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Unraveling Molecular Causes of Primary Immunodeficiency in a Cohort of Palestinian Children

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Thesis Approval

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Declaration

I declare that this thesis has been composed by my own research work as a master's student in the Immunohematology program at the Arab American University of Palestine and has not been previously submitted for any other degree or professional qualification, and every effort has been made to appropriately indicate, mention, and acknowledge other contributions.

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Dedication

To my beloved family, whose endless support and love have been my greatest source of strength throughout this journey. To the soul of my dear aunt, who first introduced me to the fascinated world of immunology. My aunt lived with rheumatoid arthritis, an autoimmune disease in which the immune system attacks the body's own tissues. Despite her condition, she remained positive and deeply interested in understanding more about the disease and dedicated herself to helping others get diagnosed early by sharing her knowledge. Unfortunately, such knowledge was not readily available when her symptoms first began, so she was diagnosed late and that led to numerous severe complications. Her determined dedication to community support has inspired me to become who I am today and her memory in my heart will keep me motivated to complete her story and write my own one.

Jana Basheer Khaled Zaid

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Abstract

Primary immunodeficiencies (PIDs) are genetic disorders that result in dysregulation of the immune system, causing a wide range of symptoms ranging from recurrent infections to autoimmunity and malignancy. Early detection of PIDs helps avoid complications and enables targeted therapies such as bone marrow transplantation. The Palestinian population is not only highly consanguineous but also underserved, meaning that disease-causing variants in PIDs are not well characterized. In this study, we investigated the genetic variants of 67 patients with PIDs from 54 families and did in-depth analysis of five selected cases. The patients had symptoms suggesting primary immunodeficiency, with some of them overlapping with immune dysregulation symptoms. Clinical characterization of patient's symptoms was done for all patients. Exome or whole genome sequencing was done for the index in each family followed by deep analysis of the resulting data. The most common manifestations were recurrent infections and fever, while skin abnormalities were observed in 49% (33/67) of patients and 41% (28/67) of the patients had failure to thrive. The patients had variable laboratory testing results with White Blood Cell (WBCs) abnormalities seen in 52% (35/67), immunoglobulin levels abnormalities in 52% (35/67) and elevated inflammatory markers in 46% (31/67). Genetic diagnoses were identified in 17 families (31%). Additionally, three novel variants were found in new disease genes, and one known variant revealed a potentially new disease mechanism while, six patients had variants of uncertain significance identified in immune-related genes. Notably, none of these variants were detected in a diagnostic facility in Palestine. Eight patients found to have pathogenic variants in the MEFV gene, associated with the autoinflammatory disorder Familial Mediterranean Fever. Comprehensive analysis of the selected cases identified important variants in the following genes: *ZNF341, RTEL1, DOCK11, IL36RN* and *TNFRSF13B*. Our findings indicate that advanced genomic diagnostics and robust variant analysis are crucial for early detection and improving outcomes in PID patients, especially those with severe forms like SCID. It also highlights the importance of developing population-specific genetic screening programs and the need to equip the next generation of scientists with the variant's interpretation skills required for advancing precision medicine in underserved populations.

Keywords: Primary Immunodeficiency, Variant interpretation, Next-Generation Sequencing, Palestinian Children.

Table of Contents

List of Tables

List of Supplementary tables

List of Figures

List of Supplementary Figures

List of Abbreviations

Abbreviation	Full Term
ACMG	American College of Medical Genetics and Genomics
ACGS	Association for Clinical Genomic Science
AMP	Molecular Pathology
CBC	Complete Blood Count
CADD	Combined Annotation-Dependent Depletion
cDNA	Complementary DNA
CGD	Chronic Granulomatous Disease
CID	Combined Immunodeficiency
CRP	C-Reactive Protein
CVID	Common Variable Immunodeficiency
DGS	DiGeorge Syndrome
EDA-ID	Anhidrotic Ectodermal Dysplasia with Immunodeficiency
EBV	Epstein-Barr Virus
ES	Exome Sequencing
FMF	Familial Mediterranean Fever
fSCIG	Facilitated Subcutaneous Immunoglobulin
FTT	Failure to Thrive
gnomAD	The Genome Aggregation Database
GoF	Gain of Function
HIES3	Hyper-IgE Syndrome 3
HLH	Hemophagocytic Lymphohistiocytosis
HSCT	Hematopoietic Stem Cell Transplantation
IEI	Inborn Errors of Immunity
IgAD	Selective IgA Deficiency

IgE	Immunoglobulin E
IRB	Institutional Review Board
IUIS	International Union of Immunological Societies
IVIG	Intravenous Immunoglobulin
KREC	Kappa-Deleting Recombination Excision Circles
LAD	Leukocyte Adhesion Deficiency
LINEs	Long Interspersed Retrotransposable Elements
LoF	Loss-of-Function
NGS	Next-Generation Sequencing
NMD	Nonsense-Mediated Decay
OMIM	Online Mendelian Inheritance in Man
PCR	Polymerase Chain Reaction
PIDs	Primary Immunodeficiency Disorders
PIDDs	Primary Immunodeficiency and Immune Dysregulation Disorders
pHaplo	Probability of Haploinsufficiency
pLI	Probability of Loss-of-Function Intolerance
RBCs	Red Blood Cells
REVEL	Rare Exome Variant Ensemble Learner
RNA-seq	RNA Sequencing
SCID	Severe Combined Immunodeficiency
SCIG	Subcutaneous Immunoglobulin
SIN	Self-Inactivating
SINEs	Short Interspersed Retrotransposable Elements
SNPs	Single Nucleotide Polymorphisms
SoH	'Stories of Hope, Stories from Palestine' research project
TAE Buffer	Tris-Acetate-EDTA

TLR	Toll-Like Receptor
TREC	T-Cell Receptor Excision Circle
UK	United Kingdom
UTIs	Urinary Tract Infections
VUS	Variants of Uncertain Significance
WBCs	White Blood Cells
WGS	Whole-Genome Sequencing

Chapter 1: Introduction

1.1 Background

Primary Immunodeficiency Disorders (PIDs) are inherited genetic conditions that impair the immune system's normal function. They are also known as Primary Immunodeficiency and Immune Dysregulation Disorders (PIDDs) and Inborn Errors of Immunity (IEI). These disorders typically had three main presentations: recurrent infections, immune dysregulation characterized by autoimmunity or autoinflammation, and/or immunodeficiency features occurring alongside syndromic features or malignancies. In addition to these main presentations, there are other signs that indicate the need for IEI testing. These warning signs include positive family history, failure to thrive, lymphopenia, hypogammaglobulinemia, and prolonged need for intravenous antibiotic treatment (Seidel et al., 2019).

Primary immunodeficiencies (PIDs) involve a wide range of subtypes, each presenting with distinct phenotypes. However, they can generally be classified into six main categories: immunodeficiencies affecting cellular and humoral immunity, predominantly antibody deficiencies, diseases of immune dysregulation, congenital defects of phagocyte number or function, defects in intrinsic and innate immunity, and complement deficiencies. A summary of these categories with examples of each is presented in Table 1.1.

Table 1.1 : Summary of all types and subtypes of primary immunodeficiencies.

Immunodeficiency type	Subtype	Examples
Immunodeficiencies affecting cellular and humoral immunity	T cell - B cell + severe combined immunodeficiency (SCID)	e.g.: *JAK3* deficiency, CD45 deficiency, and *IL-7Rα* deficiency
	T cell - B cell - severe combined immunodeficiency (SCID)	e.g.: *RAG* deficiency, *DCLRE1C* deficiency, and *ADA* deficiency
	Combined immunodeficiency (CID)	*DOCK8* deficiency, *MALT1* deficiency, and *CARD11* deficiency
Predominantly antibody deficiencies	Agammaglobulinemia	*BTK* deficiency, *PAX5* deficiency, and *SLC39A7* deficiency
	Common variable immune Deficiency (CVID) phenotype	e.g.: *PIK3CG* deficiency, *NFKB2* deficiency, and TACI deficiency
	hyper-IgM	e.g.: *CTNNBL1* deficiency, APRIL deficiency, and *MSH6* deficiency
Diseases of immune dysregulation	Familial hemophagocytic lymphohistiocytosis (FHL) syndromes	e.g.: *SLC7A7* deficiency, *STXBP2* deficiency, and *RHOG* deficiency
	FHL syndromes with hypopigmentation	e.g.: Chediak–Higashi syndrome, Griscelli syndrome type 2, and Hermansky–Pudlak syndrome type 2
	Regulatory T-cell defects	e.g.: *CTLA4* haploinsufficiency, *STAT3* GOF, and *BACH2* deficiency
	Autoimmunity with or without lymphoproliferation	e.g.: *JAK1* GOF, Prolidase deficiency, and *TLR7* monogenic lupus
	Immune dysregulation with colitis	e.g.: *IL-10R* deficiency, *TGFB1* deficiency, and *DOCK11* deficiency
	Autoimmune lymphoproliferative syndrome (ALPS; Canale-Smith syndrome)	e.g.: *FADD* deficiency and ALPS-Caspase 8
	Susceptibility to EBV and lymphoproliferative conditions	e.g.: *RLTPR* deficiency, *IL-27RA* deficiency, and *PRKCD* deficiency
Congenital defects of phagocyte number or function	Congenital neutropenias	e.g.: G-CSF receptor deficiency, *SMARCD2* deficiency, and *JAGN1* deficiency
	Defects of motility	e.g.: Leukocyte adhesion deficiency type1(LAD1), *WDR1* deficiency, and Rac 2 deficiency
	Defects of respiratory burst	e.g.: X-linked chronic Granulomatous disease (CGD)

Defects in intrinsic and innate immunity	Mendelian susceptibility to mycobacterial disease (MSMD)	e.g.: IFN-γ receptor deficiency, *STAT1* deficiency, and *JAK1* deficiency
	Epidermodysplasia verruciformis (HPV)	e.g.: EVER1 deficiency and *CIB1* deficiency
	Predisposition to severe viral infection	e.g.: *STAT2* deficiency, *ZNFX1* deficiency, and RNA polymerase III deficiency
	Herpes simplex encephalitis (HSE)	e.g.: *TLR3* deficiency, *RIPK3* deficiency, and *IKBKE* deficiency
	Predisposition to invasive fungal diseases	e.g.: *CARD9* deficiency
	Predisposition to mucocutaneous candidiasis	e.g.: ACT1deficiency, *IL-17RA* deficiency, and JNK1 haploinsufficiency
	TLR signaling pathway deficiency	e.g.: *TLR7* deficiency, *TLR4* deficiency, and *TLR8* GOF
	Other IEIs related to nonhematopoietic tissues	e.g.: Isolated congenital asplenia
	Other IEIs related to leukocytes	e.g.: IL-18BP deficiency and *GATA2* deficiency
Complement deficiencies	Complement deficiencies	e.g.: C2 deficiency, C1 inhibitor deficiency, and *MASP2* deficiency

Summary of main Immunodeficiency types and subtypes with examples for each subtype. Adapted from Poli et al., 2025, Human inborn errors of immunity: 2024 update on the classification from the International Union of Immunological Societies Expert Committee, Journal of Human Immunity, 1(1), e20250003. https://doi.org/10.70962/jhi.20250003

1.2 Scope of Primary immunodeficiency in consanguineous populations

Although PIDs are classified as rare diseases, their prevalence is significantly higher in communities with high rates of consanguinity due to the increased probability of autosomal recessive inheritance (Broides et al., 2017; Darr et al., 2016). On the other hand, most of the currently available epidemiological data originated from western populations such as Europe and America. These populations have a low rate of consanguineous marriages (Kwan et al., 2014). The available studies of PIDs in consanguineous populations show that the prevalence ranges from 0.81 to 30.5 per 100,000 individuals. These data can be different depending on the makeup of the population studied and the diagnostic criteria used (Al-Mousa & Al-Saud, 2017).

Palestinian population is underserved and have limited accessibility to genetic and healthcare services. This means that immunophenotyping analysis is not readily available, systematic genetic testing is not covered by most health coverage insurances, and patients with diagnostic challenges have limited access to scientific research. All of this can cause diagnosis delays that might be life-threatening, creating a huge burden on families with multiple affected but undiagnosed individuals (El Naofal et al., 2023).

1.3 Next-generation sequencing and PIDs

Over the last decade, the number of known monogenic causes of PIDs has increased from nearly three hundred to over five hundred genes (Bousfiha et al., 2025; Poli et al., 2025). This increase is primarily because of the advances in molecular technologies, such as next-generation sequencing (NGS), long-read sequencing, RNA sequencing (RNA-seq), among others. These technologies enhanced our ability to diagnose many monogenic diseases, including PIDs. The increasing accessibility of this technology has made identifying disease-causing variants more efficient, resulting in clinical benefits and the adaptation of more personalized treatment approaches (Yska et al., 2019).

However, access to genetic testing and research is limited in Palestine as it is strongly associated with socioeconomic status and countries' resources (Gentili et al., 2018). As a result, the Palestinian population is underrepresented in genomic and population databases. That lead to the inability of applying certain American College of Medical Genetics and Genomics (ACMG) criteria effectively, such as PM criteria, making the interpretation of genomic variants very challenging (Matalon et al., 2023). The absence of nationwide genomic testing and data in this context limits the ability to develop screening programs. With all these disparities in mind, countries in similar situations do not have established academic routes to prepare a generation of clinical scientists with strong and efficient variant interpretation skills essential for genomic data interpretation, and collaborate with clinicians to help them interpret genetic results effectively (Ritter et al., 2025).

1.4 Study gap

There are not enough genotype-phenotype correlations data for immunodeficiencies in Palestine and there is no comprehensive database exists to pool variants PIDs disease-causing variants. This gap is critical, as such data is essential for clinical decision-making, treatment optimization, and genetic counseling. In addition, further efforts should focus on designing a Palestinian-specific genetic panel for PIDs that reflects the unique group of variants within the population. There is also an absence of comprehensive Palestinian genome project where scientist and clinicians work side by side to better define genomic interpretation through in-depth bio-curation and employing standardized guidelines, such as those from the ACMG and AGCS. This gap is further compounded by absence of population databases that have comprehensive clinical and genetic profiling to further assist the immunodeficiency variants Applying the variant interpretation process with comprehensive clinical phenotyping outlined in my thesis can contribute meaningfully toward addressing this gap.

1.5 Study's significance

Studying the genetic variants associated with primary immunodeficiency and their clinical manifestations in the Palestinian population will focus the attention to the importance of developing population-specific gene panels making genetic screening efficient and cost-effective. The study result will set the foundation for neonatal national screening programs for pediatric PIDs which are important to get earlier diagnosis and management. Additionally, our findings in Palestine, which have a highly consanguineous population, will enhance the understanding of PIDs in the Palestinians and give lessons for other underrepresented consanguineous populations.

1.6 Study's objectives

The objective of this study is to assess the clinical and genetic profiles of 67 patients from 54 families using Exome Sequencing (ES) or whole-genome sequencing (WGS) to identify potential disease-causing variants. This study will specifically focus on deeply analyzing the genetic variants and its clinical correlation of five families having prominent immunological manifestation and clinical correlated variants.

1.7 Study's questions

What are the genetic variants our cohort of Palestinian PIDs patients have, and how can clinical and scientific approaches be effectively applied to interpret these variants to help in establishing an accurate diagnosis?

1.8 Limitations

First, the diagnostic yield regarding PIDs is generally low (Sogkas et al., 2022; Thaventhiran et al., 2020). However, applying enhanced variant interpretation skills through collaboration with a multidisciplinary team and utilizing genomic databases such as Genomics England and the UK Biobank can improve diagnostic yield. In addition, identifying variants of uncertain significance (VUS) presents a challenge as functional studies and detailed immune phenotyping are necessary to determine their pathogenicity. Although functional validation is beyond the scope of this thesis, the findings will provide a foundation for future investigations.

Another significant challenge is the limited availability of population-specific allele frequency data among underrepresented populations, including Palestinians, in population databases like The Genome Aggregation Database (gnomAD) (Gudmundsson et al., 2022). This challenge can be addressed through collaborating with SoH 'Stories of Hope, Stories from Palestine' research project, a collaborative research project between Arab American University and the University of Exeter. This project gather data from Palestinian patients affected with rare genetic diseases. Sequencing is done in a platform that includes other patients from underrepresented populations including Egypt, Pakistan, and Oman. In addition, we have collaborations with local Palestinian genetic labs that offer NGS services and have extensive genomic databases which also help address this limitation.

Chapter 2 : Literature Review

2.1 Clinical Manifestations of PIDs

The symptoms of PIDs exhibit variable age of onset. Combined immunodeficiency diseases (CID) patients typically start showing symptoms before 6 months of age while B-cell dysfunction symptoms usually start between 6 and 12 months, as maternal antibodies decline. Infants with delayed separation of the umbilical cord usually beyond 4-6 weeks alongside with an elevated white blood cell count may suggest leukocyte adhesion deficiency (LAD) (Devonshire & Makhija, 2019).

The hallmark clinical feature of PIDs are frequent infections that reflect the underlying immune system dysfunction. For example, opportunistic infections caused by viruses, fungi, or protozoa indicate T lymphocyte dysfunction. Similarly, Multiple Staphylococcus skin infections and fungal infections can suggest neutrophil dysfunction or hyper-IgE syndrome. Recurrent Neisseria infections are typically associated with defects in the late complement components (C5–C9, or the membrane attack complex), whereas recurrent viral or pyogenic bacterial infections point to abnormalities in Toll-like receptor (TLR) signaling. In addition, mycobacterial infections are indicative of defects in the IL-12 and interferon-γ pathway. (Bonilla et al., 2015).

Gastrointestinal symptoms are often early indicators of PIDs. These manifestations can be infectious, noninfectious chronic diarrhea, or autoimmune-related conditions. They are difficult to treat without a diagnosis and can lead to severe malnutrition, failure to thrive (FTT), and premature death due to unique immune-related mechanisms, making their management challenging (Agarwal & Cunningham-Rundles, 2019).

Distinct dysmorphic features could indicate a specific syndrome such as facial dysmorphisms and congenital heart disease indicate 22q11.2 deletion syndrome (McDonald-McGinn et al., 2015) and partial albinism indicate Chèdiak-Higashi syndrome (Ajitkumar et al., 2025). Telangiectasias of the skin combined with bulbar conjunctiva are seen in ataxia-telangiectasia patients (Liptai, 2018). Severe eczematous rashes may be present in disorders such as hyper-IgE syndrome (Tsilifis et al., 2021), IPEX syndrome (Bekis Bozkurt et al., 2024) and Wiskott-Aldrich syndrome (Albert & Freeman, 2019). Additionally, signs of recurrent

infections, such as digital clubbing, wheezing, or rhonchi may also be evident during physical examination and suggest underlying pulmonary damage (Grenier et al., 2023; Nonas, 2015).

PIDs patients can present with neurological manifestations due to genetic defects disrupting DNA repair or essential metabolic pathways for neuronal function. These manifestations may also develop secondary or as a complication of infections, autoimmune processes, or neurologic treatment. These manifestations include cognitive delay, learning disabilities, seizures, meningitis, ataxia, and neuropathy (Dehkordy et al., 2012; Kose et al., 2024). Early diagnosis and intervention in neurological phenotypes are essential to prevent irreversible neurological damage and improve the outcome (Yildirim et al., 2018).

2.2 Laboratory tastings in PIDs

Complete Blood Count (CBC) can support the diagnosis of PIDs as it can show variable indicators of the disease such as pancytopenia and abnormalities in white blood cells (WBCs), red blood cells (RBCs), and platelets counts. These abnormalities can indicate certain immunological defects. For example, patient with LAD have leukocytosis (Almarza Novoa et al., 2018; Dvorak et al., 2023) and patients with IgA deficiency and Wiskott-Aldrich Syndrome (WAS) have thrombocytopenia (Mohtashami et al., 2022). Additionally, Patient with autoinflammatory-related symptoms can present with high inflammatory markers, such as C-Reactive Protein (CRP) (Delplanque et al., 2023).

Immunoglobulins levels represent an important laboratory testing in PIDs evaluation and it’s readily accessible to patients from Palestine. Immunoglobulins levels are significantly reduced in X-linked agammaglobulinemia (XLA) and some forms of SCID. In addition, In patients with Selective IgA deficiency (IgAD) only IgA subclass is reduced (Herriot & Sewell, 2008). In contrast, certain PIDs are marked with present of elevated Immunoglobulin E (IgE) levels, including STAT3-deficient Job’s syndrome. Elevated IgE has been also reported in other PIDs syndromes such as CARD11 and ZNF341 deficiencies (Ponsford et al., 2018). It's important to note that immunoglobulin defects not only arise from PIDs as it can be secondary to various pathological conditions and environmental factors (Otani et al., 2022).

Flow cytometry serves as a standard diagnostic tool widely used for the initial evaluation of PIDs and detailed analysis of immune cell populations. It can indicate disorders such as SCID, X-linked agammaglobulinemia, hyper IgM syndromes, and Wiskott-Aldrich syndrome, among others. Flow cytometry is used to quantify lymphocyte subsets and

characterize their specific cell surface markers and intracellular proteins (Kanegane et al., 2018; Oliveira et al., 2008). Unfortunately, only basic immune cell subsets profiling is available to patients from Palestine.

2.3 Genetic Basis of PIDs

2.3.1 Inheritance Patterns and Population Genetics

PIDs can have different inheritance patterns depending on their genetic origin. PIDs can be autosomal recessive, autosomal dominant, or X-linked (Bousfiha et al., 2025) with autosomal recessive forms being prevalent in populations with high rates of consanguinity (Al-Herz et al., 2014). Founder variant effect can play an important role in the prevalence of PIDs across different populations. This effect arises from population bottlenecks, geographic isolation, and cultural practices. Founder variants are remarkable in consanguineous middle eastern and North African populations, where autosomal recessive PIDs are especially common. For example, founder variants in *DOCK8* gene have been reported in Turkish and Lebanese cohorts (Aydin et al., 2015). In addition, founder variants of *PGM3* gene are prevalent in Tunisia (Ben-Khemis et al., 2017). SCID-causing founder variants are common in North African Berber populations (Ouadani et al., 2016). It is critical to Recognize population-specific founder variants as it help in developing targeted genetic panels that improve carrier testing and counseling programs (Meyts et al., 2016; Monies et al., 2017)

2.3.2 Molecular Mechanisms of Disease

Pathogenic variants in PIDs genes can result in either loss-of-function or gain-of-function effects, each of which lead to distinct consequences. For instance, loss-of-function variants in *RAG1* or *RAG2* impair V(D)J recombination, resulting in SCID (Greenberg-Kushnir et al., 2020). In contrast, gain-of-function variants in *STAT1* lead to dysregulating of interferon signaling causing chronic mucocutaneous candidiasis (Karim et al., 2024).

Another molecular mechanism of pathogenicity in PIDs is haploinsufficiency. For example, haploinsufficiency of *GATA2* gene disrupts hematopoietic stem cell maintenance and myeloid cell differentiation. Subsequently, this can progress to bone marrow failure and predispose the patient to myelodysplastic syndrome and acute myeloid leukemia (Shimizu & Yamamoto, 2016). Similarly, *CTLA4* haploinsufficiency reduce the expression of immune regulators and lead to severe immune dysregulation. Heterozygous loss-of-function mutations

in *CTLA4* cause autosomal dominant immune dysregulation with variable penetrance (Jamee et al., 2021).

Another mechanism of disease is dominant negative mutations that result in dysfunctional proteins that interfere with normal cellular processes. For instance, pathogenic variants in *TNFRSF13B* result in production of dysfunctional proteins that interfere with normal B-cell activation and class switching. Patients with these variants present with common variable immunodeficiency (CVID) (Mohammadi et al., 2009).

2.3.3 Structural Variants and Copy Number Variations (CNV)

Structural variants can have several mechanisms of pathogenicity. An example of CNV-associated immunodeficiency is the 22q11.2 deletion syndrome which is caused by heterozygous deletions of approximately 3 million base pairs encompass over 40 genes. The immunological consequences are primarily because of *TBX1* haploinsufficiency. *TBX1* is one of the deleted genes and haploinsufficiency of this gene disrupts thymic organogenesis during embryonic development, leading to reduced T cell production (Sullivan, 2019). Similarly, structural variants in the *SERPING1* gene, which encodes C1 inhibitor, are associated with hereditary angioedema. Both small and large deletions or duplications of this gene are common and can contribute to pathogenicity (Drouet et al., 2022).

CNVs can cause different phenotypes depending on the size and location of the deleted region. An example is that heterozygous large deletions in *IKBKG* gene result in Incontinentia pigmenti in females and are lethal in hemizygous males. However, smaller deletions affecting only specific exons or regulatory regions is not lethal and cause anhidrotic ectodermal dysplasia and immunodeficiency (EDA-ID) (Bret Puvilland et al., 2021).

Although CNV detection is classically done using WGS, it is currently possible to detect some CNVs using ES data but it require special bioinformatic pipelines (Tilemis et al., 2023). However, it is still important to apply special methods to detect and interpret inversions, and other complex CNVs. Additionally, validation of CNVs for diagnostic purposes is important and it is usually done using methods including Droplet Digital Polymerase Chain Reaction (ddPCR) that is not readily available in Palestine (Mazaika & Homsy, 2014).

2.4 Complication of PIDs

Infectious complications are very common in PIDs and can cause serious health issues. Patients with PIDs can develop chronic opportunistic infections that lead to bronchiectasis, chronic lung disease, and sepsis (Baumann et al., 2018). Additionally, chronic gastrointestinal infections can cause permanent damage of the mucosal layers, leading to malnutrition and consequently failure to thrive (Schwimmer & Glover, 2019).

Moreover, complications of chronic inflammation such as granulomatous disease, lymphoproliferation disorders, and multi-organ inflammation can cause significant organ damage over time (Bakhtiar et al., 2019; Hurst et al., 2017; Leiding et al., 2023). Growth and developmental delays are also common complications of PIDs, particularly in SCID (Yildirim et al., 2018).

Some PIDs result in life-threatening complications that can lead to premature death. Examples of these complications are malignancies, very severe infections, and Hemophagocytic Lymphohistiocytosis (HLH). In such cases, a definitive therapy is required to prevent these conditions from progressing to mortality (Fox & Booth, 2021).

Malignancies are a well-recognized complication in patients with PIDs. The most reported related malignancies are hematological malignancies. They are caused by impaired immune surveillance due to recurrent viral infections, particularly Epstein-Barr virus (EBV). recurrent viral infections can cause recurrent antigenic stimulation driving oncogenesis (Ballow et al., 2022)

Autoimmunity is also a common complication of PIDs and sometimes it is one of the first clinical signs. These autoimmune manifestations can arise from defects in immunological tolerance, affecting the central and peripheral tolerance. In addition, affected Treg/Th17 balance may cause a proinflammatory environment that increases the possibility of developing autoimmunity. Chronic and recurrent infections can also trigger autoimmunity through mechanisms such as molecular mimicry, bystander activation, and superantigen stimulation (Amaya-Uribe et al., 2019; Conteduca et al., 2018; Rojas et al., 2018).

2.5 Autoinflammatory Diseases in Primary Immunodeficiencies

According to the International Union of Immunological Societies (IUIS) classification of IEI, Inflammatory disease represents a distinct category that include diseases lead to recurrent episodes of systemic inflammation (Bousfiha et al., 2025). Monogenic defects in genes responsible for controlling inflammatory pathways, inflammasome function, and cellular

stress responses lead to excessive production of pro-inflammatory cytokines. The clinical presentation typically includes recurrent fever episodes, skin rashes, arthritis, serositis, and organ-specific inflammation which overlap with symptoms of PIDs (Pathak et al., 2017).

One of the most common autoinflammatory diseases is Familial Mediterranean Fever (FMF) that results from mutations in the *MEFV* gene. FMF is prevalent disorder in Mediterranean countries and is characterized by early onset recurrent episodes of fever and serositis affecting the chest, abdomen, and joints (Balcı-Peynircioğlu et al., 2020).

Although autoinflammatory disorders are distinct from PIDs, they significantly overlap in symptoms at initial presentation. This poses a diagnostic challenge in deciphering causes of recurrent fevers and high inflammatory markers in consanguineous populations particularly in the Mediterranean populations.

2.6 Management and treatment of patients with PIDs

2.6.1 Prophylactic antibiotics and antimicrobials

Prophylactic antimicrobial therapy is considered the first choice of management of people with PIDs, particularly for patients with neutrophil defects and complement deficiencies. The selection of prophylactics depends on the specific immunological defect and associated infection patterns. For instance, patients with chronic granulomatous disease (CGD) typically receive antibiotics, antifungals, and immunosuppressive therapy for autoinflammation (Staudacher & von Bernuth, 2024). Similarly, individuals with complement deficiencies receive prophylactic antibiotics against encapsulated organisms, especially Neisseria species (Brodszki et al., 2020).

2.6.2 Immunoglobulin Replacement Therapy

One of the important ways of managing patients with antibody deficiencies is to replace the deficient antibodies by giving exogenous antibodies. This will provide patients with passive immunity, reducing the frequency and severity of infections they have (Nguyen & Aquino, 2024). There are different routes for Immunoglobulins administration, each with their own advantages and considerations. The administration can be either intravenous or subcutaneous. Intravenous Immunoglobulin (IVIG) directly and rapidly delivers high concentrations of Immunoglobulins to bloodstream. In contrast, Subcutaneous Immunoglobulin (SCIG) offers convenient administration through subcutaneous injections that can be done from home. The

choice between IVIG and SCIG depends on the type and severity of the antibody deficiency and patients' preference and convenience. To allow infusion of larger volumes of immunoglobulins, Facilitated Subcutaneous Immunoglobulin (fSCIG) is used. fSCIG is a recombinant human hyaluronidase that temporarily breaks down hyaluronan in the subcutaneous tissue. It follows the same absorption and distribution pathway as SCIG but enables less frequent and higher-volume dosing (Sriaroon & Ballow, 2015; Wasserman, 2019).

2.6.3 Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment option for patients with PIDs, with survival rates reaching up to 90%. Fully HLA-matched and early transplantations before significant infections or organ damage must be done to improve the transplantation success rates (Castagnoli et al., 2019; Morris & Albert, 2019; Slatter & Gennery, 2013). HSCT is also becoming a treatment option for adults with PIDs, with survival rates and low incidences of graft-versus-host disease has been seen in (Dávila Saldaña, 2018).

Although HSCT is now considered a promising treatment for PIDs, it has several limitations. Late diagnosis often leads to irreversible organ damage caused by infections or chronic inflammation limiting the ability to perform HSCT. Other limitations include the need for alternative donors, the toxicity of conditioning regimens, and the risk of graft-versus-host disease (Castagnoli et al., 2019; Dávila Saldaña, 2018; Morris & Albert, 2019; Slatter & Gennery, 2013).

2.6.4 Gene Therapy

Gene Therapy is a curative option in PIDs which is done by transplantation of autologous hematopoietic stem cells that are genetically corrected through viral vector-mediated gene transfer. Early gene therapy trials in the 1990s and 2000s used murine gamma-retroviral vectors. Although they demonstrated clinical success, they raised safety concerns due to genotoxicity and myeloproliferative disorders in some patients. Recently, Self-inactivating (SIN) lentiviral vectors have been used and improved safety profile with no major genotoxic events in clinical studies were seen. Several PIDs have been treated using this method, including SCID, Wiskott-Aldrich syndrome, and chronic granulomatous disease (Kohn & Kohn, 2021).

2.7 Neonatal Screening for PIDs and The Role of NGS

Newborn screening in PIDs enables early intervention that can prevent complications of recurrent or severe infections. Current screening methodologies include T-cell receptor excision circle (TREC), which can identify SCID and other forms of T cell lymphopenia, and kappa-deleting recombination excision circles (KREC) assays (Kobrynski, 2021). Recently, newborn screening for PIDs has been conducted using genetic testing through NGS, which enables earlier and more accurate diagnosis of immunodeficiencies (King & Hammarström, 2018; Solis et al., 2024).

NGS technologies such as gene panels, ES, and WGS are now the most effective tools to diagnose patients with PIDs. Each of these technologies has advantages and disadvantages (Meyts et al., 2016). Panel testing is considered cost-effective and takes less time but it is limited to the predefined genes in the panel so it can miss novel or rare variants (Nijman et al., 2014; Stoddard et al., 2014). ES covers all coding regions enabling the discovery of new variants so it is more comprehensive than panels and can be considered cost-effective compared to WGS, but it can miss non-coding, deep intronic, and structural variants and it has uneven coverage. WGS covers the entire genome including non-coding regions so it can detect CNVs, intronic, and intergenic variants and it has uniform coverage, but it has a high cost and results in complex data to analyze and store and results in more incidental findings (Raje et al., 2014).

2.8 Variant interpretation of NGS data using international guidelines

Genetic variants interpretation can be challenging as not having a consistent approach causes confusion among clinicians and patients which may result in inappropriate clinical decisions. As a result, standardized guidelines are important for accurate classifications, clear reporting hence suitable clinical management. In 2008, (Plon et al., 2008) published a group of recommendations for classifying sequence variants in seven cancer susceptibility genes.

In 2015, ACMG and the Association for Molecular Pathology (AMP) released a collaborative guideline setting a structured approach to interpreting sequence variants (Richards et al., 2015). To unify the variant interpretation system in the United Kingdom (UK), Association for Clinical Genomic Science (ACGS) has also developed guidelines aligning with the ACMG framework for sequence variant classification but incorporating additional evidence from clinical phenotypes to enhance the accuracy of interpretation (Durkie et al., 2023).

The guidelines outlined 28 specific criteria based on loss of function evidence at the level of the protein, frequency in healthy population databases, functional impact, evidence of deleterious effect of the variant based on available in silico tools, specificity of the clinical phenotype, and segregation data. Each criterion is given a unique code to evaluate different types of variant evidence. Each code was designated as either benign (B) or pathogenic (P) and was assigned a strength level as follows: stand-alone (A), very strong (VS), strong (S), moderate (M), or supporting (P). Additionally, the guideline indicates rules for combining these criteria to assess the predicted pathogenicity outcome of a sequence variant. It recommends standardized terms like "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign" for describing variants in genes linked to Mendelian disorders (Richards et al., 2015).

(Tavtigian et al., 2018) converted the ACMG guidelines into a quantitative Bayesian framework. This Bayesian approach increases flexibility, allowing combinations beyond those specified by (Richards et al., 2015), such as combining two strong and one moderate criterion to reach a pathogenic classification. Each criterion is assigned a specific point value: Very Strong (8), Strong (4), Moderate (2), and Supporting (1) for pathogenicity, while points for benignity are negative equivalents (e.g., Strong = -4). Variants are classified based on total scores: Pathogenic (≥10), Likely Pathogenic (6-9), VUS (0-5), Likely Benign (-1 to -5), and Benign (≤-6). Except for BA1 (stand-alone), at least two criteria are required for a definitive classification, meaning a variant with only one piece of strong or supporting evidence remains a VUS.

Chapter 3 : Methods

3.1 Study population data collection

Institutional Review Board (IRB) approval for this study was obtained from the AAUP IRB Committee-Ramallah under protocol number R-2024/A/33/N. Fifty-four families with one or more children with suggestive symptoms of primary immunodeficiency were referred by pediatric immunologists. The inclusion criteria included individuals with recurrent fever and infections, abnormal inflammatory responses and/or abnormal Immunoglobulins levels. After families reviewed the project information sheet and provided informed consent, clinicians working with the SoH project performed detailed clinical phenotyping in a research genomic clinic. Patient records and medical reports were kept in a secure data sharing platform.

Whole blood samples and buccal swabs were collected from patients and healthy controls in their families. DNA was extracted from blood and buccal samples. DNA samples of the affected individuals were sent for Exome or Genome Sequencing at the Genomics of Rare Disease Laboratory at Exeter University's Medical School as part of a collaborative translational genomic project "Stories of Hope Stories from Palestine". Sequencing and data processing followed the bioinformatics pipeline detailed in (Khalaf-Nazzal et al., 2021). Detailed methods used for next generation sequencing data generation including variant calling, filtration, and annotation lies outside the scope of this thesis. Variants were systematically filtered by the pipeline to exclude those with a high allele frequency (>1%), low allele depth, or synonymous changes, as these are less likely to contribute to disease phenotypes. I analyzed results included in Excel files of the annotated variants, with annotations about functional impact of the variants, population frequency in gnomAD and in-house dataset of 8,000 exomes and genomes and conservation/intolerance of the genes.

3.2 Variant interpretation process

Comprehensive analysis of the resulting sequencing data was conducted, relevant genetic variants found were sorted and presented in tables highlighting the most important information about them. I focused my work on five families selected and described in the result section for detailed clinical description and genomic variant analysis and downstream segregation combined with genotype-phenotype correlation. These five families were selected based on the distinct clinical phenotypes and diagnostic relevance.

The genetic variant analysis process involves examining three groups of variants remaining after the filtration and prioritization process. These groups include homozygous,

compound heterozygous, and heterozygous variants. Each group was analyzed and prioritized based on the expected mode(s) of inheritance determined from pedigree analysis. Variants were assessed if they belong to known disease associated genes using Online Mendelian Inheritance in Man (OMIM) website. Then, inheritance patterns were assessed using OMIM and gnomAD v4.1.0 to ensure consistency with established modes of inheritance. Afterwards, the variant is assessed for its rarity by evaluating its frequency in a diverse range of populations in gnomAD v4.1.0 including Middle Eastern. These frequency data can be used as control groups. Variants in genes known to cause autosomal recessive disease are expected to have no occurrence in the homozygous state in population databases. For genes linked to autosomal dominant conditions, the frequency of variants in the heterozygous state should be checked for its rarity. For X-linked recessive conditions, variant frequency is evaluated in the hemizygous state.

Gene and regional constraint metrics are used to estimate gene's tolerance to variation. These metrics include probability of loss-of-function intolerance (pLI) score that indicates the gene tolerance to loss-of-function (LoF) variants with higher values suggest potential of being pathogenic variation, the missense Z score that evaluates how the gene is constrained to missense variants with higher scores suggest reduced tolerance to non-synonymous changes and Probability of haploinsufficiency (pHaplo) scores which is a prediction score for haploinsufficiency indicates the likelihood that a single functional copy of a gene is insufficient for normal function. These metrics among others are available on Decipher and gnomAD v4.1.0 websites and help prioritize variants by assessing the importance of specific genes and regions.

Disease databases such as Human Gene Mutation Database (HGMD), Decipher, ClinVar and OMIM were used to evaluate the occurrence of the variant in patients. Data from these databases combined with data obtained from literature were used to determine mechanisms of the disease and genotype-phenotype associations. In addition, ClinVar and literature were searched for Well-established *in vitro* or *in vivo* functional studies that indicate a damaging effect of the variant on the gene or gene product. Given that ClinVar is a public archive, careful revision of evidence of pathogenicity was performed.

predictive *In-Silico* score helps in assessing the potential pathogenicity of a variant. SpliceAI is used to predict the splicing effect of splicing variants with higher scores suggesting strong evidence of a resulting splice effect. Other predictive tools such as AlphaMissense, Rare Exome Variant Ensemble Learner (REVEL), and Combined Annotation-Dependent Depletion (CADD) give informative scores about the potential deleterious effects of missense variants (Cheng et al., 2023; Garcia et al., 2022).

Analysis of the variants was enriched with thorough search of relevant literature to ensure clinical relevance of identified variants, gene functions, protein functional domains and disease mechanisms. Tissue gene expression was also considered to better understand the disease mechanism and variant consequences using Genotype-Tissue Expression (GTEx) Portal (Lonsdale et al., 2013).

There are additional things to consider depending on the variant type. For variants predicted to result in complete loss of protein function such as stop-gain, nonsense, frameshift, deletion, duplication, start-loss and canonical splice site variants, it's important to ensure that loss of function is a known genetic mechanism of the disease and consider other factors such as variant predicted effect, variant position within the gene, and the affected exon. It is also important to determine whether the variant is in a region where nonsense-mediated decay (NMD) is predicted by checking the Decipher database (Abou Tayoun et al., 2018). For missense variants, The DECIPHER and UCSC websites are used to check if the changed amino acid is conserved among species. The DECIPHER website can be used to check if the missense variants are in a position with a high frequency of mutations, hot spot, with the absence of benign variants (Karczewski et al., 2019).

Although the variant interpretation method described here outlines the core steps I followed in my analysis process, the full process can be more detailed and complex. It is also important to note that different tools, databases, and software platforms can be used to get the same information regarding studying variant pathogenicity.

3.3 Segregation analysis

Segregation analysis was performed for the selected families to confirm the mode of inheritance and evaluate the pathogenicity of the variants, except for family one (P1), where no family samples were available. Polymerase chain reaction (PCR) was performed using DNA samples following the conditions listed in Supplementary table S3 in Appendix A. Primers for each variant were designed using Primer3Plus (Untergasser et al., 2007) and melting temperature and GC content were verified the UCSC In-Silico PCR tool (Lee et al., 2022). Primer specificity was confirmed using BLAST against the GRCh38/hg38 reference genome (Mangan et al., 2014) to ensure target regions were free of repetitive elements like short interspersed retrotransposable elements (SINEs), long interspersed retrotransposable elements (LINEs) or Single Nucleotide Polymorphisms (SNPs).

PCR products were detected using Agarose gel at 1.5% concentration prepared using Tris-acetate-EDTA (TAE Buffer) and stained with Ethidium Bromide (0.1%). Then, PCR products were purified using Eppic Fast clean-up reagent (A&A Biotechnology) before Sanger sequencing with the BigDye™ Direct Cycle Sequencing Kit (Thermo Fisher Scientific, USA). Sequencing data were analyzed using the Applied Biosystems 3500 Genetic Analyzer, and chromatograms were viewed with FinchTV software.

Complementary DNA (cDNA) study was also conducted as a trial to assess *IL36RN* splicing variant (c.244-5G>A) impact on splicing. RNA extraction was performed using the RNeasy Mini Kit (Qiagen), followed by cDNA synthesis using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™). PCR conditions are mentioned in Supplementary table S3 in Appendix A, and DNA loading dye was used to visualize gel electrophoresis results.

Figure 3.1: Schematic representation of the methods

Schematic overview of the methodological workflow, including patient recruitment, informed consent, clinical data and sample collection, DNA preparation, and data analysis. Inheritance patterns were confirmed through family-based segregation analysis in selected cases.

3.4 Variant classification according to ACMG and ACGS guidelines

ACMG and ACGS guidelines were used to classify the variants. In the case of having sufficient evidence scores, the variant is categorized as Pathogenic (P) or Likely Pathogenic (LP). On the other hand, variants with insufficient evidence scores or conflicting evidence are classified as VUS.

Chapter 4 : Results

4.1 Study Population Overview

The study population consisted of 67 patients from 54 families, distributed geographically as follows: 75% of patients are from the north of West Bank, 15% from the central regions of West Bank, 7% from the south of West Bank, and 3% from Gaza. Regarding sex distribution, 58% of the patients were males. 62% of the families are consanguineous, which is consistent with the high proportion of autosomal recessive disorders observed (81%) in patients who obtained a diagnosis. In contrast, autosomal dominant and X-linked recessive disorders accounted for 13% and 6% of the cases, respectively (Fig. 4.1). Interestingly, 47.5% (19/40) patients from consanguineous families have genetic diagnosis while only 7% (2/27) patients from non-consanguineous families get genetic diagnosis.

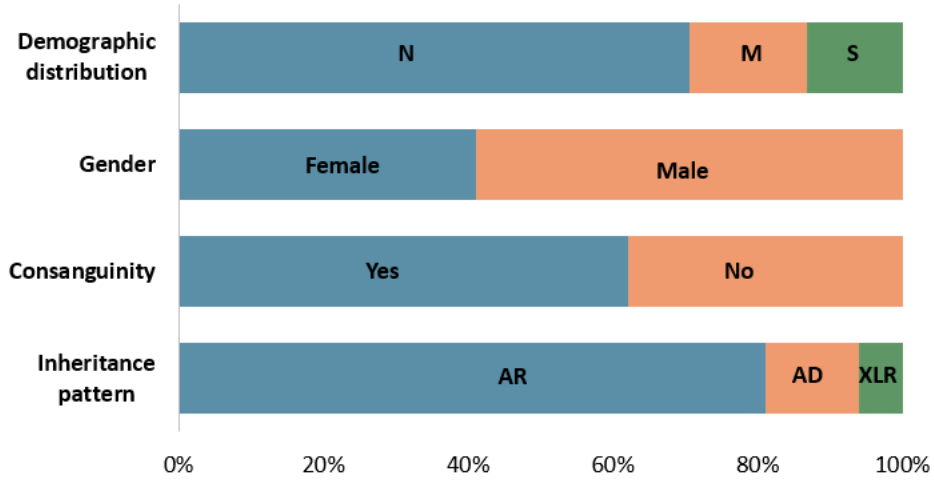

Figure 4.1: Population characteristics and distribution among the patient cohort

Bar chart illustrating general characteristics of the patient cohort, including regional distribution across the North, Middle, and South of the West Bank and Gaza, gender, consanguinity rates, and inheritance patterns among genetically diagnosed cases. The x-axis shows the percentage of patients; the y-axis lists the evaluated features. AD: Autosomal Dominant, AR: Autosomal Recessive, G: Gaza, M: Middle of West Bank, N: North of West Bank, S: South of West Bank, XLR: X-linked Recessive.

The patient cohort has a range of different clinical manifestations. The most frequent manifestations were recurrent infections (88%) and fever (75%). Skin-related abnormalities including skin infections, eczema, and abscesses were observed in 49% of the patients. Additionally, 41% presented with failure to thrive, while 12% had neonatal sepsis, which contributed to early neonatal death in some cases (Fig. 4.2).

Laboratory investigations revealed abnormalities in WBCs counts in 52% of patients, including both elevated and reduced levels. Notable abnormalities included neutropenia, lymphocytosis, or lymphopenia. Abnormal immunoglobulin levels were also detected in 52% of the cohort, ranging from generalized increases or decreases across all immunoglobulin classes to selective elevations, such as hyper-IgE. Elevated inflammatory markers were noted in 46% of patients. Immunophenotyping was available for only 22% of the cohort, of which 7% demonstrated abnormal findings (Fig. 4.2). Detailed clinical information for each participant can be found in table 4.1.

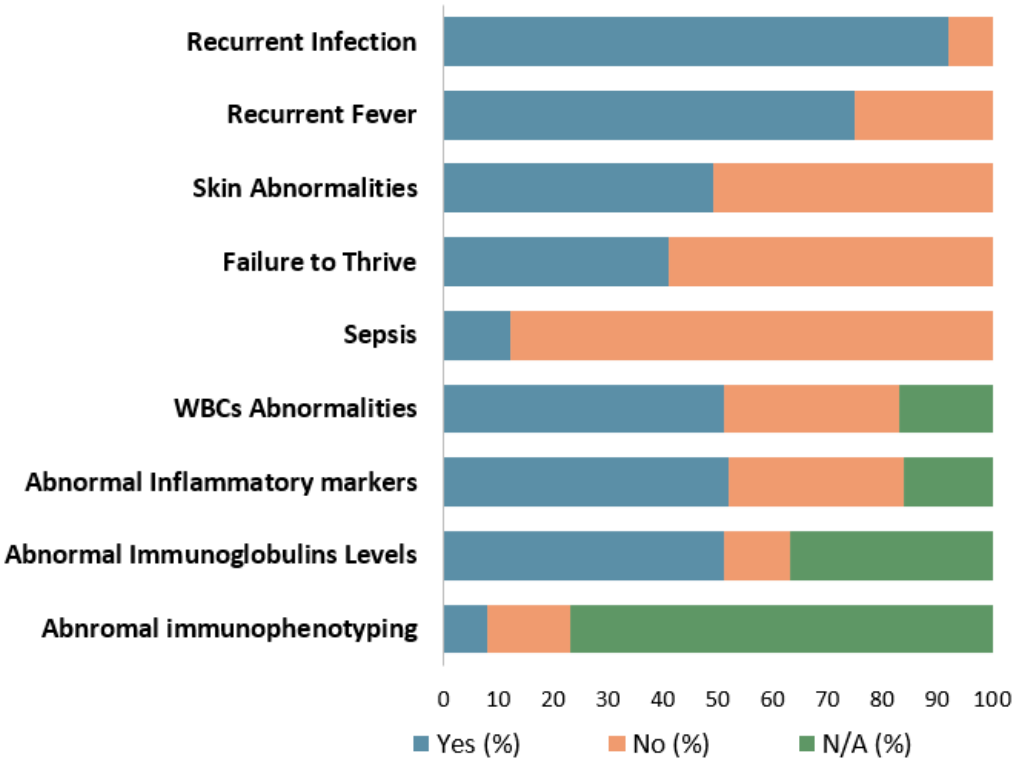

Figure 4.2: Clinical presentation and lab abnormalities among the patient cohort

Bar chart illustrates the distribution of common clinical symptoms and laboratory abnormalities in the patient cohort. The x-axis indicates the percentage of patients, while the y-axis lists the evaluated features. Blue bars represent patients with the feature, orange bars represent those without, and green bars indicate unavailable data.

Table 4.1: Clinical presentation of the patient cohort

Patient Number*	Consanguinity	Recurrent Fever	Skin Abnormalities	FTT	UTIs	OM	Chest Infections	Sepsis	gastroenteritis	Oral /Ulcer Thrush	Hepatosplenomegaly	Abnormal WBCs	Abnormal Inflammatory markers	Abnormal Ig-G	Abnormal Ig-M	Abnormal Ig-A	Abnormal Ig-E	Abnormal Immunophenotyping
P1	-	+	-	-	-	+	+	-	-	-	-	-	-	↓	↓	↓	↓	**na**
P2	+	+	+	+	+	-	-	+	+	+	-	↓	↑	↓	↓	↓	↓	+
P3	-	+	+	-	-	-	-	-	+	-	-	↑	-	-	-	-	-	-
P4	+	-	+	-	+	+	-	-	-	+	-	-	**na**	↑	↑	↑	↑↑↑	**na**
P5.1	+	+	+	-	-	+	-	-	+	+	-	↑	↑↑↑	↑	↑	↑	↑	**na**
P5.2	+	-	+	+	-	-	+	-	-	-	-	**na**	**na**	**na**	**na**	**na**	**na**	**na**
P6	+	+	-	+	-	-	-	+	-	-	-	↓	↑	↓	↓	↓	↓	-
P7	+	+	+	-	+	-	-	+	+	-	+	↑	↑	-	-	-	-	-
P8	-	+	-	-	-	+	+	-	+	+	-	-	-	↑	↑	↑	↑	**na**
P9	-	+	+	+	-	+	-	-	+	-	-	↑	-	-	-	-	-	-
P10.1	+	+	+	-	-	+	+	+	-	+	-	↑	↑	-	-	-	↑	**na**
P10.2	+	+	-	-	-	-	+	-	+	-	-	↑	↑	**na**	**na**	**na**	**na**	**na**
P11	-	+	+	+	+	+	+	-	+	-	-	↑	-	↓	↓	↓	↓	-
P12	-	+	-	+	+	+	-	-	-	-	-	-	-	↓	↓	↓	-	**na**
P13.1	+	+	-	-	-	+	-	-	+	-	-	-	↑	↓	↓	↓	↑	**na**
P13.2	-	+	-	-	-	-	+	-	-	-	-	**na**	**na**	**na**	**na**	**na**	**na**	**na**
P14.1	+	+	-	-	-	-	+	-	-	+	-	-	**na**	**na**	**na**	**na**	**na**	**na**
P14.2	+	+	-	-	-	-	-	-	-	-	-	↑	↑	**na**	**na**	**na**	**na**	**na**
P15	+	+	-	-	-	-	-	-	+	-	-	↑	↑	**na**	**na**	**na**	**na**	-
P16	-	-	+	+	-	-	+	-	-	-	-	-	**na**	-	-	-	↑	**na**
P17.1	+	+	+	+	-	+	+	-	+	-	-	↑	↑	-	↓	-	↑	+

P17.2	+	+	+	+	-	-	+	-	+	+	-	↑	↑	**na**	**na**	**na**	**na**	**na**
P18	-	+	+	-	-	-	-	-	+	+	-	-	**na**	↓	↓	↓	↓	**na**
P19	-	+	+	-	+	-	-	+	+	-	-	↓	↑	-	-	-	↑	**na**
P20	-	+	+	-	+	-	+	-	+	-	-	↑	↑	-	-	-	-	**na**
P21	-	+	+	-	-	-	-	-	-	-	-	-	↑	-	-	↓	-	**na**
P22.1	-	+	-	+	-	-	-	-	+	-	-	-	-	↓	↓	↓	↓	-
P22.2	-	-	-	+	-	-	-	-	+	-	-	**na**	**na**	**na**	**na**	**na**	**na**	**na**
P23	+	+	-	+	-	-	+	-	-	-	-	↓	↑	↓	↓	↓	↓	-
P24.1	+	-	+	+	-	-	+	-	-	-	+	-	↑	↑	↑	↑	↑	+
P24.2	+	-	+	+	-	-	+	-	-	-	-	**na**	**na**	**na**	**na**	**na**	**na**	**na**
P25	+	+	-	-	-	-	+	-	-	-	-	-	**na**	-	-	-	↑	**na**
P26	+	+	+	-	-	-	-	-	+	-	-	↑	↑	-	-	-	↑	**na**
P27	+	+	+	-	+	+	+	-	-	-	-	↓	**na**	↓	↓	↓	↓	**na**
P28	+	+	+	-	+	-	-	-	-	-	-	↑	↑	-	-	-	-	**na**
P29	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	↑	**na**
P30	-	+	+	+	-	-	-	-	+	-	-	↑	↓	↑	↓	↓	↓	**na**
P31	-	+	+	-	-	-	-	-	-	-	-	↑	↑	-	-	↓	↑	**na**
P32.1	+	+	+	-	-	-	-	-	+	-	-	**na**	**na**	**na**	**na**	**na**	**na**	**na**
P32.2	+	+	+	-	-	-	-	-	-	-	-	**na**	**na**	**na**	**na**	**na**	**na**	**na**
P33	-	-	+	-	-	-	-	-	+	-	-	-	-	**na**	**na**	**na**	**na**	**na**
P34	+	+	-	+	-	+	+	-	+	-	-	↓	↑	↓	↓	↓	↓	-
P35	-	+	+	+	+	-	-	-	+	-	+	-	↑	-	-	-	-	**na**
P36	+	-	+	-	-	-	+	-	-	-	-	↑	**na**	-	-	-	↑	**na**
P37	+	-	+	+	-	-	+	-	+	-	-	↑	↑	↓	↓	↓	↓	+
P38	-	+	-	+	-	-	+	-	+	-	-	-	↑	↓	↓	↓	↓	+
P39.1	-	+	-	-	-	-	+	-	+	-	-	-	↑	-	-	↓	-	**na**
P39.2	-	+	-	-	-	-	-	+	-	-	-	↑	↑	**na**	**na**	**na**	**na**	**na**
P39.3	-	+	-	-	-	-	+	-	-	-	-	**na**	**na**	**na**	**na**	**na**	**na**	**na**

P39.4	-	-	+	-	-	-	-	-	-	-	-	**na**	**na**	**na**	**na**	**na**	**na**	**na**
P40	+	+	-	-	-	+	+	-	-	-	-	**na**	**na**	**na**	**na**	**na**	**na**	**na**
P41	+	-	-	+	-	-	-	-	+	-	-	-	**na**	-	-	-	↑	**na**
P42.1	+	-	-	-	-	-	-	-	-	-	+	-	**na**	**na**	**na**	**na**	**na**	**na**
P42.2	+	-	-	-	-	-	-	-	-	-	+	-	**na**	**na**	**na**	**na**	**na**	**na**
P43	+	+	-	-	-	+	+	-	-	-	-	↓	↑	-	↓	-	-	**na**
P44	+	-	-	-	-	-	+	-	-	-	-	**na**	**na**	**na**	**na**	**na**	**na**	**na**
P45	+	+	-	+	-	-	-	-	-	+	-	↓	**na**	↓	**na**	↓	**na**	**na**
P46	+	-	-	+	+	-	-	+	+	-	-	↓	↑	-	-	-	-	-
P47	+	+	+	-	-	-	-	-	-	+	-	↑	↑	**na**	**na**	**na**	**na**	**na**
P48	-	+	+	+	-	-	-	-	+	-	-	**na**	**na**	**na**	**na**	**na**	**na**	**na**
P49.1	+	+	-	+	-	-	+	-	-	-	-	↓	**na**	**na**	**na**	**na**	**na**	**na**
P49.2	+	+	-	+	-	-	+	-	-	-	-	↓	**na**	**na**	**na**	**na**	**na**	**na**
P50	+	-	-	-	-	-	+	+	-	-	-	↓	↑	**na**	**na**	**na**	**na**	**na**
P51	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	**na**
P52	+	+	-	-	-	-	+	-	+	-	+	↓	**na**	↓	↓	↓	↓	**na**
P53	+	+	-	-	-	-	-	-	+	-	-	↓	**na**	-	-	↑	↑	**na**
P54	+	+	-	+	-	+	+	-	-	-	+	↑	↑	-	**na**	-	**na**	**na**
Total: 67	***42/67***	***50/67***	***33/67***	***28/67***	***11/67***	***15/67***	***31/67***	***8/67***	***31/67***	***11/67***	***7/67***	***35/67***	***31/67***	***20/67***	***21/67***	***24/67***	***29/67***	***5/67***
Percent	***62%***	***75%***	***49%***	***41%***	***16%***	***22%***	***46%***	***12%***	***46%***	***16%***	***10%***	***52%***	***46%***	***30%***	***31%***	***36%***	***43%***	***7%***

Summary of the most common clinical symptoms and laboratory abnormalities observed in the patient cohort, along with the percentage of patients exhibiting each feature. "+" indicates presence of the symptom or abnormality; "–" indicates absence. FTT: Failure To Thrive, HET: Heterozygous, HOM: Homozygous, Ig-A: Immunoglobulin A, Ig-E: Immunoglobulin E, Ig-G: Immunoglobulin G, Ig-M: Immunoglobulin M, LP: Likely Pathogenic, N/A: Not Available, OM: Otitis Media, P: Pathogenic, UTIs: Urinary Tract Infections, VUS: Variant of Uncertain Significance, WBCs: White Blood Cells, ↑: High/Increased, ↑↑↑: Extremely High, ↓: Low/Decreased.

4.2 Genetic data overview:

The genetic results identified in each of the tested individuals are detailed in Table 4.2. Twenty-one patients had a definitive or very probable genetic diagnosis. Of these, 33% were novel variants, 48% were shared variants that have been shared with multiple family members, and 19% had been previously reported in scientific literature or genetic databases. Six patients had VUS variants identified in immune-related genes, of which 4 were previously reported and 2 were novel. In addition, novel candidate disease genes were identified in three novel variants were identified in three patients, and one patient had a reported variant suggesting a potential new disease mechanism.

Pathogenic variants associated with FMF were identified in eight patients, including three cases in the homozygous state and five in the heterozygous state (see Supplementary Table S1 in Appendix A). Furthermore, nine patients were found to have incidental variants (see Supplementary Table S2 in Appendix A).

Table 4.2: Genetic variants found in the patient cohort

Classification of Variants	Patient number *	Gene	Zygosity	Variant type	Novelty of variants	Nucleotide change	Protein change	OMIM	GnomAD v4.1.0 (Het, Hom)	ClinVar	In-silico Prediction
Variants with definitive diagnosis	P2	*RTEL1*	Hom	Missense	Reported	c.1940C>T	p.Pro647Leu	Dyskeratosis congenita 5, AR, #615190	4,0	4, VUS, VCV000553611.35	REVEL: 0.914, Alphamissense: 0.96
	P3	*DOCK11*	Hom	LoF	Novel	c.4344del	p.Gln1448Hisfs*6	Autoinflammatory disease multisystem with immune dysregulation, XLR, #301109	na	na	na
	P4	*ZNF341*	Hom	LoF	Reported	c.1156C>T	p.Arg386*	Hyper-IgE syndrome 3 autosomal recessive with recurrent infections, AR, #618282	4,0	2, P, VCV000599411.3	na

	P17.1 and P17.2	***MALT1***	**Hom**	**LoF**	**Recurrent**	**c.2T>G**	**na**	**Immunodeficiency 12, AR, #615468**	**4,0**	**na**	**na**
	P22.1	***ACSL5***	**Hom**	**LoF**	**Novel**	**c.1374_1380del**	**p.Asp459Hisfs*14**	**Diarrhea 13, #620357**	**Absent**	**na**	**na**
	P24.1 and P24.2	***PEPD***	**Hom**	**LoF**	**Recurrent**	**c.504-1G>A**	**na**	**Prolidase deficiency, AR, #170100**	**1,0**	**2,P/LP, VCV000804428.9**	**SpliceAI: 0.99**
	P32.1 and P32.2	***IL17RA***	**Hom**	**LoF**	**Recurrent**	**c.1388del**	**p.Ala463Glyfs*21**	**Immunodeficiency 51, AR, #613953**	**Absent**	**na**	**na**
	P42.1	***SERPING1***	**Het**	**LoF**	**Novel**	**g.57361837-57365651del**	**na**	**Angioedema, hereditary, 1 and 2, #106100**	**Absent**	**na**	**na**
	P44	***SERPING1***	**Het**	**missense**	**Reported**	**c.589C>G**	**p.Leu197Val**	**Angioedema, hereditary, 1 and 2, #106100**	**Absent**	**2 VUS, VCV000418485.4**	**REVEL: 0.822, Alphamissense: 0.304**
	P49.2	***INTS11***	**Hom**	**missense**	**Recurrent**	**c.50G>T**	**p.Arg17Leu**	**Neurodevelopmental disorder with motor and language delay, ocular defects, and brain abnormalities #620428**	**1,0**	**1, P, VCV002506983.1**	**REVEL: 0.66, Alphamissense: 0.997**
	P50	***STXBP2***	**Hom**	**LoF**	**Reported**	**c.864G>A**	**p.Trp288***	**Hemophagocytic lymphohistiocytosis, familial, 5, with or without microvillus inclusion disease #613101**	**3,0**	**1, P, VCV001988382.3**	**SpliceAI: 0.930**

Variants with highly probable diagnosis	**P43**	***CSF3R***	**Hom**	**LoF**	**Novel**	**c.1475-3C>G**	**na**	**Neutropenia severe congenital 7, AR, #617014**	**Absent**	**na**	**SpliceAI: 0.94**
	P5.1 and P5.2	***IL36RN***	**Hom**	**LoF**	**Recurrent**	**c.244-5**	**na**	**Psoriasis 14, pustular, AR, 614204**	**126,0**	**3, VUS, VCV000836349.12**	**SpliceAI: 0.97**
	P23	***CEP57***	**Hom**	**LoF**	**Novel**	**c.1447_1457del**	**p.Asp483Hisfs*9**	**Mosaic variegated aneuploidy syndrome 2, AR, #614114**	**3,0**	**na**	**na**
	P40	***DCLRE1C***	**Hom**	**LoF**	**Recurrent**	**c.1543C>T**	**p.Gly515***	**Severe combined immunodeficiency Athabascan type, AR, #602450**	**Absent**	**1, P, VCV001378789.7**	**na**
	P53	***CSF3R***	**Hom**	**LoF**	**Novel**	**na**	**p.His54Profs*34**	**Neutropenia severe congenital 7, AR, #617014**	**Absent**	**na**	**na**
	P54	***MCM4***	**Hom**	**Missense**	**Novel**	**c.2511A>T**	**p.Lys837Asn**	**Immunodeficiency 54 #609981**	**1,0**	**na**	**REVEL: 0.12 Alphamissense: 0.863**
Low to moderate variants with uncertain significance	**P1**	***TNFRSF13B***	**Het**	**Missense**	**Reported**	**c.515G>A**	**p.Cys172Tyr**	**Immunodeficiency, common variable, 2**	**283,1**	**6 VUS+ 1 P, VCV000449548.48**	**REVEL: 0.710, Alphamissense: 0.533**
	P1	***TLR3***	**Het**	**LoF**	**Novel**	**c.763_764del**	**p.Leu255Glufs*2**	**{Immunodeficiency 83, susceptibility to viral infections}**	**2,0**	**na**	**na**
	P12	***NIPBL***	**Het**	**Missense**	**Novel**	**c.1096A>G**	**p.Arg366Gly**	**Cornelia de Lange syndrome 1, AD, #122470**	**Absent**	**na**	**REVEL: 0.585, Alphamissense: 0.69**

	P27	***TNFAIP3***	**Het**	**Missense**	**Reported**	**c.227C>T**	**p.Thr76Ile**	**Autoinflammatory syndrome familial Behcet-like 1, AD, #616744**	**Absent**	**3, VUS, VCV001031619.4**	**REVEL: 0.12, Alphamissense: 0.157**
	P34	***TNFRSF13B***	**Hom**	**Missense**	**Reported**	**c.310T>C**	**p.Cys104Arg**	**Immunodeficiency, common variable 2, AD+AR, #240500**	**8783, 32**	**27, P/LP, VCV000005302.85**	**REVEL: 0.918, Alphamissense: 0.944**
	P45	***CARMIL2***	**Compound Het**	**Compound Het**	**Reported**	**c.1429G>A / c.4226C>G**	**p.Asp477Asn / p.Pro1409Arg**	**Immunodeficiency 58**	**30,1 / 43,1**	**4 VUS, VCV000636842.11 / 5, VUS, VCV000636843.12**	**REVEL: 0.160, 0.0250 Alphamissense: 0.163, 0.06**
Variant with novel mechanism of disease	**P7**	***SCN11A***	**Hom**	**LoF**	**Reported for sensory neuropathy**	**c.4610_4611del**	**p.Phe1537Cysfs*31**	**Neuropathy, hereditary sensory and autonomic, type VII, AD, #615548**	**16,0**	**2, VUS, VCV001741888.8**	**na**
Variants in novel disease genes	**P16**	***RNF145***	**Compound Het**	**LoF/Missense**	**Novel**	**c.941del/ c.281A>G**	**p.Gly314Alafs*2/ p.His94Arg**	**Not a disease associated gene**	**Absent**	**na**	**REVEL: 0.820, Alphamissense: 0.939**
	P28	***ITGAL***	**Het**	**LoF**	**Novel**	**c.2293-10G>A**	**na**	**Not a disease associated gene**	**Absent**	**na**	**SpliceAI: 1.0**
	P37	***C3AR1***	**Hom**	**LoF**	**Novel**	**c.1216G>T**	**p.Glu406***	**Not a disease associated gene**	**5,0**	**na**	**na**

Overview of key genetic variants identified in the patient cohort, including zygosity, variant type and details, associated disease, ClinVar and gnomAD database entries, and in-silico prediction scores. In the novelty column, “Novel” indicates variants not reported in disease databases and not shared within the cohort; “Shared” refers to variants shared with other family members; and “Reported” means variants previously documented in disease databases. AD: Autosomal Dominant, AR: Autosomal Recessive, Hemi: Hemizygous, Het: Heterozygous, Hom: Homozygous, LoF: Loss of Function, LP: Likely Pathogenic, na: Not Available, P: Pathogenic, VUS: Variant of Uncertain Significance.

4.3: Clinical and genetic results of selected families

For the purposes of this thesis, the clinical and genetic findings of selected families will be described, with particular focus on five cases chosen for detailed presentation.

4.3.1 Case One

Case number one consists of one affected girl (P4) born to consanguineous parents and three other healthy siblings. P4 presented with a history of recurrent infections such as otitis media, genital infections, and urinary tract infections (UTIs), but no recurrent fever. She also had atopic dermatitis and a scalp abscess leading to hair loss at the abscess site, in addition to cheilosis and recurrent mouth thrush. The patient's IgE levels were very high, exceeding 10,000 kU/L, along with a reduced WBC count. Further complicating the clinical picture, the patient showed signs of developmental regression and learning difficulties. She also exhibited dysmorphic features, including posteriorly rotated ears, resorbed primary and dome-shaped teeth, and a high-arched palate (Fig. 4.3 (a)).

Genetic analysis revealed that she has a homozygous stop-gain variant in the *ZNF341* gene, located on chromosome 20, resulting in a premature stop codon (Chr20(GRCh38):g.33761989C>T;NM_001282933:c.1156C>T; NM_001282933:p.Arg386*) (Fig. 4.3 (c)). The stop codon occurred at amino acid number 386, meaning that 468 amino acids are predicted to be lost from the protein. NMD is expected to occur because of this variant.

According to OMIM (MIM: 618282), biallelic pathogenic *ZNF341* variants are associated with hyper-IgE syndrome 3 (HIES3), an autosomal recessive condition characterized by high serum IgE levels, recurrent infections, and chronic eczema. According to gnomAD v4.1.0, this stop-gain variant was found only in four individuals in the heterozygous state, with no reported homozygous cases. Segregation analysis showed that the patient had the variant in the homozygous state, while her parents were heterozygous carriers (Fig. 4.3 (b)).

There were two submissions of the variant of the *ZNF341* gene in ClinVar under accession number VCV000599411, classified as pathogenic (last checked August 2025). Functional studies conducted by (Frey-Jakobs et al., 2018) showed that cells with the p.Arg386* variant lacked the full-length *ZNF341* protein. Although the mutant protein could still be localized to the nucleus, its ability to bind chromatin was reduced compared to the wild-type protein.

Because of the variant's rarity and its confirmed effect as a LoF variant in a gene where loss of function is a known disease mechanism, it is classified as pathogenic according to ACGS and ACMG guidelines.

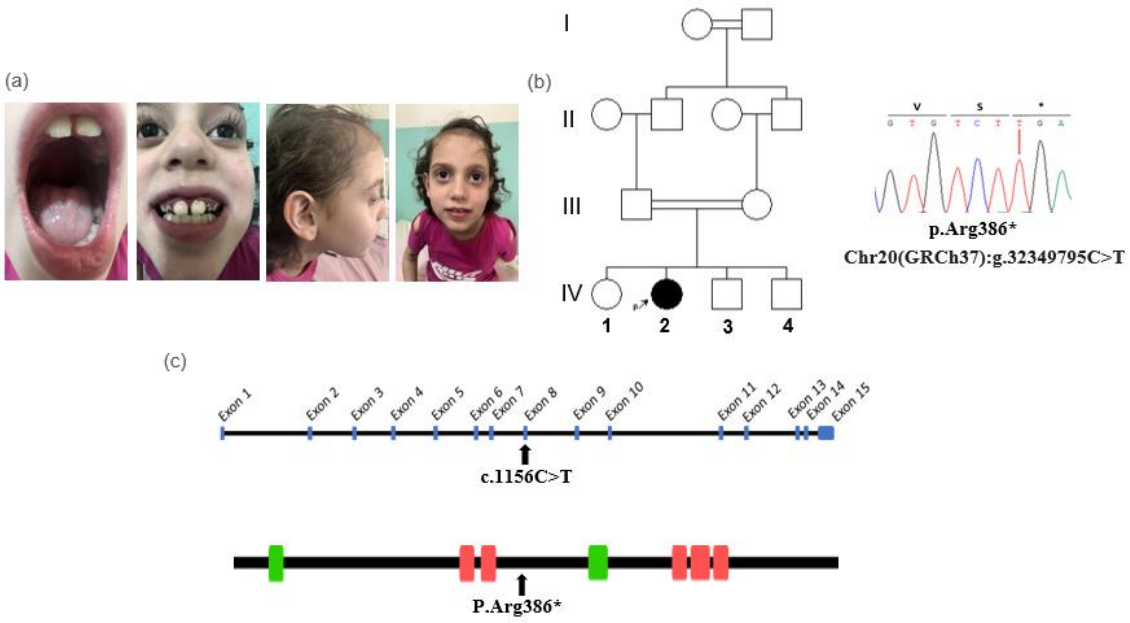

Figure 4.3: Summary of clinical and genetic results of case one

(a) show the dysmorphic features of P4: high-arched palate, dental anomalies and low set ears. (b) the pedigree indicates the consanguinity of the parents that led to a homozygous variant in patient 4 causing a premature stop codon at amino acid 386 of ZNF341 protein. (c) the variant is substitution of C nucleotide number 1156 of exon 8 to T causing a stop codon at amino acid number 386 possibly leading to a truncated protein with a loss of 468 amino acids.

4.3.2 Case Two:

Case number two included two affected children born to first-cousin parents. The first child, designated as IV:1 in Fig. 4.4(b), died at the age of 9 months and did not undergo genetic analysis. The second affected child (P2), designated as IV:5 in Fig. 4.4 (b), was admitted to the hospital following multi-organ failure caused by septicemia and HLH. She also presented with failure to thrive, recurrent fever and severe infections, anemia, thrombocytopenia and bleeding tendency from the gum, pancytopenia due to hypocellular bone marrow, Fanconi anemia, and thrombocytopenia, which made her transfusion dependent. Chromosomal breakage studies showed abnormalities, with 50% of cells exhibiting chromosomal breakage, while telomere studies indicated the presence of shortened telomeres. She was also diagnosed with left ventricular apical cardiomyopathy. Brain MRI showed that she had cerebellar atrophy.

Genetic analysis of P2 identified a homozygous missense variant in the *RTEL1* gene (Chr20(GRCh38):g.63689563C>T;NM_001283009:c.1940C>T; NM_001283009:p.Pro647Leu). The variant affects a canonical transcript, leading to the substitution of a highly conserved proline with leucine at position 647 (Fig. 4.4 (c)). This substitution is in the functional domain Helicase_C_2 of the *RTEL1* protein. The variant has high *in-silico* prediction scores (REVEL score of 0.914 and AlphaMissense score of 0.96).

Variants in the *RTEL1* gene are associated with autosomal recessive dyskeratosis congenita 5 (MIM: 615190), characterized by telomere dysfunction, which manifests as skin abnormalities, bone marrow failure, and immunodeficiency. Population frequency data from gnomAD v4.1.0 indicated that only four heterozygous individuals reported and no homozygous cases. Segregation analysis confirmed that P2 inherited the variant in the homozygous state from her heterozygous parents, confirming the autosomal recessive pattern. Unfortunately, a sample from her deceased brother was unavailable, preventing confirmation of his genetic status.

The variant is absent from gnomAD v4.1.0 in the homozygous state and 4 individuals have it in the heterozygous state. The variant is listed in ClinVar under VCV000553611.35 as a VUS, with five submissions, three of which associate the variant with dyskeratosis congenita.

According to ACGS guidelines, this variant is classified as likely pathogenic, supported by strong functional evidence of its effect on telomeres, moderate evidence for its rarity in the healthy population, and supportive evidences for its high in-silico prediction and its consistent clinical presentation.

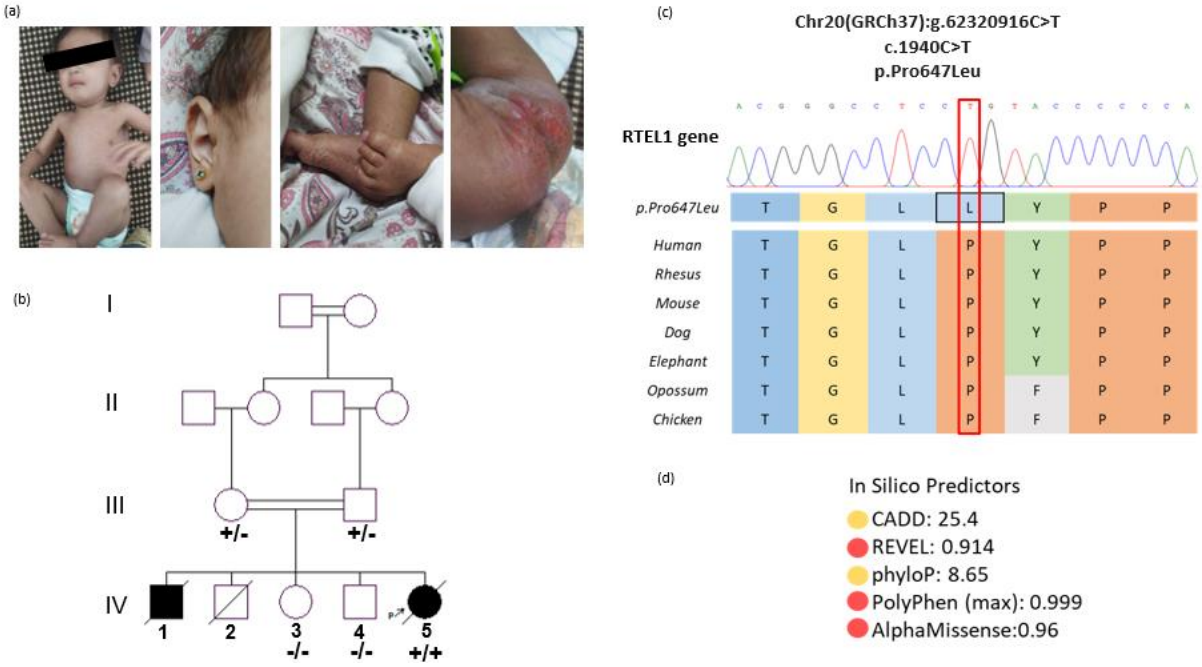

Figure 4.4: Summary of clinical and genetic results of case Two

(a) photos of ulcers and dysmorphic features seen in the patient. (b) family pedigree shows the consanguinity of the parent and a deceased brother with similar clinical history. (c) The patient is homozygous for p. Pro647Leu variant of *RTEL1* gene where a highly conserved amino acid, Proline, is converted to Leucine. (d) indicates that the variant is predicted to be deleterious by different In-Silico predictors.

4.3.3 Case Three:

Case number three consisted of an affected patient (P3) born for a non-consanguineous parent. P3 presented with severe eczema (Fig. 4.5 (a)) and a history of recurrent fevers. His WBCs counts were persistently elevated, with eosinophilia and lymphocytosis. Additionally, he had severe developmental delays, hypotonia, recurrent episodes of diarrhea, and a known milk allergy.

Genetic analysis identified a hemizygous frameshift deletion in the *DOCK11* gene (ChrX(GRCh38):g.118643540del; NM_144658.4:c.4344del; NM_144658.4:p.Gln1448Hisfs*6) (Fig. 4.5 (b)). The deletion introduced a premature stop codon predicted to result in a truncated *DOCK11* protein, missing 619 amino acids from the C-terminal region and is predicted to trigger NMD (Fig. 4.5 (d)).

Disease-causing variants in the *DOCK11* are newly described causes of X-linked recessive autoinflammatory disease with multi-system involvement and immune dysregulation (MIM: 301109). Cases reported in the literature describe a range of clinical presentations, with variable severity and age of onset among patients carrying *DOCK11* variants (Table S4 in Appendix A).

Mutations in the *DOCK11* gene are associated with an X-linked recessive autoinflammatory disease with multi-system involvement and immune dysregulation (MIM: 301109). Population data from gnomAD v4.1.0 indicated that this specific deletion was absent. While the variant was not reported in ClinVar, different publications indicate that LoF mutations in *DOCK11* are known to cause disease (Block et al., 2023; Boussard et al., 2023; Elsayed et al., 2025). Segregation analysis revealed that patient has maternally inherited the variant, while his sister is a healthy carrier (Fig. 4.5 (c)).

According to ACGS and ACMG guidelines, this variant was classified as pathogenic. This classification was supported by its absence in the healthy population and strong evidence for pathogenicity due to its loss-of-function effect.

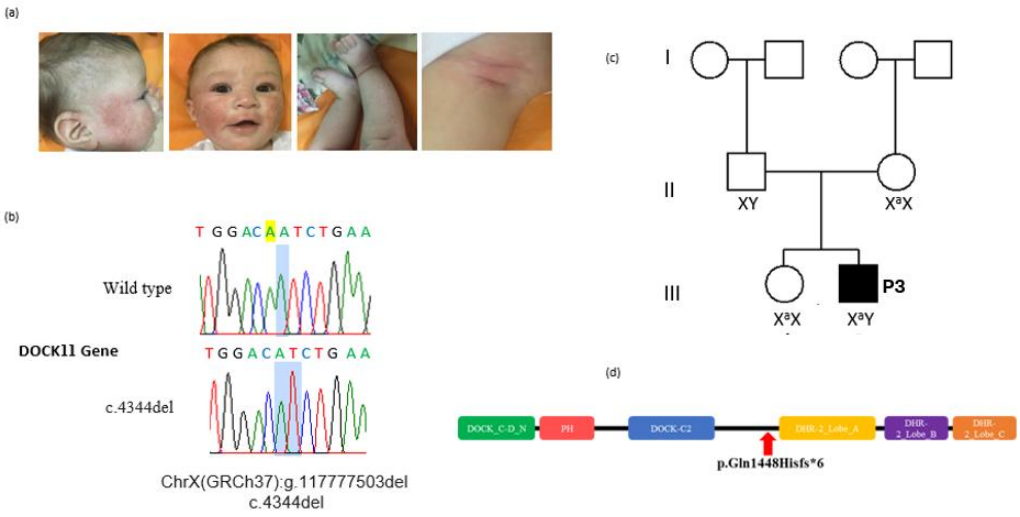

Figure 4.5: Summary of clinical and genetic results of case Three

(a) indicates the skin rashes of patient 3 covering his face, legs and armpit. (b) genetic analysis reveals a deletion variant in the *DOCK11* gene. (c) segregation analysis shows that the variant is maternally inherited. (d) Indicates the variant effect on protein where an early stop codon is predicted.

4.3.4 Case Four:

Case number four involves two affected girls (P5.1 and P5.2) born to a consanguineous family (Figure 4.6 (d)). P5.1 presented with episodic nodular skin lesions (Figure 4.6 (a)) and lung infiltration. She had a history of recurrent fevers and elevated inflammatory markers alongside leukocytosis. She also exhibited chronic mucosal inflammation, including angular stomatitis and recurrent aphthous ulcers. The parents were healthy at the time of evaluation but reported having skin rash and pustular acne during their youth. Her sister (P5.2) showed milder symptoms.

Genetic analysis of patient P5.1 identified a homozygous intronic splice site variant in intron 4 of the *IL36RN* gene, located on chromosome 2. The variant affected a canonical transcript of the gene near a critical splice site (Chr2(GRCh38):g.113062448G>A; NM_012275.2:c.244-5G>A). This change is predicted to disrupt normal splicing of the gene and consequently alter its function, as it is predicted to gain a new acceptor site with a high predictive score (SpliceAI = 0.97).

According to OMIM, Biallelic pathogenic variants in the *IL36RN* gene cause autosomal recessive conditions (MIM: 614204) characterized by severe skin inflammation, pustules, and recurrent episodes of painful rashes. Although this variant is found in the heterozygous state in 126 individuals in gnomAD v4.1.0, it is not present in the homozygous state. The variant is reported in ClinVar three times under the ID VCV000836349.11 and classified as a VUS. Two submissions were associated with generalized pustular psoriasis and one with autoinflammatory syndrome.

Segregation analysis showed that the parents were heterozygous for the variant, while their two daughters carried the variant in the homozygous state (Figure 4.6 (d)). A cDNA study of a whole blood sample failed to detect the transcript, as this gene is poorly expressed in blood. Due to the lack of a splicing assay, the variant lacked sufficient evidence and therefore remained classified as a VUS according to ACGS and ACMG guidelines.

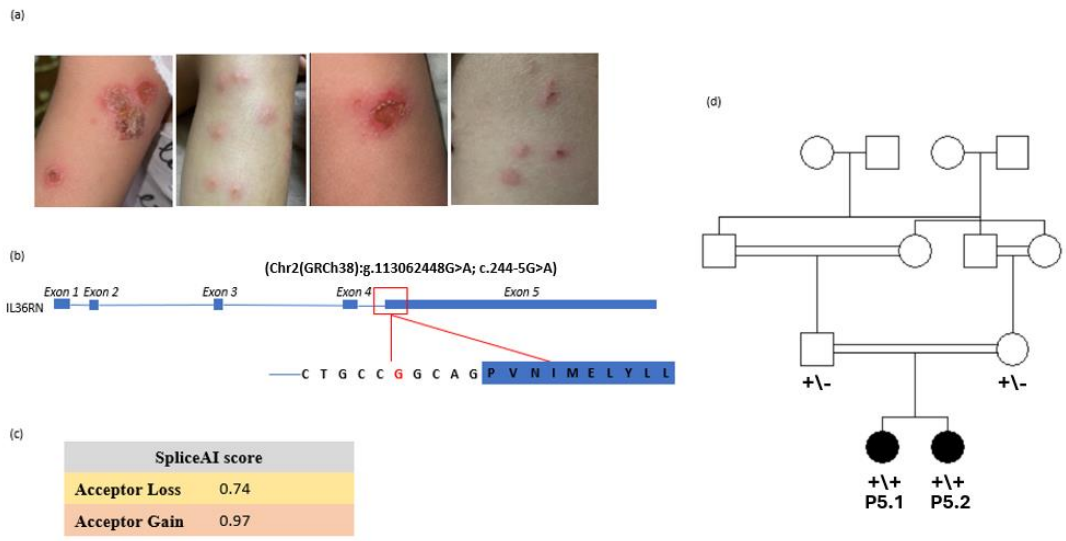

Figure 4.6: Summary of clinical and genetic results of Case Four

(a) shows the skin manifestation of the patients while having an episode. (b) elaborate the variant effect where a substitution of C to G at the fourth intron. (c) indicate the SpliceAI predictive high score. (d) the family pedigree shows that her parents are first cousins.

4.3.5 Case five:

Case number five included one affected girl (P1) born to non-consanguineous parents. The patient began having recurrent infections at the age of five, including recurrent chest infections, recurrent otitis media, and chronic draining ears. She also experienced chronic cough, bronchiectasis, pneumonia, and recurrent fevers, along with limited exercise tolerance. Laboratory investigations revealed persistently low immunoglobulin levels, consistent with a diagnosis of CVID.

Genetic analysis of patient identified a heterozygous missense variant in the *TNFRSF13B* gene, located on chromosome 17 (Chr17(GRCh38):g.16940442C>T; NM_012452.3:c.515G>A; NM_012452.3:p.Cys172Tyr). The variant affects a canonical transcript and results in the substitution of cysteine, which is a highly conserved amino acid, with tyrosine at position 172. In-silico prediction tools supported a potential deleterious effect, with a REVEL score of 0.710 and an AlphaMissense score of 0.533. The regional missense Z-score was 3.3, suggesting that this region is evolutionary constrained.

Mutations in the *TNFRSF13B* gene are associated with common variable immunodeficiency type 2 (MIM: 240500), which can follow either autosomal dominant or autosomal recessive inheritance patterns (Salzer et al., 2009). Population frequency data from gnomAD v4.1.0 indicated that this variant was present in 283 individuals in the heterozygous state and only one in the homozygous state. There are multiple submissions of this variant in ClinVar under accession number VCV000449548.48 classifying it as VUS.

Protein modeling of p.Cys172Tyr variant performed at Invitae (ClinVar accession: SCV000769956.6) indicated that it is expected to disrupt TNFRSF13B protein function. Additionally, functional assays showed that this variant affects TNFRSF13B activity by decreasing the activation of nuclear factor kB (NF-κB) and nuclear factor of activated T cells (NFAT) (Fried et al., 2011). Furthermore, this variant has been reported multiple times in individuals diagnosed with CVID (Mohammadi et al., 2009; Pulvirenti et al., 2016; Zhang et al., 2007). Based on ACGS and ACMG guidelines, the variant is classified as a VUS for its regional constraint (PP2), in-silico prediction support (PP3), and the patient's phenotype (PP4).

The patient also has another important variant in *TLR3* gene, located on chromosome 4 (Chr4(GRCh38):g.186082449_186082450del; NM_003265.3:c.763_764del; NM_003265.3: p.Leu255Glufs*2). Variants of this gene are associated with Immunodeficiency 83, susceptibility to viral infections (MIM: 613002). Although variants of this gene cause highly

variable phenotype and Incomplete penetrance, it worth to mention that some patients have recurrent and sever episodes of infections (Lim et al., 2019; Partanen et al., 2020). For this variant NMD is expected. In gnomAD v4.1.0 there are two heterozygous individuals with this variant and no homozygous.

Chapter 5 : Discussion

This study represents the first effort to establish genotype-phenotype correlations in patients with immunological dysregulations in Palestine. We achieved diagnoses in 17 patients and identified clinically important variants in 10 other cases, reflecting a strong overall diagnostic yield. These diagnostic findings helped in making informed decisions regarding definitive and preventive treatment strategies. It was notable that Patients presenting with milder phenotypes showed a lower rate of genetic diagnosis. A genetic diagnosis was achieved in 47.5% (19/40) of patients from consanguineous families, compared with only 7% (2/27) of patients from non-consanguineous families. This difference underscores how higher consanguinity rates increase the likelihood of identifying a genetic diagnosis (Cheema et al., 2020).

We also identified eight patients with pathogenic *MEFV* variants which reflect the high frequency of FMF in our population and the overlapping between autoinflammatory and immunodeficiency symptoms. Five of the patients with *MEFV* variants had them in the heterozygous state, which emphasizes the clinical significance of heterozygous *MEFV* variants. In fact, ClenGen has recently reported FMF to have a semi-dominant inheritance pattern. This means that we should consider both heterozygous and homozygous variants of *MEFV* gene as both can contribute to disease manifestation,

We have also identified a homozygous loss-of-function variant in *SCN11A* (p.Phe1537Cysfs*31), typically associated with autosomal dominant hereditary sensory and autonomic neuropathy and episodic pain syndrome. This variant was present in patient P7 with an immunodeficiency phenotype, suggesting a novel disease mechanism for a known disease gene.

Three VUS variants were identified in non-disease-associated genes, and six VUS variants were identified in immune-related genes, indicating the challenges in their interpretation. Reporting VUS to clinicians and families is ethically and clinically complex, as the uncertainty can lead to anxiety of the family or inappropriate management decisions by clinicians.

VUS represents a diagnostic bottleneck, as they cannot yet be confidently linked to disease despite their strong clinical correlation and functional evidence. The absence of

Palestinian-specific allele frequency data makes interpretation more challenging, as variants that appear rare in global databases may be benign polymorphisms in this population. Establishing a local variant frequency reference is therefore essential to support VUS classification.

Neonatal sepsis was identified in 10% of the patients and led to early neonatal death in some cases. This emphasizes the importance of early recognition and intervention in PID patients. Unfortunately, early recognition can be challenging as immunophenotyping data is limited in Palestine. Only 21% of our patients' cohort had immunophenotyping results. The lack of functional phenotyping is largely due to technical limitations, insufficient expertise, and resource constraints in Palestine, making NGS-based panel testing the most practical and accessible approach.

Some countries like the UK have implemented WGS as a newborn screening tool ("Newborn Genomes Programme," 2025). Genetic screening can detect conditions before symptom onset, preventing disease-related complications. However, ES and WGS are costly and require specialized expertise. As a result, population-targeted gene panels can offer an efficient screening tool. This approach, however, requires extensive research and development, which is a core objective of establishing SoH 'Stories of Hope, Stories from Palestine' research project and conducting this study. Panel design must be tailored to the genetic makeup of the community, considering founder variants and consanguinity rates. Regular updates must incorporate newly discovered genes and variants.

Genetic analysis of case number one revealed a stop-gain variant in the *ZNF341* gene (p.Arg386*), which plays a key role in regulating the JAK-STAT pathway. Dysfunction of this pathway contributes to various diseases, as it is essential for cell proliferation, differentiation, apoptosis, and immune responses. Reduced signaling through this pathway leads to immunodeficiency (Casanova et al., 2012). Pathogenic mechanisms within the JAK-STAT pathway vary depending on the variant type and molecular mechanism involved. For example, autosomal dominant LoF variants in *STAT1* gene increase the susceptibility to mycobacterial infections while gain-of-function (GoF) variants cause autoimmunity (Boisson-Dupuis et al., 2012). LoF variants in *STAT3* impair IL-17 production, resulting in immunodeficiency (Milner et al., 2008), while somatic GoF variants contribute to the pathogenesis of large granular lymphocytic leukemia (Koskela et al., 2012). On the other hand, Germline GoF variants in *STAT3* are associated with immune dysregulation (Flanagan et al., 2014; Milner et al., 2015).

Knowing that enabled targeting the JAK-STAT pathway for the treatment of autoimmune diseases (Schwartz et al., 2016) and for reducing infection rates (Mössner et al., 2016) and design a management plan (Tsilifis et al., 2021).

Case number two was diagnosed with dyskeratosis congenita, caused by a pathogenic variant in *RTEL1* gene (p.Pro647Leu). RTEL1 is essential for telomere maintenance and genomic stability, encoding an ATP-dependent DNA helicase involved in telomere length regulation, DNA repair, and genome integrity. A severe form of dyskeratosis congenita, Hoyeraal-Hreidarsson syndrome (HH), can present in early childhood and is characterized by cerebellar hypoplasia, developmental delay, intrauterine growth restriction, immune deficiency, and progressive bone marrow failure, along with typical dyskeratosis congenita features (Le Guen et al., 2013). Patients with dyskeratosis congenita are at increased risk for bone marrow failure, myelodysplastic syndrome, acute myeloid leukemia (AML), and solid tumors (Alter et al., 2009; Egan et al., 2011; Wrensch et al., 2009).

Dyskeratosis congenita can be inherited in an autosomal dominant (Ballew et al., 2013) or autosomal recessive manner (Walne et al., 2013). In addition, Heterozygous *RTEL1* variants are associated with a predisposition to pulmonary fibrosis (Cogan et al., 2015). Dyskeratosis congenita exhibits genetic anticipation, where successive generations show earlier onset and more severe manifestations due to progressive telomere shortening (Gutierrez-Rodrigues et al., 2019). So, individuals with heterozygous *RTEL1* variants may develop late-onset autosomal dominant dyskeratosis congenita or pulmonary fibrosis, meaning that being asymptomatic does not exclude disease. Therefore, telomere length testing is recommended for all heterozygous and homozygous carriers within the extended family. In addition, relatives should be monitored and advised to adopt preventive lifestyle measures, such as avoiding smoking.

Similarly, patients P43 and P53 carry homozygous variants in *CSF3R* (c.1475-3C>G), which are associated with neutropenia in homozygous individuals and neutrophilia in heterozygous carriers, with a predisposition to myelodysplastic syndrome. Regular monitoring and genetic screening of carriers in these families are strongly recommended.

In case number three the patients have LoF variant in *DOCK11* gene (p.Gln1448Hisfs*6). DOCK11 protein is a member of the DOCK-D subfamily of Guanine Nucleotide Exchange Factors (GEFs). It plays a critical role in Rho-GTPase signaling, cytoskeletal remodeling, cell migration, and adhesion. Deficiencies in DOCK proteins, particularly affecting B and T cell function, have been linked to actinopathies with immunological manifestations (Boussard et al., 2023).

Similarly to our patient that has severe developmental delay and hypotonia, neurodevelopmental abnormalities were also noted in two patients described by (Block et al., 2023). One of them has delayed developmental milestones and hypotonia, while the other has facial nerve palsy. Given that DOCK11 deficiency impairs CDC42 activity and CDC42 mutations are known to cause syndromic neurodevelopmental disorders (Lam et al., 2019; Martinelli et al., 2018), this possibly explains the overlap between DOCK11 deficiency phenotypes and CDC42-associated central nervous system involvement.

Case number four demonstrates inflammatory pathway dysfunction resulting in Deficiency of the Interleukin-36 Receptor Antagonist (DIRTA) disease. The patient has a splicing variant in *IL36RN* (c.244-5G>A) that encodes interleukin-36 receptor antagonist (IL-36Ra). Under physiological conditions, interleukin-36α, β, and γ bind to the interleukin-36 receptor, subsequently activating NF-κB signaling cascades that mediate inflammatory responses. IL-36Ra competitively binds to the receptor to attenuate inflammatory signaling and prevent excessive inflammation. IL-36Ra deficiency results in dysregulated inflammation and manifestations of generalized pustular psoriasis (Diaz, 2019). Patients have flares of skin rash because of excessive production of interleukin-8 in response to both interleukin-36 proteins and viral-like stimulation. This suggests that common infections may trigger pustular flares.

Despite autosomal recessive inheritance, *IL36RN* mutations may contribute to disease pathogenesis in heterozygous carriers through complex oligogenic mechanisms (Mössner et al., 2018). This explains the skin rash and pustular acne observed in the heterozygous parents. Similar patterns occur in FMF, another autoinflammatory disorder, suggesting potential tri-allelic inheritance patterns that may explain these cases.

Monoclonal antibody targeting the IL-36 receptor has demonstrated efficacy in improving generalized pustular psoriasis symptoms across two clinical trials (Gwillim & Nichols, 2024). Anti-IL-36R monoclonal antibodies have also shown therapeutic benefit in Crohn's disease by reducing intestinal inflammation (Hecker et al., 2025). As IL36RN belongs to the IL-1 cytokine family, IL-36 receptor stimulation upregulates IL-1 production (Onoufriadis et al., 2011), suggesting IL-1 as an additional therapeutic target.

Case number five represents a complex form of PID characterized by both phenotypic and genetic heterogeneity with p.Cys172Tyr variant of *TNFRSF13B*. Patients with CVID often present with highly variable clinical features, even among those sharing the same genotype. Many cases occur sporadically and show delayed onset of symptoms. Additionally, some pathogenic variants are found in the general population at frequencies exceeding expected thresholds (Bogaert et al., 2016).

CVID is most commonly a polygenic disorder, though monogenic causes account for a notable proportion. The *TNFRSF13B* gene encodes TACI, a B cell-specific protein that regulates activation, proliferation, and differentiation. Defects in TACI are associated with increased susceptibility to encapsulated respiratory bacteria and a higher risk of autoimmune complications in CVID patients (Fernando et al., 2021) . The pathogenesis of CVID involves diverse mechanisms, including dominant-negative effects and haploinsufficiency, further contributing to its clinical variability.

Importantly, TACI dysfunction is observed in both homozygous and heterozygous carriers, including individuals without symptoms or with only mildly reduced immunoglobulin levels (Martinez-Gallo et al., 2013). *TNFRSF13B* variants are relatively common in the general population and may not be sufficient alone to cause disease. This suggests the involvement of additional modifier genes. For example, (Abolhassani et al., 2025) indicates that heterozygous *TNFRSF13B* variants may require coexisting polymorphisms, HLA susceptibility haplotypes, or other pathogenic mutations in PID-related genes to manifest clinically. In our patient, a variant of uncertain significance in the *TLR3* (p.Leu255Glufs*2) gene was also identified.

Conclusion

Early detection and timely intervention critical for improving PID patient's outcomes especially in patients with severe immunodeficiencies, such as SCID that are associated with high mortality rates or in cases where genetic diagnosis has therapeutic implications. The use of advanced diagnostic techniques with robust variant analysis offers a high diagnostic yield, facilitating the identification of underlying genetic causes. However, diagnosing conditions like Common Variable Immunodeficiency (CVID) and interpreting Variants of Uncertain Significance (VUS) remain challenging. In such cases, population databases like the UK Biobank can support variant interpretation.

Recommendations

Several key recommendations can be taken out of this thesis. First, variants of uncertain significance identified through genetic testing should be prioritized for functional validation studies to definitively establish their pathogenicity and facilitate clinical decision-making. Second, patients who receive negative results from targeted genetic testing should be considered for advanced sequencing approaches, including whole genome sequencing and RNA sequencing, to detect pathogenic variants in non-coding regions and structural variations

that conventional methods may miss. Third, genetic testing panels for primary immunodeficiencies is more effective and should be specifically designed to reflect the unique genetic makeup of Palestinian population. Finally, comprehensive training programs in variant interpretation must be established to build capacity among clinicians, laboratory scientists, and researchers.

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Appendices

Appendix A: Supplementary tables

Supplementary table S1: *MEFV* variants in the patient's cohort

Inheritance Pattern	Patient number	Gene	Zygosity	Variant type	Nucleotide change	Protein change	OMIM	GnomAD v4.1.0 (Het, Hom)	ClinVar	In silico
Bi-allelic	P10	*MEFV*	Hom	missense	c.2177T>C	p.Val726Ala	Familial Mediterranean fever, AR, #249100	2115,26	39,P/LP, VCV000002540.146	REVEL: 0.35, Alphamissense: 0.0578
	P15, P34	*MEFV*	Hom	missense	c.2080A>G	p.Met694Val	Familial Mediterranean fever, AR, #249100	285,3	41, P/LP, VCV000002538.120	REVEL: 0.406, Alphamissense: 0.146
Mono-allelic	P21, P26	*MEFV*	Het	missense	c.2177T>C	p.Val726Ala	Familial Mediterranean fever, AD, #134610	2115,26	39,P/LP, VCV000002540.146	REVEL: 0.35, Alphamissense: 0.0578
	P23	*MEFV*	Het	missense	c.2282G>A	p.Arg761His	Familial Mediterranean fever, AD, #134610	151,0	25, P/LP, VCV000002549.92	REVEL: 0.352, Alphamissense: 0.061
	P35, P48	*MEFV*	Het	missense	c.2040G>A	p.Met680Ile	Familial Mediterranean fever, AD, #134610	19,0	18, P, VCV000002550.93	REVEL: 0.35, Alphamissense: 0.2569

Overview of the *MEFV* variants the patients have including zygosity, variant type and details, associated disease, ClinVar and gnomAD database entries, and in silico prediction scores. AD: Autosomal Dominant, AR: Autosomal Recessive, Het: Heterozygous, Hom: Homozygous, LoF: Loss of Function, LP: Likely Pathogenic, na: Not Available, P: Pathogenic, VUS: Variant of Uncertain Significance.

Supplementary table S2: Incidental findings in the patient's cohort

Patient number	Gene	Zygosity	Variant type	Nucleotide change	Protein change	OMIM	GnomAD v4.1.0 (Het, Hom)	ClinVar	In silico
P22	*HIVEP2*	Het	LoF	c.2959G>T	p.Glu987*	Intellectual developmental disorder, autosomal dominant 43, #616977	Absent	na	na
P26	*GDF1*	Het	LoF	c.909dup	p.Val304Argfs*48	Congenital heart defects multiple types 6, AD, #613854	153,0	3, P/LP, VCV000065389.29	na
P28	*ST3GAL3*	Hom	missense	c.736G>A	p.Glu246Lys	Developmental and epileptic encephalopathy 15, 615006	5,0	na	REVEL: 0.242, Alphamissense: 0.702
P29	*CHD7*	Het	missense	c.3272G>A	p.Cys1091Tyr	CHARGE syndrome, AD, #214800	Absent	na	REVEL: 0.814, Alphamissense: 0.917
P30	*FLG*	Het	LoF	c.4420C>T	p.Arg1474*	ichthyosis vulgaris, AR+AD, #146700	60,0	3, P/LP, VCV000488829.36	na
P33	*PRKAR1B*	Het	missense	c.391G>C	p.Ala131Pro	Marbach-Schaaf neurodevelopmental syndrome, AD, #619680	Absent	na	REVEL: 0.896, Alphamissense: 0.999
P33	*TYR*	Het	missense	c.518A>G	p.Tyr173Cys	[Skin/hair/eye pigmentation 3, light/dark/freckling skin], AD, #601800	1,0	na	REVEL: 0.976, Alphamissense: 0.757
P35	*SGCE*	Hom	missense	c.743G>A	p.Cys248Tyr	Dystonia-11 myoclonic, AD, #159900	Absent	1, VUS, VCV001523865.5	REVEL: 0.952, Alphamissense: 0.99
P41	*SLC25A13*	Hom	missense	c.74C>A	p.Ala25Glu	Citrullinemia type II neonatal-onset, AR, #605814	Absent	1, P, VCV002678843.1	REVEL: 0.916, Alphamissense: 0.992

Overview of incidental genomic findings in the patient cohort including zygosity, variant type and details, associated disease, ClinVar and gnomAD database entries, and in silico prediction scores. AD: Autosomal Dominant, AR: Autosomal Recessive, Het: Heterozygous, Hom: Homozygous, LoF: Loss of Function, LP: Likely Pathogenic, na: Not Available, P: Pathogenic, VUS: Variant of Uncertain Significance

Supplementary table S3: Summary of described DOCK11 cases

	This study	(Block et al., 2023)				(Elsayed et al., 2025)	(Boussard et al., 2023)						(Boussard et al., 2023)		(Gilton et al., 2024)
Patient	P3	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient A	Patient B	Patient C	Patient D1	Patient D2	Patient E	Patient F	Patient G	Patient UPN6b/M
Genetic variant	p.Gln1448Hisfs*6	p.Glu26Ter	p.Pro533_Lys573del	p.Trp1707Ser	p.Tyr108Cys	p.Gln1252*	p.Leu1298Arg	p.His1336Arg	p.Thr275Ser	p.Asp414Tyr	p.Asp414Tyr	p.Leu1706Ser	p.Arg1366Gln	p.Arg1885Cys	p.Leu298Arg
Age of onset	1 month	40 days	At birth	4 months	2 years	1 month	1.4 years	5 years	5 years	14 years	9 years	3 years	5 years	5 years	1 years
Anemia	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+
Recurrent infection	+	+	+	+	-	+	-	-	-	-	+	-	-	-	-
CNS involvement	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
GI Manifestation	+	-	-	+	+	-	+	+	-	+	-	-	+	-	+
Skin Manifestation	+	+	-	-	-	-	+	-	+	-	+	-	+	-	-
FTT	-	+	+	-	-	-	+	-	-	+	+	-	-	-	-
Inflammatory conditions	-	-	+	+	-	-	+	+	-	+	+	-	+	-	-
autoimmunity	na	na	na	na	na	na	na	+	+	na	na	+	na	+	+
Abnormal Immunoglobulin	na	+	na	+	+	na	+	na	na	+	na	na	+	+	+

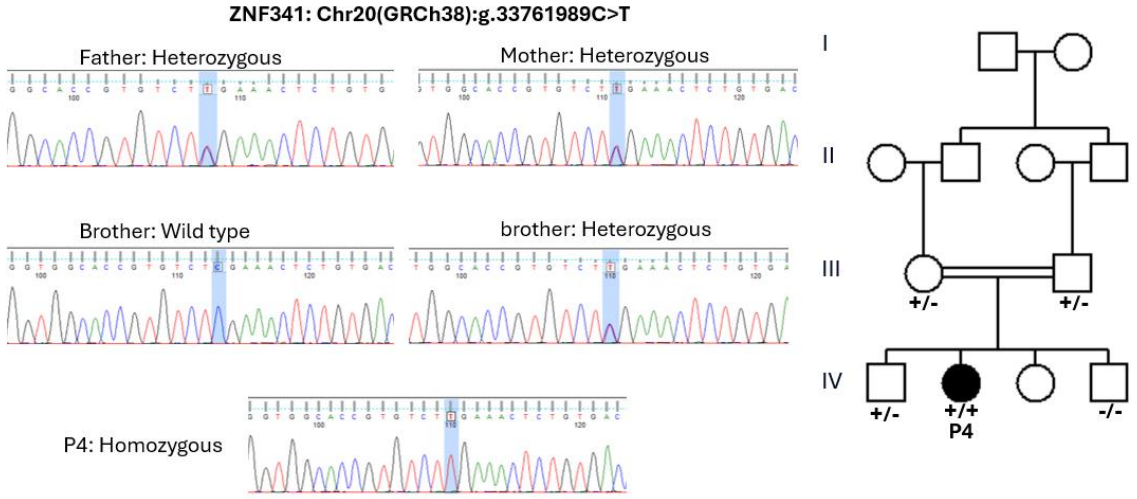
Clinical data of patients with loss-of-function variants in *DOCK11* reported in the literature, presented for phenotypic comparison. CNS: Central Nervous System, FTT: Failure to Thrive, GI: Gastrointestinal,

Supplementary table S4: Conditions for selected families PCR

Patient/s number	Gene	Primers sequence	Initial Denaturation	Denaturation	Annealing	cycles	Extension
P2	*RTEL1*	*FP:CTGACCCACAGATGGAGCTT RP:ACTGCATCTTGAGGACAACC*	94°C for 2 min	95° for 15 sec	56°C for 1 minutes	30 cycles	72°C for 5 minutes
P3	*DOCK11*	*FP:TCTCAGTGGGGCATAAACCA RP:CCCCATTGTCCCATCTTTCC*	94°C for 2 min	95° for 15 sec	58°C for 1 minutes	30 cycles	72°C for 5 minutes
P4	*ZNF341*	*FP:AACACATGCAGACCCACAAG RP:GAAGAGAGGTGTGGGAAGCA*	94°C for 2 min	95° for 15 sec	57°C for 1 minutes	30 cycles	72°C for 5 minutes
P5.1 and P5.2	*IL36RN*	*FP:GTGAAGAGATCAGCGTGGTC RP:ATTCCAGCCACCATTCTCGG*	94°C for 2 min	95° for 15 sec	57°C for 1 minutes	30 cycles	72°C for 5 minutes

Primer sequences and PCR conditions used for amplification of variants identified in selected families for segregation. FP: Forward Primer, FR: Reverse Primer, sec: seconds.

Appendix B: Supplementary figures

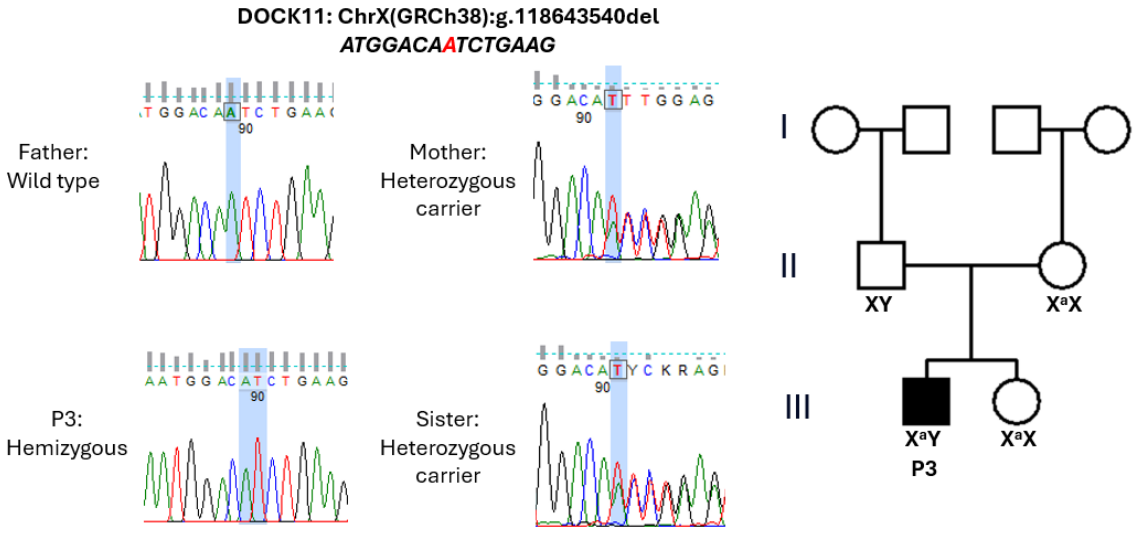


Supplementary figure 1: Segregation results of *ZNF341* in case number one

Segregation analysis of the *ZNF341* variant, showing chromatogram results for the affected individual, parents, and siblings, alongside the corresponding pedigree. Genotypes are indicated as follows: +/+: homozygous; +/-: heterozygous; - /-: wild type.

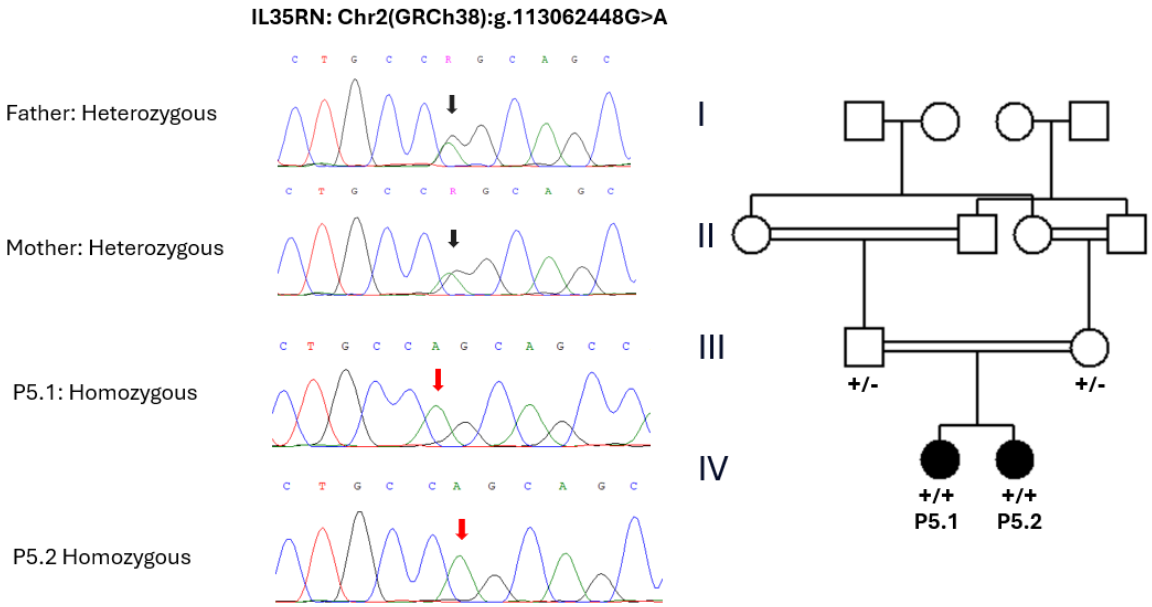
Supplementary figure 2 : Segregation results of *RTEL1* in case number two

Segregation analysis of the *RTEL1* variant, showing chromatogram results for the affected individual, parents, and siblings, alongside the corresponding pedigree. Genotypes are indicated as follows: +/+: homozygous; +/-: heterozygous; - /-: wild type.



Supplementary figure 3 : Segregation results of *DOCK11* in case number three

Segregation analysis of the *DOCK11* variant, showing chromatogram results for the affected individual, parents, and siblings, alongside the corresponding pedigree. Genotypes are indicated as follows: XᵃY: hemizygous male; XY: wild type male; XX: wild type female; XᵃX: carrier/heterozygous female.



Supplementary figure 4 : Segregation results of *IL36RN* in case number four

Segregation analysis of the *IL36RN* variant, showing chromatogram results for the affected individual, parents, and siblings, alongside the corresponding pedigree. Genotypes are indicated as follows: +/+: homozygous; +/-: heterozygous; - /-: wild type.

الملخص

نقص المناعة الأولي هي اضطرابات وراثية تؤدي إلى خلل في تنظيم الجهاز المناعي، مما يسبب عدداً من الأعراض التي تتراوح بين العدوى المتكررة وأمراض المناعة الذاتية وحتى الأورام الخبيثة. ويسهم الكشف المبكر عن هذه الاضطرابات في تجنب المضاعفات وتمكين تطبيق علاجات موجهة مثل زراعة نخاع العظم. ينتشر زواج الأقارب بشكل مرتفع في المجتمع الفلسطيني، كما أنه يعد من المجتمعات غير المخدومة بحثيًا، مما يعني أن الطفرات المسببة للأمراض في حالات نقص المناعة الأولي لم تُدرس بشكل كافٍ. في هذه الدراسة، قمنا بتحليل المتغيرات الجينية لدى 67 مريضًا من 54 عائلة يعانون من نقص المناعة الأولي، مع إجراء تحليل تفصيلي لخمس حالات مختارة. أظهرت الأعراض السريرية لدى المرضى مؤشرات على وجود نقص مناعي أولي، مع تداخل بعض الحالات مع أعراض اضطراب في تنظيم المناعة. تم أخذ الأعراض والسجل المرضي و نتائج الفحوص المخبرية لجميع المرضى، وأُجري تسلسل الإكسوم أو الجينوم الكامل للحالة المرجعية في كل عائلة، تلاه تحليل معمّق للبيانات الناتجة. كانت الأعراض الأكثر شيوعًا هي العدوى المتكررة والحمى، في حين لوحظت اضطرابات جلدية لدى 49%(67/33) من المرضى، وفشل في النمو لدى 41%(67/28). أظهرت الفحوصات المخبرية تنوعًا في النتائج، حيث سُجلت اضطرابات في عدد كريات الدم البيضاء لدى 52%(67/35) ، واضطرابات في مستويات الغلوبولين المناعي لدى 52%(67/35)، وارتفاع في مؤشرات الالتهاب لدى 46%(67/31). تم التوصل إلى تشخيص جيني في 17 عائلة (31%). بالإضافة إلى ذلك، تم اكتشاف ثلاث متغيرات جديدة في جينات لم تُربط سابقًا بأمراض، كما كشفت متغير معروف عن آلية مرضية جديدة محتملة، وتم تحديد متغيرات VUS في جينات مرتبطة بالمناعة لدى ستة مرضى. ومن الجدير بالذكر أن أياً من هذه المتغيرات لم يُكتشف في مختبرات تشخيصية داخل فلسطين. كما تم تحديد طفرات مرضية في جين *MEFV* المرتبط بمرض الحمى البحر الأبيض المتوسط العائلية لدى ثمانية مرضى. وقد أظهر التحليل الشامل للحالات المختارة متغيرات مهمة في الجينات التالية: *ZNF341*، *RTEL1*، *DOCK11*، *IL36RN*، و*TNFRSF13B*. تشير نتائجنا إلى أن التشخيص الجينومي المتقدم والتحليل الدقيق للطفرات ضروريان للكشف المبكر وتحسين نتائج العلاج لدى مرضى نقص المناعة الأولي، خاصة في الحالات الشديدة مثل SCID. كما تؤكد النتائج على أهمية تطوير برامج فحص جيني مخصصة، والحاجة إلى تأهيل جيل جديد من العلماء بمهارات تفسير المتغيرات اللازمة لتطوير التشخيص الجيني في المجتمعات غير المخدومة.