Functionally distinct groups of inherited PTEN mutations in autism and tumour syndromes

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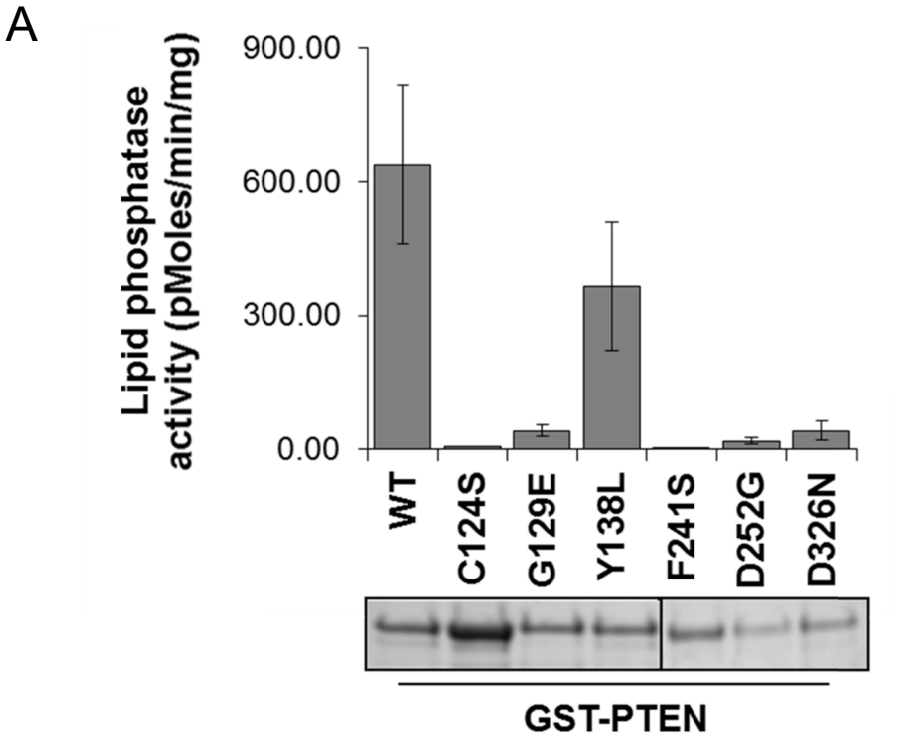
Figure S1


A
Lipid phosphatase activity (pMoles/min/mg)
900.00
600.00
300.00
0.00
WT
C124S
G129E
Y138L
F241S
D252G
D326N
GST-PTEN

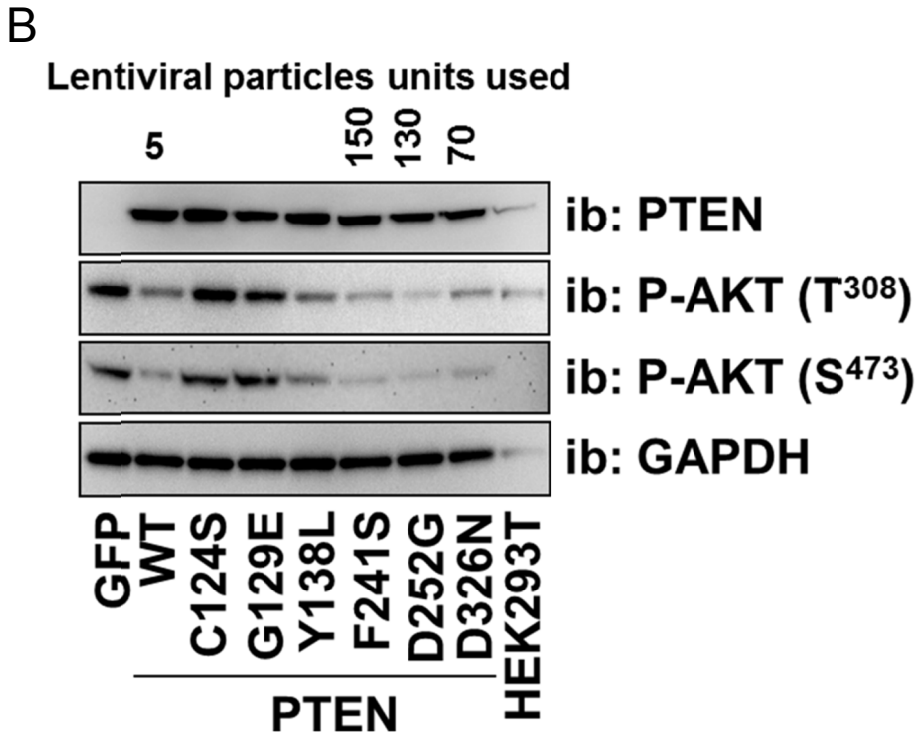

B
Lentiviral particles units used
5
150
130
70
ib: PTEN
ib: P-AKT (T308)
ib: P-AKT (S473)
ib: GAPDH
GFP
WT
C124S
G129E
Y138L
F241S
D252G
D326N
HEK293T
PTEN

Figure S1. PTEN mutants characterisation in bacteria and U87MG cells

Autism-related mutations proteins drastically reduce PTEN phosphatase activity when expressed in bacteria but they are able to regulate AKT when expressed in U87MG cells. PTEN WT and PTEN mutants (C124S, G129E, Y138L, F241S, D252G and D326N) were expressed in bacteria and purified as GST-fusion proteins. (A) The proteins were separated by SDS-PAGE and stained with Coomassie Brilliant Blue R-250. Proteins were assayed against ^{33}P radiolabelled PIP_3 for 1 hour at 37ºC. The activity is presented as the mean activity ± s.e.m from three experiments performed in duplicates. (B) PTEN null U87MG cells were transduced for 48 hours with lentiviruses encoding GFP, PTEN WT or C124S, G129E, Y138L, F241S, D252G and D326N. PTEN expression and AKT phosphorylation were investigated by western blotting of total cell lysates using total and phospho-specific antibodies. Units of lentiviruses particles used to express PTEN are indicated (5 units are equal to 50 µl of viral supernatant).

Figure S2

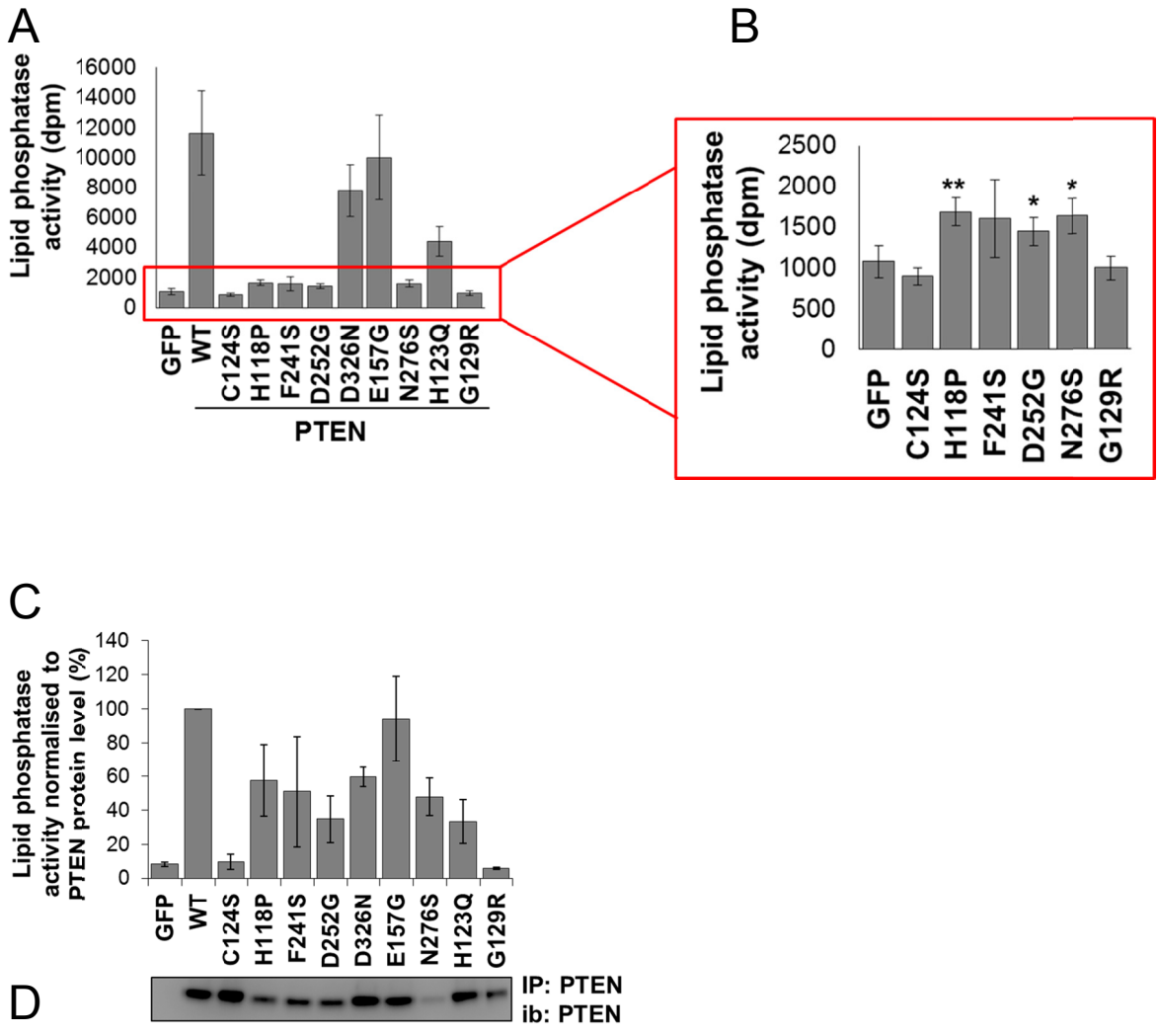

A
B
Lipid phosphatase activity (dpm)
16000
14000
12000
10000
8000
6000
4000
2000
0
GFP
WT
C124S
H118P
F241S
D252G
D326N
E157G
N276S
H123Q
G129R
PTEN
2500
2000
1500
1000
500
0
**
*
*
C
Lipid phosphatase activity normalised to PTEN protein level (%)
140
120
100
80
60
40
20
0
D
IP: PTEN
ib: PTEN

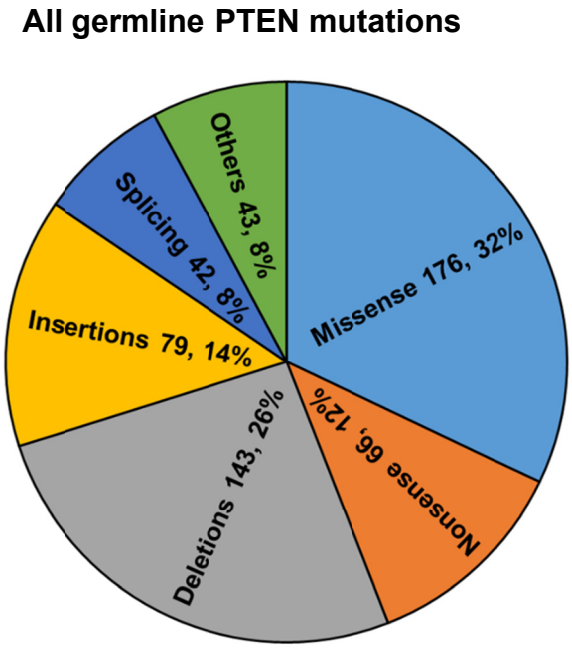
Figure S2. Raw data from the PTEN lipid phosphatase activity assay

PTEN proteins or GFP were transiently over-expressed in U87MG cells and after cell lysis, immunoprecipitated using an antibody raised against the N-terminus of PTEN. Immune complexes were assayed against radiolabelled PIP_3 vesicles. (A) Mean raw phosphatase activity in dpm released phosphate is shown, derived from six experiments performed on different days, ± s.e.m.

(B) This shows the same mean raw phosphatase activity data as (A) rescaled to show the differences between raw values with low recovered activity. Across the six experiments there was no overlap between the released phosphate values for any of the seven mutants and the data obtained using the inactive PTEN C124S control. Accordingly, for six of the seven mutants the measured released phosphate was significantly higher than detected with this inactive control (the exception being F241S). (**p< 0.01, *p<0.05 compared to PTEN C124S; Student's *t*-test using Excel and GraphPad Prism software). (C) This shows the mean activity from six independent experiments ± s.e.m as a % of wild-type PTEN activity after normalisation to the amount of protein immunoprecipitated. Immunoprecipitated protein was determined by densitometry (ImageQuant TL software) after immunoblotting and direct chemiluminiscence detection using CCD camera. (D) A representative immunoblot of the immunoprecipitated proteins from one of the experiments.

Figure S3

A



B

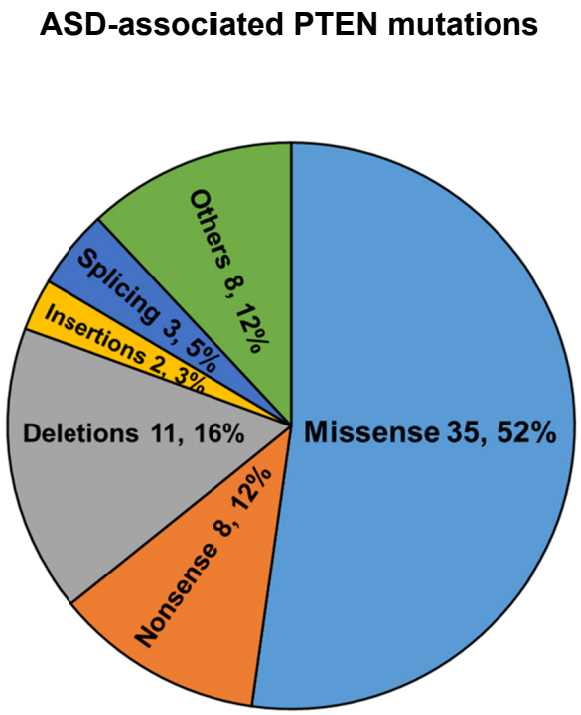
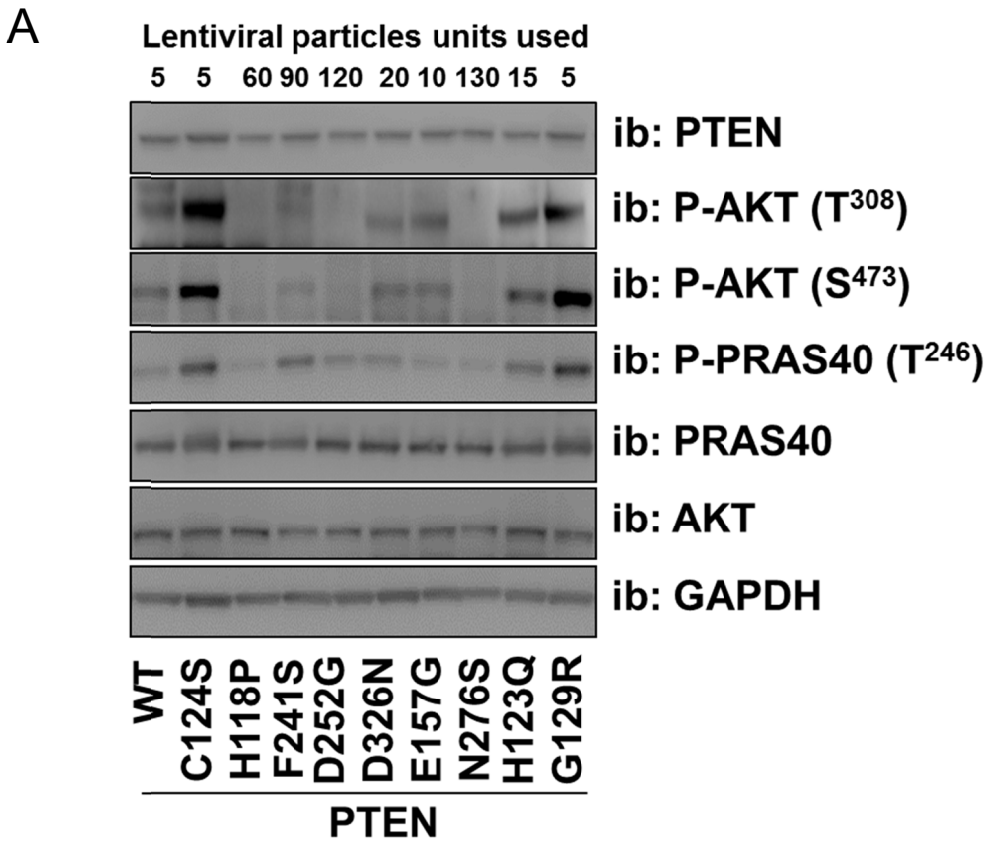


Figure S3. Classification of all inherited PTEN mutations (A) and selectively those associated with ASD (B). The germline PTEN mutations detailed in Tables S1, S3 and S4 were classified into the categories shown. (A) displays the number of apparently independent patients carrying PTEN mutations and (B) the classification of PTEN mutations in independent patients described with ASD, either in the presence or absence of other symptoms including PHTS. A significantly larger fraction of ASD associated mutations are missense mutations 35/67 and 176/549 ($p<0.005$, Fishers Exact Test)

Figure S4


A
Lentiviral particles units used
5 5 60 90 120 20 10 130 15 5
ib: PTEN
ib: P-AKT (T308)
ib: P-AKT (S473)
ib: P-PRAS40 (T246)
ib: PRAS40
ib: AKT
ib: GAPDH
WT
C124S
H118P
F241S
D252G
D326N
E157G
N276S
H123Q
G129R
PTEN

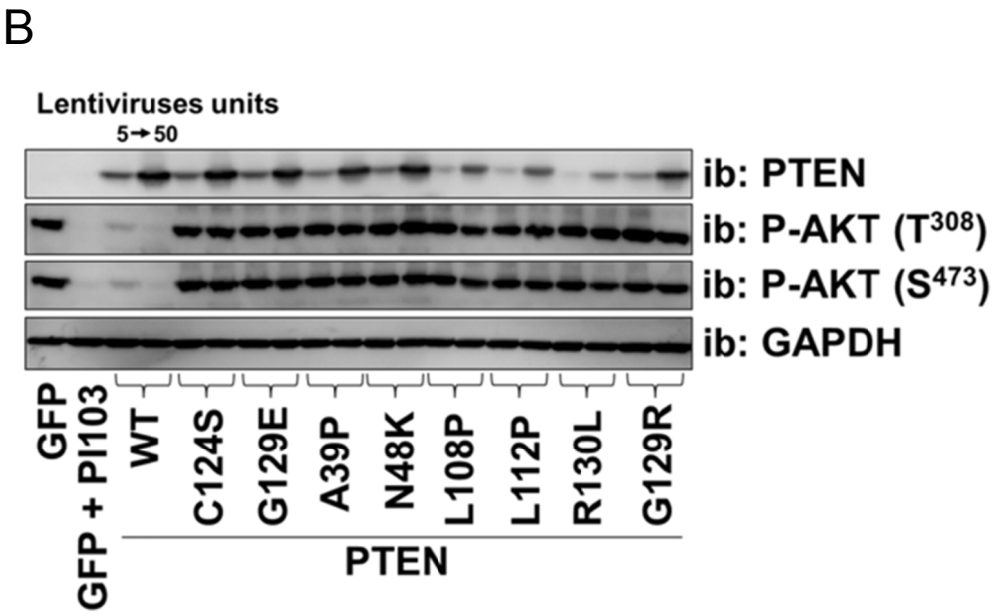

B
Lentiviruses units
5→50
ib: PTEN
ib: P-AKT (T308)
ib: P-AKT (S473)
ib: GAPDH
GFP
GFP + PI103
WT
C124S
G129E
A39P
N48K
L108P
L112P
R130L
G129R
PTEN

Figure S4. Regulation of AKT and downstream pathways by autism- and Cowden-related mutant proteins in U87MG cells

PTEN null U87MG cells were transduced for 48 hours with lentiviruses encoding PTEN WT or mutants using (A) different amount of lentiviral particles, as shown, for each mutant in order to obtain the same PTEN expression level or (B) two concentrations (low and high) of lentiviruses particles (5 units are equal to 50 µl of viral supernatant). Cell expressing GFP and cells treated with the PI3K inhibitor PI103 (1 µM for 30 minutes) were used as control. The evaluation of PTEN and phosphorylated and total AKT and PRAS40 in the lysates from transduced cells was carried out by western blotting using total and phospho-specific antibodies. The blotting panels shown are representative of three independent experiments.

Table S1. PTEN mutations associated with ASD

Missense	Nonsense	Deletions	Insertions	Splicing	Others	References
c.278A>G H93R						Butler et al., 2005
c.722T>C F241S						Butler et al., 2005
c.755A>G D252G						Butler et al., 2005
c.66C>G D22E						Buxbaum et al., 2007
					c._1088C>T	Buxbaum et al., 2007
					c._1084C>T	Buxbaum et al., 2007
					c. IVS3-9T>C	Buxbaum et al., 2007
c.976G>A D326N						Buxbaum et al., 2007
					c._1026C>A	Buxbaum et al., 2007
					c._903G>A	Buxbaum et al., 2007
c.353A>C H118P						Orrico et al., 2009
c.530T>G Y176C						Orrico et al., 2009
c.824A>G N276S						Orrico et al., 2009
			c.519-520insT			Varga et al., 2009
	c.388C>T R130*					Varga et al., 2009
	c.416T>A L139*					Varga et al., 2009
c.470A>G E157G						Varga et al., 2009
c.369C>G H123Q						McBride et al., 2010
c.518G>A R173H						McBride et al., 2010
c.232A>G T78A						Schaaf et al., 2011
c.75G>T L25F						Mester et al., 2012
	c.1003C>T R335*					Mester et al., 2012
		Whole gene deletion				Mester et al., 2012
			c.420-421insA			Mester et al., 2012
c.392C>T T131I						O'Roak et al., 2012
c.500C>A T167N						O'Roak et al., 2012
		c.158-159delTA				Bubien et al., 2013
		c.284delC				Bubien et al., 2013

Missense	Nonsense	Deletions	Insertions	Splicing	Others	References
	c.388C>T R130*					Bubien et al., 2013
	c.388C>T R130*					Bubien et al., 2013
c.392C>T T131I						Bubien et al., 2013
c.403A>G I135V						Bubien et al., 2013
c.406T>C C136R						Bubien et al., 2013
c.518G>A R173H						Bubien et al., 2013
		c.586delC				Bubien et al., 2013
		c.586delC				Bubien et al., 2013
		c.586delC				Bubien et al., 2013
				c.635-1G>C		Bubien et al., 2013
				c.635-3C>G		Bubien et al., 2013
c.830C>G T277R						Bubien et al., 2013
		c.1004-1005delGA				Bubien et al., 2013
c.202T>A Y68N						Klein et al., 2013
c.113C>A P38H						Klein et al., 2013
c.764T>C V255A						Klein et al., 2013
	c.959T>G L320*					Vanderver et al., 2014
c.17A>T L6I						Vanderver et al., 2014
c.194A>G Y65C						Vanderver et al., 2014
c.511C>G Q171E						Vanderver et al., 2014
	c.388C>T R130*					Vanderver et al., 2014
c.737C>T P246L						Vanderver et al., 2014
c.633C>G C211W						Vanderver et al., 2014
		c.43delA				Marchese et al., 2014
c.208C>G L70V						Hobert et al., 2014
					c.3G>T M1I	Hobert et al., 2014
					c.3G>T M1I	Hobert et al., 2014
	c.1003C>T R335*					Hobert et al., 2014
				c.209+5G>A		Hobert et al., 2014

Missense	Nonsense	Deletions	Insertions	Splicing	Others	References
c.40C>G R14G						Frazier et al., 2014
c.755A>T D252V						Frazier et al., 2014
c.202T>C Y68H						Frazier et al., 2014
c.37A>C N12T						Frazier et al., 2014
c.395G>A G132D						Frazier et al., 2014
c.277C>T H93Y						Frazier et al., 2014
		c.597delGTT				Frazier et al., 2014
					c._1177C>T	Frazier et al., 2014
		4-Mb deletion				Frazier et al., 2014
		10q deletion				Frazier et al., 2014

Tables S2 and S3. Severity index scoring system.

Based on the clinical description of the patients carrying PTEN germline mutations, the severity scores were created according to the number of sites affected as well as the presence of mental retardation/ development delay (MR/DD). The presence of macrocephaly was recorded but not included in the severity index score, as macrocephaly was seen in almost every subject, with the chance that it was not recorded in others. For each site affected by benign tumours or lesions it was assigned one point, two points for each site affected by malignant tumours, and one point for the presence of mental retardation. The severity index score is the sum of these numbers. Patients who scored one to three were classified as “mild”, four to five as “moderate” and six or above as “severe”.

The choice of mutations to analyse has been made based on the “index score average” resulting from the average of the scores of all patients carrying the same PTEN mutation. It should be noted that the characterised PTEN mutants associated with severe phenotypes (see figure 4) were selected at a mid-point of the progression of the project and that the data tabulated here contain very recently published information added since that time. Efforts have been made to minimise the chances of double counting but this cannot be excluded.

Table S2

Severity index scoring system			
Site affected	**Benign lesion/tumour**	**Malignant tumour/cancer**	**MR/DD**
CNS	**1 point** assigned for each site affected	**2 points** assigned for each site affected	**1 point** assigned for the presence of Mental Retardation/ Developmental Delay
Thyroid			
Breast			
Skin/Mucosa			
Gastrointestinal tract			
Others			
Total score	**1-3 MILD** **4-5 MODERATE** **6 + SEVERE**		

Table S3

Mutations	Index score average	Index score patients	Assigned Phenotype	References
M1I	1	1	ASD	[1] Hobert et al., 2014
		1	ASD	[1] Hobert et al., 2014
K6I	1	1	ASD	[2] Vanderver et al., 2014
K6E	1	1	ASD	[2] Vanderver et al., 2014
K13E	3	4	CS	[3] Bubien et al., 2013
		4	CS	[3] Bubien et al., 2013
		1	CS	[3] Bubien et al., 2013
R14G	0	0	ASD	[4] Frazier et al., 2014
R15S	3	3	CS	[5] Nagy et al., 2011
Y16H	4	4	CS	[6] Ngeow et al., 2014
D22E	0	0	ASD	[7] Buxbaum et al., 2007
D24Y	5	2	BRRS	[8] Celebi et al., 1999
		8	CS	[3] Bubien et al., 2013
D24G	4	4	CS	[6] Ngeow et al., 2014
D24H	6	6	CS	[6] Ngeow et al., 2014
D24V	5	5	CS	[6] Ngeow et al., 2014
L25F	0	0	ASD	[9] Mester et al., 2012
T26P	2	2	Unknown	[9] Mester et al., 2012
Y27N	4	4	CS	[6] Ngeow et al., 2014
Y27C	1	1	Unknown	[2] Vanderver et al., 2014
P30L	1	1	CS	[3] Bubien et al., 2013
A34D	2	2	BRRS	[10] Marsh et al., 1999
		2	BRRS	[10] Marsh et al., 1999
M35R	1	1	JPC	[11] Olschwang et al., 1998
M35T	4	4	PLS	[12] Zhou et al., 2001
G36R	5	5	CS	[13] Celebi et al., 2000
P38H	1	1	ASD	[14] Klein et al., 2013
A39P	7	7	CS	[15] Tate et al., 2008
G44D	2	2	Unknown	[16] Varga et al., 2009
N48K	8	8	CS	[17] Vega et al., 2003
I50T	1	1	Unknown	[2] Vanderver et al., 2014
H61D	0	0	VATER	[18] Reardon et al., 2001
H61R	4	4	CS	[6] Ngeow et al., 2014
Y65C	0	0	ASD	[2] Vanderver et al., 2014
		0	ASD	[2] Vanderver et al., 2014
I67R	7	7	CS	[3] Bubien et al., 2013
Y68N	2	2	ASD	[14] Klein et al., 2013
Y68D	0.5	1	PS	[19] Loffeld et al., 2006
		0	CS	[19] Loffeld et al., 2006
Y68H	1.33	1	BRRS	[20] Marsh et al., 1998
		3	CS	[20] Marsh et al., 1998
		0	ASD	[4] Frazier et al., 2014
Y68C	2	2	CS	[21] Lobo et al., 2009
L70P	2	2	CS	[20] Marsh et al., 1998
L70V	3	3	ASD	[1] Hobert et al., 2014
T78A	0	0	ASD	[22] Schaaf et al., 2011
A79T	3	3	BC	[23] Figer et al., 2002
N82T	3	3	BC	[23] Figer et al., 2002
Y88C	1	1	PHTS	[9] Mester et al., 2012
D92A	5	5	CS	[3] Bubien et al., 2013
H93Y	2.66	4	CS	[24] Kohno et al., 1998
		4	CS	[6] Ngeow et al., 2014
		0	ASD	[4] Frazier et al., 2014

Mutations	Index score average	Index score patients	Assigned Phenotype	References
H93R	1	1	ASD	[25] Butler et al., 2005
P96Q	2.5	3	CS	[26] Bussaglia et al., 2002
		2	CS	[26] Bussaglia et al., 2002
P96R	3.5	3	CS	[9] Mester et al., 2012
		4	CS	[27] Busch et al., 2013
C105Y	2	2	BRRS	[10] Marsh et al., 1999
D107G	1	1	Unknown	[2] Vanderver et al., 2014
L108P	6.7	6	PHTS	[28] Tan et al., 2007
		7	PHTS	[3] Bubien et al., 2013
		7	CS	[29] Ngeow et al., 2013
L112P	11	11	CS	[30] Tsou et al., 1998
H118P	1	1	ASD	[31] Orrico et al., 2009
V119I	4	4	Multiple cancers	[32] De Vivo et al., 2000
		4	Multiple cancers	[32] De Vivo et al., 2000
H123Q	2.5	1	ASD	[33] McBride et al., 2010
		4	CS	[34] Kersseboom et al., 2012
H123R	4	4	CS	[35] Nelen et al., 1997
H123D	3	3	CS	[26] Bussaglia et al., 2002
C124R	4	2	CS	[35] Nelen et al., 1997
		4	CS	[35] Nelen et al., 1997
		6	CS	[35] Nelen et al., 1997
A126P	3	3	CS	[9] Mester et al., 2012
K128E	4	4	CS	[27] Busch et al., 2013
K128N	3	3	CS	[29] Ngeow et al., 2013
G129R	3.5	5	CS	[36] Elia et al., 2012
		2	Unknown	unpublished patient
G129E	4	4	CS	[37] Liaw et al., 1997
		5	CS	[37] Liaw et al., 1997
		7	CS	[37] Liaw et al., 1997
		2	CS	[37] Liaw et al., 1997
		2	CS	[29] Ngeow et al., 2013
R130Q	3.9	5	CS	[38] Kurose et al., 1999
		4	CS/BRRS	[38] Kurose et al., 1999
		3	CS/BRRS	[38] Kurose et al., 1999
		3	CS/BRRS	[38] Kurose et al., 1999
		5	CS	[39] Heindl et al., 2012
		4	CS	[39] Heindl et al., 2012
		5	CS	[29] Ngeow et al., 2013
		4	CS	[6] Ngeow et al., 2014
		2	CS	[6] Ngeow et al., 2014
R130G	1.8	2	CS	[21] Lobo et al., 2009
		4	Unknown	[9] Mester et al., 2012
		1	CS	[3] Bubien et al., 2013
		1	CS	[3] Bubien et al., 2013
		1	Unknown	[3] Bubien et al., 2013
		2	CS	[3] Bubien et al., 2013
R130L	5	3	CS	[3] Bubien et al., 2013
		3	CS	[3] Bubien et al., 2013
		10	CS	[20] Marsh et al., 1998
		4	CS	[6] Ngeow et al., 2014
T131I	1.7	1	CS/ASD	[3] Bubien et al., 2013
		3	Unknown	[3] Bubien et al., 2013
		1	ASD	[40] O'Roak et al., 2012
G132A	1	1	PHTS	[28] Tan et al., 2007

Mutations	Index score average	Index score patients	Assigned Phenotype	References
G132D	3.16	2	CS	[41] Derrey et al., 2004
		6	CS	[3] Bubien et al., 2013
		4	CS	[3] Bubien et al., 2013
		3	CS	[3] Bubien et al., 2013
		4	CS	[39] Heindl et al., 2012
		0	ASD	[4] Frazier et al., 2014
G132V	3	3	PHTS	[42] Tekin et al., 2006
M134R	0.75	0	BRRS	[23] Figer et al., 2002
		0	BRRS	[23] Figer et al., 2002
		0	BRRS	[23] Figer et al., 2002
		3	Unknown	[9] Mester et al., 2012
M134I	0	0	Unknown	[43] Busa et al., 2013
		0	Unknown	[43] Busa et al., 2013
		0	Unknown	[43] Busa et al., 2013
		0	Unknown	[43] Busa et al., 2013
M134T	4.66	1	MR	[33] McBride et al., 2010
		5	CS	[29] Ngeow et al., 2013
		8	Unknown	[27] Busch et al., 2013
I135R	3	3	BRRS	[44] Boccone et al., 2006
I135V	2.7	1	BRRS	[10] Marsh et al., 1999
		3	Unknown	[3] Bubien et al., 2013
		2	Unknown	[3] Bubien et al., 2013
		5	Unknown	[3] Bubien et al., 2013
		3	Unknown	[3] Bubien et al., 2013
		1	Unknown	[3] Bubien et al., 2013
		4	CS	[3] Bubien et al., 2013
C136R	5.6	7	CS	[45] Kubo et al., 2000
		7	CS	[3] Bubien et al., 2013
		2	CS	[3] Bubien et al., 2013
		6	CS	[3] Bubien et al., 2013
		6	CS	[6] Ngeow et al., 2014
R142P	3	3	CS	[3] Bubien et al., 2013
A151B	3	3	CS	[3] Bubien et al., 2013
Y155N	4	4	CS	[3] Bubien et al., 2013
Y155C	3.66	2	CS	[46] Gicquel et al., 2003
		7	CS	[3] Bubien et al., 2013
		2	CS	[29] Ngeow et al., 2013
E157G	1	1	ASD	[16] Varga et al., 2009
V158I	3	4	Unknown	[32] De Vivo et al., 2000
		2	Unknown	[32] De Vivo et al., 2000
D162E	1	1	CS	[9] Mester et al., 2012
G165E	5	5	CS	[47] Nelen et al., 1999
G165V	4	4	CS	[20] Marsh et al., 1998
G165R	2	2	CS	[48] Banneau et al., 2010
T167N	1	1	ASD	[40] O'Roak et al., 2012
S170R	4	2	BRRS	[49] Marsh et al., 1997
		7	CS	[3] Bubien et al., 2013
		4	Unknown	[3] Bubien et al., 2013
		3	CS	[3] Bubien et al., 2013
S170I	4	4	CS	[6] Ngeow et al., 2014
Q171E	0	0	ASD	[2] Vanderver et al., 2014
R173C	2.5	1	BRRS	[50] Lachlan et al., 2007
		4	CS	[6] Ngeow et al., 2014
R173P	3.5	3	LDD	[51] Kirches et al., 2010
		4	Unknown	[51] Kirches et al., 2010

Mutations	Index score average	Index score patients	Assigned Phenotype	References
R173H	0.75	0	Unknown	[50] Lachlan et al., 2007
		2	Unknown	[50] Lachlan et al., 2007
		1	CS/ASD	[3] Bubien et al., 2013
		0	ASD	[33] McBride et al., 2010
R173G	2	2	CS	[6] Ngeow et al., 2014
Y176C	1	1	ASD	[31] Orrico et al., 2009
L181P	1	1	CS	[52] Thiffault et al., 2004
T202I	2	2	Unknown	[16] Varga et al., 2009
C211W	1	1	ASD	[2] Vanderver et al., 2014
V217D	2	2	CS	[53] Kim et al., 2005
R234Q	2	2	Multiple cancers	[54] Staal et al., 2002
F241S	0	0	ASD	[25] Butler et al., 2005
P246L	1	2	BRRS	[10] Marsh et al., 1999
		1	BRRS	[9] Mester et al., 2012
		0	ASD	[2] Vanderver et al., 2014
D252G	1	1	ASD	[25] Butler et al., 2005
		1	CS	[3] Bubien et al., 2013
D252V	0	0	ASD	[4] Frazier et al., 2014
V255A	0	0	ASD	[14] Klein et al., 2013
W274L	1	1	DD	[33] McBride et al., 2010
N276S	1	1	ASD	[31] Orrico et al., 2009
T277R	1.66	1	CS	[3] Bubien et al., 2013
		2	CS	[3] Bubien et al., 2013
		2	CS	[3] Bubien et al., 2013
		3	CS	[3] Bubien et al., 2013
		0	CS/ASD	[3] Bubien et al., 2013
		2	CS	[48] Banneau et al., 2010
K289E	1	1	CS	[55] Chi et al., 1998
D326N	1	1	AUTISM	[7] Buxbaum et al., 2007
R335L	4	4	CS	[56] Sawada et al., 2000
F337S	2	2	CS	[50] Lachlan et al., 2007
K342N	2	3	HTS	[3] Bubien et al., 2013
		1	CS	[3] Bubien et al., 2013
V343E	3.5	4	CS	[57] Lynch et al., 1997
		4	CS	[57] Lynch et al., 1997
		5	CS	[57] Lynch et al., 1997
		3	CS	[57] Lynch et al., 1997
		2	CS	[57] Lynch et al., 1997
		3	CS	[57] Lynch et al., 1997
L345V	4.5	4	CS	[6] Ngeow et al., 2014
		5	CS	[27] Busch et al., 2013

ASD, Autism spectrum disorder; BC, Breast cancer; BRRS, Bannayan-Riley-Ruvalcaba Syndrome; CS, Cowden Syndrome; DD, Developmental Delay; HTS, Hamartoma Tumour Syndrome; JPS, Juvenile Polyposis Coli; LDD, Lhermitte-Duclos disease; MR, Mental Retardation; PHTS, PTEN Hamartoma-Tumour Syndrome; PLS, Proteus-Like Syndrome; PS; Proteus Syndrome.

MILD	MODERATE	SEVERE

Table S4 Clinical features of the 204 patients carrying germline-derived PTEN missense mutations.

Mutation	M1I	M1I	K6I	K6E	N12T	K13E	K13E	K13E	R14G	R15S
Diagnosis	ASD	ASD	ASD		ASD	CS	CS	CS	ASD	CS
Sex	M	M	M	F	M				F	
Age	11y	12y	3y	11m	5y2m				3y3m	
CNS										
Macrocephaly	X	X	X	X	X	X	X		X	X
LDD										
Benign Tumour										
Malignant Tumour										
MR/DD			X	X						
THYROID										
Benign (adenoma, goitre)						X	X			
Malignant										X
BREASTS										
Benign										
Malignant Tumour										
SKIN/MUCOSA										
Trichilemmona										
MCL, FP, AK, F	X	X				X	X	X		
OP	X	X				X	X	X		
SP								X		
Lipomas								X		
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps						X				
Benign (hamartoma)										X
Malignant Tumour										
OTHER BENIGN						GU les				
OTHER CANCERS							LUNG			
SI SCORE	1	1	1	1	1	0	4	4	1	0	3
REFERENCES	Hobert et al., 2014	Hobert et al., 2014	Vanderver et al., 2014	Vanderver et al., 2014	Frazier et al., 2014	Bubien et al., 2013	Bubien et al., 2013	Bubien et al., 2013	Frazier et al., 2014	Nagy et al., 2011

Mutation	**Y16H**	**D22E**	**D24Y**	**D24Y**	**D24G**	**D24H**	**D24V**	**L25F**	**T26P**	**Y27N**
Diagnosis	CS	ASD	BRRS	CS	CS	CS	CS	ASD		CS
Sex	F	M	M		F	F	F	M	F	F
Age	65y		4y		49y	46y	28y	7y	19y	46y
CNS										
Macrocephaly		X	X				X	X	X	
LDD									X	
Benign Tumour										
Malignant Tumour										
MR/DD			X							
THYROID										
Benign (adenoma, goitre)				X			X		X	
Malignant										
BREASTS										
Benign				X						
Malignant Tumour	X			X	X	X	X			X
SKIN/MUCOSA										
Trichilemmona										
MCL, FP, AK, PPK, F				X			X			
OP				X						
SP			X							
Lipomas			X							
Malignant Tumour				X						
GASTROINTESTINAL TRACT										
GI polyps										
Benign (hamartoma)										
Malignant Tumour										
OTHER BENIGN				GU les			UTLM			
OTHER CANCERS	ENCA				ENCA	ENCA-RC				RC
SI SCORE	4	0	2	8	4	6	5	0	2	4
REFERENCES	Ngeow et al., 2014	Buxbaum et al., 2007	Celebi et al., 2009	Bubien et al., 2013	Ngeow et al., 2014	Ngeow et al., 2014	Ngeow et al., 2014	Mester et al., 2012	Mester et al., 2012	Ngeow et al., 2014

Mutation	**Y27C**	**P30L**	**A34D**	**A34D**	**M35R**	**M35T**	**G36R**	**P38H**	**A39P**	**G44D**
Diagnosis		CS	BRRS	BRRS	JPC	PLS	CS	ASD	CS	
Sex	F		M	M	M	M	F	M	F	F
Age	3y10m			10y	10y	7y	47y	15y	50-59y	27m
CNS										
Macrocephaly		X		X		X	X	X	X	X
LDD	X									
Benign Tumour										
Malignant Tumour										
MR/DD	X			X		X				X
THYROID										
Benign (adenoma, goitre)									X	
Malignant										
BREASTS										
Benign									FBD	
Malignant Tumour							X		X	
SKIN/MUCOSA										
Trichilemmona							X			
MCL, FP, AK, PPK, F		X					X		X	
OP							X		X	
SP		X	X	X		X		X		
Lipomas			X	X		X		X		
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps						X	X		X	
Benign (hamartoma)					JPC					
Malignant Tumour										
OTHER BENIGN			VASC les			RH,NP,HN	UF		UTLM	Hreflux
OTHER CANCERS										
SI SCORE	1	1	2	2	1	4	5	1	7	2
REFERENCES	Vanderver et al., 2014	Bubien et al., 2013	Marsh et al., 1999	Marsh et al., 1999	Olschwang et al., 1998	Zhou et al., 2001	Celebi et al., 2000	Klein et al., 2013	Tate et al., 2008	Varga et al., 2009

Mutation	**N48K**	**I50T**	**H61D**	**H61R**	**Y65C**	**Y65C**	**I67R**	**Y68N**	**Y68D**	**Y68D**	**Y68H**
Diagnosis	CS		VATER	CS	ASD	ASD	CS	ASD	PS	CS	BRRS
Sex	F	M	M	F	M	F			M	F	M
Age	38y	8m	18m	47y	4y5m	11m		4y	3y		
CNS											
Macrocephaly		X	X		X	X		X	X	X	
LDD											
Benign Tumour											
Malignant Tumour											
MR/DD		X									
THYROID											
Benign (adenoma, goitre)	X										
Malignant											
BREASTS											
Benign							X				
Malignant Tumour	X			X			X				
SKIN/MUCOSA											
Trichilemmona	X										
MCL, FP, AK, PPK, F	X						X				
OP	X						X				
SP								X			X
Lipomas							X	X	X		X
Malignant Tumour											
GASTROINTESTINAL TRACT											
GI polyps	X						X	X			
Benign (hamartoma)											
Malignant Tumour											
OTHER BENIGN	OVC										
OTHER CANCERS	ENCA			CRC			ENCA				
SI SCORE	8	1	0	4	0	0	7	2	1	0	1
REFERENCES	Vega et al., 2003	Vanderver et al., 2014	Reardon et al., 2001	Ngeow et al., 2014	Vanderver et al., 2014	Vanderver et al., 2014	Bubien et al., 2013	Klein et al., 2013	Loffeld et al., 2006	Loffeld et al., 2006	Marsh et al., 2008

Mutation	**Y68H**	**Y68H**	**Y68C**	**L70P**	**L70V**	**T78A**	**A79T**	**N82T**	**Y88C**	**D92A**
Diagnosis	CS	ASD	CS	CS	ASD	ASD	BC	BC	PHTS	CS
Sex	F	M	F	M	M		F	F	M	
Age	75y	2y	46y	31y	13y		36y	35y	1y	
CNS										
Macrocephaly		X			X	X			X	X
LDD										
Benign Tumour										
Malignant Tumour										
MR/DD					X				X	
THYROID										
Benign (adenoma, goitre)	X				X					X
Malignant			X	X						
BREASTS										
Benign	X						X	X		X
Malignant Tumour			X				X	X		
SKIN/MUCOSA										
Trichilemmona	X									
MCL, FP, AK, PPK, F	X									X
OP	X				X					X
SP										
Lipomas					X					X
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps										
Benign (hamartoma)										
Malignant Tumour										
OTHER BENIGN										
OTHER CANCERS										OVCA
SI SCORE	3	0	4	2	3	0	3	3	1	5
REFERENCES	Marsh et al., 1998	Frazier et al., 2014	Ngeow et al., 2014	Marsh et al., 1998	Hobert et al., 2014	Schaaf et al., 2011	Figer et al., 2002	Figer et al., 2002	Mester et al., 2012	Bubien et al., 2013

Mutation	**H93Y**	**H93Y**	**H93Y**	**H93R**	**P96Q**	**P96Q**	**P96R**	**P96R**	**C105Y**	**D107G**
Diagnosis	CS	CS	ASD	ASD	CS	CS	CS	CS	BRRS	
Sex	M	F	M	M	M	F	F	F		M
Age	46y	48y	4y4m	4y	72y	42y	30y	26y		3y
CNS										
Macrocephaly	X		X	X			X	X		X
LDD										
Benign Tumour										
Malignant Tumour										
MR/DD				X						X
THYROID										
Benign (adenoma, goitre)	X				X	X	X	X		
Malignant										
BREASTS										
Benign							X			
Malignant Tumour		X								
SKIN/MUCOSA										
Trichilemmona	X				X		X			
MCL, FP, AK, PPK, F					X	X				
OP					X		X			
SP									X	
Lipomas							X	X	X	
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps	X				X			X	X	
Benign (hamartoma)										
Malignant Tumour										
OTHER BENIGN	VASC les									
OTHER CANCERS		ENCA					VASC les			
SI SCORE	4	4	0	1	3	2	4	3	2	1
REFERENCES	Kohno et al., 1998	Ngeow et al., 2014	Frazier et al., 2014	Butler et al., 2005	Bussaglia et al., 2002	Bussaglia et al., 2002	Busch et al., 2013	Mester et al., 2012	Marsh et al., 1999	Vanderver et al., 2014

Mutation	**L108P**	**L108P**	**L108P**	**L112P**	**H118P**	**V119I**	**V119I**	**H123Q**	**H123Q**	**H123R**
Diagnosis	PHTS	PHTS	CS	CS	ASD	Mcancer	Mcancer	ASD	CS	CS
Sex			F	F		F	F	F	F	M
Age			50y	26y				2y6m	28y	
CNS										
Macrocephaly	X			X	X			X	X	X
LDD				X						
Benign Tumour										
Malignant Tumour				X						
MR/DD	X			X	X			X		
THYROID										
Benign (adenoma, goitre)		X	X	X						X
Malignant										X
BREASTS										
Benign	FBD	X	X	X					X	
Malignant Tumour		X	X			X	X			
SKIN/MUCOSA										
Trichilemmona				X						
MCL, FP, AK, PPK, F				X						X
OP				X						
SP										
Lipomas	X	X								
Malignant Tumour				X						
GASTROINTESTINAL TRACT										
GI polyps		X								
Benign (hamartoma)										
Malignant Tumour			X						X	
OTHER BENIGN	VASC les	GU les							H of leg	
OTHER CANCERS	OVCA		ENCA	ENCA		LUNG	ENCA			
SI SCORE	6	7	7	11	1	4	4	1	4	4
REFERENCES	Tan et al., 2007	Bubien et al., 2013	Ngeow et al., 2013	Tsou et al., 1998	Orrico et al., 2009	deVivo et al., 2000	de Vivo et al., 2000	McBride et al., 2010	Kersseboom et al., 2012	Nelen et al., 1997

Mutation	**H123D**	**C124R**	**C124R**	**C124R**	**A126P**	**K128E**	**K128N**	**G129R**	**G129R**	**G129E**
Diagnosis	CS	CS	CS	CS	CS	CS	CS	CS		CS
Sex	F	F			M	M	M	F	M	
Age	53y				35y	23y	35y	14y7m		
CNS										
Macrocephaly		X	X		X	X		X	X	
LDD										
Benign Tumour						X		X		
Malignant Tumour								X		
MR/DD								X		
THYROID										
Benign (adenoma, goitre)	X	X	X	X	X	X			X	X
Malignant										
BREASTS										
Benign			X	X						X
Malignant Tumour										
SKIN/MUCOSA										
Trichilemmona	X									
MCL, FP, AK, PPK, F	X	X	X	X						X
OP	X					X				
SP					X					
Lipomas					X	X				
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps								X		
Benign (hamartoma)			X	X	X		X			X
Malignant Tumour				X			X			
OTHER BENIGN	OC					VASC les			VASC les	
OTHER CANCERS										
SI SCORE	3	2	4	6	3	4	3	5	2	4
REFERENCES	Bussaglia et al., 2002	Nelen et al., 1997	Nelen et al., 1997	Nelen et al., 1997	Mester et al., 2012	Busch et al., 2013	Ngeow et al., 2013	Elia et al, 2012	UNPUB	Liaw et al., 1997

Mutation	G129E	G129E	G129E	G129E	R130Q	R130Q	R130Q	R130Q	R130Q	R130Q
Diagnosis	CS	CS	CS	CS	CS	CS/BRRS	CS/BRRS	CS/BRRS	CS	CS
Sex		M	M	F	M	M	M	M	F	M
Age				60y	35y	23y	25y	22y	48y	17y
CNS										
Macrocephaly				X		X	X	X	X	X
LDD										
Benign Tumour										
Malignant Tumour		X								
MR/DD						X	X			
THYROID										
Benign (adenoma, goitre)			X	X	X	X		X		X
Malignant										
BREASTS										
Benign									X	
Malignant Tumour	X	X							X	
SKIN/MUCOSA										
Trichilemmona		X	X							
MCL, FP, AK, PPK, F	X				X	X	X			X
OP						X	X			
SP						X	X	X		X
Lipomas				X			X			X
Malignant Tumour	X	X								
GASTROINTESTINAL TRACT										
GI polyps					X			X		
Benign (hamartoma)						X	X	X	X	X
Malignant Tumour								X		
OTHER BENIGN									UF	LPH
OTHER CANCERS					LUNG					
SI SCORE	5	7	2	2	5	4	3	3	5	4
REFERENCES	Liaw et al., 1997	Liaw et al., 1997	Liaw et al., 1997	Ngeow et al., 2013	Kurose et al., 1999	Kurose et al., 1999	Kurose et al., 1999	Kurose et al., 1999	Heindl et al., 2012	Heindl et al., 2012

Mutation	**R130Q**	**R130Q**	**R130Q**	**R130G**	**R130G**	**R130G**	**R130G**	**R130G**	**R130G**	**R130L**
Diagnosis	CS	CS	CS	CS		CS	CS		CS	CS
Sex	F	F	F	F	F					
Age	42y	29y	48y		40y					
CNS										
Macrocephaly	X				X	X	X	X	X	X
LDD										
Benign Tumour										
Malignant Tumour										
MR/DD										
THYROID										
Benign (adenoma, goitre)	X				X				X	X
Malignant										
BREASTS										
Benign	X									
Malignant Tumour	X	X	X	X	X					
SKIN/MUCOSA										
Trichilemmona										
MCL, FP, AK, PPK, F						X		X	X	X
OP						X			X	X
SP									X	X
Lipomas	X						X	X	X	
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps										X
Benign (hamartoma)										
Malignant Tumour										
OTHER BENIGN					UF					
OTHER CANCERS		OVCA								
SI SCORE	5	4	2	2	4	1	1	1	2	3
REFERENCES	Ngeow et al., 2013	Ngeow et al., 2014	Ngeow et al., 2014	Lobo et al., 2009	Mester et al., 2012	Bubien et al., 2013	Bubien et al., 2013	Bubien et al., 2013	Bubien et al., 2013	Bubien et al., 2013

Mutation	**R130L**	**R130L**	**R130L**	**T131I**	**T131I**	**T131I**	**G132A**	**G132D**	**G132D**	**G132D**
Diagnosis	CS	CS	CS	CS/ASD		ASD	PHTS	CS	CS	CS
Sex			F			F		M	M	
Age			21y			4y1m		27y	53y	
CNS										
Macrocephaly		X		X	X	X	X		X	X
LDD										
Benign Tumour										
Malignant Tumour								DGCC		
MR/DD				X		X	X			
THYROID										
Benign (adenoma, goitre)	X	X			X				X	
Malignant		X	X							X
BREASTS										
Benign		X			X					X
Malignant Tumour		X								X
SKIN/MUCOSA										
Trichilemmona										
MCL, FP, AK, PPK, F	X	X							X	X
OP	X								X	X
SP	X									
Lipomas										
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps	X	X			X					
Benign (hamartoma)										
Malignant Tumour		X								
OTHER BENIGN										
OTHER CANCERS			UBC						RC	
SI SCORE	3	10	4	1	3	1	1	2	4	6
REFERENCES	Bubien et al., 2013	Marsh et al., 1998	Ngeow et al., 2014	Bubien et al., 2013	Bubien et al., 2013	O'Roak et al., 2012	Tan et al., 2007	Derrey et al., 2004	Heindl et al., 2012	Bubien et al., 2013

Mutation	**G132D**	**G132D**	**G132D**	**G132V**	**M134R**	**M134R**	**M134R**	**M134R**	**M134I**	**M134I**
Diagnosis	CS	CS	ASD	PHTS	BRRS	BRRS	BRRS			
Sex			M	M	M	M	M	F	F	M
Age			4y	4y6m	18y	5y		46y	38y	17y
CNS										
Macrocephaly	X	X	X	X	X	X	X	X	X	X
LDD	X									
Benign Tumour										
Malignant Tumour										
MR/DD				X						
THYROID										
Benign (adenoma, goitre)	X	X								
Malignant										
BREASTS										
Benign	X	X								
Malignant Tumour								X		
SKIN/MUCOSA										
Trichilemmona										
MCL, FP, AK, PPK, F		X								
OP		X								
SP					X	X	X			
Lipomas	X			X						
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps				X				X		
Benign (hamartoma)										
Malignant Tumour										
OTHER BENIGN										
OTHER CANCERS										
SI SCORE	4	3	0	3	0	0	0	3	0	0
REFERENCES	Bubien et al 2013	Bubien et al., 2013	Frazier et al., 2014	Tekin et al., 2006	Figer et al., 2002	Figer et al., 2002	Figer et al., 2002	Mester et al., 2012	Busa et al., 2013	Busa et al., 2013

Mutation	M134I	M134I	M134T	M134T	M134T	I135R	I135V	I135V	I135V	I135V
Diagnosis			MR	CS		BRRS	BRRS			
Sex	F	F	F	F	F					
Age	15y	8y	9y3m	44y	46y					
CNS										
Macrocephaly	X	X		X	X	X		X	X	X
LDD										
Benign Tumour										
Malignant Tumour										
MR/DD			X			X			X	
THYROID										
Benign (adenoma, goitre)				X	X			X		X
Malignant					X					
BREASTS										
Benign				X	X			X		X
Malignant Tumour				X	X					
SKIN/MUCOSA										
Trichilemmona					X					
MCL, FP, AK, PPK, F					X			X	X	X
OP				X	X			X	X	X
SP							X			
Lipomas							X			X
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps						X				X
Benign (hamartoma)										
Malignant Tumour										
OTHER BENIGN					VASC les	LH				GU les
OTHER CANCERS										
SI SCORE	0	0	1	5	8	3	1	3	2	5
REFERENCES	Busa et al., 2013	Busa et al., 2013	McBride et al., 2010	Ngeow et al., 2013	Busch et al., 2013	Boccone et al., 2006	Marsh et al., 2009	Bubien et al., 2013	Bibien et al., 2013	Bubien et al., 2013

Mutation	I135V	I135V	I135V	C136R	C136R	C136R	C136R	C136R	R142P	A151D
Diagnosis		ASD	CS	CS	CS	CS/MR/AS D	CS	CS	CS	CS
Sex				F				F		
Age				30y				33y		
CNS										
Macrocephaly	X	X	X			X			X	X
LDD							X			
Benign Tumour										
Malignant Tumour										
MR/DD						X				
THYROID										
Benign (adenoma, goitre)	X		X	X			X		X	X
Malignant					X		X	X		
BREASTS										
Benign			X	X	X					
Malignant Tumour					X			X		
SKIN/MUCOSA										
Trichilemmona										
MCL, FP, AK, PPK, F	X		X	X	X	X	X		X	X
OP	X		X	X			X		X	X
SP							X			
Lipomas		X	X			X				X
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps	X			X	X		X		X	X
Benign (hamartoma)										
Malignant Tumour				X						
OTHER BENIGN			GU les	OC						
OTHER CANCERS								ENCA		
SI SCORE	3	1	4	7	7	2	6	6	3	3
REFERENCES	Bubien et al., 2013	Bubien et al., 2013	Bubien et al., 2013	Kubo et al., 2000	Bubien et al., 2013	Bubien et al., 2013	Bubien et al., 2013	Ngeow et al., 2014	Bubien et al., 2013	Bubien et al., 2013

Mutation	Y155N	Y155C	Y155C	Y155C	E157G	V158I	V158I	D162E	G165R	G165E
Diagnosis	CS	CS	CS	CS	ASD			CS	CS	CS
Sex		F		F		F	F	M		F
Age		50y		66y	3y8m			3y	59y	
CNS										
Macrocephaly			X	X	X			X		X
LDD										
Benign Tumour										
Malignant Tumour										
MR/DD					X			X		
THYROID										
Benign (adenoma, goitre)	X		X							X
Malignant										X
BREASTS										
Benign			X	X						X
Malignant Tumour			X			X			X	
SKIN/MUCOSA										
Trichilemmona										
MCL, FP, AK, PPK, F	X		X							X
OP	X		X							
SP										
Lipomas			X	X						
Malignant Tumour	X									
GASTROINTESTINAL TRACT										
GI polyps			X							
Benign (hamartoma)										
Malignant Tumour										
OTHER BENIGN			GU les							
OTHER CANCERS		MT				LGTC	L			
SI SCORE	4	2	7	2	1	4	2	1	2	5
REFERENCES	Bubien et al., 2013	Gicquel et al., 2003	Bubien et al., 2013	Ngeow et al., 2013	Varga et al., 2009	de Vivo et al., 2000	de Vivo et al., 2000	Mester et al., 2012	Bannean et al., 2010	Nelen et al., 1999

Mutation	**G165V**	**T167N**	**S170R**	**S170R**	**S170R**	**S170R**	**S170I**	**Q171E**	**R173C**	**R173C**
Diagnosis	CS	ASD	BRRS	CS		CS	CS	ASD	BRRS	CS
Sex		F					F	F		F
Age		8y2m					17y	3y10m		54y
CNS										
Macrocephaly	X	X	X	X	X	X		X	X	
LDD						X				
Benign Tumour	X									
Malignant Tumour										
MR/DD		X							X	
THYROID										
Benign (adenoma, goitre)	X				X	X				
Malignant				X	X		X			X
BREASTS										
Benign	X			X						
Malignant Tumour				X						X
SKIN/MUCOSA										
Trichilemmona										
MCL, FP, AK, PPK, F	X				X	X				
OP					X	X				
SP			X						X	
Lipomas			X	X	X	X				
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps				X						
Benign (hamartoma)										
Malignant Tumour										
OTHER BENIGN			VASC les- LM							
OTHER CANCERS							ENCA			
SI SCORE	4	1	2	7	4	3	4	0	1	4
REFERENCES	Marsh et al., 1998	O'Roak et al., 2012	Marsh et al., 1997	Bubien et al., 2013	Bubien et al., 2013	Bubien et al., 2013	Ngeow et al., 2014	Vanderver et al., 2014	Lachlan et al., 2007	Ngeow et al., 2014

Mutation	**R173G**	**R173P**	**R173P**	**R173H**	**R173H**	**R173H**	**R173H**	**Y176C**	**L181P**	**T202I**
Diagnosis	CS	LD		CS			ASD	ASD	CS	
Sex	F	F	F		M	M	M			M
Age	44y		42y		9y	43y	4y5m			1y8m
CNS										
Macrocephaly			X	X	X	X	X	X	X	
LDD		X	X							
Benign Tumour										
Malignant Tumour										
MR/DD			X	X				X		X
THYROID										
Benign (adenoma, goitre)		X	X							
Malignant										
BREASTS										
Benign										
Malignant Tumour	X									
SKIN/MUCOSA										
Trichilemmona										
MCL, FP, AK, PPK, F		X	X							X
OP						X				
SP						X				
Lipomas										
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps						X				
Benign (hamartoma)										
Malignant Tumour										
OTHER BENIGN									VASC les	
OTHER CANCERS										
SI SCORE	2	3	4	1	0	2	0	1	1	2
REFERENCES	Ngeow et al., 2014	Kirches et al., 2010	Kirches et al., 2010	Bubien et al., 2013	Lachlan et al., 2007	Lachlan et al., 2007	McBride et al., 2010	Orrico et al., 2009	Thiffault et al., 2004	Varga et al., 2009

Mutation	**C211W**	**V217D**	**R234Q**	**F241S**	**P246L**	**P246L**	**P246L**	**D252V**	**D252G**	**D252G**
Diagnosis	ASD	CS	Mcancer	ASD	BRRS	BRRS	ASD	ASD	ASD	CS
Sex	M		M	M		M	M	F	M	
Age	3y10m		38y	2y5m		2y	5y	15y5m	3y5m	
CNS										
Macrocephaly	X					X	X	X	X	X
LDD										
Benign Tumour										
Malignant Tumour			OD, MnG							
MR/DD	X					X			X	
THYROID										
Benign (adenoma, goitre)										
Malignant										
BREASTS										
Benign										
Malignant Tumour										
SKIN/MUCOSA										
Trichilemmona										
MCL, FP, AK, PPK, F										X
OP		X								X
SP				X						
Lipomas					X					
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps		X								
Benign (hamartoma)										
Malignant Tumour										
OTHER BENIGN					VASC les					
OTHER CANCERS										
SI SCORE	1	2	2	0	2	1	0	0	1	1
REFERENCES	Vanderver et al., 2014	Kim et al., 2005	Staal et al., 2002	Butler et al., 2005	Marsh et al., 1999	Mester et al., 2012	Vanderver et al., 2014	Frazier et al., 2014	Butler et al., 2005	Bubien et al., 2013

Mutation	**V255A**	**W274L**	**N276S**	**T277R**	**T277R**	**T277R**	**T277R**	**T277R**	**T277R**	**K289E**
Diagnosis	ASD	DD	ASD	CS	CS	CS	CS	CS/ASD	CS	CS
Sex	M	F							F	
Age	4y4m	8m							27y	
CNS										
Macrocephaly	X	X	X	X	X	X	X	X		
LDD										
Benign Tumour										
Malignant Tumour										
MR/DD		X	X		X					
THYROID										
Benign (adenoma, goitre)						X	X			
Malignant										
BREASTS										
Benign							X			
Malignant Tumour									X	
SKIN/MUCOSA										
Trichilemmona										
MCL, FP, AK, PPK, F				X	X	X	X			
OP				X	X	X	X			
SP										
Lipomas				X			X			
Malignant Tumour										
GASTROINTESTINAL TRACT										
GI polyps										X
Benign (hamartoma)										X
Malignant Tumour										
OTHER BENIGN										
OTHER CANCERS										
SI SCORE	0	1	1	1	2	2	3	0	2	1
REFERENCES	Klein et al., 2013	McBride et al., 2010	Orrico et al., 2009	Bubien et al., 2013	Bubien et al., 2013	Bubien et al., 2013	Bubien et al., 2013	Bubien et al., 2013	Banneau et al., 2010	Chi et al., 1998

Mutation	**D326N**	**R335L**	**F337S**	**K342N**	**K342N**	**V343E**	**V343E**	**V343E**	**V343E**	**V343E**	**V343E**	**L345V**	**L345V**
Diagnosis	ASD	CS	CS	HTS	CS	CS	CS	CS	CS	CS	CS	CS	CS
Sex		F	M			F	F	F	M	F	F	F	F
Age		48y	9y									36y	58y
CNS													
Macrocephaly	X		X	X	X								
LDD													
Benign Tumour													
Malignant Tumour													
MR/DD	X												
THYROID													
Benign (adenoma, goitre)		X				X	X	X					X
Malignant				X							X		
BREASTS													
Benign							X	X			X		X
Malignant Tumour		X				X				X		X	X
SKIN/MUCOSA													
Trichilemmona		X											
MCL, FP, AK, PPK, F		X					X						X
OP		X	X	X									X
SP			X	X									
Lipomas			X	X	X								
Malignant Tumour													
GASTROINTESTINAL TRACT													
GI polyps			X				X	X	X				
Benign (hamartoma)													
Malignant Tumour													
OTHER BENIGN						NE							
OTHER CANCERS								ENCA	PGT			ENCA	
SI SCORE	1	4	2	3	1	4	4	5	3	2	3	4	5
REFERENCES	Buxbaum et al., 2007	Sawada et al., 2000	Lachlan et al., 2007	Bubien et al., 2013	Bubien et al., 2013	Lynch et al., 1997	Lynch et al., 1997	Lynch et al., 1997	Lynch et al., 1997	Lynch et al., 1997	Lynch et al., 1997	Ngeow et al., 2014	Busch et al., 2013

Abbreviations

AK	Acral Keratosis
CNS	Central Nervous System
CRC	Colorectal Cancer
DGCC	Dysplastic Gangliocytoma of the Cerebellum
ENCA	Endometrial Cancer
F	Fibromas
FBD	Fibrocystic disease
FP	Facial papules
GgNa	Ganglioneuroma
GI	Gastointestinal
GU les	Genitourinary lesions
H	Hemangioma
H reflux	Hernia reflux
HN	Hydronephrosis
JPC	Juvenile Polyposis Coli
L	Lymphoma
LDD	Lhermitte-Duclos disease
LGTC	Lower Genital Tract Cancer
LH	Lymphoid Hyperplasia
LM	Leiomyoma
LPH	Lipomatous Hemangioma
MCL	Mucocutaneus Lesions
MnG	Meningioma
MR/DD	Mental Retardation/Developmental Delay
MT	Multiple Tumours
NE	Neuroma
NP	Nephrolithiasis
OC	Ovaric Cysts
OD	Oligodendrioma
OP	Oral mucosal Papillomatosis
OVCA	Ovarian cancer
PGT	Papillary Carcinoma
PLS	Proteus-Like Syndrome
PPK	Palmoplantar Keratoses
RC	Renal Carcinoma
RH	Renal Hamartoma
SP	Speckled Penis
UBC	Urinary Bladder Cancer
UF	Uterin Fibroids
UNPUB	Unplublished
UTLM	Uterine Leiomyoma
VASC les	Vascular lesions

Supporting Materials and Methods

Expression vectors and recombinant PTEN protein expression

Plasmid and lentiviral expression vectors for untagged and glutathione S-transferase (GST)-tagged human PTEN have been previously described [58 59]. The mutant expression vectors derived from pGEX6P1 PTEN and pHR-SIN PTEN were prepared by site-directed mutagenesis of the corresponding PTEN WT constructs with primers containing missense mutations. Recombinant wild-type and mutant forms of PTEN were expressed in Escherichia coli and purified by glutathione-affinity chromatography.

Immunoblotting

Protein gel electrophoresis was conducted with 10 μg of total soluble protein per sample using NuPage Bis-Tris 4-12% gradient polyacrylamide gels (Invitrogen Life Technologies) following manufacturers protocols. Proteins were transferred onto PVDF membrane (Perkin-Elmer) and membranes blocked in 5% milk powder/TBST for 1 hour at room temperature (RT). Blocked membranes were incubated overnight with primary antibodies. Antibodies used were: anti-P(Ser473)Akt and anti-P(Thr308)Akt (Cat. n.9271L and 9275L, Cell Signaling Technology), anti-PTEN (A2B1 Cat. n. sc-7974, Santa Cruz Biotechnology and PTEN 138G6, Cat. n. 9559S, Cell Signaling Technology), anti-GAPDH (Cat. n. AB2302, Millipore), anti-Akt (Cat. n. 9272, Cell Signaling Technology), anti-P(Thr246)PRAS40 (Cat. n. 29975, Cell Signaling Technology), anti-PRAS40 (Cat. n. 2691S, Cell Signaling Technology). Antibody complexes were detected by 1 hour incubation at RT with HRP conjugated secondary antibodies (Vector Labs). Blots were developed with ECL plus (Millipore)

and chemiluminescence imaged directly using an ImageQuant LAS4000 imaging system.

Lipid phosphatase assay

The preparation of 3-^{33}P labelled phosphoinositide substrate has been described previously ([58 59]. To test the lipid phosphatase activity of PTEN against PtdIns(3,4,5)P_3, lipid vesicles were prepared by sonication containing 100 μM phosphatidylcholine (PC), 1 μM unlabelled diC16 PtdIns(3,4,5)P_3 and a volume of ^{33}P-PtdIns(3,4,5)P_3 to give 100,000 cpm per assay. PTEN was immunoprecipitated using anti-PTEN A2B1 antibody in lysis buffer (50 mM Tris-HCL pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 5 mM Sodium pyrophosphate, 10 mM β-glycerophosphate, 50 mM Sodium fluoride, 1% NP-40 and protease inhibitors) and washed twice with high salt (300 mM NaCl) lysis buffer and then with reaction buffer (50 mM Hepes pH 7.4, 1 mM EGTA, 10 mM dithiothreitol (DTT) and 150 mM NaCl). Substrate vesicles were incubated in the reaction buffer with PTEN immune complexes or with 500 ng of recombinant PTEN protein. After 1 hour of incubation at 37ºC, the reactions were stopped through the addition of 10 μl of Bovine Serum Albumin (essentially fatty acid free- Roche) (10 mg/ml) and 500 μl of 1M ice cold perchloric acid (PCA). The samples were mixed and incubated for 30 minutes on ice, followed by centrifugation at 14,000 rpm for 10 minutes at 4ºC to remove lipid and protein. 10% (w/v) ammonium molybdate was added to the supernatant to allow the inorganic phosphate to partition into the organic phase. After incubation of 10 minutes at room temperature, 1 ml Toluene:Isobutyl alcohol (1:1 v/v) was added. A two-phase mix was formed, and the upper organic phase, containing the phosphate complex, was then removed, mixed to liquid scintillation cocktail (Scint Safe 3, Fisher Chemical) and radioactivity was counted using a Beckman scintillation counter.

Reverse Transcriptase quantitative PCR (qPCR)

Total cellular RNA was isolated from U87MG cells expressing PTEN WT and mutants by using TRIzol (Life Technologies - Invitrogen) and RNeasy Mini kit (Qiagen), reversed transcribed into random primed cDNA (RNA to cDNA EcoDry Premix – Random Hexamers Clontech) and used as a template in quantitative PCR reactions to measure PTEN mRNA levels. GAPDH was used as internal control and Power Sybr green as the fluorescent reporter (Applied Biosystems). All reactions were performed according to the manufacturer's instructions on a Step One Plus real time PCR instrument (Life Technologies).

Primers were designed to be able to measure PTEN mRNA levels only and not its pseudogene. PTEN Primers: F: 5'-CCCGGGGGGGATCCACTAG; R: 5'-GGATAAATATAGGTCAAG. GAPDH Primers: F: 5'-GTGAAGGTCGGAGTCAACGG; R: 5'-GAGGGATCTCGCTCCTGGAA.

Immunocytochemistry

Neuronal cultures treated as described above were fixed using 4% paraformaldehyde for 30 minutes and then blocked with 3% BSA and 0.1% Triton x100 for 1 hour. Fixed neurons were incubated with anti-GFP antibody (1:1,000, Abcam), anti-PTEN antibody (1:500, PTEN138G6 Cell Signaling Technology) or anti-RFP (1:1,000, Cat. n. ab62341 Abcam) antibody diluted in 3% BSA, 1% goat serum and 1% sodium azide followed by incubation with Alexa488, Alexa568 and Alexa647 conjugated secondary antibodies (1:1,000, Invitrogen). Cells were stained with DAPI (1 µg/ml) for 10 minutes prior to mounting. The soma of the neurons, density and length of the spines were measured using ImageJ and were represented in µm.

Animal procedures

All animal procedures (breeding and sacrifice) were conducted in accordance with local ethical guidelines and approved animal care protocols (Berlin: Institutional Animal Care and Use Committee (IACUC) and the Landesamt für Gesundheit und Soziales (LAGeSO) – license T 0347/11.

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