Phenotypic Heterogeneity and Genotypic Spectrum of Primary Immunodeficiencies with Whole Exome Sequencing in a Thai Patient Cohort

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August 6, 2021

Abstract

Background: Primary immunodeficiency diseases (PIDs) 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, immunological and genetic features of Thai pediatric patients with PIDs. The use of whole exome sequencing (WES) in diagnosis and clinical decision making was also assessed. Methods: 36 unrelated patients with clinical and laboratory findings consistent with PIDs 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 PID genes were identified. The clinical usefulness of WES in PIDs was demonstrated, emphasizing it as an effective diagnostic strategy in these genetically heterogeneous disorders.

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Keywords : Primary immunodeficiency diseases, Next generation sequencing, Whole exome sequencing; Novel variants, Thai

Abstract

Background: Primary immunodeficiency diseases (PIDs) 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, immunological and genetic features of Thai pediatric patients with PIDs. The use of whole exome sequencing (WES) in diagnosis and clinical decision making was also assessed. **Methods:** 36 unrelated patients with clinical and laboratory findings consistent with PIDs 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 PID genes were identified. The clinical usefulness of WES in PIDs was demonstrated, emphasizing it as an effective diagnostic strategy in these genetically heterogeneous disorders.

Key Message

Primary immunodeficiency diseases (PIDs) 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 PID 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 PIDs 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 PIDs.

INTRODUCTION

Primary immunodeficiency diseases (PIDs) are a heterogeneous group of more than 400 monogenic disorders caused by defects in genes responsible for different components of the immune system. PIDs had 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 PIDs has been increasing rapidly.[3-5] This has expanded our understanding of genotype-phenotype correlations and provided better insights into the pathogenesis of PIDs. 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 with PIDs. 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 counselling 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 PIDs 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 PIDs with diagnostic yield ranged from 15% to 79%.[13] It has been demonstrated that patients with PIDs 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[5,14]In this study, we aimed to characterize the clinical and genetic features of PIDs in the Thai pediatric population. WES was performed in all cases. Our findings have expanded the phenotypic and genotypic spectrum of PIDs.

METHODS

2.1 Patients

A total of 36 unrelated patients with clinical and laboratory findings suspected of PIDs were recruited in the study. Most of the patients were evaluated at King Chulalongkorn Memorial Hospital from January 2010 to December 2020.

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 were taken from the patients and their available parents. Genomic DNA was extracted from peripheral blood leukocytes using the Puregene blood kit (Qiagen, Hilden, Germany). Whole exome sequencing (WES) was performed by Macrogen, Inc (Seoul, Korea) as previously described.[15] 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.source forge.net/). The single-nucleotide polymorphisms (SNPs) and Indels were detected by SAMTOOLS (http://samtools.source forge.net/) and annotated by dbSNP&1000G. The variants were subsequently filtered out if they were present in our in-house database of 2,166 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, 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 MCAP (http://bejerano.stanford.edu/mcap/), were 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.

RESULTS

A total of 36 patients clinically diagnosed with PIDs 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.

Whole exome sequencing (WES) was performed to investigate the genetic defects in all 36 cases. According to American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guideline for variant classification, 20 variants including pathogenic, likely pathogenic and variants of uncertain significance explaining the patients' phenotypes were identified in 18 cases (50%) (Table 2). Of the 20 variants identified, 6 in 20 (30%) were novel (Table 3). These variants included eight missense, six frameshift, three splicing, two nonsense and one in-frame. 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: disease of immune dysregulation,

and one in category V: congenital disorders of phagocyte number or function. No variants were identified in genes classified in categories VI-X (Figure 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 PIDs (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 classified as likely pathogenic.

Patient 2 had recurrent skin infections, chronic eczema and recurrent pneumonia since the age of two years. He subsequently developed pulmonary tuberculosis at eight years old. His immunoglobulin test revealed an elevated IgE level (1,661 IU/ml). We identified a novel 1-bp insertion variant, c.3201dupT (p.Val1068Cysfs*3) in *DOCK8* (Figure 2a, Table 3). 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. The leukocyte count was low. The immunoglobulin test that was performed after intravenous immunoglobulin (IVIG) administration revealed normal levels. 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). 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, Supplementary table 1). T-B-NK+ severe combined immunodeficiency was suspected. WES revealed a known homozygous variant, c.1871G>A (p.Arg624His) in *RAG1* .

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. 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).

Patient 6 developed recurrent sinopulmonary tract infections, severe atopic dermatitis, chronic diarrhea, and multiple food allergies since the age of two years. Immunoglobulin levels were normal and immunophenotyping revealed low levels of CD3, CD4, CD8 (Table 1, Supplementary table 1). Using WES, two compound heterozygous variants, c.1527delC (p.Asn510Metfs*4) and c.1087A>G (p.Thr363Ala) in*PGM3* were identified, confirming the diagnosis of PGM3 deficiency.[16]

Patients 7, 8, 9, and 10 developed recurrent skin infections and recurrent pneumonia. They all had extremely elevated levels of IgE (more than 2,000 IU/ml) (Table 1, Supplementary table 1). The heterozygous variants, c.1110-2A>G, and c.1397A>G (p.Asn466Ser) in *STAT3* were found in patients 8 and 9, respectively. Two patients (patients 7 and 10) harbored the similar variant (c.1909G>A; p.Val637Met).

Patient 11 had persistent diarrhea, failure to thrive with light-colored, brittle hair starting at the age of one month. The flow cytometry showed low levels of CD4 and CD56 while the immunoglobulin test revealed normal levels of IgG and elevated levels of IgM and IgA (Table 1, Supplementary table 1). WES revealed two compound heterozygous variants, c.2689delT (p.Cys897Alafs*27) and c.154G>T (p.Glu52Ter; PMID:

29527791) in *TTC37* . The c.2689delT (p.Cys897Alafs*27) inherited from the father has not been previously described (Figure 2d, Table 3). Trichohepatoenteric syndrome (THES) was diagnosed in this case.

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 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 heterozygous 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 c.974+5G>A and c.179_181del (p.Lys60del) were previously described.

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.

Patient 16 was a 4-month-old boy born to consanguineous parents. He was found to have fever, progressive splenomegaly, pancytopenia, hyperferritinemia (1,856 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. It is classified as pathogenic (Table 3).

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 one year. An elevated white blood cell count with neutrophil predominance (WBC: 46,000 cells/µL, neutrophil 80%). Dihydrorhodamine (DHR) test showed abnormal results. WES identified a known hemizygous variant, c.586C>T, p.Arg196Cys in *G6PD* (Table 3). His G6PD activity was 0 U/gHb (4.6-13.5).

DISCUSSION

We studied 36 pediatric patients with clinical and immunologic features consistent with PIDs. With whole exome sequencing, as a first-tier diagnostic tool, we successfully identified pathogenic, likely pathogenic variants and variants of uncertain significance 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 PIDs using WES in the Thai population.

Due to the phenotypic and genetic heterogeneity of PIDs, 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. 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 afterwards. 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 are approximately 70%; however, poor T-cell engraftment and immune function could develop unless conditioning prior to cell infusion was given.[17-19]

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 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 1,330 cells/μL), CD3 (0.009 x 10^9/L; normal range 2.50-5.50), CD4 (0.009 x 10^9/L; normal range 1.60-4.00), CD8 (0.009 x 10^9/L; normal range 0.56-1.70), CD19 (0.76 x 10^9/L; normal range 0.30-2.00), and CD56 (0.08 x 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 two months and showed favorable outcomes. IVIG has been administered monthly and his laboratory findings at the age of one year and four months old revealed a normal absolute lymphocyte count (3,697 cells/μL), CD3 (2.96 x 10^9/L; normal range 1.46-5.44), CD4 (1.70 x 10^9/L; normal range 1.02-3.60), CD8 (0.89 x 10^9/L; normal range 0.57-2.23), CD19 (0.52 x 10^9/L; normal range 0.50-1.50), CD56 (0.15 x 10^9/L), and IgG (8.33 g/l; normal range 3.44-11.8). He is currently one year and five 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.[20,21]The reduction of granulocyte NADPH oxidase leads to the impairment of neutrophil extracellular trap (NET) formation, resulting in susceptibility to infections.[22] Our patient currently receives co-trimoxazole prophylaxis with no episodes of severe infections.

There were 18 cases suspected of PIDs with negative WES results (50%). As WES 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.[3,23,24]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 PID-associated genes could provide molecular insights into the pathway involved in the human immune system and expand our knowledge of the molecular mechanism underlying PIDs 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 PID 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 PIDs supporting the use of WES in diagnosis and clinical decision making.

ACKNOWLEDGMENTS

We would like to thank the patients and their families for participating in this study. This work was supported by Thailand Research Fund (BRG5980001, DPG6180001, MRG6080172), Health Systems Research Institute, TSRI Fund (CU_FRB640001_01_30_10), and Grants for Development of New Faculty Staff, Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

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

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Figure legend

Figure 1 Molecular findings in the patients with PIDs categorized by IUIS classification.

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

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