Predicted Truncating Variants in *SMARCA4* May be Innocent: The Importance of Multi-Institutional Collaboration

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Germline variants in *SMARCA4* are known to predispose women to small cell carcinoma of the ovary, hypercalcemic type (SCCOHT). However, there are many variants of unknown significance (VUSs) found in this gene, and the penetrance of the pathogenic variants remains unknown. Although this cancer is relatively uncommon, it is extremely aggressive, with a mean age of onset of diagnosis at age 24. It is currently known that loss of function (LoF) of the gene is a mechanism of disease, and loss of SMARCA4 protein expression on immunohistochemistry in an ovarian tumor is essentially pathognomonic for SCCOHT. However, due to the aggressive nature and rarity of the disease, the pathogenicity of some LoF variants in *SMARCA4* has been difficult to determine, leaving women who carry these variants with very difficult, and potentially dangerous, decisions to make. We aimed to collect data on *SMARCA4* variants found in affected and unaffected women and their relatives, in order to better determine the pathogenicity of these variants.

We collected data from six sequencing centers on 2848 individuals previously sequenced for *SMARCA4* who were found to carry a variant in *SMARCA4* that was classified as VUS, Likely Pathogenic, or Pathogenic. The data collected included the following details: variant details, proband's indication for testing, details on personal and family history of cancer, and segregation details. In addition, we reviewed *SMARCA4* variants that were previously published in patients with SCCOHT. We prioritized variants that were predicted LoF (nonsense, frameshift, canonical splice) variants, as well as other types of variants seen in patients with SCCOHT. Upon reviewing the data, we noticed numerous probands with loss of function variants in exons 27, 28, and 30, none of whom had a personal or family history of SCCOHT (Table 1). To investigate further, we sequenced DNA and RNA from blood and healthy ovarian tissue from these patients, as well as cloned cDNA from the ovarian tissue.

Sequencing of RNA from patients with the c.4180_4181delinsC (p.Gly1394fs) variant in exon 30 showed that this exon is not expressed in blood. Exon 30 is only present in one of 11 transcripts in the UCSC genome browser; however, as the transcript that is used for clinical sequencing is often the longest one, and this exon is present in the most commonly sequenced transcript (NM_001128849), this variant was classified as pathogenic. Although we were not able to acquire RNA from all patients with variants in exon 30, we expect that the other nonsense and frameshift variants will not be expressed as well. Similarly, when studying the c.3774+2T>A variant in exon 27, sequencing across exon 27 in cDNA from control samples revealed that this exon is not expressed in blood. Like exon 30, exon 27 is only present in 3 of 11 *SMARCA4* transcripts, and therefore LoF variants in this exon do not appear to be leading to complete loss of the SMARCA4 protein.

Additionally, we investigated a variant in intron 28: c.3951+2T>C. This variant is predicted to splice out exon 28, a small exon that is in frame. However, sequencing of cDNA from probands with this variant showed that this variant was not causing any splicing defects in cDNA from lymphoblastoid cell lines after nonsense mediated decay (NMD) was inhibited with colchicine. However, another variant at this splice site, c.3951+1G>A, was seen in a patient with SCCOHT. Therefore, care needs to be taken when classifying splicing variants without RNA sequencing data.

Women who are found to have pathogenic variants in *SMARCA4* are at risk for the development of SCCOHT and are faced with a difficult decision for themselves and for their family members. There is currently no good screening test for SCCOHT and without knowing the penetrance of these variants, clinical management is difficult. To help address this issue, an international SCCOHT consortium has been created and the development of an SCCOHT patient registry is in progress. The work here highlights the importance of transcript selection and exon expression in classification of variants, as misclassification of variants as pathogenic can be detrimental to patients' physical and mental health.

Table 1. Variants classified as pathogenic or likely pathogenic in *SMARCA4* exons 27, 28, and 30.

Patient ID	Sex	Exon	cDNA	Amino acid	Variant type	Indication for testing	Results of RNA studies
1	Male	27	c.3774+2T>A	NA	Splice	Unknown	Exon 27 is not expressed in blood. Ovarian sequencing is pending.
2	Male	27	c.3774+2T>A	NA	Splice	Neuroblastoma	
4	Female	28	c.3951+2T>C	NA	Splice	Family history of non-ovarian cancers	This variant is not expressed in the cDNA after inhibition of NMD.
5	Female	28	c.3951+2T>C	NA	Splice	Family history of non-ovarian cancers	
6	Female	28	c.3951+2T>C	NA	Splice	Arthrogryposis	
7	Female	28	c.3951+2T>C	NA	Splice	Family history of non-ovarian cancers	
8	Female	28	c.3951+2T>C	NA	Splice	Family history of non-ovarian cancers	
9	Female	30	c.4180_4181delinsC	p.Gly1394Glnfs*101	Frameshift	Epithelial ovarian cancer	Exon 30 is not expressed in blood. Ovarian sequencing is pending.
10	Male	30	c.4180_4181delinsC	p.Gly1394Glnfs*101	Frameshift	Family history of ovarian cancer, NOS	
11	Female	30	c.4208delG	p.Ser1403Metfs*92	Frameshift	DCIS	
12	Female	30	c.4226_4227insATTC	p.Gln1411Ilefs*77	Frameshift	Suspected Charcot-Marie-Tooth disease	
13	Female	30	c.4266+1G>C	NA	Splice	Myxofibrosarcoma	
14	Female	30	c.4266+1G>T	NA	Splice	Family history of multiple cancers, pathogenic BRCA2 variant found	
15	Female	30	c.4266+1G>T	NA	Splice	Invasive ductal carcinoma	