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Original Article

Molecular diagnostic success of targeted next-generation sequencing (NGS) in 101 pediatric patients with inborn errors of immunity

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ABSTRACT

Objective: Inborn errors of immunity (IEIs) comprise a genetically heterogeneous group of disorders predisposing individuals to recurrent and severe infections, autoimmunity, and immune dysregulation. Next-generation sequencing (NGS) has greatly improved diagnostic efficiency by allowing simultaneous analysis of multiple genes. This study aimed to evaluate the molecular diagnostic yield and characterize the variant spectrum in patients with suspected IEIs using a targeted NGS panel.

Methods: A total of 101 pediatric patients clinically diagnosed with IEIs and referred to the Pediatric Genetics Department Ümraniye Training and Research Hospital between 2018 and 2021 were included in the study. Genetic analysis was performed using a targeted NGS panel encompassing 260 genes associated with IEIs. Variants were interpreted according to American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) and Clinical Genome (ClinGen) Sequence Variant Interpretation (SVI) guidelines. Single-nucleotide variants (SNVs) were confirmed by Sanger sequencing, and copy number variants (CNVs) were validated using array-based comparative genomic hybridization (array-CGH).

Results: Pathogenic or likely pathogenic (P/LP) variants were identified in 25 of 101 patients (24.7%), yielding 26 distinct variants across 21 genes, including one patient with two variants in a compound heterozygous state. Among these, 18 were homozygous, 3 heterozygous, 3 hemizygous, and 1 compound heterozygous. The most frequently affected genes were *RAG1*, *DCLRE1C*, *SPINK5*, and *STAT1*. Three novel variants were identified, expanding the known mutational spectrum of IEIs. In addition, several variants initially identified in this cohort were later reported by our group, highlighting the contribution of our study to the expanding genetic spectrum of IEIs in Türkiye. Most variants exhibited autosomal recessive inheritance, consistent with the high consanguinity rate in the study population.

Conclusion: In our IEI cohort, targeted NGS achieved a 24.7% molecular diagnostic yield and successfully identified both known and novel pathogenic variants across a broad spectrum of genes. These findings highlight the diagnostic value of targeted NGS in genetically and clinically heterogeneous conditions such as IEIs and underscore the importance of population-specific variant databases for improving variant interpretation and optimizing patient care.

Keywords: Inborn errors of immunity, next-generation sequencing, children, diagnosis

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INTRODUCTION

Inborn errors of immunity (IEIs) are a heterogeneous group of disorders caused by genetic defects in immune system development and function.[1,2] These conditions predispose affected individuals to recurrent and severe infections, autoimmunity, malignancies, and autoinflammatory complications. The 2024 classification by the International Union of Immunological Societies (IUIS) Expert Committee recognizes 508 distinct genetic defects and further documents 17 phenocopy conditions. This update incorporates 67 newly defined monogenic IEIs and 2 novel phenocopies, thereby expanding both genotypic and phenotypic categorization and providing an updated framework for the design of diagnostic panels.[3]

The global prevalence of IEIs is approximately 1 in 10,000 live births, affecting more than 6 million individuals worldwide. However, 70–90% of patients remain undiagnosed due to clinical heterogeneity and limited access to advanced genetic testing.[4,5] In populations with high consanguinity, such as Türkiye (20–35%), the frequency of autosomal recessive IEIs is significantly increased.[5,6] While this contributes to a higher disease burden, it also enhances the likelihood of identifying homozygous or compound heterozygous variants, thereby improving the diagnostic yield of genetic testing. Given the extensive genetic heterogeneity of IEIs, conventional single-gene sequencing approaches are often slow and insufficient for diagnosis.

Next-generation sequencing (NGS) has transformed the field by enabling simultaneous analysis of multiple genes.[7] Targeted NGS panels have been shown to be cost-effective first-line tests, offering high coverage, shorter turnaround times, and fewer incidental findings.[8] More recently, large-scale studies indicate that whole-exome sequencing (WES) provides diagnostic yield equal to or greater than that of other approaches and is increasingly cost-effective, with the added advantages of reanalysis potential and novel gene discovery. Targeted panels, however, remain useful in well-defined clinical scenarios requiring rapid results, deeper coverage, or in settings where access to WES is limited.[9] Reported diagnostic yields of targeted NGS panels in PID cohorts range from 15% to 70%, depending on patient selection and disease category. Notably, higher yields are observed in severe combined immunodeficiency (SCID), with reported rates of 58–90%.[7] Türkiye's high consanguinity rate may further enhance diagnostic yield, as suggested by several studies: targeted sequencing in SCID patients yielded a 32% diagnostic rate, while WES-based approaches achieved 41–63%.[5,10,11] Globally, recent large-scale analyses have reported a 42% overall diagnostic yield, rising to 58% in patients with a positive family history, and cost-effectiveness analyses often favor WES approaches.[12] In smaller clinical exome studies, complementary analyses further increased diagnostic yield from 31% to 42%.[2]

In this study, we aimed to evaluate the molecular diagnostic success rate, characterize the spectrum of identified variants, and assess the clinical implications of genetic diagnosis for patient management and counseling, based on our experience at our institution using a targeted NGS panel in a cohort of 101 pediatric patients with suspected IEIs.

MATERIALS and METHODS

Study population

A total of 101 pediatric patients with a clinical diagnosis of IEIs who were referred for genetic testing between 2018 and 2021 were included in this study. Among these, 25 patients had likely pathogenic or pathogenic (P) variants according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) and Clinical Genome (ClinGen) guidelines (see Genetic and Variant Analysis section). Ethical approval for the study was obtained from the Health Science University, Ümraniye Training and Research Hospital Ethics Committee (approval number: B10.1.T.K.H.4.34.H.GP.0.01/392).

Genetic and variant analysis

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood using a semi-automated system (Qiagen) in accordance with the manufacturer's protocol. DNA quality and concentration were assessed using spectrophotometric and fluorometric methods. Library preparation was performed with the Clinical Exome Solution Kit (Sophia Genetics, Switzerland), targeting 260 genes associated with primary immunodeficiency (gene list provided in Supplementary Material). Sequencing was conducted on the NextSeq 500 platform (Illumina, San Diego, CA, USA).

Bioinformatic analysis, including alignment, variant calling, and annotation, was performed using Sophia DDM software (version 5.2) with the NCBI Build 37 (hg19) human genome reference. Variants within ±10 bp of exon–intron boundaries

and with ≥50× read depth were analyzed, while low-quality or off-target variants were excluded. All called variants were visually verified in Integrative Genomics Viewer (IGV). Detected single nucleotide (SNV) and copy number (CNV) variants were classified as LP or P according to the ACMG/AMP guidelines,[13] updated recommendations by the ClinGen Sequence Variant Interpretation (SVI) Working Group (https://clinicalgenome.org/working-groups/sequence-variant-interpretation), and gene and disease-specific specifications developed by ClinGen Expert Panels available through the ClinGen Clinical Specification Portal (https://cspec.genome.network/cspec/ui/svi/). Sanger sequencing was performed for variant confirmation, with primer details and reaction settings available upon request.

All CNVs identified by NGS were confirmed by array-based comparative genomic hybridization (array-CGH) analysis.

RESULTS

A total of 101 pediatric patients with a clinical diagnosis of IEIs were analyzed using a targeted NGS panel encompassing 260 genes associated with immune disorders. Genetic analysis identified LP or P variants in 25 patients (24.7%), representing the molecular diagnostic yield of the study cohort. Of the 25 genetically confirmed patients, 13 were male, and 12 were female. The median age at symptom onset was 6 months (range: 0–180 months) among patients with available data. Parental consanguinity was documented in 10 of the 25 families (40.0%), consistent with the high background consanguinity rate reported for Türkiye. In total, 26 distinct variants were detected among these 25 patients, one of whom carried two variants in a compound heterozygous state. Among the identified variants, 18 (69.2%) were homozygous, 3 (11.5%) were heterozygous, and 3 (11.5%) were hemizygous. Most variants were SNVs or small insertions/deletions (indels), while CNVs were detected in two patients (8.0%) and confirmed using array-based comparative genomic hybridization (array-CGH).

The identified disease-causing variants were distributed across 21 genes. The most frequently affected genes were *RAG1*, *DCLRE1C*, *SPINK5*, and *STAT1*, each detected in more than one individual. Most variants showed an autosomal recessive inheritance pattern, consistent with the high rate of parental consanguinity documented in our cohort, and this likely contributed to the relatively high proportion of homozygous variants identified. Of the 26 variants, 8 (30.8%) were classified as LP and 18 (69.2%) as P according to the ACMG/AMP and ClinGen SVI guidelines. Three novel variants (11.5%) were identified. The major clinical diagnostic categories observed among genetically confirmed patients were combined immunodeficiency (CID) (8 patients), severe combined immunodeficiency (SCID) (5 patients), and Mendelian susceptibility to mycobacterial disease (MSMD) (4 patients), followed by immune dysregulation, agammaglobulinemia, and autoinflammatory disorders. For an overview of the immunological phenotype categories (e.g., T-B+NK+ SCID, T-B-NK+ SCID, CID, agammaglobulinemia, immune dysregulation, autoinflammatory disease) and their corresponding genes, the 25 genetically confirmed patients are summarized in Supplementary Table 1.

Segregation analysis was performed in all available family members, confirming biallelic inheritance, X-linked transmission, or de novo occurrence in each case. Identified variants and corresponding disease categories are summarized in Table 1, which also includes detailed clinical and laboratory findings to enable genotype–phenotype correlation.

DISCUSSION

In this study, we evaluated the molecular diagnostic yield and variant spectrum of a targeted NGS panel in 101 pediatric patients with clinically diagnosed IEIs. P or LP variants were detected in 25 patients (24.7%), distributed across 21 genes, including 3 novel variants. In addition, five variants (patients 2, 4, 16, 21, and 22) were initially identified in this cohort and later reported by our group, highlighting the contribution of our study to the expanding genetic spectrum of IEIs in Türkiye. The findings confirm the diagnostic utility of targeted NGS panels as a first-line genetic test in IEIs cohorts, particularly in populations with high rates of consanguinity or in regions where access to comprehensive approaches such as WES or whole genome sequencing (WGS) is limited due to cost or infrastructure constraints. In our cohort, combined immunodeficiencies, including SCID, represented the largest subgroup, in line with previous reports from Türkiye.[5,10,11] Patients with *RAG1*/*RAG2*, *DCLRE1C*, *CIITA*, *DOCK8*, and *FOXN1* defects illustrated the broad clinical spectrum of CID/SCID, ranging from classic T-B−NK+ SCID to leaky or syndromic forms with dysmorphic features, growth delay, or skeletal and ectodermal anomalies. Immune dysregulation phenotypes were also prominent, particularly in patients with *FAS*, *LRBA*, *FOXP3*, and *NFKB2* variants, reflecting the growing

Table 1. Summary of identified gene variants, clinical features, and laboratory findings

Patient	Primary Diagnosis	Gene/ Transcript	Variant	Zygosity	Inheritance †	ACMG classification	Clinical Findings	Laboratory Findings	Reported Previously (Reference)
1	ALPS	*FAS* NM_000043.6	c.869C>T p.(Ala290Val)	homozygous	biallelic	Likely Pathogenic	Recurrent fever and infections, lymphoproliferation, hepatosplenomegaly, and T-cell lymphoma.	Elevated IgG and IgE levels, hypergammaglobulinemia, increased DNT cells, high vitamin B12, abnormal CD4/ CD8 ratio.	[14]
2	Immune dysregulation	*LRBA* NM_001364905.1	c.6372del p.(F2124Lfs*29)	homozygous	biallelic	Pathogenic	Recurrent respiratory infections, chronic diarrhea, malabsorption, recurrent otitis media, bronchiectasis, hyperthyroidism, food allergy, arthralgia, and splenomegaly.	Panhypogammaglobulinemia, lymphopenia, pancytopenia, inverted CD4/CD8 ratio, anemia.	*[15]
3	SCID	*RAG2* NM_000536.4	c.233G>C p.(Cys78Ser)	homozygous	biallelic	Likely Pathogenic	Eczema, recurrent wheezing, chronic diarrhea, recurrent respiratory infections, otitis media, arthritis, ptosis, adrenal mass, oral candidiasis, aphthous ulcers, and history of hemolytic anemia.	Decreased IgG, IgE, and IgA levels; normal IgM; lymphopenia with reduced $CD3^+$, $CD4^+$, $CD8^+$ T lymphocytes and NK cells.	[16]
4	MHC class II deficiency	*CIITA* NM_000246.4	c.2879T>A p.(Leu960Gln)	homozygous	biallelic	Likely Pathogenic	Upper respiratory tract infection and cytomegalovirus infection.	Decreased $CD4^+$ T lymphocytes, reduced recent thymic emigrants, and low human leukocyte antigen–DR expression.	*[17]
5	SCID	*DCLRE1C*	Exon1-3 deletion[&]	homozygous	biallelic	Pathogenic	Recurrent respiratory infections, diarrhea, and eczema; small perimembranous ventricular septal defect; two siblings died due to Artemis-SCID; underwent HSCT at 9 months of age	Decreased IgA and IgG levels, normal IgM; lymphopenia, reduced $CD4^+$ T cells, $CD8^+$ T cells, and B cells.	[18,19]
6	SCID	*DCLRE1C* NM_001033855.3	c.632G>T p.(Gly211Val)	homozygous	biallelic	Pathogenic	Recurrent infections, recurrent otitis media, frequent febrile episodes, thrombus in the left middle cerebral artery, moyamoya disease, growth retardation, microcephaly, and congenital hypothyroidism; underwent HSCT at 46 months of age.	Decreased IgA and IgE levels; IgG unknown; reduced $CD4^+$ T cells, $CD8^+$ T cells, and B lymphocytes.	[9,19]
7	MSMD	*STAT1* NM_007315.4	c.1154C>T p.(Thr385Met)	heterozygous	*de novo*	Pathogenic	Chronic mucocutaneous candidiasis, recurrent aphthous ulcers, recurrent respiratory infections, pertussis, acneiform rash, growth retardation, dermatomycosis, and autoimmune hemolytic anemia.	Lymphopenia, low IgM, and inverted CD4/CD8 ratio.	[20]
8	MSMD	*STAT1* NM_007315.4	c.71A>G p.(Asp24Gly)	heterozygous	*de novo*	Likely Pathogenic	Recurrent fever, moniliasis, history of seizures, miliary tuberculosis, onychomycosis, and cheilitis.	Lymphopenia, elevated IgE, and decreased IgG.	Novel
9	Agammaglobulinemia	*BTK* NM_000061.3	c.900_903del p.(Gly302Valfs*28)	hemizygous	maternal	Pathogenic	Recurrent upper respiratory tract infections and sinusitis, short stature due to growth hormone deficiency, and chronic diarrhea.	Panhypogammaglobulinemia and decreased $CD19^+$ B lymphocytes.	[21]
10	SCID	*RAG1* NM_000448.3	c.1682G>A p.(Arg561His)	homozygous	biallelic	Pathogenic	Recurrent upper respiratory tract infections, diaper dermatitis, cutaneous granulomas, recurrent warts, chronic diarrhea, moniliasis, and history of molluscum contagiosum; underwent HSCT.	Lymphopenia, panhypogammaglobulinemia, and T-, B-, and NK-cell lymphopenia.	[16,22]

ALPS: Autoimmune lymphoproliferative syndrome, MSMD: Mendelian susceptibility to mycobacterial disease, SCID: Severe combined immune deficiency. MHC: Major Histocompatibility Complex CID: Combined immune deficiency. CMC: chronic mucocutaneous candidiasis IVIG: Intravenous immunoglobulin; IgG: Immunoglobulin G; IgA: Immunoglobulin A; IgE: Immunoglobulin E; IgM: Immunoglobulin M; DNT: Double negative T.
HSCT: Hematopoietic stem cell transplantation. BCG: Bacillus Calmette–Guérin; CD: Cluster of differentiation
[&]Chromosomal microarray done for confirmation.
[†]Segregation analysis (parental ± sibling testing) was performed in all cases to confirm the reported mode of inheritance (biallelic, X-linked, or de novo
[*]Patients 15, 17, 27, 31, and 32 were first identified in our cohort and subsequently reported in the literature by our group or collaborators. These cases are therefore not classified as novel but were unpublished at the time of detection.

Table 1. Continued

Patient	Primary Diagnosis	Gene/ Transcript	Variant	Zygosity	Inheritance †	ACMG classification	Clinical Findings	Laboratory Findings	Reported Previously (Reference)
11	SCID	*RAG1* NM_000448.3	c.1767C>G p.(Tyr589*)	homozygous	biallelic	Pathogenic	Generalized dermatitis, erythroderma, chronic diarrhea, diaper dermatitis, axillary lymphadenopathy, and history of omphalitis; underwent HSCT from an unrelated donor at 8 months of age.	Decreased $CD19^+$ B cells and $CD45RA^+$ naïve T cells, increased memory T cells ($CD45RO^+$), eosinophilia, and decreased IgG, IgA, and IgE levels	[9]
12	Immuno-osseous dysplasia	*SMARCAL1* NM_014140.4	c.1939A>C p.(Lys647Gln)	homozygous	biallelic	Likely Pathogenic	Recurrent otitis media, history of small for gestational age birth, skeletal dysplasia, short stature, recurrent upper respiratory tract infections, cheilitis, hyperpigmented skin lesions, herpes zoster infection, proteinuria, focal segmental glomerulosclerosis, chronic kidney disease and hypertension.	Lymphopenia, decreased $CD4^+$ T cells, decreased IgG levels, and proteinuria.	[23]
13	MSMD	*IFNGR1* NM_000416.3	c.523del p.(Tyr175Metfs*2)	homozygous	biallelic	Pathogenic	BCG-associated lymphadenitis, hepatosplenomegaly, anemia, and history of disseminated BCG infection and pulmonary tuberculosis; interferon-gamma therapy was attempted without clinical benefit; underwent HSCT complicated by acute graft-versus-host disease and fulminant hepatitis.	Normal complete blood count, immunoglobulin levels, and immunophenotyping; Mycobacterium tuberculosis complex isolated from axillary and abdominal lymph nodes.	[24]
14	CID	*SPINK5* NM_006846.4	c.238dup p.(Ala80Glyfs*19) and c.1888-1G>A	Compound heterozygous	biallelic	Pathogenic/ Pathogenic	Eczema, erythroderma, alopecia, bamboo hair, congenital cytomegalovirus infection, growth retardation, chronic mucoid diarrhea, periorbital erythema, prolonged neonatal jaundice, oral candidiasis, and seborrheic dermatitis of the scalp.	Elevated IgE levels, eosinophilia, and decreased $CD19^+$ B cells.	[25,26]
15	CID	*SPINK5* NM_006846.4	c.1351dup p.(Cys451Leufs*6)	homozygous	biallelic	Pathogenic	Generalized erythroderma, recurrent otitis media, recurrent gram-negative sepsis.	Eosinophilia, elevated IgE levels, and mildly decreased $CD8^+$ T cells.	Novel
16	Immune dysregulation	*FOXP3* NM_014009.4	c.1040G>A p.(Arg347His)	hemizygous	maternal	Likely Pathogenic	Autoimmune hepatitis, jaundice, hepatosplenomegaly, chronic diarrhea, recurrent fever, cervical, mediastinal, and axillary lymphadenopathy, eczema, and asthma.	Lymphopenia, positive autoantibodies (antinuclear antibody and anti-smooth muscle antibody), decreased $CD3^+$, $CD4^+$, and $CD8^+$ T cells, and elevated IgG levels.	*[27]
17	CID	*TTC37* NM_014639.4	c.66C>G, p.(Tyr22*)	homozygous	biallelic	Pathogenic	Thin, fragile hair, growth retardation, interatrial septal aneurysm, and delayed neuromotor development.	Decreased $CD4^+$ T cells, negative vaccine responses, and normal immunoglobulin levels.	Novel
18	CMC	*AIRE* *NM_000383.4*	c.769C>T p.(Arg257*)	homozygous	biallelic	Pathogenic	Chronic mucocutaneous candidiasis, scalp dermatophyte infection, moniliasis, recurrent upper respiratory tract infections, bronchiectasis, and asthma.	Eosinophilia with normal immunophenotyping results.	[28]

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[&]Chromosomal microarray done for confirmation.
[†]Segregation analysis (parental ± sibling testing) was performed in all cases to confirm the reported mode of inheritance (biallelic, X-linked, or de novo
[*]Patients 15, 17, 27, 31, and 32 were first identified in our cohort and subsequently reported in the literature by our group or collaborators. These cases are therefore not classified as novel but were unpublished at the time of detection.

Table 1. Continued

Patient	Primary Diagnosis	Gene/ Transcript	Variant	Zygosity	Inheritance †	ACMG classification	Clinical Findings	Laboratory Findings	Reported Previously (Reference)
19	Autoinflammatory Disorders	*LPIN2* NM_001375808.2	c.1673G>A p.(Trp558*)	homozygous	biallelic	Pathogenic	Abdominal pain, weight loss, diffuse pain, peritoneal thickening, and generalized lymphadenopathy; diagnosed with tuberculous peritonitis; mild mental retardation, speech disturbance, amnesia, and hepatomegaly.	Normal nitro blue tetrazolium test, immunophenotyping, and immunoglobulin levels.	ClinVar: Variation ID: 2760513
20	CID	*FOXN1* NM_001369369.1	c.880G>A p.(Val294Ile)	homozygous	biallelic	Pathogenic	Recurrent fever and diarrhea, alopecia, growth retardation, hyperpigmented skin lesions, perianal abscess, moniliasis, and delayed neurological and speech development; underwent HSCT in 2020.	Severe neutropenia, panhypogammaglobulinemia, decreased $CD3^+$, $CD4^+$, $CD8^+$, and NK lymphocytes, with increased B lymphocyte count.	[29,30]
21	CID	*MALT1* NM_006785.4	c.1202_1203insAAT p.(Leu401_ Leu402insIle)	homozygous	biallelic	Likely Pathogenic	Refractory seborrheic dermatitis, alopecia, chronic diarrhea, herpes simplex virus infection, history of sepsis, recurrent diaper dermatitis, multiple inguinal lymphadenopathies, multiple food allergies, and growth retardation; underwent HSCT at 13 months of age.	Eosinophilia, elevated IgE and IgM levels, decreased IgG and IgA levels, and reduced T regulatory cells and $CD19^+$ B cells.	*[31]
22	CID	*IKBKG* NM_001099857.5	c.64del p.(Ala22Glnfs*93)	hemizygous	maternal	Pathogenic	Chronic perforated otitis media, recurrent upper respiratory tract infections, growth retardation, pulmonary infection with atypical mycobacteria (Mycobacterium bovis), uveitis, juvenile idiopathic arthritis, and splenomegaly.	Lymphopenia, decreased class-switched B lymphocytes, $CD4^+$ T lymphopenia, elevated IgA levels, and decreased IgM levels	*[32]
23	CID	*DOCK8*	Exon2-26 deletion[&]	homozygous	biallelic	Pathogenic	Asthma, recurrent pneumonia, otitis media, sinusitis, hepatosplenomegaly, milk and egg allergy, eczema, hyperpigmented skin lesions, history of pulmonary tuberculosis, cytomegalovirus infection, Pneumocystis jirovecii pneumonia, and growth retardation; underwent HSCT from a sibling at 51 months of age.	Eosinophilia, elevated IgE levels, and decreased $CD19^+$ B cells	[9]
24	CID	*NFKB2* NM_001322934.2	c.2557C>T p.(Arg853*)	heterozygous	*de novo*	Pathogenic	History of acute immune thrombocytopenic purpura, asthma, recurrent upper respiratory tract infections, history of otitis media, past urinary incontinence with left pelvicalyceal ectasia, and adrenal insufficiency.	Panhypogammaglobulinemia with decreased class-switched B lymphocytes	[33]
25	MSMD	*TYK2* NM_003331.5	c.647del p.(Pro216Argfs*14)	homozygous	biallelic	Pathogenic	Recurrent upper respiratory tract infections, fever and generalized rash after measles-mumps-rubella vaccination, hepatosplenomegaly, and history of pulmonary tuberculosis.	Normal immunoglobulin levels and immunophenotyping results.	[34,35]

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[*]Patients 15, 17, 27, 31, and 32 were first identified in our cohort and subsequently reported in the literature by our group or collaborators. These cases are therefore not classified as novel but were unpublished at the time of detection.

recognition of primary immune regulatory disorders within the IUIS classification. These patients frequently presented with autoimmunity, lymphoproliferation, and enteropathy, underscoring the need for early genetic testing to guide targeted therapies, including hematopoietic stem cell transplantation in selected cases. The presence of *STAT1*, *IFNGR1*, and *TYK2* variants among our patients highlights the contribution of inborn errors of IFN-γ/IL-12/IL-23 signaling to Mendelian susceptibility to mycobacterial disease in our population. In addition, *AIRE*-related chronic mucocutaneous candidiasis and *LPIN2*-associated autoinflammatory disease further illustrate the diversity of IUIS categories captured by targeted NGS panels in a single-center pediatric cohort.

Comparison with previous studies

The diagnostic yield of 24.7% in our cohort is comparable to previous targeted NGS studies, which reported yields ranging from 15% to 31% depending on cohort size, gene content, and sequencing platform. Stoddard et al.[8] analyzed 173 IEI genes and achieved a 15% yield, Cifaldi et al.[36] screened 300 genes and reported 31%, while Rudilla et al.[2] applied clinical exome sequencing covering approximately 4,800 clinically relevant genes, including a large subset of IEIs-associated genes, and reported a 31% diagnostic yield that increased to 42% after complementary analyses.

A large international study by Platt et al. involving 878 patients with suspected IEIs demonstrated an overall diagnostic yield of 56–58% using a combined targeted NGS and WES strategy. While WES provided slightly higher diagnostic efficiency and allowed identification of novel disease genes, the authors emphasized that targeted panels remain a feasible first-line approach due to faster turnaround, high coverage, and lower data burden—particularly in settings where access to exome or genome sequencing is still limited.[9]

Although detailed information on consanguinity was not available for all patients, most of the identified variants displayed an autosomal recessive inheritance pattern. The predominance of autosomal recessive defects in this cohort may have contributed to the relatively high diagnostic yield, consistent with findings from populations characterized by increased parental relatedness.[6,11] This observation underscores the importance of accounting for population-specific genetic architecture when evaluating the efficiency and interpretation of NGS-based diagnostic approaches.

Novel variants and clinical implications

Three novel variants were identified in our cohort, expanding the mutational spectrum of genes associated with primary immunodeficiency. These variants were distributed across genes involved in major immunologic pathways, including *STAT1*, *SPINK5*, and *TTC37*. Variant interpretation was performed according to ACMG/AMP and ClinGen SVI guidelines and supported by literature review, segregation analysis, and close collaboration between clinical immunologists and molecular geneticists. The concordance between genotype and clinical phenotype further reinforced the pathogenicity of these variants.

The identification of novel variants in well-characterized PID genes highlights the genetic diversity of our study population and emphasizes the importance of regional studies for improving variant interpretation. Populations with high allelic heterogeneity, such as Türkiye, continue to contribute substantially to global variant databases and to the identification of population-specific mutations. Establishing collaborative diagnostic networks and integrating clinical expertise into molecular interpretation can enhance diagnostic accuracy and facilitate reclassification of uncertain variants over time.

From a clinical perspective, achieving a molecular diagnosis provides tangible benefits for patient care and family counseling. In our cohort, genetic findings directly impacted clinical management: 8 of the 25 genetically confirmed patients underwent hematopoietic stem cell transplantation, mainly those with severe or syndromic combined immunodeficiencies (including *RAG1*, *DCLRE1C*, *DOCK8*, *FOXN1*, and *MALT1* defects). Immunoglobulin replacement and/or antimicrobial prophylaxis were initiated or adjusted in 19 patients, and cascade genetic testing, together with formal genetic counseling, was offered to all families. In selected cases with immune dysregulation or MSMD (including *STAT1* and *IFNGR1*/*TYK2* defects), targeted immunomodulatory therapies such as interferon-γ or JAK inhibition were introduced, illustrating how multidisciplinary interpretation of genetic data supports precision medicine in primary immunodeficiencies and complements the case-based information summarized in Table 1.

Methodological advantages and limitations

The main advantage of targeted NGS panels lies in their ability to provide high sequencing depth, uniform coverage, and rapid turnaround time, making them particularly suitable for routine clinical diagnostics. In our study, the

260 gene panel achieved reliable coverage across all target regions and allowed the simultaneous detection of single-nucleotide variants and copy number variations, which were subsequently confirmed by array-CGH. The standardized use of ACMG/AMP and ClinGen SVI guidelines for variant classification ensured reproducibility and alignment with international diagnostic criteria. Furthermore, the collaborative workflow between clinicians and molecular geneticists facilitated accurate phenotype–genotype correlation and timely clinical reporting.

Despite these advantages, targeted NGS panels have inherent limitations. Because they are restricted to predefined gene sets, variants in genes not yet associated with IEIs at the time of panel design remain undetected. Deep intronic, regulatory, and structural variants are also beyond the reach of standard short-read sequencing methods. In addition, periodic updates are required to incorporate newly discovered IEI genes and maintain clinical relevance. Whole-exome or whole-genome sequencing can overcome some of these limitations by offering broader coverage and the possibility of reanalysis as new genes are identified. In addition, recent work has shown that systematic reanalysis of WES and WGS data with extended IEI gene panels and structural variant calling can provide an incremental increase in diagnostic yield in patients with suspected primary immunodeficiency.[37] However, these approaches remain more resource-intensive and are not yet accessible in all healthcare settings. In this context, extended IEI gene panels represent a practical alternative to whole-exome sequencing in many clinical settings, and both extended panels and clinical exome/WES, especially when combined with reanalysis and structural variant calling, can further increase diagnostic yield in patients who remain undiagnosed after initial targeted panel testing.

Nevertheless, targeted NGS panels continue to represent a practical and efficient first-line diagnostic tool for primary immunodeficiencies, especially in populations with well-defined clinical phenotypes or in regions where access to comprehensive genomic testing is limited.

Limitations

This study has several limitations. First, it was conducted at a single center with a limited cohort size, which may not fully represent the genetic heterogeneity of IEIs in the general population. Although variant interpretation followed standardized ACMG/AMP and ClinGen guidelines, functional validation of the novel variants was not performed, and their pathogenicity was inferred based on clinical correlation and segregation data.

Second, the targeted NGS panel was restricted to 260 known IEI-related genes, which represent approximately half of the currently recognized IEI genes. As a result, pathogenic variants in genes not included in this panel or discovered after the panel design, as well as deep intronic, promoter, regulatory, or structural variants, could not be detected and may partly explain the unsolved cases. Structural rearrangements and mosaic variants may also have been underrepresented due to the limitations of short-read sequencing technology.

Finally, while clinical genetic correlation was established for all reported variants, future studies incorporating functional validation, expanded gene content, and multicenter collaboration will be crucial to further enhance diagnostic yield and refine genotype–phenotype interpretation in IEIs cohorts.

CONCLUSION

Our study demonstrates that targeted next-generation sequencing is a reliable, efficient, and cost-effective approach for the molecular diagnosis of primary immunodeficiency diseases. Using a 260-gene panel, we achieved a diagnostic yield of 24.7% and identified three novel variants across 21 genes, expanding the known mutational spectrum of IEIs. The predominance of autosomal recessive inheritance patterns reflects the genetic characteristics of our population and underscores the importance of population-specific genetic studies. Through close collaboration between clinicians and molecular geneticists, the integration of genetic data with clinical evaluation enabled accurate diagnosis and informed patient management, including hematopoietic stem cell transplantation and targeted therapy. Although whole-exome and whole-genome sequencing offer broader genomic coverage and reanalysis potential, targeted NGS panels remain a practical first-line diagnostic tool, particularly in regions where access to comprehensive genomic testing is limited. Continued expansion of multicenter collaborations and inclusion of functional studies will further enhance diagnostic precision and contribute to personalized care for patients with IEIs.

Ethical approval

This study has been approved by the Health Science University, Ümraniye Training and Research Hospital Ethics Committee (approval date 23.10.2025, number B10.1.T. K.H.4.34.H.GP.0.01/392). Written informed consent was obtained from the participants.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: YKD, AK; data collection: YKD, AK, ZM, EY; analysis and interpretation of results: YKD, AK, ZM, BY, EY, VO; draft manuscript preparation: YKD, AK, ZM, BY, VO. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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