Somatic hotspot variants rarely coexist with germline drivers of Cushing's disease

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Introduction.

Recurrent somatic hotspot variants have been found in a plurality of adult and a minority of pediatric corticotropinomas. In contrast, *causative germline defects are not rare in children with Cushing's disease* (CD) but are very infrequent in adults. The overlap and interactions between germline and somatic variants in patients with CD are unknown. We sought to determine the *frequency of CD-associated somatic hotspot variants in a large pediatric-enriched cohort of patients with CD*, and to analyze the overlap of somatic and germline variants.

Methods

This analysis included *120 unrelated individuals with CD* who were evaluated at the outpatient clinic and/or admitted for clinical workup and treatment, or whose DNA samples were referred for study at the National Institutes of Health Clinical Research Center between 1997-2018. All individuals and their parents or guardians provided informed assent or consent and were recruited under protocol 97-CH-0076 (ClinicalTrials.gov: NCT00001595).

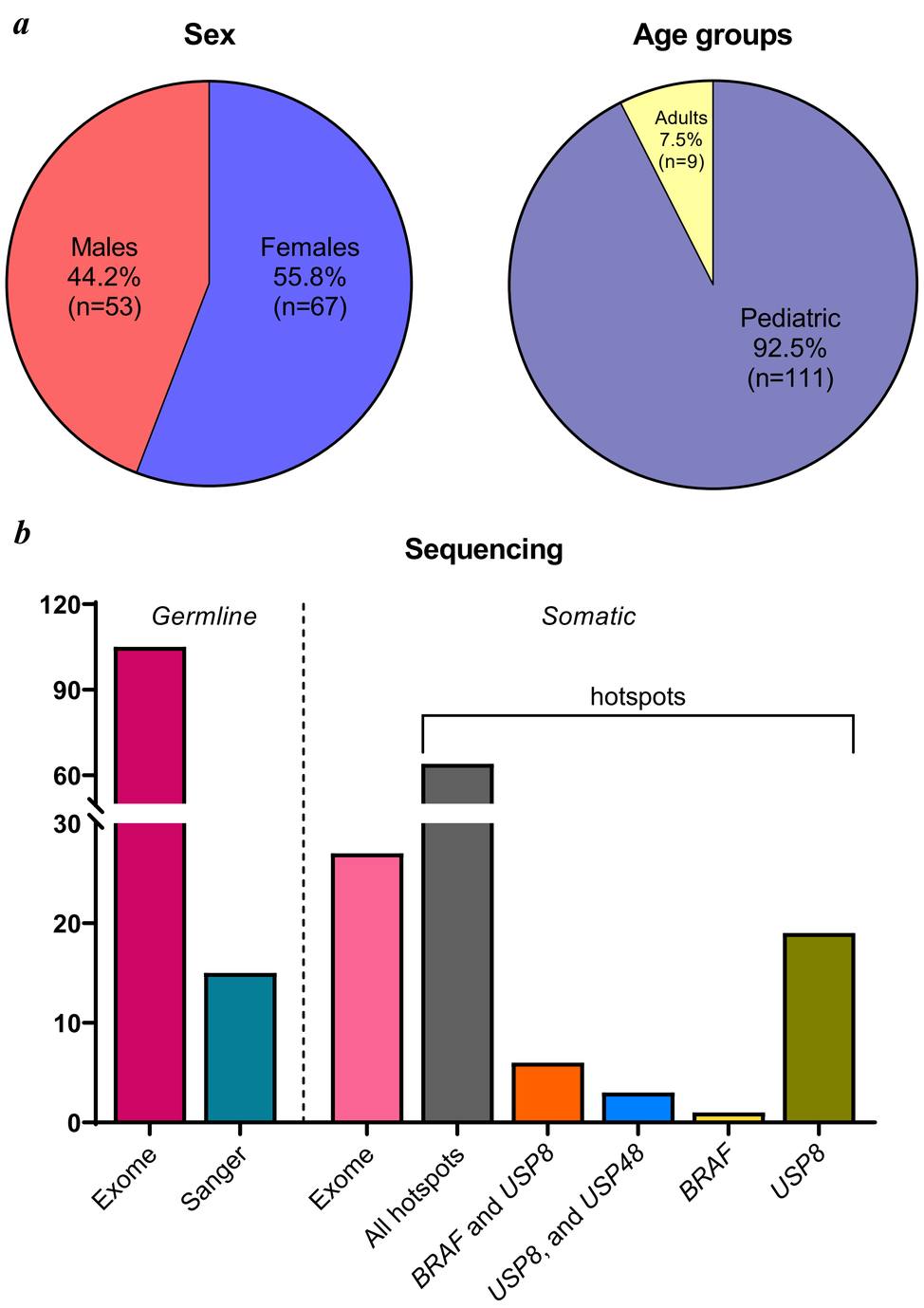


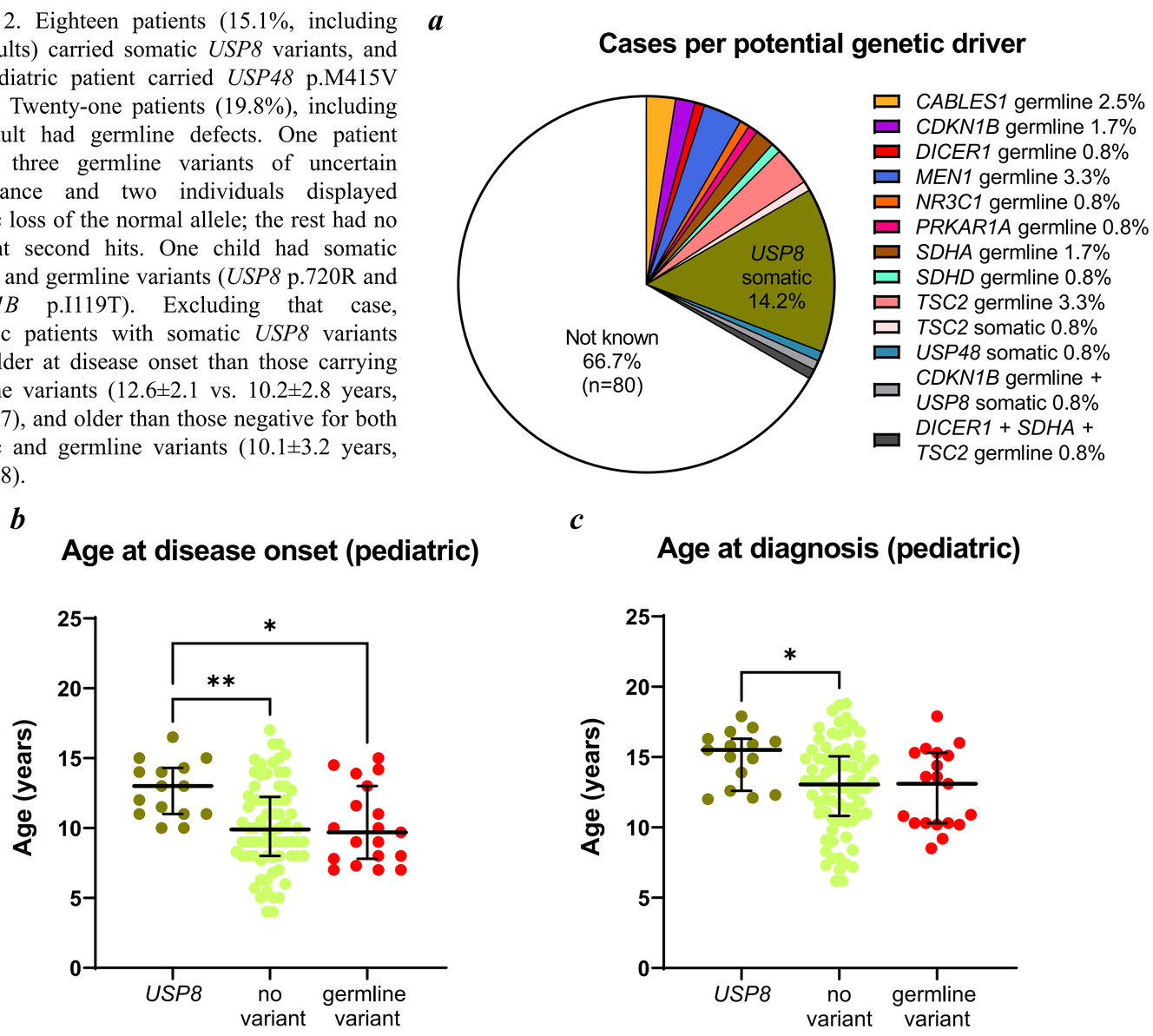
Figure 1. a) Characteristics of the study cohort. b) Strategy for genetic screening. Paired germline and corticotropinoma exome sequencing was performed in 27 cases. For 93 patients, targeted Sanger-based screening of *BRAF* (n=1), *USP8* (n=19), *BRAF/USP8* (n=6), *USP8/USP48* (n=3), or *BRAF/USP8/USP48* (n=64) somatic hotspots was performed.

Results.

Table 1. Germline and somatic variants identified in the study cohort

	Gene	Variant	ACMG/AMP category	No. of cases
Germline	*CABLES1* (NM_001100619.2)	c.92C>T, p.P31L	VUS	1
		c.935G>A, p.G312D	LP	1
		c.1388A>G, p.D463G	LP	1
	CDKN1B (NM_004064.5)	c.-29_-26del, p.?	LP	1
		c.356T>C, p.I119T	VUS	1
		c.407A>G, p.D136G	VUS	1
	DICER1 (NM_030621.4)	c.184G>A, p.V62I	VUS	1
		c.3422C>T, p.S1141F	VUS	1
	MEN1 (NM_000244.3)	Exon 1-2 deletion	Pathogenic	1
		c.251_252del, p.S84Yfs*32	Pathogenic	1
		c.1207C>T, p.Q403*	Pathogenic	1
		c.1258C>T, p.R420*	Pathogenic	1
	NR3C1 (NM_001018076.2)	c.1796C>G, p.S599C	LP	1
	PRKAR1A (NM_002734.5)	c.674del, p.G225Afs*16	Pathogenic	1
	SDHA (NM_004168.4)	c.61G>A, p.A21T	VUS	1
		c.221dup, p.L74Ffs*9	Pathogenic	1
		c.1272C>G, p.H424Q	VUS	1
	SDHD (NM_003002.4)	c.53C>T, p.A18V	VUS	2
	TSC2 (NM_000548.5)	c.1601T>G, p.V534G	VUS	1
		c.1882C>G, p.R628G	LP	1
		c.2545A>G, p.T849A	VUS	1
		c.3599G>A, p.R1200Q	LP	1
		Variant not known	Pathogenic	1
Somatic		c.4815_4816del, p.Q1605Hfs*8	Pathogenic	1
	USP8 (NM_005154.5)	c.2152T>C, p.S718P	Pathogenic	6
		c.2153C>G, p.S718C	Pathogenic	1
		c.2153C>T, p.S718F	Pathogenic	1
		c.2155_2157del, p.S719del	Pathogenic	7
		c.2155_2172del,p.S719_Q724del	Pathogenic	1
		c.2159C>G, p.P720R	Pathogenic	2
	USP48 (NM_032236.8)	c.1243A>G, p.M415V	LP	1

Figure 2. Eighteen patients (15.1%, including two adults) carried somatic *USP8* variants, and one pediatric patient carried *USP48* p.M415V (1.1%). Twenty-one patients (19.8%), including one adult had germline defects. One patient carried three germline variants of uncertain significance and two individuals displayed somatic loss of the normal allele; the rest had no apparent second hits. One child had somatic hotspot and germline variants (*USP8* p.720R and *CDKN1B* p.I119T). Excluding that case, pediatric patients with somatic *USP8* variants were older at disease onset than those carrying germline variants (12.6±2.1 vs. 10.2±2.8 years, P=0.047), and older than those negative for both somatic and germline variants (10.1±3.2 years, P=0.008).



Discussion.

In our cohort of patients with CD, the overlap between potentially pathogenic germline and somatic hotspot variant was very infrequent (0.8%). Pediatric patients carrying *USP8* hotspot variants are characterized by an older age at disease onset and at diagnosis. Our results indicate that *somatic hotspot variants and germline defects are two groups of genetic drivers that independently lead to CD.*

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