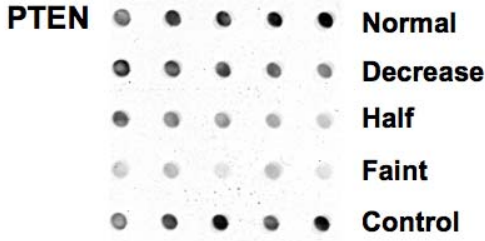


Supplemental Data

Differential Expression of *PTEN*-Targeting MicroRNAs *miR-19a* and *miR-21* in Cowden Syndrome

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Figure S1. PTEN Protein Levels in Patient and Control Samples



Whole cell protein lysates from control or patient lymphoblastoid lines were applied to nitrocellulose with a dotblot apparatus (Bio-Rad, Hercules, CA, USA). Patient protein lysates were isolated from immortalized LBCLs by the Genomic Medicine Biorepository, and protein concentrations were determined via the BCA assay, with BSA as a standard. Thirty micrograms of protein was applied to nitrocellulose with a dotblot apparatus, and equal protein loading was confirmed by staining of the nitrocellulose blots with Ponceau S. Nitrocellulose blots were then subjected to western blot analysis with α -PTEN (monoclonal antibody clone 6H2.1 [Cascade Biosciences, Portland, OR, USA]) at 1:1000 dilution, and they were then incubated with appropriate secondary antibody as previously described. Proteins were visualized with enhanced chemiluminescence. The series of dotblot western blots illustrate the PTEN protein levels of *PTEN*-mutation-negative patients with Cowden or Cowden-like syndrome and those of the control group.

“Normal” indicates normal PTEN protein levels.

“Decreased” indicates decreased PTEN protein levels relative to control levels.

“Half” indicates PTEN protein levels that are half those of control levels.

“Faint” indicates faint PTEN protein levels relative to control levels.

“Control” indicates the protein levels of control samples.

Table S1. Clinical Features, PTEN Protein*, and Relative *PTEN* mRNA, *miR-19a*, and *miR-21* Expression among Patients with R130X, R233X, or R335X Mutations

Mutation/ Sample	Gender	Age at Diagnosis	Phenotype	PTEN Protein Expression	<i>PTEN</i> Transcript Expression	<i>miR-19a</i> Expression	<i>miR-21</i> Expression	Clinical Manifestations
R130X								
0047-01	M	46	CS	Faint	0.893	1.567	2.128	Macrocephaly, trichilemmomas, acral keratoses, papillomatous papules, lipomas, GI cancer
0491-01	F	70	CS	Normal	0.870	1.510	1.181	Macrocephaly, fibrocystic breast disease, breast cancer, endometrial fibroids, endometrial cancer
0521-01	M	15	CS	Decrease	0.805	1.880	2.937	Macrocephaly, LDD, benign thyroid, multiple papules on lips, tongue, and esophagus
0857-01	F	63	CS	Half	0.604	1.876	1.381	Macrocephaly, benign thyroid, colon polyps, GU abnormality
1111-01	M	56	CS	Half	1.020	3.900	2.532	Macrocephaly, LDD, thyroid cancer

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1432-01	M	59	CS	Normal	0.637	0.829	0.918	Macrocephaly, lipomas, trichilemmomas, papillomatous papules, benign thyroid
4396-01	F	47	CS	Normal	0.817	1.231	0.646	Macrocephaly, lipomas, benign thyroid, breast cancer, endometrial fibroids
11578-01	F	61	CS	Half	0.634	1.861	1.447	Macrocephaly, papillomatous papules, breast cancer, hemangioma, lipomas, benign thyroid, endometrial fibroids
12644-01	F	47	CS	Decrease	0.828	1.456	1.502	Macrocephaly, trichilemmomas, papillomatous papules, breast cancer, benign thyroid
2381-01	F	2	CSL	Normal	0.984	1.158	1.271	Macrocephaly, lipomas, developmental delay
15413-01	M	50	CS/BRRS	Decrease	0.913	1.510	2.249	Macrocephaly, papillomatous papules, lipomas, hemangioma, benign thyroid, LDD, pigmented macules on penis
0268-01	M	9	BRRS	Normal	1.219	0.959	1.315	Macrocephaly, lipomas, pigmented macules on penis
4498-01	M	9	BRRS	Normal	0.932	1.016	1.006	Macrocephaly, oral papillomatous papules, pigmented macules on penis
R233X								
12961-01	F	48	CS	Half	0.528	1.419	1.421	Macrocephaly, trichilemmomas, papillomatous papules, acral keratoses, lipomas, hemangioma, thyroid cancer
4026-01	F	3	CSL	Normal	0.514	1.696	1.625	Macrocephaly, developmental delay
4389-01	F	40	CSL	Half	0.527	2.078	2.180	Macrocephaly, breast cancer, lipomas
17727-01	F	6	CSL	Decrease	0.619	1.310	1.133	Macrocephaly, lipomas, hemangioma, developmental delay
972-01	M	7	BRRS	Decrease	0.602	1.443	1.028	Macrocephaly, developmental delay, hemangioma
R335X								
1113-01	F	32	CS	Faint	1.275	1.073	1.235	Macrocephaly, trichilemmomas, acral keratoses, lipomas, fibromas, breast cancer
1194-01	F	60	CS	Faint	1.146	2.210	1.537	Macrocephaly, papillomatous papules, lipomas, benign thyroid, developmental delay
1495-01	F	50	CS	Decrease	1.202	2.225	1.964	Fibrocystic breast disease, papillomatous papules, lipomas, benign thyroid, endometrial fibroids
4292-01	F	56	CS	Half	1.381	0.463	0.950	Fibrocystic breast disease, acral keratoses, lipomas, trichilemmomas, papillomatous papules, endometrial fibroids
15394-01	F	50	CS	Decrease	1.284	1.549	1.929	Fibrocystic breast disease, trichilemmomas, papillomatous papules, lipomas, trichilemmomas, breast cancer, benign thyroid, endometrial cancer, endometrial fibroids
16224-01	M	38	CS	Decrease	1.661	1.628	1.609	Macrocephaly, trichilemmomas, papillomatous papules, lipomas

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19885-01	F	10	CS	Decrease	1.067	1.562	1.222	Macrocephaly, acral keratoses, lipomas, hemangioma
6230-01	M	39	CSL	Half	1.260	2.118	1.574	Macrocephaly, lipomas
0232-01	M	44	BRRS	Normal	1.008	0.678	0.597	Macrocephaly, trichilemmomas, papillomatous papules, lipomas, benign thyroid, pigmented macules on penis
0531-01	M	1	BRRS	Faint	1.333	0.762	1.107	Macrocephaly, developmental delay

*PTEN protein status “calls” are illustrated in Figure S1.

