Clinical, immunologic, and genetic spectrum of 696 patients with combined immunodeficiency

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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 patients with CIDs in Iran clinically and genetically.

Methods: Clinical and laboratory data were obtained from 696 patients with CIDs. Patients were subdivided into those with

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syndromic (344 patients) and nonsyndromic (352 patients) CIDs. Targeted DNA sequencing was performed on 243 (34.9%) patients.

Results: The overall diagnostic yield of the 243 sequenced patients was 77.8% (189 patients). The clinical diagnosis of hyper-IgE syndrome ($P < .001$), onset of disease at greater than 5 years ($P = .02$), and absence of multiple affected family members ($P = .04$) 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 ataxia-telangiectasia mutated *(ATM)*, autosomal dominant mutations in signal transducer and activator of transcription 3 *(STAT3)*, and microdeletions in *22q11.21* were the most commonly affected genomic loci. Patients with syndromic CIDs had a significantly lower 5-year survival rate rather than those with nonsyndromic CIDs.

Conclusions: This study provides proof of principle for the application of targeted next-generation sequencing panels in countries with limited diagnostic resources. The effect of genetic diagnosis on clinical care requires continued improvements in therapeutic resources for these patients. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Combined immunodeficiencies, next-generation DNA sequencing, whole-exome sequencing, targeted gene panel sequencing*

Combined immunodeficiencies (CIDs) are characterized by defective development or function of T cells. As the most severe form of primary immunodeficiencies (PIDs), CIDs are characterized by a susceptibility to infection, particularly from opportunistic organisms, which leads to severe morbidity and mortality.[1,2] A subpopulation of patients also has syndromic features caused by the function of the affected gene in nonimmune cells.[3] The reported incidence of CIDs is 1:100,000 to 1:5,000 live births worldwide[4-8]; however, this is considered an underestimation of the actual incidence because of the mortality of patients before diagnosis, misdiagnoses in patients with atypical clinical manifestations, and incomplete national registries documenting CID incidence.[9] The diagnostic delay between the age of disease onset and diagnosis has been reported to range from a few days to several years in patients with CIDs.[10]

A genetic diagnosis provides the rationale for initiating the only curative interventions for CIDs, hematopoietic stem cell transplantation (HSCT) and gene therapy, both of which can incur a high risk of morbidity and mortality.[11] Early diagnosis enables initiation of curative therapies at a young age, which significantly increases the survival rate.[12-14] The diagnosis of CID is challenging because of the wide variability in clinical phenotypes and the limited availability of clinical laboratory tests for characterizing defects in immune systems.[3] Neonatal screening for severe combined immunodeficiency (SCID) through quantification of T-cell receptor excision circles has expedited the early diagnosis of patients with SCID by identifying defective T-cell generation.[15] 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, and whole-genome sequencing have facilitated the identification of genetic defects.[16] 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 accepted.[17]

Abbreviations used

AT:	Ataxia telangiectasia
BCG:	Bacillus Calmette–Guérin
BCGosis:	Disseminated BCG infection
CID:	Combined immunodeficiency
HIES:	Hyper-IgE syndrome
HIGM:	Hyper-IgM phenotype
HSCT:	Hematopoietic stem cell transplantation
NGS:	Next-generation DNA sequencing
PID:	Primary immunodeficiency
SCID:	Severe combined immunodeficiency
STAT:	Signal transducer and activator of transcription
WAS:	Wiskott-Aldrich syndrome

CIDs constitute a group of clinically and genetically heterogeneous disorders, necessitating a comprehensive molecular approach for a definitive diagnosis.[18] The proportion of specific gene defects varies among countries because of differences in rates of consanguinity and effects of founder mutations. DiGeorge syndrome is the most commonly reported CID in Western countries but accounts for less than 30% of CIDs in the Middle East.[19] 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 China.[20-25] In the European Society for Immunodeficiencies online database, causative mutations were identified in 36.2% of patients with PIDs.[26] The overall rate of genetic diagnosis was less than 33.7% among 77,193 PID patients reported from Jeffrey Modell centers worldwide in 2014.[19] Despite the fact that nearly 300 genes are known to cause PIDs,[3] these data demonstrate that many patients with PIDs lack a genetic diagnosis.

Previously, we reported demographic and phenotypic data from patients enrolled in the Iranian national registry.[27] With the increasing availability of NGS technologies, we now present genetic findings from 696 Iranian patients with CIDs. This is the largest cohort of genetically defined patients with CIDs from a single country. Although consanguineous populations in the Middle East are commonly thought to have a high incidence of autosomal recessive CIDs, we show that autosomal dominant mutations in signal transducer and activator of transcription 3 *(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 of targeted NGS panels in countries with limited diagnostic resources.

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, their parents, or both.

This study was conducted as a retrospective study of patients enrolled in the Iranian national registry for PIDs[27] from the "National PID Network," which comprises 25 medical centers in Iran. The Iranian national registry for PIDs was established in 1997 and is managed by the Research Centre for Immunodeficiencies. The registry currently includes approximately 2500

patients with clinically diagnosed PIDs (<2% of expected patients according to the probable prevalence), and only 35% of these patients have received a genetic diagnosis.[28] A questionnaire surveyed the patients' demographic information, age of disease onset, age of diagnosis, family history, detailed clinical history that included vaccine history and associated adverse reactions, recurrent infections, physical examination findings, laboratory testing, and treatment history.[27] Diagnostic laboratory data obtained included complete and differential blood counts, serum immunoglobulin levels, immunophenotyping of peripheral blood lymphocytes, α-fetoprotein measurement, and assays of T-cell function, which include proliferative assays (using phytohaemagglutinin [PHA], Bacillus Calmette–Guérin [BCG], and *Candida* species), the tuberculosis skin test, and the radiosensitivity test.[27,29-32] The delay in diagnosis is defined as the period of time between the age of disease onset and the age at diagnosis. Patients were given a diagnosis of CID based on the standard criteria introduced by the European Society for Immunodeficiencies and Pan-American Group for Immunodeficiency (http://esid.org/Working-Parties/Registry/Diagnosis-criteria, see Table E1 in this article's Online Repository at www.jacionline.org).

The collected data from patients were reviewed by expert clinical immunologists in the Children's Medical Center Hospital in Iran, the largest referral center for PIDs 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 PID Committee's updated classification.[3] According to this classification, patients with isolated immune-related manifestations were located in nonsyndromic CIDs (including SCID, Omenn phenotype, hyper-IgM phenotype [HIGM], and partial T-cell defects) and the remaining had additional nonimmune complications classified as syndromic CIDs (including hyper-IgE syndrome [HIES], Wiskott-Aldrich syndrome [WAS], DiGeorge syndrome, DNA repair defect syndromes, dyskeratosis congenital, ectodermal dysplasia, and 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.

Genetic analysis

Genomic DNA was extracted from whole blood, as previously described.[35] For patients with classical clinical presentations suggestive of a specific CID, Sanger sequencing was performed on the most likely genes.[36-39] Patients with thymic defects (see Table E1), were examined by using fluorescent *in situ* hybridization (FISH) for *22q11.2* deletion and a comparative genomic hybridization array.[40] For patients in whom Sanger sequencing failed or who had a clinical presentation resembling several genetic defects, targeted NGS was performed with the PID v2 panel and Ion Torrent S5 sequencer (Thermo Fisher, Waltham, Mass), with an average coverage of 335×. Variant calling and coverage analysis was performed with Ion Reporter software.[41] The detection of large deletions was performed by using normalized mean coverage of individual exons.[42] In patients with less profound CIDs (see Table E1), whole-exome sequencing was performed by using a pipeline described previously, with an average on-target coverage of 50×.[43]

Statistical analysis

Statistical analysis was performed with a commercially available software package (SPSS Statistics 17.0.0; SPSS, Chicago, Ill). The 1-sample Kolmogorov-Smirnov test was applied to estimate whether data distribution is normal. Parametric and nonparametric analyses were performed based on the findings of this evaluation. Kaplan-Meier curve and log-rank tests were used to compare different survival estimates. A P value of .05 or less was considered statistically significant.

RESULTS

Patient population

A cohort of 696 patients with CIDs (408 male and 288 female patients from 624 unrelated families) was evaluated. The diagnosis of CID was made by using established clinical criteria developed by the European Society of Immunodeficiency (see Table E1). Three hundred eighty-seven (55.7%) patients were given a diagnosis from 2013 to 2016, and 309 (44.3%) were given a diagnosis before 2013.[27] Patients were followed for a total of 3841 patient years, with a median follow-up of 3 years per patient (range, 0.1-29 years). The median age at onset was 1.5 years (range, 1 day to 31 years); 93% of patients had disease onset before 13 years of age. The median age at diagnosis was 3.6 years (range, 2 weeks to 45 years); 41.8% of patients received a diagnosis before 1 year of age. The median delay in diagnosis was 2.4 years (range, 0-24 years). Parental consanguinity was present in 474 (75.9%) of the 624 families, which was substantially greater than the 40% prevalence of consanguineous marriage in Iran.[44] A positive family history of stillbirth or unknown death was identified in 331 (52.9%) unrelated families. A positive history of premature birth was reported in 14.9% (n = 104) of patients, which is 38% higher than overall premature birth rate in Iran.[45] The overall mortality rate among our cohort of patients with CIDs was 50%.

We categorized the patients as nonsyndromic (n = 352) and syndromic (n = 344) by using criteria established by the International Union of Immunologic Societies (Table I).[3] The 5-year survival rate of patients with syndromic CIDs was significantly higher than that of patients with nonsyndromic CIDs (approximately 75% vs 40%, P = .001). Although the survival rate of patients with syndromic CIDs progressively diminished to 15% (P = .004; see Fig E1, *A*, in this article's Online Repository at www.jacionline.org), the 15-year survival rate of patients with nonsyndromic CIDs plateaued at approximately 40%. Among nonsyndromic patients, those with SCID had a significantly greater mortality rate than those with less profound CIDs during the first 2 years of follow-up (P < .001; see Fig E1, *B*). Infections and infectious complications accounted for almost all causes of death in patients with nonsyndromic CIDs (168/189 deceased patients); only 36% of patients with syndromic CIDs died from infectious causes (58/159 patients, P < .001), whereas the remainder of the patients died from multiorgan failure, neurologic abnormalities, and malignancies.

Patients with nonsyndromic CIDs

The most common clinical presentations of patients with nonsyndromic CIDs were recurrent pneumonia (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 (11.3%) patients dermatologic lesions, such as severe generalized eczema, skin infections, and abscesses, were the earliest clinical presentations. Disseminated BCG infection (BCGosis) accounted for the primary presentation in 23 (6.5%) patients, whereas BCGosis was documented in 34 (9.6%) cases after 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%), urinary tract infections in 59 (16.7%), and cutaneous infections in 51 (14.4%) patients. Fifty-six percent (197) of the patients had multisite infections requiring intensive medical treatment. Vaccine-derived poliovirus was isolated in 6 patients with acute flaccid paralysis. Five of the patients had fulminant

TABLE I. Characteristics and molecular diagnosis in a cohort of 696 Iranian patients with CIDs

Disorders*	No. of patients (%)	Sex (M/F)	Age (y), ± SD	Consanguinity (%)	Mortality (%)	Patients evaluated for genetic diagnosis (% of total)	Patients with confirmed genetic diagnosis	Diagnostic yield (%)
Total patients with CID	696	408/288	4.2 (3.6)	546 (78.4)	348 (50.0)	243 (34.9)	189	77.8
Patients with nonsyndromic CIDs	352 (50.5)	208/144	3.4 (2.9)	291 (82.6)	189 (53.6)	103 (29.2)	84	81.5
Severe nonsyndromic CIDs	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 nonsyndromic CID	172 (24.7)	105/67	8.8 (7.1)	139 (80.8)	49 (28.4)	58 (33.7)	47	81.0
HIGM	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
Patients with syndromic CIDs	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
AT 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
HIES	101 (14.5)	53/48	7.7 (7.0)	68 (67.3)	29 (28.7)	62 (61.3)	37	59.6
WAS	29 (4.1)	29/0	3.6 (1.5)	11 (37.9)	8 (27.5)	19 (65.5)	15	78.9
Thymic defect 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

F, Female; *M*, male.
*For defined clinical criteria, please see Table E1.

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 in 15 patients, *Pneumocystis jirovecii* in 13 patients, and varicella-zoster virus in 6 patients. Severe EBV-associated lymphoproliferative disorders were present in 8 patients, and *Cryptosporidium* species infection was present in 6 patients.

A clinical diagnosis of SCID was identified in 169 patients, of whom 63 (37.2%) had a $T^-B^+NK^-$, 55 (32.4%) had a $T^-B^-NK^+$, 43 (25.4%) had a $T^-B^+NK^+$, and 8 (4.7%) had a $T^-B^-NK^-$ immunologic phenotype (see Table E1). Details of the clinical and immunologic manifestations of patients with SCID are listed in Table E2 in this article's Online Repository at www.jacionline.org. The Omenn phenotype was diagnosed in 11 (1.5%) patients (Table I). The remaining 172 patients were classified as having CIDs (less profound nonsyndromic CIDs). Of these, 69 (19.6%) had an HIGM phenotype associated with T-cell defects, 41 (11.6%) had CD4 deficiency, 19 (5.3%) had CD8 deficiency, and 43 (12.2%) had late-onset functional T-cell defects (with normal T-cell counts but defective proliferative assays).

The percentage (5.7% ± 4.0% vs 35.5% ± 24.3%, $P = .01$) and absolute numbers (85.9 ± 40.0 vs 1149.5 ± 540.7 cells/μL, $P < .001$) of T cells were significantly lower in patients with SCID compared with those in patients with CIDs, as was the age of onset (2.3 ± 2.0 vs 18.5 ± 14.3 months, $P < .001$), age of diagnosis (0.4 ± 0.2 vs 3.5 ± 2.9 years, $P < .001$), and follow-up period (0.5 ± 0.4 vs 5.8 ± 4.5 years, $P < .001$). The hospitalization rate was significantly higher in patients with SCID compared with that in patients with CIDs (242.4 ± 170.3 vs 32.4 ± 25.8 d/y, $P < .001$).

With available resources, molecular diagnosis was performed in 103 patients with nonsyndromic CIDs and identified the causative mutation in 84 (81.5%) patients. A molecular diagnosis was achieved in 37 (82.2%) of 45 patients with SCID or Omenn 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 II and see Fig E2 and Table E3 in this article's Online Repository at www.jacionline.org). Thus autosomal recessive forms of SCID accounted for 91.8% of patients with an identified molecular diagnosis, whereas 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 patients with nonsyndromic CIDs 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-cell defects (see Table E1). The diagnostic yield in the 22 patients with HIGM was 81.8%, with all 18 patients having mutations affecting *CD40L* in male subjects. The diagnostic yield in the 36 patients with CIDs 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 II and see Table E3).

Patients with syndromic CIDs

In the 344 patients with syndromic CIDs, the most frequent nonimmunologic manifestations were neurologic. Two hundred five (59.5%) of the patients had ataxia, microcephalus, hydrocephalus, intellectual disability, mental retardation, or cognitive impairment. The second most frequent nonimmunologic manifestations were dermatologic abnormalities, which

TABLE II. Inheritance pattern of the 23 genes identified in 84 Iranian patients with nonsyndromic CIDs (for details, see Table E3)

Nonsyndromic CIDs	Inheritance	No. of genes affected	No. of patients affected	Gene ID (no. of patients)
SCIDs and Omenn phenotype	Autosomal recessive/compound heterozygous	2	4	*IL7R* (2), *RAG2* (2)
	Autosomal recessive/homozygous	10	30	*JAK3* (3), *CD3E* (1), *CD3D* (1), *RAG1* (13), *RAG2* (4), *DCLRE1C* (3), *ADA* (2), *PRKDC* (1), *NHEJ1* (1), *PTPRC* (1)
	X-linked recessive	1	3	*IL2RG* (3)
CIDs with generally less profound immunodeficiency	Autosomal recessive/compound heterozygous	0	0	—
	Autosomal recessive/homozygous	9	29	*ZAP70* (1), *RFXANK* (2), *CIITA* (1), *STK4* (3), *MALT1* (1), *ITK* (1), *ICOS* (2), *LRBA* (15), *CD27* (3)
	X-linked recessive	1	18	*CD40L* (18)

were present in 187 (54.3%) of the patients and included telangiectasia, ectodermal dysplasia, sparse hair, hyperkeratosis, congenital ichthyosis, atopic diathesis, café-au-lait spots, nail dystrophy, and hypopigmented/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 patients with syndromic CIDs. Additional syndromic features included musculoskeletal abnormalities, malignancies, cardiac malformation, endocrine defects, and intestinal atresia (see Table E4 in this article's Online Repository at www.jacionline.org).

Based on clinical and immunologic criteria (see Table E1), the diagnosis of a DNA repair defect syndrome was made in 193 (56.1%) patients as follows: 145 with ataxia telangiectasia (AT), 5 with Nijmegen breakage syndrome, 3 with Bloom syndrome, and 40 with an unspecific syndromic CID characterized by radiosensitivity. There were 101 (29.3%) patients with the clinical diagnosis of HIES; of these, 68 patients had consanguineous parents, suggesting an autosomal recessive model of HIES. Twenty-nine patients were given a diagnosis of WAS, and 13 patients were given a diagnosis of DiGeorge syndrome. Eight patients were categorized as having other syndromic immunodeficiencies that included dyskeratosis congenita, ectodermal dysplasia, and other atypical and incomplete syndromic CIDs (Table I). Summaries of the 2 main categories of patients in this group (AT and HIES) are shown in Tables E5 and E6 in this article's Online Repository at www.jacionline.org. Patients with thymic defects (with clinical or imaging evidence of absent or hypoplastic thymus) had the highest mortality rate (61.5%, mainly caused by congenital heart disease) of all main categories of syndromic CIDs, with all patients harboring the *22q11.21* microdeletion determined by using cytogenetic studies.

A molecular defect was identified in 105 (75%) of the 140 patients with syndromic CIDs in whom NGS was performed. A molecular diagnosis was achieved in 21 (86.3%) of 24 patients with AT and 13 (81.2%) of 16 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 III and see Fig E2 and Table E7 in this article's Online Repository at www.jacionline.org). All 13 patients with DiGeorge syndrome studied had a microdeletion encompassing *TBX1*. Of the 62 patients with HIES, disease-causing variants were identified in 37 (59.6%) patients. They included 19 (51.3%) patients with autosomal dominant HIES and 18 (49.7%) patients with autosomal recessive HIES 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 patients with WAS studied had a hemizygous mutation in the X-linked *WAS* gene. One patient with pathogenic mutations in each of the *EPG5*, *PNP, TTC7A, IKBKG, DCK1*, and *SMARCAL1* genes was also identified (Table III and see Table E7).

Overall diagnostic yield

Genetic sequencing was performed on 243 (34.9%) of the 696 patients. The overall diagnostic yield of the 243 sequenced patients was 77.8% (189 patients). Table E8 in this article's Online Repository at www.jacionline.org stratifies 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 comprised 20.1% of the genetic diagnoses, and 17% of patients had 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*, *DLCRE1C*, and *PRKDC*) accounted for 16.7% of the total disease-causing etiologies, whereas defects in DNA repair (*ATM*, *DNMT3B*, and *ZBTB24*) accounted for 19% of genetic defects. Defects in costimulatory molecules (eg, CD40 ligand, inducible costimulator, and 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 patients with HIES (37/62 [59.6%] tested). 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

TABLE III. Inheritance pattern of the 17 genes identified in 105 Iranian patients with syndromic CIDs (for details, see Table E7)

Syndromic CIDs	Inheritance	No. of genes affected	No. of patients affected	Gene ID (no. of patients)
DNA repair defects syndromes	Autosomal recessive/compound heterozygous	1	8	*ATM* (8)
	Autosomal recessive/homozygous	3	26	*ATM* (13), *DNMT3B* (8), *ZBTB24* (5)
	X-linked recessive	0	0	—
HIES	Autosomal recessive/compound heterozygous	0	0	—
	Autosomal recessive/homozygous	4	18	*DOCK8* (13), *TYK2* (3), *PGM3* (1), *SPINK5* (1)
	Autosomal dominant/loss of function	1	19	*STAT3* (19)
	X-linked recessive	0	0	—
WAS	X-linked recessive	1	15	*WAS(15)*
Thymic defects syndromes	Autosomal dominant/loss of function	1	13	Microdeletion in *22q11.21* (13)
Other syndromic CIDs	Autosomal recessive/compound heterozygous	4	4	—
	Autosomal recessive/homozygous	0	0	*EPG5* (1), *PNP* (1), *TTC7A* (1), *SMARCAL1* (1)
	X-linked recessive	2	2	*IKBKG* (1), *DCK1* (1)

diagnosis of HIES ($P < .001$), a late age of presentation (onset of disease >5 y, $P = .02$), and absence of multiple affected family members ($P = .04$) were significantly more frequent in the patients who had no genetic defects identified.

DISCUSSION

We report the largest cohort of patients with CIDs 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 patients with CIDs either globally or regionally from Turkey, Kuwait, and Saudi Arabia, suggesting a lack of founder effects within the currently reported CID cohorts from the Middle East.

Recent genetic diagnostic studies on patients with undefined CIDs have identified a diagnostic yield of 15% in white patients and up to 56% in a multinational CID cohort (Table IV).[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 absence of affected family members as 2 factors associated with a lower yield of identified genetic variants. The majority of patients in our cohort were young (mean age, 4.2 years), and 25.6% had affected family members with a clinical diagnosis of CID. Because nearly all CIDs are autosomal recessive diseases, the high percentage of consanguineous marriages (78%) expedites NGS data analysis by increasing the likelihood that the disease-causing variants are homozygous mutations that constitute the minority of variants in the human exome. These characteristics might have improved the diagnostic yield in our cohort. The yield for molecular diagnosis was highest in patients with DiGeorge syndrome (100% of the 13 sequenced patients). Patients with HIES had the lowest diagnostic yield (approximately 60%), which is likely multifactorial. Mutations in *ZNF341* have been reported very recently to cause HIES,[53] but this gene is not part of the targeted NGS panel for this study. Neither whole-exome sequencing 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 might be higher for patients with specific phenotypes.

Reduced levels of mortality and morbidity were observed compared with the previous decade.[54] In our cohort patients with syndromic CIDs had a significantly higher mortality rate than those with nonsyndromic CIDs. Furthermore, only a minority of patients with syndromic CIDs (36%) died from infectious causes (58/159 patients, $P < .001$). Multiorgan failure, neurologic abnormalities, and malignancies accounted for the remainder of these patients' deaths, thus indicating the need for multidisciplinary care, including malignancy screening, for these patients. Therefore extensive efforts are required for improving the knowledge of first-line physicians, 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 patients with CIDs and the breadth of genetic findings in our cohort indicate 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 are operational in Iran, but this 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 a CID requires prompt intervention because 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 PIDs,[11] HSCT remains the mainstay of curative treatment in patients with CIDs. Gene therapy is not available in Iran, and HSCT is available only for a limited number of patients because

TABLE IV. Comparison of NGS in studies of patients with CIDs

Parameters	Stoddard et al[46]	Nijman et al[47]	Moens et al[48]	Al-Mousa et al[49]	Stray-Pedersen et al[50]	Yu et al[41]	Gallo et al[51]	Erman et al[52]	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
No. 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 (National Institutes of Health, United States)	White (The Netherlands, Germany)	White (Poland, Sweden)	Arab (Saudi Arabia)	Twenty-two different countries	Three different countries (United States, Norway, Saudi Arabia)	White (Italy)	Turkish	Persian, 212 Turkish, 28 Arab, 13
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 nonsyndromic, 23 syndromic	66 nonsyndromic, 17 syndromic	30 nonsyndromic	Not mentioned	19 nonsyndromic	103 nonsyndromic, 140 syndromic
Solved CID cases	Not mentioned	16 nonsyndromic, 7 syndromic	Not mentioned	19 nonsyndromic, 6 syndromic	26 nonsyndromic, 9 syndromic	17 nonsyndromic	2 nonsyndromic, 1 syndromic	6 nonsyndromic	84 nonsyndromic, 105 syndromic

CNV, Copy number variation.

of limitations in expertise and financial resources. The findings of the current study indicate that overall survival during the first year of life observed before 2008[54] changed from 0% to more than 60% 1-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 CIDs. Additionally, 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 with nonsyndromic CIDs are susceptible to BCG and vaccine-derived poliovirus infections, suggesting a risk of live vaccines in this cohort.[54] Although the BCG and oral polio vaccines are routinely given to all Iranian children at birth, we recommend that families with a history of PIDs or early childhood death should undergo evaluation in specialized centers with facilities for PID diagnostics. Moreover, surveillance for poliovirus excretion among patients with CIDs 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 because the standard approaches for newborn screening do not provide a molecular diagnosis.

We respectfully dedicate this work to our patients and their families.

Key messages

- **The overall rate of molecular diagnosis was more than 78%, ranging from 60% for patients with HIES to 100% for patients with DiGeorge syndrome.**
- **Patients with syndromic CIDs had a significantly lower 5-year survival rate rather than those with nonsyndromic CIDs, indicating the continued need for improved therapeutic interventions in these patients.**
- **Our results provide proof of principle for the application of targeted next-generation sequencing panels in countries with limited diagnostic resources.**

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