Letter to the Editor

Increased PI3K/Akt activity and deregulated humoral immune response in human PTEN deficiency

To the Editor:

Autosomal-dominant germline mutations in *PTEN* are associated with phosphate and tensin homologue deleted on chromosome 10 (PTEN) hamartoma tumor syndromes (PHTS), including Cowden syndrome, characterized by hamartomas, malignant tumors, macrocephaly, and neurodevelopmental delay.[1] Immunodeficiency has recently been reported in PHTS,[2] but the mechanism of disease is not clear. Therefore, we performed a detailed study of the peripheral B-cell development of 9 patients with PHTS, to explore the role of phosphatidyl inositol 3-kinase (PI3K)/Akt signaling in the humoral immune response in these patients (details are described in this article's Methods section in the Online Repository at www.jacionline.org).

We included 9 patients bearing heterozygous germline mutations in *PTEN* (clinical and genetic details are given in Table E1, Fig E1, and the Methods section in this article's Online Repository at www.jacionline.org). Three patients suffered from hypogammaglobulinemia, fulfilling the common variable immunodeficiency (CVID) disorders diagnostic criteria, of whom 1 has been previously reported as a case of hypogammaglobulinemia and macrocephaly.[3] Peripheral B-cell subset distribution was studied by analyzing the relative and absolute B-cell subset size of 2 naive and 3 memory B-cell subsets (Fig 1). Patients with PHTS were compared with 45 age-matched healthy controls. Absolute counts of transitional B cells were increased (Fig 1, *A*). Data of 3 memory B-cell subsets

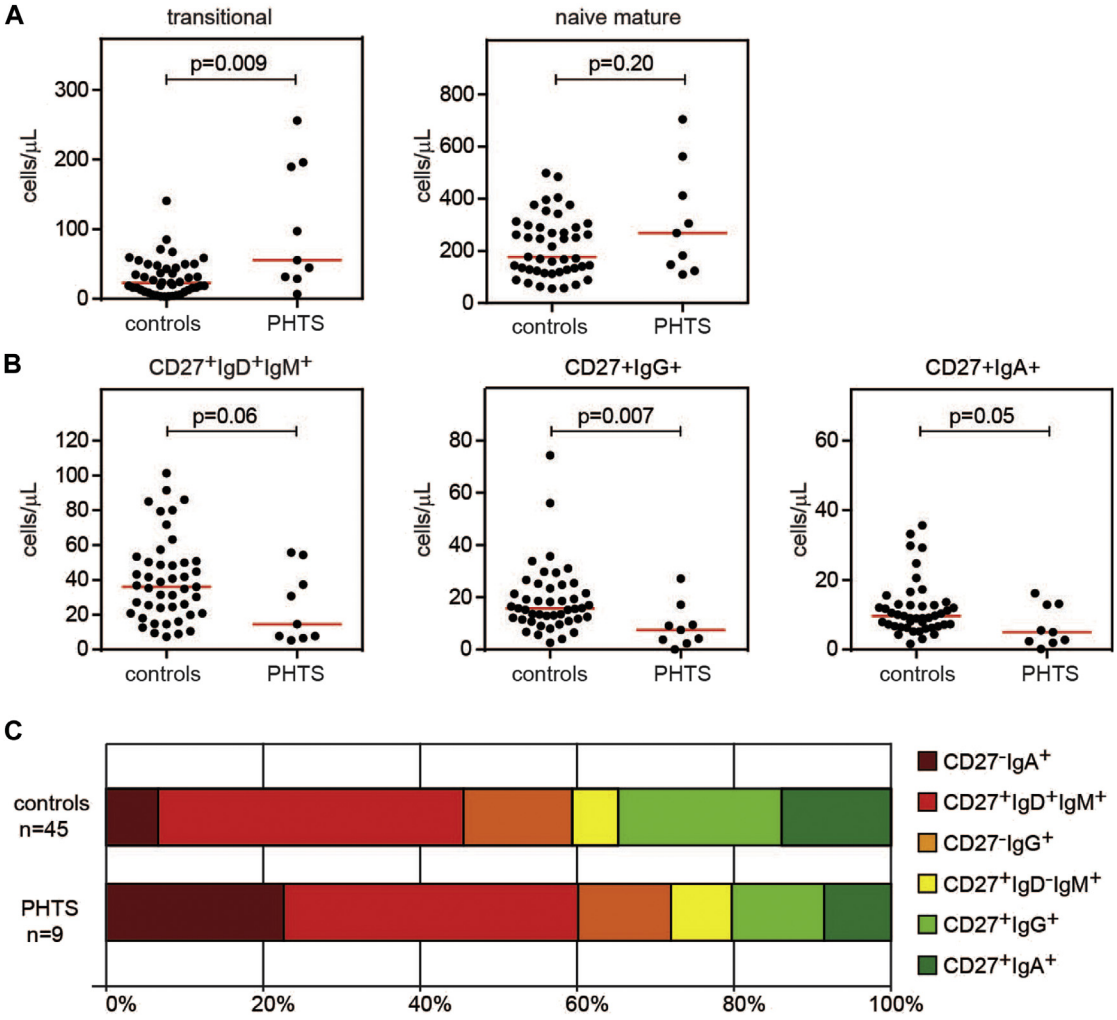


FIG 1. Naive and memory B-cell subsets in patients with PHTS. Absolute counts of transitional and naive mature B cells **(A)** and of 3 memory B-cell subsets **(B)**. **C,** Relative distribution of 6 memory B-cell subsets. Data are compared with age-matched normal controls using the Mann-Whitney test. Individual data points are displayed and bars indicate medians. Significant values are indicated: $P < .01$ after Bonferroni correction for multiple comparison.

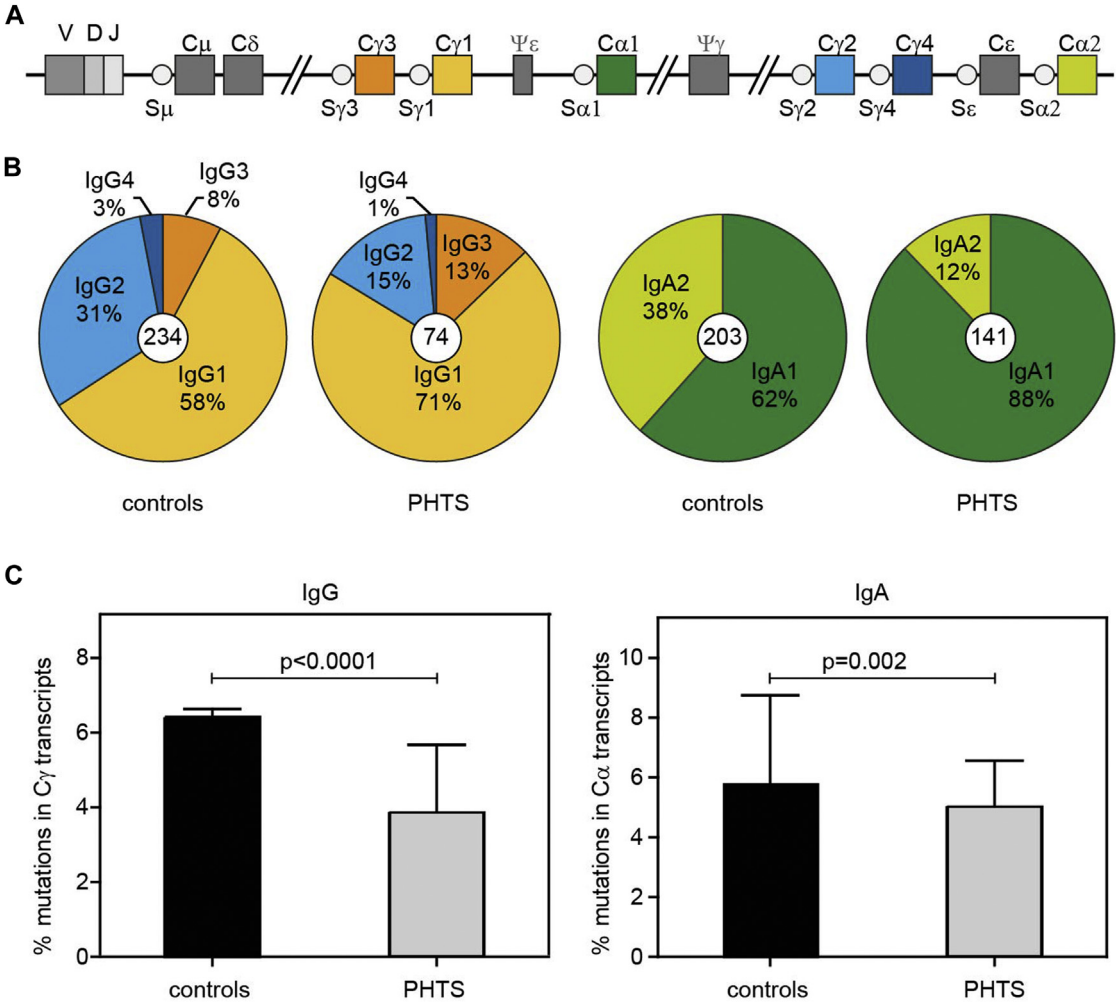


FIG 2. IgA and IgG class switching and somatic hypermutation in *IGH* transcripts of patients with PHTS. **A,** Schematic representation of the constant regions in the *IGH* locus. **B,** Frequencies of *IGG* and *IGA* transcripts in PHTS (n = 9) were compared with controls (n = 8). In the center of each plot, the number of analyzed transcripts is depicted. **C,** Somatic hypermutation analysis of *IGG* and *IGA* transcripts. *Gray* bars represent the 9 patients with PHTS and *black* bars represent controls.

showed that absolute counts of class-switched $CD27^+IgG^+$ memory B cells, which originate from a T-cell–dependent differentiation pathway,[4] were decreased in PHTS (Fig 1, *B*). We observed a trend toward a more severe reduction in switched memory B-cell subsets in patients with hypogammaglobulinemia (data not shown). In summary, the T-cell–dependent B-cell response is deregulated in PHTS. Analysis of peripheral T-cell subsets revealed that the absolute counts of naive, memory, and effector $CD4^+$ and $CD8^+$ T cells were comparable to healthy controls (see Fig E2 in this article's Online Repository at www.jacionline.org).

Class switch recombination (CSR) was studied at the molecular level by analyzing the IGG and IGA subclass distribution of *IGH* transcripts (Fig 2). We compared the IGG and IGA subclass distribution between patients and controls by chi-square analysis. In PHTS, IgG subclass distribution differed significantly from that in controls ($P = .002$), which was the result of an increase in CSR to IgG_1 and a decrease to IgG_2. CSR distribution seemed more clearly affected in patients with PHTS with hypogammaglobulinemia compared with patients with PHTS without hypogammaglobulinemia (data not shown). Similar results were obtained for CSR to IgA_2, which was significantly reduced in PHTS ($P < .0001$). So, mutations in *PTEN* cause abnormalities in CSR to distal IgG and IgA subclass regions.

The somatic hypermutation (SHM) frequency was determined by mutational analysis of the *IGG* and *IGA* transcripts (Fig 2, *C*). Mutational analyses showed a significantly decreased SHM frequency in both *IGG* and *IGA* transcripts of patients with PHTS, indicating that SHM is affected in PTEN deficiency. In mice, levels of activation-induced cytidine-deaminase expression are regulated by PI3K/Akt signaling.[5-7] We hypothesize that in patients with PHTS, decreased SHM is caused by a deregulating effect of Akt signaling on activation-induced cytidine-deaminase expression and function, which has to be established in future studies. PTEN deficiency results in abnormalities in B-cell development indicative of CSR deficiency and SHM deficiency, which is clinically apparent as hypogammaglobulinemia in part

of the patients with PHTS. Our data provide evidence that increased PI3K/Akt signaling deregulates the human humoral immune response, which is in accordance with recent observations in patients with autosomal-dominant activating mutations in the *PIK3CD* gene, coding for the P110δ subunit of PI3K.[8,9] These patients suffer from recurrent infections and have a variable severe antibody deficiency, similar to patients with PHTS. The clinical outcome of an individual patient most likely depends on interindividual differences in any of the multiple factors that regulate PI3K/Akt signaling and the complex interplay between immune function, (auto)antigen exposure, and the timing and cell types involved in immune activation.

Patients with PHTS with hypogammaglobulinemia fulfilled the CVID diagnostic criteria. We explored whether mutations in *PTEN* were present in a cohort of 42 patients with CVID, but no mutations could be detected. Patients with PHTS show similarities to patients with activation mutations in *PIK3CD* gene[8,9]: a variable antibody deficiency, a tendency to develop malignancies, respiratory infections, and as far as immunological findings are concerned, increased Akt activity, an increase in the proportion of transitional B cells, and a decreased proportion of memory B cells. Differences are also present, such as the absence of hamartomas and macrocephaly in patients with activating mutations of the *PIK3CD* gene, which can be explained by the fact that P110δ is lymphoid specific. Despite these differences, our study and the study of Angulo et al[8] and Lucas et al[9] show that PI3K/Akt signaling is important for the human B-cell response to antigens and that deregulated signaling is implicated in the pathophysiology of antibody deficiency. We propose that deregulated PI3K/Akt signaling is an attractive disease-causing mechanism to be further explored in patients with CVID, because heterozygous germline mutations in *PTEN* have been associated with autoimmunity, lymphoproliferation, an increased risk to develop malignancies, and intestinal nodular interstitial hyperplasia, which are all commonly encountered in CVID.

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METHODS

Patients

We included 9 patients bearing heterozygous germline mutations in the *PTEN* gene. Three patients suffered from hypogammaglobulinemia and 6 did not have a clinically apparent antibody deficiency. Data of these patients were compared with data of 45 age-matched normal controls. Furthermore, we screened 42 patients with CVID[E1] for the presence of mutations in *PTEN*. The research was approved by the Medical Ethical Committee of the ErasmusMC.

Patient histories

Patient 1 is a 43-year-old woman who suffered from recurrent upper and lower respiratory tract infections from childhood and was diagnosed with hypogammaglobulinemia at the age of 12 years. Immunoglobulin replacement was initiated. At the age of 30 years, a hemithyroidectomy was performed because of nodular hyperplasia. At the age of 31 years, a melanoma in situ was removed. Thereafter, she suffered from several episodes of lobar pneumonia, resulting in bronchiectasis despite immunoglobulin replacement. Switched memory B cells were virtually absent. Furthermore, she was treated for a candida esophagitis. Dermatologic evaluation showed keratotic plugs hand palms, lipomas, and café-au-lait maculae. At the age of 40 years, the diagnosis of Cowden disease was made, on the basis of clinical history, including macrocephaly, and heterozygous mutations in *PTEN*. Patient 2 is 12 years old and is a daughter of patient 1. She was known with developmental delay and macrocephaly, without specific diagnosis. Cerebral magnetic resonance imaging (MRI) showed cortical dysplasia. She appeared to have the same *PTEN* mutation as her mother and the diagnosis of Cowden disease was made at the age of 10 years. She suffered from recurrent ear, nose, and throat infections in childhood, which improved after adenotomy. Screening for immunodeficiency revealed IgA deficiency, IgG_2 deficiency, and specific polysaccharide antibody deficiency (decreased response to pneumococcal polysaccharide vaccination; response to serotype 1,3,4,5,23 measured 4 weeks after Pneu23 vaccination <0.1 mg/mL). Within 2 years of follow-up, she developed a mild hypogammaglobulinemia at the age of 12 years. She is not receiving immunoglobulin replacement therapy because of a stable clinical condition. She is suffering from recurrent upper respiratory tract infections during the winter periods, which respond well to antibiotic treatment.

Patient 3 is a 6-year-old boy whose case has previously been published as a case of macrocephaly and hypogammaglobulinemia (case 2).[E2] This child of nonconsanguineous parents suffered from recurrent febrile episodes from the age of 3 months. Several dysmorphic features were observed, including macrocephaly. There was a mild developmental delay and brain MRI revealed delay in myelinization of the periventricular white matter. He suffered from frequent upper respiratory tract infections. At the age of 21 months, a diagnosis of hypogammaglobulinemia was made, in the presence of a decreased response to tetanus (<100 IU/mL). Immunoglobulin replacement was initiated, after which the frequency and severity of infections improved. At the age of 6 years, a de novo heterozygous *PTEN* mutation was detected.

Patients without antibody deficiency:

Patient 4: Macrocephaly, delayed motor and mental development.
Patient 5: Macrocephaly, delayed motor and mental development.
Patient 6: Macrocephaly, delayed motor and mental development, periventricular white matter abnormalities on cerebral MRI.
Patient 7: Macrocephaly, delayed motor and mental development, lipomas.
Patient 8: Macrocephaly, resection of sigmoid carcinoma.
Patient 9: Macrocephaly, resection borderline ovarian tumor, fibroadenoma breast.

Flow cytometry

Six-color flow cytometric immunophenotyping of peripheral blood was performed on a CantoII (BD Biosiences) and data were analyzed using FACS Diva software (BD Biosiences, San Jose, Calif). The following mAbs were used: CD19-PerCP-Cy5.5, CD19-PE-Cy7, CD19-APC (all SJ25C1), CD5-APC (L17F12), CD45-PerCP (2D1), CD19-APC (SJ25C1), CD38-PE, CD38-APC, and CD38-PE-Cy7 (HB7), CD27-APC (L128), CD3-PerCP-Cy5.5 (SK7), and CD8-APC-Cy7 (SK1) all from BD Biosciences, polyclonal IgD-FITC, IgD-PE, and IgM-PE (all from Southern Biotechnologies, Birmingham, Ala), polyclonal Kallestad IgG-FITC (Biorad, Herculas, Calif), IgA-FITC and IgA-PE (IS11-8E10; Miltenyi Biotech, Bergisch Gladbach, Germany), CD24-FITC (gran-B-ly-1; Sanquin, Amsterdam, The Netherlands), CD21-PE (LB21; Biorad), CD45RO-FITC (UCHL1; DAKO, Agilent technologies, Glostrup, Denmark), CD4-PC7 (SFCI12T4D11), and CD45-RA-RD1 (2H4; all from Beckman Coulter, Brea, Calif). The cell counts of peripheral B-cell subsets (transitional B cells, naive mature B cells, and 6 memory B-cell subsets) and T-cell subsets were compared with age-matched controls.

Analysis of SHM and CSR

SHM were analyzed as described previously.[E3] In short in V_H3-Cα, V_H4-Cα, V_H3-Cγ, and V_H4-Cγ, transcripts were amplified, cloned into pGEM-T easy vector (Promega, Madison, Wis), and sequenced using Sanger sequencing on the ABI Prism 3130 XL fluorescent sequencer (Applied Biosystems, Foster City, Calif). Sequences were analyzed with the International ImMunogGeneTics database (IMGT, http://imgt.cines.fr) V-quest analysis tool to not only assign the V, D, and J segments but also identify SHMs. The mutation frequency and *IGHV* isotype and subclass distribution were determined for V_H gene of each transcript in 7 controls and 9 patients. The number of transcripts and mutations frequency per individual can be found in Table E2.

Statistical analysis

Statistical analysis was performed with Graphpad Prism 5.0 software (Graphpad Software, San Diego, Calif). Whenever 2 groups with continuous outcomes were compared, the Mann-Whitney test was applied. For categorical variables, the chi-square test or Fisher exact test was used. For groups with unequal variances, an unpaired *t* test with Welsh correction was applied. Statistical significance was set at 2-sided $P < .05$. In case of multiple comparisons, we performed Bonferroni correction for multiple testing.

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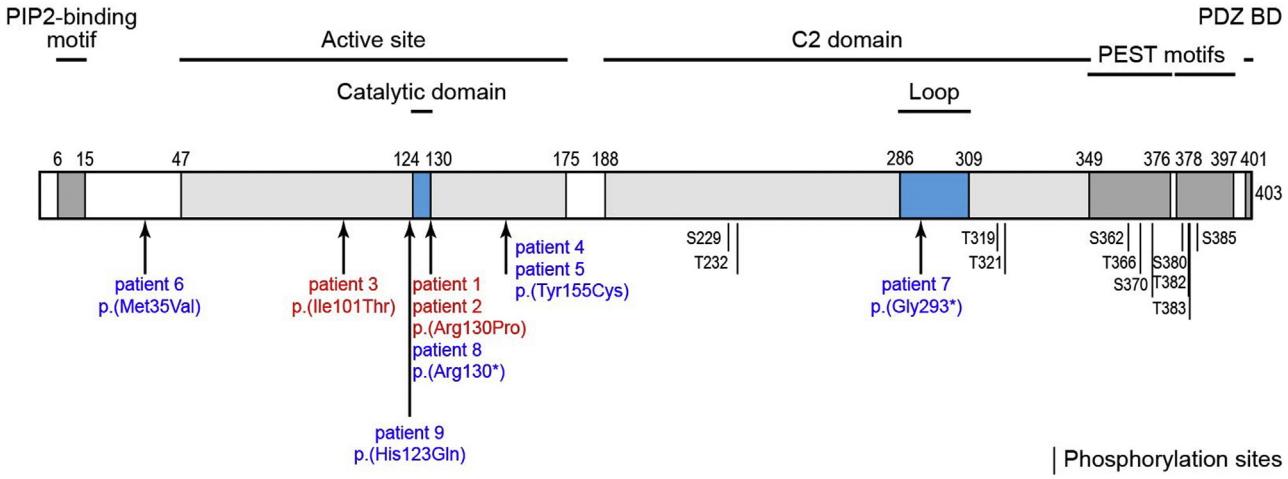


FIG E1. A structural overview of the PTEN protein with type of mutations. The overview includes distinct domains, phosphorylation sites, and the locations as well as the type of mutations within the patient cohort. *Red* color font indicates patients with hypogammaglobulinemia, whereas *blue* color font indicates patients without hypogammaglobulinemia.

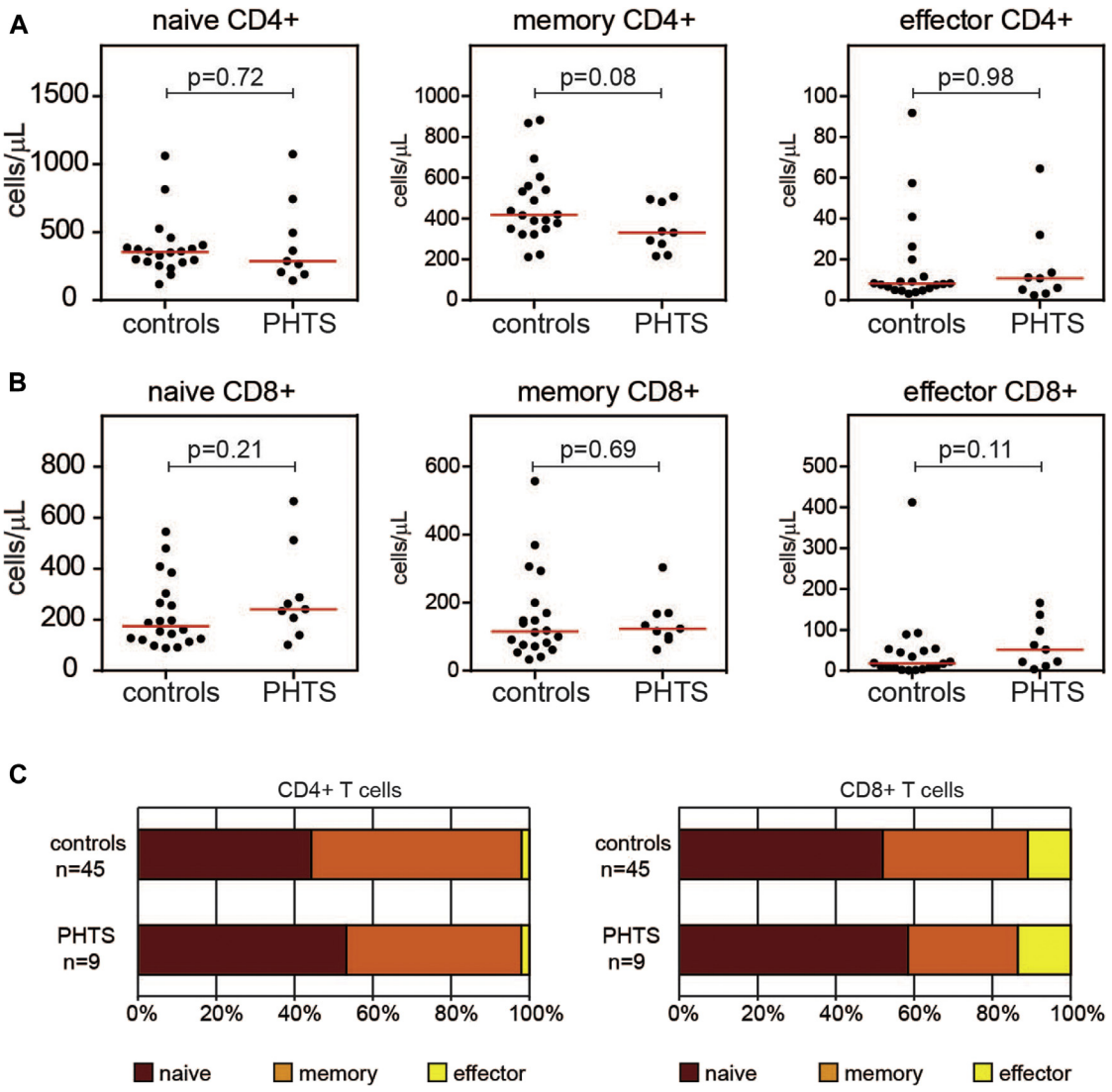


FIG E2. Naive, memory, and effector T-cell subsets in patients with PHTS. Absolute numbers of $CD4^+$ **(A)** and $CD8^+$ **(B)** T-cell subsets. **C.** Relative distributions of $CD4^+$ and $CD8^+$ T-cell subsets. Naive T cells ($CD45RA^+CD27^+$); memory T cells ($CD45RA^-CD27^+$), and effector T cells ($CD45RA^{+/-}CD27^-$).

TABLE E1. Characteristics of patients with PHTS

Patient no.	Sex	Age (y)	*PTEN* mutation	Clinical diagnosis	B cells*	T cells*	CD4*	CD8*	CD4/CD8 ratio	NK cells*	IgG (g/L)	IgA (g/L)	IgM (g/L)
1	F	43	c.389G>C p. Arg130Pro	PHTS, hypogammaglobulinemia	0.16 (0.1-0.4)	1.37 (0.7-2.1)	0.58 (0.3-1.4)	0.71 (0.2-1.2)	0.82	0.06 (0.09-0.6)	**4.7** (6.0-12.3)	<**0.07** (0.3-1.6)	**0.30** (0.5-2.0)
2	F	12	c.389G>C p.Arg130Pro	PHTS, hypogammaglobulinemia	0.38 (0.2-2.1)	1.18 (0.8-3.5)	0.53 (0.4-2.1)	0.51 (0.2-1.2)	1.03	0.26 (0.07-1.20)	**5.5** (6.0-12.3)	**0.16** (0.3-1.6)	0.65 (0.5-2.0)
3	M	6	c.302T>C p.Ile101Thr	PHTS, hypogammaglobulinemia	0.41 (0.2-1.6)	1.77 (0.7-4.2)	0.93 (0.3-2.0)	0.68 (0.3-1.8)	1.38	0.36 (0.09-0.9)	**1.5** (4.0-11.0)	**0.07** (0.1-1.6)	0.69 (0.5-1.8)
4	M	6	c.464A>G p.Tyr155Cys	PHTS	1.01 (0.2-1.6)	3.20 (0.7-4.2)	1.69 (0.3-2.0)	0.95 (0.3-1.8)	1.78	1.29 (0.09-0.9)	9.0 (4.0-11.0)	0.80 (0.3-1.6)	0.90 (0.5-1.8)
5	M	37	c.464A>G p.Tyr155Cys	PHTS	0.18 (0.1-0.4)	1.01 (0.7-2.1)	0.68 (0.3-1.4)	0.28 (0.2-1.2)	2.41	0.43 (0.09-0.6)	14.0 (7.0-16.0)	2.60 (0.7-4.0)	1.80 (0.4-2.3)
6	M	8	c.103A>G p.Met35Val	PHTS	0.76 (0.2-1.6)	2.25 (0.7-4.2)	1.25 (0.3-2.0)	0.85 (0.3-1.8)	1.48	0.16 (0.09-0.9)	10.0 (4.0-11.0)	0.70 (0.3-2.0)	0.50 (0.5-2.0)
7	M	7	c.877G>T p.Gly293X	PHTS	0.91 (.2-2.6)	1.50 (0.7-4.2)	0.97 (0.3-2.0)	0.42 (0.3-1.8)	2.32	0.33 (0.09-0.9)	7.0 (4.0-11.0)	0.50 (0.3-2.0)	1.70 (0.5-2.0)
8	F	18	c.388C>T p.Arg130X	PHTS	0.20 (0.1-0.4)	1.28 (0.7-2.1)	0.78 (0.3-1.4)	0.23 (0.2-1.2)	1.83	0.11 (0.09-0.6)	12.4 (7.0-16.0)	0.82 (0.7-4.0)	1.68 (0.4-2.3)
9	F	32	c.369C>G p.His123Gln	PHTS	0.23 (0.1-0.4)	1.07 (0.7-2.1)	0.70 (0.3-1.4)	0.31 (0.2-1.2)	2.27	0.12 (0.09-0.6)	13.7 (7.0-16.0)	1.12 (0.7-4.0)	1.58 (0.4-2.3)

Mutation nomenclature is according to HGVS guidelines (http://www.hgvs.org). Lymphocyte subset counts are given as 10^9/L; bold values are below the normal range. Immunoglobulin levels of patients 1 and 3 are before immunoglobulin replacement was started.

F, Female; *M*, Male.

*Age-related normal values are indicated between parentheses. Age-related normal values of lymphocyte subsets are adapted from Comans-Bitter et al.[E4]

TABLE E2. Number of analyzed IgG and IgA transcripts and percentage somatic hypermutations per sample

Sample	IgG_1	IgG_2	IgG_3	IgG_4	IgA_1	IgA_2	% SHM IgG	% SHM IgA
Control 1	12	6	0	4	20	9	7.2	6.2
Control 2	25	6	0	0	23	7	5.9	4.8
Control 3	19	8	1	0	14	4	7.1	6.3
Control 4	14	4	4	1	23	2	6.1	7.7
Control 5	25	18	3	2	21	19	6.5	7.1
Control 6	20	19	4	0	21	33	4.9	5.2
Control 7	16	12	6	0	24	9	7.9	7.6
Control 8	43	17	6	0	27	23	6.2	4.3
Patient 1	1	0	7	0	0	0	0.6	ND
Patient 2	12	0	1	0	10	0	3.4	4.8
Patient 3	14	0	3	0	7	1	2.8	4.3
Patient 4	14	3	1	0	9	3	4.2	4.1
Patient 5	11	1	1	0	9	0	6.8	6.1
Patient 6	12	1	2	0	12	0	5.9	3.2
Patient 7	18	5	3	0	7	3	3.3	3.3
Patient 8	3	2	0	0	4	1	4.2	8.5
Patient 9	15	9	0	2	8	2	3.4	5.9

ND, Not determined.