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Molecular diagnosis of childhood immune dysregulation, polyendocrinopathy and enteropathy, and implications for clinical management

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Abstract

Background.—Most patients with childhood-onset immune dysregulation, polyendocrinopathy and enteropathy have no genetic diagnosis for their illness. These patients may undergo empirical immunosuppressive treatment with highly variable outcomes.

Objective.—To determine the genetic basis of disease in patients referred with "IPEX-like" disease, but with no mutation in *FOXP3*; then to assess consequences of genetic diagnoses for clinical management.

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Methods.—Genomic DNA was sequenced using a panel of 462 genes implicated in inborn errors of immunity. Candidate mutations were characterized by genomic, transcriptional, and (for some) protein analysis.

Results: Of 123 patients with *FOXP3*-negative IPEX-like disease, 48 (39%) carried damaging germline mutations in one of 27 genes including *AIRE, BACH2, BCL11B, CARD11, CARD14, CTLA4, IRF2BP2, ITCH, JAK1, KMT2D, LRBA, MYO5B, NFKB1, NLRC4, POLA1, POMP, RAG1, SH2D1A, SKIV2L, STAT1, STAT3, TNFAIP3, TNFRSF6/FAS, TNRSF13B/TACI, TOM1, TTC37*, and *XIAP.* Many of these had not been previously associated with an IPEX-like diagnosis. For 42 of the 48 patients with genetic diagnoses, knowing the critical gene may have altered therapeutic management, including recommendations for targeted treatments and for or against hematopoietic cell transplantation.

Conclusion: Many childhood disorders now bundled as “IPEX-like” disease are caused by individually rare, severe mutations in immune regulation genes. Most genetic diagnoses of these conditions yield clinically actionable findings. Barriers are lack of testing or lack of repeat testing if older technologies failed to provide a diagnosis.

Clinical Implication: Pediatric immune dysregulation would benefit from a genetics-first approach to diagnosis: for >80% of these patients with genetic diagnoses, the genetic information offers critical guidance to clinical management.

CAPSULE SUMMARY

Immune dysregulation, polyendocrinopathy or enteropathy in many pediatric patients results from a mutation with severe clinical effect. Identification of these causal mutations enables treatment based on genotype, supporting a genetics-first approach to diagnosis.

Keywords

Immune dysregulation; molecular diagnosis; genetics; sequencing; pediatric; precision medicine; autoimmunity; inborn errors of immunity; primary immunodeficiency disorders

INTRODUCTION

Clinical presentations of immune dysregulation in children are notoriously complex[1,2,3]. Genetic diagnoses can help disentangle this complexity and can also guide clinical management of these patients, suggesting targeted therapies or prompting hematopoietic cell transplantation (HCT)[4]. Despite excellent studies revealing genetic causes of primary immunodeficiency diseases[5], there are as yet no widely accepted recommendations for genetic testing of pediatric patients with immune dysregulation. Even now, a decade after next-generation sequencing became widespread, genetic testing for inborn errors of immunity often proceeds only following functional testing and in targeted panels, with the choice of gene frequently based on phenotype and serology. This approach misses genetic diagnoses of a large proportion of patients.

Among inborn errors of immunity, IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked) syndrome, caused by mutations in *FOXP3,* a lineage-defining gene in regulatory T cells, is a prototype of disorders affecting regulation of immune

responses[6,7]. IPEX manifests most commonly with severe diarrhea, dermatitis, and autoimmune endocrinopathies such as type 1 diabetes or thyroid disease[8]. However, these clinical features are widespread in pediatric diseases of immune dysregulation, and many patients with these symptoms have no mutation in *FOXP3*. These patients have been termed "IPEX-like," a distinction that is clinically significant, because patients with *FOXP3* mutations usually undergo HCT[9], whereas patients with IPEX-like syndrome are generally monitored and treated with immunomodulatory treatments aimed at the affected organ system. Patients with IPEX-like syndrome receive transplantation only if their disease progresses and becomes life-threatening. If HCT is ultimately undertaken, the delay in treatment can result in higher morbidity and mortality.

The scope, prevalence, and distribution of genes and mutations responsible for *FOXP3*-negative pediatric immune dysregulation, polyendocrinopathy or enteropathy (IPE) are not known. The goal of this project was to determine the underlying genetic causes of these conditions in pediatric patients with no mutation in *FOXP3*, to assess the diagnostic yield of genetic testing in these patients, and to evaluate the implications of genetic diagnosis for treatment.

METHODS

Study subjects

The study was approved by the institutional review boards of Seattle Children's Hospital (SCH) and the University of Washington (UW). Patients were eligible for the study if referred to SCH with clinical signs suggestive of IPEX, and hence for genetic analysis of *FOXP3*, but with no *FOXP3* mutation identified. DNA was available for 123 such patients.

Gene panel development

We designed an oligonucleotide-based sequencing panel encompassing the coding exons, 5' and 3' untranslated regions, and flanking intronic regions of 462 known and candidate genes for immune-mediated disease (Table EI). Genes were chosen from the classification of immune genes by the International Union of Immunological Societies (IUIS)[10], and by review of the literature and consensus expert opinion of colleagues from immunology, rheumatology, and genetics. Of the 462 genes, 337 genes harbor mutations leading to inborn errors of immunity in humans; the other 125 genes are involved in immune tolerance, many with compelling murine models. Total length of the targeted genomic region was 1.5 MB. The panel enables simultaneous identification of single base pair mutations, small insertions and deletions, and exon-impacting copy number variants (CNVs) for all targeted genes. The panel was validated by blinded analysis of 33 patients with independently identified mutations. All genotypes were concordant with previous results. In addition to its research use, the panel is now in clinical use by the UW Department of Laboratory Medicine[11].

Genomics

Genomic DNA was isolated from whole blood, from peripheral blood mononuclear cells (PBMCs), or from expanded PBMCs. For each sample, 500–750ng DNA was captured using the oligonucleotide panel. Molecular barcodes were added after hybridization, and 32–48

samples were multiplexed and sequenced in a single flow-cell on an Illumina HiSeq2500 instrument to obtain 100bp paired-end reads at >400x median coverage. Identification of variants was carried out as previously described[12]. Copy number variants (CNVs) were confirmed by TaqMan analysis and breakpoints identified by whole genome sequencing, also as described[12].

Interpretation of genetic variants

Variant interpretation was based on the guidelines of the American College of Medical Genetics (ACMG)[13], as applied to conditions of pediatric immune dysregulation. As described above, the challenges of these conditions are, on the one hand, phenotypes defined by an exceptionally wide range of clinical presentations and, on the other hand, candidate genes with an exceptionally wide range of biological functions, with no straightforward alignment of phenotypes and genes. For each patient, candidate variants were evaluated by several criteria and experiments: by *in silico* predictive tools; by published functional studies; by similarities of clinical presentations of our patient with any patients previously reported with mutations in the same gene; by transcriptional analysis in our lab when appropriate and feasible[14]; and by testing for *de novo* inheritance, if DNA from parents could be obtained. For each variant that we reported as pathogenic, likely pathogenic, or of unknown significance (VUS), we provided a narrative explanation of published evidence and of experiments carried out in our lab, as well as reporting *in silico* predictions and prior classification by ClinVar[15]. The VUS classification was used sparingly, only for mutations with plausible but uncertain links to the patient's phenotype, for which further functional or genetic studies could add to evidence for or against causality.

RESULTS

Genetic diagnoses

Demographic and clinical features of the cohort.—Demographic and clinical characteristics of the 123 patients are shown in Table I. Most patients were male, consistent with original referral for X-linked IPEX syndrome. Patients were referred by physicians from six continents and represented a wide variety of ancestral populations, identified by self-report and by ancestral SNPs[16]. Clinical diagnoses were heterogeneous, including enteropathy, dermatitis, autoimmune hemolytic anemia, type I diabetes, and other autoimmune conditions.

Genomic sequencing.—Genomic analysis of DNA samples from the 123 patients yielded median coverage across the targeted region of 406X, with 95.9% of targeted bases having >40X coverage and 99.1% of targeted bases having >8X coverage. This depth of coverage enabled identification of point mutations and small insertions and deletions in coding or regulatory regions and exon-impacting CNVs.

Frequencies and features of genetic diagnoses.—Genomic analysis yielded genetic diagnoses for 39% of patients (48 of 123), involving 27 different genes (Table II). All variants considered pathogenic, likely pathogenic, or of unknown significance but warranting further study, are indicated in Table III, with evidence bearing on causality

for the child's phenotype. Of the 28 different conditions in the 48 patients with genetic diagnoses, 19 conditions were inherited as autosomal dominant, 7 as autosomal recessive, and 2 as X-linked recessive. Damaging mutations included truncating, splice altering, and missense mutations, each private or extremely rare (Table III). Five patients carried multi-kilobase genomic deletions or amplifications in single genes (Figs E1, E2). Of the 56 different variants contributing to these 48 genetic diagnoses, 36 appeared for the first time in a patient in this series and 20 had been previously reported[15] (Table III). Of the 58 reported variants, 52 were classified as pathogenic or likely pathogenic and 4 as VUS with recommendation for further functional analysis (Table III). Proportions of patients with genetic diagnoses were similar for males (37/94, or 39%) and for females (11/29, or 38%). Average age at referral for patients with a genetic diagnosis was 6.2y and for patients with no genetic diagnosis was 6.6y.

Biological functions of genes mutant in patients

Mutant genes in the IUIS classification system.—Genes responsible for patients' diagnoses are involved in both adaptive and immune response (Table II), and in biological functions including signaling, cell differentiation, apoptosis, debris handling, and epithelial integrity (Figure 1). The 27 genes appeared in multiple categories of the phenotypic classification system for inborn errors of immunity of the International Union of Immunological Societies' (IUIS) (Table II)[10]. Causal genes in the IUIS Immune Dysregulation category would be expected, given original clinical suspicion for IPEX. However, an equal number of genetic diagnoses were due to genes in other IUIS phenotypic categories, including Autoinflammatory Disorders, Antibody Deficiencies, Combined Immunodeficiencies, and Defects in Intrinsic and Innate Immunity. Most of the 27 genes were not previously reported in the context of "IPEX-like" disease.

For some genes, the mutations of these patients provide additional support for the role of the genes in IPE (Tables II, III). For example, somatic mutations in the *JAK1* kinase are common in tumors, but a germline mutation in *JAK1* has previously been documented in only one family[17]. Patients S66, S67, and S170, with severe IPE phenotypes, carry three different damaging mutations in *JAK1*, strongly supporting a role for *JAK1* germline mutations in IPE (Figure S2). Similarly, *IRF2BP2* encodes a transcriptional regulator of type I interferon and has a well-documented role in immune regulation, angiogenesis, and apoptosis[18,19], but a germline mutation in *IRF2BP2* has been previously documented in only one family[20]. Patient S125, with a similar phenotype to the previously reported patient, carries a *de novo* damaging mutation in *IRF2BP2*, supporting a role for this gene in IPE.

Two genes responsible for diagnoses of our patients were not previously included in the IUIS classification. *TOM1* encodes a protein of endocytosis, with a missense mutation previously reported in a family with autosomal dominant immune dysregulation and impaired autophagy[21]. Patient S15, with a severe IPE phenotype, is heterozygous for a *TOM1* splice mutation that produces a stable product lacking the vesicular trafficking domain, so likely yielding a dominant negative effect (Figure E3). Mutations in *MYO5B* are well documented in patients with microvillus inclusion disease[22]. Like patient SDH, patients

with *MYO5B* deficiency can have extraintestinal manifestations, including hematuria, lung disease, and increased susceptibility to infection[23].

Immune regulation and dysmorphology.—For four patients, immune dysregulation appeared in combination with dysmorphology. Three patients with mildly dysmorphic features harbored mutations in *TTC37* or *SKIV2L*, both of which are responsible for tricho-hepato-enteric (THE) syndrome[24,25]. One patient, with a splice mutation in *KMT2D*, presented with features of Kabuki syndrome in addition to autoimmune dysregulation and severe enteropathy, both of which are rare but reported features of Kabuki syndrome[26,27]. Genetic analysis is quite frequently undertaken for patients with dysmorphic features. However, for several patients with mutations in dysmorphism genes, these features were subtle and not recognized until after the genetic diagnosis.

Genetic diagnoses and treatment

For 42 of the 48 patients with genetic diagnoses, knowing the critical gene could have guided treatment (Figure 2). Specific management implications for each patient with a genetic diagnosis are indicated in Table EII. Some patients for whom the causal gene was identified could have been treated with appropriately targeted immune modulatory drugs. Patients whose genetic diagnoses suggested life-limiting disease may have been considered for HCT early in their disease course. Conversely, patients with genetic diseases having less severe outcomes or no effective therapies may have avoided high-risk treatments.

Targeted therapeutics.—For 25 patients, genetic diagnoses suggested specific targeted therapies. For example, abatacept, a CTLA-Ig fusion protein, has been reported to be an effective targeted therapy for patients with *CTLA4* haploinsufficiency or *LRBA* deficiency[28]. JAK inhibitors have been shown to be effective in patients with *JAK1, STAT1,* or *STAT3* gain-of-function mutations[29]. Patients with *NLRC4* gain-of-function mutations have been treated successfully in preliminary studies with recombinant IL-18-binding protein[30], and a clinical trial is underway evaluating this treatment in patients with *XIAP* mutations[31]. Patients with *CARD14* and *TNFAIP3* mutations often respond to TNF-alpha inhibitors and to IL12/IL23 inhibitors[32,33].

Screening.—For 29 patients, genetic diagnoses would have altered recommendations for screening. Loss of function mutations in *STAT3*, *FAS*, and *SH2D1A* predispose to lymphoma, and gain of function mutations in STAT3 predispose to leukemia[34]. Patients with *CLTA4* haploinsufficiency and *LRBA* deficiency also appear to be at a higher risk for cancer[35,36]. In our series, two of four patients with *LRBA* deficiency developed malignancies: patient S45 died at age 16 from gastric adenocarcinoma, and patient S14 developed lymphoma at age 3. Patients with mutations in *AIRE, RAG1* and *NFKB1* can develop a broad range of autoimmune phenomena that call for regular thyroid, blood, liver, renal, and pulmonary screening[2,37,38.] Patients with *STAT3* mutations should undergo pulmonary screening[39], and although there are limited cases reported, current evidence also supports pulmonary screening for patients with mutations in *BACH2*, *ITCH* and *TOM1*[21,40,41]. Patients with *POLA1* deficiency (figure E4) should undergo regular

ophthalmologic exams, given the risk of sterile inflammation leading to cataracts, scarring and blindness[42].

Hematopoietic cell transplantation (HCT).—For 18 patients, genetic diagnoses represent potential indications for HCT. The 8 patients with *CTLA4* haploinsufficiency and *LRBA* deficiency would be strongly considered for HCT given the increasing evidence of high morbidity and mortality in these diseases. For example, patient S88 developed inflammatory brain lesions while on abatacept; because he had failed appropriate targeted therapy and had a genetic diagnosis, he underwent HCT. Patients with mutations in *XIAP, RAG1,* and *SH2D1A* are also frequently considered for early HCT[43,44,45]. Patient S21, who had hypogammaglobulinemia and autoimmune enteropathy beginning in early childhood, was referred for genetic testing at age 16y. She was found to have compound heterozygous *RAG1* mutations, often considered an indication for HCT. She died at age 16 from a fungal infection prior to genetic diagnosis.

Genetic diagnosis can benefit patients even if gene-specific therapeutic guidelines are not yet available. For instance, severe childhood illness may lead physicians to attempt HCT in the absence of a genetic diagnosis. If genetic diagnosis reveals the cause of disease to be a gene of the hematopoietic system, bone marrow transplantation is more likely to be effective. Conversely, for the 6 patients with mutations in *MYO5B*, *TTC37*, *SKIV2L*, and *CARD14,* HCT would be unlikely to ameliorate disease, since these include epithelial defects that do not respond to HCT[46]. Moreover, a genetic diagnosis can be crucial to guide diagnosis in patients' relatives. For instance, patient S92, with *CTLA4* haploinsufficiency, underwent HCT due to the severity of his disease despite lacking a genetic diagnosis. He now has an infant son who can be screened for the same mutation.

DISCUSSION

Enabling precision medicine for children with immune dysregulation, polyendocrinopathy or enteropathy requires embracing genetic heterogeneity. That is, despite all patients in this project being referred for the same clinical concern (IPEX), many different genes were responsible for their illnesses, including some genes known to cause IPEX-like disease and others not previously associated with this syndrome. The diversity of IUIS phenotypes among these patients highlights the difficulty of inferring genotype from phenotype and the value of comprehensive genomic analysis early in the course of disease.

This genetic heterogeneity reflects the clinical reality that the features of childhood immune disorders are widely encountered and overlapping. The search for a genetic cause cannot realistically be based only on clinical phenotype, because immune dysregulation conditions have ill-defined phenotypic boundaries and can be due to any of multiple different genes. A genetics-first paradigm avoids unhelpful classifications that may lead to errors in diagnosis or therapy. To assist this practice, we suggest that when a causal link between gene and phenotype is established, the gene responsible for an immune disorder be included in its name (e.g. *LRBA* immunodeficiency). Diagnoses impact treatment options and define cohorts for scientific studies. By including the responsible gene in the name of each disease,

clinicians can avoid ambiguity and immediately access treatments most promising for the patient.

Genetic testing platforms vary widely in capacity and limitations[47]. Given rapidly improving technology, it is important both to seek early genetic diagnosis and to re-evaluate if original results are negative. Sequencing to high coverage and including critical intronic and regulatory regions among the targets greatly increases detection sensitivity for copy number variants (CNVs), which for our patients represented 10% of all mutations. CNVs were particularly frequent in *LRBA* and represent a growing class of defects for which genetic diagnosis dictates targeted therapy. Custom-designed gene panels detect this class of mutations, but most exome sequencing does not. In practice, most commercial clinical sequencing is now exome sequencing, with “gene panels” in fact simply exome sequence data with only a subset of genes reported to the physician. Approximately 10% of the mutations of our patients would have been missed by exome sequencing.

If genetic disease remains a consideration despite a negative genetic test, it is worth considering more complete and current genomic analysis. False negatives on genetic tests can result from coverage that is inadequate to detect structural variants, from incorrect variant interpretation, from limitations in knowledge of gene function and hence of pathogenic variants, or from lab error. Errors of variant interpretation can be subtle and include failure to detect effects on transcription caused by mutations that do not alter amino acid sequence, failure to detect mutations at sites other than exon-intron boundaries that alter splicing, failure to distinguish variation in true genes from variation in pseudogenes, and so on. The pace of gene discovery, knowledge about individual genes, knowledge of classes of cryptic mutations, and technological advance all support additional genetic testing as platforms improve. Immunologists will likely find it useful to consult with academic centers to interpret increasingly complex genetic information. Conversely, clinically well-characterized patients will very greatly contribute to functional analysis of new mutations.

In our experience, negative results from tests of single genes or narrow panels have almost no value, and furthermore may impair the ability of clinicians to do follow-up testing due to insurance limitations. The solution is for the first genetic test to include as many genes and as many classes of mutations as possible, either from a large gene panel or from whole exome sequencing.

This project had several limitations, largely as the result of some patients being originally referred several years ago. First, for some patients, clinical information was limited and referring physicians were no longer available. Second, only probands were available for initial analysis, although a few families were re-contacted through the referring providers. Analysis of a child and both parents is critical to identifying *de novo* mutations, an important part of genetic diagnosis[48]. Analysis of the complete patient-parent-parent trio is particularly important in the evaluation of mutations in genes such as CTLA4. Severe mutations in CTLA4 can lead to severe phenotypes, as in our patients (Tables II and III), but in some families, mutations in CTLA4 have been identified in relatives with no, or late-onset, clinical signs[49,50]. It is possible that *CTLA4* mutations with severe, early onset phenotypes are *de*

novo in affected children, but this speculation can only be tested with DNA from parents. Both these limitations reduced the number of genetic diagnoses in which we had confidence.

A third limitation was that our gene-panel sequencing strategy detected all mutations in coding sequence and all gene-impacting copy number variants but did not detect mutations in distant non-coding genomic regions. Such distant non-coding mutations would be revealed by whole genome sequencing. The cost of whole genome sequencing is rapidly decreasing, but the cost of interpreting non-coding variants in whole genome sequence is very high. Insurance reimbursement is the practical limitation to applying whole genome sequencing to genetic diagnosis to these or other complex conditions.

Despite these limitations, the yield of reportable mutations among the children in this series was quite high (39%). Analysis of an active clinical population, with patient-parent-parent trios available for testing, would yield more solved cases with correspondingly greater benefit for management.

In conclusion, genetic diagnoses of children with immune dysregulation, polyendocrinopathy and enteropathy, but no mutation in *FOXP3*, revealed a wide array of clinical immune diseases and disruption of a wide array of biological pathways. More than 80% of genetic diagnoses were clinically actionable. We suggest that these results support a “genetics first” approach for patients with severe, early-onset immune dysregulation. We propose that all patients with childhood-onset immune dysregulation undergo comprehensive genomic analysis, rather than single-gene testing or no genetic testing at all. Treatments targeting immune pathways are rapidly advancing. To harness the promise of these treatments, it is critical to fully exploit genetic testing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

IPEX	Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked
CNV	copy number variant
IPE	Immune dysregulation, polyendocrinopathy, enteropathy
IUIS	International Union of Immunological Societies
UW	University of Washington
SCH	Seattle Children’s Hospital
HCT	hematopoietic cell transplantation

PBMC	peripheral blood mononuclear cell
ACMG	American College of Medical Genetics
THE	trichohepatoenteric

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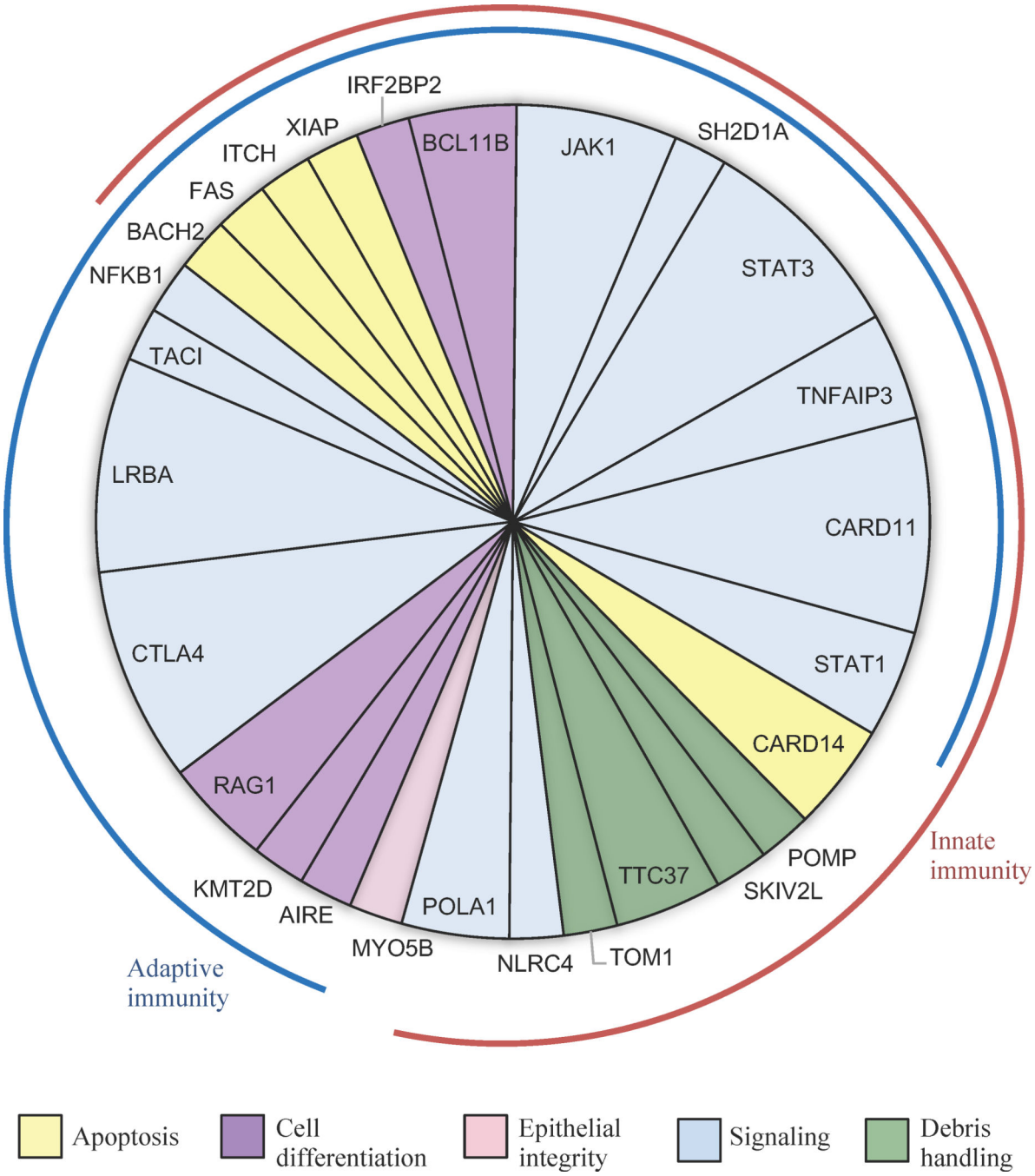

Figure 1:

Genes responsible for childhood immune dysregulation, polyendocrinopathy, and enteropathy. Genes responsible for childhood IPE in 48 patients by principal biological function, based on literature review and Gene Ontogeny annotation. Genes may act primarily within the adaptive arm of the immune system (blue arc), the innate arm (maroon arc), or both.

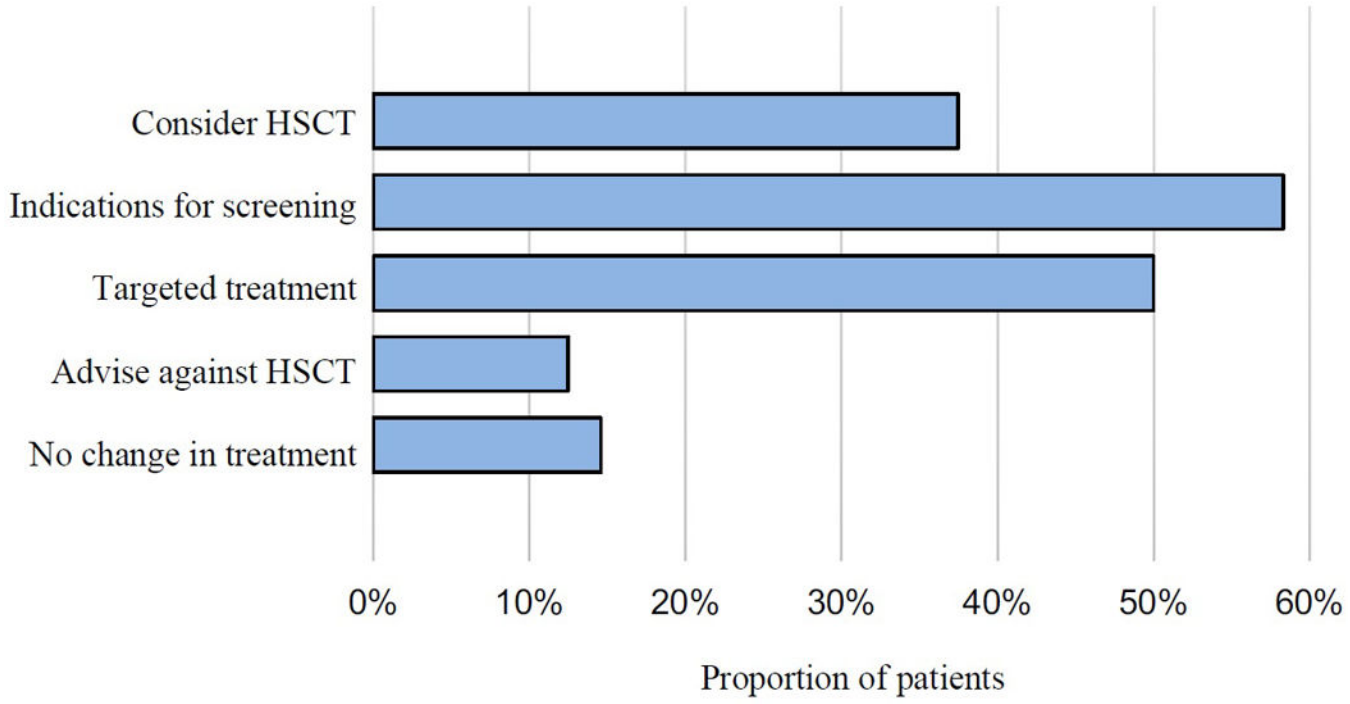

Figure 2:

IPE disease management recommendations based on genetic diagnoses. Recommendations for therapy for each gene are presented in supplementary Table EII. Percentages add to more than 100%, because for some patients, more than one recommendation was available. HCT is hematopoietic cell transplant.

Table I.

Patient characteristics

	N	Proportion
Age at referral		
0 – 2 y	40	0.33
2 – 4 y	26	0.21
5 – 9 y	18	0.15
10 – 14 y	13	0.11
15+ y	17	0.14
not recorded	9	0.07
Total	123	1.00
Gender		
Male	94	0.76
Female	29	0.24
Total	123	1.00
Ancestry		
Caucasian, not Hispanic	78	0.63
Caucasian, Hispanic	24	0.20
East Asian	9	0.07
African American	5	0.04
Middle East/West Asian	4	0.03
Central/South Asian	2	0.02
Native American	1	0.01
Total	123	1.00
Major reported clinical features		
Enteropathy	69	0.56
Dermatitis	30	0.24
Autoimmunity	45	0.37
Immunodeficiency; recurrent infections	37	0.30
“IPEX” only	29	0.24
No information recorded	11	0.09

Table II.

Genetic diagnoses with phenotypic classifications of the International Union of Immunological Societies (IUIS)

Patient	Age	Gender	Gene	AI / II*	"IPEX-like" gene**	Inh^	Clinical features
IUIS: Diseases of immune dysregulation							
S048	11 y	M	*AIRE*	AI	no	AR	Addison's disease and recurrent oral thrush
S093	22 m	F	*BACH2*	AI, II	no	AD	Chronic diarrhea
S092	12 y	M	*CTLA4*	AI	yes	AD	Enteropathy, total parenteral nutrition dependence, alopecia totalis; HSCT
S175	9 y	M	*CTLA4*	AI	yes	AD	Onset 3y. Watery diarrhea, type 1 diabetes, anti-thyroid antibodies, hemolytic anemia, thrombocytopenia, lymphadenopathy, splenomegaly, alopecia; HSCT
S190	16 y	M	*CTLA4*	AI	yes	AD	Colitis, eczema, hypothyroidism, common variable immunodeficiency, cytopenias
S018	11 y	F	*CTLA4, CD28, CASP8*	AI	yes	AD	Autoimmune enteropathy, eczema, interstitial pneumonitis, seizures, DD, pulmonary hypertension, vitiligo, thrombocytopenia, pan-hypogammaglobulinemia; recurrent infections including esophageal candidiasis, otitis media, and sinusitis
S184	13 y	M	*FAS/TNFRSF6*	AI, II	no	AD	Enteropathy with villous atrophy from 1y, eczema, type 1 diabetes, alopecia, recurrent severe infections, short stature, seizures. Low IgA and IgG levels, and B cell lymphopenia
S071	3 y	M	*ITCH*	AI, II	yes	AR	Autoimmune thyroid disease, FTT, DD, chronic secretory and bloody diarrhea, alopecia, hemolytic anemia, eczema, recurrent fungal and bacterial infections. Low IgM levels; positive pANCA and anti-smooth muscle antibodies
S066	7 m	F	*JAK1*	AI, II	no	AD	Dysmorphisms, enteropathy, hypogammaglobulinemia, failure to thrive, hepatitis, and decreased FOXP3 T-cells. Died from sepsis. Five siblings died in infancy.
S067	2 y	M	*JAK1*	AI, II	no	AD	Enteropathy and immunodeficiency, with decreased FOXP3 expression.
S170	4 m	M	*JAK1*	AI, II	no	AD	Exfoliative dermatitis, enteropathy, anemia, thrombocytopenia, neutropenia, hepatosplenomegaly.
S014	4 y	M	*LRBA*	AI	yes	AR	Type 1 diabetes, autoimmune enteropathy, autism, lymphoma
S045	14 y	M	*LRBA*	AI	yes	AR	Diarrhea from age 3 mo., malabsorption, type 1 diabetes, esophageal candidiasis, and delayed puberty. Died age 16y of intestinal adenocarcinoma
S129	17 y	F	*LRBA*	AI	yes	AR	Hypothyroidism, inflammatory bowel disease, hepatosplenomegaly, hemolytic anemia, and hypogammaglobulinemia
S088	10 y	M	*LRBA*	AI	yes	AR	Early onset type 1 diabetes mellitus, autoimmune cytopenias, arthritis, interstitial lung disease, autoimmune enteropathy, uveitis, eczema, failure to thrive, recurrent warts, inflammatory brain lesions. Hypogammaglobulinemia, low T-regulatory cells
S123	11 y	M	*SH2D1A*	AI, II	no	AD	Alopecia totalis, neutropenia, recurrent infections, mucositis, dermatitis, colitis; HSCT

Patient	Age	Gender	Gene	AI / II*	"IPEX-like" gene**	Inh^	Clinical features
S041	6 m	M	*STAT3*	AI, II	yes	AD	Chronic diarrhea, severe eczema, recurrent respiratory infections and developmental delay; HSCT
S068	8 y	M	*STAT3*	AI, II	yes	AD	"IPEX"
S112	9 y	M	*STAT3*	AI, II	yes	AD	Recurrent infections, type 1 diabetes, immune thrombocytopenic purpura, hypothyroidism, short stature, and mild hypogammaglobulinemia
S108	20 m	M	*XIAP*	AI, II	no	XR	Severe malabsorption, failure to thrive
IUIS: Autoinflammatory disorders							
S030	4 m	M	*CARD14*	II	no	AD	Severe eczema, multiple food allergies, enteropathy
S055	3 y	F	*CARD14*	II	no	AD	Colitis
S098	1 m	M	*NLRC4*	II	no	AD	Intractable diarrhea, distended bowel with mucosal autolysis and erosions, severe pulmonary congestion and hemorrhage, ascites, viral meningitis
S102	2 y	M	*POLA1*	II	no	XR	Immune dysregulation
S114	ni	M	*POLA1*	II	no	XR	Unknown
S160	2 m	M	*POMP*	II	no	AD	Enteropathy, dermatitis, pulmonary nodules, recurrent respiratory infections, mild thrombocytopenia, and elevated IgE
S027	3 y	M	*TNFAIP3*	AI, II	no	AD	Watery diarrhea, eczema, failure to thrive, autoantibodies to thyroid (without frank thyroiditis), elevated IgE.
S087	12 y	F	*TNFAIP3*	AI, II	no	AD	"IPEX"
IUIS: Predominantly antibody deficiency							
S125	4 y	F	*IRF2BP2*	AI, II	no	AD	Chronic diarrhea, severe eczema, anemia, failure to thrive, fevers, short stature, recurrent infections, cataracts, hypodontia, hypotrichosis alopecia, hypogammaglobulinemia
S043	6 y	M	*TACI*	AI	no	AD	Eczema, enteropathy with complete villous atrophy; HSCT
IUIS: Combined immunodeficiency with associated features							
S025	6 y	M	*BCL11B*	AI, II	no	AD	Failure to thrive, nutritional deficiencies, chronic emesis, pyloric stenosis, focal villous blunting of duodenum
S029	1 mo.	M	*BCL11B*	AI, II	no	AD	Severe dermatitis
S040	6 m	M	*CARD11*	AI, II	yes	AD	Watery diarrhea with villous atrophy, eczema, type 1 diabetes, failure to thrive, alopecia areata, skin tags, club foot

Patient	Age	Gender	Gene	AI / II*	"IPEX-like" gene**	Inh^	Clinical features
S046	8 y	M	*CARD11*	AI, II	yes	AD	Enteropathy, severe atopic dermatitis, failure to thrive, high IgE levels
S178	11 m	M	*CARD11*	AI, II	yes	AD	Unknown
S179	1 m	F	*CARD11*	AI, II	yes	AD	"IPEX"
S100	25 y	F	*KMT2D*	AI	no	AD	Short stature, high arched palate; autoimmune disease, enteropathy. Small intestine transplant.
S024	4 m	M	*NFKB1*	AI	no	AD	"IPEX"
S021	16 y	F	*RAG1*	AI	no	AR	Hypogammaglobulinemia, aplastic anemia, autoimmune enteropathy. Died at age 16y of fungal infection
S090	2 m	M	*RAG1*	AI	no	AR	"IPEX"
S182	4 m	M	*SKIV2L*	II	no	AR	Severe enteropathy, failure to thrive, total parenteral nutrition dependence, psoriatic-type rash, watery diarrhea with villous atrophy, dilated aortic root, developmental delay. Died age 4y.
S097	2 y	M	*STAT3*	AI, II	yes	AD	Chronic enteropathy and chronic liver disease.
S070	7m	M	*TTC37*	II	yes	AR	Failure to thrive, small triangular face, sparse hair. Death not related to immune disease.
S186	5 y	F	*TTC37*	II	yes	AR	Enteropathy with villous atrophy from age 1 y, eczema, recurrent severe upper respiratory infections and gastrointestinal infections, hemolytic anemia, thrombocytopenia, alopecia, developmental delay, trichorrhexis nodosa
IUIS: Defects in intrinsic and innate immunity							
S120	3 y	M	*STAT1*	AI, II	yes	AD	"IPEX"
S103	19 y	M	*STAT1*	AI, II	yes	AD	Enteropathy, dermatitis, failure to thrive, autoimmune hemolytic anemia, recurrent infections including oropharyngeal candidiasis and herpes zoster, hypogammaglobulinemia with low T-regulatory cells
IUIS: Not included in IUIS categories							
SDH	19 y	M	*MYO5B*	-	no	AR	Enteropathy and failure to thrive; reduced expression of FOXP3 and of CD25. Two previously deceased siblings.
S015	2 y	M	*TOM1*	II	no	AD	Congenital autoimmune enteropathy, exocrine pancreatic insufficiency, failure to thrive, hepatitis, atopic dermatitis, pityriasis alba, hypogammaglobulinemia, lymphopenia

* Role of the gene in adaptive immunity (AI), innate immunity (II), or both

** Genes previously associated with diagnoses of "IPEX-like" syndrome

^ Inheritance of the phenotype: autosomal dominant (AD), autosomal recessive (AR), or X-linked recessive (XR). Complete genotypes are indicated in Table S2.

Table III.

Variant alleles and interpretations

Pt	*Gene*	Mutation	hg19 position	Type / PPH2 / gerp	Clin Var	This study	Geno-type	Functional consequence of mutation; evidence for causality	gnomAD **
S048	*AIRE*	c.967_979del, p.D323fs	chr21:45711063	LoF	P	P	cpd het *	Well-documented pathogenic mutation (PMID: 9837820)	138
S048	*AIRE*	c.1249insC, p.L417fs	chr21:45713024	LoF	P/LP	P	cpd het *	Well-documented pathogenic mutation (PMID: 10677297)	5
S093	*BACH2*	c.C1586T, p.A529V	chr6:90660239	1.00 / 5.2	ni	LP	het	Completely conserved site. Reduced FOXP3 expression in patient cells, consistent with BACH2 haploinsufficiency (PMID: 28530713)	5
S025	*BCL11B*	c.C779T, p.T260M	chr14:99642394	1.00 / 3.8	ni	LP	het	Destroys completely conserved phosphorylation site	9
S029	*BCL11B*	c.C2421G, p.N607K	chr14:99640752	1.00 / 3.7	P	P	het	Well-documented pathogenic mutation (PMID: 27959755)	0
S040	*CARD11*	c.C1483T, p.P495S	chr7:2974122	0.58^ / 5.2	ni	LP	het	Highly conserved site; enhanced TCR-induced NFKB activation (PMID:30170123)	3
S046	*CARD11*	c.C88T, p.R30W	chr7:2987341	1.00 / 4.5	P/VUS	LP	het	Completely conserved site; cosegregates with combined immunodeficiency (PMID: 28826773); leads to reduced secretion of IFN-gamma and IL-2 (PMID: 28826773)	0
S178	*CARD11*	c.G2510A, pR837Q	chr7:2959006	1.00 / 4.1	ni	LP	het	Predict loss of donor splice, transcriptional deletion of exon 18 and stop at codon 764	0
S179	*CARD11*	c.G1670A, p.R557H	chr7:2968316	1.00 / 4.9	VUS	VUS	het	Highly conserved site; same mutation reported in another immunodeficiency patient, but no functional effect detected by saturation genome editing (PMID: 32302260)	4
S030	*CARD14*	c.G669C, p.Q223H	chr17:78158031	1.00 / na	ni	LP	het	Completely conserved site; phenotype is excellent fit	4
S055	*CARD14*	c.C605T, p.S202L	chr17:78157967	1.00 / 3.6	ni	VUS	het	Highly conserved site; no functional information	4
S092	*CTLA4*	c.G118A; p.V40M	chr2:204735317	1.00 / 5.3	LP/ VUS	LP	het	Completely conserved site in homodimerization domain; reported in patients with primary immunodeficiency (PMID: 29077208, 30048690)	0

Pt	*Gene*	Mutation	hg19 position	Type / PPH2 / gerp	Clin Var	This study	Geno-type	Functional consequence of mutation; evidence for causality	gnomAD **
S175	*CTLA4*	c.523dupT; p.L174fs	chr2:204736165	LoF	ni	P	het	Truncating mutation; haploinsufficiency is pathogenic (PMID: 25329329)	0
S190	*CTLA4*	c.C208T; p.R70W	chr2:204735407	1.00 / 4.3	P	P	het	One of original CTLA4 pathogenic mutations; decreased CTLA4 T-regulatory cells (PMID: 25329329)	0
S018	*CTLA4, CD28, CASP8*	2.83MB deletion	chr2:201994549–204824394 del	LoF	ni	P	het	Complete deletion of gene	0
S125	*IRF2BP2*	c.1606insTTT, p.Q536delinsX	chr1:234743040	LoF	ni	P	het	*De novo* truncating mutation at codon 536 of 587; we confirmed experimentally that the mutant message is stable	0
S071	*ITCH*	c.393dupA; p.T131fs	chr20:33001602	LoF	ni	P	homoz	Truncating mutation. Original *ITCH* pathogenic mutation, cosegregating with disease (PMID: 20170897)	0
S066	*JAK1*	c.T2414A, p.F805Y	chr1:65307274	1.00 / 4.6	ni	LP	het	Private mutation at completely conserved site in kinase domain; family includes 6 children with JAK1 phenotype	0
S067	*JAK1*	c.C1901A, p.A634D	chr1:65312418	1.00 / 3.9	LP	P	het	Activating somatic mutation (PMID: 20167706); not previously observed in germline	0
S170	*JAK1*	25kb triplication	chr1:65337211–65362210	inframe tripl	ni	LP	het	Tandem triplication of exons 2–5, encoding aa 1–151 of membrane-binding FREM domain, stable mutant message experimentally confirmed (Fig S2)	0
S100	*KMT2D*	c.4132–1G>A	chr12:49441853	inframe del	ni	P	het	Clinically dx as Kabuki. Transcriptional loss of exon 14, encoding aa 1378–1412, stable mutant message experimentally confirmed	0
S014	*LRBA*	2347bp del	chr4:151792136–151794482	LoF	ni	P	cpd het *	Transcriptional loss of exons 18–19 (202 bp), stop codon 725 of 2864; maternal allele; experimentally confirmed (Fig S1)	0
S014	*LRBA*	c.8349+4_8349+7del	chr4:151203595	LoF	ni	P	cpd het *	Transcriptional loss of exon 56 (197 bp), stop codon 2755 of 2864; paternal allele; experimentally confirmed (Fig S1)	0
S045	*LRBA*	c.C6640T, p.R2214X	chr4:151392836	LoF	P/LP	P	homoz	Known pathogenic mutation (PMID: 29867916)	1
S129	*LRBA*	c.C5047T, p.R1683X	chr4:151749456	LoF	P	P	cpd het *	Known pathogenic mutation (PMID: 22608502)	0

Pt	*Gene*	Mutation	hg19 position	Type / PPH2 / gerp	Clin Var	This study	Geno-type	Functional consequence of mutation; evidence for causality	gnomAD **
S129	*LRBA*	>277bp deletion	chr4:151935548–151935825 (min del)	LoF	ni	P	cpd het*	Genomic deletion of exon 2 including translation start; experimentally confirmed (Fig S1)	0
S088	*LRBA*	79 kb deletion	chr4:151599677–151678661 del	inframe del	ni	P	cpd het*	Deletion of exons 36–37, encoding aa 1882–1974, region highly conserved across all BEACH proteins; maternal allele; experimentally confirmed (Fig S1)	0
S088	*LRBA*	c.G2736A, p.W912X	chr4:151788853	LoF	ni	P	cpd het*	Truncating mutation; experimentally confirmed as paternal allele (Fig S1)	0
SBDH	*MYO5B*	c.G946A, p.G316R	chr18:47511088	0.99 / 5.5	VUS	LP	homoz	Predict loss of donor splice site and transcriptional deletion of exon 8, encoding aa 280–355 of myosin motor domain; same mutation in patient with microvillus atrophy (PMID: 21206382)	18
S024	*NFKB1*	c.A2494C, p.N832H	chr4:103533665	1.00 / 5.1	ni	LP	het	Completely conserved site in death domain, critical to programmed cell death (PMID: 10786798). Also predicted loss of splice enhancer and transcriptional deletion of exon 22 (173bp), stop at codon 829 of 969.	0
S098	*NLRC4*	c.T1022C, p.V341A	chr2:32475911	0.99 / 3.3	P	P	het	Documented gain of function, pathogenic mutation (PMID: 25217960)	0
S114	*POLA1*	c.4243+5G>A	chrX:24948671	LoF	ni	LP	hemiz	Splice effect experimentally confirmed, transcriptional deletion of exon 36 (97bp), stop at codon 1385 of 1463.	2 hemiz
S102	*POLA1*	c.G304T, p.D102Y	chrX:24722562	1.00 / 2.2	ni	VUS	hemiz	Basepair completely conserved; predict loss of exonic splice enhancer, transcriptional deletion exon 4 encoding aa 83–109, no RNA available to validate splice effect	0 hemiz
S160	*POMP*	c.342del14, p.F114fs	chr13:29246553	LoF	P	P	het	Original pathogenic mutation for *POMP,* escapes NMD (PMID: 29805043)	0
S021	*RAG1*	c.A1336G, p.N446D	chr11:36596190	0.99 / 5.6	ni	LP	cpd het*	Completely conserved in DNA-binding domain. Missense at A444V is pathogenic.	0
S021	*RAG1*	c.C2095T, p.R699W	chr11:36596949	1.00 / na	P	P	cpd het*	Documented pathogenic mutation with reduced T and B-cells (PMID:20956421)	4
S090	*RAG1*	c.G2147A, p.R716Q	chr11:36597001	1.00 / 6.1	LP	LP	cpd het*	Completely conserved site in RAG1 domain	1

Pt	*Gene*	Mutation	hg19 position	Type / PPH2 / gerp	Clin Var	This study	Geno-type	Functional consequence of mutation; evidence for causality	gnomAD **
S090	*RAG1*	c.G2924A, p.R975Q	chr11:36597778	1.00 / 5.6	P	P	cpd het *	Multiple reports as damaging (PMID:20956421); impairs recombination activity (PMID: 18768869)	12
S123	*SH2D1A*	c.T160A, p.Y54N	chrX:123499633	1.00 / 4.8	ni	LP	hemiz	Completely conserved site in SH2 domain; Y54C protein has reduced stability and binding affinity (PMID: 14674764)	0
S182	*SKIV2L*	c.A1907G, p.H636R	chr6:31932055	1.00 / 6.0	ni	LP	cpd het *	Completely conserved site in helicase domain; cpd het with truncating mutation; good fit to phenotype	2
S182	*SKIV2L*	c.C3187T, p.R1063X	chr6:31936654	LoF	ni	LP	cpd het *	Truncating mutation	33
S120	*STAT1*	c.T1627C, p.C543R	chr2:191845351	0.99 / 5.1	ni	VUS	het	Completely conserved site in STAT-binding domain consistent with autosomal dominant immunodeficiency; no sample available for functional analysis and no DNA available from parents to test if *de novo*	0
S103	*STAT1*	c.C820T, p.R274W	chr2:191859911	1.00 / 5.7	ni	LP	het	R274G is known gain of function allele, increases STAT1 dependence on cytokines (PMID:21727188)	0
S041	*STAT3*	c.G1032C, p.Q344H	chr17:40485708	0.99 / 4.2	P	P	het	Documented gain of function pathogenic mutation (PMID: 25359994)	0
S068	*STAT3*	c.C2144T, p.T715M	chr17:40469200	1.00 / 5.0	P	P	het	Documented gain of function pathogenic mutation (PMID: 25359994)	0
S097	*STAT3*	c.G1909A, p.V637M	chr17:40474492	1.00 / 3.7	P	P	het	Documented gain of function pathogenic mutation (PMID:21727188)	0
S112	*STAT3*	c.T937C, p.F313L	chr17:40485928	0.99 / 5.6	ni	LP	het	Completely conserved site in STAT-alpha domain; private allele; good fit to phenotype	0
S043	*TACI / TNFRSF13B*	c.G311A, p.C104Y	chr17:16852186	1.00 / 5.0	LP	LP	het	Completely conserved site; documented in families and patients with CVID (PMID:18981294, 27123465, 22884984); C104R leads to reduced immunoglobulin production (PMID: 21458042, 20889194)	33
S027	*TNFAIP3*	c.1127delC, p.S376fs	chr6:138199709	LoF	ni	P	het	Truncating mutation; haploinsufficiency is a causal mechanism for TNFAIP3 syndromes (PMID: 26642243)	0
S087	*TNFAIP3*	c.G2300A, p.C767Y	chr6:138202383	1.00 / 5.7	ni	LP	het	Completely conserved site at critical cysteine in zinc finger; haploinsufficiency is causal	0

Pt	*Gene*	Mutation	hg19 position	Type / PPH2 / gerp	Clin Var	This study	Geno-type	Functional consequence of mutation; evidence for causality	gnomAD **
								mechanism for TNFAIP3 syndromes (PMID: 26642243)	
S184	*TNFRSF6 / FAS*	c.A260G, p.E87G	chr10:90767520	1.00 / 3.1	ni	LP	het	Completely conserved site at critical turn in disulfide binding domain	0
S015	*TOM1*	c.267+2T>C	chr22:35719172	LoF / inframe del	ni	P	het	Splice mutation yielding two mutant transcripts: (1) intron 4 retained, leading to premature stop; (2) cryptic donor splice leading to deletion of aa 20-152 of vesicular trafficking domain. Experimentally confirmed (Fig S3)	4
S070	*TTC37*	c.C409T, p.R137X	chr5:94876528	LoF	P	P	homoz	Documented pathogenic mutation	2
S186	*TTC37*	c.994+1G>A	chr5:94864704	inframe del	ni	LP	cpd het *	Predict loss of donor splice site, transcript deletion of exon 12 and aa 298–331	1
S186	*TTC37*	c.402+2T>G	chr5:94877007	LoF	ni	P	cpd het *	Predict loss of donor splice site and transcriptional deletion of exon 7 and stop at codon 142 of 1564	0
S108	*XIAP*	c.949del11, p.W317fs	chrX:123022540	LoF	ni	P	hemiz	Truncating mutation	0

* All paired alleles of compound heterozygous (cpd het) genotypes were experimentally confirmed to be in trans in the patient

** All gnomAD entries are heterozygotes unless otherwise indicated

^ Completely conserved as proline through mammals; no species has serine as reference sequence

ni: no information on ClinVar; PP2: polyphen-2 score; gerp: gerp score; LoF: loss of function

P: pathogenic; LP: likely pathogenic; VUS: unknown significance, warranting further evaluation