

## Supplementary Online Content

Bennett KL, Mester J, Eng C. Germline epigenetic regulation of *KILLIN* in Cowden and Cowden-like syndrome. *JAMA*. 2010;304(24):2724-2731.

**eTable 1.** Clinical Phenotypes of the CS/CSL Patients Analyzed for Germline Methylation

**eTable 2.** International Cowden Consortium Operational Diagnostic Criteria for Cowden Syndrome (2000)

**eTable 3.** Primer Sequences Used in the Study

This supplementary material has been provided by the authors to give readers additional information about their work.

**eTable 1.** Clinical Phenotypes of the CS/CSL Patients Analyzed for Germline Methylation

Patient ID	Methylation	Classification	Macrocephaly	MR/autism/DD	Lhermitte-Duclos	Breast-Malignant	Breast-Benign	Thyroid-Malignant	Thyroid-Benign	Path. mucocutaneous lesions	Lipomas	Fibromas	GI hamartomas	GI polyps-other	Uterine cancer	Uterine fibroids	Renal cell cancer	Other G/U tumor/malformation
21	Y	CSL				X	X			X						X		
31	Y	CSL				X		X										
32	Y	CSL				X	X									X		
40	Y	CSL		X														
114	Y	CSL				X	X	X								X		
276	Y	CS	X				X		X	X						X		
366	Y	CSL						X			X						X	
397	Y	CSL				X	X								X		X	
443	Y	CS	X			X	X			X								
446	Y	CSL					X		X									X
450	Y	CSL				X		X							X			
469	Y	CS	X			X	X	X	X	X	X			X		X		
471	Y	CS	X			X												
488	Y	CSL				X	X		X									
513	Y	CS	X			X				X					X			
532	Y	CSL				X		X	X									
534	Y	CSL				X	X									X		
542	Y	CSL				X	X	X								X		
545	Y	CSL					X	X									X	
580	Y	CSL				X	X								X	X		
594	Y	CS				X	X				X					X		X
606	Y	CS	X				X		X		X							X
616	Y	CS					X	X	X	X				X		X		
750	Y	CS				X	X	X	X	X				X				
785	Y	CSL				X									X			
1039	Y	CSL				X	X				X							
1272	Y	CSL				X		X										
1296	Y	CS				X	X		X			X		X		X		
1322	Y	CS				X	X		X							X		X
1350	Y	CS					X		X		X			X		X		
1584	Y	CS				X	X				X				X		X	
1596	Y	CSL				X									X			
1767	Y	CS	X		X				X	X	X							
1896	Y	CSL				X		X	X									
1938	Y	CS	X			X	X							X		X		
1946	Y	CS				X			X		X					X		
2025	Y	CSL				X			X						X			

Patient ID	Methylation	Classification	Macrocephaly	MR/autism/DD	Lhermitte-Duclos	Breast-Malignant	Breast-Benign	Thyroid-Malignant	Thyroid-Benign	Path. mucocutaneous lesions	Lipomas	Fibromas	GI hamartomas	GI polyps-other	Uterine cancer	Uterine fibroids	Renal cell cancer	Other G/U tumor/malformation
2056	Y	CS	X			X	X											
2275	Y	CS				X	X		X							X		
2355	Y	CSL				X	X								X			
2357	Y	CS	X			X										X		
2402	Y	CSL	X				X									X		
2540	Y	CSL				X					X			X		X		
2568	Y	CS	X			X												
2619	Y	CSL				X	X				X							
13	N	CS				X	X		X		X			X		X		
15	N	CS				X	X		X		X			X		X		
17	N	CS					X		X		X					X		
25	N	CS				X	X	X		X	X					X		
33	N	CSL				X	X		X									
42	N	CSL				X	X								X			
66	N	CSL				X	X											X
124	N	CSL				X	X									X		
130	N	CS	X			X	X		X	X						X		
132	N	CSL				X	X	X								X		
142	N	CSL					X	X	X									
153	N	CSL				X		X										
155	N	BRR		X					X	X								
156	N	CSL				X	X	X										
158	N	CS					X	X			X					X		
179	N	CSL				X		X								X		
182	N	CSL				X			X						X			
213	N	CSL				X								X	X			
215	N	CS	X			X	X							X		X		
233	N	CSL	X				X		X					X				
249	N	CSL					X	X			X							
261	N	CSL				X	X	X			X							
262	N	CSL				X	X	X										
263	N	CSL				X	X									X		
265	N	CSL					X		X						X			
267	N	CSL					X		X	X				X				
274	N	CS				X	X			X	X							
290	N	CSL				X		X						X	X			
291	N	CSL				X	X							X	X			
303	N	CSL				X	X							X		X		X

Patient ID	Methylation	Classification	Macrocephaly	MR/autism/DD	Lhermitte-Duclos	Breast-Malignant	Breast-Benign	Thyroid-Malignant	Thyroid-Benign	Path. mucocutaneous lesions	Lipomas	Fibromas	GI hamartomas	GI polyps-other	Uterine cancer	Uterine fibroids	Renal cell cancer	Other G/U tumor/malformation
334	N	CSL				X	X		X									
335	N	CSL				X	X		X		X							
339	N	CSL							X	X						X		
358	N	CSL				X	X		X									
363	N	CSL					X	X			X							
365	N	CS				X	X		X							X		
378	N	CSL				X		X								X		
380	N	CSL				X		X										
390	N	CS	X			X	X									X		
398	N	CSL				X			X				X			X		
458	N	CS	X				X								X	X		
459	N	CSL				X				X								
494	N	CSL	X										X				X	
524	N	CSL					X		X							X		
539	N	CS	X			X	X		X							X		
561	N	CSL					X	X								X		
572	N	CSL					X						X			X		
583	N	CS					X		X		X					X	X	
627	N	CS	X				X		X							X		
757	N	CS	X		X	X			X	X	X					X		
763	N	CSL					X								X	X		
820	N	CS				X	X			X	X	X	X					
860	N	CSL				X			X							X		
981	N	CS	X			X	X		X			X				X	X	X
1132	N	CS	X			X										X		
1141	N	CS				X	X	X	X	X								
1286	N	CSL				X		X										
1330	N	CSL				X		X										
1539	N	CSL				X	X	X					X					
1606	N	CS				X	X				X					X		
1733	N	CSL				X		X								X		
1831	N	CS	X			X			X							X		
1844	N	CSL				X	X									X		
1998	N	CS				X	X			X	X					X	X	
2000	N	CSL						X			X					X		
2012	N	CSL				X									X			
2059	N	CSL				X			X		X							
2126	N	CSL				X		X					X					

<b>Patient ID</b>	<b>Methylation</b>	<b>Classification</b>	<b>Macrocephaly</b>	<b>MR/autism/DD</b>	<b>Lhermitte-Duclos</b>	<b>Breast-Malignant</b>	<b>Breast-Benign</b>	<b>Thyroid-Malignant</b>	<b>Thyroid-Benign</b>	<b>Path. mucocutaneous lesions</b>	<b>Lipomas</b>	<b>Fibromas</b>	<b>GI hamartomas</b>	<b>GI polyps-other</b>	<b>Uterine cancer</b>	<b>Uterine fibroids</b>	<b>Renal cell cancer</b>	<b>Other G/U tumor/malformation</b>
2180	N	CSL				X		X	X									
2204	N	CS				X	X		X		X					X		
2207	N	CS				X	X		X		X							X
2221	N	CS	X			X												X
2276	N	CSL				X	X									X		
2331	N	CS				X	X	X			X					X		
2525	N	CSL					X		X							X		
2560	N	CS				X	X		X		X					X		
2639	N	CSL	X				X											X
2673	N	CS					X	X	X					X		X		

\*Familial per JM denotes genetic counselor review for familial case defined as at least one other family member who has features of CS. Family in 8458 denotes whether family member of proband consented to participate in the study.

**eTable 2. International Cowden Consortium Operational Diagnostic Criteria for Cowden Syndrome (2000)**

**Pathognomonic criteria**

Mucocutaneous lesions:

- Mucosal lesions
- Trichilemmomas (facial)
- Papillomatous lesions
- Acral keratoses

**Major criteria**

- Breast cancer
- Thyroid cancer, especially follicular thyroid cancer
- Macrocephaly (occipital frontal circumference  $\geq$  97th percentile)
- Endometrial carcinoma
- Lhermitte-Duclos disease, defined as presence of a cerebellar dysplastic gangliocytoma

**Minor criteria**

- Other thyroid lesions (e.g., goiter)
- Mental retardation (IQ  $\leq$  75)
- Hamartomatous intestinal polyps
- Fibrocystic disease of the breast
- Lipomas
- Fibromas
- Genito-urinary tumors (for example, uterine fibroids, renal cell carcinoma) or genito-urinary malformation

**Operational diagnosis if an individual meets any one of the following criteria:**

- Pathognomonic mucocutaneous lesions alone if there are:
  - Six or more facial papules, of which three or more must be trichilemmoma, or
  - Cutaneous facial papules and oral mucosal papillomatosis, or
  - Oral mucosal papillomatosis and acral keratoses, or
  - Six or more palmo plantar keratoses
- Two or more major criteria (one must be macrocephaly or Lhermitte-Duclos disease)
- One major and at least three minor criteria
- At least four minor criteria

**Relatives of individuals with Cowden syndrome are considered to have a diagnosis of CS if they meet any of the following criteria:**

- The pathognomonic criteria
- Any one major criterion with or without minor criteria
- Two minor criteria

**eTable 3. Primer Sequences Used in the Study**

<b>Primer</b>	<b>Sequence (5' to 3')</b>	<b>T<sub>m</sub></b>	<b>Product</b>
<b>Size</b>			
PTEN_ChIP_F	AAAGCTAGCCAGACTCGAGTCAGTGA	55	343 bp
PTEN_ChIP_R	AAAAGATCTCGAGGCGGACGGGAC	55	
KILLIN_ChIP_F	GATGGAAGTTTGAGAGTTGAG		62
245 bp			
KILLIN_ChIP_R	CCACGGCTTCCACCTTCCC	62	
KILLIN_RT_F	AAAAGAATTCCGGGGCTGGCGCTTGGGG	60	162 bp
KILLIN_RT_R	AAAAGCGGCCGCGTCCTTTGGCTTGCTCTTAGG	60	
PTEN_RT_F	CAGAAAGACTTGAAGGCGTAT	60	624 bp
PTEN_RT_R	AACGGCTGAGGGAATC	60	
BS_PCR_F	GTTGTAGTTTTAGGGAGGGGGT	60	283 bp
BS_PCR_R	CTACTTCTCCTCAACAACCAAAAAC	60	