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Phenotypic heterogeneity and genotypic spectrum of inborn errors of immunity identified through whole exome sequencing in a Thai patient cohort

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Abstract

Background: Inborn errors of immunity (IEI) comprise more than 400 rare diseases with potential life-threatening conditions. Clinical manifestations and genetic defects are heterogeneous and diverse among populations. Here, we aimed to characterize the clinical, immunologic, and genetic features of Thai pediatric patients with IEI. The use of whole-exome sequencing (WES) in diagnosis and clinical decision making was also assessed.

Methods: Thirty six unrelated patients with clinical and laboratory findings consistent with IEI were recruited from January 2010 to December 2020. WES was performed to identify the underlying genetic defects.

Results: The median age of disease onset was 4 months (range: 1 month to 13 years), and 24 were male (66.7%). Recurrent sinopulmonary tract infection was the most common clinical presentation followed by septicemia and severe pneumonia. Using WES, we successfully identified the underlying genetic defects in 18 patients (50%). Of the 20 variants identified, six have not been previously described (30%). According to the International Union of Immunological Societies (IUIS), 38.9% of these detected cases (7/18) were found to harbor variants associated with genes in combined immunodeficiencies with associated or syndromic features (Class II).

Conclusion: The diagnostic yield of WES in this patient cohort was 50%. Six novel genetic variants in IEI genes were identified. The clinical usefulness of WES in IEI was demonstrated, emphasizing it as an effective diagnostic strategy in these genetically heterogeneous disorders.

KEYWORDS
inborn errors of immunity, next-generation sequencing, novel variants, primary immunodeficiency diseases, Thai, whole-exome sequencing

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1 | INTRODUCTION

Inborn errors of immunity (IEI) are a heterogeneous group of more than 400 monogenic disorders caused by defects in genes responsible for different components of the immune system. IEI have phenotypic and genetic heterogeneity with varying degrees of immunodeficiency and immune dysregulation.[1,2] With the advent of next-generation sequencing (NGS), the number of novel variants in known genes and newly identified genes responsible for IEI has been increasing rapidly.[3-5] This has expanded our understanding of genotype-phenotype correlations and provided better insights into the pathogenesis of IEI. Currently, these diseases are classified into 10 different categories by the International Union of Immunological Societies (IUIS).[6,7]

Genetic testing plays a vital role in the diagnosis and management of patients suspected of IEI. It facilitates rapid and timely diagnosis, making more precise treatment planning, leading to better patient outcomes.[4,8-10] In addition, knowing exact genetic defects can help determine the inheritance pattern and family members at risk. This genetic information could provide the basis for counseling on family planning.

The application of next-generation sequencing (NGS) including whole-exome sequencing (WES) and whole-genome sequencing (WGS) has accelerated the discovery of novel disease-associated genes causing IEI and helped unravel disease-associated variants in several unresolved cases.[5,11,12] NGS has become a valid and cost-effective tool for diagnosis of IEI with diagnostic yield ranging from 15% to 79%.[13,14] It has been demonstrated that patients with IEI have a wide spectrum of clinical manifestations including atypical presentation and overlapping features. Therefore, WES could be used as a first-tier test for such cases[5,15] In this study, we aimed to characterize the clinical and genetic features of IEI in the Thai pediatric population. WES was performed in all cases. Our findings have expanded the phenotypic and genotypic spectrum of IEI.

Key Message

Inborn errors of immunity (IEI) are a heterogeneous group of more than 400 monogenic disorders caused by defects in genes responsible for different components of the immune system. This study is the first and largest to investigate genetic causes in pediatric IEI cases in the Thai population. Exome sequencing was successfully performed to identify the genetic defects in 50% of cases. Our findings demonstrated the genetic and phenotypic heterogeneity of IEI supporting the use of WES in diagnosis and clinical decision making. In addition, of all the 20 variants found to be associated with the diseases, six (30%) were novel expanding the genotypic spectrums of IEI.

2 | METHODS

2.1 | Patients

A total of 36 unrelated patients with clinical and laboratory findings suspected of IEI were recruited in the study. Most of the patients were evaluated at King Chulalongkorn Memorial Hospital from January 2010 to December 2020. Serum levels of immunoglobulins IgG, IgA, and IgM were measured using nephelometry. Serum IgE was measured using Elecsys® IgEII immunoassay (Roche). Flow cytometry for lymphocyte subpopulations was performed using BD Tritest™ CD3 FITC/CD4 PE/CD45 PERCP, CD3 FITC/CD8 PE/CD45 PERCP, CD3 FITC/CD19 PE/CD45 PERCP, CD3 FITC/CD16, and CD56 PE/CD45 PERCP (BD). T-lymphocyte proliferation responses to phytohemagglutinin (PHA) were measured. The amount of 3H-thymidine detected in cells stimulated with PHA was divided by 3H-thymidine detected in unstimulated cells. The result was demonstrated as the stimulation index (SI). Dihydrorhodamine (DHR) flow cytometry test was used to evaluate granulocyte oxidative bursts. The SI was calculated as the ratio of geometric mean channel fluorescence intensity of phorbol myristate acetate–stimulated and unstimulated granulocytes. A healthy control was also used along with the patients' samples in the PHA stimulation test and DHR test.

Written informed consent was obtained from the patients and/or their parents. This study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (IRB No.264/62), and conducted in accordance with the Declaration of Helsinki.

2.2 | Whole-exome sequencing and data analysis

After informed consent, three milliliters of peripheral blood was taken from the patients and their available parents. Genomic DNA was extracted from peripheral blood leukocytes using the Puregene Blood Kit (Qiagen). Whole-exome sequencing (WES) was performed by Macrogen, Inc. as previously described.[16] In brief, DNA samples were prepared as an Illumina sequencing library, and in the exome capture step. The sequencing libraries were enriched by SureSelect Human All Exon V7 Kit. The captured libraries were sequenced using Illumina HiSeq 4000 Sequencer. Sequence reads were mapped against UCSC hg19 using Burrows-Wheeler Alignment (BWA) software (http://bio-bwa.sourceforge.net/). The single nucleotide polymorphisms (SNPs) and indels were detected by SAMtools (http://samtools.sourceforge.net/) and annotated by dbSNP&1000G. A total of 1648 genes listed in abnormality of the immune system (HP:0002715) were applied in the analysis. The variants were subsequently filtered out if they were present in our in-house database of 2166 unrelated Thai exomes. The variants would be called novel if they were not listed in the ClinVar Miner database (https://clinvarminer.genetics.utah.edu/) and the Genome Aggregation Database (GnomAD) (https://gnomad.broadinstitute.org). Prediction software including PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (Sorting Intolerant

From Tolerant; http://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html), and M-CAP (http://bejerano.stanford.edu/mcap/) was used to analyze the potential pathogenicity of the missense variants. In addition, for insertion and deletion variants, PROVEAN (Protein Variation Effect Analyzer; http://provean.jcvi.org) was used for protein function prediction. All novel potential causative variants were confirmed by PCR-Sanger sequencing.

3 | RESULTS

A total of 36 patients clinically diagnosed with IEI were included and underwent WES. The median age of disease onset was 4 months (range: 1 month to 13 years), and 24 (66.7%) were male. The most frequent clinical presentation was recurrent sinopulmonary tract infections (33.3%), followed by septicemia (30.6%) and severe pneumonia (16.7%). The clinical manifestations and immunologic findings are summarized in Table 1. The clinical diagnosis distribution of all patients based on IUIS classification is shown in Figure 1A.

Whole-exome sequencing (WES) was performed to investigate the genetic defects in all 36 cases. According to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guideline for variant classification, 20 variants including pathogenic, and likely pathogenic explaining the patients' phenotypes were identified in 18 cases (50%) (Figure 1B, Table 2). Of the 20 variants identified, 6 of 20 (30%) were novel (Table 3). These variants included eight missense, six frameshift, three splicing, two nonsense, and one in-frame deletion. According to IUIS classification, seven cases were found to harbor variants associated with genes in category II: combined immunodeficiencies with associated or syndromic features; four in category I: immunodeficiencies affecting cellular and humoral immunity; three in both category III: predominantly antibody deficiencies, and category IV: diseases of immune dysregulation; and one in category V: congenital defects of phagocyte number, function, or both. No variants were identified in genes classified in categories VI-X (Figure 1B). Of all seven and three patients classified into categories II and IV by clinical features, respectively, the molecular defects were successfully identified in all of them (Figure 1). Nevertheless, of the 14 patients with clinical diagnosis of immunodeficiencies affecting cellular and humoral immunity, four (29%) were found to harbor causative variants (Figure 1). This could be due to the broad spectrum and variety of clinical presentations in this category. There were also three patients diagnosed with defects in intrinsic and innate immunity; however, no disease-associated variants could be identified.

3.1 | Class I: Immunodeficiencies affecting cellular and humoral immunity

Four variants in different genes including *CD40LG*, *DOCK8*, *IL2RG*, and *RAG1* were identified in four unrelated patients with IEI (Table 2).

Patient 1 presented with disseminated cryptococcosis affecting skin, gastrointestinal tract, and blood. The leukocyte count and flow cytometry were in normal range, while the immunoglobulin test showed an elevated IgM level (5.07 g/L). The diagnosis of hyper-IgM syndrome was made by identifying a hemizygous variant c.514T > C (p.Tyr172His) in *CD40LG*. This variant was inherited from the mother and previously reported in individuals with hyper-IgM syndrome.[17]

Patient 2 had chronic eczema, recurrent bacterial skin infections, and recurrent pneumonia since the age of 2 years. He subsequently developed pulmonary tuberculosis at 8 years old. His immunoglobulin test revealed an elevated IgE level (1661 IU/ml). We identified a novel 1-bp insertion variant c.3201dupT (p.Val1068Cysfs*3) in *DOCK8* (Figure 2A, Table 3). The valine residue 1068 is highly conserved among different species, from zebrafish to humans (Figure S1). Loss-of-function mutations in *DOCK8* are associated with autosomal recessive hyper-IgE syndrome.

Patient 3 was found to have chronic diarrhea at the age of 3 months and subsequently developed severe pneumonia and sepsis. T-B + NK + severe combined immunodeficiency was suspected. WES revealed a novel hemizygous variant c.722G > A (p.Ser241Asn) in *IL2RG*, which was inherited from the mother, confirming the diagnosis of X-linked severe combined immunodeficiency (SCID) (Figure 2B). The serine residue 241 is conserved (Figure S1). This variant was classified as likely pathogenic (Table 3).

Patient 4 developed pneumonia since the age of 4 months and later developed rotavirus gastroenteritis, fungal skin infections, and BCGitis. The leukocyte count and immunoglobulin levels were extremely low (Table 1). T-B-NK + severe combined immunodeficiency was suspected. WES revealed a known homozygous variant c.1871G > A (p.Arg624His) in *RAG1*.[18,19]

3.2 | Class II: Combined immunodeficiencies with associated or syndromic features

Seven patients were found to carry variants in genes associated with combined deficiencies with associated or syndromic features. The variants were identified in *KMT2D*, *PGM3*, *STAT3*, and *TTC37* (Table 2).

Patient 5 was found to have recurrent sinopulmonary tract infections and pancytopenia. She also had arched eyebrows, long palpebral fissures, and depressed nasal tip. WES revealed a de novo novel heterozygous 1-bp deletion, c.453delG (p.Gln152Argfs*56) in *KMT2D* that was classified as pathogenic from ACMG classification and associated with Kabuki syndrome (Figure 2C, Table 3). The glutamine residue 152 is also conserved (Figure S1).

Patient 6 developed recurrent sinopulmonary tract infections, severe atopic dermatitis, chronic diarrhea, and multiple food allergies since the age of 2 years. Using WES, two compound previously reported heterozygous variants c.1527delC (p.Asn510Metfs*4) and c.1087A > G (p.Thr363Ala) in *PGM3* were identified, confirming the diagnosis of PGM3 deficiency.[20]

TABLE 1 Clinical features and immunologic findings identified in patients with inborn errors of immunity (IEI)

ID	SEX	Age at onset (Y)	Clinical manifestation	CD3 (10^9/L)	CD4 (10^9/L)	CD8 (10^9/L)	CD19 (10^9/L)	CD56 (10^9/L)	IgG (g/L)	IgM (g/L)	IgA (g/L)	IgE (IU/ml)	IUIS classification by clinical manifestation
1	M	13	Disseminated cryptococcosis	↑ 4.76 (1.40–2.00)	↑ 3.20 (0.70–1.10)	↓ 0.30 (0.60–0.90)	Normal 0.30 (0.30–0.50)	Normal 0.10 (0.07–0.48)	↓ 2.89 (6.98–11.94)	↑ 5.07 (0.59–0.99)	↓ 0.10 (0.22–2.74)	Normal 100 (<200)	1
2	M	2	Recurrent skin and sinopulmonary tract infections	Normal 1.80 (1.61–4.23)	↓ 0.77 (0.90–2.86)	Normal 0.84 (0.63–1.91)	↑ 15.78 (0.70–1.30)	↑ 4.69 (0.18–0.92)	↑ 15.50 (3.44–11.80)	Normal 0.67 (0.12–1.04)	↑ 1.90 (0.02–0.98)	↑↑ 1661 (<60)	2[b]
3	M	3 months	Severe pneumonia and chronic diarrhea	↓ 0.26 (2.07–6.54)	↓ 0.10 (1.46–5.12)	↓ 0.06 (0.65–2.45)	↑ 9.93 (0.50–1.50)	Normal 0.18 (0.17–0.83)	NA	NA	NA	NA	1
4	M	4 months	Recurrent pneumonia and fungal infections	↓↓ 0.01 (2.28–6.45)	↓↓ 0.01 (1.69–4.60)	↓↓ 0.01 (0.72–2.49)	↓↓ 0.01 (0.50–1.50)	↑ 1.86 (0.17–0.83)	↓↓ 0.04 (0.55–7.99)	↓↓ 0.01 (0.06–0.77)	↓↓ 0.01 (0–0.64)	Normal 0.2 (<15)	1
5	F	2 months	Recurrent sinopulmonary tract infections	↓ 2.02 (2.07–6.54)	↓ 1.45 (1.46–5.12)	Normal 1.12 (0.65–2.45)	↑ 1.69 (0.50–1.50)	Normal 0.36 (0.17–1.10)	↑ 12.60 (1.92–6.68)	↑ 1.29 (0.08–0.50)	↑ 0.76 (0–0.47)	↑ 184 (<15)	1[b]
6	M	2 months	Recurrent sinopulmonary tract, fungal infections, severe atopic dermatitis	↓ 0.73 (2.07–6.54)	↓ 0.30 (1.46–5.12)	↓ 0.43 (0.65–2.45)	↓ 0.30 (0.50–1.50)	Normal 0.31 (0.17–1.10)	↑ 13.20 (1.92–6.68)	Normal 0.50 (0.08–0.50)	Normal 0.30 (0–0.47)	↑↑ 13,877 (<15)	2
7	M	1 month	Recurrent skin and sinopulmonary tract infections	↓ 0.98 (2.07–6.54)	↓ 0.40 (1.46–5.12)	↓ 0.56 (0.65–2.45)	↓ 0.01 (0.50–1.50)	Normal 0.29 (0.17–1.10)	↑ 24.86 (1.92–6.68)	↑ 1.53 (0.08–0.50)	↑ 0.50 (0–0.47)	↑↑ 48,332 (<15)	2
8	M	4 months	Recurrent skin and sinopulmonary tract infections	Normal 3.66 (2.28–6.45)	Normal 2.08 (1.69–4.60)	Normal 0.86 (0.72–2.49)	Normal 1.02 (0.50–1.50)	Normal 0.41 (0.17–0.83)	↑ 12.30 (0.55–7.99)	↑ 1.77 (0.06–0.77)	↑ 1.46 (0–0.64)	↑↑ 2000 (<15)	2
9	M	1	Recurrent skin abscess	Normal 4.92 (1.46–5.44)	Normal 2.58 (1.02–3.60)	Normal 1.94 (0.57–2.23)	↑ 7.03 (0.50–1.50)	↑ 1.30 (0.16–0.95)	↑ 20.00 (2.23–10.99)	↑ 1.62 (0.08–1.00)	↑ 1.39 (0.01–0.73)	↑↑ 38,500 (<60)	2
10	M	8	Recurrent skin and sinopulmonary tract infections	↓ 0.83 (1.40–2.00)	↓ 0.24 (0.70–1.10)	↑ 0.99 (0.60–0.90)	↑ 6.51 (0.30–0.50)	↑ 1.09 (0.10–0.48)	Normal 14.19 (4.11–14.35)	↑ 1.26 (0.15–1.15)	Normal 0.74 (0.34–2.14)	↑↑ 6987 (<90)	2
11	F	1 month	Chronic diarrhea with brittle light-colored hair	Normal 2.84 (2.07–6.54)	↓ 1.44 (1.46–5.12)	Normal 1.22 (0.65–2.45)	↑ 2.01 (0.50–1.50)	↓ 0.07 (0.17–1.10)	Normal 5.05 (1.92–6.68)	↑ 0.90 (0.08–0.50)	↑ 1.59 (0–0.47)	NA	2
12	M	1	Gram negative septicemia	↑ 7.41 (1.46–5.44)	↑ 5.21 (1.02–3.60)	↑ 5.96 (0.57–2.23)	↓↓ Lower limit of detection	NA	↓ 0.07 (2.23–10.99)	Normal 0.58 (0.08–1.00)	Normal 0.27 (0.01–0.73)	NORMAL 4.00 (<60)	3

(Continues)

TABLE 1 (Continued)

ID	SEX	Age at onset (Y)	Clinical manifestation	CD3 (10^9/L)	CD4 (10^9/L)	CD8 (10^9/L)	CD19 (10^9/L)	CD56 (10^9/L)	IgG (g/L)	IgM (g/L)	IgA (g/L)	IgE (IU/ml)	IUIS classification by clinical manifestation
13	M	4	Recurrent sinopulmonary tract infections and bronchiectasis	Normal 2.30 (1.61–4.23)	Normal 1.30 (0.90–2.86)	↓ 0.62 (0.63–1.91)	↓ 0.10 (0.70–1.30)	Normal 0.67 (0.13–0.72)	↓ 0.07 (4.73–13.85)	↓ 0.18 (0.20–0.92)	↓ 0.06 (0.39–1.47)	normal 5.00 (<60)	3
14	M	7	Recurrent sinopulmonary tract infections	↓ 0.92[a] (1.40–2.00)	↓ 0.40[a] (0.70–1.10)	↓ 0.56[a] (0.60–0.90)	↓↓ 0.01[a] (0.30–0.50)	Normal 0.35 (0.10–0.48)	↓ 1.29 (4.11–14.35)	Normal 0.17 (0.15–1.15)	↓ 0.23 (0.34–2.14)	Normal 18.50 (<90)	3
15	F	2 months	Hemophagocytic lymphohistiocytosis	NA	NA	NA	NA	NA	NA	NA	NA	NA	4
16	M	4 months	Hemophagocytic lymphohistiocytosis with silver-colored hair and eyebrows	↓ 1.13 (2.28–6.45)	↓ 0.66 (1.69–4.60)	↓ 0.45 (0.72–2.49)	NA	NA	↑ 9.09 (0.55–7.99)	↑ 1.51 (0.06–0.77)	↑ 1.12 (0–0.64)	↑ 32 (<15)	4
17	M	11 months	Hemophagocytic lymphohistiocytosis	NA	NA	NA	NA	NA	NA	NA	NA	NA	4
18	M	1	Necrotizing pneumonia	Normal 1.79 (1.46–5.44)	↓ 0.95 (1.02–3.60)	Normal 0.81 (0.57–2.23)	↑ 3.71 (0.50–1.50)	↑ 7.19 (0.18–0.92)	Normal 10.90 (2.23–10.99)	↑ 1.09 (0.08–1.00)	Normal 0.61 (0.01–0.73)	NA	5
19	F	1 month	Subdural empyema	↓ 0.25 (2.07–6.54)	↓ 1.19 (1.46–5.12)	Normal 1.24 (0.65–2.45)	Normal 0.51 (0.50–1.50)	Normal 0.32 (0.17–1.10)	Normal 3.97 (1.92–6.68)	↑ 0.55 (0.08–0.50)	Normal 0.26 (0–0.47)	NA	1
20	F	3 months	Disseminated tuberculosis	Normal 5.60 (2.07–6.54)	Normal 1.93 (1.46–5.12)	↑ 3.17 (0.65–2.45)	Normal 1.16 (0.50–1.50)	↑ 1.16 (0.17–1.10)	↑ 11.70 (1.92–6.68)	↑ 1.90 (0.08–0.50)	Normal 0.41 (0–0.47)	↑ 53.30 (<15)	1
21	M	5	Disseminated varicella infection	Normal 1.95 (1.61–4.23)	↓ 0.88 (0.90–2.86)	Normal 0.91 (0.63–1.91)	↓ 0.54 (0.70–1.30)	↑ 2.70 (0.13–0.72)	Normal 11.37 (4.73–13.85)	↓ 0.12 (0.20–0.92)	↑ 2.23 (0.39–1.47)	↑ 476 (<60)	1
22	F	8 months	Meningitis	Normal 2.40 (2.28–6.45)	Normal 1.99 (1.69–4.60)	↓ 0.38 (0.72–2.49)	Normal 1.37 (0.50–1.50)	NA	↓ 0.22 (2.23–10.99)	Normal 0.19 (0.08–1.00)	Normal 0.24 (0.01–0.73)	NA	1
23	M	6 months	Recurrent sinopulmonary tract infections and bronchiectasis	↓ 0.37 (2.28–6.45)	↓ 0.15 (1.69–4.60)	↓ 0.19 (0.72–2.49)	↓ 0.36 (0.50–1.50)	↓ 0.16 (0.17–0.83)	↑ 15.50 (0.55–7.99)	↑ 0.78 (0.06–0.77)	↑ 0.76 (0–0.64)	↑ 12.50 (<15)	1
24	F	1	Severe pneumonia	NA	NA	NA	NA	NA	NA	NA	NA	NA	1
25	F	4 months	Pneumocystis pneumonia	↓ 1.96 (2.28–6.45)	↓ 1.12 (1.69–4.60)	Normal 0.78 (0.72–2.49)	↑ 2.20 (0.50–1.50)	↑ 3.20 (0.17–0.83)	Normal 7.22 (0.55–7.99)	Normal 0.17 (0.06–0.77)	Normal 0.06 (0–0.64)	↑ 39.90 (<15)	1

(Continues)

TABLE 1 (Continued)

ID	SEX	Age at onset (Y)	Clinical manifestation	CD3 (10^9/L)	CD4 (10^9/L)	CD8 (10^9/L)	CD19 (10^9/L)	CD56 (10^9/L)	IgG (g/L)	IgM (g/L)	IgA (g/L)	IgE (IU/ml)	IUIS classification by clinical manifestation
26	M	11 months	Recurrent sinopulmonary tract infections	↓ 0.70 (1.46–5.44)	↓ 0.54 (1.02–3.60)	↓ 0.14 (0.57–2.23)	Normal 1.43 (0.50–1.50)	Normal 0.60 (0.18–0.92)	↓ 2.10 (2.23–10.99)	Normal 0.17 (0.08–1.00)	Normal 0.10 (0.01–0.73)	Normal 11.6 (<60)	1
27	F	1 month	Disseminated staphylococcal infection	↑ 8.68 (2.07–6.54)	↑ 6.21 (1.46–5.12)	Normal 2.04 (0.65–2.45)	Normal 0.86 (0.50–1.50)	Normal 0.96 (0.17–1.10)	Normal 3.22 (1.92–6.68)	Normal 0.42 (0.08–0.50)	Normal 0.06 (0–0.47)	↑↑ 32,100 (<15)	1
28	M	3 months	Severe pneumonia with psoriasis	↓ 0.91 (2.07–6.54)	↓ 0.67 (1.46–5.12)	↓ 0.44 (0.65–2.45)	↓ 0.13 (0.50–1.50)	NA	↓ 1.60 (1.92–6.68)	↑ 0.51 (0.08–0.50)	Normal 0.26 (0–0.47)	↑ 284 (<15)	1
29	F	1 month	Recurrent skin abscess	↓ 0.89 (2.07–6.54)	↓ 0.75 (1.46–5.12)	↓ 0.14 (0.65–2.45)	Normal 0.70 (0.50–1.50)	NA	Normal 5.08 (1.92–6.68)	↓ 0.05 (0.08–0.50)	Normal 0.05 (0–0.47)	NA	3
30	M	1	Recurrent sinopulmonary tract infections and bronchiectasis	Normal 2.54 (1.46–5.44)	Normal 1.53 (1.02–3.60)	Normal 0.88 (0.57–2.23)	NA	NA	Normal 3.05 (2.23–10.99)	↓↓ 0.01 (0.08–1.00)	Normal 0.20 (0.01–0.73)	NA	3
31	M	1	Severe pneumonia	NA	NA	NA	NA	NA	NA	NA	NA	NA	5
32	F	1 month	Disseminated staphylococcal infection	↓↓ Lower limit of detection	↓↓ Lower limit of detection	↓↓ Lower limit of detection	↓↓ Lower limit of detection	↓↓ Lower limit of detection	Normal 6.14 (1.92–6.68)	Normal 0.40 (0.08–0.50)	Normal 0.26 (0–0.47)	↑ 90.2 (<15)	5
33	F	6 months	Multiple liver and splenic abscesses secondary to Chromobacterium violaceum bacteremia	↑ 6.68 (2.28–6.45)	Normal 3.34 (1.69–4.60)	↑ 3.09 (0.72–2.49)	↓ 0.34 (0.50–1.50)	↓ 0.12 (0.17–0.83)	↑ 10.09 (0.55–7.99)	↑ 1.04 (0.06–0.77)	↑ 1.74 (0–0.64)	NA	5
34	M	4 months	Disseminated tuberculosis and candidiasis	Normal 2.81 (2.28–6.45)	↓ 1.54 (1.69–4.60)	Normal 1.07 (0.72–2.49)	↓ 0.11 (0.50–1.50)	↓ 0.16 (0.17–0.83)	↑ 14.65 (0.55–7.99)	↑ 0.89 (0.06–0.77)	↑ 1.21 (0–0.64)	↑↑ 2246 (<15)	6
35	F	13	Recurrent Salmonella septicemia with osteomyelitis	NA	NA	NA	NA	NA	NA	NA	NA	NA	6
36	M	1 month	Recurrent herpes infections	Normal 2.61 (2.07–6.54)	↓ 1.36 (1.46–5.12)	Normal 0.78 (0.65–2.45)	↑ 3.08 (0.50–1.50)	Normal 0.23 (0.17–1.10)	↑ 18.26 (1.92–6.68)	↑ 1.95 (0.08–0.50)	↑ 2.44 (0–0.47)	↑↑ 1297 (<15)	6

Note: ↓ low; ↓↓: extremely low (with CD and Ig levels less than or equal to 0.05 × 10^9/L and 0.05 g/L); ↑: high; ↑↑: extremely high (with IgE levels greater than or equal to 1000 IU/ml). Age-related reference ranges of lymphocyte populations and serum levels of immunoglobulins IgG, IgA, and IgM.

Abbreviations: F, female; M, male; NA, not available; Y, year.

[a]The immunologic studies were performed while the patient had severe sepsis. The subsequent studies were done 2 weeks later and revealed CD3 2.80 (1.40–2.00 × 10^9/L), CD4 2.88 (0.70–1.10 × 10^9/L), CD8 3.40 (0.60–0.90 × 10^9/L), and CD19 0.01 (0.30–0.50 × 10^9/L).

[b]IUIS classification is changed by molecular results.

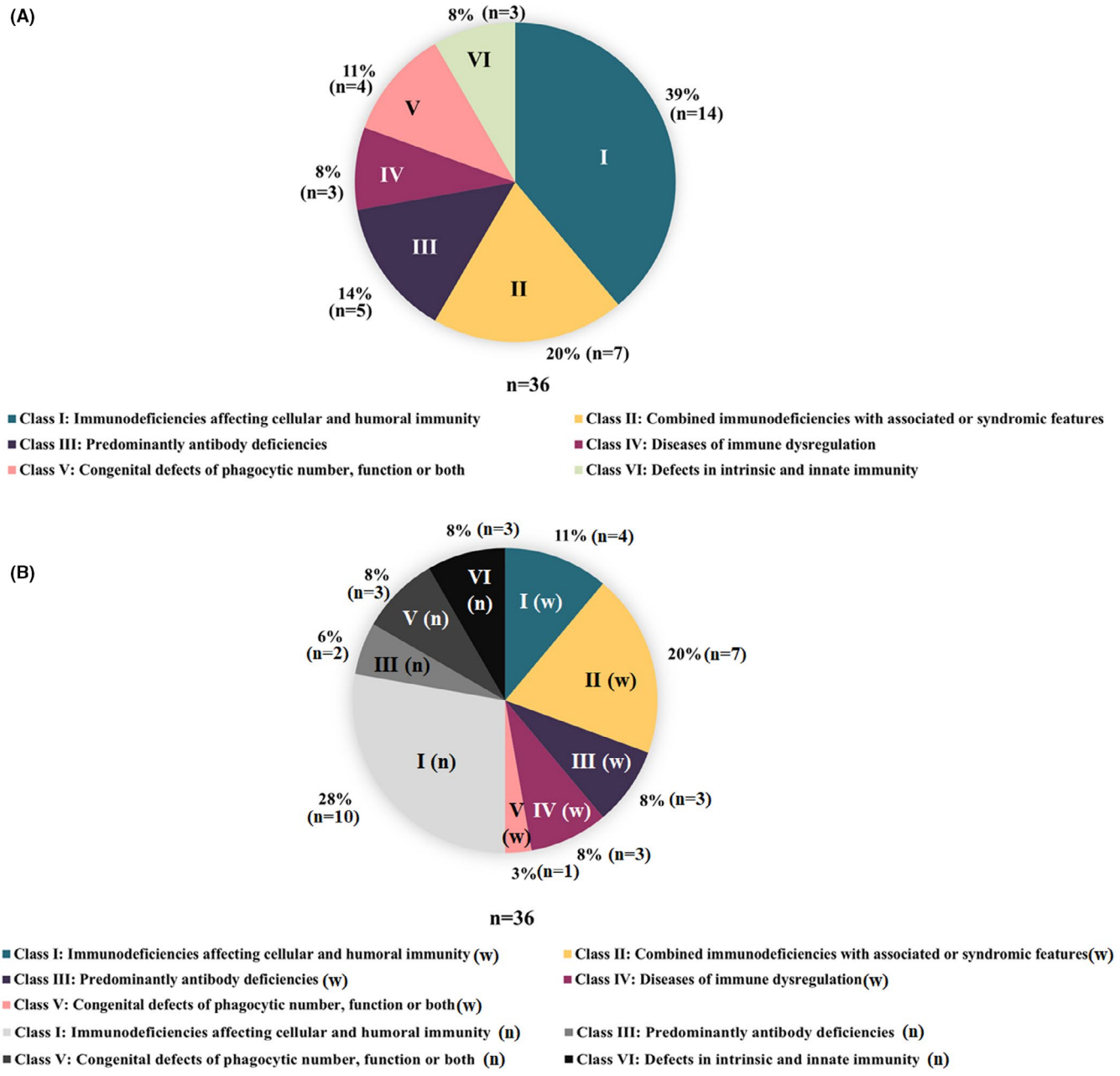


FIGURE 1 IEI categorized by IUIS classification. (A) Clinical diagnosis distribution of all patients. (B) Molecular findings in patients with IEI categorized by IUIS classification. IEI, Inborn errors of immunity; IUIS, International Union of Immunological Societies; n, negative WES; w, positive WES; WES, whole-exome sequencing

Patients 7, 8, 9, and 10 developed recurrent skin infections and recurrent pneumonia. They all had extremely elevated levels of IgE (more than 2000 IU/ml) (Table 1). The known heterozygous variants c.1110-2A > G and c.1397A > G (p.Asn466Ser) in *STAT3* were found in patients 8 and 9, respectively.[21,22] Two patients (patients 7 and 10) harbored the same variant (c.1909G > A; p.Val637Met), which has been reported previously.[23]

Patient 11 had persistent diarrhea, with failure to thrive with light-colored, brittle hair starting at the age of 1 month. PHA stimulation test was markedly decreased (SI = 2, control SI = 533). WES revealed two compound heterozygous variants, c.2689delT (p.Cys897Alafs*27) and c.154G > T (p.Glu52Ter) in *TTC37*. The c.154G > T (p.Glu52Ter) inherited from the mother was reported in a 3-year-old girl with failure to thrive, recurrent infections, and chronic diarrhea starting at 2 years of age.[24] The c.2689delT (p.Cys897Alafs*27) inherited from the father has not been previously described (Figure 2D, Table 3). The cysteine residue 897 is highly conserved (Figure S1). Trichohepatoenteric syndrome (THES) was diagnosed in this case.

3.3 | Class III: Predominantly antibody deficiencies

Three patients (patients 12, 13, and 14) were found to harbor disease-associated variants in the *BTK* gene associated with predominantly

TABLE 2 Diagnostic variants identified in patients with inborn errors of immunity (IEI)

ID	Gene	Transcript ID	Variant (s)	Source	Inheritance	Type(s)	Reported in	ACMG	Molecular diagnosis	Zygosity	IUIS
1	*CD40LG*	NM_000074.2	c.514T > C (p.Tyr172His)	Maternal	XL	Missense	Athipongarporn A et al. 2021	LP	Hyper IgM syndrome	Hemi	1
2	*DOCK8*	NM_203447.3	c.3201dupT (p.Val1068Cysfs*3)	NA	AR	Frameshift	Novel	P	DOCK8 deficiency	Homo	1[a]
3	*IL2RG*	NM_000206.2	c.722G > A (p.Ser241Asn)	Maternal	XL	Missense	Novel	LP	Severe combined immunodeficiency	Hemi	1
4	*RAG1*	NM_000448.3	c.1871G > A (p.Arg624His)	Paternal Maternal	AR	Missense	Cifaldi C et al. 2016	LP	Severe combined immunodeficiency	Homo	1
5	*KMT2D*	NM_003482.3	c.453delG (p.Gln152Argfs*56)	De novo	AD	Frameshift	Novel	P	Kabuki syndrome	Het	2[a]
6	*PGM3*	NM_001199917.2	c.1527delC (p.Asn510Metfs*4)	Paternal	AR	Frameshift	Ittiwut C et al. 2020	P	PGM3 deficiency	Comp het	2
			c.1087A > G (p.Thr363Ala)	Maternal		Missense	Ittiwut C et al. 2020	P			
7	*STAT3*	NM_139276.3	c.1909G > A (p.Val637Met)	De novo	AD	Missense	Saikia B et al. 2021	P	Hyper IgE syndrome	Het	2
8	*STAT3*	NM_139276.3	c.1110-2A > G	De novo	AD	Splicing	Woellner C et al. 2010	P	Hyper IgE syndrome	Het	2
9	*STAT3*	NM_139276.3	c.1397A > G (p.Asn466Ser)	Paternal	AD	Missense	Minakawa S et al. 2016	P	Hyper IgE syndrome	Het	2
10	*STAT3*	NM_139276.3	c.1909G > A (p.Val637Met)	De novo	AD	Missense	Saikia B et al. 2021	P	Hyper IgE syndrome	Het	2
11	*TTC37*	NM_014639.4	c.2689delT (p.Cys897Alafs*27)	Paternal	AR	Frameshift	Novel	P	Trichohepatoenteric syndrome	Comp het	2
			c.154G > T (p.Glu52Ter)	Maternal		Nonsense	Chong JH et al. 2015	P			
12	*BTK*	NM_000061.2	c.974 + 5G > A	Maternal	XL	Splicing	Conley ME et al. 2005	P	X-linked agammaglobulinemia	Hemi	3
13	*BTK*	NM_000061.2	c.179_181delAGA (p.Lys60del)	Maternal	XL	In-frame	Doğruel D et al. 2019	P	X-linked agammaglobulinemia	Hemi	3
14	*BTK*	NM_000061.2	c.1635T > A (p.Tyr545*)	NA	XL	Nonsense	Novel	P	X-linked agammaglobulinemia	Hemi	3
15	*PRF1*	NM_005041.4	c.658G > A (p.Gly220Ser)	Paternal Maternal	AR	Missense	Pronicka E et al. 2016	LP	Familial hemophagocytic lymphohistiocytosis	Homo	4
16	*RAB27A*	NM_004580.4	c.377delC (p.Pro126Glnfs*3)	Maternal	AR	Frameshift	Novel	P	Griscelli syndrome	Homo/ hemi	4
17	*UNC13D*	NM_199242.2	c.2709 + 1G > A	Paternal	AR	Splicing	Liu D et al. 2017	P	Familial hemophagocytic lymphohistiocytosis	Comp het	4
			c.446delG (p.Gly149Alafs*13)	Maternal		Frameshift	https://www.ncbi.nlm.nih.gov/snp/rs758813224?vertical_tab=true	P			
18	*G6PD*	NM_001042351.1	c.496C > T (p.Arg166Cys)	Maternal	XL	Missense	Moradkhani K et al. 2012	LP	G6PD deficiency	Hemi	5

Abbreviations: AD, autosomal dominance; AR, autosomal recessive; Comp het, compound heterozygous; Hemi, hemizygous; Het, heterozygous; Homo, homozygous; LP, likely pathogenic; NA, not available; P, pathogenic; XL, X-linked.

[a]IUIS classification is changed by molecular results.

TABLE 3 Novel variants identified in patients with inborn errors of immunity (IEI)

	Patient 2	Patient 3	Patient 5	Patient 11	Patient 14	Patient 16
Gene	*DOCK8*	*IL2RG*	*KMT2D*	*TTC37*	*BTK*	*RAB27A*
Chromosome location	chr9:399226dupT	chrX:70329113 C > T	chr12:49448147 delC	chr5:94850573 delA	chrX:100608973 A > T	chr15:55516177 delG
Variant	c.3201dupT (p.Val1068Cysfs*3)	c.722G > A (p.Ser241Asn)	c.453delG (p.Gln152Argfs*56)	c.2689delT (p.Cys897Alafs*27)	c.1635T > A (p.Tyr545*)	c.377delC (p.Pro126Glnfs*3)
SIFT	NA	Damaging (0.00)	NA	NA	NA	NA
PROVEAN	NA	Deleterious (−2.88)	NA	NA	NA	NA
PolyPhen−2	NA	Probably damaging (1.00)	NA	NA	NA	NA
M-CAP	NA	Possibly pathogenic (0.818)	NA	NA	NA	NA
CADD	33	26.4	23.2	33	35	33
GnomAD	None	None	None	None	None	None
In-house database	None	None	None	None	None	None
Classification†	Pathogenic	Likely pathogenic	Pathogenic	Pathogenic	Pathogenic	Pathogenic
Expected consequences	Frameshift leading to a valine to cysteine substitution at 1068 and a premature stop codon at position 3 of the new reading frame	A serine to asparagine substitution at 241 (fibronectin III domain)	Frameshift leading to a glutamine to arginine substitution at 152 and premature stop codon at position 56 of the new reading frame	Frameshift leading to a cysteine to alanine substitution at 897 and premature stop codon at position 27 of the new reading frame	A tyrosine to stop codon change at position 545 predicted to result in truncated protein products	Frameshift leading to a proline to glutamine substitution at 126 and premature stop codon at position 3 of the new reading frame

Abbreviations: CADD, combined annotation-dependent depletion (https://cadd.gs.washington.edu/; gnomAD, genome aggregation database (http://gnomad.broadinstitute.org/); M-CAP, Mendelian clinically applicable pathogenicity score (http://bejerano.stanford.edu/mcap/); NA, not applicable; PolyPhen-2, prediction of functional effects of human SNPs (http://genetics.bwh.harvard.edu/pph2/); PROVEAN, protein variation effect analyzer (http://provean.jcvi.org/); recommended pathogenicity threshold >20); SIFT, sorting intolerant from tolerant (http://sift.jcvi.org/).

†According to the American College of Medical Genetics and Genomics interpretation guidelines (PMID 25741868).

antibody deficiencies. All were male with recurrent sinopulmonary tract infections and low levels of immunoglobulins requiring monthly intravenous immunoglobulin. One of them (patient 13) developed chronic bronchiectasis. Mutation analysis showed three hemizygous variants, c.974 + 5G > A, c.179_181del (p.Lys60del), and c.1635T > A (p.Tyr545*) in *BTK* in patients 12, 13, and 14, respectively. The c.1635T > A (p.Tyr545*) was novel and classified as pathogenic (Figure 2E, Table 3). The tyrosine 545 is highly conserved (Figure S1). The c.974 + 5G > A and c.179_181del (p.Lys60del) were previously described.[25,26]

3.4 | Class IV: Disease of immune dysregulation

There were three patients with PIDs carrying variants in the *PRF1*, *RAB27*, and *UNC13D* genes.

Patients 15 and 17 had sepsis with splenomegaly and cytopenia. Their clinical features were consistent with hemophagocytic lymphohistiocytosis (HLH). WES revealed a homozygous variant c.658G > A, p.Gly220Ser in *PRF1* in patient 15 and compound heterozygous variants c.446delG (p.Gly149Alafs*13) and c.2709 + 1G > A in *UNC13D* in patient 17. Both *PRF1* and *UNC13D* genes were known to be associated with familial hemophagocytic lymphohistiocytosis syndrome (Table 2).[27-29]

Patient 16 was a 4-month-old boy born to consanguineous parents. He was found to have fever, progressive splenomegaly, pancytopenia, hyperferritinemia (1856 μg/L), hypofibrinogenemia (<100 mg/dl), and hypertriglyceridemia (598 mg/dl). Bone marrow aspiration and biopsy revealed hemophagocytosis. He also had oculocutaneous albinism with silver-colored hair and eyebrows. The microscopic examination of his hair showed pigment clumps in the medullary area. A novel deletion variant c.377delC (p.Pro126Glnfs*3) in *RAB27A* was identified by WES confirming the diagnosis of Griscelli syndrome type 2 (Figure 2F). The mother was heterozygous for this variant. The paternal DNA was unavailable. The proline residue 126 is highly conserved (Figure S1). It is classified as pathogenic (Table 3).

3.5 | Class V: Congenital defects of phagocytic number, function, or both

One patient was found to carry a variant in the *G6PD* gene.

Patient 18 developed Chromobacterium violaceum skin infection and necrotizing pneumonia at the age of 1 year. An elevated white blood cell count with neutrophil predominance was seen (WBC: 46,000 cells/μl, neutrophil 80%). Dihydrorhodamine (DHR) test showed reduced oxidative burst (SI = 17.4, control SI = 110.7) with broad histograms. The DHR pattern from the mother showed bimodal distribution. WES identified a known hemizygous variant c.496C > T, p.Arg166Cys in *G6PD* (Table 2) in the patient.[30] His G6PD activity was 0 U/gHb (4.6–13.5). The variant was also identified in the mother.

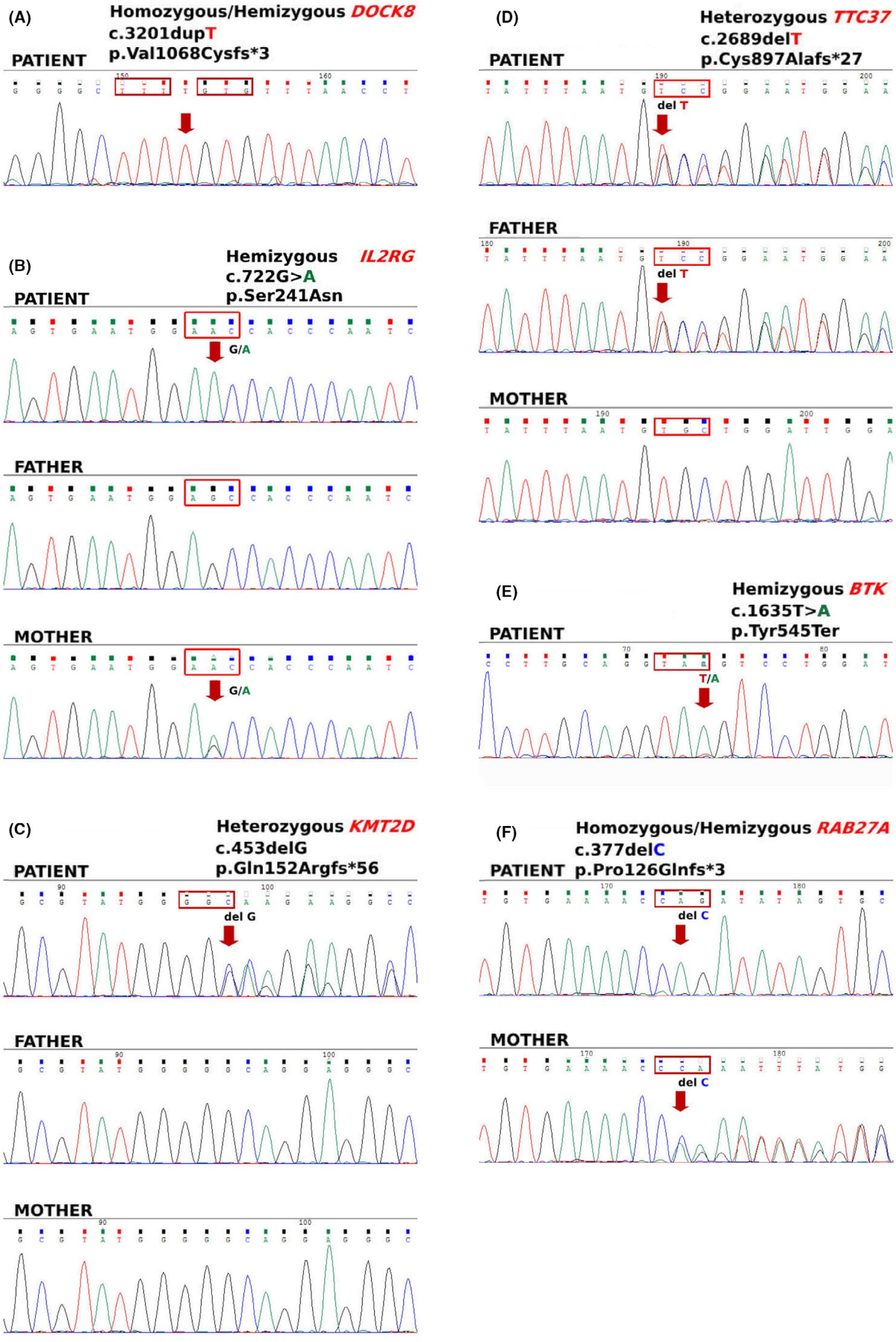
4 | DISCUSSION

We studied 36 pediatric patients with clinical and immunologic features consistent with IEI. With whole-exome sequencing, as a first-tier diagnostic tool, we successfully identified pathogenic and likely pathogenic variants in 18 patients (50%). 30% of these identified variants have not been previously described. In addition, 38.9% of these detected variants were found in genes responsible for combined immunodeficiencies with associated or syndromic features (Class II). Our study is the first and largest to investigate the genetic defects underlying IEI using WES in the Thai population.

Due to the phenotypic and genetic heterogeneity of IEI, molecular diagnosis by NGS has become a crucial part for evaluating these patients with complex condition. In addition, cases with atypical features or severe manifestations would require rapid and definitive diagnosis that could be possibly made by using NGS. These results can lead to appropriate decision and life-saving treatment in some patients. There were two patients whose clinical diagnosis was changed after having molecular results. Patient 2 was initially diagnosed with combined immunodeficiencies with associated syndromic features. WES revealed a pathogenic variant c.3201dupT (p.Val1068Cysfs*3) in *DOCK8*, a gene associated with immunodeficiencies affecting cellular and humoral immunity. Patient 5 was initially diagnosed to have immunodeficiencies affecting cellular and humoral immunity at the age of 2 months. The patient was found to harbor a heterozygous c.453delG (p.Gln152Argfs*56) in *KMT2D*, the gene responsible for Kabuki syndrome. Patients 4 and 16 were found to carry a homozygous variant in *RAG1* and *RAB27A*, respectively. Patient 4 underwent hematopoietic stem cell transplantation (HSCT) shortly afterward. Due to severe infection in patient 16, he has not received HSCT. Previous studies have demonstrated that overall survival rate of HLA-matched HSCT in both diseases is approximately 70%; however, poor T-cell engraftment and immune function could develop unless conditioning prior to cell infusion was given.[31-33]

We also identified a hemizygous variant in *IL2RG*, confirming the diagnosis of X-linked severe combined immunodeficiency (SCID) in patient 3 (Table 2). He was the second child with a healthy brother. The mother was found to be a carrier. Genetic counseling was then provided. Subsequently, the mother was pregnant for the third time. The baby boy was born at term after an uneventful pregnancy. He was found to harbor the similar variant. T-cell receptor excision

FIGURE 2 Electropherograms showing all six novel variants identified in the PID genes. (A) The 1-bp insertion variant c.3201dupT (p.Val1068Cysfs*3) in *DOCK8* was present in patient 2. (B) The hemizygous variant c.722G > A (p.Ser241Asn) in *IL2RG* was detected in patient 3 and his mother. (C) The de novo heterozygous 1-bp deletion c.453delG (p.Gln152Argfs*56) in *KMT2D* was identified in patient 5. (D) The c.2689delT (p.Cys897Alafs*27) in the *TTC37* gene was found in patient 11 and her father. (E) The c.1635T > A (p.Tyr545*) in *BTK* was identified in patient 14. (F) The deletion variant c.377delC (p.Pro126Glnfs*3) in *RAB27A* was present in patient 16 and his mother


(A)
Homozygous/Hemizygous DOCK8
c.3201dupT
p.Val1068Cysfs*3
PATIENT
(B)
Hemizygous IL2RG
c.722G>A
p.Ser241Asn
PATIENT
G/A
FATHER
MOTHER
G/A
(C)
Heterozygous KMT2D
c.453delG
p.Gln152Argfs*56
PATIENT
del G
FATHER
MOTHER
(D)
Heterozygous TTC37
c.2689delT
p.Cys897Alafs*27
PATIENT
del T
FATHER
del T
MOTHER
(E)
Hemizygous BTK
c.1635T>A
p.Tyr545Ter
PATIENT
T/A
(F)
Homozygous/Hemizygous RAB27A
c.377delC
p.Pro126Glnfs*3
PATIENT
del C
MOTHER
del C

circles (TRECs) obtained from peripheral blood at birth were undetectable. At age nine days, flow cytometry was performed and revealed lymphopenia with markedly low T-cell and NK cell numbers (lymphocyte 1330 cells/μl), CD3 (0.009×10^9/L; normal range 2.50–5.50), CD4 (0.009×10^9/L; normal range 1.60–4.00), CD8 (0.009×10^9/L; normal range 0.56–1.70), CD19 (0.76×10^9/L; normal range 0.30–2.00), and CD56 (0.08×10^9/L; normal range 0.17–1.10). The immunoglobulin testing also showed normal levels of serum IgG (7.68 g/L; normal range 6.31–14.31) and IgA (<0.05 g/L; normal range 0–0.08) with high IgM (0.25 g/L; normal range 0.01–0.21). Intravenous immunoglobulin (IVIG) and prophylactic medications including fluconazole, acyclovir, and co-trimoxazole were given starting at the age of 19 days. He received HSCT from his healthy 4-year-old brother at the age of 2 months and showed favorable outcomes. IVIG has been administered monthly, and his laboratory findings at the age of 1 year and 4 months revealed a normal absolute lymphocyte count (3697 cells/μl), CD3 (2.96×10^9/L; normal range 1.46–5.44), CD4 (1.70×10^9/L; normal range 1.02–3.60), CD8 (0.89×10^9/L; normal range 0.57–2.23), CD19 (0.52×10^9/L; normal range 0.50–1.50), CD56 (0.15×10^9/L), and IgG (8.33 g/L; normal range 3.44–11.8). He is currently 1 year and 2 months old without history of severe infection.

As shown in patient 18, patients with severe G6PD deficiency could present with recurrent infections mimicking the phenotype of chronic granulomatous disease.[34,35] The reduction in granulocyte NADPH oxidase leads to the impairment of neutrophil extracellular trap (NET) formation, resulting in susceptibility to infections.[36] Our patient currently receives co-trimoxazole prophylaxis with no episodes of severe infections.

There are six disease-associated variants that have not been previously described. The functional consequences of these newly identified variants require further studies. Functional validation of variants remains fundamental to attribute pathogenicity with certainty.[37]

There were also 18 cases suspected of IEI with negative WES results (50%) (Figure 1B). There were no differences in severity between children who had a positive and negative WES (Table 1). Of note, WES was able to identify molecular defects in all patients classified as combined immunodeficiencies with associated or syndromic features, and diseases of immune dysregulation. Nevertheless, of the 14 patients with clinical diagnosis of immunodeficiencies affecting cellular and humeral immunity, four (29%) were found to harbor disease-associated variants (Figure 1). This could be due to the broad spectrum and variety of clinical presentations in this category.

Whole-exome sequencing and WGS have markedly increased the number of newly identified disease-associated genes; re-analysis of the exome data for those novel genes could lead to diagnosis in some patients.[3,38,39] There is also a possibility that copy number or structural variants, variants located deep within introns, and repeat expansions could be missed by WES. It has been demonstrated that whole-genome sequencing (WGS) could be used for further evaluation if the cases remained undiagnosed after WES. If potential new disease-causing genes could be identified, evaluating the validity and performing functional studies to confirm disease-gene association and elucidate the pathophysiology underlying diseases are required. Discovering novel IEI-associated genes could provide molecular insights into the pathway involved in the human immune system and expand our knowledge of the molecular mechanism underlying IEI or immune-related disorders. This could bring new therapeutic opportunities leading to improved patient outcomes.

In conclusion, this study is the first and largest to investigate genetic causes in pediatric IEI cases in the Thai population. WES was successfully performed to identify the genetic defects in 50% of cases. Of all the 20 variants found to be associated with the diseases, six (30%) were novel. Our findings also demonstrated the genetic and phenotypic heterogeneity of IEI supporting the use of WES in diagnosis and clinical decision making.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Maliwan Tengsujaritkul: Data curation (equal); Formal analysis (equal); Writing – original draft (equal). **Narissara Suratannon:** Formal analysis (supporting); Investigation (lead); Writing – original draft (equal). **Chupong Ittiwut:** Formal analysis (equal); Methodology (supporting); Software (equal). **Rungnapa Ittiwut:** Formal analysis (equal); Methodology (supporting); Software (equal). **Pantipa Chatchatee:** Conceptualization (supporting); Writing – review & editing (equal). **Kanya Suphapeetiporn:** Conceptualization (equal); Funding acquisition (equal); Supervision (equal); Writing – review & editing (equal). **Vorasuk Shotelersuk:** Conceptualization (equal); Funding acquisition (equal); Writing – review & editing (equal).

PEER REVIEW

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