

1 **Biallelic somatic *SMARCA4* mutations in small cell carcinoma of the ovary,**
2 **hypercalcemic type (SCCOHT).**

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26 **Abstract**

27 Small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is a rare,
28 aggressive tumor that primarily affects young women. SCCOHT has recently been
29 identified as a monogenic disorder caused by germline and/or somatic *SMARCA4*
30 mutations. We describe a 15-year-old Caucasian girl with a SCCOHT harboring a
31 previously unreported somatic mutation in the *SMARCA4* gene
32 (c.1757delA;p.K586.fs) with loss of heterozygosity. No germline mutation was
33 identified. Subsequent immunohistochemical staining confirmed loss of SMARCA4
34 protein. These molecular findings will aid with SCCOHT diagnosis through
35 immunohistochemical staining for SMARCA4 and in the future may have
36 implications for the management of this disease.

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Background

Small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is a rare ovarian tumor that primarily affects adolescent and younger women, with a mean age at presentation of 23.4 years [1]. Treatment schedules are currently based on a combination of surgery and multi-agent chemotherapy but despite this the majority of patients relapse, and long-term survival is less than 33%, even in early-stage disease [1]. A better understanding of the biology of this rare tumor could assist diagnosis, rationalise treatment strategies and improve patient outcomes.

The SMARCA4 protein (also known as BRG1) is a central catalytic component of the human ATP-dependent switching and sucrose non-fermenting (SWI/SNF), a chromatin-remodeling complex critically involved in the control of gene transcription within the cell [2]. Recently, deleterious somatic and germline *SMARCA4* mutations have been identified in the vast majority of patients with SCCOHT, with three independent groups demonstrating immunohistochemical loss of tumoral SMARCA4 protein expression in a combined total of 64/69 (92.8%) SCCOHTs [3-5]. In contrast, *SMARCA4* mutations have been identified at much lower frequencies in other solid tumors [5], including Burkitt's lymphoma and medulloblastoma [6].

SCCOHT have been noted to share similarities with another rare malignancy, namely rhabdoid tumors. Both SCCOHT and rhabdoid tumors show similar histopathological appearances and demonstrate poor responses to conventional therapy [1,7]. Mutations in another member of the SWI/SNF complex, *SMARCB1*, occur in 95% of all rhabdoid tumors, resulting in loss of staining for the protein product SMARCB1 (also known as INI1). The cancer genomes of both SCCOHT and rhabdoid tumors are

otherwise relatively mutationally silent, with few other mutations identified in addition to those in the SWI/SNF complex [3]. Immunohistochemical staining in rhabdoid tumors generally appears to be mutually exclusive for these proteins, in that those not showing SMARCB1 loss demonstrate SMARCA4 loss instead, and *vice versa* [8,9]. This clinical, pathological and molecular evidence of commonality between SCCOHT and rhabdoid tumors (both intracranial atypical teratoid/rhabdoid tumors [10] and extracranial malignant rhabdoid tumors [7]) has led to the recommendation that SCCOHT be re-classified within the rhabdoid tumor family and renamed ‘malignant rhabdoid tumor of the ovary’ (MRTO) [3,11]. Here, we report the case of a teenage girl with a SCCOHT (MRTO) harboring novel somatic biallelic *SMARCA4* mutations.

Case Report

A previously well 15-year-old Caucasian girl presented to her local hospital with a two-week history of nausea and vomiting, lethargy and abdominal pain. Other family members were well and there was no family history of cancer. Examination revealed a large abdominal mass arising from the pelvis, confirmed by ultrasound imaging. Initial blood investigations revealed marked hypercalcemia, with a corrected calcium level of 4.79 mmol/l (reference range 2.1-2.5 mmol/l), hypomagnesemia and hypophosphatemia. Levels for all measured tumor markers were within the institutional reference ranges; namely alpha-fetoprotein (AFP) 2 kU/l (0-10 kU/l), human chorionic gonadotrophin (HCG) <2.0 U/l (0-4 U/l), carcinoembryonic antigen (CEA) <0.5 µg/l (0-5 µg/l), cancer antigen 125 (CA-125) 24 kU/l (0-30 kU/l) and lactate dehydrogenase 287 U/l (164-290 U/l). The hypercalcemia was treated with intravenous hyperhydration and disodium pamidronate, plus magnesium and phosphate supplementation. Computed tomography (CT) scan of the abdomen (Figure 1A) revealed a large 11 x 9 x 10 cm solid and cystic mass centered on the left ovary, with no evidence of metastatic disease. The patient underwent a left salpingo-oophorectomy and omental biopsy. At surgery, the tumor was noted to demonstrate a capsular breach. Post-operative recovery was only complicated by symptomatic rebound hypocalcemia requiring calcium gluconate infusions.

Histopathological analysis showed that the ovary was replaced by haphazardly arranged small tumor cells with small nucleoli (Figure 1B). Immunohistochemical staining for inhibin was negative (Figure 1C) and CD99 demonstrated patchy positivity (Figure 1D). SMARCB1 staining was retained (Figure 1E). The omental biopsy showed no tumor involvement. Together, the clinical, surgical and

histopathological findings confirmed an International Federation of Gynaecological Oncologists (FIGO) Stage 1c SCCOHT [12].

Chemotherapy was initially commenced with two cycles of EP (etoposide and cisplatin), taking into account recent evidence [1,13] and expert opinion. Treatment was then modified in line with a protocol developed by the German MAKEI group following their report of improved outcomes with intensified multi-agent therapy [14]. The patient therefore received one course of cisplatin, ifosfamide and doxorubicin (PIA) and two subsequent courses with the carboplatin substituted for cisplatin due to impaired renal function. This was followed by consolidation with high dose carboplatin and etoposide and peripheral blood stem cell rescue. Treatment was completed seven months following presentation. End of treatment MRI abdomen/pelvis showed no evidence of disease. The patient remains well, now nine months off-treatment, with a scheduled follow-up MRI program instigated for disease surveillance.

Mutation analysis of DNA from lymphocytes of the patient, and from both her parents, revealed no germline *SMARCA4* mutations. Tumor DNA was extracted and analyzed using next generation and then confirmatory conventional Sanger sequencing. A (c.1757delA; p.K586.fs) mutation was identified (Figure 1F), together with intragenic loss of heterozygosity, indicating absence of the second allele. Primers used to sequence across this *SMARCA4* mutation were: 5'-AGTGCGCTTCTGGATTGACT -3' and 3'-GGAAATAGAGAGAGACACGGTCA-5'. Polymerase chain reaction (PCR) thermocycler conditions were used as previously described [2]. The mutation results in a deletion of amino acid 586, between the

125 helicase/SANT-associated (HAS) and Brahma and Kismet (BRK) domains of the
126 SMARCA4 protein [3]. Subsequent confirmatory immunohistochemical staining
127 revealed complete tumoral loss of SMARCA4 protein (Figure 1G).

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Discussion and Conclusion

We report the case of a teenage girl presenting with a SCCOHT (‘malignant rhabdoid tumor of the ovary’; MRTO), harboring a novel *SMARCA4* mutation with resultant loss of tumoral SMARCA4 staining. The patient remains well over a year following initial diagnosis. However, overall outcomes for this disease are poor following conventional treatment, which involves surgery and chemotherapy [1]. As SCCOHT (MRTO) largely affects young women, late effects, including subfertility, are a concern in surviving patients.

The identification of a novel somatic *SMARCA4* mutation in this case contributes to the growing body of evidence that SCCOHT (MRTO) is a monogenic disorder. The implication of a single gene in SCCOHT (MRTO) will not only aid with diagnosis through immunohistochemical staining for SMARCA4 [4], but may have implications for the management of this disease. Three independent groups are currently working on targeted therapies for SCCOHT (MRTO) [15], in order to improve overall survival and reduce the current dependence on high-dose conventional cytotoxic agents in this young patient population, with their associated long-term sequelae in survivors.

Figure Legend

Figure 1. Imaging, histopathology and mutation findings in the SCCOHT case.

A) Transverse CT image of the pelvis at the time of diagnosis, demonstrating a large mass; B) Haematoxylin and eosin (H&E) stain demonstrating haphazardly arranged SCCOHT cells; C) Negative inhibin staining of the tumor cells; D) Patchy CD99 staining of the tumor cells; E) Retained nuclear SMARCB1 staining of the tumor cells; F) Electrophoretogram showing the somatic homozygous *SMARCA4* mutation c.1757delA; p.K586.fs; G) Loss of nuclear SMARCA4 staining of the tumor cells.

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