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Neurofibromatosis type 1 mosaicism in Constitutional Mismatch Repair Deficiency patients

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Competing interests

The authors declare that they have no conflicts of interest directly related to the topic of this article.

Author Contributions

Study concept and design, collection and assembly of data: LGR, LB, JG

Molecular somatic and germline data analyses: EP, MM, TAB, OC, SC, EG, ER, SZ, DV

Neuropathological examinations: PV

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ABSTRACT

Differential diagnosis between Constitutional Mismatch Repair Deficiency (CMMRD) and Neurofibromatosis type 1 (NF1) is crucial as treatment and surveillance differ. We report the case of a girl with a clinical diagnosis of sporadic NF1 who developed a glioblastoma. Immunohistochemistry for MMR proteins identified PMS2 loss in tumor and normal cells and WES showed the tumor had an ultra-hypermuted phenotype, supporting the diagnosis of CMMRD. Germline analyses identified two variants (one pathogenic (PV) and one classified as VUS) in the *PMS2* gene and subsequent functional assays on blood lymphocytes confirmed the diagnosis of CMMRD. The large plexiform neurofibroma of the thigh and the freckling were however more compatible with NF1. Indeed, a *NF1* PV (VAFs of 20%, 3% and 9% and in blood, skin and saliva samples, respectively) was identified confirming a mosaicism for NF1. Retrospective analysis of a French cohort identified NF1 mosaicism in blood DNA in 2/22 patients with CMMRD, underlining the existence of early post-zygotic PV of *NF1* gene in CMMRD patients whose tumors have been frequently reported to exhibit somatic *NF1* mutations. It highlights the potential role of this pathway in the pathogenesis of CMMRD-associated gliomas and argues in favor of testing MEK inhibitors in this context.

KEYWORDS

Glioma predisposition; CMMRD; NF1; RAS-MAPK pathway; Oncogenesis; Glioblastoma; MMR; Mosaic

Word Count: 2539

INTRODUCTION

Neuro-cutaneous syndromes are associated with the risk of brain tumor, the most frequent being neurofibromatosis type 1 (NF1)[1] for which café-au-lait macule (CALM) is often the most obvious clinical sign in young patients. Other conditions such as Constitutional Mismatch Repair Deficiency (CMMRD) syndrome[2–4] can also be associated with brain tumors and CALMs. Pilocytic astrocytoma[5] (WHO grade I), often located in the optic pathway (optic pathway glioma), is the typical brain tumor observed in an NF1 child or adolescent, whereas CMMRD patients of this age develop malignant tumors (mainly high-grade gliomas and medulloblastomas).

In CMMRD, patient's prognosis is worse, the risk of successive tumors is considerably higher and therapeutic strategies clearly differ. Unfortunately, the phenotypic overlap between these two cancer predisposing syndromes may lead to a delayed diagnosis of CMMRD in some patients[6–8]. We describe here the case of a patient, initially diagnosed with NF1, in whom CMMRD syndrome was suspected after the occurrence of a glioblastoma. Genetic testing identified germline pathogenic variants (PV) of both *PMS2* alleles. Nevertheless, a PV in *NF1* was detected in a mosaicism form in DNAs from blood, skin, and saliva. Our observation raises important issues with respect to diagnosis and pathogenesis of CMMRD-associated glial tumors.

PATIENTS AND METHODS

Patients

After the identification of a mosaic NF1 in a CMMRD patient, we questioned the frequency of *NF1* mosaicism in a series of 22 French patients diagnosed with CMMRD and for whom blood

samples were available for DNA analysis. For all the patients, consents for genetic testing have been obtained from the parents/patients, according to research ethics requirements during a genetic counseling. CMMRD was confirmed in case of the identification of two variants in any of the four MMR genes, classified as (likely) PV and confirmed to be in *trans* by genetic testing. For patients with mono or biallelic variant(s) of unknown significance (VUS), confirmation of constitutional microsatellite instability (MSI) by a functional test (see below) was performed to confirm the diagnosis of CMMRD. Clinical and molecular data of CMMRD-patients were collected in the "Observatory of Genetic Cancer Predisposition Syndromes in Children and Adolescents" French database (*Observatoire des syndromes de prédisposition génétique au cancer des enfants et des adolescents*, PREDCAP, IRB00003888).

Nucleic acids extractions and next generation sequencing

DNA extraction and experiments were performed at the next-generation sequencing (NGS) panel facility of Gustave Roussy, Villejuif and Cochin Hospital, Paris (Assistance Publique-Hôpitaux de Paris, AP-HP). *NFI* was sequenced in the patient and in a large series of 22 CMMRD-patients for whom blood samples were available, using a targeted NGS panel, as previously described[9,10]. Sequencing libraries were prepared from 10ng of DNA per sample according to the TruSeq Custom Amplicon Library Preparation Guide (Illumina, San Diego, CA). The custom primer panel targets the entire *NFI*-coding exons, intron boundaries (25 bp), and the 5' and 3' untranslated regions (UTRs) with amplicons with an average size of 150 bp. The pooled libraries were paired-end (2×150) and sequenced with NextSeq 500 Mid Output Kit v2 on a NextSeq500 instrument (Illumina). The number of samples tested per run was adapted to allow an increase in sequencing depth (mean sequencing depth per sample: 1,500X). After demultiplexing and generation of FASTQ files, the sequence analysis was performed according to the Genome Analysis Tool Kit (GATK) guidelines. Sequence alignment, variant

calling, and variant annotation were performed using the MOABI pipeline and Polyweb pipeline (Paris Cité University). The VAF threshold used for the analysis was set at 3% to detect low mosaic variations. Variants were named at the coding DNA, RNA, and protein levels according to the *Human Genome Variation Society* recommendations. An assessment of variants' pathogenicity was performed according to the *American College of Medical Genetics and Genomics and the Association for Molecular Pathology* guidelines. Assessment of variants implication was mainly performed based on population databases (gnomAD), variant databases (ClinVar, HGMD, LOVD, and COSMIC), and prediction software. *In silico* predictions of the effect of the variant were performed with CADD, SPiP, dbSCSNV, Human Splice Finder, and PROVEAN. For the patients in whom a mosaic was identified in *NF1*, the PV was also searched by sequencing in samples of different origin (i.e saliva and skin).

Additional and functional tests for CMMRD diagnosis

An immunohistochemical (IHC) analysis of PMS2, MLH1, MSH2 and MSH6 protein expression in non-neoplastic cells was performed using formalin-fixed paraffin-embedded (FFPE) tumor sections available to assess loss of the affected MMR protein.

Due to the presence of a VUS of the *PMS2* gene, functional tests, i.e methylation tolerance (MT) and *ex vivo* microsatellite instability (evMSI) assays[11] were performed on immortalized lymphocytes. Studies were completed with NGS-based analysis of the large panel of selected markers, recently published by Gallon et al.[12].

RESULTS

1. Case presentation (patient 1)

We describe the case of a girl who presented numerous CALMs, bilateral axillar freckling, and a plexiform neurofibroma of the right thigh (Figure 1.a, b, c), suggesting NF1[13], but no

molecular screening for *NF1* was performed at the time of diagnosis. No history of tumor or NF1 phenotype in relatives have been reported. She had been treated for scoliosis since the age of 4. She had an ophthalmological screening at the age of 6 which did not find neither Lisch nodules nor visual alteration, but no brain MRI was performed during childhood.

At the age of 16, she was diagnosed with a right posterior parieto-occipital brain tumor revealed by headaches accompanied by hypoesthesia of the left upper limb. Brain MRI showed two synchronous contiguous lesions with a contrast enhancement, peripheral edema and infiltration towards the corpus callosum. Multiple developmental venous brain anomalies were also reported but no focal area of signal intensity on T2/FLAIR was observed. A partial excision of the occipital part of the tumor was performed allowing for pathological diagnosis and somatic molecular analyzes. The histopathology examination of the tissue biopsy revealed a glioblastoma with a few giant or multinucleated cells. IHC staining demonstrated expression of OLIG2, ATRX, and P53 in the tumor cells. The occurrence of a high-grade glioma, a typical CMMRD-associated pediatric tumor, raised doubts about the NF1 diagnosis. Thus immunostaining for MMR protein expression was performed and showed PMS2 loss in normal and tumor cells. Whole-tumor-exome sequencing revealed a typical pattern for CMMRD associated tumor[14] with an ultra-high tumor burden (229 mut/Mb; above the threshold of 100 mut/Mb defined for ultra-hypermutator phenotype), a somatic *POLE* driver PV (c.857C>G p.Pro286Arg), multiple PVs in genes such as *NF1* (two variants: c.2033dup p.(Ile679Aspfs*21) and c.532G>T p.(Glu178*)), *PMS2* (two variants: c.695G>T p.(Gly232Val) and c.2275+1G>A), *TP53*, *ATR* and *FANCA* (Table 1). Neither *IDH1* p.Arg132His somatic mutation, nor histone H3K27M or H3G34R/V somatic mutation were found. No microsatellite instability (MSI) (2.4% i.e., 202/7940) was reported according to MSI sensor (<https://github.com/ding-lab/msisensor>).

In view of the patient's phenotypic and tumor characteristics, constitutional genetic analyzes were performed after genetic counselling. Germline genetic analysis revealed two different variants (compound heterozygous) in the *PMS2* gene identified by NGS on two independent blood samples: one (c.2275+1G>A) classified as pathogenic inherited from the mother and the second (c.695G>T,p.(Gly232Val)), initially classified as VUS inherited from the father. In addition, the germline genetic analysis also identified a PV in the exon 18 of the *NF1* gene (NM_001042492.3): c.2033dup, p.(Ile679Aspfs*21) (variant allele frequency (VAF): ~20% in a blood sample, 3% in a skin sample, and 9% in a saliva sample) confirming the diagnosis of a mosaic NF1 associated with CMMRD in the patient (Supp Figure S1). Cultured fibroblasts were enriched with cells with the *NF1* PV (VAF=50% by Sanger sequencing).

Since one *PMS2* variant was classified as VUS, we performed additional ancillary testing in order to confirm the diagnosis of CMMRD. Functional assays demonstrated that the cells of the patient displayed methylation tolerance and *ex vivo* MSI[11], confirming the diagnosis of CMMRD although analyzes showed clear microsatellite instability only after prolonged culture. The patient was included in the study of R. Gallon et al.[12], her cMSI score confirmed the diagnosis of CMMRD in this patient although she has one of the lowest cMSI score among the *PMS2*-associated CMMRD samples, probably due to the *PMS2* missense variant causing their CMMRD syndrome. Based on these additional analyses, the c.695G>T p.(Gly232Val) *PMS2* variant was finally reclassified as likely pathogenic (class 4), according to the ACMG-AMP criteria[15].

After the partial surgery, the patient received adjuvant treatment combining radiotherapy to the right occipital lobe (54Gy at the rate of 1.8Gy per session) and immune check-point inhibition therapy with anti-PD1 as per the NIVOGLIO protocol (NCT04267146), without Temozolomide. She had a good clinical and radiological response with a significant decrease of the residual brain tumor and, as per protocol, immunotherapy was discontinued after one

year of treatment. She is in continuous remission 1.5 years after immunotherapy withdrawal and 2.5 years after the initial diagnosis. The identification of the CMMRD tumor predisposition syndrome enabled appropriate oncological surveillance to be proposed to this patient and her parents. Upper gastrointestinal endoscopy was normal. Colonoscopy at the age of 17, revealed a single 3 mm sessile polyp from the middle rectum, which has been excised. The pathological examination was in favor of a low-grade adenoma.

2. Cohort analysis

Among a series of 22 patients, we identified two additional patients with a *NF1* mosaicism in blood DNA: c.4751dup, p.(Lys1585Glnfs*37) with VAF ~4% (100/2560 reads) for patient 2 and c.184_185dup, p.(Leu62Phefs*2) with VAF ~16% (191/1167 reads) for patient 3 (Supp Figure S1). Patient 2 has previously been published in the C4CMMRD report on brain tumors[3]. He was diagnosed with CMMRD after occurrence of T lymphoblastic lymphoma, with identification of *PMS2* biallelic germline PV (c.2007-2A>G, p.?). Subsequently, during childhood, he developed a brain anaplastic astrocytoma grade III (IDH-wild type with angiocentric features) with a loss of *PMS2* expression by immunostaining and a high mutation load (rate = 184 coding SNV/Mb). He is still alive with stable disease after a recurrence of his brain tumor treated with a further focal radiation therapy 2.5 years ago. Clinically, he presents multiple CALMs with hypopigmented skin lesions but no neurofibroma or other NF1-associated features.

Patient 3 is the brother of a boy who developed an acute leukemia in the first years of life and a subsequent colonic adenocarcinoma, from which he died. Both brothers are carriers of compound heterozygous *PMS2* PVs, (c.1020_1021del p.(Arg341Alafs*23) and exons 13-15 deletion). CMMRD was identified in patient 3 prior to the occurrence of an acute B-lymphoblastic leukemia (as young teenager) treated according to the French CAALL protocol.

He is in complete remission 2 years after the end of the treatment. He presents with thoracic CALMs with segmental distribution exclusively on the right thoracic side and disseminated hypopigmented skin lesions. Like his brother, he also has multiple pilomatricomas, some of which have been surgically removed.

DISCUSSION

We report 3 patients who carry a combination of a CMMRD syndrome caused by bi-allelic germline *PMS2* PVs and a post-zygotic *NF1* PV. For the patient 1, initially diagnosed with NF1, CMMRD was suspected after the occurrence of a glioblastoma. The tumor characteristics (i.e. IHC and high mutation burden) and additional functional assays confirm CMMRD in this patient. Accordingly, both parents had a Lynch syndrome. Identification of the CMMRD and Lynch underlying diseases enables appropriate oncological surveillance for the patient and her parents, to be proposed according to published recommendations[4,16,17].

Since the first descriptions, the occurrence of CALM, especially with a segmental distribution, in patients with CMMRD is intriguing[18,19]. To date, only one patient carrying both a biallelic MMR PV and a *de novo* germline *NF1* PV (c.3721C>T p.(Arg1241*)) has been reported[6,20], while no *NF1* PV was reported in any of the other CMMRD cases in whom *NF1* analysis was performed[6,19]. Our report highlights that *NF1* postzygotic PVs exist in CMMRD-patients, and 3/23 (13%) patients have this combined genotype in the CMMRD cohort studied. Although tumor spectrum of CMMRD and NF1 may partly overlaps, tumor histologies are different between these two conditions. This observation underlines the fact that the identification of a mosaic *NF1* PV does not rule out the diagnosis of CMMRD. Considering the rarity of such a situation associating CMMRD and a mosaic *NF1* mutation, additional explorations to confirm or exclude CMMRD in patients with mosaic NF1 should however be proposed for patients with

one or more additional features strongly suggestive of CMMRD such as a typical CMMRD-associated tumor, digestive polyps or a diagnosis of Lynch syndrome in one of the parents[2,7]. The large size of the *NF1* gene and its high mutation rate, reflected in the fact that at least 50% of all NF1 cases are sporadic, may render the *NF1* gene highly susceptible to postzygotic mutations[18]. In addition, the c.2033dup variant identified in patient 1, is a hotspot variant in a 7 cytosine stretch which is the longest mononucleotide repeat in the *NF1* coding regions. We can speculate that in CMMRD patients, post-zygotic mutations of *NF1* may occur more frequently than in the normal population due to a non-functional MMR system. It is worth noting that the three *NF1* mosaic variants identified in our study are all duplications (c.2033dup, c.4751dup, and c.184_185dup), suggesting a mechanism of replicative slippage mutagenesis favored by the MMR system deficiency. The very high frequency of *NF1* somatic mutations in MMR-deficient tumors[21,22] and the lack of recurrent mutations reported in other large genes, also support the hypothesis that *NF1* is a target of MMR deficiency. *NF1* haploinsufficiency has been shown to increase astrocyte proliferation[23], is associated with increased angiogenesis[24] and perturbations of cell cycle and DNA repair pathways[25]. It is conceivable that *NF1* heterozygous mutations may confer a growth advantage and a positive selection of *NF1*-mutated cells in a CMMRD context. We suggest that CMMRD may alter *NF1* at different developmental times and heightens the pressure to acquire *NF1* postzygotic mutations early in development. For patient 1, the mutation probably appeared very early before the individualization of the neuro-ectodermal and mesenchymal leaflets.

These three cases support the hypothesis that NF1-associated phenotypic features in CMMRD patients are a consequence of early *NF1* mutations. In 2 out the 3 patients described, CALMs were segmental, which is consistent with the clinical features of usual mosaic NF1. In patient 1, molecular analysis of the glioblastoma showed two hits in *NF1* (including the mosaic PV), indicating the contribution of *NF1* inactivation to the development of this cancer. The actual

impact of such genetic conditions on oncological risks for patients who carry both MMR and *NF1* PVs remains to be determined. The risk of neurofibroma transformation in the context of *NF1* is not negligible[26]. Even though no malignant peripheral nerve sheath tumors (MPNST) have been reported as far as we know in CMMRD patients, it could be speculated that the risk of transformation could be higher in the context of MMR-deficiency. There is currently no evidence of the need for specific surveillance guidelines for plexiform neurofibroma in the context of CMMRD. Future study will have to analyze whether the current guidelines for patients with *NF1*-associated plexiform neurofibroma[27] are adapted for CMMRD patients.

This report demonstrates that the phenotypic overlap between CMMRD and *NF1* syndromes can sometimes be explained by mosaic *NF1* and suggests the involvement of the RAS-MAPK pathway in the pathogenesis of CMMRD-associated glial neoplasms. These observations support the previous suggestion[22] to explore the combination of MEK inhibitors with immune checkpoint inhibitors for the treatment of these tumors.

REFERENCES

- 1 Kratz CP, Jongmans MC, Cavé H, *et al.* Predisposition to cancer in children and adolescents. *Lancet Child Adolesc Health* 2021;**5**:142–54. doi:10.1016/S2352-4642(20)30275-3
- 2 Wimmer K, Kratz CP, Vasen HFA, *et al.* Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium ‘care for CMMRD’ (C4CMMRD). *J Med Genet* 2014;**51**:355–65. doi:10.1136/jmedgenet-2014-102284
- 3 Guerrini-Rousseau L, Varlet P, Colas C, *et al.* Constitutional mismatch repair deficiency-associated brain tumors: report from the European C4CMMRD consortium. *Neurooncol Adv* 2019;**1**:vdz033. doi:10.1093/noajnl/vdz033
- 4 Aronson M, Colas C, Shuen A, *et al.* Diagnostic criteria for constitutional mismatch repair deficiency (CMMRD): recommendations from the international consensus working group. *J Med Genet* Published Online First: 23 February 2021. doi:10.1136/jmedgenet-2020-107627

- 5 Louis DN, Perry A, Wesseling P, *et al.* The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro Oncol* 2021;**23**:1231–51. doi:10.1093/neuonc/noab106
- 6 Wimmer K, Rosenbaum T, Messiaen L. Connections between constitutional mismatch repair deficiency syndrome and neurofibromatosis type 1. *Clin Genet* 2017;**91**:507–19. doi:10.1111/cge.12904
- 7 Suerink M, Ripperger T, Messiaen L, *et al.* Constitutional mismatch repair deficiency as a differential diagnosis of neurofibromatosis type 1: consensus guidelines for testing a child without malignancy. *J Med Genet* 2019;**56**:53–62. doi:10.1136/jmedgenet-2018-105664
- 8 Guerrini-Rousseau L, Suerink M, Grill J, *et al.* Patients with High-Grade Gliomas and Café-au-Lait Macules: Is Neurofibromatosis Type 1 the Only Diagnosis? *AJNR Am J Neuroradiol* 2019;**40**:E30–1. doi:10.3174/ajnr.A6058
- 9 Pasmant E, Parfait B, Luscan A, *et al.* Neurofibromatosis type 1 molecular diagnosis: what can NGS do for you when you have a large gene with loss of function mutations? *Eur J Hum Genet* 2015;**23**:596–601. doi:10.1038/ejhg.2014.145
- 10 Lobón-Iglesias MJ, Laurendeau I, Guerrini-Rousseau L, *et al.* NF1-like optic pathway gliomas in children: clinical and molecular characterization of this specific presentation. *Neurooncol Adv* 2019;**2**:i98–106. doi:10.1093/oaajnl/vdz054
- 11 Bodo S, Colas C, Buhard O, *et al.* Diagnosis of Constitutional Mismatch Repair-Deficiency Syndrome Based on Microsatellite Instability and Lymphocyte Tolerance to Methylating Agents. *Gastroenterology* 2015;**149**:1017-1029.e3. doi:10.1053/j.gastro.2015.06.013
- 12 Gallon R, Phelps R, Hayes C, *et al.* Constitutional microsatellite instability, genotype, and phenotype correlations in Constitutional Mismatch Repair Deficiency. *Gastroenterology* 2022;;S0016-5085(22)01444-5. doi:10.1053/j.gastro.2022.12.017
- 13 Legius E, Messiaen L, Wolkenstein P, *et al.* Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. *Genet Med* 2021;**23**:1506–13. doi:10.1038/s41436-021-01170-5
- 14 Campbell BB, Light N, Fabrizio D, *et al.* Comprehensive Analysis of Hypermutation in Human Cancer. *Cell* 2017;**171**:1042-1056.e10. doi:10.1016/j.cell.2017.09.048
- 15 Richards S, Aziz N, Bale S, *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;**17**:405–24. doi:10.1038/gim.2015.30
- 16 Vasen HFA, Ghorbanoghli Z, Bourdeaut F, *et al.* Guidelines for surveillance of individuals with constitutional mismatch repair-deficiency proposed by the European Consortium ‘Care for CMMR-D’ (C4CMMR-D). *J Med Genet* 2014;**51**:283–93. doi:10.1136/jmedgenet-2013-102238

- 17 Seppälä TT, Latchford A, Negoï I, *et al.* European guidelines from the EHTG and ESCP for Lynch syndrome: an updated third edition of the Mallorca guidelines based on gene and gender. *Br J Surg* 2021;**108**:484–98. doi:10.1002/bjs.11902
- 18 Wang Q, Lasset C, Desseigne F, *et al.* Neurofibromatosis and early onset of cancers in hMLH1-deficient children. *Cancer Res* 1999;**59**:294–7.
- 19 Auclair J, Leroux D, Desseigne F, *et al.* Novel biallelic mutations in MSH6 and PMS2 genes: gene conversion as a likely cause of PMS2 gene inactivation. *Hum Mutat* 2007;**28**:1084–90. doi:10.1002/humu.20569
- 20 Alotaibi H, Ricciardone MD, Ozturk M. Homozygosity at variant MLH1 can lead to secondary mutation in NF1, neurofibromatosis type I and early onset leukemia. *Mutat Res* 2008;**637**:209–14. doi:10.1016/j.mrfmmm.2007.08.003
- 21 Wang Q, Montmain G, Ruano E, *et al.* Neurofibromatosis type 1 gene as a mutational target in a mismatch repair-deficient cell type. *Hum Genet* 2003;**112**:117–23. doi:10.1007/s00439-002-0858-4
- 22 Campbell BB, Galati MA, Stone SC, *et al.* Mutations in the RAS/MAPK Pathway Drive Replication Repair-Deficient Hypermutated Tumors and Confer Sensitivity to MEK Inhibition. *Cancer Discov* 2021;**11**:1454–67. doi:10.1158/2159-8290.CD-20-1050
- 23 Gutmann DH, Loehr A, Zhang Y, *et al.* Haploinsufficiency for the neurofibromatosis 1 (NF1) tumor suppressor results in increased astrocyte proliferation. *Oncogene* 1999;**18**:4450–9. doi:10.1038/sj.onc.1202829
- 24 Wu M, Wallace MR, Muir D. Nf1 haploinsufficiency augments angiogenesis. *Oncogene* 2006;**25**:2297–303. doi:10.1038/sj.onc.1209264
- 25 Pemov A, Park C, Reilly KM, *et al.* Evidence of perturbations of cell cycle and DNA repair pathways as a consequence of human and murine NF1-haploinsufficiency. *BMC Genomics* 2010;**11**:194. doi:10.1186/1471-2164-11-194
- 26 Brems H, Beert E, de Ravel T, *et al.* Mechanisms in the pathogenesis of malignant tumours in neurofibromatosis type 1. *Lancet Oncol* 2009;**10**:508–15. doi:10.1016/S1470-2045(09)70033-6
- 27 Carton C, Evans DG, Blanco I, *et al.* ERN GENTURIS tumour surveillance guidelines for individuals with neurofibromatosis type 1. *EClinicalMedicine* 2023;**56**:101818. doi:10.1016/j.eclinm.2022.101818

LEGENDS

Figure 1

Skin features of the patient 1

(a) axillar frecklings, (b) plexiform neurofibroma of the right thigh, (c) numerous café-au-lait macules.

Table 1

Molecular analysis of patient 1: Germline and somatic alterations identified by RNA sequencing and whole exome sequencing of blood and tumor (glioblastoma, percentage of tumor cells: 80%).

Table 1

Gene name	Variant classification	HGVSc	HGVSp	Consequence	Constitutional DNA VAF (%)	Tumor DNA VAF (%)	Tumor RNA VAF (%)
<i>PMS2</i>	PV / SNV	NM_000088.4: c.695G>T	NP_000526.2: p.(Gly232Val)	Missense variant	55	50	68
<i>NF1</i>	PV / insertion	NM_000267.3: c.2033dup	NP_000258.1: p.(Ile679Aspfs*21)	Frameshift variant	20	47	0
<i>PMS2</i>	PV / SNV	NM_000535.7: c.2275+1G>A	.	Splice donor variant	45	29	0
<i>TP53</i>	PV / SNV	NM_000546.6: c.817C>T	NP_000537.3: p.(Arg273Cys)	Missense variant	.	48	77
<i>FANCA</i>	PV / SNV	NM_000135.4: c.3624C>T	NP_000126.2: p.(Ser1208=)	Splice region & synonymous variant	.	47	0
<i>TGFBR2</i>	PV / SNV	NM_003242.6: c.1336G>A	NP_001020018.1: p.(Asp446Asn)	Missense variant	.	46	15
<i>SETD2</i>	PV / insertion	NM_001349370.3: c.4087dup	NP_001336299.1: p.(Arg1363Lysfs*8)	Frameshift variant	.	45	22
<i>SMO</i>	PV / SNV	NM_005631.5: c.1965G>A	NP_005622.1: p.(Trp655*)	Nonsense	.	44	42
<i>KDM6A</i>	PV / SNV	NM_001291415.2: c.4207C>T	NP_001278344.1: p.(Arg1403*)	Nonsense	.	41	0
<i>POLE</i>	PV / SNV	NM_006231.4 c.857C>G	NP_006222.2: p.(Pro286Arg)	Missense variant	.	41	58
<i>ATR</i>	PV / SNV	NM_001184.4: c.7597C>T	NP_001175.2: p.(Arg2533*)	Nonsense	.	38	31
<i>NF1</i>	PV / SNV	NM_000267.3: c.532G>T	NP_000258.1: p.(Glu178*)	Nonsense	.	37	50
<i>APC</i>	PV / SNV	NM_000038.6: c.2626C>T	NP_000029.2: p.(Arg876*)	Nonsense	.	34	50
<i>TP53</i>	PV / SNV	NM_001126112.3: c.586C>T	NP_001119584.1: p.(Arg196*)	Nonsense	.	30	0
<i>PPP2R1A</i>	PV / SNV	NM_001363656.2: c.7C>T	NP_001350585.1: p.(Arg3Trp)	Missense variant	.	25	28
<i>ARID1B</i>	PV / SNV	NM_001346813.1: c.2455C>T	NP_001333742.1: p.(Gln819*)	Nonsense	.	14	16

Abbreviation: VAF: variant allele frequency, PV: pathogenic variant, SVN : small nucleotide variant

