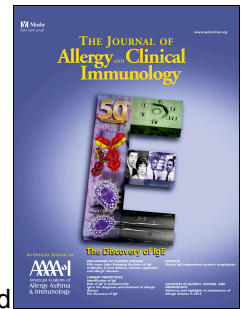


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Clinical, Immunological and Genetic Spectrum of 696 Patients with Combined Immunodeficiency

Hassan Abolhassani, MD, PhD, Janet Chou, MD, Wayne Bainter, PhD, Craig Platt, PhD, Mahmood Tavassoli, MD, Toba Momen, MD, Marzieh Tavakol, MD, Mohammad Hossein Eslamian, MD, Mohammad Gharagozlou, MD, Masoud Movahedi, MD, Mohsen Ghadami, PhD, Amir Ali Hamidieh, MD, Gholamreza Azizi, PhD, Reza Yazdani, PhD, Mohsen Afarideh, MD, Alireza Ghajar, MD, Arash Havaei, MD, Zahra Chavoushzadeh, MD, Seyed Alireza Mahdavian, MD, Taher Cheraghi, MD, Nasrin Behniafard, MD, Reza Amin, MD, Soheila Aleyasin, MD, Reza Faridhosseini, MD, Farahzad Jabbari-Azad, MD, Mohammad Nabavi, MD, Mohammad Hassan Bermanian, MD, Saba Arshi, MD, Rasol Molatefi, MD, Roya Sherkat, MD, Mahboubeh Mansouri, MD, Mehrnaz Mesdaghi, MD, Delara Babaie, MD, Iraj Mohammadzadeh, MD, Javad Ghaffari, MD, Alireza Shafiei, MD, Najmeddin Kalantari, MD, Hamid Ahanchian, MD, Maryam Khoshkhui, MD, Habib Soheili, MD, Abbas Dabbaghzadeh, MD, Afshin Shirkani, MD, Rasoul Nasiri Kalmarzi, MD, Seyed Hamidreza Mortazavi, MD, Javad Tafaraji, MD, Abbas Khalili, MD, Javad Mohammadi, MD, PhD, Babak Negahdari, MD, PhD, Mohammad-Taghi Joghataei, PhD, Basel K. al-Ramadi, PhD, Capucine Picard, MD, PhD, Nima Parvaneh, MD, Nima Rezaei, MD, PhD, Talal Chatila, MD, Michel J. Massaad, PhD, Sevgi Keles, MD, Lennart Hammarström, MD, PhD, Raif S. Geha, MD, Asghar Aghamohammadi, MD, PhD



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Clinical, Immunological and Genetic Spectrum of 696 Patients with Combined Immunodeficiency

Hassan Abolhassani, MD, PhD^{1,2*}, Janet Chou, MD^{3*}, Wayne Bainter, PhD³, Craig Platt, PhD³, Mahmood Tavassoli, MD⁴, Toba Momen, MD⁴, Marzieh Tavakol, MD⁵, Mohammad Hossein Eslamian, MD⁶, Mohammad Gharagozlou, MD⁷, Masoud Movahedi, MD⁷, Mohsen Ghadami, PhD⁸, Amir Ali Hamidieh, MD⁹, Gholamreza Azizi, PhD¹⁰, Reza Yazdani, PhD¹¹, Mohsen Afarideh, MD¹, Alireza Ghajar, MD¹, Arash Havaei, MD¹, Zahra Chavoushzadeh, MD¹², Seyed Alireza Mahdavian, MD¹³, Taher Cheraghi, MD¹⁴, Nasrin Behniafard, MD¹⁵, Reza Amin, MD¹⁶, Soheila Aleyasin, MD¹⁶, Reza Faridhosseini, MD¹⁷, Farahzad Jabbari-Azad, MD¹⁷, Mohammad Nabavi, MD¹⁸, Mohammad Hassan Bemanian, MD¹⁸, Saba Arshi, MD¹⁸, Rasol Molatefi, MD^{18,19}, Roya Sherkat, MD²⁰, Mahboubeh Mansouri, MD¹², Mehrnaz Mesdaghi, MD¹², Delara Babaie, MD¹², Iraj Mohammadzadeh, MD²¹, Javad Ghaffari, MD²², Alireza Shafiei, MD²³, Najmeddin Kalantari, MD²⁴, Hamid Ahanchian, MD¹⁷, Maryam Khoshkhui, MD¹⁷, Habib Soheili, MD²⁵, Abbas Dabbaghzadeh, MD²¹, Afshin Shirkani, MD²⁶, Rasoul Nasiri Kalmarzi, MD²⁷, Seyed Hamidreza Mortazavi, MD²⁸, Javad Tafaroji, MD²⁹, Abbas Khalili, MD³⁰, Javad Mohammadi, MD, PhD³¹, Babak Negahdari, MD, PhD³², Mohammad-Taghi Joghataei, PhD³³, Basel K. al-Ramadi, PhD³⁴, Capucine Picard, MD, PhD³⁵, Nima Parvaneh, MD¹, Nima Rezaei MD, PhD^{1,36}, Talal Chatila, MD³, Michel J. Massaad, PhD³, Sevgi Keles, MD³, Lennart Hammarström MD, PhD², Raif S. Geha MD^{3,#}, Asghar Aghamohammadi, MD, PhD^{1,36,#}

¹ Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Science, Tehran, Iran

² Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska Institute at Karolinska University Hospital Huddinge, Stockholm, Sweden

³ Division of Immunology Boston Children's Hospital, and Department of Pediatrics, Harvard Medical School, Boston, USA

⁴ Department of Allergy and Clinical Immunology, Isfahan University of Medical Sciences, Isfahan, Iran

⁵ Department of Allergy and Clinical Immunology, Shahid Bahonar Hospital, Alborz University of Medical Sciences, Karaj, Iran.

⁶ Department of Pediatrics, Hamadan University of Medical Sciences, Hamadan, Iran

⁷ Department of Immunology, Asthma and Allergy Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Science, Tehran, Iran

⁸ Department of Medical Genetics, Tehran University of Medical Sciences, Tehran, Iran

⁹ Hematology, Oncology and Stem Cell Transplantation Research Centre, Tehran University of Medical Sciences, Tehran, Iran

¹⁰ Department of Laboratory Medicine, Imam Hassan Mojtaba Hospital, Alborz University of Medical Sciences, Karaj, Iran

¹¹ Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

¹² Pediatric Infectious Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

¹³ Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

¹⁴ Department of Pediatrics, 17th Shahrivar Children's Hospital, Guilan University of Medical Sciences, Rasht, Iran

¹⁵ Department of Allergy and Clinical Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

¹⁶ Department of Pediatric Immunology and Allergy, Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

¹⁷ Department of Allergy and Clinical Immunology, Mashhad University of Medical Sciences, Mashhad, Iran

¹⁸ Department of Allergy and Clinical Immunology, Rasool e Akram Hospital, Iran University of Medical Sciences, Tehran, Iran

¹⁹ Department of Pediatrics, Bo-Ali children's Hospital of Ardabil University of Medical Sciences, Ardabil, Iran.

²⁰ Acquired Immunodeficiency Research Center, Al-Zahra Hospital, Isfahan University of Medical Sciences, Isfahan, Iran

²¹ Noncommunicable Pediatric Diseases Research Center, Amirkola Hospital, Babol University of Medical Sciences, Babol, Iran

²² Department of Pediatrics, Mazandaran University of Medical Sciences, Sari, Iran

²³ Department of Immunology, Bahrami Hospital, Tehran University of Medical Sciences, Tehran, Iran

²⁴ Department of Immunology and Allergy, Golestan University of Medical Sciences, Gorgan, Iran

²⁵ Department of Pediatrics, School of Medicine, Arak University of Medical Sciences, Arak, Iran

²⁶ Bushehr University of Medical Science, School of Medicine, Allergy and Clinical Immunology Department, Bushehr, Iran

²⁷ Cellular & Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran

²⁸ Department of Pediatrics, Kermanshah University of Medical Sciences, Kermanshah, Iran

²⁹ Department of Pediatrics, Qom University of Medical Sciences, Qom, Iran

³⁰ Department of Pediatrics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

³¹ Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran

³² School of Advanced Technologies in Medicine, Department of Medical Biotechnology, Tehran University of Medical Sciences, Tehran, Iran

³³ Cellular and Molecular Research Center & Department of Anatomy and Neuroscience, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

³⁴ Department of Medical Microbiology & Immunology, College of Medicine and Health Sciences, United Arab University, Al-Ain, UAE.

³⁵ Study Center for Primary Immunodeficiencies, AP-HP, Necker Enfants Malades Hospital, Paris, France.

³⁶ Primary Immunodeficiency Diseases Network (PIDNet), Universal Scientific Education and Research Network (USERN), Tehran, Iran

* Equal contribution

Equal contribution

Corresponding authors:

Asghar Aghamohammadi, Children's Medical Center Hospital, 62 Qarib St., Keshavarz Blvd., Tehran 14194, Iran. Tel: +98 21 66438622. Fax: +98 21 66923054. Email: aghamohammadi@tums.ac.ir.

Raif S. Geha, Division of Immunology Boston Children's Hospital, and Department of Pediatrics, Harvard Medical School, Boston MA 0211, USA. Tel: +1 617-919-2482. Fax: +1 617-730-0528. Email: raif.geha@childrens.harvard.edu

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Abstract

Background: Combined immunodeficiencies (CIDs) are diseases of defective adaptive immunity with diverse clinical phenotypes. Although CIDs are more prevalent in the Middle East than Western countries, the resources for genetic diagnosis are limited.

Objectives: This study aims to characterize the categories of CID patients in Iran clinically and genetically.

Methods: Clinical and laboratory data were obtained from 696 patients with CIDs. Patients were subdivided into those with syndromic (344 patients) and non-syndromic CIDs (352 patients). Targeted DNA sequencing was performed on 243 patients (34.9%).

Results: The overall diagnostic yield of the 243 sequenced patients was 77.8% (189 patients). The clinical diagnosis of HIES ($p<0.001$), onset of disease $> 5y$ ($p=0.02$), and the absence of multiple affected family members ($p=0.04$), were significantly more frequent in the patients without a genetic diagnosis. An autosomal recessive disease was found in 62.9% of patients, reflecting the high rate of consanguinity in this cohort. Mutations impairing VDJ recombination and DNA repair were the most common underlying causes of CIDs. However, in patients with syndromic CIDs, autosomal recessive mutations in *ATM*, autosomal dominant mutations in *STAT3* and microdeletions in *22q11.21* were the most commonly affected genomic loci. Patients with syndromic CIDs had a significantly lower five-year survival rate rather than those with non-syndromic CIDs.

Conclusions: This study provides proof of principle for the application of targeted next-generation sequencing panels in countries with limited diagnostic resources. The impact of genetic diagnosis on clinical care requires continued improvements in therapeutic resources for these patients.

Key messages:

1. The overall rate of molecular diagnosis was more than 78%, ranging from 60% for patients with hyper-IgE syndrome to 100% for patients with DiGeorge syndrome.

2. Patients with syndromic CIDs had a significantly lower five-year survival rate rather than those with non-syndromic CIDs, indicating the continued need for improved therapeutic interventions in these patients.

3. Our results provide proof of principle for the application of targeted next-generation sequencing panels in countries with limited diagnostic resources.

Capsule Summary: In this study of 696 patients with CID, molecular diagnosis was achieved in ~78% of the patients.

Keywords: combined immunodeficiencies; next-generation DNA sequencing; whole exome sequencing; targeted gene panel sequencing

Abbreviations:

AT: Ataxia telangiectasia

BCG: Bacillus Calmette–Guérin

CID: Combined immunodeficiency

CMV: Cytomegalovirus

EBV: Epstein-Barr virus

ESID: European Society for Immunodeficiencies

FISH: Fluorescent in situ hybridization

HIES: Hyper IgE syndrome

HIGM: Hyper IgM phenotype

HSCT: Hematopoietic stem cell transplantation

KRECs: Kappa-deleting recombination excision circles

NGS: Next-generation DNA sequencing

PHA: Phytohemagglutinin

PID: Primary immunodeficiency

153 **SCID:** Severe combined immunodeficiency
154 **TRECs:** T cell receptor excision circles
155 **VDPV:** Vaccine-derived polio virus
156 **VZV:** Varicella-zoster virus
157 **WES:** Whole exome sequencing
158 **WGS:** Whole genome sequencing
159 **WAS:** Wiskott-Aldrich syndrome
160

Introduction

Combined immunodeficiencies (CIDs) are characterized by the defective development or function of T cells. As the most severe form of primary immunodeficiencies (PID), CIDs are characterized by a susceptibility to infections, particularly from opportunistic organisms, which leads to severe morbidity and mortality^{1, 2}. A subpopulation of patients also has syndromic features due to the function of the affected gene in non-immune cells³. The reported incidence of CID is 1:100,000 to 1:5,000 live births worldwide⁴⁻⁸; however, this is considered an underestimation of the actual incidence due to the mortality of patients before diagnosis, misdiagnoses in patients with atypical clinical manifestations, and incomplete national registries documenting CID incidence⁹. The diagnostic delay between the age of disease onset and diagnosis has been reported to range from few days to several years in patients with CIDs¹⁰.

A genetic diagnosis provides the rationale for initiating the only curative interventions for CIDs, hematopoietic stem cell transplant and gene therapy, both of which can incur a high risk of morbidity and mortality¹¹. Early diagnosis enables initiation of curative therapies at a young age, which significantly increases the survival rate¹²⁻¹⁴. The diagnosis of CIDs is challenging due to the wide variability in clinical phenotypes and the limited availability of clinical laboratory tests for characterizing defects in immune systems³. Neonatal screening for severe CID (SCID) through quantification of T cell receptor excision circles (TRECs) has expedited the early diagnosis of patients with SCID by identifying defective T cell generation¹⁵. However, this approach does not identify T cell dysfunction in the setting of normal T cell numbers or provide a genetic diagnosis. The increasing availability of targeted next-generation DNA sequencing (NGS) panels, whole exome sequencing (WES), and whole genome sequencing (WGS), have facilitated the identification of genetic defects¹⁶. However, as demonstrated by the reluctance of commercial insurers to cover these tests, the utility of NGS as a diagnostic tool in clinical medicine is not uniformly accepted¹⁷.

CIDs constitute a group of clinically and genetically heterogeneous disorders, necessitating a comprehensive molecular approach for a definitive diagnosis¹⁸. The proportion of specific gene defects varies among countries, due to differences in rates of consanguinity and effects of founder

mutations. DiGeorge syndrome is the most commonly reported combined immunodeficiency in Western countries, but accounts for less than 30% of CIDs in the Middle East¹⁹. The published frequencies of genetic diagnosis in patient registries varies significantly among countries from 8% in Turkey and 13% in Tunisia to 40.4% in India and 60.5% in China²⁰⁻²⁵. In the European Society for Immunodeficiencies (ESID) online database, causative mutations were identified in 36.2% of the PID patients²⁷. The overall rate of genetic diagnosis was less than 33.7% among 77,193 patients reported from Jeffrey Modell centers worldwide in 2014¹⁹. Despite the fact that nearly 300 genes are known to cause PIDs³, these data demonstrate that many patients with PIDs lack a genetic diagnosis.

We have previously reported the demographic and phenotypic data from patients enrolled in the Iranian national registry²⁸. With the increasing availability of next-generation DNA sequencing technologies, we now present the genetic findings from 696 Iranian patients with CIDs. This is the largest cohort of genetically defined CID patients from a single country. Although consanguineous populations in the Middle East are commonly thought to have a high incidence of autosomal recessive CID, we show that autosomal dominant mutations in *STAT3* and microdeletions in *22q11.21* are the most prevalent mutations in patients with syndromic CIDs. These results stress the need for a comprehensive genetic approach for the diagnosis of PIDs and provide proof of principle for the application targeted NGS panels in countries with limited diagnostic resources.

Patients and Methods

Patients

This study was approved by the Ethics Committee of the Faculty of Medicine of Tehran University of Medical Sciences. Written informed consent has been obtained from all patients and/or their parents.

This study was conducted as a retrospective study of patients enrolled in the Iranian national registry for PIDs²⁸ from “National PID network” that comprises 25 medical centers in Iran. The Iranian national registry for PID was established in 1997 and is managed by the Research Centre for Immunodeficiencies. The registry currently includes approximately 2,500 clinically diagnosed PID patients (<2% of expected patients according to the probable prevalence) and only 35% of these patients have been diagnosed genetically²⁶. A questionnaire surveyed the patients’ demographic information, age of disease onset, age of diagnosis, family history, a detailed clinical history that included vaccine history and associated adverse reactions, recurrent infections, physical examination findings, laboratory testing, and treatment history²⁸. Diagnostic laboratory data obtained included complete and differential blood count, serum immunoglobulin levels, immunophenotyping of peripheral blood lymphocytes, alpha-fetoprotein, assays of T cell function, which including proliferative assays (using phytohemagglutinin [PHA], Bacillus Calmette–Guérin [BCG] and Candida), Tuberculosis skin test, and radiosensitivity test²⁸⁻³². The delay in diagnosis is defined as a period of time between the age of disease onset and the age at diagnosis. Patients were diagnosed with CID based on the standard criteria introduced by the ESID and Pan-American Group for Immunodeficiency (<http://esid.org/Working-Parties/Registry/Diagnosis-criteria>, **Table S1**).

The collected data from the patients were reviewed by expert clinical immunologists in the Children’s Medical Center Hospital in Iran, the largest referral center for PID in the country, to confirm that the diagnosis made at different centers met the standard criteria^{33, 34}. After confirmation of diagnosis, patients were classified according to the International Union of Immunological Societies (IUIS) PID Committee updated classification³. According to this classification, patients with only immune-related manifestations were located in non-syndromic CID (including SCID, Omenn

phenotype, hyper IgM phenotype [HIGM] and partial T cell defects) and the remaining with additional non-immune complications classified as syndromic CID (including hyper-IgE syndrome [HIES], Wiskott-Aldrich syndrome [WAS], DiGeorge syndrome, DNA repair defect syndromes, dyskeratosis congenital, ectodermal dysplasia, as well as other atypical and incomplete syndromic CIDs).

A computerized database program (new registry section in <http://rcid.tums.ac.ir/>) was designed for data entry and direct statistical analysis of data. Patients with incomplete diagnostic criteria were excluded.

Methods

Genomic DNA was extracted from whole blood as previously described³⁵. For patients with classical clinical presentations suggestive of a specific CID, Sanger sequencing was performed on the most likely genes³⁶⁻³⁹. Patients with thymic defects (**Table S1**), were examined by fluorescent in situ hybridization (FISH) for 22q11.2 deletion and comparative genomic hybridization array⁴⁰. For patient with failed Sanger sequencing or with clinical presentation resembling several genetic defects, targeted NGS was performed using the PID v2 panel and Ion Torrent S5 sequencer (ThermoFisher), with an average coverage of 335X. Variant calling and coverage analysis was performed using Ion Reporter software⁴¹. The detection of large deletions was performed using the method of normalized mean coverage of individual exons⁴². In patients with less profound CID (**Table S1**), whole exome sequencing was performed using a pipeline described previously, with an average on-target coverage of 50X⁴³.

Statistical analysis

Statistical analysis was performed using a commercially available software package (SPSS Statistics 17.0.0, SPSS, Chicago, Illinois). One-sample Kolmogorov-Smirnov test was applied to estimate whether data distribution is normal. Parametric and nonparametric analyses were performed based on the finding of this evaluation. Kaplan-Meier curve and log-rank test were utilized to compare different survival estimates. A *p*-value of 0.05 or less was considered statistically significant.

Results

Patient population

A cohort of 696 patients with CID (408 males and 288 females from 624 unrelated families) was evaluated. The diagnosis of CID was made using the established clinical criteria developed by the European Society of Immunodeficiency (**Table S1**). 55.7% (n=387) of patients were diagnosed from 2013 to 2016, while 44.3% (n=309) were diagnosed prior to 2013²⁸. Patients were followed for a total of 3841 patient years with a median follow-up of 3 years per patient (range 0.1 to 29 years). The median age at onset was 1.5 years (range 1 day to 31 years); 93% of patients had onset of their disease before a 13 years of age. The median age at diagnosis was 3.6 years (range 2 weeks to 45 years); 41.8% of patients were diagnosed before 1 year of age. The median delay in diagnosis was 2.4 years (range 0 to 24 years). Parental consanguinity was present in 474 of the 624 families (75.9%), substantially higher than the 40% prevalence of consanguineous marriage in Iran⁴⁴. A positive family history of stillbirth or unknown death was identified in 331 unrelated families (52.9%). A positive history of premature birth was reported in 14.9% of patients, (n=104) which is 38% higher than overall premature birth in Iran⁴⁵. The overall mortality rate among our cohort of CID patients was 50%.

We categorized the patients as non-syndromic (n=352) and syndromic (n=344) using criteria established by the International Union of Immunologic Societies³ (**Table 1**). The 5-year survival rate of syndromic CID patients was significantly higher than that of non-syndromic CID patients individuals (~75% versus 40%, $p=0.001$). While the survival rate of patients with syndromic CIDs progressively diminished to 15% ($p=0.004$, **Figure S1 A**), the 15 year survival rate of patients with non-syndromic CIDs plateaued at ~40%. Among non-syndromic patients, those with SCID had a significantly greater mortality rate than those with less profound CID during first 2 years of follow-up ($p<0.001$, **Figure S1 B**). Infections and infectious complications accounted for almost all causes of death in patients with non-syndromic patients (168 of 189 deceased patients); only 36% of patients with syndromic CID died from infectious causes (58 of 159 patients, $p<0.001$); the remainder of the patients died from multi-organ failure, neurological abnormalities, and malignancies.

Non-syndromic CID Patients

The most common clinical presentations of non-syndromic CID patients were recurrent pneumonias (n=130, 36.9%), failure to thrive (n=93, 26.4%), chronic diarrhea (n=84, 23.8%), and recurrent oral candidiasis (n=42, 11.9%). In 40 patients (11.3%), dermatologic lesions such as severe generalized eczema, skin infections, and abscesses were the earliest clinical presentations. Disseminated Bacillus Calmette-Guerin infection (BCGosis) accounted for the primary presentation in 23 (6.5%) patients, while BCGosis was documented in 34 cases (9.6%) following vaccination.

Respiratory tract and gastrointestinal tract infections were the main complications identified in 242 (68.7%) and 209 (59.3%) patients, respectively. Oral candidiasis was documented in 81 (23.0%) patients, urinary tract infections in 59 (16.7%), and cutaneous infections in 51 (14.4%). Fifty-six percent of the patients (n=197) developed multi-site infections requiring intensive medical treatment. Vaccine-derived polio virus (VDPV) was isolated in 6 patients with acute flaccid paralysis. Five of the patients developed fulminant viral hepatitis B during the neonatal period that later developed into cirrhosis. Three patients were affected by pulmonary tuberculosis. Other pulmonary opportunistic infections included cytomegalovirus (CMV) in 15 patients, *Pneumocystis jiroveci* in 13 patients, and Varicella-zoster virus (VZV) in six patients. Severe Epstein-Barr virus (EBV)-associated lymphoproliferative disorders were present in eight patients and cryptosporidium infection was present in six patients.

A clinical diagnosis of SCID was identified in 169 patients of whom 63 (37.2%) had a T⁻B⁺NK⁻ 55 (32.4%) a T⁻B⁻NK⁺, 43 (25.4%) a T⁻B⁺NK⁺, and 8 (4.7%) a T⁻B⁻NK⁻ immunologic phenotype (**Table S1**). Details of the clinical and immunologic manifestations of SCID patients are listed in **Table S2**. Omenn phenotype was diagnosed in 11 (1.5%) patients (**Table 1**). The remaining 172 patients were classified as CID (less profound, non-syndromic CID). Of these, 69 patients (19.6%) had a HIGM phenotype associated with T cell defects, 41 (11.6%) had CD4 deficiency, 19 (5.3%) had CD8 deficiency, and 43, and (12.2%) had late onset functional T-cell defects (with normal count of T-cells but defective proliferative assays).

The percentage of T cells ($5.7 \pm 4.0\%$ vs. $35.5 \pm 24.3\%$; $p=0.01$) and absolute numbers of T cells (85.9 ± 40.0 cells / μ L vs. 1149.5 ± 540.7 cells / μ L; $p<0.001$) were significantly lower in SCID patients compared to CID patients, as was the age of onset (2.3 ± 2.0 months vs. 18.5 ± 14.3 months; $p<0.001$), age of diagnosis (0.4 ± 0.2 years vs. 3.5 ± 2.9 years; $p<0.001$), and follow-up period (0.5 ± 0.4 years vs. 5.8 ± 4.5 years; $p<0.001$). The hospitalization rate was significantly higher in SCID patients compared to CID patients (242.4 ± 170.3 days/year vs. 32.4 ± 25.8 days/year; $p<0.001$).

With available resources, molecular diagnosis was performed in 103 patients with non-syndromic CID and identified the causative mutation in 84 patients (81.5%). A molecular diagnosis was achieved in 37 of 45 (82.2%) patients with SCID or Omenn's syndrome. Three had mutations in X-linked genes, and 34 had mutations in autosomal recessive genes. The genetic defects included mutations in *RAG1* (n=13), *RAG2* (n=6), *IL2RG* (n=3), *JAK3* (n=3), *DCLRE1C* (n=3), *ADA* (n=2), *IL7R* (n=2), *CD3E* (n=1), *CD3D* (n=1), *PRKDC* (n=1), *NHEJ1* (n=1) and *PTPRC* (n=1) (**Table 2**, **Table S3**, **Figure S2**). Thus, autosomal recessive forms of SCID accounted for 91.8% of patients with identified molecular while X-linked SCID, which represents the most common form of SCID in Western countries, accounted for only 9.2% of the cases. The high incidence of autosomal recessive SCID correlates with the high rate of consanguinity (78.4%) in the patients' families. Of the 58 non-syndromic CID patients without SCID or Omenn syndrome, disease-causing variants were identified in 47 (81%) patients. Twenty two had a phenotype consistent with HIGM and 36 had partial T cells defects (**Table S1**). The diagnostic yield in the 22 HIGM patients was 81.8% with all 18 patients having mutations affecting *CD40L* in males. The diagnostic yield in the 36 CID patients with a partial T cell defect was 80.5% (29 patients) as follows: LRBA deficiency (n=15), CD27 deficiency (n=3), STK4 deficiency (n=3), ICOS deficiency (n=2), MHC class II deficiency (n=3: 2 mutations in *RFXANK* and 1 mutation in *CIITA* genes), ZAP70 deficiency (n=1), ITK deficiency (n=1) and MALT1 deficiency (n=1) (**Table 2**).

Syndromic CID Patients

In the 344 syndromic CID patients, the most frequent non-immunologic manifestations were neurologic. 59.5% of the patients (n=205) had ataxia, microcephalus, hydrocephalus, intellectual disability, mental retardation, or cognitive impairment. The second most frequent non-immunologic manifestations were dermatologic abnormalities, which were present in 54.3% of the patients (n=187) and included telangiectasia, ectodermal dysplasia, sparse hair, hyperkeratosis, congenital ichthyosis, atopic diathesis, café-au-lait spots, nail dystrophy, and hypo/hyperpigmented lesions. Facial dysmorphic features (bird-like facies, broad or flat nasal bridge, hypertelorism, low-set ears, macroglossia, and cleft lip) were present in 31.0 % of the syndromic CID patients. Additional syndromic features included musculoskeletal abnormalities, malignancies, cardiac malignancies, endocrine defects, and intestinal atresia (**Table S4**).

Based on the clinical and immunologic criteria (**Table S1**), the diagnosis of a DNA repair defect syndrome was made in 193 patients (56.1%) as follows: 145 with ataxia telangiectasia (AT), 5 with Nijmegen breakage syndrome, 3 with Bloom syndrome, and 40 with unspecific syndromic CID characterized by radiosensitivity. There were 101 patients (29.3%) with the clinical diagnosis of HIES; of these, 68 patients had consanguineous parents, suggesting an autosomal recessive model of HIES. Twenty-nine patients were diagnosed with WAS, and 13 patients with DiGeorge syndrome. Eight patients were categorized as other syndromic immunodeficiencies that included dyskeratosis congenita, ectodermal dysplasia, as well as other atypical and incomplete syndromic CIDs (**Table 1**). The summaries of the two main categories of patients in this group (AT and HIES) are shown in **Tables S5** and **S6**. Patients with thymic defects (with clinical or imaging evidence of absent or hypoplastic thymus) had the highest mortality rate (61.5%, mainly due to congenital heart disease) of all main categories of syndromic CID, with all patients harboring the 22q11.21 microdeletion determined using cytogenetic studies.

A molecular defect was identified in 105 of the 140 (75%) syndromic CID patients in whom NGS was performed. A molecular diagnosis was achieved in 21 of 24 (86.3%) patients with AT and 13 of 16 (81.2%) other radiosensitive patients studied. All the mutations were in genes associated with autosomal recessive CIDs and included *ATM* (n=21), *DNMT3B* (n=8) and *ZBTB24* (n=5) (**Table 3**,

Table S7, Figure S2). All 13 DiGeorge patients studied had a microdeletion encompassing *TBX1*. Of the 62 HIES patients, disease-causing variants were identified in 37 (59.6%) patients. They included 19 patients with autosomal dominant HIES (51.3%) and 18 patients with autosomal recessive HIES (49.7%) as follows: STAT3 deficiency (n=19), DOCK8 deficiency (n=13), TYK2 deficiency (n=3), PGM3 deficiency (n=1) and SPINK5 deficiency (n=1). Fifteen of 15 of WAS patients studied (100%) had a hemizygous mutation in X-linked gene of WAS. One patient with pathogenic mutation in each of *EPG5*, *PNP*, *TTC7A*, *IKBKG*, *DCK1* and *SMARCA1* genes was also identified (**Table 3, Table S7**).

Overall diagnostic yield

Genetic sequencing was performed on 243 of the 696 patients (34.9 %). The overall diagnostic yield of the 243 sequenced patients was 77.8% (189 patients). **Table S8** stratified the genetic analysis based on the age and sex of the patients. The majority of patients (62.9%) had an autosomal recessive disease, with 56.6% having homozygous mutations and only 6.3% having compound heterozygous mutations. X-linked diseases compromised 20.1% of the genetic diagnoses and 17% of patients had an autosomal dominant disease. In the 189 patients with a genetic diagnosis, defects in genes that encode proteins involved in the DNA recombination pathways (*RAG1*, *RAG2* and *DLCRE1C*, *PRKDC*) accounted for 16.7% of the total disease-causing etiologies, while defects in DNA repair (*ATM*, *DNMT3B* and *ZBTB24*) accounted for 19% of genetic defects. Defects in costimulatory molecules (e.g. CD40L, ICOS, CD27 deficiencies in 12.6% of patients) and in the STAT3 signaling pathway (in 10% of patients) were also the other frequently observed defects in our CID cohort.

The highest diagnostic yield was obtained in patients with DiGeorge syndrome (100% of 13 tested) and the lowest was in HIES patients (37 of 62 tested; 59.6%). We performed stratification on the patients who underwent sequencing to determine the parameters associated with a diagnosis. Of note, consanguinity and the severity of clinical presentation were similar between those who had a molecular defect identified (n=189) and those who did not (n=64). However the clinical diagnosis of HIES ($p<0.001$), a late age of presentation (onset of disease > 5y; $p=0.02$), and the absence of multiple

391 affected family members ($p=0.04$) were significantly more frequent in the patients who had no genetic
392 defect identified.

393

Discussion

We report the largest cohort of CID patients in whom a molecular diagnosis was sought and achieved to date, with a follow-up exceeding 30 years for patients with less severe phenotypes. These data detail the increasingly diverse genetic landscape of CIDs in Iran. None of the variants identified were found in the Exome Aggregation Consortium or Greater Middle East Variome. Furthermore, less than 5% of identified variants within our cohort were previously reported in CID patients either globally or regionally from Turkey, Kuwait and Saudi Arabia, suggesting a lack of founder effects within the currently reported cohorts of CID from Middle East.

Recent genetic diagnostic studies on undefined CID patients have identified a diagnostic yield of 15% in Caucasian patients and up to 56% in a multinational CID cohort (**Table 4**)^{41, 46-52}. The diagnostic yield did not significantly differ between whole exome sequencing and targeted gene panels^{41, 46-52}. A genetic defect was identified in 78% of our cohort, which represents the highest published diagnostic yield to date. We identified late age of onset and the absence of affected family members as two factors associated with a lower yield of identified genetic variants. The majority of patients in our cohort were young (mean age of 4.2 years) and 25.6% had affected family members with clinical diagnosis of CID. Since nearly all CIDs are autosomal recessive diseases, the high percentage of consanguineous marriages (78%) expedites the analysis of the NGS data by increasing the likelihood that the disease-causing variants are homozygous mutations, which constitute the minority of variants in the human exome. These characteristics may have improved the diagnostic yield in our cohort. The yield for molecular diagnosis was highest in DiGeorge syndrome (100% of the 13 sequenced patients). Patients with HIES had the lowest diagnostic yield (~60%), which is likely multifactorial. Mutations in *ZNF341* have been very recently reported to cause HIES⁵³, but this gene is not part of the targeted NGS panel for this study. Neither WES nor the targeted NGS panel will identify intronic mutations in *DOCK8* or *STAT3*, which have been reported to cause HIES. There is no standard in the field for prioritizing NGS as a first-line approach for patients with specific phenotypes, despite the financial limitations in research and clinical arenas. Our data provide the first evidence that the diagnostic yield may be higher for patients with specific phenotypes. A reduced level of mortality and morbidity were

observed compared to the previous decade⁵⁴. In our cohort, patients with syndromic CIDs had a significantly higher mortality rate than those with non-syndromic CIDs. Furthermore, only a minority of patients with syndromic CIDs (36%) died from infectious causes (58 of 159 patients, $p<0.001$). Multi-organ failure, neurological abnormalities, and malignancies accounted for the remainder of these patients' deaths, thus indicating the need for multidisciplinary care, including malignancy screening, for these patients. Extensive efforts are therefore required for improving the knowledge of first-line physicians, the development of a national newborn screening program, establishment of PID therapeutic centers, expansion of the registry of potential HSC donors, and modifications in national vaccination program at least for high-risk families.

The volume of CID patients and breadth of genetic findings in our cohort indicates the critical need for centers dedicated to HSCT of patients with PIDs. Newborn screening using quantification of T-cell receptor excision circles and kappa-deleting recombination excision circles (TRECs/KRECs) are operational in Iran but it remains a pilot program that is not yet integrated into national screening programs. This screening test cannot identify T cell dysfunction or provide the genetic diagnoses essential for therapeutic decisions.

A diagnosis of CID requires prompt intervention since the prognosis for children with SCID is poor due to the susceptibility to opportunistic infections in these patients. Although gene therapy is available at selected medical centers for patients with specific PIDs¹¹, HSCT remains the mainstay of curative treatment in patients with CID. Gene therapy is not available in Iran, and HSCT is available only for a limited number of patients due to limitations in expertise and financial resources. The findings of the current study indicate that the overall survival during the first year of life observed prior to 2008⁵⁴ changed from 0% to more than 60% one year-survival and 20% 3 year-survival in recent years. Although the resources for HSCT in Iran are limited, genetic diagnoses are pivotal for confirming clinical diagnoses and identifying new genetic causes of CID. Additionally, the identification of pathogenic mutations in genes associated with radiosensitivity prompted regular screening for malignancies and minimizing exposure to irradiation. Molecular diagnosis is essential

for genetic counseling, carrier detection, and prenatal diagnosis, all of which are essential in countries with limited resources for HSCT and gene therapy.

In agreement with previous studies, our data indicate that Iranian patients non-syndromic CID are susceptible to BCG and vaccine-derived polio virus infections suggesting a risk of live vaccines in this cohort⁵⁴. While the BCG and oral polio vaccines are routinely given to all Iranian children at birth, we recommend that families with a history of PID, or early childhood death, should undergo evaluation in specialized centers with facilities for PID diagnostics. Moreover, surveillance for poliovirus excretion among CID patients should be reinforced until polio eradication is certified and the use of oral poliovirus vaccine is stopped.

Our results provide proof of principle for the application of targeted next-generation sequencing panels in countries with limited diagnostic resources. This is particularly relevant to efforts driving the expansion of newborn screening for SCID, as the standard approaches for newborn screening do not provide a molecular diagnosis.

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Author contributions

(1) The conception and design of the study

(2) Acquisition of data,

(3) Analysis and interpretation of data,

(4) Drafting the article

(5) Revising it critically for important intellectual content,

(6) Final approval of the version to be submitted

HA (1,2,3,4,6), JC, WB, CP (2,3,5,6) and MT, TM, MTa, MHE, MG, MM, MGh, AAH, GA, RY, MA, AG, AH, ZC SAM, TC ,RA, SA, RF, FJ, RS, MN, MHB, MMa, MMe, DB, IM, JG, AS, NK, SAr, JM, BN, NP and NR (2,3,5) BKA, CP, TCh, MJM, SK and LH, (2,3,5,6) RSG and AA (1,3,4,5,6).

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Table1. Characteristics and molecular diagnosis in a cohort of 696 Iranian patients with combined immunodeficiencies.

Disorders ^a	Number of Patients (%)	Sex (M/F)	Age, years, (±SD)	Consanguinity (%)	Mortality (%)	Patients evaluated for genetic diagnosis (% of total)	Patients with confirmed genetic diagnosis	Diagnostic yield %
Total CID patients	696	408/288	4.2 (3.6)	546 (78.4)	348 (50.0)	243 (34.9)	189	77.8
Non-syndromic CID patients	352 (50.5)	208/144	3.4 (2.9)	291 (82.6)	189 (53.6)	103 (29.2)	84	81.5
Severe non-syndromic CID	169 (24.2)	99/70	0.47 (0.3)	143 (84.6)	133 (78.6)	44 (26.0)	36	81.8
Omenn phenotype	11 (1.5)	4/7	1.2 (0.7)	9 (81.8)	7 (63.6)	1 (9.0)	1	100
Less profound non-syndromic CID	172 (24.7)	105/67	8.8 (7.1)	139 (80.8)	49 (28.4)	58 (33.7)	47	81.0
Hyper IgM phenotype	69 (9.9)	69/0	7.6 (2.0)	41 (59.4)	32 (46.3)	22 (31.8)	18	81.8
Partial T cell defects	103 (14.7)	36/67	13.5 (9.3)	98 (95.1)	17 (16.5)	36 (34.9)	29	80.5
Syndromic CID patients	344 (49.4)	197/144	5.3 (4.0)	255 (74.1)	159 (46.2)	140 (40.6)	105	75.0
DNA repair defects syndrome	193 (27.7)	104/89	7.2 (4.6)	164 (84.9)	110 (56.9)	40 (20.7)	34	85.0
Ataxia telangiectasia syndrome	145 (20.8)	76/69	8.8 (6.4)	121 (83.4)	87 (60.0)	24 (16.5)	21	87.5
Nijmegen breakage syndrome	5 (0.7)	2/3	5.3 (4.2)	5 (100)	4 (80.0)	0	-	-
Bloom syndrome	3 (0.4)	1/2	10.4 (4.0)	3 (100)	3 (100)	0	-	-
Radiosensitive CID syndrome	40 (5.7)	25/15	5.6 (5.1)	35 (87.5)	16 (40.0)	16 (40.0)	13	81.2
Hyper IgE syndrome	101 (14.5)	53/48	7.7 (7.0)	68 (67.3)	29 (28.7)	62 (61.3)	37	59.6
Wiskott-Aldrich syndrome	29 (4.1)	29/0	3.6 (1.5)	11 (37.9)	8 (27.5)	19 (65.5)	15	78.9
Thymic defects syndrome	13 (1.9)	7/6	0.8 (0.2)	8 (61.5)	8 (61.5)	13 (100)	13	100
Other syndromic CID	8 (1.1)	7/1	1.3 (1.1)	4 (50.0)	4 (50.0)	6 (75.0)	6	100

^a For defined clinical criteria please see **Table S1**

CID: combined immunodeficiencies

Table 2. Inheritance pattern of the 23 genes identified in 84 Iranian patients with non-syndromic combined immunodeficiencies (For details see **Table**

S3)

non-syndromic combined immunodeficiencies	Inheritance	No. of genes affected	No. of patients affected	Gene ID (Number of patients)
Severe combined immunodeficiencies and Omenn phenotype	Autosomal recessive/ compound heterozygous	2	4	<i>IL7R(2), RAG2(2)</i>
	Autosomal recessive/ homozygous	10	30	<i>JAK3(3), CD3E(1), CD3D(1), RAG1(13), RAG2(4), DCLRE1C(3), ADA(2), PRKDC(1), NHEJ1(1), PTPRC(1)</i>
	X-linked recessive	1	3	<i>IL2RG(3)</i>
Combined immunodeficiencies with generally less profound immunodeficiency	Autosomal recessive/ compound heterozygous	0	0	-
	Autosomal recessive/ homozygous	9	29	<i>ZAP70(1), RFXANK(2), CIITA(1), STK4(3), MALT1(1), ITK(1), ICOS(2)</i>
	X-linked recessive	1	18	<i>LRBA(15), CD27(3), CD40L(18)</i>

Table 3. Inheritance pattern of the 17 genes identified in 105 Iranian patients with syndromic combined immunodeficiencies (For details see **Table S7**).

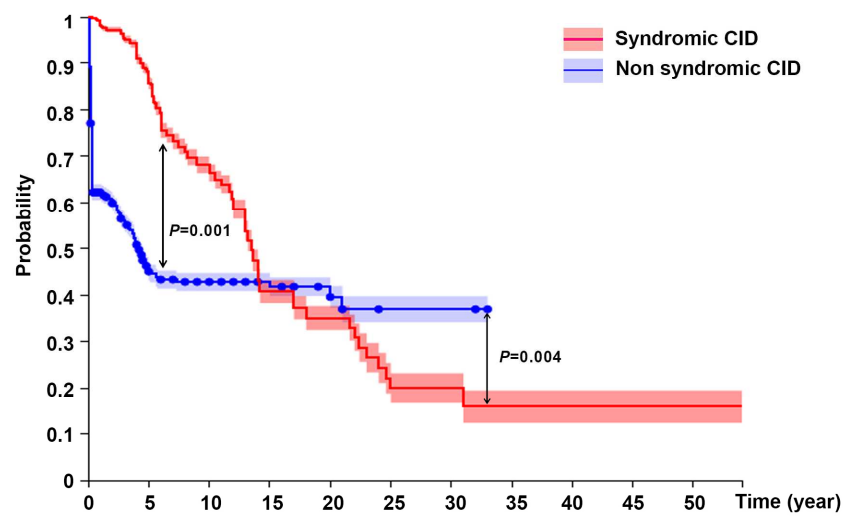
Syndromic combined immunodeficiencies	Inheritance	No. of genes affected	No. of patients affected	Gene ID (Number of patients)
DNA repair defects syndromes	Autosomal recessive/ compound heterozygous	1	8	<i>ATM</i> (8)
	Autosomal recessive/ homozygous	3	26	<i>ATM</i> (13), <i>DNMT3B</i> (8), <i>ZBTB24</i> (5)
Hyper IgE syndrome	X-linked recessive	0	0	-
	Autosomal recessive/ compound heterozygous	0	0	-
	Autosomal recessive/ homozygous	4	18	<i>DOCK8</i> (13), <i>TYK2</i> (3), <i>PGM3</i> (1), <i>SPINK5</i> (1)
	Autosomal dominant/ loss-of-function	1	19	<i>STAT3</i> (19)
Wiskott-Aldrich syndrome	X-linked recessive	0	0	-
	X-linked recessive	1	15	<i>WAS</i> (15)
Thymic defects syndromes	Autosomal dominant/ loss-of-function	1	13	<i>Microdeletion in 22q11.21</i> (13)
Other syndromic combined immunodeficiencies	Autosomal recessive/compound heterozygous	4	4	-
	Autosomal recessive/ homozygous	0	0	<i>EPG5</i> (1), <i>PNP</i> (1), <i>TTC7A</i> (1), <i>SMARCA1</i> (1)
	X-linked recessive	2	2	<i>IKBKG</i> (1), <i>DCK1</i> (1)

653 **Table 4.** Comparison of NGS in combined immunodeficiencies patients' studies.

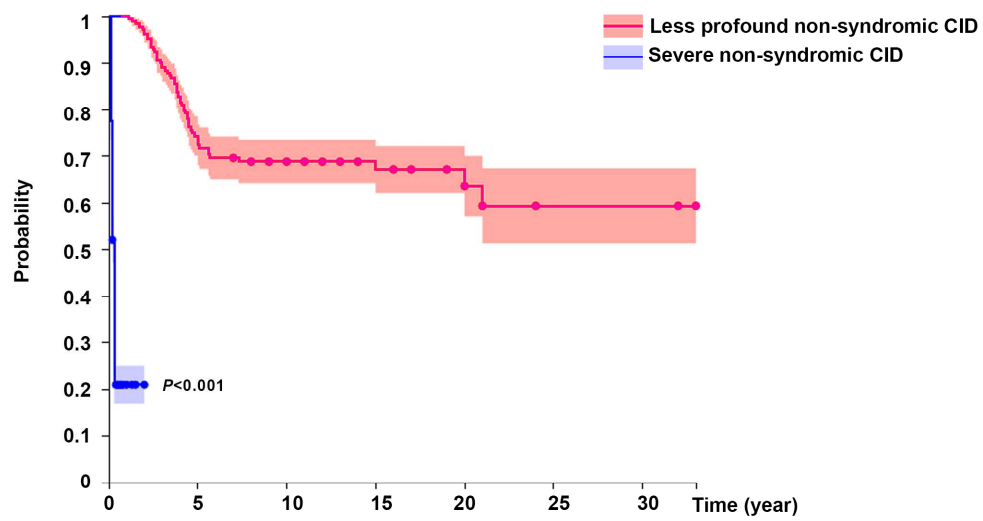
Parameters	Stoddard et al. ⁵²	Nijman et al. ⁵¹	Moens et al. ⁵⁰	Al-Mousa et al. ⁴⁹	Stray-Pedersen et al. ⁴⁶	Yu et al. ⁴¹	Gallo et al. ⁴⁸	Erman et al. ⁴⁷	Current Study
Year	2014	2014	2014	2016	2016	2016	2016	2017	2017
Method	Targeted sequencing	Targeted sequencing	Targeted sequencing	Targeted sequencing	Whole exome sequencing	Targeted sequencing	Targeted sequencing / whole exome sequencing	Targeted sequencing	Targeted sequencing / whole exome sequencing
Computational CNV analysis	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Number of prioritized known PID genes	173	170	179	162	475	200	571	356	200/365
Total undefined PID cases	120	26	15	139	278	30	45	19	243
Consanguinity	Not mentioned	3.8%	20%	90%	6.4%	10%	Not mentioned	68.4%	76%
Ethnicity	Not mentioned (NIH, US)	Caucasian (the Netherlands, Germany)	Caucasian (Poland, Sweden)	Arab (Saudi Arabia)	22 different countries	3 different countries (USA, Norway, Saudi Arabia)	Caucasian (Italy)	Turkish	Persian 212 Turkish 28 Arab 13
Percentage of solved cases	15%	15%	40%	25%	40%	56%	15.5%	33%	77.8%
Total undefined CID cases	Not mentioned	32(32 CID)	Not mentioned	50 non-syndromic 23 syndromic	66 non-syndromic 17 syndromic	30 non-syndromic	Not mentioned	19 non-syndromic	103 non-syndromic 140 syndromic
Solved CID cases	Not mentioned	16 non-syndromic 7 syndromic	Not mentioned	19 non-syndromic 6 syndromic	26 non-syndromic 9 syndromic	17 non-syndromic	2 non-syndromic 1 syndromic	6 non-syndromic	84 non-syndromic 105 syndromic

CID: combined immunodeficiencies; CNV: copy number variation; PID: primary immunodeficiencies.

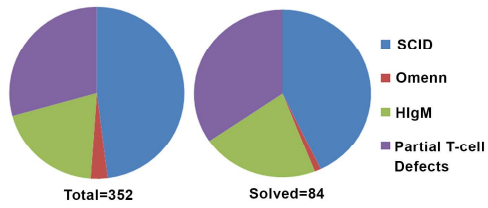
A.



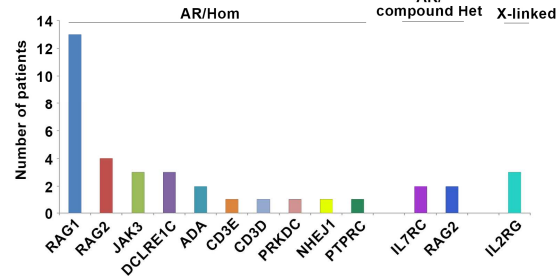
B.



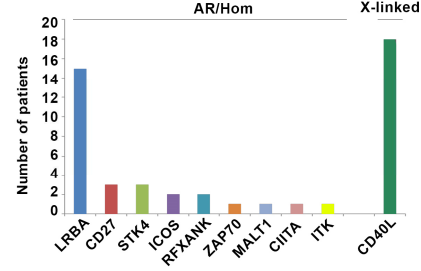
A. Non-syndromic CID



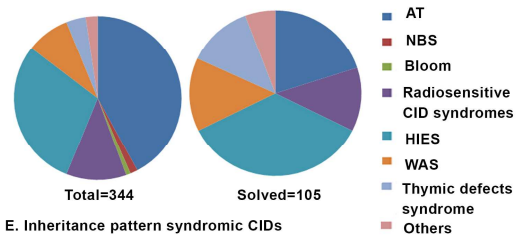
B. Inheritance pattern non-syndromic SCID



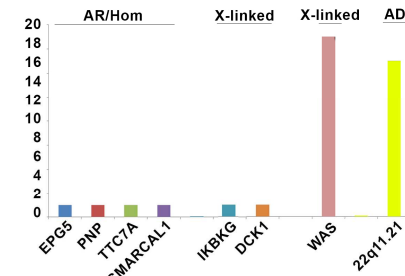
C. Inheritance pattern non-syndromic CIDs



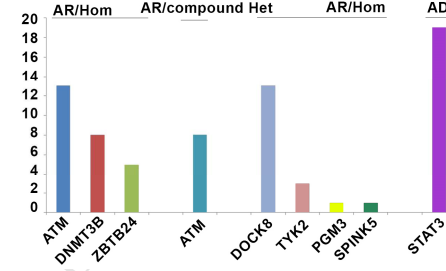
D. Syndromic CID



E. Inheritance pattern syndromic CIDs



F. Inheritance pattern syndromic DNA repair and HIES CIDs



Supplementary data

**Clinical, Immunological and Genetic Spectrum of 696 Patients with Combined
Immunodeficiency in Iran**

Abolhassani et al.

Supplementary figure legends

Figure S1- Survival rate analysis. **A.** Comparison of syndromic and non-syndromic CID patients. **B.**

Comparison of SCID and CID patients in the non-syndromic group.

Figure S2- Summary of genetic study on syndromic and non-syndromic CID patients. **A.** Total and

solved patients' number in non-syndromic CID group. **B.** The inheritance pattern and genes affected

in patients with non-syndromic SCID and **C.** non-syndromic CIDs. **D.** Total and solved patients'

number in syndromic CID group. **E.** The inheritance pattern and genes affected in patients with

syndromic CID and **F.** DNA repair defects and hyper IgE syndromes. AR: autosomal recessive, AD:

autosomal dominant, Hom: homozygous, Het: heterozygous, HIgM: Hyper IgM phenotype, AT:

ataxia telangiectasia, NBS: Nijmegen breakage syndrome, HIES: hyper IgE syndrome, WAS:

Wiskott-Aldrich syndrome.

Table S1- Criteria used for clinical diagnosis of combined immunodeficiency patients.

Diagnosis	Clinical criteria
Combined immunodeficiency (CID)	At least one of: - At least one severe infection (requiring hospitalization) - One manifestation of immune dysregulation (autoimmunity, severe eczema, lymphoproliferation, granuloma) - Malignancy - Affected family member AND 2 of 4 T cell criteria fulfilled: - Reduced CD3 or CD4 or CD8 T cells - Reduced naive CD4 and/or CD8 T cells - Elevated g/d T cells - Reduced proliferation to mitogen or T cell receptor stimulation AND HIV excluded
Severe non-syndromic combined immunodeficiency (SCID)	CID At least one of the following: - Invasive bacterial, viral or fungal/opportunistic infection - Persistent diarrhea and failure to thrive - Affected family member AND manifestation in the first year of life AND Absence or very low number of T cells (CD3 T cells < 300/ul)
Omenn phenotype	Desquamating erythroderma in the first year of life AND one of the following: - Lymphoproliferation - Failure to thrive - Chronic diarrhea - Recurrent pneumonia AND Eosinophilia or elevated IgE AND T-cell deficiency AND Maternal engraftment
Leaky severe non-syndromic combined immunodeficiency	CID AND Normal number of CD3 T cells at the time of diagnosis AND Gradual reduction in number of CD3 T cells AND Maternal engraftment excluded
Less profound non-syndromic combined immunodeficiency HIGM phenotype	CID AND SCID excluded AND Syndromic CID excluded At least one of the following: - Increased susceptibility to infections (recurrent and/or opportunistic, including) - Immune dysregulation (autoimmunity, lymphoproliferation, sclerosing cholangitis) - Cytopenia (neutropenia or autoimmune) - malignancy (lymphoma) - Affected family member AND marked decrease of IgG (measured at least twice) AND normal or elevated IgM (measured at least twice) AND no evidence of profound T-cell deficiency AND no evidence of Ataxia telangiectasia
Partial non-syndromic T cell deficiency	CID AND SCID excluded AND Syndromic CID excluded AND HIGM phenotype excluded
Syndromic CID	CID AND at least one of the following: - Dysmorphic features such as short stature, facial abnormalities, microcephaly, skeletal abnormalities - Other organ manifestations such as albinism, hair or tooth abnormalities, heart or kidney defects, hearing abnormalities, primary neurodevelopmental delay, seizures AND at least one numeric or functional abnormal finding upon immunological investigation

DNA repair defects syndrome	AND exclusion of secondary causes for immunological abnormalities CID AND at least two of the following: - Defined neurological disorders - Facial abnormalities - Functional T cell defect - Radiosensitivity - Malignancy
Ataxia telangiectasia	Ataxia AND at least two of the following: - Oculocutaneous telangiectasia - Elevated alphafetoprotein (tenfold the upper limit of normal) - Lymphocyte A-T caryotype (translocation 7;14) - Cerebellum hypoplasia on MRI
Nijmegen breakage syndrome	Microcephaly AND reduced T cell number and/or elevated percentage of memory CD4 and CD8 cells and/or reduced T cell function AND at least two of the following: - Typical facial appearance - Variable hypogammaglobulinemia, dysgammaglobulinemia and/or reduction of B cells - opportunistic and/or chronic, recurrent infections, predominantly of the respiratory tract - Café-au-lait spots and/or hypopigmented areas and/or skin granulomas - Lymphoma/leukemia or other malignancy - Chromosomal instability increased sensitivity towards ionizing radiation and alkylating agents
Bloom syndrome	Short stature AND - Immunodeficiency (hypogammaglobulinemia, variably reduced lymphocyte proliferation, lower respiratory tract infections) - Cytogenetics: high sister-chromatid exchange rate, chromosomal breaks AND at least one of the following - Skin: photosensitivity, butterfly erythema, café-au-lait maculae - Head: microcephaly, dolichocephaly, prominent ears and nose - Hands: syndactyly, polydactyly, fifth finger clinodactyly - Malignancy: leukemia, lymphoma, adenocarcinoma, squamous cell carcinoma
Hyper IgE syndrome (HIES)	CID AND IgE > 10 times the norm for age AND pathologic susceptibility to infectious diseases
Wiskott-Aldrich Syndrome (WAS)	At least one of the following: - Eczema - Recurrent bacterial or viral infections - Autoimmune diseases (including vasculitis) - Malignancy - Reduced WASP expression in a fresh blood sample - Abnormal antibody response to polysaccharide antigens and/or low isohaemagglutinins - Positive maternal family history of XLT/WAS AND male patient with thrombocytopenia (less than 100,000 platelets/mm³) (measured at least twice) AND small platelets (platelet volume < 7,5 fl)
Thymic defects syndrome	CID AND at least two of the following: - Defined by congenital heart disease - Facial anomalies - Laryngotracheoesophageal anomalies - Hypocalcemia - Growth hormone deficiency - Absent or hypoplastic thymus in chest X-ray

22 *CID: Combined immunodeficiency; XLT: X linked thrombocytopenia, WAS: Wiskott-Aldrich*
23 *syndrome, HIES: Hyper IgE syndrome, HIGM: hyper IgM phenotype, SCID: Severe non-syndromic*
24 *combined immunodeficiency*
25

Table S2. Characteristics of 169 severe combined immunodeficiency patients.

Parameters	Severe combined immunodeficiency					p-value
	Total	T-B+NK+	T-B+NK-	T-B-NK+	T-B-NK-	
Number of patients	169	43	63	55	8	-
Gender, female/male	73/96	25/18	23/40	23/32	2/6	0.36 ^a
Consanguinity, Y/N	143/26	39/4	50/13	49/6	5/3	0.17 ^a
Family history of PID, Y/N	31/138	8/35	16/47	5/50	2/6	0.55 ^a
Family history of recurrent infection, Y/N	47/122	8/35	20/43	17/38	2/6	0.68 ^a
Family history of early-age death, Y/N	76/93	14/29	32/31	28/27	2/6	0.59 ^a
Status, alive/dead	133/36	34/9	48/15	45/10	6/2	0.27 ^a
Current age, years, (±SD)	1.0±0.9	1.0±0.2	0.9±0.2	1.3±0.68	0.9±2.8	0.27 ^b
Age at onset of symptoms, months, (±SD)	4.2±3.0	2.1±0.6	4.5±2.6	4.1±0.7	0.6±0.1	0.35 ^b
Age at diagnosis, months, (±SD)	8.6±5.6	4.2±0.3	6.7±5.4	7.9±2.0	3.4±2.0	0.23 ^b
Diagnostic delay, months, (±SD)	4.2±3.7	1.9±1.0	2.1±0.9	3.1±0.6	2.8±2.0	0.38 ^b
Period of follow-up, months, (±SD)	4.7±3.9	6.8±3.8	3.7±0.5	4.9±1.8	1.5±1.0	0.48 ^b
Course of the disease, months, (±SD)	7.4±5.2	1.8±0.8	4.2±3.0	9.9±5.9	6.0±2.4	0.054 ^b
Hospitalization episodes, (±SD)	2.4±1.7	2.2±0.5	2.4±2.2	2.7±1.4	2.3±0.7	0.063 ^b
Immunoglobulins						
IgG, mg/ml, (±SD)	381.5±79.8	266.1±63.9	402.8±80.4	447.7±151.7	381.5±210.9	0.45 ^b
IgM, mg/ml, (±SD)	102.0±22.9	113.0±100.7	108.2±71.9	88.1±43.0	90.0±70.0	0.87 ^b
IgA, mg/ml, (±SD)	48.9±15.5	52.7±32.8	43.4±20.5	54.0±28.3	36.8±5.7	0.93 ^b
IgE, IU/ml, (±SD)	526.1±387.9	812.8±131.5	433.3±218.1	425.8±51.6	394.8±85.7	0.44 ^b
Complete blood count						
Leukocytes, cell/ml, (±SD)	8933.9±864.4	1044.9±812.8	8548.8±5452.7	8370.1±2618.3	7650.9±895.3	0.31 ^b
Lymphocytes, %, (±SD)	25.6±22.9	30.2±18.0	25.0±7.0	23.2±4.0	21.6±3.2	0.36 ^b
Neutrophils, %, (±SD)	55.9±43.5	52.1±41.3	58.6±6.6	56.0±8.9	55.0±47.1	0.63 ^b
Eosinophil, %, (±SD)	6.4±5.6	7.8±4.8	5.4±3.3	5.7±4.78	11.6±1.3	0.006 ^b
Lymphocyte subtypes						
CD3 ⁺ , % of lymphocytes (±SD)	17.3±12.4	17.2±6.1	17.9±9.2	17.5±7.5	10.1±2.5	0.84 ^b
CD4 ⁺ , % of lymphocytes (±SD)	9.1±6.0	7.3±5.4	9.8±9.7	10.3±4.2	4.9±1.2	0.63 ^b
CD8 ⁺ , % of lymphocytes (±SD)	10.2±7.3	9.4±5.1	12.2±3.2	9.3±2.6	5.1±1.5	0.43 ^b
CD16 ⁺ , % of lymphocytes (±SD)	28.1±26.9	29.5±8.0	25.6±2.6	31.4±7.5 ^c	17.6±1.7 ^c	0.009 ^b
CD19 ⁺ , % of lymphocytes (±SD)	26.6±21.3	28.4±10.9	35.6±2.7 ^d	16.9±2.8 ^d	13.5±2.0	0.008 ^b
CD56 ⁺ , % of lymphocytes (±SD)	8.7±6.0	8.5±8.1	8.6±5.3	9.0±7.4	8.8±2.0	0.92 ^b

^a Represents the p-value of difference between the all four groups of SCID patients, as revealed by Chi-square analysis

^b Represents the p-value of difference between the all four groups of SCID patients, as revealed by ANOVA analysis

^c Indicated the significant difference in CD16 between T-B⁺NK⁺ and T-B⁺NK⁻ patients, as revealed by Scheffe's Post hoc test.

^d Indicates the significant difference in CD19 between T-B⁺NK⁻ and T-B⁺NK⁺ patients, as revealed by Scheffe's Post hoc test.

Age-matched reference levels based on the age of diagnosis: IgM, 55-210 mg/dL; IgG, 700-1600 mg/dL; IgA, 19-220 IU/mL; IgE, <100 IU/mL; Leukocytes, 6,000-11,000 cell/μL; Neutrophils, 1,500-8,500 cell/μL; Lymphocyte, 4,000-10,000 cell/μL; CD3⁺, 51-74%; CD4⁺, 35-53%; CD8⁺, 13-27%; CD16/CD56, 4-15%; CD19⁺, 17-37%.

39 **Table S3.** Deleterious changes detected in 84 Iranian patients with non-syndromic combined
 40 immunodeficiencies

Patients ID	Age	Sex	Immunologic phenotype	Gene	Deleterious variants	Zygosity	Inheritance	New/Reported
Severe combined immunodeficiencies and Omenn phenotype								
P1	6d	M	T-B+NK- SCID	<i>IL2RG</i>	p.S225R	Hemizygous	X-linked	¹
P2	4m	M	T-B+NK- SCID	<i>IL2RG</i>	p.E284X	Hemizygous	X-linked	New patient
P3	5m	M	T-B+NK- SCID	<i>IL2RG</i>	p.Q61VfsX79	Hemizygous	X-linked	New patient
P4	14m	M	Leaky SCID	<i>JAK3</i>	p.V722I	Homozygous	AR	²
P5	7m	F	Omenn phenotype	<i>JAK3</i>	p.R775H	Homozygous	AR	New patient
P6	6m	F	T-B+NK+SCID	<i>JAK3</i>	p.W709R	Homozygous	AR	New patient
P7	1.5y	M	T-B- NK+SCID	<i>IL7R</i>	p.T66I	Compound heterozygous	AR	³
P8	6m	F	T-B- NK+SCID	<i>IL7R</i>	p. I138V p. T56A p. C57W	Compound heterozygous	AR	³
P9	5m	M	T-B+NK+ SCID	<i>CD3E</i>	p. E70X	Homozygous	AR	New patient
P10	7m	M	T-B+NK+ SCID	<i>CD3D</i>	p.E83X	Homozygous	AR	New patient
P11	10m	F	T-B- NK+SCID	<i>RAG1</i>	p.W896X	Homozygous	AR	³
P12	1.2y	F	T-B- NK+SCID	<i>RAG1</i>	p. W204R	Homozygous	AR	³
P13	7m	M	T-B- NK+SCID	<i>RAG1</i>	p.M324V	Homozygous	AR	³
P14	4m	F	T-B- NK+SCID	<i>RAG1</i>	p.T731I	Homozygous	AR	³
P15	9m	F	T-B- NK+SCID	<i>RAG1</i>	p.M1006V	Homozygous	AR	³
P16	14y	M	Leaky SCID	<i>RAG1</i>	p.C358Y	Homozygous	AR	⁴
P17	10y	M	T-B- NK+SCID	<i>RAG1</i>	p.N855S	Homozygous	AR	New patient
P18	1.5y	F	T-B- NK+SCID	<i>RAG1</i>	p.R397W	Homozygous	AR	New patient
P19	7m	F	T-B- NK+SCID	<i>RAG1</i>	p.R397W	Homozygous	AR	New patient
P20	8m	M	Leaky SCID	<i>RAG1</i>	p.A857D	Homozygous	AR	New patient
P21	9m	F	Leaky SCID	<i>RAG1</i>	p.W995X	Homozygous	AR	New patient
P22	1y	M	Leaky SCID	<i>RAG1</i>	p.V469L	Homozygous	AR	New patient
P23	7m	F	T-B-NK+ SCID	<i>RAG1</i>	p.W995X	Homozygous	AR	New patient
P24	3m	M	T-B- NK+SCID	<i>RAG2</i>	p.R229W	Homozygous	AR	³
P25	6m	F	T-B- NK+SCID	<i>RAG2</i>	p.R229W	Homozygous	AR	³
P26	6m	M	T-B-NK+ SCID	<i>RAG2</i>	p.S194X	Compound heterozygous	AR	⁵
P27	7m	F	T-B-NK+ SCID	<i>RAG2</i>	p.T1784G p.S194X p.T1784G	Compound heterozygous	AR	⁵
P28	6m	M	T-B-NK+ SCID	<i>RAG2</i>	p.R229W	Homozygous	AR	New patient
P29	5m	F	T-B-NK+ SCID	<i>RAG2</i>	p.G44R	Homozygous	AR	New patient
P30	10y	M	Leaky SCID	<i>DCLRE1C</i>	p.G14V	Homozygous	AR	⁶
P31	8m	M	T-B-NK+ SCID	<i>DCLRE1C</i>	p.E388X	Homozygous	AR	New patient
P32	11y	M	Leaky SCID	<i>DCLRE1C</i>	p.S417YfsX459	Homozygous	AR	New patient
P33	4m	M	T-B-NK+ SCID	<i>ADA</i>	p.E139X	Homozygous	AR	New patient
P34	3m	M	T-B-NK+ SCID	<i>ADA</i>	p.R235Q	Homozygous	AR	New patient
P35	1y	M	Leaky SCID	<i>PRKDC</i>	p.F1244LfsX2855	Homozygous	AR	New patient
P36	2y	F	Leaky SCID	<i>NHEJ1</i>	p.R176X	Homozygous	AR	New patient
P37	3m	F	T-B+NK+	<i>PTPRC</i>	p.S834R	Homozygous	AR	⁷
Combined immunodeficiencies with generally less profound immunodeficiency								
P38	18y	M	Hyper IgM profile	<i>CD40LG</i>	Ivs1+2T>C	Hemizygous	X-linked	^{8,9}
P39	8y	M	Hyper IgM profile	<i>CD40LG</i>	p.F63L	Hemizygous	X-linked	New patient
P40	7y	M	Hyper IgM profile	<i>CD40LG</i>	p.G219R	Hemizygous	X-linked	New patient
P41	8y	M	Hyper IgM profile	<i>CD40LG</i>	p.T29fsX36	Hemizygous	X-linked	^{8,9}

P42	9y	M	Hyper IgM profile	CD40LG	p.G167R	Hemizygous	X-linked	8,9
P43	4y	M	Hyper IgM profile	CD40LG	p.D62fsX79	Hemizygous	X-linked	8,9
P44	6y	M	Hyper IgM profile	CD40LG	p.L161P	Hemizygous	X-linked	8,9
P45	4y	M	Hyper IgM profile	CD40LG	p.G167R	Hemizygous	X-linked	8,9
P46	6y	M	Hyper IgM profile	CD40LG	p.M36T	Hemizygous	X-linked	New patient
P47	5y	M	Hyper IgM profile	CD40LG	p.Q186X	Hemizygous	X-linked	New patient
P48	2y	M	Hyper IgM profile	CD40LG	p.Q186X	Hemizygous	X-linked	New patient
P49	2y	M	Hyper IgM profile	CD40LG	p.Q186X	Hemizygous	X-linked	New patient
P50	1.5y	M	Hyper IgM profile	CD40LG	p.G252D	Hemizygous	X-linked	9,10
P51	9y	M	Hyper IgM profile	CD40LG	p.G167R	Hemizygous	X-linked	New patient
P52	7y	M	Hyper IgM profile	CD40LG	p.Q186X	Hemizygous	X-linked	New patient
P53	1y	M	Hyper IgM profile	CD40LG	p.L81X	Hemizygous	X-linked	New patient
P54	3y	M	Hyper IgM profile	CD40LG	p.G144V	Hemizygous	X-linked	New patient
P55	5y	M	Hyper IgM profile	CD40LG	p.I171T	Hemizygous	X-linked	7
P56	3y	M	CD8 deficiency	ZAP70	p.D521N	Homozygous	AR	11
P57	7y	M	MHC class II deficiency	RFXANK	p.R189X	Homozygous	AR	New patient
P58	4y	M	MHC class II deficiency	RFXANK	c.438+5G>A	Homozygous	AR	7
P59	2y	F	MHC class II deficiency	CIITA	p.N1082del	Homozygous	AR	New patient
P60	2y	F	CD4 and CD19 deficiency, neutropenia	STK4	p.W250X	Homozygous	AR	12
P61	10y	M	CD4 and CD19 deficiency, neutropenia	STK4	p.W250X	Homozygous	AR	12
P62	4y	F	CD4 and CD19 deficiency, neutropenia	STK4	p.W250X	Homozygous	AR	12
P63	5m	F	Early onset CID	MALT1	p.N485S	Homozygous	AR	New patient
P64	14y	F	Late onset CID	ITK	p.L157fsX265	Homozygous	AR	13
P65	35y	F	Late onset CID	ICOS	p.V151L	Homozygous	AR	New patient
P66	28y	M	Late onset CID	ICOS	p.V151L	Homozygous	AR	New patient
P67	14y	F	Late onset CID	LRBA	c.4729+2dupT	Homozygous	AR	14
P68	14y	M	Late onset CID	LRBA	p.E59X	Homozygous	AR	14
P69	10y	M	Late onset CID	LRBA	p.E59X	Homozygous	AR	14
P70	15y	F	Late onset CID	LRBA	c.1014+1G>A	Homozygous	AR	14
P71	17y	M	Late onset CID	LRBA	p.R182X	Homozygous	AR	14
P72	22y	F	Late onset CID	LRBA	c.4729+2dupT	Homozygous	AR	14
P73	13y	F	Late onset CID	LRBA	p.I1875SfsX1889	Homozygous	AR	New patient
P74	13y	M	Late onset CID	LRBA	Exon 41 del	Homozygous	AR	6
P75	2y	F	Late onset CID	LRBA	Exon 41 del	Homozygous	AR	14
P76	22y	F	Late onset CID	LRBA	p.S1605X	Homozygous	AR	New patient
P77	35y	M	Late onset CID	LRBA	p.S1605X	Homozygous	AR	14
P78	28y	M	Late onset CID	LRBA	p.S1605X	Homozygous	AR	14
P79	1y	F	Late onset CID	LRBA	Exon1 del	Homozygous	AR	14
P80	2y	M	Late onset CID	LRBA	p.D248EfsX250	Homozygous	AR	15
P81	6y	F	Late onset CID	LRBA	p.S462LfsX469	Homozygous	AR	New patient
P82	38y	M	Late onset CID	CD27	p.C96Y	Homozygous	AR	16
P83	20y	F	Late onset CID	CD27	p.C96Y	Homozygous	AR	16
P84	21y	F	Late onset CID	CD27	p.R78W	Homozygous	AR	16

41 M: male, F: female, SCID: Severe combined immunodeficiencies, CID: Combined immunodeficiencies, AR:

42 autosomal recessive

Table S4. Characteristics of non-immunologic clinical manifestation patients with syndromic combined immunodeficiencies.

Involved non-immunologic system	Manifestations	Number of affected patients (%)
Neurologic abnormalities	Ataxia, microcephalus, hydrocephalus, intellectual disability, mental retardation, or cognitive impairment	205 (59.5)
Dermatologic abnormalities	Telangiectasia, ectodermal dysplasia, sparse hair, hyperkeratosis, congenital ichthyosis, atopic diathesis, café-au-lait spots, nail dystrophy, and hypo/hyperpigmented lesions	187 (54.3)
Facial dysmorphic features	Bird-like facies, broad or flat nasal bridge, hypertelorism, low-set ears, macroglossia, and cleft lip	107 (31.0)
Musculoskeletal disorders	Hyperextensible joints, osteoporosis, pathologic bone fractures, scoliosis, retention of primary teeth, and short stature	61 (17.7)
Malignancies	Hodgkin's and Non-Hodgkin Lymphoma, leukemias, Multiple myeloma, Breast cancer, Gastric adenocarcinoma	23 (6.7)
Cardiac malformation	Tetralogy Fallot, conotruncal cardiac defects, peculiar anatomical, deficiency of the infundibular septum and malalignment of the aortic arch and pulmonary arteries.	15 (4.3)
Endocrine congenital disorders	Hypocalcemia, hypoparathyroidism, growth hormone deficiency, congenital abnormalities of the thyroid, hypogonadism	11 (3.0)
Gastrointestinal malformation	Intestinal atresias	10 (2.9)

Table S5. Characteristics of 145 patients with ataxia telangiectasia.

Clinical and demographic parameters	Results	Immunologic parameters	Results
Number of patients	145	Immunoglobulins	
Gender, female/male	76/69	IgG, mg/dl ($\pm$ SD)	772.5 $\pm$ 568.1
Current age, years, ($\pm$SD)	8.8 $\pm$ 6.4	IgM, mg/dl ($\pm$ SD)	296.9 $\pm$ 238.1
Age at onset of symptoms, months, ($\pm$SD)	1.1 $\pm$ 0.7	IgA, mg/dl ($\pm$ SD)	53.6 $\pm$ 43.8
Age at diagnosis, years, ($\pm$SD)	6.7 $\pm$ 5.5	IgE, mg/dl ($\pm$ SD)	10.4 $\pm$ 6.7
Diagnostic delay, years, ($\pm$SD)	4.5 $\pm$ 4.2	IgG1, mg/dl ($\pm$ SD)	684.6 $\pm$ 378.0
Recurrent infections, Y/N	121/24	IgG2, mg/dl ($\pm$ SD)	117.3 $\pm$ 109.1
Respiratory tract infections, Y/N	113/32	IgG3, mg/dl ($\pm$ SD)	47.3 $\pm$ 41.9
Upper respiratory tract infection, Y/N	55/90	IgG4, mg/dl ($\pm$ SD)	8.8 $\pm$ 7.8
Pneumonia, Y/N	66/79	Complete blood count and lymphocyte subtypes	
Infectious diarrhea, Y/N	27/118	Leukocytes, cell/ μ L ($\pm$ SD)	7310 $\pm$ 3779
Mucocutaneous infection, Y/N	48/97	Lymphocyte, cell/ μ L ($\pm$ SD)	2254 $\pm$ 2017
Lymphoproliferation, Y/N	17/128	Neutrophils, cell/ μ L ($\pm$ SD)	4389 $\pm$ 2494
Autoimmunity, Y/N	21/124	CD3 ⁺ , % of lymphocytes ($\pm$ SD)	44.7 $\pm$ 16.3
Allergy, Y/N	20/125	CD4 ⁺ , % of lymphocytes ($\pm$ SD)	21.5 $\pm$ 10.0
Enteropathy, Y/N	18/127	CD8 ⁺ , % of lymphocytes ($\pm$ SD)	24.1 $\pm$ 11.1
Malignancy, Y/N	3/143	CD19 ⁺ , % of lymphocytes ($\pm$ SD)	11.7 $\pm$ 8.4
		Serum AFP level, ng/dL ($\pm$SD)	267.6 $\pm$ 232.1

AFP: alpha fetoprotein.

Age-matched reference levels based on the age of diagnosis: IgM, 40-230 mg/dL; IgE, <100 IU/mL ; IgA, 41-297 mg/dL; IgG, 700-1600 mg/dL; IgG1, 250-1000 mg/dL; IgG2, 55-435 mg/dL; IgG3, 10-100 mg/dL; IgG4, 1-100 mg/dL; Leukocytes, 4,000-11,000 cell/ μ L; Neutrophils, 1,500-7,000 cell/ μ L; Lymphocyte, 1,500-6,000 cell/ μ L; CD3⁺, 30-78%; CD4⁺, 22-58%; CD8⁺, 10-37%; CD19⁺, 8-14%; AFP level <10 ng/dL.

Table S6. Characteristics of 101 patients with Hyper IgE syndrome

Parameters	Total (n=101)	STAT3 deficiency	DOCK8 deficiency	p-value
Number of patients	101	19	13	-
Gender, female/male	46/55	10/9	7/6	1.0
Current age, years, ($\pm$ SD)	14.1 $\pm$ 9.5	9.5 $\pm$ 7.4	15.7 $\pm$ 10.1	0.08
Age at onset of symptoms, months, ($\pm$ SD)	21.4 $\pm$ 6.8	37.4 $\pm$ 11.0	18.7 $\pm$ 9.6	0.002 ^a
Age at diagnosis, years, ($\pm$ SD)	9.5 $\pm$ 7.8	9.7 $\pm$ 8.1	3.4 $\pm$ 2.0	0.001 ^a
Diagnostic delay, years, ($\pm$ SD)	7.72 $\pm$ 4.2	8.1 $\pm$ 7.8	1.5 $\pm$ 0.9	0.003 ^a
Period of follow-up, years, ($\pm$ SD)	4.71 $\pm$ 3.3	6.0 $\pm$ 5.4	4.0 $\pm$ 3.5	0.004 ^a
Recurrent upper respiratory infections, Y/N	79/22	15/4	6/7	0.07
Recurrent pneumonia, Y/N	70/31	19/0	5/8	<0.001 ^a
Pneumatocele, Y/N	25/76	13/6	0/13	<0.001 ^a
Bronchiectasis, Y/N	16/85	3/16	1/12	0.62
Eczema, Y/N	94/7	19/0	13/0	0.59
Recurrent skin abscess, Y/N	78/23	19/0	13/0	1.0
Candidiasis, Y/N	46/55	7/12	6/7	0.72
Other unusual infection, Y/N	57/44	8/10	12/1	0.008 ^a
Lymphoma, Y/N	2/99	0/19	0/13	1.0
Eosinophilia, Y/N	65/36	13/6	8/5	0.72
NIH Score ($\pm$ SD)	47.1 $\pm$ 17.3	60.7 $\pm$ 13.7	47.2 $\pm$ 21.6	<0.001 ^a
SCORAD ($\pm$ SD)	68.4 $\pm$ 15.4	78.0 $\pm$ 61.7	60.1 $\pm$ 29.3	0.08
Immunoglobulins				
IgG, mg/ml, ($\pm$ SD)	1392.6 $\pm$ 770.0	1726.8 $\pm$ 763.8	1120.6 $\pm$ 509.2	0.007 ^a
IgM, mg/ml, ($\pm$ SD)	140.7 $\pm$ 118.7	149.6 $\pm$ 75.3	110.3 $\pm$ 49.0	0.06
IgA, mg/ml, ($\pm$ SD)	201.5 $\pm$ 49.8	142.7 $\pm$ 94.8	121.4 $\pm$ 43.2	0.97
IgE, IU/ml, ($\pm$ SD)	9045.0 $\pm$ 1260.9	10247.2 $\pm$ 1562.0	30500.0 $\pm$ 11420.2	<0.001 ^a

^a Significant difference between STAT3 deficiency and DOCK8 deficiency patients <0.05.

SCORAD: Scoring atopic dermatitis, NIH: National Institutes of Health.

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Age-matched reference levels based on the age of diagnosis: 3-5 years—IgM, 40-230 mg/dL; IgG,

700-1600 mg/

dL; IgA, 48-345 mg/dL; 8-10 years—IgM, 40-230 mg/dL; IgG, 700-1600 mg/dL; IgE, <100 IU/mL.

62 **Table S7-** Deleterious changes detected in 105 Iranian patients with syndromic combined
 63 immunodeficiencies.

Patients ID	Age	Sex	Clinical Presentation	Gene	Deleterious variants	Zygoty	inheritance	New/Reported
DNA repair defects syndromes								
P85	7y	M	Ataxia telangiectasia syndrome	ATM	p.S2761LfsX2805 p.I2628fsX2630	Compound heterozygous	AR	17
P86	8y	F	Ataxia telangiectasia syndrome	ATM	p.S2761LfsX2805 p.I2628fsX2630	Compound heterozygous	AR	17
P87	4y	M	Ataxia telangiectasia syndrome	ATM	p.S2761LfsX2805 p.I2628fsX2630	Compound heterozygous	AR	17
P88	6y	F	Ataxia telangiectasia syndrome	ATM	p.K2756T p.I2628fsX2630	Compound heterozygous	AR	17
P89	10y	F	Ataxia telangiectasia syndrome	ATM	p.E2778DfsX2805 p.H2552PfsX2563	Compound heterozygous	AR	17
P90	7y	M	Ataxia telangiectasia syndrome	ATM	p.R2792TfsX2795	Homozygous	AR	17
P91	8y	M	Ataxia telangiectasia syndrome	ATM	p.I2628fsX2630	Homozygous	AR	17
P92	5y	F	Ataxia telangiectasia syndrome	ATM	p.I2628fsX2630	Homozygous	AR	17
P93	8y	M	Ataxia telangiectasia syndrome	ATM	p.I2628fsX2630	Homozygous	AR	17
P94	7y	F	Ataxia telangiectasia syndrome	ATM	p.I2628fsX2630 p.H2552PfsX2563	Compound heterozygous	AR	17
P95	4y	M	Ataxia telangiectasia syndrome	ATM	p.I2628fsX2630 p.T2556fsX2563	Compound heterozygous	AR	17
P96	9y	F	Ataxia telangiectasia syndrome	ATM	p.I2628fsX2630	Homozygous	AR	17
P97	7y	F	Ataxia telangiectasia syndrome	ATM	p.I2628fsX2630	Homozygous	AR	17
P98	7y	F	Ataxia telangiectasia syndrome	ATM	p.I2628fsX2630	Homozygous	AR	17
P99	10y	F	Ataxia telangiectasia syndrome	ATM	p.L1851fsX1856	Homozygous	AR	6
P100	8y	F	Ataxia telangiectasia syndrome	ATM	p.A2622V	Homozygous	AR	18
P101	4y	M	Ataxia telangiectasia syndrome	ATM	p.E1622X	Homozygous	AR	New patient
P102	7y	F	Ataxia telangiectasia syndrome	ATM	p.E277X	Homozygous	AR	New patient
P103	6y	F	Ataxia telangiectasia syndrome	ATM	p.Y2969X c.2639-1G>A	Compound heterozygous	AR	New patient
P104	4y	M	Ataxia telangiectasia syndrome	ATM	p.A1299PfsX1348	Homozygous	AR	New patient
P105	5y	F	Ataxia telangiectasia syndrome	ATM	p.R1875X	Homozygous	AR	7
P106	10y	M	Atypical ICF syndrome	DNMT3B	p.D722E	Homozygous	AR	New patient
P107	8y	F	Atypical ICF syndrome	DNMT3B	p.G810C	Homozygous	AR	New patient
P108	22y	F	Atypical ICF syndrome	DNMT3B	p.D722E	Homozygous	AR	New patient
P109	3y	F	Atypical ICF syndrome	DNMT3B	p.Y624C	Homozygous	AR	New

P110	21y	F	Atypical ICF syndrome	<i>DNMT3B</i>	p.D722E	Homozygous	AR	patient
P111	13y	M	Atypical ICF syndrome	<i>DNMT3B</i>	p.D722E	Homozygous	AR	New patient
P112	7y	F	Atypical ICF syndrome	<i>DNMT3B</i>	p.D722E	Homozygous	AR	New patient
P113	6y	M	Atypical ICF syndrome	<i>DNMT3B</i>	c.2397-11 G>A	Homozygous	AR	New patient
P114	33y	F	Atypical ICF syndrome	<i>ZBTB24</i>	p.C408E	Homozygous	AR	New patient
P115	7y	F	Atypical ICF syndrome	<i>ZBTB24</i>	p.D266RfsX28	Homozygous	AR	New patient
P116	17y	M	Atypical ICF syndrome	<i>ZBTB24</i>	p.C383S	Homozygous	AR	New patient
P117	41y	M	Atypical ICF syndrome	<i>ZBTB24</i>	p.C383S	Homozygous	AR	New patient
P118	39y	F	Atypical ICF syndrome	<i>ZBTB24</i>	p.C383S	Homozygous	AR	New patient
Hyper IgE syndrome								
P119	28y	M	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382Q	Heterozygous	AD	New patient
P120	21y	M	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382Q	Heterozygous	AD	New patient
P121	24y	F	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R18fsX34	Heterozygous	AD	New patient
P122	30y	M	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382W	Heterozygous	AD	New patient
P123	21y	M	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382Q	Heterozygous	AD	New patient
P124	19y	M	AD-HIES (Job syndrome)	<i>STAT3</i>	p.V637M	Heterozygous	AD	New patient
P125	26y	F	AD-HIES (Job syndrome)	<i>STAT3</i>	p.N466T	Heterozygous	AD	New patient
P126	27y	M	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382Q	Heterozygous	AD	New patient
P127	33y	F	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382Q	Heterozygous	AD	New patient
P128	13y	F	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382W	Heterozygous	AD	New patient
P129	30y	F	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382W	Heterozygous	AD	New patient
P130	7y	F	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382W	Heterozygous	AD	New patient
P131	12y	F	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382W	Heterozygous	AD	New patient
P132	5y	F	AD-HIES (Job syndrome)	<i>STAT3</i>	p.T657X	Heterozygous	AD	New patient
P133	23y	F	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382W	Heterozygous	AD	New patient
P134	45y	M	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382W	Heterozygous	AD	New patient
P135	9y	M	AD-HIES (Job syndrome)	<i>STAT3</i>	p.T657X	Heterozygous	AD	New patient
P136	5y	M	AD-HIES (Job syndrome)	<i>STAT3</i>	p.R382W	Heterozygous	AD	New patient
P137	2y	F	AD-HIES (Job syndrome)	<i>STAT3</i>	p.F493LfsX508	Heterozygous	AD	New patient
P138	7y	F	AR-HIES syndrome	<i>DOCK8</i>	p.S1711X	Homozygous	AR	New

P139	23y	F	AR-HIES syndrome	<i>DOCK8</i>	Large deletion, exon 1–48	Homozygous	AR	patient 19, 20
P140	19y	F	AR-HIES syndrome	<i>DOCK8</i>	Large deletion, exon 1–44	Homozygous	AR	19, 20
P141	8y	M	AR-HIES syndrome	<i>DOCK8</i>	Large deletion, exon 11–14	Homozygous	AR	19, 20
P142	8y	M	AR-HIES syndrome	<i>DOCK8</i>	Large deletion, exon 11–13	Homozygous	AR	19, 20
P143	4y	M	AR-HIES syndrome	<i>DOCK8</i>	Large deletion, exon 25–26	Homozygous	AR	19, 20
P144	1.5y	M	AR-HIES syndrome	<i>DOCK8</i>	Large deletion, exon 25–26	Homozygous	AR	19, 20
P145	17y	F	AR-HIES syndrome	<i>DOCK8</i>	Large deletion, exon 2	Homozygous	AR	20
P146	7y	F	AR-HIES syndrome	<i>DOCK8</i>	Large deletion, exon 2	Homozygous	AR	20
P147	2y	F	AR-HIES syndrome	<i>DOCK8</i>	p.R1370PfsX1395	Homozygous	AR	New patient
P148	6y	M	AR-HIES syndrome	<i>DOCK8</i>	p.S1711X	Homozygous	AR	New patient
P149	8y	F	AR-HIES syndrome	<i>DOCK8</i>	Large deletion, exon 1 and 25–26	Homozygous	AR	New patient
P150	9y	M	AR-HIES syndrome	<i>DOCK8</i>	Large deletion, exon 1 and 27–28	Homozygous	AR	New patient
P151	5y	M	AR-HIES syndrome	<i>TYK2</i>	p. E154X	Homozygous	AR	21
P152	3y	F	AR-HIES syndrome	<i>TYK2</i>	p. E154X	Homozygous	AR	21
P153	9y	F	AR-HIES syndrome	<i>TYK2</i>	p.S50HfsX1	Homozygous	AR	21
P154	8y	F	Leaky CID syndrome	<i>PGM3</i>	p.A548T	Homozygous	AR	New patient
P155	2y	F	Comel-Netherton syndrome	<i>SPINK5</i>	p.N755MfsX782	Homozygous	AR	7
Wiskott-Aldrich syndrome								
P156	0.5y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.R13X	Hemizygous	X-linked	22
P157	1y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.R41X	Hemizygous	X-linked	22
P158	3y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.G8X	Hemizygous	X-linked	22
P159	2y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.G70R	Hemizygous	X-linked	22
P160	1y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.A56V	Hemizygous	X-linked	22
P161	1y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.A56V	Hemizygous	X-linked	22
P162	0.6y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.N127K	Hemizygous	X-linked	22
P163	1y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.D283E	Hemizygous	X-linked	22
P164	8y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.P412fsX446	Hemizygous	X-linked	22
P165	1y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.G358fsX494	Hemizygous	X-linked	22
P166	4y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.E464X	Hemizygous	X-linked	22
P167	0.7y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.N335X	Hemizygous	X-linked	23
P168	7y	M	Wiskott-Aldrich syndrome	<i>WAS</i>	c.777+1 G>A	Hemizygous	X-linked	New patient
P169	9m	M	Wiskott-Aldrich syndrome	<i>WAS</i>	c.777+1 G>C	Hemizygous	X-linked	New patient
P170	18m	M	Wiskott-Aldrich syndrome	<i>WAS</i>	p.K230fsX262	Hemizygous	X-	New

							linked	patient
Thymic defects syndromes								
P171	1.5y	M	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	²⁴
P172	0.3y	F	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
P173	1y	F	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
P174	1y	F	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
P175	0.5y	F	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
P176	1y	M	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
P177	3y	M	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
P178	0.1y	F	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
P179	1.3y	M	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
P180	0.4y	M	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
P181	2y	F	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
P182	2y	M	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
P183	0.2y	M	DiGeorge syndrome	<i>TBX1</i>	microdeletion in 22q11.21	Heterozygous	AD	New patient
Other syndromic combined immunodeficiencies								
P184	3.5y	M	Vici syndrome	<i>EPG5</i>	p.R2483X	Homozygous	AR	²⁵
P185	10y	F	Leaky CID syndrome	<i>PNP</i>	p.C70R	Homozygous	AR	¹⁴
P186	8y	M	Leaky CID syndrome	<i>TTC7A</i>	p.A55V	Homozygous	AR	¹⁴
P187	4y	M	Schimke 15immune-osseous dysplasia	<i>SMARCAL1</i>	p.R561H	Homozygous	AR	²⁶
P188	10y	M	Anhidrotic ectodermal dysplasia syndrome	<i>IKBKG</i>	p.D311G	Hemizygous	X-linked	New patient
P189	15y	M	Leaky CID syndrome	<i>DCK1</i>	p.S280R	Hemizygous	X-linked	New patient

64 M: male, F: female, CID: Combined immunodeficiencies, AR: autosomal recessive, AD: autosomal
 65 dominant, ICF: Immunodeficiency, Centromeric region instability, Facial anomalies syndrome, HIES:
 66 hyper IgE syndrome.

67 **Table S8-** Stratification of genetic diagnosis based on age and sex on combined immunodeficiency

68 patients

Gender (N)	Genetic study (N)	Age of patients			
		<6m (N)	6m-5y (N)	5y-15y (N)	>15y (N)
Male (408)	Not performed (261)	77	143	26	15
	Not solved (33)	11	18	3	1
	Solved (114)	<i>IL2RG</i> (3), <i>CD3E</i> (1), <i>RAG2</i> (1), <i>ADA</i> (2), <i>TBX1</i> (2)	<i>JAK3</i> (1), <i>IL7R</i> (1), <i>CD3D</i> (1), <i>RAG1</i> (3), <i>RAG2</i> (2), <i>DLRE1C</i> (1), <i>PRKDC</i> (1), <i>CD40LG</i> (8), <i>ZAP70</i> (1), <i>RFXANK</i> (1), <i>STK4</i> (1), <i>LRBA</i> (3), <i>ATM</i> (4), <i>DOCK8</i> (2), <i>WAS</i> (15), <i>TBX1</i> (5), <i>EPG5</i> (1), <i>SMARCA1</i> (1)	<i>DCLRE1C</i> (2), <i>RAG1</i> (2), <i>CD40LG</i> (9), <i>RFXANK</i> (1), <i>LRBA</i> (7), <i>ATM</i> (4), <i>DNMT3B</i> (3), <i>STAT3</i> (2), <i>DOCK8</i> (4), <i>TYK2</i> (1), <i>TTC7A</i> (1), <i>IKBKG</i> (1), <i>DCK1</i> (1)	<i>CD40LG</i> (1), <i>ICOS</i> (1), <i>LRBA</i> (3), <i>CD27</i> (1), <i>ZBTB24</i> (2), <i>STAT3</i> (7)
Female (288)	Not performed (192)	56	90	19	27
	Not solved (21)	6	12	3	0
	Solved (75)	<i>RAG1</i> (1), <i>RAG2</i> (1), <i>PTPRC</i> (1), <i>MALT1</i> (1), <i>TBX1</i> (2)	<i>JAK3</i> (2), <i>IL7R</i> (1), <i>RAG1</i> (7), <i>RAG2</i> (2), <i>NHEJ1</i> (1), <i>CIITA</i> (1), <i>STK4</i> (1), <i>ITK</i> (1), <i>DNMT3B</i> (1), <i>STAT3</i> (1), <i>DOCK8</i> (1), <i>TYK2</i> (1), <i>SPINK5</i> (1), <i>TBX1</i> (4)	<i>STK4</i> (1), <i>ATM</i> (13), <i>DNMT3B</i> (2), <i>ZBTB24</i> (1), <i>STAT3</i> (4), <i>DOCK8</i> (3), <i>TYK2</i> (1), <i>PGM3</i> (1), <i>PNP</i> (1)	<i>ICOS</i> (1), <i>LRBA</i> (2), <i>CD27</i> (2), <i>DNMT3B</i> (2), <i>ZBTB24</i> (2), <i>STAT3</i> (5), <i>DOCK8</i> (3)

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