

# Whole-Exome Sequencing of Syndromic Adrenocortical Carcinoma Reveals Distinct Mutational Profile From Sporadic ACC

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Next-generation sequencing has provided genetic profiles of a large number of sporadic adrenocortical carcinomas (ACCs), but the applicability of these results to ACC cases associated with tumor predisposition syndromes is unclear. Although the germline features of these syndromes have been well described, the somatic mutational landscape of the tumors they give rise to is less clear. Our group obtained germline and tumor tissue from a pediatric patient who developed ACC during her first year of life, which was treated successfully. She was subsequently diagnosed with additional tumors later in childhood. Whole exome sequencing analysis was performed followed by *in silico* protein function prediction, revealing a probably deleterious germline *TP53* L265P mutation. The somatic mutational burden was comparable between the index case and a previously published cohort of 40 sporadic cases, but the mutational spectrum was distinct in terms of raw base-change frequency as well as in a trinucleotide context-specific analysis. No canonical somatic genetic drivers of ACC were identified in the reported case, suggesting that syndromic adrenocortical tumors may represent a genetically distinct entity from sporadic tumors.

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**Freeform/Key Words:** adrenocortical carcinoma, Beckwith-Wiedemann, Li-Fraumeni, p53, mutational spectrum

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Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with a poor prognosis [1]. Although these tumors are rare overall, they are somewhat more common in several known predisposition syndromes, including Beckwith-Wiedemann syndrome and Li-Fraumeni syndrome [2]. The somatic genomic landscapes of sporadic adrenocortical carcinoma have been well described, but these previous studies have typically excluded syndromic cases [3–5]. As a result, although the germline events leading to tumor predisposition disorders have been well described, the somatic genetic profiles of these syndromic tumors are not well understood, which calls into question their tumor biology, and potential response to available targeted therapies. A previous series has examined the landscape of pediatric ACC in a cohort with a high prevalence of a founder Brazilian *TP53* R337H germline variant using whole-genome, whole-exome, and transcriptome profiling techniques, but did not examine mutational signature of syndromic cases specifically [6]. We therefore performed whole exome sequencing (WES) of tumor and germline tissue from a pediatric patient with ACC with suspected tumorigenic syndrome.

The patient initially presented at five months of age when she developed progressive hemiparesis and was found to have a large left adrenal mass with apparent spinal metastasis.

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Abbreviations: ACC, adrenocortical carcinoma; SNV, single nucleotide variants; WES, whole exome sequencing.

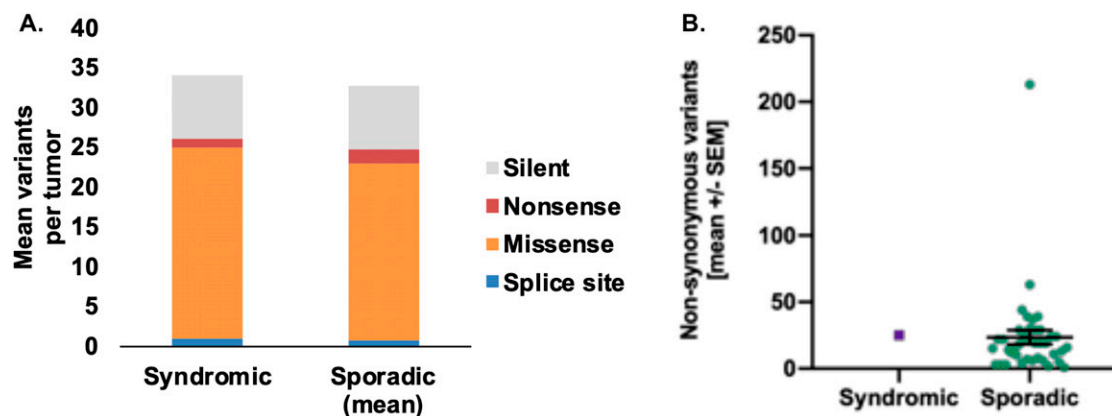
She had been noted previously to have some atypical developmental features at birth, including macroglossia and hemihypertrophy. There was no family history in either parent or any other relatives of childhood cancers or other apparent tumor syndromes. The child underwent resection of the adrenal and spinal masses. Pathology revealed a 4.5 cm, 19.5 g adrenocortical tumor with increased mitotic rate, atypical mitoses, and nuclear pleomorphism without necrosis, vascular, or capsular invasion or lymph node metastases; in light of the spinal metastasis, the tumor was noted to be an European Network for the Study of Adrenal Tumors stage IV adrenocortical carcinoma. The patient was treated postoperatively with adjuvant chemotherapy including ifosfamide, carboplatin, etoposide, and intrathecal methotrexate, after which she was apparently cured of her ACC, which never recurred. Unfortunately, she subsequently developed a pelvic osteosarcoma at the age of 12 years, which was refractory to multiple resections and local and systemic therapies, and she ultimately died of that tumor four years later.

## 1. Materials and Methods

The patient's family signed informed consent for research and the project was approved by the Yale institutional review board. DNA was extracted from archival formalin-fixed paraffin-embedded tissue samples representing both the ACC tumor and uninvolved normal adrenal, using a proprietary enzymatic deparaffinization and extraction protocol. Sequencing was performed on the Illumina (Illumina, Inc., San Diego, CA) platform using the same approaches described previously for our group's sporadic ACC cohort [4]. Somatic and germline single nucleotide variants (SNVs) were called using an in-house pipeline, and variants with an allele fraction of 5% or more were retained. Functional protein consequences of mutations were predicted by MutationTaster [7]. Somatic and germline nonsynonymous mutational burdens were compared with a previously published cohort of sporadic ACCs. Mutational signatures taking into account the tribase context of each SNV (including silent, splice-site, and nonsynonymous) were tabulated for each tumor using the deconstructSigs algorithm normalized to the whole exome [8]. Cases with five or fewer SNVs were excluded from signature analysis.

## 2. Results

WES revealed a comparable mutational burden in the syndromic case relative to the previously investigated cohort ( $n = 40$ ) of sporadic cases (Fig. 1). However, the base-change spectrum of the syndromic case's mutations relative to the sporadic cases was distinct ( $P < 0.001$  by  $\chi^2$  analysis of syndromic vs pooled sporadic variants). The syndromic case had fewer



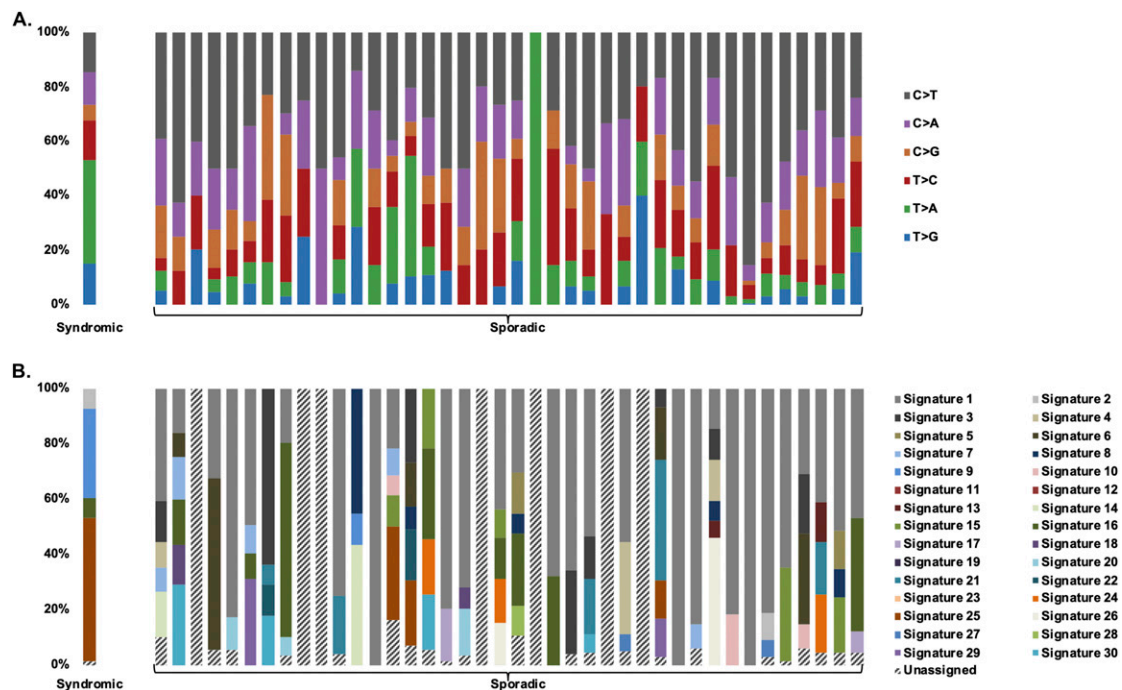
**Figure 1.** SNV burden in syndromic vs sporadic ACC. A, Average burden of variants in syndromic ( $n = 1$ ) vs sporadic ( $n = 40$ ) cases. B, Total burden of nonsynonymous SNVs in syndromic (25,  $n = 1$ ) and sporadic (mean 23.5 per tumor, SE 5.3,  $n = 40$ ) ACC.

C>T variants and relatively more T>A variants (Fig. 2A). These corresponded to a relative lack of Signature 1 and an abundance of Signature 25 in the syndromic case (Fig. 2B) [9]. Nonsynonymous somatic SNVs were identified in 25 genes, including several annotated in the Catalogue of Somatic Mutations in Cancer Cancer Gene Census, although none are known to be drivers in sporadic ACC (Table 1) [10]. Many of these were predicted to be damaging *in silico* by the MutationTaster algorithm [7]. Nonsynonymous germline variants were identified in 8 genes, including a probably damaging heterozygous *TP53* variant not identified in the previous pediatric ACC cohort (Table 2) [6].

### 3. Discussion

In this study, we performed WES of an ACC occurring in a pediatric patient initially suspected to have Beckwith-Wiedemann syndrome. The genetic findings in our study (and indeed the patient's ultimate clinical course) would suggest that the more appropriate diagnosis was likely Li-Fraumeni syndrome. In any case, it is clear that the somatic genetic events in syndromic ACC are likely somewhat distinct from those events that occur in sporadic cases.

We identified a similar overall burden of somatic mutations, but a distinct mutational signature, in the syndromic case. In particular, the tumor carried relatively few Signature 1 mutations (associated with aging) and an unusually high number of Signature 25 mutations (a signature of unknown etiology that has been previously identified in Hodgkin lymphoma cells). Admittedly, the lack of Signature 1 mutations is possibly a consequence of the patient's young age, rather than the tumor predisposition syndrome *per se*. Of course, many pediatric cases of ACC are associated with germline predisposition syndromes, so pediatric and syndromic ACC are overlapping categories.



**Figure 2.** Base change spectrum and mutational signatures of syndromic vs sporadic ACC. A, Mutational spectra of syndromic vs sporadic ACC. The base-change spectrum of syndromic ACC was distinct from the pooled spectrum of sporadic ACC ( $P < 0.001$  by  $\chi^2$  test). All base changes are referred to by the original pyrimidine of the base pair. B, There was substantial heterogeneity in the mutational signatures of the examined ACCs. The syndromic case was notable for the lack of Signature 1 (aging-associated) mutations, accompanied by an increase in Signature 25 mutations.

**Table 1. Nonsilent Somatic Mutations in Reported Syndromic ACC Case**

Gene	Base Change	AA Change	LOH	MutationTaster	COSMIC
<i>AFF3</i>	A>T	L1088I	*	D	*
<i>BTF3</i>	A>T	K158M		D	
<i>CACNA2D3</i>	G>T	L571F		D	
<i>CCDC14</i>	C>T	E347K		B	
<i>CLASP1</i>	C>G	E489Q		D	
<i>CWC22</i>	A>T	S106R	*	B	
<i>DIAPH2</i>	A>T	K293I	*	D	
<i>DMD</i>	A>C	Y425D		D	
<i>DYSF</i>	A>T	R293W		D	
<i>FGF13</i>	A>T	C52S	*	D	
<i>FLT3</i>	A>C	S762A		D	*
<i>HPRT1</i>	A>T	D18V		D	
<i>KLHDC2</i>	A>G	Y163C		D	
<i>LARS</i>	G>T	A820D	*	D	
<i>LUC7L3</i>	T>C	I61T		D	
<i>PDHA2</i>	T>G	N245K	*	B	
<i>PHLDA1</i>	C>G	Q383H	*	B	
<i>PLEK2</i>	A>T	F159Y		D	
<i>PLEKHD1</i>	A>T	K150X	*	D	
<i>PSMA8</i>	G>T	G46V	*	D	
<i>PSMD1</i>	A>T	T755S	*	D	
<i>SLC24A1</i>	T>C	L513P	*	D	
<i>SMS</i>	A>C	K230T	*	D	
<i>SYNRG</i>	G>T	P518H		D	
<i>ZMPSTE24</i>	A>T	T174S		D	

Asterisk indicates LOH at this locus. Asterisks in the final column indicate that the mutation is listed in the COSMIC Cancer Gene Census as of February 2019 [10].

Abbreviations: AA, amino acid; B, benign or D, damaging by the MutationTaster algorithm [7]; COSMIC, Catalogue of Somatic Mutations in Cancer; LOH, loss of heterozygosity.

Furthermore, despite the patient's pathologically advanced disease, she experienced an apparent cure after aggressive treatment, with more than a decade ACC-free, which would be highly unusual in sporadic adult ACC. Indeed, prior reports of pediatric ACC have demonstrated superior survival in tumors presenting in the first few years of life, compared with those presenting in older children or in adults [6]. These molecular (and clinical) findings may call into question the appropriateness of taking a "one size fits all" approach for the use of newer therapeutic agents in syndromic compared with sporadic ACC.

It may therefore be particularly important to separate these patients from sporadic ACC in future studies in the field, as the carcinogenesis and progression of these tumors may likely

**Table 2. Germline Mutations in Reported Syndromic ACC Case**

Gene	Base Change	AA Change	LOH	MutationTaster
<i>ATP1A4</i>	G>C	D685H		D
<i>FAM114A2</i>	T>C	K433E		D
<i>GBP5</i>	C>T	R221H		D
<i>MMACHC</i>	T>C	F158L		D
<i>MUC12</i>	G>C	R4631T		B
<i>RAPGEF4</i>	T>C	L204P	*	D
<i>TP53</i>	A>G	L265P		D
<i>TPH2</i>	G>T	M432I		D

Asterisk indicates LOH.

Abbreviations: AA, amino acid; B, benign or D, damaging by the MutationTaster algorithm [7]; LOH, loss of heterozygosity.

proceed via different mechanisms than those found in sporadic ACC. Furthermore, an improved understanding of the somatic events leading to tumors in the setting of germline predisposition disorders may provide insight for early detection or even prevention of these life-limiting cancers in patients and families suffering with these diseases.

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## Additional Information

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**Data Availability:** The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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## References and Notes

- Nicolson NG, Man J, Carling T. Advances in understanding the molecular underpinnings of adrenocortical tumors. *Curr Opin Oncol*. 2018;**30**(1):16–22.
- Else T. Association of adrenocortical carcinoma with familial cancer susceptibility syndromes. *Mol Cell Endocrinol*. 2012;**351**(1):66–70.
- Assié G, Letouzé E, Fassnacht M, Jouinot A, Luscip W, Barreau O, Omeiri H, Rodriguez S, Perlemonne K, René-Corail F, Elarouci N, Sbiera S, Kroiss M, Allolio B, Waldmann J, Quinkler M, Mannelli M, Mantero F, Papanthomas T, De Krijger R, Tabarin A, Kerlan V, Baudin E, Tissier F, Dousset B, Groussin L, Amar L, Clauser E, Bertagna X, Ragazzon B, Beuschlein F, Libé R, de Reyniès A, Bertherat J. Integrated genomic characterization of adrenocortical carcinoma. *Nat Genet*. 2014;**46**(6):607–612.
- Juhlin CC, Goh G, Healy JM, Fonseca AL, Schöll UI, Stenman A, Kunstman JW, Brown TC, Overton JD, Mane SM, Nelson-Williams C, Bäckdahl M, Suttrop AC, Haase M, Choi M, Schlessinger J, Rimm DL, Höög A, Prasad ML, Korah R, Larsson C, Lifton RP, Carling T. Whole-exome sequencing characterizes the landscape of somatic mutations and copy number alterations in adrenocortical carcinoma. *J Clin Endocrinol Metab*. 2015;**100**(3):E493–E502.
- Zheng S, Cherniack AD, Dewal N, Moffitt RA, Danilova L, Murray BA, Lerario AM, Else T, Knijnenburg TA, Ciriello G, Kim S, Assie G, Morozova O, Akbani R, Shih J, Hoadley KA, Choueiri TK, Waldmann J, Mete O, Robertson AG, Wu HT, Raphael BJ, Shao L, Meyerson M, Demeure MJ, Beuschlein F, Gill AJ, Sidhu SB, Almeida MQ, Fragoso MCBV, Cope LM, Kebebew E, Habra MA, Whitsett TG, Bussey KJ, Rainey WE, Asa SL, Bertherat J, Fassnacht M, Wheeler DA, Hammer GD, Giordano TJ, Verhaak RGW; Cancer Genome Atlas Research Network. Comprehensive pan-genomic characterization of adrenocortical carcinoma [published correction appears in *Cancer Cell*. 2016;**30**(2):363]. *Cancer Cell*. 2016;**29**(5):723–736.
- Pinto EM, Chen X, Easton J, Finkelstein D, Liu Z, Pounds S, Rodriguez-Galindo C, Lund TC, Mardis ER, Wilson RK, Boggs K, Yergeau D, Cheng J, Mulder HL, Manne J, Jenkins J, Mastellaro MJ, Figueiredo BC, Dyer MA, Pappo A, Zhang J, Downing JR, Ribeiro RC, Zambetti GP. Genomic landscape of paediatric adrenocortical tumours. *Nat Commun*. 2015;**6**(1):6302.
- Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods*. 2010;**7**(8):575–576.
- Rosenthal R, McGranahan N, Herrero J, Taylor BS, Swanton C. DeconstructSigs: delineating mutational processes in single tumors distinguishes DNA repair deficiencies and patterns of carcinoma evolution. *Genome Biol*. 2016;**17**(1):31.

9. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Børresen-Dale AL, Boyault S, Burkhardt B, Butler AP, Caldas C, Davies HR, Desmedt C, Eils R, Eyfjörd JE, Foekens JA, Greaves M, Hosoda F, Hutter B, Ilicic T, Imbeaud S, Imielinski M, Jäger N, Jones DT, Jones D, Knappskog S, Kool M, Lakhani SR, López-Otín C, Martin S, Munshi NC, Nakamura H, Northcott PA, Pajic M, Papaemmanuil E, Paradiso A, Pearson JV, Puente XS, Raine K, Ramakrishna M, Richardson AL, Richter J, Rosenstiel P, Schlesner M, Schumacher TN, Span PN, Teague JW, Totoki Y, Tutt AN, Valdés-Mas R, van Buuren MM, van 't Veer L, Vincent-Salomon A, Waddell N, Yates LR, Zucman-Rossi J, Futreal PA, McDermott U, Lichter P, Meyerson M, Grimmond SM, Siebert R, Campo E, Shibata T, Pfister SM, Campbell PJ, Stratton MR; Australian Pancreatic Cancer Genome Initiative, ICGC Breast Cancer Consortium, ICGC MML-Seq Consortium, ICGC PedBrain. Signatures of mutational processes in human cancer [published correction appears in *Nature*. 2013;502(7470):258]. *Nature*. 2013;500(7463):415–421.
10. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, Rahman N, Stratton MR. A census of human cancer genes. *Nat Rev Cancer*. 2004;4(3):177–183.