HHS Public Access

Author manuscript

J Med Genet. Author manuscript; available in PMC 2024 October 01.

Published in final edited form as:

J Med Genet. 2023 October ; 60(10): 987–992. doi:10.1136/jmg-2022-108854.

Germline pathogenic SMARCA4 variants in neuroblastoma

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AUTHOR CONTRIBUTIONS

LW and KEN contributed equally to this paper. LW, KEN, SEP, and WDF designed the study and contributed to manuscript preparation and editing. MJ, LT, NvE, JHM, HM, ALD, MJA provided study patients. RRDJ, AB, TR, MH performed pathology review on the cases. All authors critically reviewed and approved the final manuscript.

COMPETING INTERESTS

MJA is a full-time, salaried employee of Invitae Corporation. JHM is a full-time, salaried employee of Ambry Genetics. RRDK is a council member European Society of Pathology, unpaid. MCJ is a Board member of the SIOP Europe host genome working group, unpaid. LW is a consultant for PierianDx and Precision Rx-Dx Inc. SEP is a member of the Board of Directors, American Society of Human Genetics and a member of the Scientific Advisory Panel Baylor Genetics.

ETHICS APPROVAL

This study involves human participants and was approved by the following Ethics Committees or Institutional Boards: CIUSSS West-Central Montreal Research Ethics Board: study number 15–140; WCG Institutional Review Board (Study ID CR-001-02); Royal Melbourne Hospital Human Research Ethics Committee (HREC) (previously Melbourne Health HREC) as part of the Australian Genomics Research Protocol (HREC Reference Number: HREC/16/MH/251); Ambry Genetics Corporation IRB

Abstract

Heterozygous germline pathogenic variants (GPVs) in *SMARCA4,* the gene encoding the ATP-dependent chromatin remodeling protein SMARCA4 (previously known as BRG1), predispose to several rare tumour types, including small cell carcinoma of the ovary, hypercalcemic type, atypical teratoid and malignant rhabdoid tumor, and uterine sarcoma. The increase in germline testing of *SMARCA4* in recent years has revealed putative GPVs affecting *SMARCA4* in patients with other cancer types. Here we describe 11 patients with neuroblastoma, including four previously unreported cases, all of whom were found to harbour heterozygous germline variants in *SMARCA4*. Median age at diagnosis was 5 years (range 2 months to 26 years), nine were males and eight of nine cases with tumour location information were in an adrenal gland. Eight of the germline variants were expected to result in loss of function of *SMARCA4* (large deletion, truncating, canonical splice variants), while the remaining four were missense variants. Loss of heterozygosity of the wild-type *SMARCA4* allele was found in all eight cases where somatic testing was performed, supporting the notion that SMARCA4 functions as a classic tumour suppressor. Altogether, these findings strongly suggest that neuroblastoma be included in the spectrum of *SMARCA4*-associated tumours.

Keywords

Genetic Predisposition to Disease; Genetic Testing; Germ-Line Mutation; Pediatrics; Sequence Analysis; DNA

INTRODUCTION

Germline pathogenic variants (GPVs) in *SMARCA4,* which encodes the ATP-dependent chromatin remodeling protein SMARCA4 (previously known as BRG1), are associated with rhabdoid tumor predisposition syndrome type 2 (RTPS2), a rare cancer predisposition associated with development of small cell carcinoma of the ovary, hypercalcemic type (SCCOHT), atypical teratoid/rhabdoid tumour (ATRT), malignant rhabdoid tumor (MRT), and uterine sarcoma.[1–3] The increasing application of genomic testing in the context of research and clinical settings has revealed variants in *SMARCA4* in patients with other cancer phenotypes. Interestingly, several of these have involved cases of neuroblastoma (NBL), the most common extracranial pediatric tumour.[4–6] Of relevance to the data presented here, prior studies have shown somatic biallelic loss of *SMARCA4* in approximately 1% of NBL and that loss of chromosome 19p (the region where *SMARCA4* is located) could confer a poorer prognosis.[4 7] Among the published reports, most *SMARCA4* variants were of somatic or undetermined origin, with only rare studies describing single cases in which *SMARCA4* alterations were confirmed to be of germline origin. In one report, it was noted that *SMARCA4* variants are enriched in patients with NBL versus controls; however, this report did not definitively determine that the variants originated in the germline.[4] Thus, it remains to be determined how often NBL truly arises in association with a *SMARCA4* GPVs. Here, we describe 11 patients with NBL who harboured putative GPVs in *SMARCA4*, including four novel cases, one case presented in abstract form and six that have been previously reported. We provide information on clinical

features and tumour genomic data, which taken together suggest that *SMARCA4* represents a bona fide NBL predisposition gene.

METHODS

We performed a literature search with the goal of identifying all cases of NBL reported to arise in persons with GPVs in *SMARCA4*. Additionally, cases were gathered through internal data at participating institutions and via personal communications. Where available, we collected information on patient age, sex, tumour location, germline and somatic *SMARCA4* variant details, SMARCA4 immunohistochemical (IHC) staining results, and tumour MYCN status (Table 1). *SMARCA4* variants were classified per the Richards et al., 2015 ACMG/AMP guidelines.[8]

RESULTS

Through literature review, internal case review, and personal communication, we collected 11 cases of patients with NBL in whom a heterozygous germline *SMARCA4* variant was found upon multi-gene panel testing, exome, or genome sequencing of patients diagnosed with NBL. The variants were scattered across the gene (Figure 1A). In eight cases (73%), germline variants (frameshift, nonsense, canonical splicing, large deletion) were predicted to cause loss-of-function (LoF), typical of those reported in RTPS2, while in three cases they were missense variants (Table 1, Figure 1A). We classified two of these LoF variants as Pathogenic: c.535C>T, p.(Gln179Ter) seen in Case 5 and c.493C>T; p.(Gln165Ter) seen in Case 6 (Table 1). The tumour in Case 5 showed loss of SMARCA4 expression by IHC and we therefore used PP4 as suggested by Walsh et al.[13] The variant in Case 6 has been seen in an SCCOHT tumour (internal data), and we therefore used the code PS4 at a supporting level, as has been suggested by previous ClinGen Variant Curation Expert Panels for other genes specifications (www.clinicalgenome.org). Five additional LoF variants are predicted to undergo nonsense-mediated decay but have not been reported elsewhere, and therefore were classified as Likely Pathogenic (Table 1). One variant was a large deletion on chromosome 19 that included *SMARCA4*. This was classified as Pathogenic according to ClinGen's CNV pathogenicity calculator (http://cnvcalc.clinicalgenome.org/cnvcalc/cnv-loss) based on classification rules from Riggs et al., 2020.[14]

The remaining three variants were missense variants. Two (c.2372C>T, p.(Ala791Val) and c.4670T>C, p.(Leu1557Pro)) could not be definitively classified and therefore remained as variants of uncertain significance (VUSs), though both variants are predicted to be deleterious by REVEL. One of these three VUSs (c.4670T>C, p.Leu1557Pro) was seen in a case where SMARCA4 expression was lost in the tumour (Case 10). The third missense variant, c.2717G>A; p.(Arg906His) (Case 4), has been reported somatically in a case of NBL,[15] as well as in two cases of gastric carcinoma (http://cancer.sanger.ac.uk)[16] and one uterine endometrioid adenocarcinoma.[17 18] Furthermore, three other variants at this position (p.Arg906Leu, p.Arg906Cys, p.Arg906Ser) have been seen in other tumour types in COSMIC (COSV60811731, COSV60801865, COSV100758492).[16] The p.Arg906Cys variant has also been reported somatically in a NBL that harboured a second *SMARCA4* variant,[4] as well as in at least two patients with neurological disorders in which it arose *de*

novo (ClinVar ID: 421281). Furthermore, all variants at the c.2717G position are predicted to be damaging by the REVEL meta-predictor.[19] In case 4, despite evidence of LOH in the tumour (based on a VAF of 80%), there was retained SMARCA4 expression by IHC (Figure 1B, C). Although several pathogenic ACMG/AMP criteria can be applied, which suggests that the position is important for SMARCA4 protein function, the variant remains of uncertain significance in the context of cancer predisposition.

Tumour sequencing was performed in seven of 11 cases, all of which showed LOH that included at least *SMARCA4* (Table 1). Three cases had SMARCA4 IHC results available, as discussed above (Cases 4, 5, 10) (Figure 1, Table 1). For the remaining cases, IHC results were unavailable, but for the three cases with LoF variants and LOH in the tumour (Cases 1, 2, and 8), we expect that SMARCA4 protein expression would be lost.

The median age of onset for these cases was 5 years (range 2 months – 26 years), with all but one patient presenting in the pediatric setting, when NBL is most common. There was no obvious difference in age of onset between patients with missense versus LoF variants; however, this could not be statistically calculated due to the small sample size. Only one previously reported case presented with bilateral NBL, a 4-year-old child who also happened to harbour a PGV affecting the *CHEK2* gene.[5] MYCN status was available in four cases, all of which showed no amplification of the gene. These data are summarized in Table 1. Information on inheritance of the variants was only available in cases 2 and 10, both of which were inherited from the father. None of the individuals tested were known to have any family history of *SMARCA4*-related cancers.

DISCUSSION

Here we describe 11 patients with NBL who harboured heterozygous germline *SMARCA4* variants. Eight of the 11 cases had LoF variants, including one large deletion encompassing the entire *SMARCA4* gene. In seven patients for whom the tumours were evaluated, there was loss of the wildtype allele (including the case with the p.Arg906His missense variant), supporting the involvement of these variants in tumour development. For the remaining four cases, tumour sequencing information was not available, but three of these patients harboured LoF variants (Cases 3, 6, 9), which were classified as Likely Pathogenic or Pathogenic. The remaining case (Case 7) harboured a missense variant classified as VUS that was predicted to be deleterious using the REVEL meta-predictor (score: 0.764)[19] and is located in the helicase functional domain.

In all four cases where MYCN status was tested, none showed MYCN amplification, which is not surprising, as it has been shown in other cancers that MYC amplification and SMARCA4 inactivation are mutually exclusive.[20] Although IHC was not available for eight of 11 cases, based on studies in SCCOHT and ATRT where LoF variants almost always lead to loss of SMARCA4 expression,[3] we expect that the six cases with germline LoF variants would have loss of expression of SMARCA4 in the tumour, similar to the one case that had an LoF variant and IHC results available (Case 5, Figure 1D, E).

Hereditary NBL is known to be due to *ALK* and *PHOX2B* germline variants in 1–2% of cases.[21] Furthermore, NBL can rarely develop in patients with neurofibromatosis and other RASopathies, Beckwith-Wiedemann syndrome, Li-Fraumeni syndrome, Weaver syndrome, Fanconi anemia, and Familial paraganglioma/pheochromocytoma.[21] However, the cases reported here suggest that heterozygous germline *SMARCA4* variants may also contribute to NBL risk. Further supporting this possibility, LoF *SMARCA4* variants are extremely rare in GnomAD (https://gnomad.broadinstitute.org; there are less than 10 such variants), giving the *SMARCA4* gene a high pLI score (1.0), indicating extreme intolerance of the gene to LoF variants. In addition, the incidence of *SMARCA4* variants in NBL has been reported to be higher than expected by chance, as noted by Bellini et al., where they calculated an odds ratio of 4.5 in their series of cases.[4]

Germline LoF *SMARCA4* germline variants are known to predispose to SCCOHT, ATRT, and less commonly, uterine sarcoma. While SCCOHT, ATRT, and most SMARCA4-deficient uterine sarcomas have similar rhabdoid cell morphology,[3 22] the NBLs studied here did not have rhabdoid morphology, except for case 10, which was suspected to be a rhabdoid tumour on biopsy but was ultimately diagnosed as a NBL upon full tumour resection. Although PHOX2B staining was negative in this case, NB84, a known neuroblastoma marker, was positive; as well, there was strong chromogranin A IHC and diffuse synaptophysin staining. When combined with the observed ganglion cell differentiation in the resection specimen, this led to the final diagnosis of NBL. This highlights the importance of expert pathology review, as this is not the first case where a NBL was misdiagnosed as a rhabdoid tumour.[23]

Given that the LoF variants are similar in nature to those found in other SMARCA4-deficient tumours, there does not appear to be a clear genotype-phenotype relationship to predict the specific tumour type that develops. Previous *in vivo* studies in mice have shown that the development of ATRTs, more commonly caused by variants in *SMARCB1*, is highly dependent on the time in which the second hit in *SMARCB1* is acquired.[24] It has been suggested that like NBLs, rhabdoid tumours arise from neural crest cells,[25 26] and so perhaps the tumour type is dependent on the timing and cell type in which the second *SMARCA4* hit occurs.

In addition to germline variants, somatic variants in *SMARCA4* are commonly seen in multiple tumour types, including lung cancer, bladder carcinoma, and Burkitt's lymphoma.[17 18] More recently, somatic *SMARCA4* variants have been associated with NBL and while one study found that an increase in *SMARCA4* expression was necessary for NBL formation and associated with poorer survival,[27] others have found loss of *SMARCA4* to be associated with oncogenesis, an older age of onset, and poor prognosis.[4 7 15] All the patients listed here were diagnosed in the pediatric age, typical of NBLs, aside from one patient who was diagnosed at age 26, the patient with the p.Arg906His variant.

To put these results in context, we have compiled the results of large sequencing studies that included *SMARCA4* germline testing in a Supplementary Table (Table S1). These studies suggest that approximately 0.2% of patients with NBL harbour a likely pathogenic or pathogenic variant in *SMARCA4*. Based on these findings, we recommend that in patients

where a *SMARCA4* pathogenic variant is found in a NBL (especially those with loss of SMARCA4 staining by IHC), or those with a positive family history of NBL, SCCOHT, ATRT, or SMARCA4-deficient uterine sarcomas, germline testing of the gene should be considered to exclude an underlying hereditary predisposition. It remains unclear whether such patients should undergo regular screening for NBL, as the lifetime penetrance of *SMARCA4* variants for NBL development is unknown. Further research into the prevalence of *SMARCA4* germline variants in patients with NBL and the impacts of these variants on protein function, clinical manifestations and overall outcomes are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

Thank you to all patients and families who participated in the relevant studies. Thank you to Amanda B Spurdle and Nicola Waddell from QIMR Berghofer for their advice.

FUNDING

ALD is supported by an Australian Government Research Training Program (RTP) Scholarship and a QIMR Berghofer HDC PhD Top Up Scholarship. Case identified via research was funded by Australian Genomics. NHMRC (Grants GNT1113531, GNT2000001) and the Australian Government's Medical Research Future Fund. SEP is funded by NIH Grant 5 U01 HG006485. WDF is funded by the Canadian Institutes of Health Research (FDN-148390).

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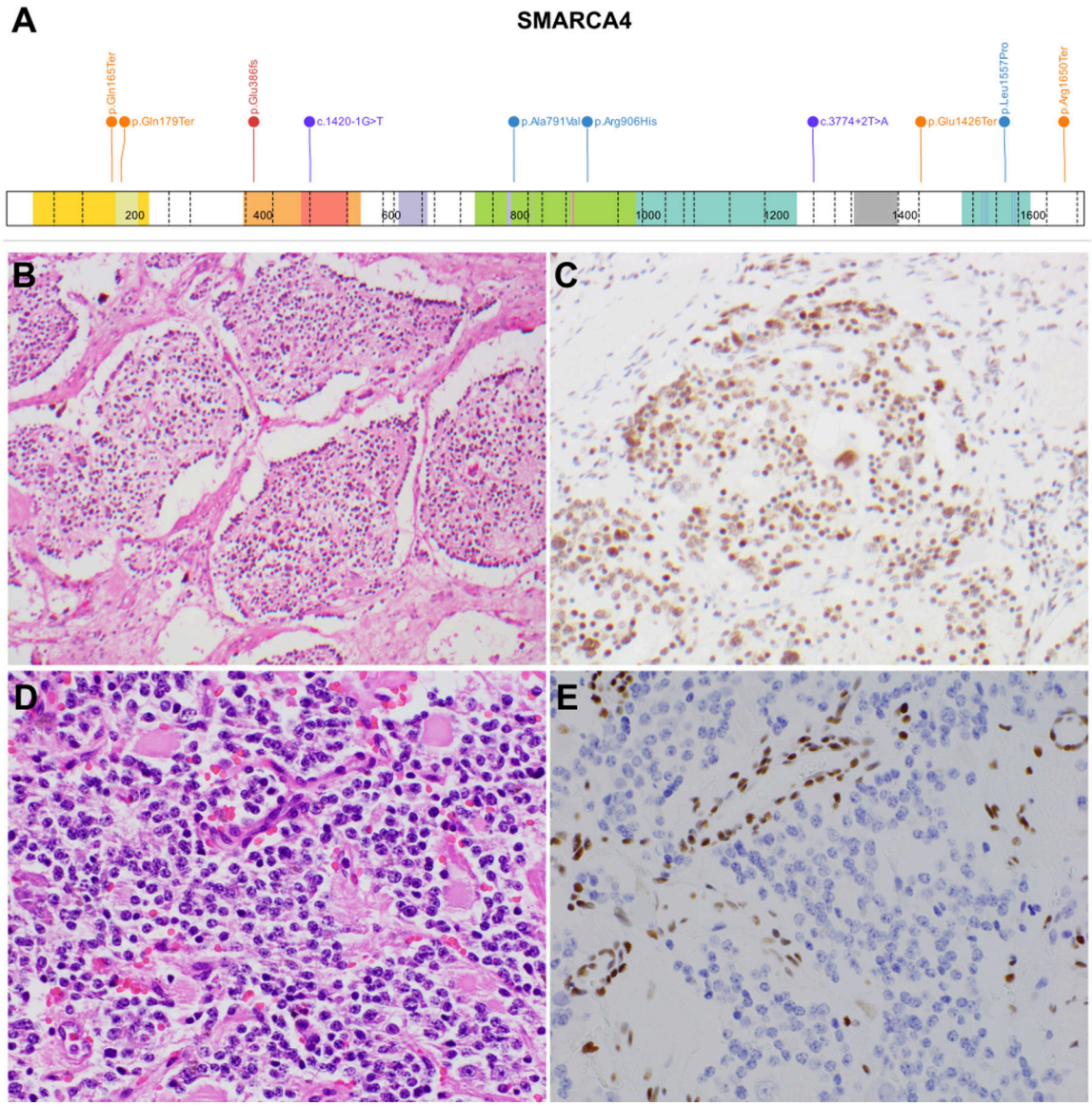

Figure 1.

A) Depiction of the *SMARCA4* gene with the variants found in the patients described in this manuscript. Variant colors refer to variant types: Orange = nonsense, Red = frameshift, Purple = splice, Blue = missense. Image created with https://pecan.stjude.cloud/pie. B-E) SMARCA4 immunostaining of 2 representative neuroblastomas. B) Case 4: Adrenal neuroblastoma, (differentiating stroma poor neuroblastoma), composed of nodules of neuroblasts and cells showing varying degrees of ganglion cell differentiation with intervening thin fibrovascular septa (H&E stain, 100X magnification). C) Case 4: Immunohistochemistry for SMARCA4 showing retained nuclear staining within the tumour (200X magnification). D) Case 5: H&E-stained image shows a poorly differentiated neuroblastoma (40X magnification). E) Case 5: SMARCA4 immunohistochemistry shows loss of nuclear staining in the neuroblastic cells (endothelial cells serving as an internal positive control; 40X magnification).

Table 1.

Neuroblastoma cases with germline *SMARCA4* variants

Case ID	Sex	Tumour	Location	Age at dx (years, unless stated)	cDNA	Protein	Variant type	Classification (evidence codes)	IHC staining for SMARCA4	Heterozygosity status in tumour	MYCN Status	Testing done	Reference
Case 1	M	NBL	Left adrenal	7	c.1156_1157del	p.(Glu386LysfsX17)	Frameshift	Likely Path (PVS1, PM2_Supp)	NA	LOH of wt allele (extent unknown)	NA	WES	Parsons et al. 2016[6]
Case 2	M	NBL	Adrenal	4	c.4276G>T	p.(Glu1426Ter)	Nonsense	Likely Path (PVS1, PM2_Supp)	NA	LOH of wt allele (chr19p loss)	MYCN-	WGS	Coorens et al. 2020[5]
Case 3	F	NBL	Adrenal	22 months	chr19: 10462524–12157782	Large deletion incl *CDKN2D, ILF3, SMARCA4, CNN1*	Deletion	Pathogenic (1B, 5A)	NA	NA	MYCN-	WES	Bellini et al. 2019[4]
Case 4	M	NBL	Right adrenal	26	c.2717G>A	p.(Arg906His)	Missense	VUS (PM2_Supp, PP3)	Retained	LOH of wt allele (extent unknown)	NA	WGS	New case
Case 5	M	NBL	Left adrenal	11	c.535C>T	p.(Gln179Ter)	Nonsense	Pathogenic (PVS1, PM2_Supp, PP4)	Absent	LOH of wt allele (chr19p loss)	MYCN-	WGS	Newman et al. 2021[9]
Case 6	M	NBL	Left adrenal	2 months	c.493C>T	p.(Gln165Ter)	Nonsense	Pathogenic (PVS1, PM2_Supp, PS4_Supp)	NA	NA	NA	MGPT	Wang et al. 2018[10]
Case 7	F	NBL	NA	NA	c.2372C>T	p.(Ala791Val)	Missense	VUS (PP3)	NA	NA	NA	MGPT	New case
Case 8	M	NBL	NA	6	c.3774+2T>A	NA	Splice	Likely Path (PVS1, PM2_Supp)	NA	LOH of wt allele (extent unknown)	NA	MGPT	New case
Case 9	F	NBL	Adrenal	4	c.4948C>T	p.(Arg1650Ter)	Nonsense	Likely Path (PVS1, PM2_Supp)	NA	NA	NA	MGPT	New case
Case 10	M	NBL	Right adrenal	11	c.4670T>C	p.(Leu1557Pro)	Missense	VUS (PP3, PP4)	Absent	LOH of wt allele (extent unknown)	MYCN-	NA	Van Engelen et al. 2022[11]
Case 11	M	NBL	NA	4	c.1420–1G>T	NA	Splice	Likely Path (PVS1, PM2_Supp)	NA	LOH of wt allele (extent unknown)	NA	MGPT	Villani et al.[12]

Variants are annotated on transcript NM_001128849.3. NBL: neuroblastoma; VUS: Variant of uncertain significance; LOH: Loss of heterozygosity; IHC: Immunohistochemistry; NA: Not available; Path: pathogenic; Supp: supporting level; WGS: Whole genome sequencing; WES: Whole exome sequencing, MGPT: Multi-gene panel testing.