2017) Association of genetic predisposition with solitary schwannoma or meningioma in children and young adults. *JAMA Neurol* 74:1123–1129. <https://doi.org/10.1001/jamaneurol.2017.1406>
13. Sahm F, Schrimpf D, Stichel D, Jones DTW, Hielscher T, Schefzyk S et al (2017) DNA methylation-based classification and grading system for meningioma: a multicentre, retrospective analysis. *Lancet Oncol* 18:682–694. [https://doi.org/10.1016/S1470-2045\(17\)30155-9](https://doi.org/10.1016/S1470-2045(17)30155-9)
14. Smith MJ, O'Sullivan J, Bhaskar SS, Hadfield KD, Poke G, Caird J et al (2013) Loss-of-function mutations in *SMARCE1* cause an inherited disorder of multiple spinal meningiomas. *Nat Genet* 45:295–298. <https://doi.org/10.1038/ng.2552>
15. Stichel D, Schrimpf D, Casalini B, Meyer J, Wefers AK, Sievers P et al (2019) Routine RNA sequencing of formalin-fixed paraffin-embedded specimens in neuropathology diagnostics identifies diagnostically and therapeutically relevant gene fusions. *Acta Neuropathol*. <https://doi.org/10.1007/s00401-019-02039-3>
16. Valouev A, Weng Z, Sweeney RT, Varma S, Le QT, Kong C et al (2014) Discovery of recurrent structural variants in nasopharyngeal carcinoma. *Genome Res* 24:300–309. <https://doi.org/10.1101/gr.156224.113>
17. Wu L, Sun T, Kobayashi K, Gao P, Griffin JD (2002) Identification of a family of mastermind-like transcriptional coactivators for mammalian notch receptors. *Mol Cell Biol* 22:7688–7700. <https://doi.org/10.1128/mcb.22.21.7688-7700.2002>
18. Zhang N, Bai H, David KK, Dong J, Zheng Y, Cai J et al (2010) The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals. *Dev Cell* 19:27–38. <https://doi.org/10.1016/j.devcel.2010.06.015>
19. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J et al (2007) Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev* 21:2747–2761. <https://doi.org/10.1101/gad.1602907>
20. Zhao B, Ye X, Yu J, Li L, Li W, Li S et al (2008) TEAD mediates YAP-dependent gene induction and growth control. *Genes Dev* 22:1962–1971. <https://doi.org/10.1101/gad.1664408>