Table S1. Baseline characteristics of PAPS, SLE/APS and healthy control

|  |  |  |  |
| --- | --- | --- | --- |
|  | PAPS | SLE/APS | healthy control |
| Number of patients, n | 26 | 19 | 10 |
| Age (years, mean ± SD) | 41.5 ± 15.7 | 42.2 ± 11.5 | 37.3 ± 6.1 |
| Females, n (%) | 23 (88.4) | 17 (89.5) | 8 (80.0) |
| Clinical manifestations associated with APS, n (%) |  |  |  |
| Arterial thrombosis, n (%) | 18 (69.2) | 12 (63.2) |  |
| Venous thrombosis, n (%) | 5 (19.2) | 8 (42.1) |  |
| Obstetrical events, n (%) | 4 (15.4) | 3 (15.8) |  |
| aPL profile |  |  |  |
| LA, n (%) | 19 (73.1) | 15 (78.9) |  |
| aCL, n (%) | 22 (84.6) | 15 (78.9) |  |
| aβ2GPI, n (%) | 14 (53.8) | 14 (73.7) |  |
| aPS/PT, n (%) | 18 (69.2) | 15 (78.9) |  |
| Medication |  |  |  |
| Antiplatelet, n (%) | 20 (76.9) | 13 (68.4) |  |
| Anticoagulant, n (%) | 11 (42.3) | 7 (36.8) |  |
| Corticosteroids, n (%) | 4 (15.3) | 16 (84.2) |  |
| Hydroxychloroquine, n (%) | 0 (0.0) | 2 (10.5) |  |

PAPS, primary antiphospholipid syndrome; SLE/APS, systemic lupus erythematosus-associated antiphospholipid syndrome; LA, lupus anticoagulant; aCL, anticardiolipin antibodies IgG and/or M; aβ2GPI, anti-β2-glycoprotein I antibodies IgG and/or M; aPS/PT, phosphatidylserine-dependent antiprothrombin antibodies IgG and/or M.

Table S2. Antibody panels for T and B cell subset analysis

|  |  |  |  |
| --- | --- | --- | --- |
|  | Treg | Th | B cells |
| FITC | Live or dead | Live or dead | Live or dead |
| PE | CD25 | CXCR3 | CD24 |
| PerCP-Cy5.5 | CD4 | CD4 | CD19 |
| PE-Cy7 | CCR7 | CCR6 | CD27 |
| APC | CD127 | CXCR5 | CD38 |
| APC-H7 | CD45RA | CD45RA | CD20 |
| V450 |  | CD3 | CD3 |
| V500 |  |  | IgD |

Table S3. Cell surface marker phenotype of T and B cell subset

|  |  |  |
| --- | --- | --- |
| Subset | Surface markers | References |
| CD4+ T cells | CD3+ CD4+ | 1 |
| Helper T cells | CD3+ CD4+ CXCR5- CD45RA- | 1 |
| Th1 cells | CD3+ CD4+ CXCR5- CD45RA- CXCR3+ CCR6- | 1 |
| Th2 cells | CD3+ CD4+ CXCR5- CD45RA- CXCR3- CCR6- | 1 |
| Th17 cells | CD3+ CD4+ CXCR5- CD45RA- CXCR3- CCR6+ | 1 |
| Follicular helper T cells | CD3+ CD4+ CXCR5+ CD45RA- | 1 |
| Central memory T cells | CD4+ CD25- CD45RA-CCR7+ | 2,3 |
| Effector memory T cells | CD4+ CD25- CD45RA-CCR7- | 2,3 |
| Naïve T cells | CD4+ CD25- CD45RA+CCR7+ | 2,3 |
| Effector T cells | CD4+ CD25- CD45RA+CCR7- | 2,3 |
| Treg cells | CD4+ CD25+ CD127-~dim | 2,3 |
| Activated Treg cells | CD4+ CD25+ CD127-~dim CD45RA- | 2,3 |
| Resting Treg cells | CD4+ CD25+ CD127-~dim CD45RA+ | 2,3 |
| Traditional B cells | CD3- CD19+ CD24++ CD38++ | 4 |
| Naïve B cells | CD3- CD19+ CD27- IgD+ | 4 |
| Total memory B cells cells | CD3- CD19+ CD27+ | 4 |
| Pre-switched memory B cells | CD3- CD19+ CD27+ IgD+ | 4 |
| Post-switched memory B cells | CD3- CD19+ CD27+ IgD- | 4 |
| CD27- IgD- B cells | CD3- CD19+ CD27- IgD- | 4 |
| Plasmablasts | CD3- CD19+ CD20- CD27++ CD38++ | 4 |

Table S4. Baseline characteristics of PAPS patients for genetic analysis

|  |  |
| --- | --- |
|  | PAPS |
| Number of patients, n | 14 |
| Age (years, mean ± SD) | 51.5 ± 12.7 |
| Females, n (%) | 14 (100) |
| Clinical manifestations associated with APS, n (%) |  |
| Arterial thrombosis, n (%) | 10 (71.4) |
| Venous thrombosis, n (%) | 3 (21.4) |
| Obstetrical events, n (%) | 1 (7.1) |
| aPL profile |  |
| LA, n (%) | 11 (78.6) |
| aCL, n (%) | 12 (85.7) |
| aβ2GPI, n (%) | 7 (50.0) |
| aPS/PT, n (%) | 9 (64.3) |
| Medication |  |
| Antiplatelet, n (%) | 11 (78.6) |
| Anticoagulant, n (%) | 6 (42.9) |
| Corticosteroids, n (%) | 0 (0.0) |
| Hydroxychloroquine, n (%) | 0 (0.0) |

Table S5. SNP list

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene | SNP | Genotype | Associated disease | References |
| TLR7 | rs179010 | C/T | SLE | 5 |
|  | rs3853839 | G/C | SLE | 5 |
| TNFAIP3 | rs13192841 | A/G | SLE | 6 |
|  | rs2230926 | T/G | SLE | 6 |
|  | rs6922466 | A/G | SLE | 6 |
| TNFSF13 | rs11552708 | G/A | SLE | 7 |
| TNFSF4 | rs10798269 | G/A | SLE | 8 |
|  | rs844644 | A/C | SLE | 9 |
| BANK1 | rs3733197 | G/A | SLE | 10 |
| TYK2 | rs2304256 | A/C | SLE | 11 |
| IRF5 | rs2004640 | G/T | SLE | 12 |
|  | rs10954213 | A/G | SLE, APS | 13,14 |
| FCGR2A | rs1801274 | C/T | SLE | 15 |
| FCGR2B | rs1050501 | C/T | SLE | 15 |
| FCG3A | rs396991 | G/T | SLE | 15 |
| CR2 | rs4308977 | G/A | SLE | 16 |
|  | rs1048971 | G/A | SLE | 16 |
|  | rs17615 | G/A | SLE | 16 |
| STAT4 | rs7574865 | G/T | SLE, APS | 17,18 |
| BLK | rs2736340 | C/T | SLE, APS | 19,14 |

Table S6. Primer list

|  |  |  |
| --- | --- | --- |
| gene | Forward primer (5’ – 3’) | Reverse primer (3’ – 5’) |
| TLR7 | AGGTGGAAGCAGACAGGAGA | TCTCCCAGACACACTTGCAG |
| LY6E | CTTACGGTCCAACATCAGAC | GCACACATCCCTACTGACAC |
| MX1 | GGGTAGCCA CTGGACTGA | AGGTGGAGCGATTCTGAG |
| IFIT1 | TCAAAGTCAGCAGCCAGTCTCA | GCCTCCTTGGGTTCGTCTATAA |
| IFIT3 | AACTACGCCTGGGTCTACTATCACTT | GCCCTTTCATTTCTTCCACAC |
| GAPDH | GGTGATGCTGGTGCTGAGTA | TCATAAGTCCCTCCACGATG |

Table S7. Serum aPL profile of the patients enrolled in the cell sorting analysis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | aCL-IgG (GPL-U/ml) | aCL-IgM (MPL-U/ml) | aβ2GPI-IgG (U/ml) | aβ2GPI-IgM (U/ml) |
| aCL detection assay (Figure 3C) |  |  |  |  |
| Patient 1 | 80< | 27 | 44 | 37 |
| Patient 2 | 80< | 0 | 1.3 | 1.4 |
| Patient 3 | 80< | 3.9 | 17 | 0 |
| Patient 4 | 80< | 0 | 0 | 1.1 |
| aβ2GPI/HLA class II complex detection assay (Figure 4C,D) |  |  |  |  |
| Patient 1 | 80< | 27 | 44 | 37 |
| Patient 2 | 42.0 | 0 | 80 | 17 |
| Patient 3 | 34.2 | 8.4 | 67 | 22 |

GPL-U, G phospholipids units; MPL-U, M phospholipids units.

Table S8. The prevalence of each listed allele among 14 PAPS patients

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene | SNP | Allele, n (%) | | |
| TLR7 | rs179010 | C/C  11 (79) | C/T  3 (21) | T/T  0 (0) |
|  | rs3853839 | G/G  8 (57) | G/C  6 (43) | C/C  0 (0) |
| TNFAIP3 | rs13192841 | A/A  11 (79) | A/G  3 (21) | G/G  0 (0) |
|  | rs2230926 | T/T  9 (64) | T/G  4 (29) | G/G  1 (7) |
|  | rs6922466 | A/A  9 (64) | A/G  4 (29) | G/G  1 (7) |
| TNFSF13 | rs11552708 | G/G  2 (14) | G/A  10 (71) | A/A  2 (14) |
| TNFSF4 | rs10798269 | G/G  10 (71) | G/A  4 (29) | A/A  0 (0) |
|  | rs844644 | A/A  5 (36) | A/C  6 (43) | C/C  3 (21) |
| BANK1 | rs3733197 | G/G  12 (86) | G/A  2 (14) | A/A  0 (0) |
| TYK2 | rs2304256 | A/A  8 (57) | A/C  4 (29) | C/C  2 (14) |
| IRF5 | rs2004640 | G/G  3 (21) | G/T  9 (64) | T/T  2 (14) |
|  | rs10954213 | A/A  3 (21) | A/G  7 (50) | G/G  4 (29) |
| FCGR2A | rs1801274 | A/A  8 (57) | A/G  6 (43) | G/G  0 (0) |
| FCGR2B | rs1050501 | C/C  12 (86) | C/T  2 (14) | T/T  0 (0) |
| FCG3A | rs396991 | G/G  6 (43) | G/T  7 (50) | T/T  1 (7) |
| CR2 | rs4308977 | G/G  10 (71) | G/A  3 (21) | A/A  1 (7) |
|  | rs1048971 | G/G  6 (43) | G/A  6 (43) | A/A  2 (14) |
|  | rs17615 | G/G  9 (64) | G/A  5 (36) | A/A  0 (0) |
| STAT4 | rs7574865 | G/G  4 (29) | G/T  8 (57) | T/T  2 (14) |
| BLK | rs2736340 | C/C  10 (71) | C/T  4 (29) | T/T  0 (0) |

Table S9. Association between disease-related SNPs and B cell subsets in patients who were not on immunosuppressive therapy

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Gene | SNP | Total memory B | pre-switch memory B | post-switch memory B | Plasma blast | Breg |
| TLR7 | rs179010 | 0.586 | 0.697 | 0.533 | 0.349 | 0.629 |
|  | rs3853839 | **0.013\*** | **0.014\*** | **0.048\*** | **0.033\*** | 0.329 |
| TNFAIP3 | rs13192841 | 0.243 | 0.101 | 0.938 | 0.533 | 0.432 |
|  | rs2230926 | 0.312 | 0.876 | 0.102 | 0.310 | 0.394 |
|  | rs6922466 | 0.660 | 0.738 | 0.388 | 0.320 | 0.296 |
| TNFSF13 | rs11552708 | 0.773 | 0.666 | 0.206 | 0.492 | 0.529 |
| TNFSF4 | rs10798269 | 0.463 | 0.121 | 0.952 | 0.389 | 0.812 |
|  | rs844644 | 0.886 | 1.000 | 0.671 | 0.156 | 0.912 |
| BANK1 | rs3733197 | 0.423 | 1.000 | 0.181 | 0.422 | 0.241 |
| TYK2 | rs2304256 | 0.737 | 0.622 | 0.778 | 0.385 | 0.291 |
| IRF5 | rs2004640 | 0.800 | 0.658 | 0.266 | 0.659 | 0.128 |
|  | rs10954213 | 0.566 | 0.835 | 0.336 | 0.231 | 0.349 |
| FCGR2A | rs1801274 | 0.181 | 1.000 | 0.092 | 0.420 | 0.938 |
| FCGR2B | rs1050501 | 0.306 | 0.592 | 0.108 | 0.593 | 0.911 |
| FCG3A | rs396991 | 0.391 | 0.316 | 0.668 | 0.617 | 0.412 |
| CR2 | rs4308977 | 0.866 | 1.000 | 0.672 | 0.933 | 0.483 |
|  | rs1048971 | 0.867 | 0.954 | 0.656 | 0.254 | 0.174 |
|  | rs17615 | 0.065 | 0.064 | 0.216 | 0.315 | 0.846 |
| STAT4 | rs7574865 | 0.430 | 0.526 | 0.603 | 0.216 | 0.237 |
| BLK | rs2736340 | 0.157 | 0.119 | 0.723 | 0.887 | 0.533 |

Values are presented as p values. \*p<0.05, using Kruskal Wallis test or Mann–Whitney U test. Breg cells, regulatory B cells.

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**Figure**



**Fig. S1. Lymphocyte subset analysis in PAPS, SLE/APS and healthy controls.** (A) The percentage of CD4+ T cells, Th1 cells, follicular helper T cells, central memory T cells, naïve T cells, effector memory T cells and effector T cells per CD4+ T cells. (B) The percentage of Traditional B cells, CD27- IgD- B cells and naïve B cells per CD19+ B cells. Th, helper T; PAPS, primary antiphospholipid syndrome; SLE/APS, systemic lupus erythematosus-associated antiphospholipid syndrome.



**Fig. S2. The correlations between each aPL titer and plasmablast counts.** (A) aCL-IgG (B) aCL-IgM (C) aβ2GPI-IgG (D) aβ2GPI-IgM.



**Fig. S3. Association of plasmablasts and total memory B cells with TLR7 SNP rs3853839 in PAPS patients.** G represents the risk allele. (A) The percentage of plasmablasts per CD19+ B cells. (B) The percentage of total memory B cells per CD19+ B cells. (C) TLR7 transcript levels in PBMCs. (D) IFN score, which represents the combination of mRNA expression levels of four IFN-regulated genes including LY6E, MX1, IFIT1, and IFIT3 in PBMCs. Each point represents one individual patient. \*p<0.05.



**Fig. S4. FACS-sorted cells.** (A) The total PBMCs fraction (B) PBMCs depleted of CD20-positive B cells (C) PBMCs depleted of CD20-negative B cells. PBMCs, peripheral blood mononuclear cells.



**Fig. S5. B cell viability and total IgG production capability of unsorted and mock-sorted PBMCs.** (A) Representative dot plots depicting dead CD19+ B cells measured by Propidium Iodide (PI). (B) The viability of CD3- CD19+ B cells. (C) Total IgG levels in the culture supernatants of PBMCs stimulated with IL-6, IL-21, CD40 ligand, APRIL. B cell viability was calculated by examining the ratio of the number of live (PI-negative) CD3- CD19+ B cells to the number of dead fluorescing (PI-positive) CD3- CD19+ B cells. Total IgG levels in the supernatants were measured by Human IgG ELISA kit (abcam). PBMCs were obtained from 5 healthy controls for these analyses.



**Fig S6. Ex-vivo aCL-IgG production in the culture supernatants of PBMCs stimulated with IL-6 (1 ng/ml), IL-21 (50 ng/ml), CD40 ligand (1 μg/ml), APRIL (300 ng/ml).** aCL-IgG detected by ELISA in the culture supernatants of total PBMCs, CD20+ B cells or CD20- B cells from four patients. Each point represents one individual patient. \*p<0.05. aCL, anticardiolipin antibodies; GPL-U, G phospholipids units; PBMCs, peripheralblood mononuclear