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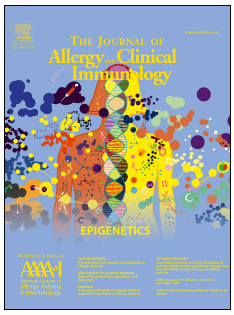

A combined immunodeficiency with severe infections, inflammation and allergy caused by ARPC1B deficiency

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Running title:

ARP2/ARP3-related actin polymerization defect results in combined immunodeficiency

The authors have no financial interests to disclose

CAPSULE SUMMARY:

We report the natural history, clinical manifestations, genetics, and immunohematological findings in 14 patients from 11 families with ARPC1B deficiency, delineating the spectrum of the disease that appears progressive and challenging to manage clinically.

KEYWORDS

Combined immunodeficiency, ARPC1B, Infection, Allergy, Inflammation, Thrombocytopenia

To the Editor:

Recently a novel syndrome of combined immunodeficiency, allergy and "auto"inflammation caused by mutations in the *ARPC1B* gene has been reported[1-4]. Analysis of patient-derived hematopoietic cells has shown a defect in actin polymerization, which resulted in a wide range of clinical manifestations and immunological-hematological features. We report on the immunological, cellular and molecular phenotypes in 14 patients with bi-allelic *ARPC1B* mutations and variable clinical presentations (**Fig.1A-B**, **Suppl.Fig.1A; Suppl.Table 1; Online Repository for Case Descriptions**), helping to delineate the broad spectrum of this novel disease and presenting unreported insights into cell-intrinsic defects involving regulatory T cells and NK cells, potential players in the immune dysregulation and susceptibility to viral infections observed in these patients. The disease causing variants are diverse and scattered throughout the gene (**Suppl.Fig.1B; Suppl.Table 1**). P4, P12 and P14 have Nepalese ancestry and share the same variant, suggesting a founder mutation. In all patient samples tested, ARPC1B protein was undetectable by Western blotting and we identified an increased - although variable - expression of the ARPC1A isoform (**Suppl.Fig.2**).

The disease is characterized by a very early clinical onset (mean 2 months, range 1-6 months) (**Suppl.Table 2**)**.** Presenting symptoms included skin rash, infections and gastrointestinal bleeding. Most patients (79%) (**Fig.1A**) suffered from recurrent or severe bleeding episodes, most frequently represented by enterorrhagia. Platelet counts were reduced (**Suppl.Table 3**), with normal volume in most cases. An increased rate and/or abnormal severity of respiratory tract infections (including pneumonia, bronchopneumonia and bronchiolitis), and skin infections (including abscesses, erysipelas, extensive warts (**Fig.1B**) and molluscum contagiosum), were observed in 71% and 50% of the patients respectively, whereas severe, protracted bacterial gastrointestinal infections have been diagnosed in a minority of individuals (**Suppl.Table 2 and 4**).

As summarized in **Fig.1A** and **Suppl.Table 5**, common manifestations of immune dysregulation included moderate-to-severe eczema which was observed in 57% of cases (**Fig.1C**), associated with food allergy (anaphylactic reactions) and asthma. Cutaneous vasculitis was noted in 69% of patients presenting as a maculopapular rash, erythema nodosum or vasculitic purpura (**Fig.1D**). In all cases investigated with a skin biopsy, leukocytoclastic vasculitis was diagnosed. Arthritis was present in 23% of patients. One child presented with two episodes of macrophage activation syndrome, followed by the appearance of enlarged lymph nodes, splenomegaly and episodes of sialadenitis (**Fig.1E**). Autoantibodies were absent in most patients.

Growth failure was noted in all patients (**Suppl.Table 2**), with growth hormone tests found to be impaired when performed (P2 and P3), compatible with a partial GH deficiency, and no catch-up growth post-hematopoietic stem cell transplantation (HSCT) (P2, P3 and P6).

Immunophenotyping showed an increased number of circulating $CD19^+$ B cells, a reduced absolute count of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells and in one patient an expansion of γδ T cells, possibly driven by CMV infection (**Fig.2A; Suppl.Table 6**). *In-vivo* immunoglobulin levels were abnormal with markedly increased IgA and IgE in almost all cases (**Suppl.Table 6**). In contrast to WAS or DOCK8 deficiency[5], the humoral response to polysaccharide vaccine was normal in most cases (**Suppl.Table 7**). The T-cell subset distribution was abnormal with low percentages of naïve $CD4^+$ and $CD8^+$ T cells (**Suppl.Fig.3A,B**). *In-vitro* T-cell proliferation in response to combination of anti-$CD3^+$ + anti-$CD28^+$, cytokines (IL-15, IL-2) and mitogens was largely normal, whilst response to low-dose CD3 and antigens were defective in some cases (**Suppl.Table 7**). The TCR repertoire was persistently oligoclonal in 2 out of 7 tested patients and transiently oligoclonal in 1 patient (**Suppl.Fig.3C; Suppl.Table 6**). The proportion and phenotype of regulatory T cells was variable (**Fig.2B; Suppl.Fig.4A**), however *in-vitro* expanded Treg cells showed decreased expression of all Treg markers including FOXP3, Helios, CD25, and CTLA-4 (**Fig.2C, Suppl.Fig.4B**). Treg suppressor activity against $CD4^+$ (**Fig.2D-E**) and $CD8^+$ T allogeneic responder cells was defective (**Suppl.Fig.4C).** An increase in the CD3-$CD56^{bright}CD16^{neg}$ NK subpopulation (27% in P2, 24% in P3 and 21% in P4) was noted when tested (P2, P3 and P4) (**Suppl.Fig.5; data not shown**). Impaired NK degranulation in the presence of K562 cells was observed and similarly to WAS patients[6] IL-2 restored degranulation and killing to normal levels (**Fig.2F; data not shown**).

Most patients received antibiotic prophylaxis (71%). One patient with recurrent oral candidiasis remained on antifungal prophylaxis. “Auto”inflammatory manifestations appear to respond to steroids, mofetil mycophenolate and sirolimus. The response to TNF-blocking agents was unsatisfactory. To date, five patients have been treated with HSCT. Two patients have a medium/long-term follow-up of 1 and 6 years respectively and are in good health and off all medication (P2 and P6) The other three patients (P3, P9 and P12) have only recently been transplanted, they are alive and well, with resolution of all “auto”inflammatory features.

In conclusion, our cohort delineates a more detailed and larger spectrum of ARPC1B deficiency phenotypes compared to previous reports. The clinical defect appears to be characterized by recurrent bacterial and viral infections, extensive eczema, allergies, thrombocytopenia and skin vasculitis, together with bleeding often manifested as early onset gastric hemorrhage and hemorrhagic colitis. The eczematous skin phenotype can be explained by immune-mediated allergic responses and the anaphylactic reactions can be avoided by elimination of food allergens from the diet. The defective Treg function is suggested to be involved in both the exaggerated Th2 responses and IgE reactivity against allergens[7]. Defects in cytoskeleton rearrangement, altered immunological synapses formation and reduced chemotaxis have been recently identified in ARPC1B-deficient patients' T cells[4], suggesting that they may play a role in the susceptibility to infections. In addition, patients NK cells show a peculiar phenotypic profile and an impaired functionality including both migration defects and NK-cell dysfunction which may well contribute to the predisposition to viral infections seen in ARPC1B-deficient patients. The neutrophil and macrophage abnormalities may explain the susceptibility of the patients to bacterial infections[1] in the presence of normal antibody levels. Although careful monitoring, antimicrobial prophylaxis and adequate treatment are mandatory to prevent and counter infections, the immunodysregulation contributing to vasculitis and arthritis requires immunosuppression. The unique and variable combination of clinical features makes ARPC1B deficiency a complex disease entity for which HSCT is considered a curative treatment option.

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LEGENDS:

Figure 1. Clinical features, imaging and histology of relevant tissues in affected patients. (**A**) Frequencies of clinical manifestations in ARPC1B-deficient patients (detailed in **Suppl.Table 8**). (B-D) Diffuse warts, eczema and skin vasculitis in P7. (E) Sialadenitis and lymph node enlargement in P3.

Figure 2. Immune cell abnormalities. (**A**) Representative plots of the altered B-lymphocyte staining (left panel), B-lymphocyte percentage (middle panel), and CD19 expression (geo-MFI, right panel) in ARPC1B-deficient patients. For $CD4^+$ and $CD8^+$ T-cell subsets (naïve, memory and effector-memory populations), see Suppl Figure 1. **(B)** T_{reg} subset analysis with a representative dot plot showing percentage of T_{regs} ($CD4^+CD25RA^{pos}CD127^{neg}$), and Treg FOXP3 and Helios expression. (**C**) FOXP3 and Helios expression of *in vitro* expanded T_{reg} cells from patient and control (lines connect each patient with their own healthy relative used as control). (**D**) FACS plots showing the proliferation of allogeneic T-responder cells (T_{resp}) measured by cell-trace violet (CTV) dilution. The stimulated and unstimulated T_{resp} without T_{reg} are shown in blue. T_{regs} from controls (in grey) and patients (in red) were cultured at different ratios with T_{resp} cells while stimulated with anti-CD3/CD28. **(E)** Quantification of CD4 and CD8 T_{resp} -cell suppression by T_{reg}. **(F)** NK cells of patients were functionally evaluated against the K562 cell line by CD107A expression experiments. Resting NK cells (left panel) and IL2-stimulated cells (right panel) were evaluated and compared to 10 healthy controls. All controls are represented by healthy adults. Bars indicate means ± SD, a star indicates statistical significance as assessed by Mann-Whitney test ($p<0,05$).

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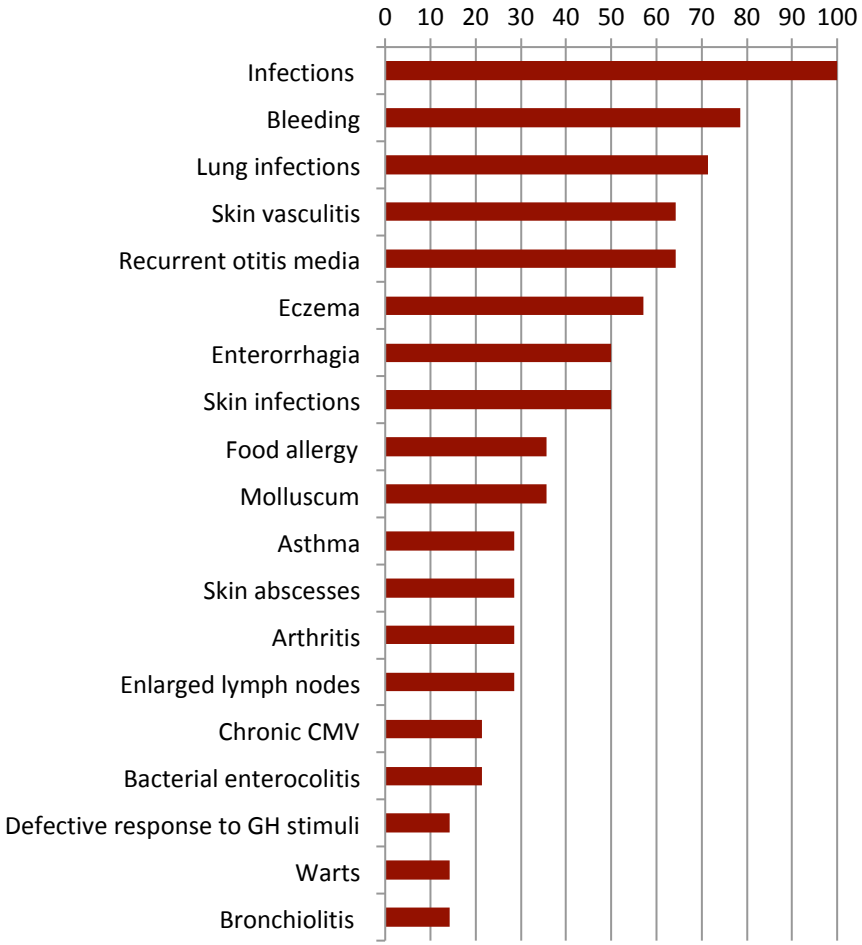

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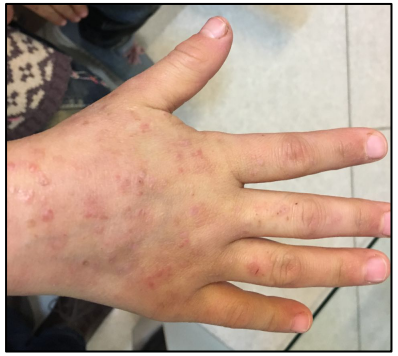

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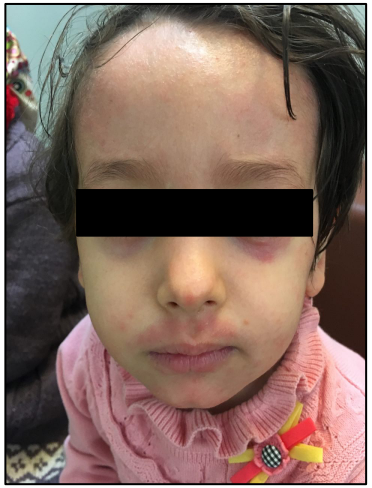

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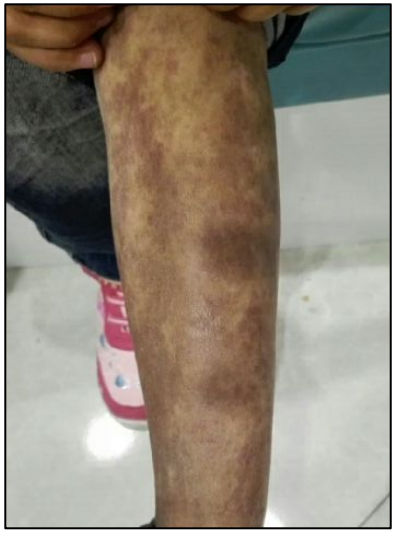

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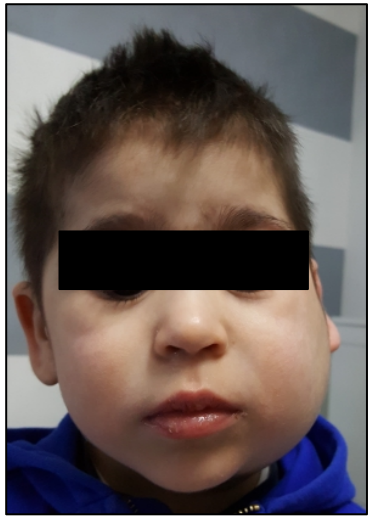

Figure 1

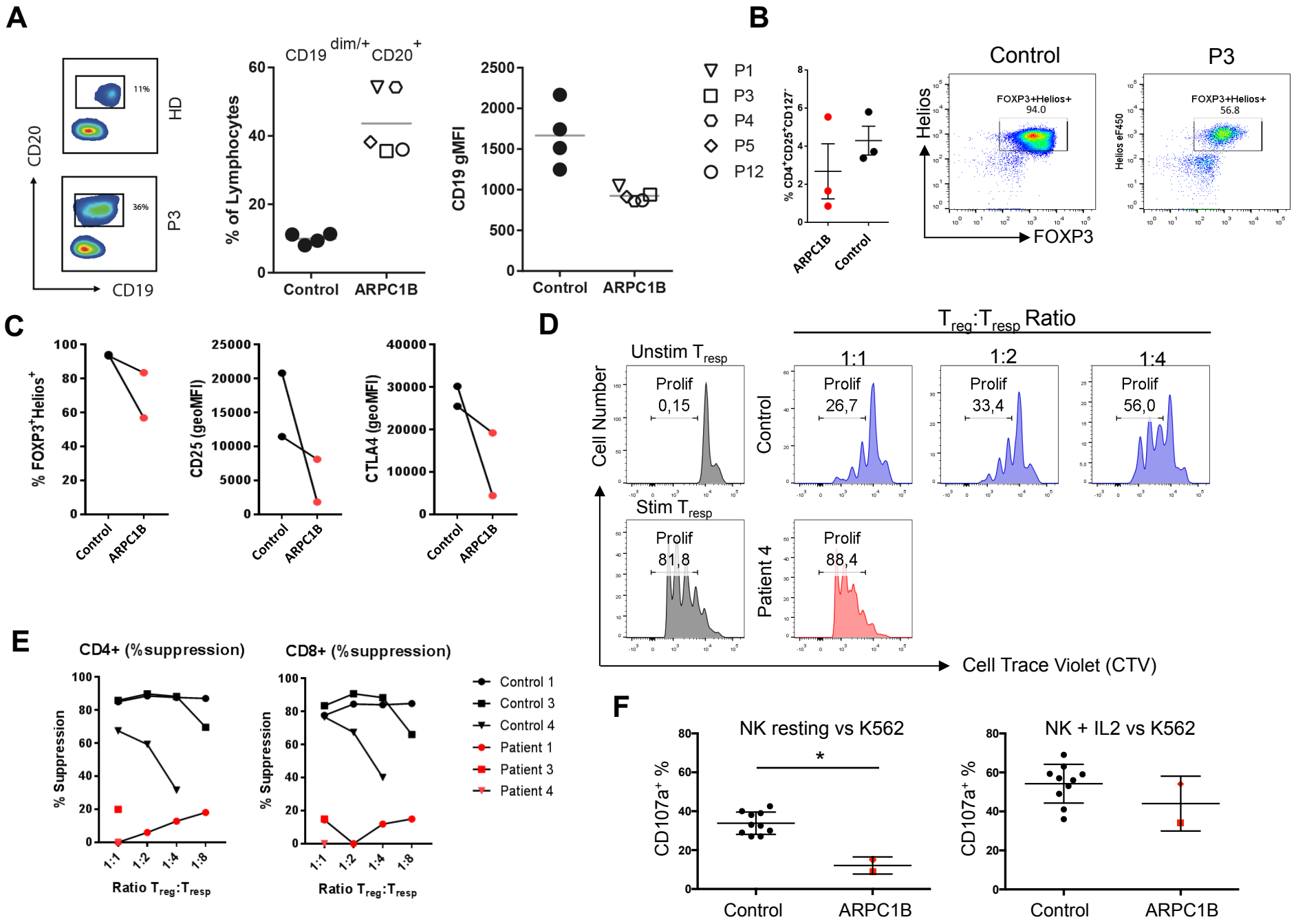
A
CD20
CD19
HD
P3
11%
36%
CD19 dim/+ CD20 +
% of Lymphocytes
Control
ARPC1B
CD19 gMFI
B
P1
P3
P4
P5
P12
% CD4+CD25+CD127-
ARPC1B
Control
Control
P3
Helios
FOXP3
FOXP3+Helios+
94.0
FOXP3+Helios+
56.8
Helios eF450
C
% FOXP3+Helios+
CD25 (geoMFI)
CTLA4 (geoMFI)
D
Treg:Tresp Ratio
Unstim Tresp
Stim Tresp
Cell Number
Control
Patient 4
1:1
1:2
1:4
Prolif
0,15
26,7
33,4
56,0
81,8
88,4
Cell Trace Violet (CTV)
E
CD4+ (%suppression)
CD8+ (%suppression)
% Suppression
Ratio Treg:Tresp
Control 1
Control 3
Control 4
Patient 1
Patient 3
Patient 4
F
NK resting vs K562
NK + IL2 vs K562
CD107a+ %
*
Control
ARPC1B

Figure 2

ONLINE REPOSITORY INFORMATION

Author contributions

SV collected and analyzed clinical data, supervised experiments, followed some of the patients and wrote the manuscript. MPC collected and analyzed clinical and contributed writing the manuscript. PT, AB performed FACS experiments, analyzed data and contributed writing the manuscript. AT and MA performed WB and proteomic experiments. EC performed Treg experiments, analyzed data and contributed writing the manuscript. EM, EMJ, JM, EMvL performed immunophenotyping and functional assays. IB analyzed genetic data and performed experiments. SC and SM performed NK cell experiments. CB supervised NK cell experiments. AE, AR, AS, CAZ, CP, HA, HMA, PP, RC, LF, PQ, RL, UP, TN, GD and RY collected clinical data and followed the patients. FB, LDN, BB, HT, IKC, EA, EGK, SG, RM and ASP analyzed genetic data. TG designed the bioinformatics pipeline for the genetic data at BHCMG enabling detection of the mutations. JLC and JRL supervised genetic data analysis. MG, JO supervised the project and followed the patients. AA, RY analyzed data, designed and supervised the project. KS, TWK collected and analyzed data, designed and supervised the project and wrote the manuscript.

Acknowledgements

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ONLINE REPOSITORY METHODS

Isolation of blood cells

Heparinized venous blood was collected from healthy donors and patient after informed consent had been obtained. The study was approved by the AMC Institutional Medical Ethics Committee in accordance with the 1964 Declaration of Helsinki, protocol number NL40331.078 for PID studies.

Human neutrophils were isolated using a Percoll gradient with a density of 1.076 g/ml. Erythrocytes were lysed with isotonic $NH_4Cl/KHCO_3$, washed twice in PBS and resuspended in Hepes buffer (132 mM NaCl, 6 mM KCl, 1 mM CaCl2, 1 mM MgSO4, 1.2 mM potassium phosphate, 20 mM HEPES, 5.5 mM glucose and 0.5% (w/v) human serum albumin, pH 7.4) for further functional testing[8].

PBMCs were separated as interphase from the Percoll gradients for subsequent experiments, after two washes and resuspended in PBS containing 0.5% (w/v) BSA for immunophenotyping or for further functional studies in IMDM supplemented with 10% fetal calf serum and antibiotics[9].

Flow cytometry, culture conditions for T- and B-cell analysis and repertoire analysis

Immunophenotyping and functional tests were performed as described previously[10]. Fluorescently-labeled conjugated monoclonal antibodies (mAbs) were obtained from BD-biosciences (San Jose, USA), Biolegend (San Diego USA), eBioscience (San Diego, USA), Sanquin (Amsterdam, the Netherlands) and Beckman Coulter (Brea, USA), analyzed using a FACSCanto-II flowcytometer and FlowJo software. For proliferation assays, PBMCs were labeled with 0.5 μM CFSE (Molecular Probes), resuspended in IMDM supplemented with 10% fetal calf serum (BioWhittaker), antibiotics, and 3.57×10–4%(v/v) β-mercapto-ethanol (Merck) and cultured for 6 days at 37℃ under different stimulatory conditions. T c ells: anti-CD3 (clone 1XE)+anti-CD28 (clone 15E8) or IL-15 (10 ng/ml, R&D systems), B cells: anti-IgM mAb (clone MH15; Sanquin), anti-CD40 mAb (clone 14G7; Sanquin), 20 ng/ml IL-21 (Invitrogen), or 1 μg/ml CpG oligodeoxynucleotide 2006 (Invivogen), with 100 U/ml IL-2 (R&D Systems). Proliferation was assessed by measuring CFSE dilution. For clonality assessment, PCR-amplified products of TCRb and TCRg locus[11] were separated using the capillary Genetic Analyzer 3130 (Applied Biosystem)[12].

Treg isolation and suppression assay

Naïve Tregs (CD4+, CD127-, CD25+, CD45RA+) were isolated by FACS sorting (FACS Aria III, BD Biosciences). Cells were then cultured in presence of 0.1 μg/ml of anti-CD3 mAb (M1654, clone 1XE, PeliCluster) and anti-CD28 mAb (16-0289-85, clone CD28.2, eBioscience) for 14 days in IMDM containing 10% FCS and 300 U/ml

IL-2 (Proleukin). The suppressive capacity of the *in vitro* expanded Tregs was assessed by inhibition of proliferation of co-cultured responder T cells (Tresp) labeled with CellTrace™ Violet cell proliferation kit (C34557, ThermoFisher). Briefly, cells were stimulated with 0.01 µg/ml of anti-CD3 mAb (M1654, clone 1XE, PeliCluster) and anti-CD28 mAb (16-0289-85, clone CD28.2, eBioscience) at different ratios of Tresp:Treg in absence of IL-2. On day 5, cells were stained with antibodies against CD4 and CD8 and TOPRO-3 (T3605, Invitrogen) and analyzed on a LSR Fortessa cytometer (BD Biosciences).

NK-cell function

PBMCs were thawed and incubated overnight with or without recombinant human IL-2. Degranulation assay: PBMCs were cocultured with the leukemic cell line K562 cells for 3.5 hours in an effector (NK cell): target (K562) ratio of 1:10 in the presence of a monoclonal antibody to CD107 (BD). Subsequently, the percentage of NK cells that underwent degranulation (=CD107a+) was analyzed by flowcytometry. Cytotoxicity assay: K562 cells were labeled with DDAO (Invitrogen) to allow distinction from PBMCs. PBMCs and K562 cells were then cocultured for 3 hours in different effector-to-target cell ratios, followed by staining with DioC6 (Invitrogen) to identify apoptotic cells. The percentage of cytotoxicity was expressed as the percentage of apoptotic (DioC6-negative) cells analyzed by flowcytometry.

WES, Sanger Sequencing

Whole genome sequencing (WGS) and whole exome sequencing was performed as described[1,2].

Confirmation was obtained by Sanger sequencing of genomic DNA (ABI 3130XL; Thermo Fisher Scientific) with the use of Big Dye Terminator (v.1.1) chemistry (Thermo Fisher Scientific). Primers used for sequencing were designed using NM_005720 as reference sequence.

PAGE and Western Blot analysis

Samples were separated by SDS polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. Individual proteins were detected with antibodies against ARPC1B (goat polyclonal antibodies, ThermoScientific, Rockford, IL, USA), against ARPC1A (rabbit polyclonal antibodies, Sigma, St Louis, USA) and against actin (mouse monoclonal antibody, Sigma). Secondary antibodies were either donkey-anti-goat-IgG IRDye 800CW, Goat-anti-mouse-IgG IRDye 800CW or Donkey-anti-rabbit-IgG IRDye 680CW (LI-COR Biosciences, Lincoln, NE, USA).

Quantification of bound antibodies was performed on an Odyssey Infrared Imaging system (LI-COR Biosciences, Lincoln, NE, USA).

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ONLINE REPOSITORY FIGURES

Supplementary Figure 1. Family pedigrees of the patients and *ARPC1B* mutations. (**A**) Family trees of the small consanguineous families with two multiplex families. Penetrance is 100%. (**B**) *ARPC1B* gene mutations indicated in its gene structure.

Supplementary Figure 2. Western blot of patient PBMCs, granulocytes (PMN), PHA-stimulated blasts (PHA) or EBV-B cells where indicated, showing total lack of ARPC1B protein. At the same time, a variable positive staining for ARPC1A was observed in all patient cells, mostly of increased intensity compared to controls.

Supplementary Figure 3. Lymphocyte subsets tested for patient T and B cells. Representative T-cell subset analysis of a patient and a healthy adult control. **(A)** and **(B)** Numbers indicate percentage of *N* naïve, *M* memory, *EM* effector memory, *HDEM* highly-differentiated effector memory ($CD4^+$) and DN double-negative ($CD8^+$) T cell subpopulations. **(C)** TCR-repertoire analysis indicating oligoclonality of V beta and V gamma TCRs as represented in the peripheral blood of P3.

Supplementary Figure 4. Characterization of *ex vivo* and *in vitro* expanded Tregs. In **(A)** the % of ICOS and Helios positive Tregs (upper panels) and geoMFI of CTLA4, Helios, CD25 of *ex vivo* Treg cells measured by FACS in ARPC1B-deficient patients (red) and their healthy relatives as controls (in black). **(B)** Graphs showing geoMFI of Treg cell parameters measured by FACS in *in vitro* expanded Tregs. **(C)** FACS plots showing the proliferation of T-responder cells (T_{resp}) measured by cell trace violet (CTV) dilution. The unstimulated T_{resp} are shown in blue. T_{regs} from controls (in green) and patients (in red) were cultured at different ratios with allogeneic T_{resp} cells.

Supplementary Figure 5.

Phenotype of patient NK cells. Phenotype of freshly isolated NK cell of P2 compared to a control. (1) Percentage of NK cells on PBL population; (2) Percentage of CD56bright among whole NK cells; NK surface expression of (3) 2B4, (4) NKp46 and (5) NKG2D. Similar findings were observed for P1 (data not shown).

Supplementary clinical case reports.

P1

P1 is a 7-year-old male born as the first child of consanguineous, healthy Moroccan parents, who presented at 2 months of age with gastric bleeding and a mild thrombocytopenia. From 5 months of age he developed recurrent episodes of leukocytoclastic vasculitis treated with corticosteroids, serious eczema and allergy to nuts. At the age of 4 years he suffered intestinal bleeding; colon biopsies showed neutrophil and eosinophil infiltration. Recurrent pneumonias that responded to antibiotics led to mild bilateral bronchiectasis. Increased IgA and IgE and eosinophilia were present. (As previously reported[1]).

P2

P2 was recently reported[4], a boy born from unrelated parents of Italian origin, who presented in the first month of life with severe growth failure, eczema and recurring episodes characterized by hemorrhagic enterocolitis, cutaneous leukocytoclastic vasculitis and elevation of inflammatory markers. Laboratory investigations showed mild T-cell lymphopenia, low IgG and IgM and elevated IgE and IgA, together with eosinophilia and intermittent thrombocytopenia. The patient responded well to steroid administration with however steroid dependency that was resolved following therapy with rapamycin. At the age of 7 months the patient developed a Staphylococcal right upper lobe pneumonia, with residual pneumatocele, requiring a surgical resection at the age of 5 years. Since the age of 10 years the patient presented with warts on the hands and the face. At 9 years the patient developed symptomatic thrombocytopenia requiring hospitalization and IVIG administration. At 10 years recombinant GH was started, but subsequently ceased after two years due to lack of efficacy. Recently, at 15 years of age, a haploidentical CD19/TCRα/β depleted peripheral blood stem cell transplant from his mother was performed (conditioning: Thiotepa, Fludarabine, Treosulfan, Rituximab, ATG).

P3

P3 was recently reported[4], a boy born from consanguineous parents of Italian origin, presented in the first months of life with two episodes of macrophage activating syndrome, chronic CMV infection, failure to thrive, persistent hepatosplenomegaly, recurrent pustular skin lesions and recurrent infections. Lymphocytopenia with increased CD4/CD8 double negative population, γ/δ T cell expansion and intermittent thrombocytopenia were present. From 2 years of age a progressive lymphadenopathy appeared. Recombinant GH was administered without clear improvement of the growth curve. At 4 years he presented with an acute episode characterized by fever, enlarged lymph nodes, parotiditis, increased acute phase

reactants and a painful abdominal wall lesion with macrophage muscular infiltrate on biopsy. He responded very well to steroid therapy and rapamycin that allowed steroid tapering. At 5 years of age a transplant of peripheral blood stem cells CD19/TCRα/β depleted from the haploidentical father was performed (conditioning: Thiotepa, Fludarabine, Treosulfan, Rituximab, ATG). At 1 year after the transplant the patient is in good clinical condition, with full donor chimerism, without any current therapies; follow-up is still ongoing.

P4

The proband is a boy of Nepalese ancestry; the parents have denied any consanguinity. He first presented for medical care at 1 month of age due to RSV bronchiolitis, which required intensive care unit admission for oscillator care. After discharge, he required multiple re-hospitalizations due to stridor, which was attributed to tracheomalacia. Failure to thrive was noted at 5 months of age, and he was ultimately admitted to the hospital again when he developed hematemesis with wheezing. Esophagogastroduodenoscopy was performed and revealed the presence of gastritis, which was treated with medications. At 6 months of age, he required readmission to the hospital for recurrence of hematemesis and chronic lung disease. Colonoscopy demonstrated the presence of a “chronic inflammatory intestinal process of unclear etiology”. *H. influenzae* was identified in bronchoalveolar lavage fluid specimens but was felt to represent colonization rather than infection. The patient was started on inhaled corticosteroids. The Genetics service was consulted and noted hemihypertrophy. Underlying lysosomal storage disease was considered but excluded. The Allergy/Immunology service was consulted, and food allergies were excluded as a cause of the gastrointestinal issues. At the same time, immunophenotyping was performed, which showed a low percentage (19%) and number (1,330 cells/mm^3) of T cells. By 10 months of age, the patient was formally diagnosed with asthma. In follow-up immunologic studies, he was noted to have persistently low $CD3^+$ T cell percentages, low $CD8^+$ T cell percentages and numbers, normal T-cell proliferative responses to mitogens and antigens, normal humoral responses to immunizations, and elevated serum IgG and IgA levels. At 18 months of age, the patient began to develop otitis media. After recurrences over 2 months, PE tubes were placed, which resulted in persistent otorrhea rather than resolution of infections. Ultimately, the patient was started on prophylactic antibiotics, which caused the infections to stop. At 32 months of age, eczema was reported for the first time and progressed. The patient then moved to a different city in Texas and did not return for follow-up evaluations. Interestingly, P4,

who lives in the USA, P12 and P14, who live in Australia, are of West-Nepal origin (P4 and P12) or have Nepalese ancestry (P14) and share the same variant, suggesting a founder mutation.

P5

Family from Somalia, parents and siblings are healthy. The patient is the youngest of seven siblings. She is now 10 years old and her siblings are 28, 27, 25, 23, 18, and 12 years old. She was born in Norway at term after an uneventful pregnancy and delivery. Her BW was 3390 g, BL 47, and OFC 34 cm. At a few weeks of age she developed a generalized neonatal maculopapular rash and, later, eczema. She was admitted to her local pediatric department at two months of age and was treated for mastoiditis. From then onward, she has had recurrent skin abscesses, warts, molluscum and showed poor growth. She has had periods of diarrhea in preschool years, on a few occasions with bloody stools. Gut biopsies showed non-specific inflammation, virology was negative. She has had recurrent upper respiratory tract infections and chronic otitis media from early childhood. She had periodontitis with premature loss of milk teeth. She was successfully treated for a chronic CMV viremia at 6 years of age. She has had three episodes of pneumonia when she was 3, 6 and 9 years old, the latter complicated with empyema that required surgical drainage. She presented bronchiectasis as well. Skin abscesses were mainly caused by *S.aureus*. She was treated for a skin abscess; she still has warts and molluscum but no eczema. She does not have a history of diarrhea, but some abdominal discomfort from time to time. In the last year she has been generally well and participates in sports at school.

P6

P6 is an 11-year-old male patient born spontaneously after an uneventful pregnancy (birth weight 3180 g, length 51 cm) as the second child of consanguineous (1st degree cousins) healthy Moroccan parents. His 4 year elder sister and his 4 year younger brother are asymptomatic. Since his first months of life P6 presented with increased susceptibility to infection, i.e. with recurrent episodes of obstructive bronchitis, with pneumonia at the age of 1month, 2 years and 6.5 years, RSV bronchiolitis. At the age of 3 months he presented with bloody enteritis (Campylobacter) at the age of 5 months, 7 months and 4 years, a skin abscess (*Pseudomonas aeruginosa* and *Klebsiella pneumonia*e) at the age of 11 months, with otitis media at the age of 10 months, 2.5 years and 4.8 years and with generalized, ulcerative and abscess-forming molluscae since the age of 4.5 years. All infections

listed above led to hospital admissions; the infections were treated symptomatically and with i.v. antibiotics if appropriate. The boy failed to thrive (body weight < 3rd percentile, growth parallel to 3rd percentile). Immunological investigations revealed a normal full blood count eosinophilia (up to 14%) and high IgE levels (up to 2.800 IU/ml) starting at 6 months of age; the levels of IgG, IgA, and IgM were also elevated. Following vaccination the production of specific antibodies against diphtheria and tetanus toxoid, pneumococcus and haemophilus was demonstrated. FACS analysis of peripheral blood leucocytes revealed reduced CD8+T-cells, reduced naïve CD4+T-cells, elevated B-cells and severely reduced non-switched and switched memory-B-cells (tested with antibodies against CD3, CD4, CD8, HLA/DR, CD45/RA, CD45/RO, CD4/CD25, IgD, IgM, CD27, CD56, CD11a, CD14, CD66b/CD49d, CD19 and CD20). The phenotype of P6 strongly resembled autosomal recessive Hyper IgE Syndrome, but no mutation in DOCK8 or TYK2 was identified. Given the dramatic infectious course especially with generalized, ulcerative and molluscae, HSCT with an HLA-matched related family donor was successfully conducted at 5 years of age. The conditioning regimen for HSCT included treosulfan, fludarabin, thiotepa and Campath (anti-CD52 antibody). Pathological skin changes disappeared immediately after transplantation. Due to a mixed hematopoietic chimerism at 6 months post-transplant the patient received 5 donor lymphocyte infusions (DLI). He still has mixed chimerism with a slight increase in autologous T- and non-T-cells. Currently, 6 years after HSCT, the patient is in excellent clinical condition with no signs of graft versus host disease, no susceptibility to infections, no skin abnormalities and sufficient growth and gain of weight.

P7

A 4-year-old girl presented with umbilical bleeding and generalized maculopapular rash in neonatal period, epistaxis at 2 months of age, and perianal abscess at 4 months of age. She had recurrent pneumonia, fungal otitis extern, and ecthyma gangrenosum, leading to several hospitalization from birth. She suffered from food allergy (milk, egg white) and allergy to house dust mite. Severe skin vasculitis started later in the course of disease.

P8

A 9-year-old girl presented with generalized maculopapular rash in the neonatal period. She was hospitalized for high-grade fever, cough, and seizure (meningitis and pneumonia) at 2.5 months of age, and for rectorrhagia at 3 months of age. Her colorectal endoscopy was compatible with severe eosinophilic colitis with crypt

abscesses. Because of chronic dermatitis, she was subjected to a skin biopsy that suggested chronic spongiotic dermatitis. She had a urinary tract infection with pyelocaliectasis and decreased uptake of the right kidney on a DMSA scan at 4 months of age. Since than, she developed anemia and thrombocytopenia, bone marrow aspiration and biopsy was performed in the second year of life and was normal. Her medical history included perforated otitis media at 2.5 years of age, purulent axillary lymphadenitis, leading to incision and antibiotic therapy at 3.5 years of age, failure to thrive, and recurrent pneumonia. Severe skin vasculitis started later in the course of the disease.

P9, 10, 11

Already reported[2].

P12

P12 is a 5-month-old female identified on exome sequencing to have homozygous splice site mutations in ARPC1B. Her parents were not known to be consanguineous, but were shown to be of close common ancestry by SNP array, and were both carriers. The clinical picture was characterized by erosive dermatitis with purpuric and eczematous areas (eczema onset at 3 weeks of age, purpura at 3-4 months with subsequent psoriasiform dermatitis), ulcerative lesions - perianal, eye lid, gum, vulva, lip ear and poor wound healing. Skin biopsies were variable - leukocytoclastic vasculitis on the scalp, spongiotic dermatitis with some perivascular inflammation felt to be short of vasculitis elsewhere and mixed inflammatory infiltrate mostly mononuclear with scant PMN. The patient presented with early onset of diarrhea, likely cow's milk protein allergic (CMPA) enteritis, starting from the first month of life, with severe metabolic acidosis and treated as CMPA with elemental feeds. Bloody diarrhea was noted following accidental re-exposure to CMP at 4 months of age, again settled with elemental diet, limited colonoscopy normal. Normocytic anemia with thrombocytopenia (fluctuating from 60 to the normal range) was present, with normal platelet size (MPV 6.6 - 7.1 fl). Bone marrow biopsy was normal. There was a persistent eosinophilia and an appropriate leukocytosis with infections. From the infection point of view, she developed chronic CMV (~ 30,000 copies/mL initially, then 3,000 copies/mL), enterococcus UTI, chronic oral thrush responsive to Nystatin, discharging otitis media (Staphylococcus aureus and

Candida albicans), two episodes periorbital cellulitis, recurrent skin "infections" with MSSA isolation. Pseudomonas was grown from a perianal ulcer. The clinical picture was complicated by a transient hepatitis, cystic pulmonary lesion of unclear etiology and poor feeding with initial poor growth, which improved with nasogastric tube feeding. Immune investigations have shown raised IgG (16.3 g/L), IgA (1.40 g/L), IgM (3.32 g/L) and IgE (1507 KU/), a profound neutrophil chemotaxis defect (done twice 2 weeks apart 0.15 mm and 0 mm; reference >1.19 mm) but normal NBT, DHR and staphylococcal bactericidal assay. Lymphocyte subsets were essentially normal except for a slight increase in CD3+HLA-DR+ and skewing to CD4+CD45RO+ memory phenotype, normal mitogen response to PHA and proliferation with anti-CD3/D28. TRECs were normal. ANA, ANCA and ASCA were negative. High-dose IVIG as a immunomodulatory treatment had limited effect and she progressed to immunosuppression with Mycophenolate, before ultimately undergoing successful HSCT.

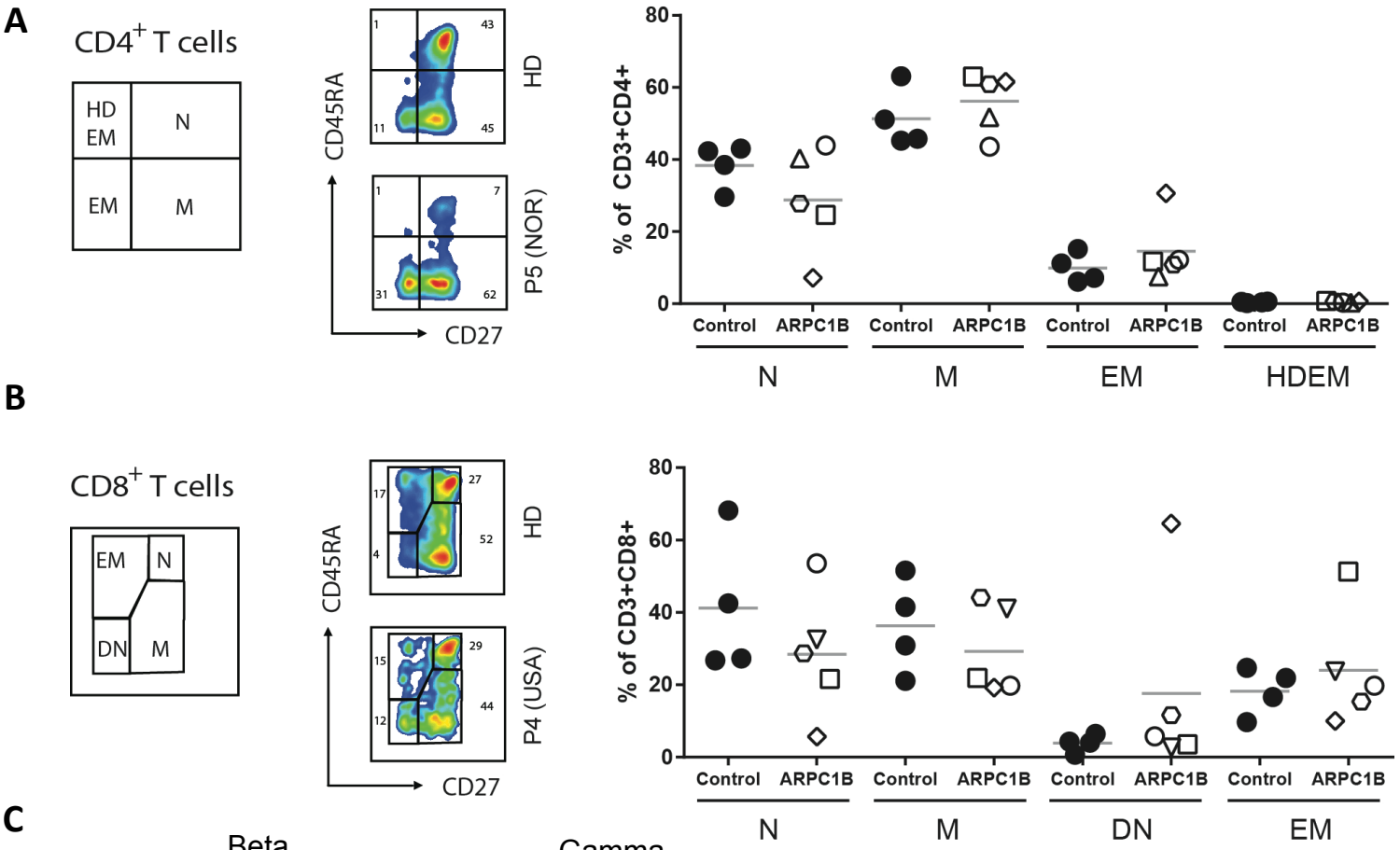
Interestingly, P12 and P14, who live in Australia and P4, who lives in the USA are of West-Nepal origin (P4 and P12) or have Nepalese ancestry (P14) and share the same variant, suggesting a founder mutation.

P13

The patient is the fifth child of parents who were second cousins. At age of 4 years, she was referred for the first time to the immunology clinic due to recurrent pulmonary infections and recurrent otitis media with onset at the age of one year. Her clinical history was remarkable for cutaneous vasculitis, her skin biopsy showed leukocytoclastic vasculitis, chronic arthritis affecting her knees and ankles bilaterally, and persistent thrombocytopenia. Hematological and immunological analyses of the blood showed very high IgA (1400 mg/dl) in two occasions, normal IgG an IgE, low platelet count (76×10^9 /L) and leukocytosis consisting of increased numbers of neutrophils (5.1×10^3 cell/µL), eosinophils (0.3×10^3 cell/µL) and lymphocytes (4.3×10^3 cell/µL). She was placed on IV immunoglobulin replacement therapy and treated with different immunosuppressant drugs such as azathioprine, steroids, and recently mycophenolate Mofetil. Although her general conditions improved, aside from her hands, to date there has not been any significant improvement of her. arthritis.

P14

This male child, now aged 8 years, was born in Australia to non-consanguineous parents from Bhutan with Nepalese ancestry. He presented at 10 months with a history of failure to thrive, mild eczema and marked lymphadenopathy with microcytic anemia and thrombocytopenia. Lymph node biopsy demonstrated non-specific granulomatous inflammation, bone marrow biopsy was non-contributory. He subsequently developed protracted diarrhea, recurrent otitis media and molluscum contagiosum. Severe, early-onset periodontal disease necessitated complete extraction of the primary dentition at age 5 years. He developed a chronic cough and recurrent bronchitis, and was found to have bilateral bronchiectasis. He experienced one episode of mild, NSAID-responsive polyarthritis. Despite polyclonal hypergammaglobulinemia, he demonstrated a poor polysaccharide vaccine response, and significant clinical improvement was noted following commencement of prophylactic immunoglobulin therapy. Interestingly, P12 and P14, who live in Australia and P4, who lives in the USA are of West-Nepal origin (P4 and P12) or have Nepalese ancestry (P14) and share the same variant, suggesting a founder mutation.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CD4+ T cells
HD
EM
N
EM
M
CD45RA
CD27
HD
P5 (NOR)
1
43
11
45
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62
% of CD3+CD4+
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60
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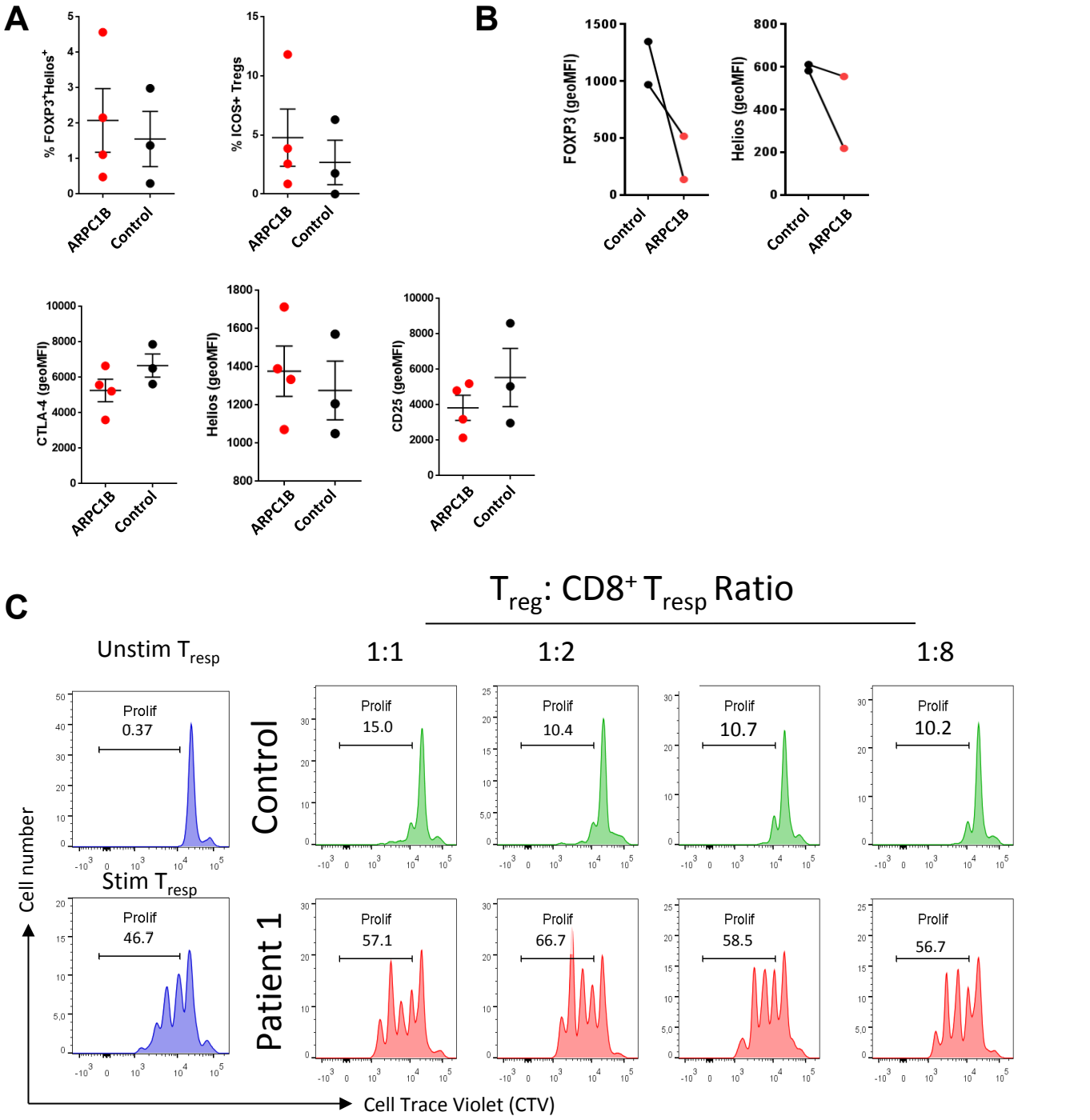
0
Control
ARPC1B
Control
ARPC1B
Control
ARPC1B
Control
ARPC1B
N
M
DN
EM
C
Beta
Gamma

Suppl. Fig. 3


A
% FOXP3+Helios+
% ICOS+ Tregs
CTLA-4 (geoMFI)
Helios (geoMFI)
CD25 (geoMFI)
ARPC1B
Control
B
FOXP3 (geoMFI)
Helios (geoMFI)
Control
ARPC1B
C
Treg: CD8+ Tresp Ratio
Unstim Tresp
1:1
1:2
1:8
Prolif
0.37
Control
15.0
10.4
10.7
10.2
Stim Tresp
46.7
Patient 1
57.1
66.7
58.5
56.7
Cell number
Cell Trace Violet (CTV)

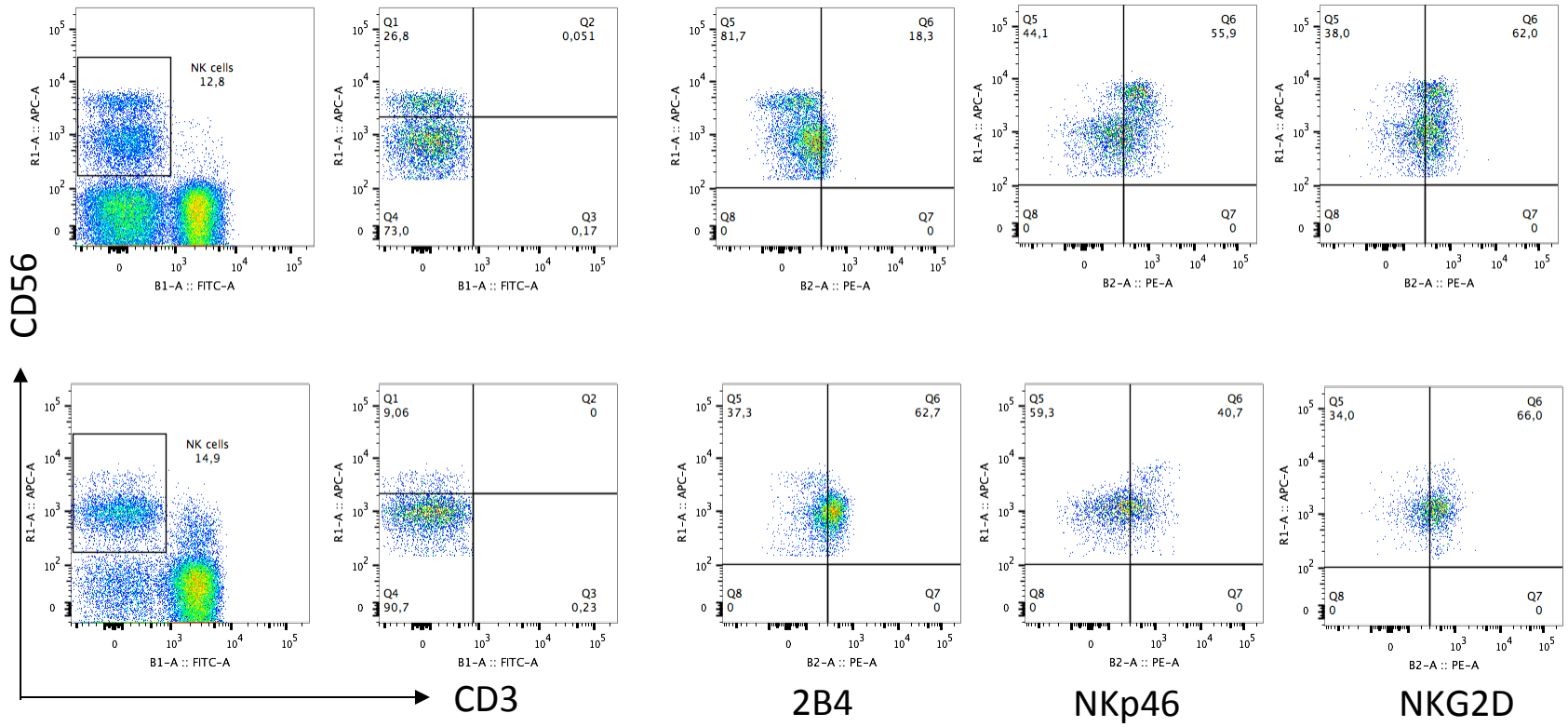
Suppl. Fig. 4

Gate: lymphocytes

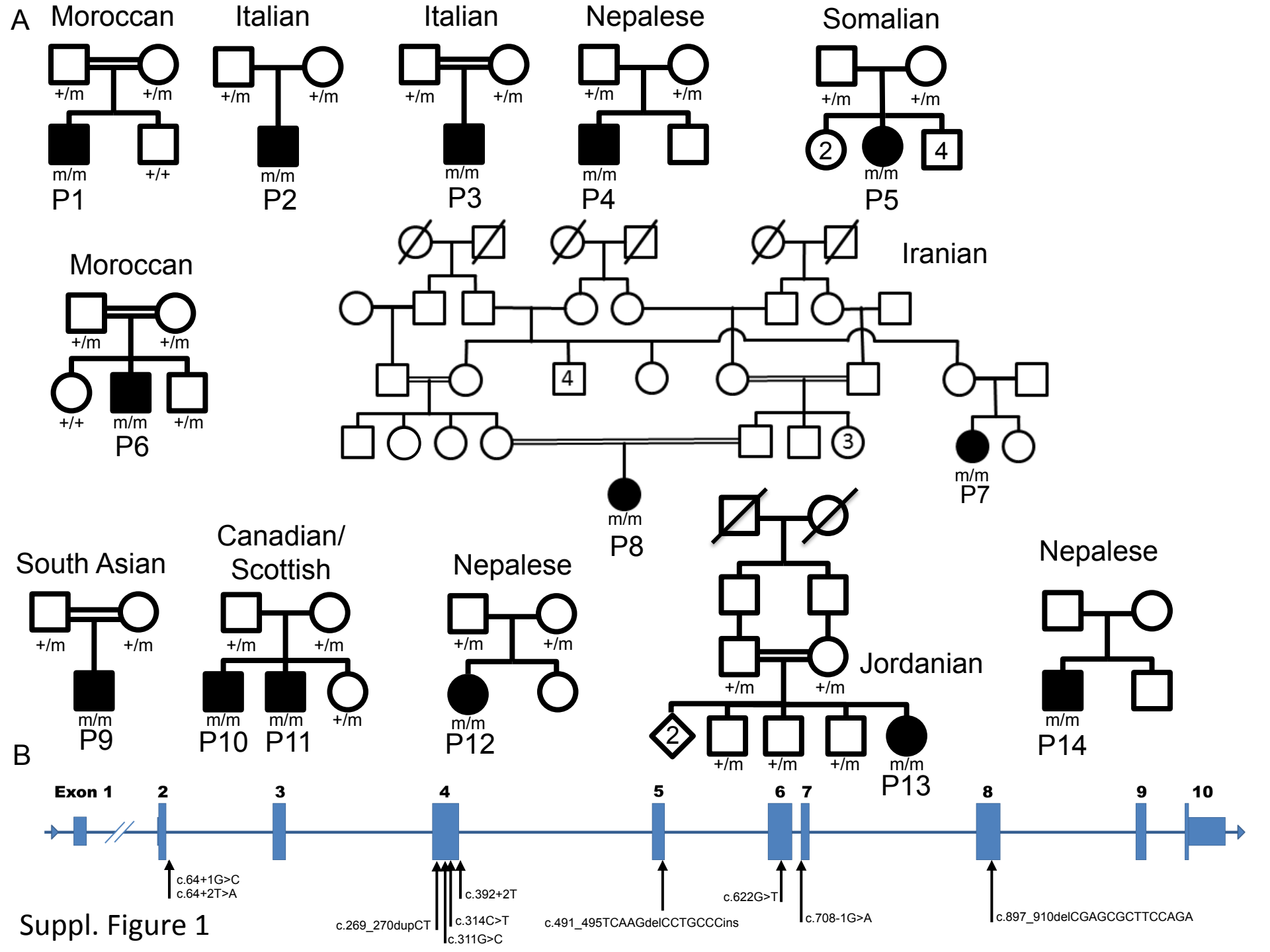
Gate: CD3- CD56+

P2

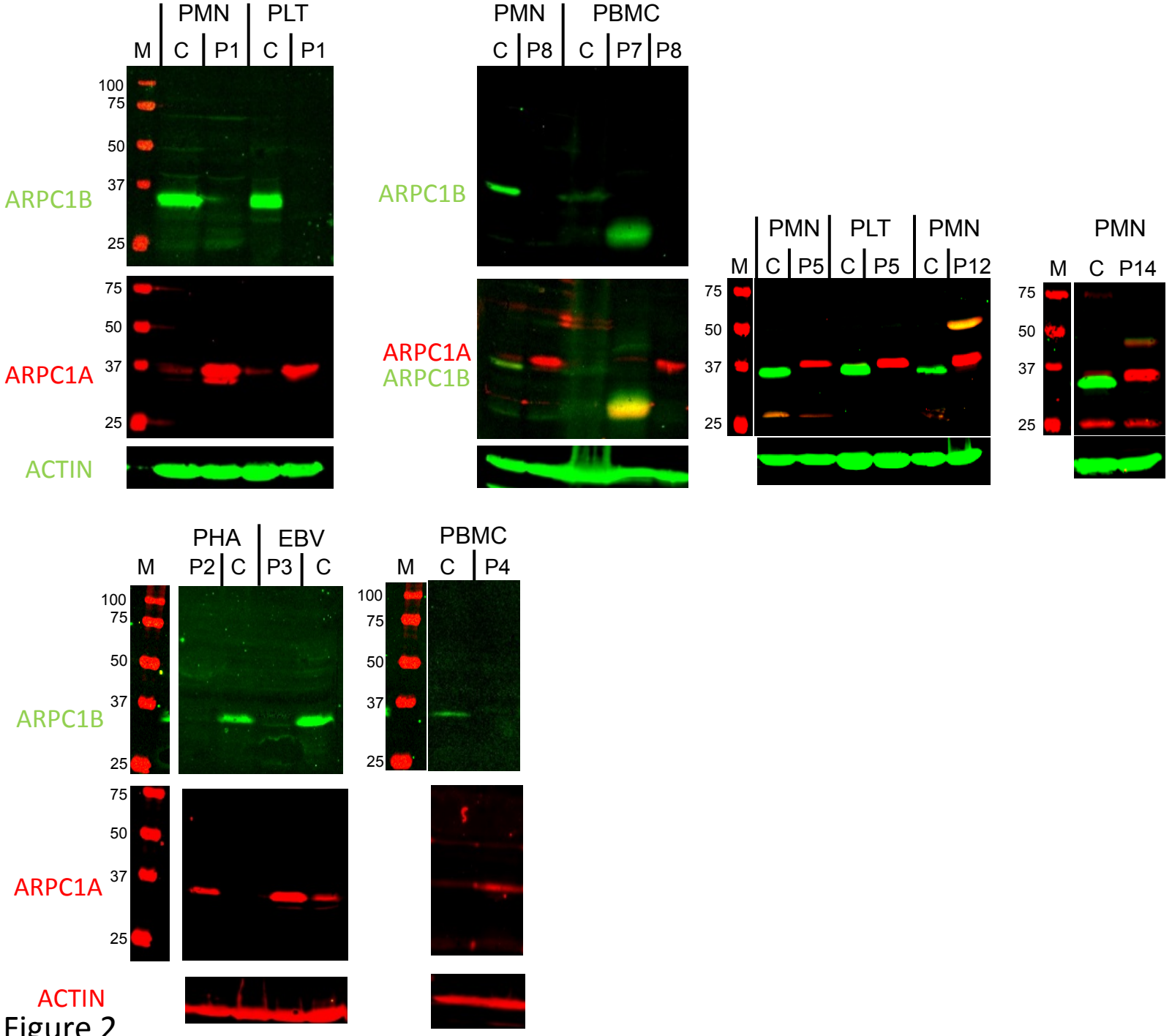
CONTROL



Suppl. Fig. 5



Suppl. Figure 1



Suppl. Figure 2

Supplementary Table 1. Genetic characteristics

	Origin	Genetic Method	Consanguinity	*ARPC1B* Genetic variant (NM_005720.3)	Zygosity	Exon	Protein variant	Protein expression	Ref
P. 1	Moroccan	WGS	Yes	c.491_495delinsC CTGCCC	Homozygous	Exon 7	p.Phe164Serfs*31	Undetectable	1
P. 2	Italian	Targeted NGS	No	c.64+1G>C	Homozygous	Donor splice site of intron 2	NA	Undetectable	4
P. 3	Italian	WES	Yes	c.622G>T	Homozygous	Exon 8	p.Val208Phe	Undetectable	4
P. 4	Nepalese	WES	No	c.64+2T>A	Homozygous	Donor splice site of intron 2	NA	Undetectable	
P. 5	Somalian	WES	No	c.392+2T>C	Homozygous	Donor splice site of intron 4	NA	Undetectable	
P. 6	Moroccan	WES	Yes	c.311G>C	Homozygous	Exon 4	p.Trp104Ser	NA	
P. 7	Iranian	WES (Following HM)	Yes	c.897_910delCGA GCGCTTCCAGA	Homozygous	Exon 10	p.Glu300Profs*153	Undetectable	
P. 8	Iranian	WES (Following HM)	Yes	c.897_910delCGA GCGCTTCCAGA	Homozygous	Exon 10	p.Glu300Profs*153	Undetectable	
P. 9	South Asian	WES	Yes	c.269_270dupCT	Homozygous	Exon 4	p.Val91Trpfs*30	Undetectable	2
P. 10	Scottish	WES	No	c.314C>T	Homozygous	Exon 4	p.Ala105Val	Reduced	2
P. 11	Scottish	WES	No	c.314C>T	Homozygous	Exon 4	p.Ala105Val	Reduced	2
P. 12	Nepalese	WES	No	c.64+2T>A	Homozygous	Donor splice site of intron 2	NA	Undetectable	
P. 13	Jordanian	WES	Yes	c.708-1G>A	Homozygous	Splice site acceptor of exon 6	NA	NA	
P. 14	Nepalese	WES	No	c.64+2T>A	Homozygous	Donor splice site of intron 2	NA	Undetectable	

NA Not available

	Height	Disease onset	Presenting clinical symptoms	Bleeding episodes	Infectious disease episodes	Other clinical manifestations*	Treatment	Past treatment	HSCT
P. 1	<3rd centile	Month 2	Gastric bleeding, infantile purpuric rash	Episodes of enterorrhagia, hemoptysis	Recurrent pulmonary infections, enterocolitis (Salmonella typhimurium), adenovirus pneumonia requiring PICU, extensive warts	Cystic pulmonary lesions at 9 years of age, bilateral bronchiectasis	Prophylactic antibiotic (azithromycin 3 times weekly), antihistamines, topical steroid, MMF, monthly 500mg/Kg IVIG		No
P. 2	<3rd centile	Month 1	Neonatal hemorrhagic enteritis and poor growth	Episodes of enterorrhagia with thrombocytopenia, onset in neonatal period	Recurrent pulmonary infections, pneumatocele (Pseudomonas), bronchiectasis, enterocolitis (Salmonella typhi), extensive warts	Lung cysts (post infective), pathologic GH response	Before HSCT: prophylactic antibiotics (TMP-SMX), inhaled steroid+B2agonist, sirolimus	MMF, sirolimus (improvement of vasculitis but interrupted for increased infection rate), steroid, rGH (no response)	Yes
P. 3	<3rd centile	Month 2	Macrophage activation syndrome (triggered by CMV infection), splenomegaly, maculopapular rash	Episodes of enterorrhagia in the first months of life	Recurrent otitis (MDR Pseudomonas), chronic CMV viremia	Pathologic GH response	Before HSCT: prophylactic antibiotics (TMP-SMX), sirolimus	rGH (no response)	Yes
P. 4	3rd centile	Month 1	Severe gastritis and gastric bleeding, severe RSV bronchiolitis, prolonged intubation	Hematemesis at 5 and 6 months of age	Recurrent otitis media and bronchiolitis	GERD, tracheomalacia, hemihypertrophy	Prophylactic antibiotics (azithromycin 3 times weekly), pulmicort BID		No
P. 5	<3rd centile	Month 1	Neonatal generalized maculopapular rash	Episodes of enterorrhagia	Mastoiditis, pneumonia (empyema), recurrent skin abscesses, chronic CMV viremia			Valganciclovir, monthly 400mg/Kg IVIG	No
P. 6	<3rd centile (no catch-up growth 4 yrs after HSCT)	Month 1	Early onset of increased susceptibility to infection with recurrent bronchitis, pneumonias, enteritis and generalized molluscum contagiosum	Enterorrhagia during Campylobacter infection	Bronchopneumonia – RSV, recurrent enteritis – Campylobacter, skin abscesses – Ps. aeruginosa + Kl pneumoniae, otitis media – Ps. aeruginosa, lymphadenitis with abscess, erysipelas, gross generalized molluscum contagiosum, EBV infection		No treatment after HSCT	Monthly 500mg/Kg IVIG	Yes
P. 7	3rd-10th centile	Month 2	Generalized maculopapular rash, epistaxis	Epistaxis, umbilical bleeding	Fungal otitis externa, ecthyma gangrenosum, perianal abscess, recurrent pneumonia		Prophylactic antibiotic (TMP-SMX), topical creams, monthly 400mg/Kg IVIG	Nystatin, fluconazole, cephalexin, ofloxacin	No
P. 8	<3rd centile	Month 1	Neonatal generalized maculopapular rash, meningitis	Episodes of enterorrhagia	Axillary abscess, eczema herpeticum (HSV), pneumonia, urinary tract infection, draining otitis media		Prophylactic antibiotic (TMP-SMX), topical creams, monthly 400mg/Kg IVIG	Clindamycin, acyclovir, cephalexin	No
P. 9	<3rd percentile	Month 1	Neonatal meningitis and stroke, bloody diarrhea attributed to rotavirus, and generalized maculopapular rash	Episodes of enterorrhagia	Meningitis, pneumonia, cellulitis and S. aureus infections of surgical wounds, finger abscess		Prophylactic antibiotic (TMP-SMX -> Clindamycin), topical creams	Corticosteroid and IVIG, and many DMARDs tried, finally stable on MMF (steroid-sparing)	Yes
P. 10	<3rd centile	Month 2	Rash on hands with target lesions, purpuric rash and swelling	Subconjunctival hemorrhage	Recurrent viral pneumonitis, conjunctivitis, recurrent otitis media	Mild torticollis	Prophylactic antibiotic (TMP-SMX), topical creams	Corticosteroid, monthly 2gr/Kg IVIG, at 9 months MTX (steroid-sparing)	No
P. 11	<10th centile	Screening 4 yrs	Eczematous rash	No bleeding	Pneumonia. Impetigo of the face	Hoarse voice	Topical creams		No
P. 12	< 3rd centile	Month 2	Seborrhoeic dermatitis, diarrhea, eczematous rash, cutaneous vasculitis, perianal ulcer, necrotic skin ulcers	Bloody diarrhea after accidental rechallenge with cow's milk protein	UTI x 4 (enterococcus), large perianal ulcer (Ps. aeruginosa), oral candidiasis responding poorly to oral therapy, suppurative otitis - S aureus, and Candida albicans, vaccine strain rotavirus, chronic CMV	Pulmonary cystic lesion with granulomatous appearance	Prophylactic antibiotic (TMP-SMX) and antifungal (Fluconazole), monthly 2g/kg IVIG, topical creams, elemental feeds, MMF		Yes
P. 13	<10th centile	Month 12	Cutaneous vasculitis, chronic arthritis	Epistaxis	Draining otitis media (4-5/year), skin abscesses (3/year). Oral mucositis		Prophylactic antibiotic (TMP-SMX), monthly 400mg/Kg IVIG, Hydroxychloroquine, MMF, Prednisolone		No
P. 14	1st centile	Month 8	FTT, recurrent URTI, necrotizing granulomatous cervical and abdominal lymphadenopathy, anemia, diarrhea	Hemoptysis and enterorrhagia	Recurrent respiratory tract infection (upper respiratory tract and pneumonia), recurrent otitis media, molluscum contagiosum, skin abscess	Severe periodontal disease, bilateral bronchiectasis	Monthly 400mg/Kg IVIG		No

Supplementary Table 2. Clinical characteristics

***see Suppl. Table 5 for Allergy, Autoimmunity and Autoinflammation**

MMF mycophenolate mofetil, TMP-SMX trimethoprim sulfamethoxazole, HSCT hematopoietic stem cell transplantation, rGH recombinant growth hormone, GERD gastroesophageal reflux disease, RSV respiratory syncytial virus, IVIG intravenous immunoglobulin, DMARD disease modifying anti-rheumatic drugs, FTT failure to thrive, URTI upper respiratory tract infections.

Supplementary Table 3. Hematological characteristics

	Age	Hb (g/dL)	MCV (fL)	Leukocytes (*10^6/mL)	Neutrophils (*10^6/mL)	Eosinophils (*10^3/mL)	Lymphocytes (*10^6/mL)	Platelets (most recent, *10^6/mL)	Platelets range (*10^6/mL)	MPV (fL)
P. 1	8	**11.5**	78	8.8	4.4	800	4.3	**98**	49 - 687	8.8
P. 2	14	14.1	82.4	12.1	7.1	**930 (max 7286)**	**0.8**	**52**	10 - 87	8.7
P. 3	4	**9.3**	68.7	8.8	4.68	90 (max **1190**)	2.9	196	97-150	8.0
P. 4	2	11.2	78.3	12.5	4.78	**1655**	4.0	130	130-322	8.4
P. 5	10	**9.7**	83	10.6	6.2	NA	3.6	475	250-690	NA
P. 6	6	**7.7***	64.3	14.9	NA	NA	NA	327	NA	8.6
P. 7	4	**10.4**	65	10.3	4.94	100	5.15	**62**	34 - 88	8.3
P. 8	10	**10.2**	72	4.4	**1.76**	410	1.98	**60**	45 - 120	8.8
P. 9	9	12.2	72	6.2	4.0	880	1	**73**	28-241	8.3
P. 10	2	12.1	78	20.7	8.4	**4120**	6.6	328	79-672	9.8
P. 11	7	11.9	83	12.1	5.7	**1089**	2.2	263	233-347	6.3
P. 12	0.5	**9.8**	73.4	9	**1.4**	**1580** (max **3590)**	5.86	**96**	74-108	6.8
P. 13	15	12.8	80.8	10.9	5.1	300	4.3	**76**	150-450	**5.9**
P. 14	7	12	78,6	7.7	4.2	800	2.1	96	33 - 188	7.0

* Affected by Beta Thalassemia
Bold is abnormal according to age-specific ranges, nv normal values, NA not available.

Supplementary Table 4: Microbiological infections (Pathogens and Manifestations)

Infections	Number of patients	% of total	Clinical manifestation
Bacterial	**12/14**	**(86%)**	
Staphylococcus aureus	5	(36%)	Skin abscess, pneumonia, meningitis
Pseudomonas aeruginosa	4	(29%)	Otitis media, pneumonia, skin abscess
Salmonella typhimurium	2	(14%)	Enterocolitis
Campylobacter jejuni	1	(7%)	Enterocolitis
Klebsiella Pneumoniae	1	(7%)	Skin abscess
Moraxella Catarralis	1	(7%)	Upper respiratory tract infections
Haemophilus Influenzae	1	(7%)	Otitis media
Enterococcus	1	(7%)	Recurrent urinary tract infections
Not Available	1	(7%)	Recurrent otitis, recurrent skin abscesses
Viral	**10/14**	**(71%)**	
Molluscum contagiosum virus	5	(36%)	Molluscum contagiosum
CMV	3	(21%)	Chronic viremia
Adenovirus	3	(21%)	Pneumonia, respiratory failure, hemoptysis
Papilloma virus	2	(14%)	Warts
RSV	2	(14%)	Bronchiolitis, bronchopneumonia
EBV	1	(7%)	Viremia
HSV	1	(7%)	Eczema herpeticum
Rotavirus	1	(7%)	Enterocolitis
Parainfluenza virus	1	(7%)	Upper respiratory tract infections
Influenza A virus	1	(7%)	Influenza
Fungal	**2/14**	**(14%)**	
Candida	2	(14%)	Oral candidiasis (due to antibiotics?)

Supplementary Table 5- Clinical characteristics: Allergy, Autoimmunity and Autoinflammation

	Allergy	**Autoinflammatory/autoimmune manifestations**	**Autoimmunity markers**
P. 1	Eczema (general, extensive), asthma attacks (prednisone-responsive), food allergy with serious anaphylaxis (peanut, nuts)	Skin vasculitis (leukocytoclastic), erythema nodosum and arthritis (years later)	ANA/ENA/ANCA negative, normal thyroid and adrenal function
P. 2	Severe eczema, food allergy (cow's milk protein intolerance), inhalant allergy (asthma attacks especially associated with URTI events)	Skin vasculitis (leukocytoclastic), panniculitis, immune enteritis (no histological findings, but remission with sirolimus), ITP	ANA 1:160
P. 3	Nil	Maculopapular skin vasculitis, MAS. An acute episode with fever, inflammatory markers, lymphadenopathy, painful abdominal wall lesion (macrophage muscular infiltrate on biopsy)	Direct Coombs test positive. ANA/ENA/ANCA negative.
P. 4	Eczema, asthma attacks	Nil	ANA/ANCA negative
P. 5	Eczema	Maculopapular skin vasculitis, periodontitis	ANA/ANCA negative
P. 6	Eczema	Nil	NA
P. 7	Food allergy (CMPA, egg white), mite allergy	Severe skin vasculitis	ANA/ANCA negative
P. 8	Food allergy (lamb, chicken, fish), mite and Russian thistle allergy	Severe skin vasculitis	ANA/ANCA negative
P. 9	Nil	Severe skin vasculitis (leukocytoclastic), immune eosinophilic enterocolitis, lymphadenopathy	ANA positive, ANCA pos (anti-MPO pattern)
P. 10	Cradle cap, eczematous lesions, target lesions	Skin vasculitis (leukocytoclastic)	ANA varied from negative to 1:160. ANCA positive 1/20 cytoplasmic. Anti-MPO pos 20 U/ML (N<10), PR-3 negative
P. 11	Eczema and asthma (age 6)	Persistently elevated CRP	ANA negative. ANCA-pos (atypical pattern 1/20 - 1/80, PR3 and MPO negative), TTG negative. Rheumatoid factor positive
P. 12	CMPA, eczematous rash	Skin vasculitis (leukocytoclastic)	All tested autoAb negative (ANA, ENA, Anti-dsDNA, pANCA/cANCA)
P. 13 P. 14	Asthma Eczema	Skin vasculitis, arthritis Arthritis, necrotizing granulomatous cervical and abdominal lymphadenopathy	Direct Coombs test positive, ANA/ENA/ANCA negative. All other tested autoAb negative (RF, SMA, TPO, cardiolipin, dsDNA, EMA, TTG)

ANA anti-nuclear antibodies, ANCA anti-neutrophil cytoplasmic antibodies, ENA anti-extractable nuclear antigens antibodies, EMA anti-endomysial antibodies, dsDNA anti-double stranded DNA antibodies, SMA anti-smooth muscle antibodies, TPO anti-thyroid peroxidase antibodies, CMPA cow's milk protein allergy, URTI upper respiratory tract infection, TTG anti-transglutaminase antibodies, PR-3 anti-proteinase 3 antibodies, MPO anti-myeloperoxidase antibodies, RF rheumatoid factor.

Supplementary Table 6- Immune Phenotype

Patient (age)	CD3+ (cells/μL)	CD3+/CD4+ (cells/μL)	CD4+CD45RA+ (cells/μL)	CD3+/CD8+ (cells/μL)	CD3-/CD56+ (cells/μL)	CD19+ (cells/μL)	IgM (g/L)	IgG (g/L)	IgA (g/L)	IgE (IE/L)	TCR repertoire
P.1 (8)	1060 (nv 700-3200)	800 (nv 300-2400)	**155** (nv 320-1000)	**213** (nv 300-1800)	624 (nv 90-1000)	**1998** (nv 100-1200)	**0.5** (nv 0.56-2.61)	8.6 (nv7.07-19.19)	**6.4** (nv 0.41-3.15)	**7400** (nv< 60)	Polyclonal on CD3+ cells.
P.2 (14)	**795** (nv 1000-2200)	**412** (nv 530-1300)	**99.5** (nv 230-770)	340 (nv 330-920)	**45** (nv 70-1200)	485 (nv 110-570)	**0.30** (nv 0.59-2.97)	7.4 (nv 6.0-19.1)	**9.3** (nv 0.61-3.01)	**689** (nv < 160)	Polyclonal on CD3+ cells.
P.3 (4)	1160 (nv 900-4500)	**417** (nv 500-2400)	**low**	**275** (nv 300-1600)	348 (nv 100-1000)	1073 (nv 200-2100)	**0.47** (nv 0.49-2.92)	10.1 (nv 5.28-19.59)	**6.2** (nv 0.37-2.57)	**45** (nv < 35)	**Oligoclonal TCR α/β, severely restricted TCR γ/δ.**
P.4 (2.5)	**759** (nv 1656-3841)	**645** (nv 871-2379)	**190** (nv 430-1500)	**114** (nv 518-1433)	379 (nv 123-785)	**2581** (nv 421-1397)	**0.21** (nv 0.62-2.57)	**11.4** (nv 5.3-10.8)	**2.7** (nv 0.27-1.73)	**197** (nv < 49)	**Decreased TCR α/β expression on CD8+ cells.**
P.5 (9.5)	701 (nv 700-3200)	333 (nv 300-2000)	**56** (nv 320-1000)	333 (nv 300-1800)	460 (nv 90-900)	877 (nv 200-1600)	**0.44** (nv 0.61-2.76)	9.6 (nv7.07-19.19)	-	-	**Oligoclonal, expansion of Vβ 5.1 in CD4+ cells and of Vβ 8 in CD8+ cells.**
P.6 (6)	**846** (nv 1200-2600)	**627** (nv 650-1500)	**94** (nv 320-1000)	**191** (nv 370-1100)	218 (nv 90-900)	**1637** (nv 200-1600)	**0.36** (nv 0.56-2.61)	14.2 (nv7.07-19.19)	**4.4** (nv 0.41-3.15)	**2811** (nv < 60)	Polyclonal on CD3+ cells.
P.7 (4)	4292 (nv 900-4500)	1858 (nv 871-2379)	NA	**2562** (nv 518-1433)	**1975** (nv 123-785)	**3708** (nv 421-1397)	**0.54** (nv 0.62-2.57)	9.76 (nv 5.3-10.8)	**2.17** (nv 0.27-1.73)	**1300** (nv < 49)	NA
P.8 (9)	1402 (nv 900-4500)	650 (nv 500-2400)	NA	748 (nv 300-1600)	**1458** (nv 100-1000)	1458 (nv 200-2100)	0.68 (nv 0.61-2.76)	12.80 (nv7.07-19.19)	**6.0** (nv 0.41-3.15)	**1780** (nv < 60)	NA
P.9 (9.5)	936 (nv 900-4500)	581 (nv 500-2400)	NA	**249** (nv 300-1600)	186 (nv 100-1000)	1887 (nv 200-2100)	**0.3** (nv 0.61-2.76)	12.5 (nv7.07-19.19)	**6.9** (nv 0.41-3.15)	**1366** (nv < 60)	Polyclonal on CD3+ cells.
P.10	5609 (nv 2400-6900)	4487 (nv1400-5100)	NA	1596 (nv 700-2500)	932 (nv 100-1000)	**8469** (nv 700-2500)	1.0 (nv 0.62-2.57)	9.7 (nv 5.3-10.8)	**5.1** (nv 0.27-1.73)	**414** (nv < 49)	NA
P.11 (7.5)	1484 (nv 700-4200)	992 (nv 300-2000)	NA	431 (nv 300-1800)	**987** (nv 90-900)	**1645** (nv 200-1600)	0.8 (nv 0.56-2.61)	11.9 (nv7.07-19.19)	**4.4** (nv 0.41-3.15)	**1799** (nv < 90)	NA
P.12 (9 mo)	2980 (nv 2300-6500)	2980 (nv 800-3500)	1400 (nv 1100– 1700)	690 (nv 500-1600)	560 (nv 100 - 1300)	2070 (nv 600-3000)	**3.32** (nv 0.2 - 1.04)	**16.3** (nv 2.07- 6.43)	**1.40** (nv 0.17-0.81)	**1507** (nv<8)	NA
P.13 (4)	1860 (nv 900-4500)	**550** (nv 871-2379)	NA	930 (nv 518-1433)	300 (nv 123-785)	**70** (nv 421-1397)	**0.35** (nv 0.62-2.57)	10.68* (nv 5.3-10.8)	**14** (nv 0.27-1.73)	**1090** (nv < 49)	NA
P14 (8)	990 (nv 700-4200)	420 (nv 300-2000)	**533** (nv 320-1000)	360 (nv 300-1800)	900 (nv 90-900)	1102 (nv 200-1600)	0.62 (nv 0.56-2.61)	14.4* (nv7.07-19.19)	**10.51** (nv 0.41-3.15)	**4065** (nv < 90)	Polyclonal

Bold is abnormal according to age-specific ranges.

References:

1) Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WC, Groeneveld K, et al. Immunophenotyping of blood lymphocytes in childhood: reference values for lymphocyte subpopulations. J Pediatr 1997;130:388-93.
2) Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm R, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. J Allergy Clin Immunol 2003;112:973-80.

* on IVIG treatment.

Supplementary Table 7- Immune function

Patient	In vitro T cell proliferation			Response to vaccination	
	PHA	**CD3, CD28**	**Antigens**	**Protein vaccines**	**Polysaccharide vaccines**
P. 1	NA	CD3+CD28 normal	NA	Normal	Normal
P. 2	Normal	Low concentration CD3: defective CD3+CD28: normal	Defective	Normal	NA
P. 3	Normal	Low concentration CD3: defective CD3+CD28: normal	NA	Defective	NA
P. 4	Normal	NA	Defective	Normal	Normal
P. 5	Normal	NA	NA	NA	NA
P. 6	Normal	CD3+CD28 normal	Defective	Normal	Normal
P. 7	NA	NA	NA	NA	NA
P. 8	NA	NA	NA	NA	NA
P. 9	Normal	CD3 normal	NA	Normal	Normal
P. 10	Normal	CD3 normal	NA	Normal	Normal
P. 11	Normal	CD3 normal	NA	Normal	Normal
P. 12	Normal	CD3+CD28 normal	NA	Normal	NA
P. 13	NA	NA	NA	NA	NA
P. 14	Normal	NA	NA	Normal	Defective

Supplementary Table 8. Frequency of clinical manifestations presented in Figure 1A

Clinical manifestation	N. of patients	% of total
Infections	14	100
Bleeding	11	78
Lung infections	10	71
Skin vasculitis	9	64
Recurrent otitis media	9	64
Eczema	8	57
Enterorrhagia	7	50
Skin infections	7	50
Food allergy	5	35
Molluscum	5	35
Asthma	4	28
Skin abscesses	4	28
Arthritis	4	28
Enlarged lymph nodes	4	28
Chronic CMV	3	21
Bacterial enterocolitis	3	21
Defective response to GH stimuli	2	14
Warts	2	14
Bronchiolitis	2	14
Candida mucositis	2	14