Autoimmunity/inflammation in a monogenic primary immunodeficiency cohort

William Rae[1, 2], Daniel Ward[3, 4], Christopher J. Mattocks[3, 4], Yifang Gao[4, 5] Reuben J. Pengelly[6], Sanjay V. Patel[7], Sarah Ennis[6], Saul N. Faust[2, 7, 8], Anthony P. Williams[1, 4, 5].

[1] Department of Immunology, University Hospital Southampton NHS Foundation Trust, UK

[2] Southampton NIHR Wellcome Trust Clinical Research Facility, University of Southampton, University Hospital Southampton, Southampton, UK

[3] Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, UK

[4] Wessex Investigational Sciences Hub Laboratory, University of Southampton, University Hospital Southampton NHS Foundation Trust, UK

[5] NIHR Cancer Research UK Experimental Cancer Medicine Centre, Southampton, UK

[6] Human Genetics and Genomic Medicine, Faculty of Medicine, University of Southampton, Southampton, UK

[7] Department of Paediatric Immunology and Infectious Diseases, University Hospital Southampton NHS Foundation Trust, UK.

[8] Faculty of Medicine and Institute for Life Sciences, University of Southampton, UK

Corresponding author

Dr William Rae

Department of Immunology, University Hospital Southampton NHS Foundation Trust, UK.

Email will.rae@nhs.net

Tele +442381 204001

Fax +442381 206646

Conflicts of Interest: The authors declare no conflicts of interest

ABSTRACT

Primary immunodeficiencies (PIDs) are rare inborn errors of immunity that have a heterogeneous phenotype that can include severe susceptibility to life threatening infections from multiple pathogens, unique sensitivity to a single pathogen, autoimmune/inflammatory disease, allergies, and/or malignancy. We present a diverse cohort of monogenic PID patients with and without autoimmune/inflammatory diseases who underwent clinical, genetic, and immunological phenotyping. Novel pathogenic variants were identified in *IKBKG, CTLA4, NFKB1, GATA2, CD40LG,* and *TAZ* as well as previously reported pathogenic variants in *STAT3, PIK3CD, STAT1, NFKB2,* and *STXBP2.* Autoimmune/inflammatory manifestations were frequently encountered in PIDs, including at presentation. Autoimmunity/inflammation was multi-system in those effected and regulatory T cell percentages were significantly decreased compared with those without autoimmune/inflammatory manifestations. Prednisolone was used as the first line immunosuppressive agent in all cases, however steroid monotherapy failed long term control of autoimmunity/inflammation in the majority of cases and additional immunosuppression was required. Patients with multi-system autoimmunity/inflammation should be investigated for an underlying PID, and in those with PID early assessment of regulatory T cells may help to assess the risk of autoimmunity/inflammation.

INTRODUCTION

Primary immunodeficiencies (PIDs) encompass a collection of rare inborn errors of immunity often with broad overlapping phenotypes that include severe susceptibility to life threatening infections from multiple pathogens, unique sensitivity to a single pathogen, autoimmune/inflammatory (AI/I) disease, allergies, and/or malignancy.[1] Over 300 monogenic causes for PIDs have now been identified which has increased the diversity of clinical phenotypes that is encountered in clinical practice.[2]

Advances in the treatment and prophylaxis of infection have improved the quality of life and prognosis for patients with PID. Treatments such as immunoglobulin replacement and antimicrobial agents are now highly effective at preventing and treating infections in many PIDs. However, with the improved management of infection, AI/I are becoming an increasing cause of morbidity and mortality.[3] AI/I manifestations are frequently observed in PIDs due to inherent impairment of regulatory functions within the immune system.[4, 5] Failure to maintain self-tolerance results in self-epitope specific adaptive immune responses and autoimmunity, and failure to regulate innate immune responses results in autoinflammation in the absence of detectable self-reactive adaptive immune responses. Many PID conditions impair one or more immunological components required for immune system regulation, and AI/I manifestations are prevalent in PID cohorts across a range of monogenic PIDs.[3]

To investigate the varied presentation and frequency of AI/I diseases in PID we recruited a cohort of monogenic PID patients as classified within the 2015 International Union of Immunological Societies.[2] We evaluated the prevalence of AI/I manifestations in this cohort, and investigated whether any immunological, genetic, or phenotypic features correlated with the development of AI/I. We also describe the treatments and outcomes for the AI/I manifestations across the cohort.

RESULTS

Genetic investigations

A phenotypically heterogeneous cohort of 16 participants with monogenic PID was recruited from a single PID centre (Supplementary Information: Clinical Phenotypes). Participants underwent either whole exome sequencing, an extended PID gene panel, or targeted single gene sequencing. Novel pathogenic variants were identified in *IKBKG, CTLA4, NFKB1, GATA2, CD40LG,* and *TAZ.* Previously reported pathogenic variants were identified in *STAT3, PIK3CD, STAT1, NFKB2,* and *STXBP2* (Table 1).

Autoimmune/inflammatory manifestations

The initial clinical presentation was due to infection in 62% (10/16) of cases and AI/I disease in 38% (6/16) of cases. During follow-up, a further 3 participants developed AI/I manifestations, resulting in a total 56% (9/16) of the participants in the cohort experiencing AI/I disease that required medical intervention. Autoimmune cytopenias were the most frequently encountered AI/I complication (n = 7). Other organ specific AI/I manifestations effected the gastrointestinal (GI) (n = 4), pulmonary (n = 3), hepatic (n=2), cutaneous (n = 2), and renal (n = 1) organ systems (Table 1). AI/I disease was multi-system in all effected participants.

T cell subsets in participants with and without autoimmunity/inflammation

Participants were grouped into those without AI/I (PID –AI/I) and those with AI/I (PID +AI/I) (Supplementary Table 1). Analysis of peripheral naïve T cells (defined as $CD3^+$ $CD4^+$ or $CD8^+$, $CD27^+$, $CD45RA^+$), memory T cells (defined as $CD3^+$,$CD4^+$ or $CD8^+$, $CD27^{+/-}$, $CD45RA^-$) and effector T cells (defined as $CD3^+$, $CD4^+$ or $CD8^+$, $CD27^-$, $CD45RA^+$) was performed (Supplementary Figure 1).[6, 7] Analysis of regulatory T cells (Tregs) (defined as $CD3^+$, $CD4^+$, $CD25^+$, $CD127^{low}$) was also performed (Supplementary Figure 2). Treg percentages were significantly decreased in the PID +AI/I group compared with PID –AI/I ($p = 0.0079$) (Figure 1). The PID +AI/I group showed a trend towards increased effector CD8+ cells (Figure 1 and Supplementary Table 2) but results were not statistically significant compared with the PID –AI/I group. Other T cell subsets were not significantly different between the groups (Figure 1 and Supplementary Table 2).

Treatment interventions for autoimmunity/inflammation

Treatment inventions for AI/I manifestations were initiated based on clinical disease and symptoms. Prednisolone was used as first line immunosuppression in all participants with AI/I (n = 9) (Figure 2). Autoimmune cytopenias occurred in 7/16 participants (Table 1), and prednisolone 1mg/kg/day resulted in an initial clinical response in 7/7 participants. All 7/7 participants subsequently required additional immunomodulation due to refractory/relapsed autoimmune cytopenias during prednisolone weaning. As second line treatment for autoimmune cytopenias, 6/7 relapsed participants received rituximab and 1/7 was given immunoglobulin 2g/kg. Of the 6 participants who required rituximab, 4/6 needed a further long term steroid sparing agent due to recurrence of autoimmune cytopenias post-rituximab. Sirolimus (1 – 2.5mg/day) was the most effective steroid sparing at maintaining remission for autoimmune cytopenias in 4/4 participants.

GI AI/I manifestations partially responded to prednisolone in 4/4 participants. On weaning prednisolone GI disease returned and sirolimus did not adequately control GI AI/I in all 3/3 participants. Pulmonary disease was not controlled by prednisolone monotherapy in any of the participants and radiological and lung function continue to decline. Liver AI/I responded to prednisolone in 2/2 participants, but relapsed shortly after withdrawal in 1/2 participants.

It was observed that a specific immunosuppressive therapy often improved one organ specific AI/I complication in an individual, but failed to effectively treat other multi-system AI/I disease in the same individual. Examples of this include that a slow weaning course of prednisolone achieved complete long-term remission of the renal tubular acidosis in P10, but did not cause any clinical response in the alopecia areata. Similarly, in P7, there was a deterioration in cutaneous and GI AI/I disease whilst on sirolimus monotherapy, despite remission of autoimmune cytopenias. This mixed response necessitated an alteration in treatment to prednisolone 1mg/kg/day in combination with methotrexate (7.5mg/week) which resolved the cutaneous AI/I (Figure 2).

DISCUSSION

As the list of PIDs grows so does the number of AI/I manifestations reported.[2, 8] AI/I disease may be the major presenting symptom for a significant proportion of PID patients. As may be

expected, the prevalence of AI/I disease appears to increase with age in PID cohorts and effects a significant proportion of patients.[3] The pathophysiology that gives rise to AI/I in PIDs is varied and proposed mechanisms include; absolute lymphopenia causing a lack of regulatory lymphocytes, apoptosis defects preventing removal of self-reactive adaptive immune responses, over-activation and dysregulation of lymphocytes, defects of central tolerance, increased and unregulated type 1 interferon responses, and complement defects impairing the removal of immune complexes and cell debris.[4]

Autoimmune cytopenias are a common AI/I manifestation encountered across PIDs and reports suggest that PID is subsequently diagnosed in up to 50% of paediatric cases of refractory multi-lineage autoimmune cytopenia (Evans syndrome).[9, 10] This high prevalence of autoimmune cytopenias in PID was also apparent within our cohort with 7/16 of participants developing autoimmune cytopenia of one or more cell lineages (Table 1). Therefore 'difficult to treat' Evans Syndrome may indicate an underlying PID and is a frequent AI/I in clinical care.

AI/I diseases can affect all sub-groups classifications of PID, but is more frequently encountered in T cell defects and predominantly antibody defects, particularly common variable immunodeficiency.[1,3] Our cohort demonstrates similar characteristics with 4/5 participants with predominantly antibody deficiencies suffering AI/I (Table 1). In those with inherent T cell defects (mutations in genes that are significantly expressed in T cells: *IKBKG, STAT3, CTLA4, STAT1, STXBP2, CD40LG, TAZ* [11]) a significant proportion (4/10) also suffered AI/I (Table 1).

The broad genetic pleiotropy of PID patients covers a diverse array of AI/I manifestations. Previous cohort and case reports describe AI/I disease observed in cases of monogenic PIDs, and we outline the similarities and differences of previous reports compared with our participants phenotypes (Supplementary Information: Clinical Phenotypes).

IKBKG (*NEMO*) deficiency (OMIM 300291): P1 (*IKBKG* p.R63Q) suffered with Evans syndrome, colitis and granulomatous hepatitis. AIHA and immune thrombocytopenia (ITP) have both been reported in *IKBKG* deficiency, and colitis is a common inflammatory complication.[12-14] Hepatic

granuloma have been only been reported in hypofunctional *IKBKG* due to disseminated mycobacterial infection.[13] A liver biopsy performed on P1 found no evidence of mycobacteria or other pathogens, suggesting that the granuloma are sterile and due to immune dysregulation. Larger studies of *IKBKG* deficiency patients will help to expand the reported phenotype in this condition.

STAT3 dominant negative Hyper IgE Syndrome (OMIM 147060): P2 (*STAT3* p.G618D) and P3 (*STAT3* p.V637M), both with Hyper IgE Syndrome due to loss-of-function variants in *STAT3* and did not demonstrate any AI/I manifestations.[15] Non-infectious complications are common in Hyper IgE Syndrome, as was the case in our participants (Supplementary Information: Clinical Phenotypes) but these are not believed to have an AI/I pathophysiology. In contrast, *STAT3* GOF variants present with a phenotype of multi-system AI/I, which may support that *STAT3* LOF patients are relatively protected from AI/I.[16, 17]

PIK3CD gain-of-function, activated PI3K syndrome (OMIM 615513): P4 and P5 (both *PIK3CD* p.E1021K) showed discordance for autoimmune diseases, with P4 having no AI/I disease and P5 suffering with AIHA and lymphocytic colitis.AI/I disease is frequent in *PIK3CD* GOF patients with 42% of patients having some form of AI/I in reported cohorts.[18]

CTLA4 insufficiency (OMIM 616100): P6 (*CTLA4* p.A54T) and P7 (*CTLA4* p.V40M) both suffered with multi-system AI/I.[19, 20] The clinical phenotype of *CTLA4* insufficiency is heterogeneous with a wide range of organ specific AI/I being described in the disease. Enteropathy is reported in up to 78% of cases and was present in both P6 and P7.[19] Interstitial lung disease (ILD) was also present in P6 and is reported in 66% of *CTLA4* cases.[19] AIHA and ITP are also commonly encountered at 28% and 35% of cases respectively, and psoriasis 21% of cases[19], all of which were also present in P7.

STAT1 gain-of-function (OMIM 614162): P8.1 and P8.2 (*STAT1* p.R274Q GOF) did not develop any AI/I disease during follow-up. A large *STAT1* GOF cohort reported AI/I in 37% of patients, with a slight preponderance in female patients.[21] Thyroid disease was the most common AI/I reported (22%), but skin disease (10%) and autoimmune cytopenias (4%) were also frequently

reported. Further reports have further broadened the phenotype of *STAT1* GOF to include 'IPEX-like' presentations with multi-system AI/I.[22] The janus kinase inhibitor ruxolitinib has shown promise in targeted AI/I in *STAT1* GOF patients as a targeted immunosuppressive, as well as having benefits on chronic mucocandidasis.[23]

NFKB1 haploinsufficiency (OMIM 616576): P9.1 and P9.2 (*NFKB1* p.S302Ffs*7) both suffered AIHA which is reported in *NFKB1* haploinsufficient patients.[24, 25] Differing AI/I is observed in patients with *NKFB1* mutations, ranging from antibody deficiency, Behcet-like disease, to an autoinflammatory phenotype.[26]

NFKB2 dominant negative immunodeficiency (OMIM 615577): P10 (*NFKB2* p.R853*) suffered autoimmune alopecia which is widely reported in patients with dominant negative *NFKB2* variants but the renal disease that was present in P10 has not been reported in *NKFB2* variants to date.[27, 28] The pituitary adrenal axis is often effected in *NFKB2,* but was normal in P10, although this is not believed to be an AI/I phenomenon; instead due to hypoplasia of the anterior pituitary.[27-29] Further large scale studies are needed to catalogue the frequencies and phenotype of AI/I in *NFKB1* and *NFKB2* patients.

GATA2 haploinsufficiency (OMIM 614172): *GATA2* haploinsufficiency is described as protean disorder that may present with a variety of clinical phenotypes.[30] Phenotypes include dendritic cell, monocyte, B and NK cell deficiency with mycobacterial infections (MonoMAC), myelodysplastic syndromes, acute myeloid leukaemia and Emberger syndrome. Viral and mycobacterial infections are the most commonly encountered pathogens in *GATA2* haploinsufficiency.[30] *GATA2* deficiency usually causes cytopenias due to impaired bone marrow haematopoiesis and myelodysplasia, but the elevated levels of autoreactive peripheral $CD38^{-}$ $CD21^{-}$ B cells described in the periphery of *GATA2* patients may increase the risk of antibody mediated autoimmunity[31], and P11 (*GATA2* p.T176P) suffered with recurrent Evans syndrome. Lung involvement with alveolar proteinosis occurs in *GATA2* haploinsufficient patients due to impairment

of alveolar macrophages, but lung fibrosis has also been reported recently and was observed in P11.[32,33]

STXBP2 deficiency (OMIM 613101): P12 (*STXBP2* c.1247-1 homozygous) developed autoimmune neutropenia primary sclerosing cholangitis with dysgammaglobulinaemia, after initially presenting with haemophagocytic lymphohistiocytosis (HLH) (Supplementary Information: Clinical phenotype). Presentations of individuals with the same homozygous *STXBP2* variant 1247-1G>C have also been described with dysgammaglobulinaemia and autoimmune liver involvement in the absence of HLH.[34, 35]

CD40LG deficiency (OMIM 308230): P13 (*CD40LG* p.A141P) presented with raised IgM, absent IgG and IgA and necrotic pseudomonal tonsillitis. Stimulated $CD4^+$ T cells showed absent expression of CD40L on the cell surface. *CD40LG* deficient patients frequently develop autoimmunity, however P13 has no evidence of AI/I disease to date. At odds with reports of reduced Treg frequency in CD40LG patients, P13 has raised Tregs at 15.4% (Supplementary Table 3) which may be relatively protective against AI/I development in this case.[36]

TAZ deficiency (OMIM 302060): P14 (*TAZ* p.K220E) has significant T cell lymphopenia which is one aetiology believed to predispose to AI/I disease in PID.[4] The intrinsic apoptosis pathway is also defective in Barth Syndrome due to impairment of mitochondria initiation of apoptosis.[37] Despite these potential mechanistic risks for AI/I development,[4] AI/I are not widely reported in Barth Syndrome patients. Recently *TAZ* has been described to regulate Th17 and Treg development, and *TAZ* deficient lymphocytes show impaired Th17 and increased Treg differentiation. [38] This lymphocyte defect may protect Barth Syndrome patients from AI/I disease.

These previous reports and comparisons with our cohort illustrate the prevalence and heterogeneity of AI/I that is encountered in the clinical care of patients with PID. It is also apparent that multi-system AI/I is frequent in PID, and that patients presenting with complex multi-sytem AI/I should be investigated for PID.

The need to identify markers of impending AI/I in PID has long been recognised[39]. Tregs appeared reduced across our cohort of PID with AI/I, and may present a potential indicator for the risk of developing AI/I in patients. However further work is required with larger studies to confirm these findings. Due to the heterogeneity of PID there are also limitations of this approach when applied to individual cases, such as raised Treg percentages with impaired function in cases of *CTLA4* insufficient patients with AI/I.

Decisions on treatment options for AI/I in PIDs are challenging due to the inherent risks of iatrogenic immunosuppression in immunocompromised individuals. Multi-system AI/I poses further challenges, as one AI/I manifestation may respond to a therapy, whereas another can remain refractory to the same therapy. It is hoped that 'precision medicines' targeted to the underlying genetic abnormality will provide a more holistic therapeutic option for multi-system AI/I.[10, 40, 41] Currently, due to the rarity of individual monogenic PIDs, there is a relative lack of large scale studies of these precision treatments, and financial limitations within healthcare systems still limit the wide scale adoption of precision medicine at the bedside.

Our experience of a heterogeneous cohort of PID patients suggests that for autoimmune cytopenias, first line prednisolone, second line rituximab and third line sirolimus is an effective treatment regime. This is a similar treatment pathway to that described for Evans syndrome in non-PID patients, autoimmune lymphoproliferative syndrome,[42, 43] and common variable immunodeficiency,[44] indicating that this regime can be extrapolated across PIDs with autoimmune cytopenia. Several guidelines for the treatment of autoimmune cytopenias include mycophenolate mofetil as the second line agent within treatment algorithms.[45, 46] Whilst mycophenolate is often including in treatment pathways, our experience of severe autoimmune cytopenias in PID is that sirolimus appears more efficacious in difficult to treat cytopenias associated with PID. Prednisolone monotherapy appears ineffective at long term control of AI/I conditions in PID. Organ specific AI/I disease in PID often requires additional immunosuppression, such as rituximab and mycophenolate in pulmonary disease to produce a clinical benefit.[47, 48] Therefore, when considering therapeutic immunosuppression it appears that the site/tissue effected by AI/I should influence treatment choices.

In conclusion multi-system AI/I manifestations are frequently encountered across a range of monogenic PIDs in clinical care. Multi-system AI/I present in PID makes treatment options challenging, and steroid monotherapy appears ineffective in the longer term for many AI/I diseases in PID. There still remains a need to develop methods of pre-empting AI/I in PID, and although Tregs were reduced in those with AI/I there are caveats to this and further studies are needed to confirm these findings.

METHODS

Human samples

Whole blood EDTA and lithium heparinised samples were collected from controls and patients with PID at a single centre. All participants with PID had monogenic diagnoses of PID listed in the International Union of Immunological Societies classification.[2] Informed consent was obtained from all participants included in the study. All studies were approved by IRB (REC reference 12/NW/0794).

Lymphocyte phenotyping

Whole blood lymphocyte immunophenotyping was performed by flow cytometry on a FACS Canto II (BDBioscience, CA, USA). T, B, and NK cell phenotyping was performed using CD45-PerCP-Cy5.5 (clone 2D1), CD3-FITC (clone SK7), CD4-PE-Cy7 (clone SK3), CD8-APC-Cy7 (clone SK1), CD19-APC (clone SJ25C1), CD16-PE (clone B73.1), CD56-PE (clone NCAM 16.2). T cell memory phenotyping; CD3-PerCP-Cy5.5 (clone 2D1), CD4 PE-Cy7 (clone SK3), CD8-APC (clone SK1), CD27-PE (clone L128), CD45RA-FITC (clone L48). B cell memory phenotyping; CD19-FITC (clone SJ25C1), CD27-APC (clone L128), IgM-PE (clone SA-DA4, Beckman Coulter, CA, USA). αβ and γδ T cells were assessed using CD3-PerCP-Cy5.5 (clone 2D1), αβ TCR-FITC (clone WT31), γδ TCR-PE (clone 11F2). Tregs were phenotyped with CD3-PerCP-Cy5.5 (clone 2D1), CD4-APC (clone SK3), CD25-PE (clone 2A3), CD127-BV450 (clone HIL-7R-M21) (all BDBioscience, CA, USA). Flow cytometry plots for naïve ($CD3^+$ $CD4^+$ or $CD8^+$, $CD27^+$, $CD45RA^+$), memory ($CD3^+$,$CD4^+$ or

272 $CD8^+$, $CD27^{-/+}$, $CD45RA^-$), effector ($CD3^+$, $CD4^+$ or $CD8^+$, $CD27^-$, $CD45RA^+$) T cells and Tregs
273 ($CD3^+$, $CD4^+$, $CD25^+$ $CD127^{low}$) were analysed using FlowJo (LLC, OR, USA).

274 **Immunoglobulins and antibody responses**

275 Immunoglobulin (Ig) G, IgA, IgM and IgE were assessed by nephelometry according to
276 manufacturer's instructions (Beckman Coulter, CA, USA). Pneumococcal and tetanus IgG responses
277 were assessed by commercial ELISA according to the manufacturer's instructions (Binding Site,
278 Birmingham, UK).

279 **Genetic analysis**

280 DNA was extracted from EDTA blood samples using QIAamp DNA blood mini kit (Qiagen,
281 Hilden, DE) according to the manufacturer's instructions. DNA quality was checked by Nanodrop
282 spectrometry (Thermofisher, MA, USA). Genetic analysis was performed by whole exome
283 sequencing (P5, P8.1, P8.2, P10), using the TruSight One panel kit (Illumina, CA, USA) (P1, P4, P6,
284 P7, P9, P11, P12) and by single candidate gene analysis (P2, P3). Data was processed according to
285 GATK best practice guidelines and aligned to GRCh37/hg19 reference genome. Variants identified in
286 this study have been submitted to ClinVar NCBI.

287 Variant interrogation was performed using in silico predictive tools Polyphen2 [49], SIFT [50], and
288 Exome Aggregation Consortium (ExAC) [51], supported by Sapientia (Congenica, Cambridge, UK) and
289 Ensembl [52]. Variants pathogenicity was grading according to the American College of Medical
290 Genetics (ACMG) criteria [53].

291 **Participant grouping**

292 Participants were grouped into those with PID and AI/I manifestations (PID +AI/I) and those
293 without AI/I (PID –AI/I). Both groups had similar characteristics including mean age (Supplementary
294 Table 1).

295 **Clinical responses**

Clinical responses were graded similarly to previous studies.[54] Remission = complete normalisation of laboratory parameters and/or complete resolution of clinical symptoms. Partial response = improvement to near normal laboratory parameters with stabilisation of results and/or improvement in clinical manifestations (e.g. reduction in diarrhoea frequency). Relapse = little or no improvement in laboratory parameters and/or no improvement in clinical symptoms (e.g. diarrhoea frequency, skin inflammation) and/or no improvement/progressive deterioration in imaging (e.g. increased infiltrates in lungs, reducing lung function).

Statistical analysis

Due to skewed distributions of T cell subsets (Supplementary Figure 3), unpaired Mann Whitney U test was used for analysis (GraphPad Software. La Jolla, USA). $p < 0.05$ was used as significance cut-off. Graphs display Mann Whitney U Ranks (Figure 1). Data distribution was calculated using SPSS v27 (IBM, USA) (Supplementary Figure 3). Figures were created using Prism: GraphPad (LA Jolla, USA).

ACKNOWLEDGEMENTS

We thank the patients and their families for participating in this study. WR is funded/supported by the National Institute for Health Research Rare Diseases Translational Research Collaboration (NIHR RD-TRC) and the Southampton NIHR Wellcome Trust Clinical Research Facility. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. This work was supported by the Wessex Investigational Sciences Hub at the University of Southampton and University Hospital Southampton NHS Foundation Trust.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

Supplementary information is available at Clinical and Translational Immunology's website.

REFERENCES

1. Bousfiha A, Jeddane L, Al-Herz W, Ailal F, Casanova JL, Chatila T, et al. The 2015 IUIS Phenotypic Classification for Primary Immunodeficiencies. *J Clin Immunol* 2015; **35**: 727-738.

2. Picard C, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. *J Clin Immunol* 2015; **35**: 696-726.

3. Fischer A, Provot J, Jais JP, Alcais A, Mahlaoui N, group motCFPs. Autoimmune and inflammatory manifestations occur frequently in primary immunodeficiencies. *J Allergy Clin Immunol* (in press).

4. Grimbacher B, Warnatz K, Yong PF, Korganow AS, Peter HH. The crossroads of autoimmunity and immunodeficiency: Lessons from polygenic traits and monogenic defects. *J Allergy Clin Immunol* 2016; **137**: 3-17.

5. Allenspach E, Torgerson TR. Autoimmunity and Primary Immunodeficiency Disorders. *J Clin Immunol* 2016; **36**: 57-67.

6. Hamann D, Baars PA, Rep MH, Hooibrink B, Kerkhof-Garde SR, Klein MR, et al. Phenotypic and functional separation of memory and effector human CD8+ T cells. *J Exp Med* 1997; **186**: 1407-1418.

7. Schatorjé EJ, Gemen EF, Driessen GJ, Leuvenink J, van Hout RW, de Vries E. Paediatric reference values for the peripheral T cell compartment. *Scand J Immunol* 2012; **75**: 436-444.

8. Azizi G, Pouyani MR, Abolhassani H, Sharifi L, Dizaji MZ, Mohammadi J, et al. Cellular and molecular mechanisms of immune dysregulation and autoimmunity. *Cell Immunol* 2016; **310**: 14-26.

9. Teachey DT, Manno CS, Axsom KM, Andrews T, Choi JK, Greenbaum BH, et al. Unmasking Evans syndrome: T-cell phenotype and apoptotic response reveal autoimmune lymphoproliferative syndrome (ALPS). *Blood* 2005; **105**: 2443-2448.

10. Walter JE, Farmer JR, Foldvari Z, Torgerson TR, Cooper MA. Mechanism-Based Strategies for the Management of Autoimmunity and Immune Dysregulation in Primary Immunodeficiencies. *J Allergy Clin Immunol Pract* 2016; **4**: 1089-1100.

11. Heng TS, Painter MW, Consortium IGP. The Immunological Genome Project: networks of gene expression in immune cells. *Nat Immunol* 2008; **9**: 1091-1094.

12. Ramírez-Alejo N, Alcántara-Montiel JC, Yamazaki-Nakashimada M, Duran-McKinster C, Valenzuela-León P, Rivas-Larrauri F, et al. Novel hypomorphic mutation in IKBKG impairs NEMO-ubiquitylation causing ectodermal dysplasia, immunodeficiency, incontinentia pigmenti, and immune thrombocytopenic purpura. *Clin Immunol* 2015; **160**: 163-171.

13. Huppmann AR, Leiding JW, Hsu AP, Raffeld M, Uzel G, Pittaluga S, et al. Pathologic Findings in NEMO Deficiency: A Surgical and Autopsy Survey. *Pediatr Dev Pathol* 2015; **18**: 387-400.

14. Klemann C, Pannicke U, Morris-Rosendahl DJ, Vlantis K, Rizzi M, Uhlig H, et al. Transplantation from a symptomatic carrier sister restores host defenses but does not prevent colitis in NEMO deficiency. *Clin Immunol* 2016; **164**: 52-56.

15. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. *N Engl J Med* 2007; **357**:1608-1619.

16. Flanagan SE, Haapaniemi E, Russell MA, Caswell R, Lango Allen H, De Franco E, et al. Activating germline mutations in STAT3 cause early-onset multi-organ autoimmune disease. *Nat Genet* 2014; **46**: 812-814.

17. Vogel TP, Milner JD, Cooper MA. The Ying and Yang of STAT3 in Human Disease. *J Clin Immunol* 2015; **35**: 615-623.

18. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: A large patient cohort study. *J Allergy Clin Immunol* 2016; **139**: 597-606.

19. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. *Nat Med* 2014; **20**: 1410-1416.

20. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT, et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. *Science* 2014; **345**: 1623-1627.

21. Toubiana J, Okada S, Hiller J, Oleastro M, Lagos Gomez M, Aldave Becerra JC, et al. Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. Blood. 2016;**127**(25):3154-3164.

22. Uzel G, Sampaio EP, Lawrence MG, Hsu AP, Hackett M, Dorsey MJ, et al. Dominant gain-of-function STAT1 mutations in FOXP3 wild-type immune dysregulation-polyendocrinopathy-enteropathy-X-linked-like syndrome. *J Allergy Clin Immunol* 2013; **131**: 1611-1623.

23. Weinacht KG, Charbonnier LM, Alroqi F, Plant A, Qiao Q, Wu H, et al. Ruxolitinib reverses dysregulated T helper cell responses and controls autoimmunity caused by a novel signal transducer and activator of transcription 1 (STAT1) gain-of-function mutation. *J Allergy Clin Immunol* 2017; **139**: 1629-1640.

24. Fliegauf M, Bryant VL, Frede N, Slade C, Woon ST, Lehnert K, et al. Haploinsufficiency of the NF-κB1 Subunit p50 in Common Variable Immunodeficiency. *Am J Hum Genet* 2015; **97**: 389-403.

25. Schipp C, Nabhani S, Bienemann K, Simanovsky N, Kfir-Erenfeld S, Assayag-Asherie N, et al. Specific antibody deficiency and autoinflammatory disease extend the clinical and immunological spectrum of heterozygous NFKB1 loss-of-function mutations in humans. *Haematologica* 2016; **101**: e392-e396.

26. Kaustio M, Haapaniemi E, Göös H, Hautala T, Park G, Syrjänen J, et al. Damaging heterozygous mutations in NFKB1 lead to diverse immunologic phenotypes. *J Allergy Clin Immunol* (in press).

27. Chen K, Coonrod EM, Kumánovics A, Franks ZF, Durtschi JD, Margraf RL, et al. Germline mutations in NFKB2 implicate the noncanonical NF-κB pathway in the pathogenesis of common variable immunodeficiency. *Am J Hum Genet* 2013; **93**: 812-824.

28. Brue T, Quentien MH, Khetchoumian K, Bensa M, Capo-Chichi JM, Delemer B, et al. Mutations in NFKB2 and potential genetic heterogeneity in patients with DAVID syndrome, having variable endocrine and immune deficiencies. *BMC Med Genet* 2014; **15**: 139.

29. Lindsley AW, Qian Y, Valencia CA, Shah K, Zhang K, Assa'ad A. Combined immune deficiency in a patient with a novel NFKB2 mutation. *J Clin Immunol* 2014; **34**: 910-915.

30. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood* 2014; **123**: 809-821.

31. Dickinson RE, Milne P, Jardine L, Zandi S, Swierczek SI, McGovern N, et al. The evolution of cellular deficiency in GATA2 mutation. *Blood* 2014; **123**: 863-874.

32. Lasbury ME, Tang X, Durant PJ, Lee CH. Effect of transcription factor GATA-2 on phagocytic activity of alveolar macrophages from Pneumocystis carinii-infected hosts. *Infect Immun* 2003; **71**: 4943-4952.

33. Ballerie A, Nimubona S, Meunier C, Gutierrez FL, Desrues B, Delaval P, et al. Association of pulmonary alveolar proteinosis and fibrosis: patient with GATA2 deficiency. *Eur Respir J* 2016; **48**: 1510-1514.

34. Esmaeilzadeh H, Bemanian MH, Nabavi M, Arshi S, Fallahpour M, Fuchs I, et al. Novel Patient with Late-Onset Familial Hemophagocytic Lymphohistiocytosis with STXBP2 Mutations Presenting with Autoimmune Hepatitis, Neurological Manifestations and Infections Associated with Hypogammaglobulinemia. *J Clin Immunol* 2015; **35**: 22-25.

35. Maffucci P, Filion CA, Boisson B, Itan Y, Shang L, Casanova JL, et al. Genetic Diagnosis Using Whole Exome Sequencing in Common Variable Immunodeficiency. *Front Immunol* 2016; **7**: 220.

36. Hervé M, Isnardi I, Ng YS, Bussel JB, Ochs HD, Cunningham-Rundles C, et al. CD40 ligand and MHC class II expression are essential for human peripheral B cell tolerance. *J Exp Med* 2007; **204**: 1583-1593.

37. Gonzalvez F, D'Aurelio M, Boutant M, Moustapha A, Puech JP, Landes T, et al. Barth syndrome: cellular compensation of mitochondrial dysfunction and apoptosis inhibition due to changes in cardiolipin remodeling linked to tafazzin (TAZ) gene mutation. *Biochim Biophys Acta* 2013; **1832**: 1194-1206.

38. Geng J, Yu S, Zhao H, Sun X, Li X, Wang P, et al. The transcriptional coactivator TAZ regulates reciprocal differentiation of TH17 cells and Treg cells. *Nat Immunol* 2017; epub ahead of print 15 May 2017; doi:10.1038/ni.3748

431 39. Mackay IR, Leskovsek NV, Rose NR. The odd couple: a fresh look at autoimmunity and
432 immunodeficiency. *J Autoimmun* 2010; **35**: 199-205.
433 40. Lenardo M, Lo B, Lucas CL. Genomics of Immune Diseases and New Therapies. *Annu Rev*
434 *Immunol* 2016; **34**: 121-149.
435 41. Zhang Y, Su HC, Lenardo MJ. Genomics is rapidly advancing precision medicine for
436 immunological disorders. *Nat Immunol* 2015; **16**: 1001-1004.
437 42. Rao VK. Approaches to Managing Autoimmune Cytopenias in Novel Immunological
438 Disorders with Genetic Underpinnings Like Autoimmune Lymphoproliferative Syndrome. *Front*
439 *Pediatr* 2015; **3**: 65.
440 43. Bride KL, Vincent T, Smith-Whitley K, Lambert MP, Bleesing JJ, Seif AE, et al. Sirolimus is
441 effective in relapsed/refractory autoimmune cytopenias: results of a prospective multi-institutional
442 trial. *Blood* 2016; **127**: 17-28.
443 44. Gobert D, Bussel JB, Cunningham-Rundles C, Galicier L, Dechartres A, Berezne A, et al.
444 Efficacy and safety of rituximab in common variable immunodeficiency-associated immune
445 cytopenias: a retrospective multicentre study on 33 patients. *Br J Haematol* 2011; **155**: 498-508.
446 45. Podjasek JC, Abraham RS. Autoimmune cytopenias in common variable immunodeficiency.
447 *Front Immunol* 2012; **3**: 189.
448 46. Miano M, Scalzone M, Perri K, Palmisani E, Caviglia I, Micalizzi C, et al. Mycophenolate
449 mofetil and Sirolimus as second or further line treatment in children with chronic refractory Primitive
450 or Secondary Autoimmune Cytopenias: a single centre experience. *Br J Haematol* 2015; **171**: 247-
451 253.
452 47. Jolles S, Carne E, Brouns M, El-Shanawany T, Williams P, Marshall C, et al. FDG PET-CT
453 imaging of therapeutic response in granulomatous lymphocytic interstitial lung disease (GLILD) in
454 common variable immunodeficiency (CVID). *Clin Exp Immunol* 2017; **187**: 138-145.
455 48. Chase NM, Verbsky JW, Hintermeyer MK, Waukau JK, Tomita-Mitchell A, Casper JT, et al.
456 Use of combination chemotherapy for treatment of granulomatous and lymphocytic interstitial lung
457 disease (GLILD) in patients with common variable immunodeficiency (CVID). *J Clin Immunol* 2013;
458 **33**: 30-39.

459 49. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method
460 and server for predicting damaging missense mutations. *Nat Methods* 2010; **7**: 248-249.
461 50. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on
462 protein function using the SIFT algorithm. *Nat Protoc* 2009; **4**: 1073-1081.
463 51. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of
464 protein-coding genetic variation in 60,706 humans. *Nature* 2016; **536**: 285-291.
465 52. Yates A, Akanni W, Amode MR, Barrell D, Billis K, Carvalho-Silva D, et al. Ensembl 2016.
466 *Nucleic Acids Res* 2016; **44**: 710-716.
467 53. Kleinberger J, Maloney KA, Pollin TI, Jeng LJ. An openly available online tool for
468 implementing the ACMG/AMP standards and guidelines for the interpretation of sequence variants.
469 *Genet Med* 2016; **18**: 1165.
470 54. Boursiquot JN, Gérard L, Malphettes M, Fieschi C, Galicier L, Boutboul D, et al.
471 Granulomatous disease in CVID: retrospective analysis of clinical characteristics and treatment
472 efficacy in a cohort of 59 patients. *J Clin Immunol* 2013; **33**: 84-95.

473

474 **TABLE AND FIGURE LEGENDS**

475 **Table 1.** Genetic, infection and AI/I characteristics in the PID cohort. *S. Aureus = Staphylococcus*
476 *Aureus, S. Pneumoniae = Streptococcus Pneumoniae, S. Agalactiae = Streptococcus Agalactiae, H.*
477 *Influenzae = Haemophilus Influenzae, H. Parainfluenzae = Haemophilus Parainfluenzae, M.*
478 *Catarrhalis = Moraxella Catarrhalis, N. meningitidis = Neisseria Meningitidis.*

479 **Figure 1.** T cell subgroups compared between the groups, PID without AI/I (PID –AI/I) and PID with
480 AI/I (PID +AI/I) (median and interquartile range). Tregs were significantly reduced in PID +AI/I
481 compared with PID –AI/I ($p = 0.0079$). n =2. * = $p < 0.01$.

482 **Figure 2**. Diagram illustrating the treatments for AI/I manifestations within the cohort. Participants
483 had multi-system AI/I and often treatments were only efficacious for a single AI/I manifestation in
484 individuals. Prednisolone monotherapy appeared ineffective for the majority of AI/I conditions

encountered in PID. Remission = Complete normalisation of laboratory parameters and/or clinical symptoms, partial response = improvement to near normal and stabilisation in laboratory parameters and/or clinical symptoms, relapse = no improvement/continue deterioration in laboratory parameters and/or clinical symptoms.