Original Article

Comparison of Common Monogenic Defects in a Large Predominantly Antibody Deficiency Cohort

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What is already known about this topic? In recent years, next-generation sequencing has clarified the genetic basis for a number of primary immunodeficiency disorders. The elucidation of the genetic basis of B-cell differentiation and identification of the genes responsible for an increasing number of predominantly antibody deficiencies (PADs) have improved our understanding of the pathogenesis and prognosis of these disorders with implication for the clinical management of these patients.

What does this article add to our knowledge? In the present study, we compared demographic, clinical, laboratory data, and outcome of the most common monogenic PADs selected from a large cohort of Iranian patients with PAD. This report highlights the similarities and differences in the clinical and immunologic spectrum of patients with monogenic PAD versus other reported cohorts.

How does this study impact current management guidelines? We evaluated clinically and genetically patients with PAD who were clinically categorized as suffering from agamaglobulinemia, common variable immunodeficiency, and hyper-IgM syndrome. Although these patients with PAD were primarily diagnosed as suffering from agamaglobulinemia, common variable immunodeficiency—like phenotype, and hyper-IgM syndrome, there were different features in each disease based on different underlying genetic defects. We were able to demonstrate the different clinical manifestations and immunological findings according to the identified genetic defects. The comprehensive comparisons of the present study are helpful for clinical decision making, resulting in a more accurate diagnosis and more effective treatment of patients with PAD-associated genetic defects.

BACKGROUND: Predominantly antibody deficiencies (PADs) are the most common primary immunodeficiencies, characterized by hypogammaglobulinemia and inability to generate effective antibody responses.

OBJECTIVE: We intended to report most common monogenic PADs and to investigate how patients with PAD who were primarily diagnosed as suffering from agammaglobulinemia, hyper-IgM (HIgM) syndrome, and common variable immunodeficiency (CVID) have different clinical and immunological findings.

METHODS: Stepwise next-generation sequencing and Sanger sequencing were performed for confirmation of the mutations in the patients clinically diagnosed as suffering from agammaglobulinemia, HIgM syndrome, and CVID.

RESULTS: Among 550 registered patients, the predominant genetic defects associated with agammaglobulinemia (48 Bruton's tyrosine kinase [BTK] and 6 μ heavy chain deficiencies), HIgM syndrome (21 CD40 ligand and 7 activation-induced cytidine deaminase deficiencies), and CVID (17 lipopolysaccharides-responsive beige-like anchor deficiency

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Abbreviations used
    ACMG- American College of Medical Genetics and Genomics
AID/AICDA- Activation-induced cytidine deaminase
    BTK- Bruton's tyrosine kinase
    CD40L- CD40 ligand
    CVID- Common variable immunodeficiency
DNMT3B- DNA (cytosine-5)-methyltransferase 3B
    HIgM- Hyper-IgM
    ICF- Immunodeficiency, Centromeric instability, and
        Facial dysmorphism
    LRBA- Lipopolysaccharides-responsive beige-like anchor
    OPV- Oral polio vaccination
    PAD- Predominantly antibody deficiency
    PID- Primary immunodeficiency
    RTI- Respiratory tract infection
    XLA- X-linked agammaglobulinemia
ZBTB24- Zinc-finger and BTB domain containing 24

and 12 atypical Immunodeficiency, Centromeric instability, and Facial dysmorphism syndromes) were identified. Clinical disease severity was significantly higher in patients with μ heavy chain and *CD40 ligand* mutations compared with patients with *BTK* ($P = .003$) and *activation-induced cytidine deaminase* ($P = .009$) mutations. Paralysis following live polio vaccination was considerably higher in patients with μ heavy chain deficiency compared with BTK deficiency ($P < .001$). We found a genotype-phenotype correlation among patients with *BTK* mutations regarding clinical manifestation of meningitis and chronic diarrhea. Surprisingly, we noticed that first presentations in most patients with Immunodeficiency, Centromeric instability, and Facial dysmorphism were respiratory complications ($P = .008$), whereas first presentations in patients with lipopolysaccharides-responsive beige-like anchor deficiency were nonrespiratory complications ($P = .008$).
CONCLUSIONS: This study highlights similarities and differences in the clinical and genetic spectrum of the most common PAD-associated gene defects. This comprehensive comparison will facilitate clinical decision making, and improve prognosis and targeted treatment. © 2018 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;7:864-78)

INTRODUCTION

Predominant antibody deficiencies (PADs) are the most prevalent forms of primary immunodeficiencies (PIDs), characterized by an inability to produce effective humoral immune responses.[1] Hypogammaglobulinemia is the major hallmark of patients with PADs, and their main manifestation is recurrent bacterial infections, predominantly occurring in the respiratory and gastrointestinal tracts.[2] The mainstay of treatment for PADs is immunoglobulin replacement.[3]

Clinically, PADs often arise as a result of defects in early B-cell development, class switch recombination, or terminal B-cell differentiation resulting in agammaglobulinemia, hyper-IgM (HIgM) syndrome, or common variable immunodeficiency (CVID), respectively.[4,5] Most patients with agammaglobulinemia have mutations in Bruton tyrosine kinase (*BTK*) located on the X chromosome, whereas a small group with autosomal-recessive inheritance have mutations in μ heavy chain, Igα (*CD79A*), Igβ (*CD79B*), λ5 (*IGGL1*), B-cell linker protein, the subunits of phosphoinositide 3-kinase (*phosphatidylinositol 3-kinase regulatory, phosphatidylinositol-3-kinase delta*, and *phosphatase and tensin homolog*), and the transcription factor E47 (*transcription factor 3*).[6,7] Defects in genes involved in class switch recombination, including CD40 ligand (*CD40L*), *CD40*, activation-induced cytidine deaminase (*AICDA*), *INO80*, MutS protein homolog 5, and uracil N glycosylase result in HIgM disorder.[8,9] Mutations in genes involved in late B-cell development, including the CD19-B-cell receptor complex (*CD19*, *CD21*, and *CD81*), tumor necrosis factor receptor superfamily members (*transmembrane activator and CAML interactor, B-cell—activating factor receptor, CCA-adding transfer RNA nucleotidyl transferase 1*, and potentially *tumor necrosis factor—like weak inducer of apoptosis*), *inducible T-cell costimulator*, lipopolysaccharides-responsive beige-like anchor (*LRBA*), *nuclear factor kappa-chain-activated B-cell members 1 and 2, mannosyl-oligosaccharide glucosidase, interferon regulatory factor 2 binding protein 2, IKAROS family zinc finger 1*, and *CD20*, result in CVID disorder.[10]

In recent years, the elucidation of the genetic basis of B-cell differentiation has improved our understanding of the pathogenesis, prognosis, and clinical management of these patients.[5,11] In the present study, we intended to report the most common monogenic PADs and to investigate how patients with PAD who were primarily diagnosed as suffering from agamaglobulinemia, HIgM syndrome, and CVID have different clinical manifestations and immunological findings. Moreover, we evaluated whether the patients with the same mutations have different clinical and/or immunological features.

METHODS
Patients

The study included patients whose data were submitted to the Iranian national registry for PIDs established by the National PID Network under the supervision of the Research Center for Immunodeficiencies.[12] A total of 550 patients with PAD (42.8% females) with the diagnoses of agamaglobulinemia, HIgM syndrome, and CVID were registered by expert clinical immunologists in the Children's Medical Center Hospital in Iran, on the basis of updated diagnostic criteria recommended by the European Society for Immunodeficiencies.[13] Among 550 registered patients with PAD with a diagnosis of agammaglobulinemia, HIgM syndrome, or CVID, a total of 111 patients with confirmed genetic mutations were selected for analyzing. The study was approved by the Ethics Committee of the Faculty of Medicine, Tehran University of Medical Sciences.

Data collection

A 2-page questionnaire was completed by reviewing medical records, and if possible direct interview of patients to collect information including demographic data, clinical manifestations, medical history, physical examination, laboratory and molecular findings, medical severity, and mortality. Patients with incomplete diagnostic criteria were excluded. Medical information was collected after obtaining written informed consent from all patients and/or their

parents. The medical severity phenotype was defined by having 2 of the following 3 criteria: early age at onset of symptoms ($\leq$6 months), frequent symptoms of infection (according to the 10 warning signs of PID), and development of severe infectious complications during the course of the disease (sepsis, central nervous system infections, osteomyelitis, and bacterial arthritis).

Mutation analysis

Genomic DNA from 323 alive/available patients was extracted from whole blood, as previously described.[14] Because among PAD disorders, CVID is the most heterogeneous disease and it is difficult to candidate 1 gene for targeted sequencing, we performed whole-exome sequencing for all the patients with CVID. But, for patients with agamaglobulinemia (targeted sequencing for the *BTK, B-cell linker protein, CD79A, CD79B, IGLL1,* and *μ heavy chain* genes) or HIgM syndrome (targeted sequencing for the *CD40L, CD40, AICDA,* and *uracil N glycosylase* genes), Sanger sequencing was performed on the most likely genes as described previously.[15] For patients in which Sanger sequencing failed (19% of patients with agamaglobulinemia and 38% of patients with HIgM syndrome), whole-exome sequencing was carried out as well as in patients with CVID, using a previously published pipeline.[15,16] After whole-exome sequencing, the genetic diagnosis could not yield in 92 patients (50 with agamaglobulinemia, 20 with HIgM syndrome, and 22 with CVID). The pathogenicity of all disease-attributable gene variants was reevaluated using the updated guideline for interpretation of molecular sequencing by the American College of Medical Genetics and Genomics (ACMG) as described previously.[15] Variants in genes with more than 5 affected patients were selected for this study, enabling statistical analysis between groups.

Grouping of patients

After confirmation of a single causative gene defect, patients with PAD were grouped into typical PAD categories. The most commonly affected genes in patients' categories as CVID (CVID-like) were *LRBA, DNMT3B,* and *ZBTB24,*[17] whereas mutations in *CD40L* and *AICDA* were the most frequent gene defects among patients with HIgM syndrome.[9] Mutations in *BTK* and *μ heavy chain deficiencies* were the most frequent defects in patients with agammaglobulinemia who were divided into these 2 groups.[7] Similarly, Patients with CVID-like diseases were categorized into those with LRBA deficiency and those with ICF (genes associated with atypical Immunodeficiency, Centromere instability, and Facial anomalies syndrome) on the basis of confirmed mutations. Patients with HIgM syndrome were categorized into 2 groups, CD40L- and activation-induced cytidine deaminase (AID)-deficient patients. In addition, selected patient groups were analyzed according to mutational scores, affected domains of the mutated protein, and disease severity. On the basis of criteria of the ACMG, we considered mutations with strong evidence of computational and predictive data (pathogenicity very strong [PVS1]) as severe and the remaining mutations were classified as nonsevere (mild).[18]

Statistical analysis

Data analysis was performed using the SPSS software package (SPSS Statistics 17.0.0; SPSS, Chicago, Ill). We used the Kolmogorov-Smirnov test to estimate whether data were normally distributed. Variables with a significant influence on monogenic diseases underlying PAD categories of agammaglobulinemia, CVID, and HIgM syndrome were subjected to multivariate logistic modeling as linear effects with the binary logistic regression model using a stepwise forward procedure. The fitted model presented as

odds ratio (OR) and 95% CI for the remaining significant variables different between studied genetic defects. Kaplan-Meier curves and log-rank tests were used to compare different survival estimates. A *P* value of less than .05 was considered statistically significant.

RESULTS

Patient classification based on genetic mutations

A total of 111 patients with confirmed genetic mutations were selected from registered patients with PAD. The selected cohort included 54 patients with agammaglobulinemia (Table I), 29 patients with a CVID phenotype (Table II), and 28 patients with HIgM syndrome (Table III). All genetic variants were sub-classified on the basis of severity of their mutations (see Tables E1-E12 in this article's Online Repository at www.jaci-inpractice.org), except for *LRBA* and *μ heavy chain deficiencies* genes, where all identified mutations were severe (PVS1 based on ACMG criteria).

BTK- and *μ heavy chain*−deficient patients

Of the 54 patients with agammaglobulinemia, 48 patients had hemizygous mutations in the *BTK* gene (including 8 novel variations) representing X-linked agammaglobulinemia (XLA) whereas the remaining 6 patients had homozygous mutations in the *μ heavy chain* gene (Table I). The most frequent *BTK* mutations were missense mutations (47.9%), and almost half the mutations (45.8%) were found in the tyrosine kinase domain (see Figure E1, *A*, in this article's Online Repository at www.jaci-inpractice.org). Nonsense mutations were the most frequent type of variants among the *μ heavy chain*−deficient patients and all mutations affected the first constant domain of the protein.

Demographic, clinical manifestations, and laboratory data for BTK (48 males) and μ heavy chain (1 female and 5 males)-deficient patients are provided in Table IV and Figure 1, *A.* The median age of diagnosis was significantly lower in the μ heavy chain−deficient patients compared with the BTK-deficient patients, respectively (*P* = .001). Pneumonia was the most frequent symptom reported in BTK- and μ heavy chain−deficient patients. Severe complications were observed more often in the μ heavy chain−deficient patients (100%) than in patients with XLA (33.3%; *P* = .003). Surprisingly, paralytic polio as a result of oral polio vaccination (OPV) was considerably more frequent in the μ heavy chain−deficient (66.6%) compared with the BTK-deficient patients (2.0%; *P* < .001). Serum immunoglobulin levels were equally low in both groups, with the exception of IgM, which appeared to be lower in patients with μ heavy chain deficiency (2.0 [0.0-4.2] mg/dL) than in patients with BTK deficiency (19.0 [5.0-36.0] mg/dL; *P* = .009). We then adjusted our analysis for the significant variables between BTK and μ heavy chain deficiency by including them as covariates in a multivariate logistic regression model, which was used to estimate multiple comparisons. Using a stepwise forward procedure, the age at diagnosis (OR, 2.4, 95% CI, 1.3-3.5), paralysis after OPV (OR, 3.8; 95% CI, 2.6-5.0), and serum IgM level (OR, 1.3; 95% CI, 1.05-1.55) fitted a model on complete variable data between these 2 monogenic diseases.

We further compared the clinical manifestations, involved organs, and laboratory data of BTK- and μ heavy chain−deficient patients with severe and mild mutations (Tables E2-E4). Diseases severity was significantly higher in BTK- (51.8% vs 9.5%; *P* = .002) and μ heavy chain−deficient patients (100% vs 0.0%; *P* = .02) with severe mutations as compared with BTK-deficient

TABLE I. Genes mutated in patients with agammaglobulinemia

Patients' ID	Disease	Gene	Zygosity	Inheritance	PMID Reported/new patient	Method	Affected domain	Type of mutation	Deleterious variants	Prediction severity	Medical severity
P1	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Missense	p.R28C	Mild	Mild
P2	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	Cys-rich	Missense	p.Y152C	Mild	Mild
P3	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Missense	p.I651T	Mild	Mild
P4	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Missense	p.R641H	Mild	Mild
P5	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Missense	p.L616F	Mild	Mild
P6	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Missense	p.R615S	Mild	Mild
P7	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Missense	p.Y551H	Mild	Mild
P8	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Missense	p.M405I	Mild	Severe
P9	XLA	*BTK*	Hemizygous	X-linked	Novel	NGS panel	TK	Missense	p.A508T	Mild	Mild
P10	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Missense	p.Y551H	Mild	Mild
P11	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	SH2	Small inframe deletion	p.G303del	Mild	Mild
P12	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	SH2	Missense	p.H350D	Mild	Mild
P13	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	SH2	Missense	p.H350D	Mild	Mild
P14	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Small inframe deletion	p.K60del	Mild	Mild
P15	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	WES	PH	Missense	p.R28C	Mild	Mild
P16	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	WES	PH	Missense	p.R28C	Mild	Mild
P17	XLA	*BTK*	Hemizygous	X-linked	New patient	NGS panel	TK	Missense	p.R525Q	Mild	Mild
P18	XLA	*BTK*	Hemizygous	X-linked	Novel	NGS panel	TK	Missense	p.R544M	Mild	Severe
P19	XLA	*BTK*	Hemizygous	X-linked	Novel	Targeted Sanger	TK	Missense	p.Y598N	Mild	Mild
P20	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Missense	p.R641H	Mild	Mild
P21	XLA	*BTK*	Hemizygous	X-linked	Novel	Targeted Sanger	TK	Missense	p.Y598N	Mild	Mild
P22	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Splice site	IVS15-13 delTTTG	Severe	Mild
P23	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Splice site	IVS14-1 G>A	Severe	Severe
P24	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Frameshift nonsense	p.N72Ifs.X49	Severe	Mild
P25	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Splice site	IVS14-1 G>A	Severe	Severe
P26	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Stopgain	p.W588X	Severe	Severe
P27	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Stopgain	p.Q496X	Severe	Severe
P28	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Splice site	IVS12 + 1G>A	Severe	Severe
P29	XLA	*BTK*	Hemizygous	X-linked	Novel	NGS panel	TK	Stopgain	p.K515X	Severe	Severe
P30	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Splice site	IVS3 + 2T > C	Severe	Mild
P31	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Stopgain	p.L405X	Severe	Severe
P32	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	SH3	Stopgain	p.R255X	Severe	Mild
P33	XLA	*BTK*	Hemizygous	X-linked	Novel	NGS panel	SH3	Splice site	IVS8-2 delA	Severe	Severe
P34	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	SH3	Stopgain	p.R255X	Severe	Severe
P35	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	SH3	Splice site	IVS9-2 delA	Severe	Mild
P36	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Missense	p.L37P	Severe	Mild
P37	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	TK	Missense	p.P619L	Severe	Severe
P38	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Splice site	IVS3+2 T>C	Severe	Mild
P39	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Splice site	IVS3 + 2 T>C	Severe	Mild
P40	XLA	*BTK*	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Splice site	IVS1+5 G>C	Severe	Mild

Patient	Disease	Gene	Zygosity	Inheritance	Reference	Method	Domain	Mutation type	Nucleotide/Protein change		
P41	XLA	BTK	Hemizygous	X-linked	Novel	Targeted Sanger	Cys-rich	Missense	p.M509L	Severe	Mild
P42	XLA	BTK	Hemizygous	X-linked	NEW patient	NGS panel	TK	Splice site	IVS17+5 G>A	Severe	Severe
P43	XLA	BTK	Hemizygous	X-linked	Novel	NGS panel	TK	Frameshift nonsense	p.D531VfsX5	Severe	Severe
P44	XLA	BTK	Hemizygous	X-linked	New patient	NGS panel	TK	Splice site	IVS3+2 T>C	Severe	Severe
P45	XLA	BTK	Hemizygous	X-linked	PMID:26993986	WES	TK	Missense	p.L652P	Severe	Severe
P46	XLA	BTK	Hemizygous	X-linked	PMID:26910880	WES	TK	Splice site	IVS17 + 5 G>A	Severe	Mild
P47	XLA	BTK	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Splice site	IVS1 + 5 G>C	Severe	Mild
P48	XLA	BTK	Hemizygous	X-linked	PMID:26910880	Targeted Sanger	PH	Splice site	IVS1 + 5 G>C	Severe	Mild
P49	ARA	μ heavy chain	Homozygous	AR	PMID:26910880	Targeted Sanger	—	Large deletion	Large deletion	Severe	Severe
P50	ARA	μ heavy chain	Homozygous	AR	PMID:26910880	Targeted Sanger	CH1	Stopgain	p.S19X	Severe	Severe
P51	ARA	μ heavy chain	Homozygous	AR	PMID:26910880	Targeted Sanger	CH1	Stopgain	p.Q70X	Severe	Severe
P52	ARA	μ heavy chain	Homozygous	AR	PMID:26910880	Targeted Sanger	CH1	Frameshift nonsense	p.Y176LfsX87	Severe	Severe
P53	ARA	μ heavy chain	Homozygous	AR	Novel	WES	CH1	Stopgain	p.Q31X	Severe	Severe
P54	ARA	μ heavy chain	Homozygous	AR	PMID:26910880	WES	CH1	Frameshift nonsense	p.W24VfsX452	Severe	Severe

AR, Autosomal recessive; *CH1*, first constant domain; *IVS*, intervening sequence or intron; *new patient*, patients reported for the first time in our cohort with a known mutation; *NGS*, next-generation sequencing; *novel*, variant not reported in IDbase, Clinvar, and HGMD databases; *PH*, pleckstrin homology domain; *SH1/TK*, Src homology 1/tyrosine kinase catalytic domain; *SH2*, Src homology 2 domain; *SH3*, Src homology 3 domain; *TH/cys-rich*, Tec homology/cys-rich domain; *TK*, tyrosine kinase; *WES*, whole-exome sequencing.

patients with mild mutations. Interestingly, BTK-deficient patients with severe mutations presented with a significantly higher rate of meningitis (48.1% vs 14.2%; $P = .01$) and chronic diarrhea (40.7% vs 14.2%; $P = .04$) compared with patients with XLA with a mild mutation. Although BTK-deficient patients with severe mutations manifested a higher incidence of meningitis in comparison with patients with μ heavy chain deficiency ($P = .02$), μ heavy chain–deficient patients reported a high incidence of paralytic polio caused by OPV ($P = .001$).

LRBA-deficient and ICF patients

The 2 main identified monogenetic defects underlying patients with a clinical diagnosis of CVID were LRBA deficiency (9 females and 8 males) and atypical ICF syndrome (7 females and 5 males). Demographic characteristics, clinical manifestations, organ involvement, and laboratory abnormalities of these patients were compared (Tables V and E5). Mutations identified in LRBA-deficient and ICF patients were homozygous. The most common type of mutation and the most frequently affected domain in LRBA-deficient patients were a nonsense mutation (47%) and the PKA-binding domain (35.2%) (Figure E1, *B*). Mutations causing ICF1 were hypomorphic variants within the catalytic domain of the DNMT3B protein. Similarly, all variants identified in ICF2 patients were missense mutations within the zinc-finger domain, which is responsible for proper intranuclear localization of the ZBTB24 protein (Table II).

The median ages at diagnosis were considerably higher in LRBA-deficient patients than in ICF patients ($P = .003$). The most common clinical presentation of LRBA-deficient patients was chronic diarrhea (88.2%), whereas ICF patients presented mainly with upper respiratory tract infections (RTIs) (100.0%; Table V and Figure 1, *B*). Surprisingly, the first presentation in ICF patients was respiratory infections ($P = .008$), whereas the first presentation in patients with LRBA deficiency was nonrespiratory complications ($P = .008$; Figure 2). Of note, bronchiectases were significantly more recorded in LRBA-deficient patients compared with ICF patients ($P = .02$). Our data indicate that noninfectious complications such as clubbing ($P = .008$), autoimmunity ($P = .02$), splenomegaly ($P = .006$), hepatomegaly ($P = .01$), lymphoproliferative disorders ($P = .006$), and chronic diarrhea ($P = .001$) were significantly more frequent in LRBA-deficient patients than in ICF patients. Various autoimmune diseases, including autoimmune hematologic anemia and endocrine, neurologic, and rheumatologic disorders, were found in 13 (76.5%) patients with LRBA deficiency. As reported previously,[17] immune parameters were similar in LRBA deficiency and ICF, with the exception of B-cell counts ($P = .009$), which were lower in LRBA-deficient patients compared with ICF patients (Table V). Multivariate logistic regression model indicated that the clinical phenotype of lymphoproliferation (OR, 2.6; 95% CI, 1.8-3.2) and chronic diarrhea (OR, 4.0; 95% CI, 3.1-5.1) fitted the best model.

The comparison of demographic data, clinical manifestations, laboratory testing, and affected organs between ICF patients with severe and those with mild mutations did not reveal significant differences (Tables E6-E8). However, LRBA-deficient patients with severe mutations had a significantly younger age at onset ($P = .01$) and a higher frequency of chronic diarrhea ($P = .02$), clubbing ($P = .01$), and lymphoproliferative disorders ($P = .04$) compared with ICF patients with severe mutations. ICF patients with severe mutations had a significantly lower lymphocyte count, CD3^{+} cell

TABLE II. Genes mutated in patients with CVID-like phenotype

Patients' ID	Disease	Gene	Zygosity	Inheritance	PMID Reported/new patient	Method	Affected domain	Type of mutation	Deleterious variants	Prediction severity	Medical severity
P1	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	BEACH	Stopgain	p.R182X	Severe	Mild
P2	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	DUF	Stopgain	p.S1605X	Severe	Severe
P3	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	Signaling	Stopgain	p.E59X	Severe	Severe
P4	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	Con A	Splice site	IVS8 +1G>A	Severe	Severe
P5	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	Signaling	Large deletion	Exon 1-2 deletion	Severe	Severe
P6	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	DUF	Splice site	IVS29 +2dupT	Severe	Severe
P7	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	Signaling	Stopgain	p.R182X	Severe	Severe
P8	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	DUF	Frameshift nonsense	p.I1875SfsX14	Severe	Severe
P9	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	Con A	Frameshift nonsense	p.S462LfsX7	Severe	Severe
P10	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	WD	Large deletion	Exon41 deletion	Severe	Severe
P11	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	DUF	Splice site	IVS29 +2dupT	Severe	Severe
P12	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	DUF	Stopgain	p.S1605X	Severe	Severe
P13	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	DUF	Stopgain	p.S1605X	Severe	Mild
P14	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	WD	Large deletion	Exon 41 deletion	Severe	Severe
P15	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	BEACH	Stopgain	p.R182X	Severe	Severe
P16	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	Signaling	Stopgain	p.E59X	Severe	Severe
P17	LRBA	*LRBA*	Homozygous	AR	PMID:28512785	WES	Con A	Frameshift nonsense	p.D248EfsX	Severe	Severe
P18	ICF1	*DNMT3B*	Homozygous	AR	PMID: 28916186	WES	SAM	Missense	p.D722E	Mild	Mild
P19	ICF1	*DNMT3B*	Homozygous	AR	PMID: 28916186	WES	SAM	Missense	p.D722E	Mild	Mild
P20	ICF1	*DNMT3B*	Homozygous	AR	PMID: 28916186	WES	SAM	Missense	p.D722E	Mild	Severe
P21	ICF1	*DNMT3B*	Homozygous	AR	PMID: 28916186	WES	SAM	Missense	p.D722E	Mild	Severe
P22	ICF1	*DNMT3B*	Homozygous	AR	PMID: 28916186	WES	SAM	Missense	p.D722E	Mild	Mild
P23	ICF1	*DNMT3B*	Homozygous	AR	PMID: 28916186	WES	SAM	Missense	p.Y624C	Mild	Severe
P24	ICF1	*DNMT3B*	Homozygous	AR	PMID: 28916186	WES	SAM	Missense	p.D722E	Severe	Severe
P25	ICF2	*ZBTB24*	Homozygous	AR	PMID: 28916186	WES	ZNF	Missense	p.C383S	Severe	Severe
P26	ICF2	*ZBTB24*	Homozygous	AR	PMID: 28916186	WES	ZNF	Frameshift nonsense	p.D266RfsX28	Severe	Severe
P27	ICF2	*ZBTB24*	Homozygous	AR	PMID: 28916186	WES	ZNF	Missense	p.C383S	Severe	Mild
P28	ICF2	*ZBTB24*	Homozygous	AR	PMID: 28916186	WES	ZNF	Missense	p.C408W	Severe	Severe
P29	ICF2	*ZBTB24*	Homozygous	AR	PMID: 28916186	WES	ZNF	Missense	p.C383S	Severe	Mild

AR, Autosomal recessive; *BEACH*, beige and CHS domain; Con A, concanavalin A−like lectin binding domain; *DUF*, PKA-binding domain; *IVS*, intervening sequence or intron; *SAM*, SAM-binding methyltransferase; *Signaling*, STAM signal transducing adaptor molecule; *WD*, WD40 domain; *WES*, whole-exome sequencing; *ZNF*, zinc-figure domain.

TABLE III. Genes mutated in patients with HIgM syndromes

Patients' ID	Disease	Gene	Zygosity	Inheritance	PMID Reported/new patient	Method	Affected domain	Type of mutation	Deleterious variants	Prediction severity	Medical severity
P1	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	IC	Splice site	IVS1+2T>C	Severe	Severe
P2	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	IC	Frameshift nonsense	p.T29fsX36	Severe	Severe
P3	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	ECU	Frameshift nonsense	p.D62fsX79	Severe	Severe
P4	XHIGM	*CD40L*	Hemizygous	X-linked	Novel	Targeted Sanger	TNFH	Frameshift nonsense	p.S89TfsX6	Severe	Severe
P5	XHIGM	*CD40L*	Hemizygous	X-linked	Novel	Targeted Sanger	TNFH	Frameshift nonsense	p.S89TfsX6	Severe	Severe
P6	XHIGM	*CD40L*	Hemizygous	X-linked	Novel	Targeted Sanger	TNFH	Frameshift nonsense	p.S89TfsX6	Severe	Severe
P7	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	TNFH	Missense	p.T254M	Mild	Severe
P8	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	TNFH	Missense	p.Q186X	Severe	Severe
P9	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	TNFH	Missense	p.G167R	Mild	Severe
P10	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	TM	Missense	p.M360T	Mild	Severe
P11	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	TNFH	Missense	p.G252D	Mild	Severe
P12	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	TNFH	Missense	p.G167R	Mild	Severe
P13	XHIGM	*CD40L*	Hemizygous	X-linked	PMID: 28916186	Targeted Sanger	TNFH	Missense	p.Q186X	Severe	Severe
P14	XHIGM	*CD40L*	Hemizygous	X-linked	PMID: 28916186	Targeted Sanger	TNFH	Missense	p.Q186X	Severe	Severe
P15	XHIGM	*CD40L*	Hemizygous	X-linked	PMID: 28916186	NGS panel	ECU	Missense	p.L81X	Severe	Severe
P16	XHIGM	*CD40L*	Hemizygous	X-linked	PMID: 28916186	Targeted Sanger	TNFH	Missense	p.Q186X	Severe	Severe
P17	XHIGM	*CD40L*	Hemizygous	X-linked	Novel	NGS panel	TNFH	Missense	p.G144V	Mild	Severe
P18	XHIGM	*CD40L*	Hemizygous	X-linked	PMID: 23653974	Targeted Sanger	TNFH	Missense	p.G252A	Mild	Severe
P19	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	TNFH	Missense	p.G219R	Mild	Mild
P20	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	TNFH	Missense	p.G167R	Mild	Mild
P21	XHIGM	*CD40L*	Hemizygous	X-linked	PMID:19575287	Targeted Sanger	TNFH	Missense	p. L161P	Mild	Mild
P22	ARHIGM	*AICDA*	Homozygous	AR	PMID:19575287	Targeted Sanger	CMP	Stopgain	p.E121X	Severe	Severe
P23	ARHIGM	*AICDA*	Homozygous	AR	Novel	NGS panel	CMP	Splice site	IVS4-1 C>A	Severe	Severe
P24	ARHIGM	*AICDA*	Homozygous	AR	Novel	WES	CMP	Missense	p.D96V	Mild	Mild
P25	ARHIGM	*AICDA*	Homozygous	AR	PMID: 22992148	Targeted Sanger	CMP	Missense	p.G125V	Mild	Mild
P26	ARHIGM	*AICDA*	Homozygous	AR	PMID: 22992148	Targeted Sanger	CMP	Missense	p.G125V	Mild	Mild
P27	ARHIGM	*AICDA*	Homozygous	AR	NEW patient	NGS panel	CMP	Missense	p.R112C	Mild	Mild
P28	ARHIGM	*AICDA*	Homozygous	AR	PMID: 27789066	Targeted Sanger	APOBEC	Missense	p.V175A	Mild	Mild

AR, Autosomal recessive; *CMP*, cytidine monophosphate deaminase domain; *ECU*, extracellular unique region; *IC*, intracellular tail; *IVS*, intervening sequence or intron; *new patient*, patients reported for the first time in our cohort with a known mutation; *novel*, variant not reported in IDbase, Clinvar, and HGMD databases; *TM*, transmembrane domain; *TNFH*, tumor necrosis factor-homology domain; *WES*, whole-exome sequencing.

TABLE IV. Demographic data, clinical manifestations, and laboratory data of patients with BTK and μ heavy chain deficiencies

Parameter	BTK deficiency (n = 48)	μ heavy chain deficiency (n = 6)	P value
Median age at the time of the study (y) (IQR)	23.0 (14.0-26.0)	13.0 (4.5-23.0)	0.07
Median age at the onset of symptoms (mo) (IQR)	12.0 (6.0-36.0)	7.0 (2.7-12.0)	0.09
Median age at diagnosis (mo) (IQR)	60.0 (36.0-105.0)	13.0 (3.2-16.5)	0.001*
Delay in diagnosis (mo) (IQR)	38.5 (19.2-77.2)	3.5 (0.7-12.2)	0.006*
Consanguinity, n (%)	18 (37.5)	6 (100)	0.001*
Medical severity, n (%)	16 (33.3)	6 (100.0)	0.003*
Otitis media, n (%)	26 (54.1)	2 (25.0)	0.1
Sinusitis, n (%)	30 (62.5)	1 (16.6)	0.1
Pneumonia, n (%)	33 (68.7)	5 (83.4)	1.0
Bronchiectasis, n (%)	15 (31.2)	1 (16.6)	1.0
Clubbing, n (%)	8 (16.6)	1 (16.6)	1.0
Autoimmunity, n (%)	5 (10.4)	2 (33.3)	0.1
Splenomegaly, n (%)	4 (8.3)	1 (16.6)	0.4
Hepatomegaly, n (%)	5 (10.4)	1 (16.6)	0.5
Lymphoproliferation, n (%)	8 (16.6)	1 (16.6)	1.0
Allergy, n (%)	3 (6.2)	1 (16.6)	0.3
Chronic diarrhea, n (%)	14 (29.1)	1 (16.6)	1.0
Conjunctivitis, n (%)	13 (27.0)	1 (16.6)	0.1
Meningitis, n (%)	16 (33.3)	0.0 (0.0)	0.1
Paralysis following vaccination, n (%)	1 (2.0)	4 (66.6)	<0.001*
Leukocytes (cells/μL) (IQR)	9,630 (7,170-14,072)	14,615.0 (6,875.0-80,908.0)	0.2
Lymphocytes (cells/μL) (IQR)	3,560.0 (2,765.0-5,005.0)	7,738.0 (2,365.0-10,679.0)	0.2
Neutrophils (cells/μL) (IQR)	5,145.0 (2,378.8-7,240.0)	2,487.1 (1,376.0-4,672.0)	0.1
Hemoglobin (g/dL) (IQR)	12.0 (10.7-13.0)	11.0 (10.0-14.0)	0.7
Platelets (10^3/μL) (IQR)	367 (280-468)	485.0 (345.0-677.0)	0.3
Total CD3 (cells/mm^3) (IQR)	3,130.0 (1,988.0-4,139.5)	7,118.0 (2,199.0-9,290.0)	0.2
Total CD4 (cells/mm^3) (IQR)	1,514.5 (794.0-2,406.0)	3,404.0 (378.0-501.9)	0.4
Total CD8 (cells/mm^3) (IQR)	1,240.0 (754.0-1,725.5)	3,404.0 (1,726.0-4,485.0)	0.02*
Total CD19 (cells/mm^3) (IQR)	0.0 (0.0-3.5)	77.0 (0.0-106.0)	0.09
IgG (mg/dL) (IQR)	122.5 (46.7-299.2)	42.0 (13.7-369.0)	0.4
IgA (mg/dL) (IQR)	5.5 (0.0-16.5)	0.0 (0.0-5.2)	0.1
IgM (mg/dL) (IQR)	19.0 (5.0-36.0)	2.0 (0.0-4.2)	0.009*

IQR, Interquartile range.
*Statistical significance set at $P < .05$.

percentage and count, CD4$^+$ cell percentage and count as well as CD19$^+$ cell percentage compared with ICF patients with a mild mutation, indicating an impact of a severe mutation on T- and B-cell numbers (Table E7). Not surprisingly, patients with ICF had a significantly higher rate of chromosomal aberrations compared with cases with LRBA deficiency (83.3% vs 47.0%, respectively, $P = .02$) after irradiation.

CD40L- and AID-deficient patients

Among the 28 patients with HIgM syndrome, 21 had hemizygous mutations in *CD40L* and 7 (2 females and 5 males) had homozygous mutations in *AICDA*. The most frequent types of variants in both CD40L- and AID-deficient patients were missense mutations (Figure E1, *C*). The tumor necrosis factor homologous domain was the most commonly affected domain among the CD40L-deficient patients, whereas the cytidine monophosphate deaminase domain was most commonly affected among patients with mutations in *AICDA* (Table III).

Demographic, clinical manifestations, and laboratory data are provided in Table VI and Figure 1, *C*. The median age at diagnosis in CD40L-deficient patients was significantly lower compared

with AID-deficient patients ($P = .02$). Disease severity was significantly higher in the CD40L-deficient patients (83.3%) compared with the AID-deficient patients (28.5%; $P = .02$). Patients with CD40L deficiency had a higher prevalence of chronic diarrhea than did AID-deficient patients, but did not reach significance (Tables VI and E10). Twelve (57.1%) CD40L-deficient patients reported neutropenia, whereas none of the AID-deficient patients was diagnosed with neutropenia ($P = .01$). The AID-deficient patients reported significantly more often adenopathy (57.1%) than did the CD40L-deficient patients (14.2%; $P = .04$). Lymphocyte counts were lower in AID-deficient patients (but still in normal range) than in CD40L-deficient patients ($P = .02$). The age at diagnosis (OR, 1.6; 95% CI, 1.4-1.8), medical severity (OR, 0.8; 95% CI, 0.64-0.96), and neutrophil counts (OR, 1.8; 95% CI, 1.2-2.4) were in the best model fitted according to the multivariate logistic regression model.

Mortality

Data of mortality rate in the different categories of patients with PAD are illustrated in Figure E2 in this article's Online
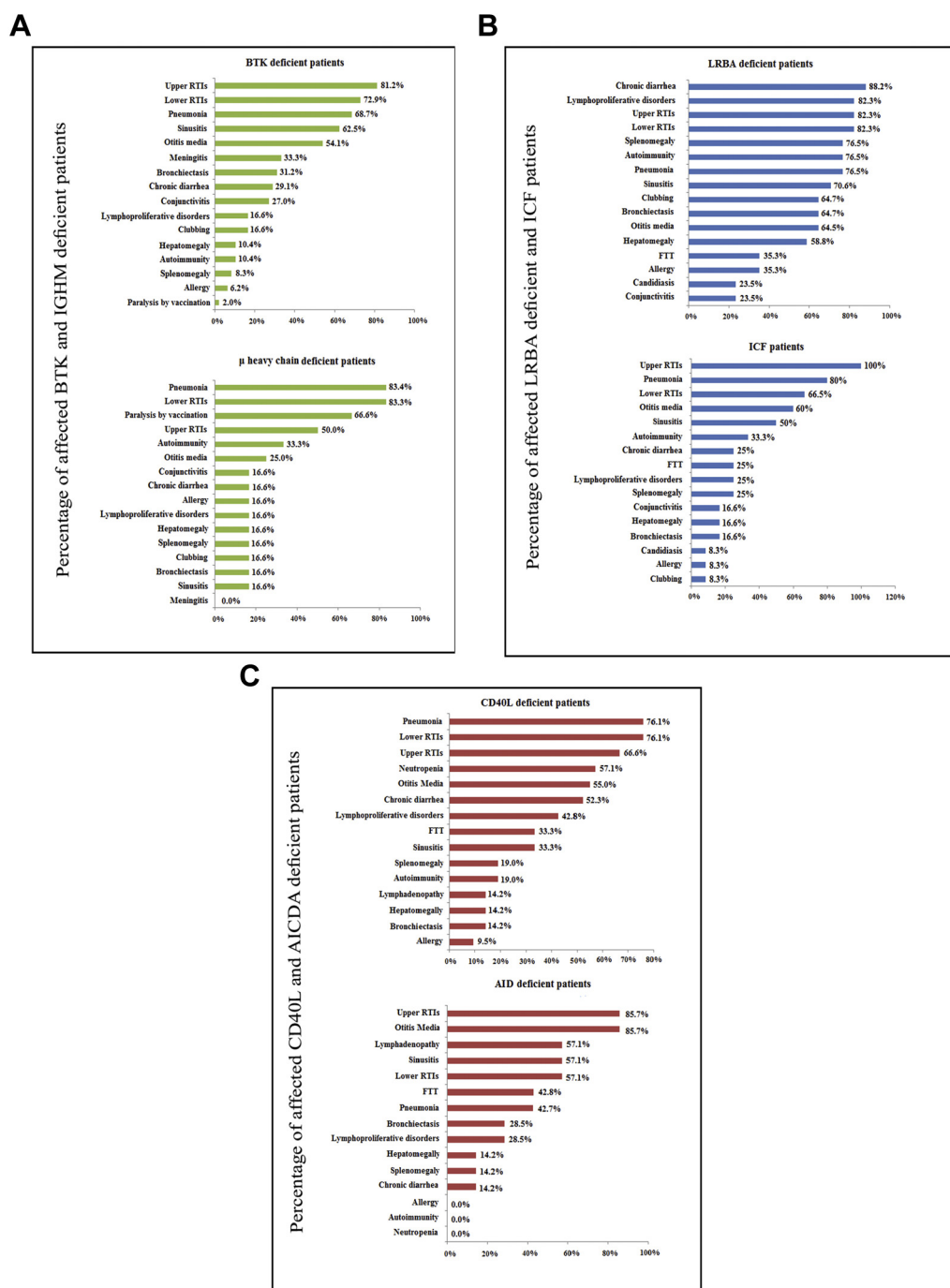
FIGURE 1. Clinical manifestations. **A,** RTIs and pneumonia are the most common manifestations in BTK- and μ heavy chain–deficient patients. **B,** Chronic diarrhea and upper RTIs were the most frequent clinical manifestations in LRBA-deficient and ICF patients, respectively. **C,** Pneumonia in CD40L-deficient patients and upper RTIs in AID-deficient patients were the most common clinical manifestations.

Repository at www.jaci-inpractice.org. Mortality was higher in the LRBA-deficient patients than in the ICF patients (35.3% vs 0.0%; $P = .05$). Kaplan-Meier analysis did not reveal statistical differences in the cumulative survival of the 2 groups ($P = .17$; the 15-year survival rate in LRBA-deficient patients was 79%, whereas in ICF patients it was 100%). Mortality was higher in the CD40L-deficient patients than in the patients with AID

deficiency (35.0% vs 0.0%; $P = .13$). There were no statistical differences in the cumulative survival rate of the 2 groups ($P = .14$; the 20-year survival rate in CD40L-deficient patients was 35%, whereas in AID-deficient patients it was 100%). Among patients with agammaglobulinaemia, mortality was higher (without statistical significance) in the μ heavy chain–deficient patients than in the BTK-deficient patients

TABLE V. Demographic data, clinical manifestations, and laboratory data of patients with LRBA deficiency and ICF syndrome

Parameter	Patients with LRBA deficiency (n = 17)	Patients with ICF syndrome (n = 12)	P value
Median age at the time of the study (y) (IQR)	16.0 (11.5-21.5)	13.0 (7.0-21.0)	0.4
Median age at the onset of symptoms (mo) (IQR)	24.0 (6.5-24)	5.0 (1.5-6.0)	0.004*
Median age at diagnosis (mo) (IQR)	84.0 (54.0-138.0)	25.0 (9.0-44.5)	0.003*
Delay in diagnosis (mo) (IQR)	60.0 (42.0-96.0)	21.7 (6.0-45.7)	0.01*
Consanguinity, n (%)	17 (100.0)	11 (91.6)	0.4
Medical severity, n (%)	15 (88.2)	7 (58.3)	0.09
Otitis media, n (%)	11 (64.5)	6 (60.0)	1.0
Sinusitis, n (%)	12 (70.6)	5 (50.0)	0.4
Pneumonia, n (%)	13 (76.5)	8 (80.0)	1.0
Bronchiectasis, n (%)	11 (64.7)	2 (16.6)	0.02*
Clubbing, n (%)	11 (64.7)	1 (8.3)	0.008*
Autoimmunity, n (%)	13 (76.5)	4 (33.3)	0.02*
Splenomegaly, n (%)	13 (76.5)	3 (25.0)	0.006*
Hepatomegaly, n (%)	10 (58.8)	2 (16.6)	0.01*
Lymphoproliferation, n (%)	14 (82.3)	3 (25.0)	0.006*
Allergy, n (%)	6 (35.3)	1 (8.3)	0.1
FTT, n (%)	6 (35.3)	3 (25.0)	0.6
Chronic diarrhea, n (%)	15 (88.2)	3 (25)	0.001*
Neutropenia, n (%)	3 (17.6)	1 (8.3)	0.4
Conjunctivitis, n (%)	4 (23.5)	2 (16.6)	1.0
Candidiasis, n (%)	4 (23.5)	1 (8.3)	0.3
Leukocytes (cells/mL) (IQR)	8,300 (5,050-11,850)	7,750 (5,450-10,475)	0.9
Lymphocytes (cells/μL) (IQR)	2,385.0 (1,878.5-3,542.0)	2,628.0 (1,825.0-4,051.5)	0.9
Neutrophils (cells/μL) (IQR)	3,450 (2,469-6,673)	2,870 (1,515-5,017)	0.1
Hemoglobin (g/dL) (IQR)	12.0 (10.5-13.0)	12.0 (9.0-13.0)	0.4
Platelets (10^3/μL) (IQR)	239.5 (107.7-294.2)	385.0 (197.5-540.2)	0.1
Total CD3 (cells/mm^3) (IQR)	1,747.5 (1,510.7-3,323.2)	2,380.0 (1,391.0-3,073.0)	0.6
Total CD4 (cells/mm^3) (IQR)	760.0 (421.0-1,177.7)	1,071.0 (600.0-1,844.0)	0.2
Total CD8 (cells/mm^3) (IQR)	1,021.0 (750.0-1,928.5)	985.0 (717.0-1,191.0)	0.6
Total CD19 (cells/mm^3) (IQR)	155.5 (88.2-393.2)	445.7 (346.8-576.3)	0.009*
IgG (mg/dL) (IQR)	310.0 (62.0-440.0)	253.0 (42.2-514.0)	0.7
IgA (mg/dL) (IQR)	7.0 (0.0-28.0)	9.0 (0.7-38.2)	0.7
IgM (mg/dL) (IQR)	44.0 (22.0-149.0)	29.0 (6.5-40.7)	0.1
IgE (IU/mL) (IQR)	1.0 (0.0-4.0)	1.0 (0.0-6.05)	0.8

FTT, Failure to thrive; *IQR*, interquartile range; *IU*, international unit.
*Statistical significance set at $P < .05$.

(16.7% vs 10.6%; $P = .53$). There was no statistical difference in the cumulative survival rate in the groups ($P = .5$; the 25 years survival rate in BTK-deficient patients was 87%, whereas in μ heavy chain–deficient patients it was 100%). The mortality rates in severe and mild mutations have been demonstrated in Figure E2.

DISCUSSION

In this first comparative review of the clinical and immunologic manifestations of the most common PAD-associated monogenic diseases from the Iranian national registry, we included patients with PAD who were primarily categorized as suffering from agamaglobulinemia, CVID-like disorders, and HIgM syndrome.

Our μ heavy chain–deficient patients were diagnosed at an earlier age and reported more complications than did those with BTK deficiency, consistent with previous findings in patients with early arrest in B-cell development.[19,20] Surprisingly, we observed that most μ heavy chain–deficient patients had developed paralytic polio infection as a result of OPV, at a significantly higher rate than was observed in BTK-deficient patients. Previously published reports suggested a much lower rate of OPV-caused paralysis in BTK- and μ heavy chain–deficient patients.[20-23] Because immunity against enteroviruses seems to be a predominant antibody-mediated mechanism, patients with major B-cell dysfunction are at increased risk for poliomyelitis.[24] Because almost approximately 67% of the patients with μ heavy chain deficiency had paralytic polio infection, we suggest genetic testing or screening programs (using kappa-deleting element recombination circle) for detecting patients with μ *heavy chain* mutation at birth, which could prevent administration of live vaccines such as OPV to these patients and their contacts and minimize the burden of adverse complications.

However, establishing screening programs is expensive and most countries are unable to perform it, making a strong cause for using inactivated polio vaccine for patients with PAD.
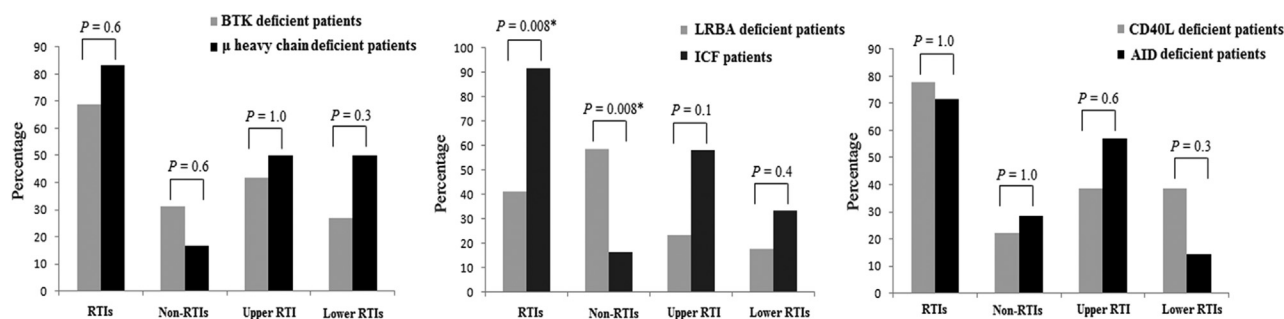
FIGURE 2. First presentations in patients with PAD. Among patients with PAD, the only significant difference was between LRBA-deficient and ICF patients. The first presentation in ICF patients was respiratory complications (P = .008), whereas the first presentation in LRBA-deficient patients was nonrespiratory complications (P = .008).

Furthermore, we found that our μ heavy chain–deficient patients were diagnosed at an earlier age and reported more complications than did those with BTK deficiency. Thus, these findings provide a hint for treating physicians that patients with the agammaglobulinaemia phenotype with low age of onset (<1 years), with parental consanguinity and severe clinical manifestation such as frequent infectious symptoms (according to the 10 warning signs of primary immunodeficiency), and development of severe infectious complications during the course of the disease have a higher chance to be due to autosomal-recessive form of the disease, mainly *μ heavy chain* mutation.

All *μ heavy chain* mutations we identified were homozygous, reflecting the high incidence (100%) of consanguinity. In our cohort, missense mutations within the first constant domain were the most frequent variants while 1 patient had a large deletion. This is in contrast to previous reports that suggest that most (∼50%)[25] harbor gross deletions. The most frequently affected BTK domain was the tyrosine kinase domain (45.8%), followed by pleckstrin homology (33.3%), SH3 (8.3%), SH2 (6.3%), and Cys-rich (6.3%) domains, in accordance with the distribution of mutations submitted to BTKbase,[26] of which 47.1% of variants were found in the tyrosine kinase domain followed by 21.5 % in the pleckstrin homology domain.

Although no significant correlation was observed between the severity of BTK mutations and age at onset, similar to previous studies[7,27] we identified a strong correlation between severity of the mutation and certain clinical manifestations such as meningitis and chronic diarrhea. Chronic diarrhea was significantly more prevalent in BTK-deficient patients with severe mutations compared with those carrying mild mutations. Overall, our BTK- deficient patients had a higher incidence of meningitis than reported in previous publications.[23,28-31] This increase was more prominent in patients with XLA with severe mutations than in those with mild mutations. These data confirm the existence of a genotype-phenotype correlation and demonstrate the importance of attempting to predict the severity of a given mutation. Thus, it suggests that identification of the type of mutation could potentially predict some clinical manifestations such as meningitis and chronic diarrhea and also a requirement for a better management of patients with XLA with severe mutations.

Most patients with a phenotype resembling CVID had mutations in *LRBA* and in genes associated with the ICF syndrome. All patients within these 2 categories were homozygous, reflecting

autosomal-recessive inheritance and mirroring the high rate of consanguinity in our population. In contrast to reported patients with typical *ZBTB24* mutations, our patients with a CVID-like phenotype had more missense mutation but still mostly within the zinc-finger domain. Among our LRBA-deficient cohort, selected patients had a progressive form of PAD and presented with HIgM syndrome (2 patients), autoimmune lymphoproliferative syndrome (1 patient), and variable autoimmune manifestations (2 patients). These data demonstrated that LRBA-deficient patients might have atypical presentations with a spectrum of immunodefieincies resembling CVID, autoimmune lymphoproliferative syndrome, immunedysregulation polyendocrinopathy enteropathy X-linked, and HIgM syndrome. Similar misdiagnoses have been observed in previous reports, and it has been suggested that a diagnosis of LRBA deficiency should be considered in patients who are negative for mutations in the genes for autoimmune lymphoproliferative syndrome, HIgM syndrome, immunedysregulation polyendocrinopathy enteropathy X-linked, and combined immunodeficiency.[32-34]

The pattern of inheritance of the most common monogenic disorders discovered underlying the CVID phenotype in our highly consanguineous cohort (LRBA deficiency and ICF syndrome) was autosomal recessive, in contrast to Western countries cohorts, with high frequency of autosomal-dominant diseases (mainly due to nuclear factor kappa-chain activated B-cell members, PI3K, CTLA4, and transmembrane activator and CAML interactor defects).[15] Age at onset and age at diagnosis were lower, and delay in diagnosis was reduced in ICF patients compared with the LRBA-deficient patients. However, the frequency of clinical complications was higher in the LRBA-deficient patients. This discrepancy might be explained by the significant difference in the presenting symptoms. ICF patients consistently presented with RTIs, whereas LRBA-deficient patients presented with symptoms other than RTIs. Because ICF patients had a higher rate of RTIs as the first manifestation, they were probably diagnosed earlier as immunodeficiency. The longer delay in diagnosing LRBA deficiency may lead to the significantly higher rate of chronic complications (eg, bronchiectasis). The most prevalent long-term clinical manifestations of LRBA-deficient and ICF patients were chronic diarrhea and upper RTIs, respectively. Both entities have similar immunologic features including hypogammaglobulinemia and variably reduced T- and B-cell counts; a higher frequency of radiosensitivity was observed in the ICF patients. Chronic diarrhea,

TABLE VI. Demographic data, clinical manifestations, and laboratory data of patients with CD40L and AID deficiencies

Parameter	Patients with CD40L deficiency (n = 21)	Patients with AID deficiency (n = 7)	P value
Median age at the time of the study (y) (IQR)	9.0 (5.0-16.0)	14.0 (10.0-25.0)	0.1
Median age at the onset of symptoms (mo) (IQR)	7.0 (6.0-12.0)	10.5 (5.5-78.0)	0.3
Median age at diagnosis (mo) (IQR)	20.5 (12.2-50.5)	78.0 (50.0-111.7)	0.01*
Delay in diagnosis (mo) (IQR)	9.0 (1.0-30.0)	43.5 (10.5-65.2)	0.1
Consanguinity, n (%)	12.0 (57.1)	7 (100.0)	0.04*
Medical severity, n (%)	18 (85.7)	2 (28.5)	0.009*
Upper RTIs, n (%)	14 (66.6)	6 (85.7)	1.0
Lower RTIs, n (%)	16 (76.1)	4 (57.1)	0.3
Sinusitis, n (%)	7 (33.3)	4 (57.1)	0.3
Otitis media, n (%)	11 (55.0)	6 (85.7)	0.2
Pneumonia, n (%)	16 (76.1)	3 (42.7)	0.5
Bronchiectasis, n (%)	3 (14.2)	2 (28.5)	0.5
Autoimmunity, n (%)	4 (19.0)	0 (0.0)	0.2
Splenomegaly, n (%)	4 (19.0)	1 (14.2)	1.0
Hepatomegaly, n (%)	3 (14.2)	1 (14.2)	1.0
Lymphoproliferation, n (%)	9 (42.8)	2 (28.5)	0.5
Lymphadenopathy, n (%)	3 (14.2)	4 (57.1)	0.04*
Allergy, n (%)	2 (9.5)	0 (0.0)	1.0
FTT, n (%)	7 (33.3)	3 (42.8)	0.6
Neutropenia, n (%)	12 (57.1)	0 (0.0)	0.01*
Chronic diarrhea, n (%)	11 (52.3)	1 (14.2)	0.1
Leukocytes (cells/μL) (IQR)	13,700 (7,800-24,265)	9,800 (8,600-12,200)	0.3
Lymphocytes (cells/μL) (IQR)	8,816.0 (4,666.0-16,280.0)	3,429.0 (2,644.5-4,578.8)	0.02*
Neutrophils (cells/μL) (IQR)	1,836 (898.1-4,900)	5,371 (3,333.2-8,637.8)	0.01*
Hemoglobin (g/dL) (IQR)	11.1 (10.5-13.3)	11.1 (11.0-12.0)	0.8
Platelets (10^3/UL) (IQR)	352.0 (235.0-381.7)	245 (182-266)	0.058
Total CD3 (cells/mm^3) (IQR)	5,715.0 (1,880.0-9,848.9)	2,484.7 (2,149.6-3,269.8)	0.3
Total CD4 (cells/mm^3) (IQR)	4,093.0 (823.0-5,253.2)	928.8 (719.5-1,328.6)	0.07
Total CD8 (cells/mm^3) (IQR)	2,141.8 (894.9-3,275.4)	987.8 (708.0-1,954.7)	0.2
Total CD19 (cells/mm^3) (IQR)	1,401.9 (125.8-2,809.0)	811.4 (442.0-2,404.4)	0.7
IgG (mg/dL) (IQR)	76.0 (5.5-152.5)	10.0 (4.0-100)	0.39
IgA (mg/dL) (IQR)	8.0 (3.0-24.0)	8.0 (4.0-16.0)	0.67
IgM (mg/dL) (IQR)	204.0 (82.0-335.5)	1,026.0 (320.0-1,467.0)	0.01*
IgE (IU/mL) (IQR)	3.0 (1.0-7.0)	13.0 (0.5-13.75)	0.3

FTT, Failure to thrive; *IQR*, interquartile range; *IU*, international unit.
*Statistical significance set at *P* < .05.

lymphoproliferative diseases, and autoimmunity were more common in LRBA deficiency, suggesting that noninfectious complications differentiate these 2 CVID-like disorders. Our LRBA-deficient and ICF patients were classified primarily as suffering from CVID before performing genetic tests due to the shared immunologic features of hypogammglubulinemia, normal B-cell counts according to the European Society for Immunodeficiencies criteria. It should be emphasized that our ICF patients showed an atypical presentation without facial abnormality or neurological problems. Therefore, we would like to inform clinical immunologists that they should be aware that ICF defects can appear with incomplete features, and *DNMT3B* and *ZBTB24* genes, as well as *LRBA*, should be investigated in patients with a tentative diagnosis of CVID.

The most frequent mutation in our patients with HIgM syndrome was a hemizygous mutation in the tumor necrosis factor-homology domain of *CD40L*, similar to the reports from Western countries.[25] The increased proportion of *AICDA* mutations in our HIgM syndrome cohort (25%) was mainly due to

the high rate of consanguinity in our cohort. All *AICDA* mutations were located in the cytidine monophosphate deaminase domain similar to previous reports from Western countries.[25]

RTIs were the most common clinical manifestations in our CD40L- and AID-deficient patients, similar to previous reports.[35-39] Although lower RTIs such as pneumonia were the most observed complications in CD40L-deficient patients,[35-40] upper RTIs were the most common presentation in our AID-deficient patients.[41,42] Chronic diarrhea was a common finding among our CD40L-deficient patients, similar to previous reports of 53%, 57%, and 65% as reported by Tang et al,[37] Madkaikar et al,[40] and Wang et al,[43] respectively. However, this complication was lower[39] or even absent[38] in some studies, possibly reflecting local differences in gastrointestinal infection rates. The frequency of different clinical manifestations in our CD40L- and AID-deficient patients was similar to that in the cohort reported by the Latin American Society for Immunodeficiencies registry.[36] Overall, most clinical manifestations and the severity of symptoms were more pronounced in CD40L-deficient patients

compared with AID-deficient patients, demonstrating that patients with *CD40L* mutation need to be given more attention for managing and to predict severe clinical manifestations, because we identified more severe clinical manifestations and complications in CD40L-deficient patients compared with AID-deficient patients. This point answers the question why CD40L-deficient patients were diagnosed earlier than the AID-deficient patients. However, lymphadenopathy was present in most AID-deficient patients, being significantly higher than in CD40L-deficient patients, similar to previous studies.[36,42] Thus, these data could discriminate diagnosis between CD40L- and AID-deficient patients, because it suggests the presence of severe clinical manifestations (especially lower RTIs) associated with neutropenia in CD40L-deficient patients, and the presence of milder clinical manifestations (upper RTIs) and lymphadenopathy in AID-deficient patients.

This could provide an explanation for the earlier diagnosis of our CD40L-deficient patients. The presence of severe clinical manifestations associated with neutropenia in CD40L-deficient patients, and lymphadenopathy in AID-deficient patients, allows differentiations of these 2 disorders. There was no phenotype-genotype correlation in our cohort nor in the Latin American Society for Immunodeficiencies report[36] or in North American and European publications.[39,41]

We have reported here 12 novel mutations in 15 patients with PAD, of which 4 were nonsense and frameshift mutations (p.K515X and p.D531VfsX5 in *BTK*, p.Q31X in *µ heavy chin*, and p.S89TfsX6 in *CD40L* genes). Moreover, 2 deleterious variants affecting the splicing site of *BTK* (IVS 8-2 delA) and *AICDA* (IVS 4-1 C>A) were categorized as the PVS1 level of evidence. Of note, the deleterious effect of both splice acceptor site variants of *BTK* and *AICDA* have been known in intron 8 and 4, respectively. Among the remaining 6 novel variants, p.A508T, p.R544M, p.Y598N, and p.M509L in the *BTK* gene and p.G144V in *CD40L* were the same amino acid change as a previously established pathogenic variant and considered as ACMG strong evidence (Tables I and III, IDbase, Clinvar, and HGMD databases). However, not only the remaining novel variant (AICDA, p.D96V) but also the above-mentioned variants should be considered for protein expression assays.

Overall, patients with PAD who presented with a high disease severity were found to have a higher mortality rate than those with less disease severity. A higher mortality was also observed in patients who had severe mutations when identified variants were analyzed on the basis of predicted severity into severe and mild groups. However, none of the comparisons for mortality rates was significant, most likely due to insufficient patient numbers. Altogether the prognostic value of genetic diagnoses observed in our study was sufficient to rationalize genetic evaluation for the patients with PAD. Our recent findings on stepwise standard genetic approach to patients with PAD showed that patients without genetic diagnosis are more with the clinical diagnosis of agammaglobulinemia and they have a late age of presentation, no other affected family members with a less progressive form of PAD, and their immunologic pattern of B-cell subset revealed postgerminal center impairment.[15]

CONCLUSIONS

We have evaluated genotype-phenotype correlation and impact of mutation severity in demographic, clinical, laboratory data, and mortality of the most common monogenic PADs. A strong correlation between the severity of the mutation and meningitis and chronic diarrhea was identified in patients with BTK deficiency. We also emphasized that paralytic polio as a result of OPV is more frequent in the µ heavy chain–deficient patients compared with the BTK-deficient patients. Medical severity was significantly higher in patients with *CD40L* mutations compared with patients with *AICDA* variants. According to the major genetic defects underlying CVID, patients should be evaluated for LRBA- and ICF-associated genes. These 2 monogenic patients with PAD can be further characterized on the basis of their first presentation (in ICF patients are respiratory infections, and in LRBA deficiency nonrespiratory complications). Regarding ICF patients, it should be noted that our results are limited to those presenting with CVID, and might not be generalized to all ICF patients. Differences in the clinical and immunologic spectrum of patients with a defect in the same gene could be due to different types of mutations or genetic and nongenetic modifiers, including modifier genes, allelic variation, environmental factors, and complex genetic and environmental interactions. The comprehensive comparisons of the present study are helpful for clinical decision making and result in a more accurate diagnosis and more effective treatment of patients with PAD-associated genetic defects.

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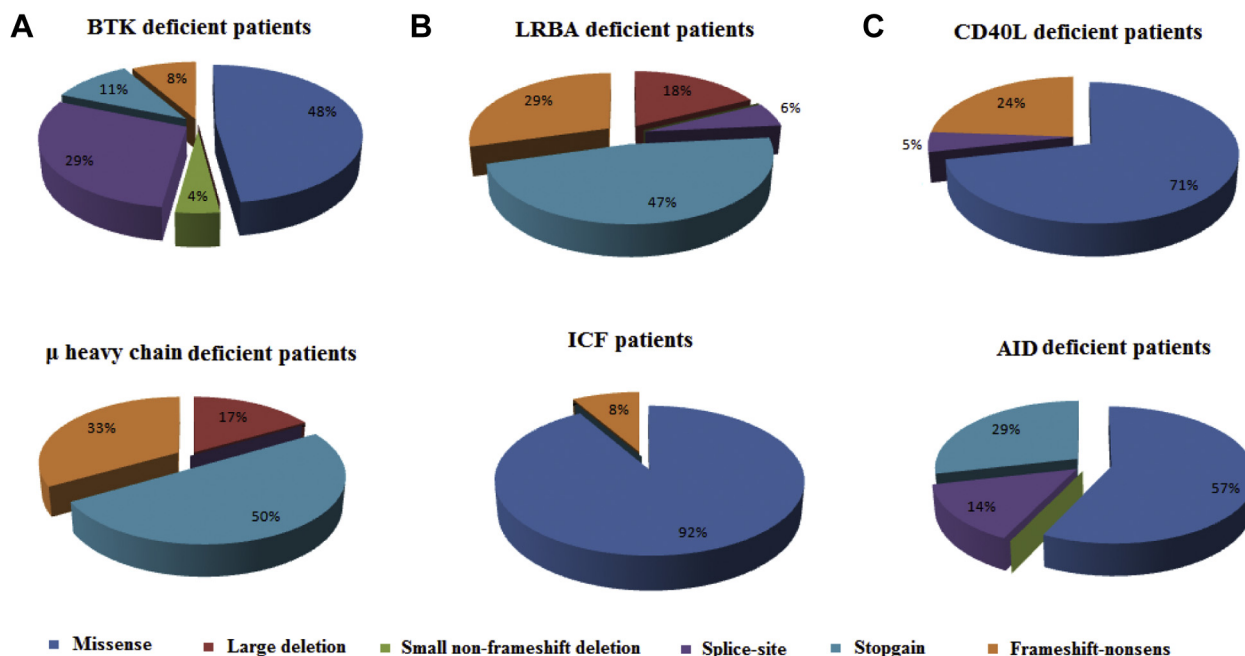
FIGURE E1. The frequency of different types of mutations. **A,** Missense and stopgain mutations are the most common type of mutations in BTK and μ heavy chain deficient patients, respectively. **B,** Stopgain and missense mutations are the most frequent type of mutations in LRBA deficient and ICF patients, respectively. **C,** Missense mutations are the most common type of mutations in CD40L and AID deficient patients, respectively.

FIGURE E2. Survival time was evaluated during the follow-up period of the study. Overall and gene defect-specific survival stratified by prediction severity were calculated using the Kaplan-Meier method. Differences among survival curves were assessed by the log-rank test.

TABLE E1. Comparison of demographic data in patients with mild and severe *BTK* and *μ heavy chain* mutations

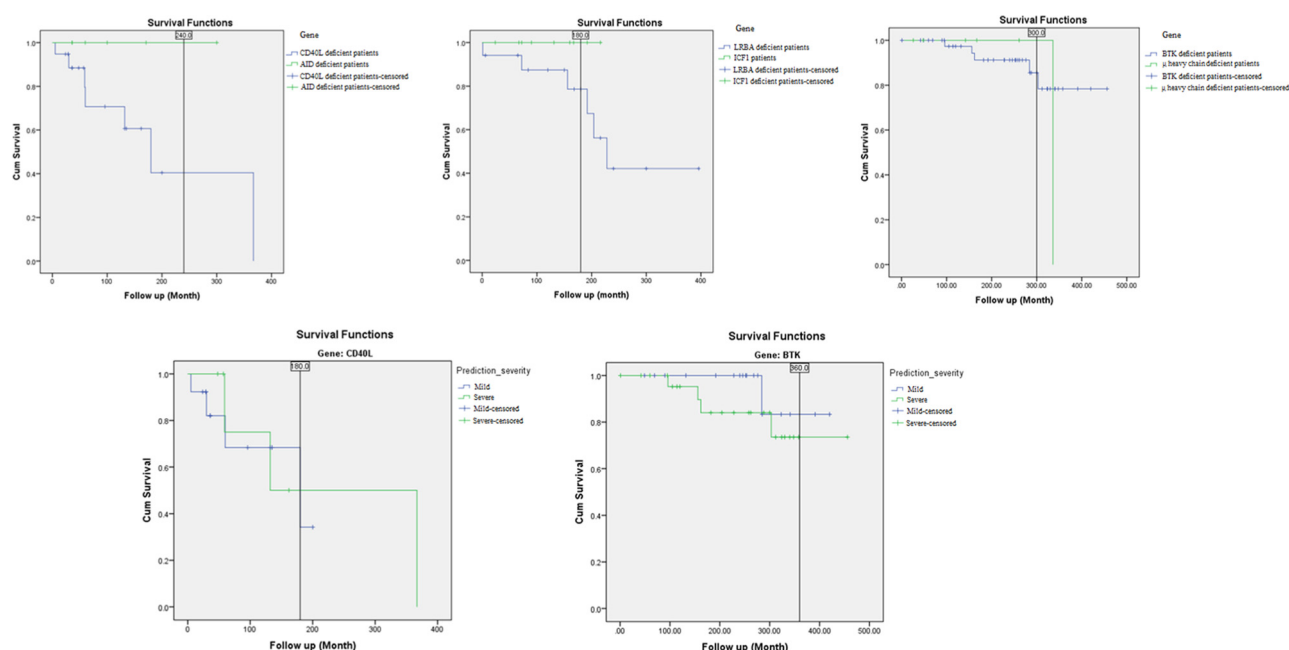| Parameter	Mild *BTK* mutations	Severe *BTK* mutations	*P* value	Severe *BTK* mutations	Severe *μ heavy chain* mutations	*P* value
Current age (y) (IQR)	22.0 (16.2-24.0)	23.0 (14.0-28.0)	0.7	23.0 (14.0-28.0)	13.0 (4.5-23.5)	0.07
Age at onset of symptoms (mo) (IQR)	12.0 (6.0-24.0)	12.0 (6.0-36.0)	1.0	15.0 (6.0-36.0)	7.0 (2.7-12.0)	0.1
Age at diagnosis (mo) (IQR)	58.0 (45.0-147.0)	64.5 (34.5-99.0)	0.83	64.5 (34.5-99.0)	13.0 (3.2-16.5)	0.001*
Diagnostic delay (mo) (IQR)	43.0 (19.5-113.2)	34.5 (16.5-65.5)	0.5	34.5 (16.5-65.5)	3.5 (0.7-12.2)	0.008*
Consanguinity, n (%)	11 (52.3)	7 (25.9)	0.14	7 (25.9)	6 (100.0)	0.002*

IQR, Interquartile range.
*Statistical significance set at *P* < .05.

TABLE E2. Comparison of involved organs in patients with BTK and μ heavy chain deficiencies

Parameter	BTK deficiency	μ Heavy chain deficiency	*P* value
Upper respiratory tract, n (%)	39 (81.2)	3 (50.0)	0.1
Lower respiratory tract, n (%)	35 (72.9)	6 (83.3)	1.0
Ophthalmologic, n (%)	17 (35.4)	1 (16.6)	0.4
Urinary tract, n (%)	4 (8.3)	1 (16.6)	0.4
Cardiovascular, n (%)	3 (6.2)	0.0 (0.0)	1.0
Hematologic, n (%)	9 (18.7)	2 (33.3)	1.0
Gastrointestinal, n (%)	20 (41.6)	3 (50.0)	1.0
Rheumatologic, n (%)	18 (37.5)	0 (0.0)	0.08
Dermatologic, n (%)	14 (29.1)	1 (16.6)	1.0
Multiple sites, n (%)	38 (79.1)	6 (100.0)	0.5

TABLE E3. Comparison of clinical and laboratory data in patients with mild and severe *BTK* and *μ heavy chain* mutations

Parameter	Mild *BTK* mutations	Severe *BTK* mutations	*P* value	Severe *BTK* mutations	Severe *μ heavy chain* mutations	*P* value
First presentation with RTIs, n (%)	14 (66.6)	18 (66.6)	1.0	18 (66.6)	5 (83.4)	0.6
First presentation with non-RTIs, n (%)	7 (36.8)	9 (36.0)	1.0	9 (36.0)	1 (16.6)	0.6
First presentation with upper RTIs, n (%)	12 (57.1)	8 (29.6)	0.05	8 (29.6)	3 (50.0)	0.3
First presentation with lower RTIs, n (%)	3 (14.2)	10 (37.0)	0.07	10 (37.0)	3 (50.0)	0.6
Otitis media, n (%)	12 (57.1)	14 (51.8)	0.57	14 (51.8)	1 (16.6)	0.3
Sinusitis, n (%)	14 (70.0)	16 (59.2)	0.44	16 (59.2)	1 (16.6)	0.1
Pneumonia, n (%)	14 (73.6)	19 (70.3)	0.8	19 (70.3)	5 (83.4)	1.0
Bronchiectasis, n (%)	6 (30.0)	9 (33.3)	0.8	9 (33.3)	1 (16.6)	1.0
Medical severity, n (%)	2 (9.5)	14 (51.8)	0.002*	14 (51.8)	6 (100)	0.06
Clubbing, n (%)	5 (25.0)	4 (14.8)	0.46	4 (14.8)	1 (16.6)	0.5
Autoimmunity, n (%)	2 (9.5)	3 (11.1)	1.0	2 (9.5)	2 (33.3)	0.2
Splenomegaly, n (%)	2 (9.5)	2 (7.4)	1.0	2 (7.4)	1 (16.6)	0.4
Hepatomegaly, n (%)	2 (9.5)	3 (11.1)	1.0	3 (11.1)	1 (16.6)	1.0
Lymphoproliferative, n (%)	5 (23.8)	3 (11.1)	0.2	3 (11.1)	1 (16.6)	1.0
Allergy, n (%)	3 (14.2)	0 (0.0)	0.07	0 (0.0)	1 (16.6)	0.1
Chronic diarrhea, n (%)	3 (14.2)	11 (40.7)	0.04*	11 (40.7)	1 (16.6)	0.3
Conjunctivitis, n (%)	5 (23.8)	8 (26.9)	0.65	8 (26.9)	1 (16.6)	0.6
Meningitis, n (%)	3 (14.2)	13 (48.1)	0.01*	13 (48.1)	0 (0.0)	0.06
Paralysis following vaccination, n (%)	1 (4.7)	0 (0.0)	0.43	0 (0.0)	4 (66.6)	<0.001*
Leukocytes (cells/μL) (IQR)	9,080.0 (7,110.0-11,675.0)	9,800.0 (7,400.0-14,517.5)	0.58	9,800.0 (7,400.0-14,517.5)	1,4615.0 (6,875.0-80,908.0)	0.2
Lymphocytes (cells/μL) (IQR)	3,565.0 (2,800.5-4,719.0)	3,500.0 (2,169.5-5,110.0)	0.5	3,500.0 (2,169.5-5,110.0)	7,738.0 (2,365.0-1,067.9)	0.1
Neutrophils (cells/μL) (IQR)	4,039.0 (2,744.7-6,498.0)	5,689.5 (2,363.7-8,768.7)	0.4	5,689.5 (2,363.7-8,768.7)	2,487.0 (1,376.0-4,672.0)	0.1
Hemoglobin (g/dL) (IQR)	11.0 (10.0-13.0)	12.0 (12.0-13.0)	0.3	12.0 (12.0-13.0)	11.0 (10.0-14.0)	0.5
Platelets (10^3/μL) (IQR)	351.0 (308.7-436.5)	381.0 (214.0-584.5)	0.84	381.0 (214.0-584.5)	485.0 (345.0)	0.4
Total CD3 (cells/mm^3) (IQR)	3,314.0 (2,516.0-4,305.0)	3,012.0 (1,623.2-4,133.7)	0.4	3,012.0 (1,623.2-4,133.7)	7,118.0 (2,199.0)	0.1
Total CD4 (cells/mm^3) (IQR)	1,619.0 (825.0-2,527.0)	1,405.0 (710.0-2,390.5)	0.5	1,405.0 (710.0-2,390.5)	3,404.0 (378.0-5,019.0)	0.4
Total CD8 (cells/mm^3) (IQR)	997.0 (795.0-2,226.0)	1,348.0 (691.0-1,707.5)	0.9	1,348.0 (691.0-1,707.5)	3,404.0 (1,726.0-4,485.0)	0.01*
Total CD19 (cells/mm^3) (IQR)	0.0 (0.0-14.2)	0.5 (0.0-1.0)	0.4	0.5 (0.0-1.0)	77.0 (0.0-106.0)	0.05
IgG (mg/dL) (IQR)	110 (39.0-320)	125.0 (82.5-298.5)	0.5	125.0 (82.5-298.5)	42.0 (13.75-369.0)	0.3
IgA (mg/dL) (IQR)	1.0 (0.0-15.0)	5.5 (0.0-17.75)	0.89	5.5 (0.0-17.75)	0.0 (0.0-5.2)	0.1
IgM (mg/dL) (IQR)	14.5 (4.0-31.5)	19.0 (2.5-39.0)	0.8	19.0 (2.5-39.0)	2.0 (0.0-4.2)	0.01*

IQR, Interquartile range.
*Statistical significance set at *P* < .05.

TABLE E4. Comparison of involved organs in patients with mild and severe *BTK* and *μ heavy chain* mutations

Parameter	Mild *BTK* mutations	Severe *BTK* mutations	*P* value	Severe *BTK* mutations	Severe *μ heavy chain* mutations	*P* value
Upper RTIs, n (%)	18 (85.7)	21 (77.7)	0.7	21 (77.7)	3 (50.0)	0.3
Lower RTIs, n (%)	15 (75.0)	20 (74.0)	0.8	20 (74.0)	5 (83.3)	1.0
Ophthalmologic, n (%)	7 (33.3)	11 (40.7)	0.5	11 (40.7)	1 (16.6)	0.3
Urinary tract, n (%)	1 (5.0)	3 (11.1)	0.6	3 (11.1)	1 (16.6)	1.0
Hematologic, n (%)	7 (33.3)	7 (25.9)	0.7	7 (25.9)	2 (33.3)	1.0
Gastrointestinal, n (%)	6 (28.5)	14 (51.8)	0.1	14 (51.8)	3 (50.0)	1.0
Rheumatologic, n (%)	9 (42.8)	10 (37.0)	0.6	10 (37.0)	0 (0.0)	0.1
Dermatologic, n (%)	8 (40.0)	6 (22.2)	0.2	6 (22.2)	1 (16.6)	1.0
Multiple sites, n (%)	15 (71.4)	24 (88.8)	0.15	24 (88.8)	6 (100)	1.0

TABLE E5. Comparison of involved organs in patients with LRBA deficiency and ICF syndrome

Complicated organs	LRBA deficiency	ICF syndrome	*P* value
Upper RTIs, n (%)	14 (82.3)	12 (100.0)	.2
Lower RTIs, n (%)	14 (82.3)	8 (66.6)	.4
Ophthalmologic, n (%)	5 (29.4)	2 (16.6)	.6
Urinary tract, n (%)	3 (17.6)	3 (25.0)	.6
Cardiovascular, n (%)	1 (5.8)	3 (25.0)	.2
Hematologic, n (%)	6 (35.2)	2 (16.6)	.4
Gastrointestinal, n (%)	16 (94.1)	4 (33.3)	.001*
Rheumatologic, n (%)	7 (41.1)	3 (25.0)	.4
Dermatologic, n (%)	9 (52.9)	4 (33.3)	.2
Multiple sites, n (%)	16 (94.1)	7 (58.3)	.05

*Statistical significance set at $P < .05$.

TABLE E6. Comparison of demographic data in patients with mild and severe ICF-associated and *LRBA* mutations

Parameter	Mild ICF-associated mutations (6)	Severe ICF-associated mutations (6)	P value	Severe *LRBA* mutations (17)	Severe ICF mutations (6)	P value
Current age (y) (IQR)	8.5 (2.3-15.0)	17.0 (12.0-26.5)	0.06	16.0 (11.5-21.5)	17.0 (12.0-26.5)	0.5
Age at onset of symptoms (mo) (IQR)	6.0 (2.0-9.0)	4.0 (0.7-5.7)	0.3	24.0 (6.5-24)	4.0 (0.7-5.7)	0.01*
Age at diagnosis (mo) (IQR)	25.0 (7.0-44.5)	23.5 (8.2-80.7)	0.8	84.0 (54.0-138.0)	23.5 (8.2-80.7)	0.05
Diagnostic delay (mo) (IQR)	21.0 (2.2-45.7)	21.7 (8.1-75.5)	0.6	60.0 (42.0-96.0)	21.7 (8.1-75.5)	0.1
Consanguinity, n (%)	6 (100.0)	5 (83.3)	1.0	17 (100.0)	5 (83.3)	0.2

IQR, Interquartile range.
*Statistical significance set at $P < .05$.

TABLE E7. Comparison of clinical and laboratory data in patients with mild and severe ICF-associated and *LRBA* mutations

Clinical manifestation	Mild ICF-associated mutations (6)	Severe ICF-associated mutations (6)	P value	Severe *LRBA* mutations (17)	Severe ICF mutations (6)	P value
First presentation with upper RTIs, n (%)	3 (50.0)	4 (66.6)	1.0	4 (23.5)	4 (66.6)	0.3
First presentation with lower RTIs, n (%)	2 (33.3)	2 (33.3)	1.0	3 (17.6)	2 (33.3)	0.5
First presentation with RTIs, n (%)	5 (83.3)	6 (100.0)	1.0	7 (41.2)	6 (100.0)	0.01*
First presentation with non-RTIs, n (%)	1 (16.6)	0 (0.0)	1.0	10 (58.8)	0 (0.0)	0.01*
Medical severity, n (%)	3 (50.0)	4 (66.6)	1.0	15 (88.2)	4 (66.6)	0.2
Otitis media, n (%)	4 (80)	2 (33.3)	1.0	11 (64.7)	2 (33.3)	0.6
Sinusitis, n (%)	3 (60)	2 (33.3)	1.0	12 (70.5)	2 (33.3)	0.3
Pneumonia, n (%)	5 (100)	3 (50.0)	1.0	13 (76.5)	3 (50.0)	0.5
Bronchiectasis, n (%)	1 (16.6)	1 (16.6)	1.0	11 (64.7)	1 (16.6)	0.06
Clubbing, n (%)	1 (16.6)	0 (0.0)	1.0	11 (64.7)	0 (0.0)	0.01*
Autoimmunity, n (%)	1 (16.6)	3 (50.0)	0.5	13 (76.4)	3 (50.0)	0.3
Splenomegaly, n (%)	1 (16.6)	2 (33.3)	1.0	13 (76.4)	2 (33.3)	0.1
Hepatomegaly, n (%)	1 (16.6)	1 (16.6)	1.0	10 (62.5)	1 (16.6)	0.1
Allergy, n (%)	0 (0.0)	1 (16.6)	1.0	6 (35.2)	1 (16.6)	0.6
FTT, n (%)	1 (16.6)	2 (33.3)	1.0	6 (35.2)	2 (33.3)	1.0
Lymphoproliferation, n (%)	1 (16.6)	2 (33.3)	1.0	14 (82.3)	2 (33.3)	0.04*
Neutropenia, n (%)	0 (0.0)	1 (16.6)	1.0	3 (17.6)	1 (16.6)	1.0
Chronic diarrhea, n (%)	1 (16.6)	2 (33.3)	1.0	15 (88.2)	2 (33.3)	0.02*
Leukocytes (cells/µL) (IQR)	9,300 (7,700-10,850)	5,700 (3,850-8,800)	0.1	8,300 (5,050-11,850)	5,700 (3,850-8,800)	0.2
Lymphocytes (cells/µL) (IQR)	3,842.0 (3,222.0-5,522.0)	1,932.0 (1,056.5-2,109.0)	0.009*	2,385.0 (1,878.0-3,542.0)	1,932.0 (1,056.5-2,109.0)	0.05
Neutrophils (cells/µL) (IQR)	4,784.0 (2,306.0-5,897.5)	2,397.0 (372.6-3,925.5)	0.1	3,450 (2,469-6,673)	2,397.0 (372.6-3,925.5)	0.05
Platelets (10^3/µL) (IQR)	495.0 (481.0-676.0)	212.0 (154.0-289.0)	0.05	239.5 (107.7-294.2)	212.0 (154.0-289.0)	0.8
Total CD3 (cells/mm^3) (IQR)	2,819.5 (2,426.5-3,190.0)	1,391.0 (1,298.0-1,504.0)	0.03*	1,747.5 (1,510.8-3,323.2)	1,391.0 (1,298.0-1,504.0)	0.04*
Total CD4 (cells/mm^3) (IQR)	1,719.5 (1,202.0-1,970.0)	600.0 (328.0-798.0)	0.03*	760.0 (421.0-1,177.8)	600.0 (328.0-798.0)	0.4
Total CD8 (cells/mm^3) (IQR)	1,180.5 (942.7-1,234.5)	717.0 (706.0-985.0)	0.07	1,021.0 (750.2-1,928.0)	717.0 (706.0-985.0)	0.3
Total CD19 (cells/mm^3) (IQR)	461.5 (287.5-810.9)	445.7 (386.4-570.0)	1.0	155.5 (88.2-393.2)	46.0 (386.0-570.0)	0.07
IgG (mg/dL) (IQR)	55.0 (33.5-539.0)	475.0 (69.5-553.0)	0.4	310.0 (62.0-440.0)	475.0 (69.5-553.0)	0.4
IgA (mg/dL) (IQR)	1.0 (0.5-27.5)	11.0 (3.5-48.5)	0.4	7.0 (0.0-28.0)	11.0 (3.5-48.5)	0.4
IgM (mg/dL) (IQR)	28.0 (12.5-45.0)	30.0 (3.5-73.5)	0.9	44.0 (22.0-149.0)	30.0 (3.5-73.5)	0.1

FTT, Failure to thrive; *IQR*, interquartile range.
*Statistical significance set at $P < .05$.

TABLE E8. Comparison of involved organs in patients with mild and severe ICF-associated and *LRBA* mutations

Complicated organs	Mild ICF- associated mutations (6)	Severe ICF- associated mutations (6)	*P* value	Severe *LRBA* mutations (17)	Severe ICF mutations (6)	*P* value
Upper RTIs, n (%)	6 (100.0)	6 (100.0)	1.0	14 (82.3)	6 (100.0)	1.0
Lower RTIs, n (%)	5 (83.3)	3 (50.0)	0.5	14 (82.3)	3 (50.0)	0.2
Ophthalmologic, n (%)	1 (16.6)	1 (16.6)	1.0	5 (29.4)	1 (16.6)	1.0
Urinary tract, n (%)	0 (0.0)	3 (50)	0.1	3 (17.6)	3 (50)	0.2
Cardiovascular, n (%)	2 (33.3)	1 (16.6)	1.0	1 (5.8)	1 (16.6)	0.4
Hematologic, n (%)	3 (50)	2 (33.3)	1.0	6 (35.2)	2 (33.3)	1.0
Gastrointestinal, n (%)	2 (50)	2 (50)	1.0	16 (94.1)	2 (50)	0.008*
Rheumatologic, n (%)	0 (0.0)	3 (50)	0.1	7 (41.1)	3 (50)	1.0
Dermatologic, n (%)	2 (50)	2 (50)	1.0	9 (52.9)	2 (50)	1.0
Multiple sites, n (%)	3 (50)	4 (66.6)	1.0	16 (94.1)	4 (66.6)	0.1

*Statistical significance set at $P < .05$.

TABLE E9. Comparison of demographic data in patients with severe and mild *CD40L* and *AICDA* mutations

Parameter	Severe *CD40L* mutations	Mild *CD40L* mutations	*P* value	Severe *AICDA* mutations	Mild *AICDA* mutations	*P* value	Severe *CD40L* mutations	Severe *AICDA* mutations	*P* value	Mild *CD40L* mutations	Mild *AICDA* mutations	*P* value
Current age (y) (IQR) and (±SD)	9.0 (5.75-20.75)	5.5 (4.25-14.75)	0.2	23.0 (14.0-30.0)	10.5 (10.0-21.0)	0.15	9.0 (5.75-20.75)	23.0 (14.0-30.0)	0.1	5.5 (4.25-14.75)	10.5 (10.0-21.0)	0.2
Age at onset of symptoms (mo) (IQR)	6.0 (3.0-11.2)	8.0 (6.5-37.0)	0.15	12.0 (4.0-132.0)	9.0 (6.0-60.0)	0.8	6.0 (3.0-11.2)	12.0 (4.0-132.0)	0.2	8.0 (6.5-37.0)	9.0 (6.0-60.0)	0.7
Age at diagnosis (mo) (IQR)	14.0 (12.0-35.7)	26.5 (14.2-70.7)	0.2	105.0 (60.0-132.0)	60.0 (20.0-96.0)	0.1	14.0 (12.0-35.7)	105.0 (60.0-132.0)	0.02*	26.5 (14.2-70.7)	60.0 (20.0-96.0)	0.3
Diagnostic delay (mo) (IQR)	7.5 (1.5-32.7)	10.0 (0.5-32.5)	0.8	56.0 (0.0-93.0)	36.0 (14.0-51.0)	0.5	7.5 (1.5-32.7)	56.0 (0.0-93.0)	0.3	10.0 (0.5-32.5)	36.0 (14.0-51.0)	0.2
Consanguinity, n (%)	5 (83.3)	7 (46.6)	0.1	3 (100)	4 (100)	1.0	5 (83.3)	3 (100)	1.0	7 (46.6)	4 (100)	0.1
Positive family history, n (%)	3 (50.0)	8 (53.3)	1.0	1 (33.3)	2 (50.0)	1.0	3 (50.0)	1 (33.3)	1.0	8 (53.3)	2 (50.0)	1.0

IQR, Interquartile range.
*Statistical significance set at $P < .05$.

TABLE E10. Comparison of involved organs in patients with CD40L and AID deficiencies

Parameter	CD40L deficiency (21)	AID deficiency (7)	*P* value
Upper RTIs, n (%)	14 (66.6)	6 (85.7)	1.0
Lower RTIs, n (%)	16 (76.1)	4 (57.1)	0.3
Urinary tract, n (%)	5 (23.0)	1 (14.2)	1.0
Hematologic, n (%)	16 (76.1)	4 (57.1)	0.37
Gastrointestinal, n (%)	12 (57.1)	1 (14.2)	0.08
Rheumatologic, n (%)	5 (23.8)	1 (14.2)	1.0
Dermatologic, n (%)	6 (28.5)	0 (0.0)	0.2
Multiple sites, n (%)	18 (76.1)	5 (71.4)	0.5

TABLE E11. Comparison of clinical and laboratory data in patients with severe and mild *CD40L* and AICDA mutations

Parameter	Severe *CD40L* mutations	Mild *CD40L* mutations	P value	Severe *AICDA* mutations	Mild *AICDA* mutations	P value	Severe *CD40L* mutations	Severe *AICDA* mutations	P value	Mild *CD40L* mutations	Mild *AICDA* mutations	P value
Medical severity, n (%)	6 (100.0)	12 (80.0)	0.5	2 (66.6)	0 (0.0)	0.1	6 (100.0)	2 (66.6)	0.3	12 (80.0)	0 (0.0)	0.009*
Sinusitis, n (%)	1 (16.6)	6 (40.0)	0.3	1 (33.3)	3 (75.0)	0.4	1 (16.6)	1 (33.3)	1.0	6 (40.0)	3 (75.0)	0.2
Otitis media, n (%)	3 (50.0)	8 (53.3)	1.0	3 (66.6)	4 (100.0)	0.4	3 (50.0)	3 (66.6)	1.0	8 (53.3)	4 (100.0)	0.2
Pneumonia, n (%)	5 (83.3)	11 (73.3)	1.0	2 (66.6)	1 (25.0)	1.0	5 (83.3)	2 (66.6)	1.0	11 (73.3)	1 (25.0)	0.4
Bronchiectasis, n (%)	1 (16.6)	1 (6.6)	0.5	1 (33.3)	1 (25.0)	1.0	1 (16.6)	1 (33.3)	1.0	1 (6.6)	1 (25.0)	0.2
Autoimmunity, n (%)	2 (33.3)	2 (13.3)	0.5	0 (0.0)	0 (0.0)	1.0	2 (33.3)	0 (0.0)	0.5	2 (13.3)	0 (0.0)	1.0
Splenomegaly, n (%)	2 (33.3)	2 (13.3)	0.5	0 (0.0)	1 (25.0)	1.0	2 (33.3)	0 (0.0)	0.5	2 (13.3)	1 (25.0)	0.5
Hepatomegaly, n (%)	2 (33.3)	1 (6.6)	0.1	0 (0.0)	1 (25.0)	1.0	2 (33.3)	0 (0.0)	0.1	1 (6.6)	1 (25.0)	1.0
Lymphoproliferation, n (%)	3 (50.0)	6 (26.6)	1.0	1 (33.3)	1 (25.0)	0.8	3 (50.0)	1 (33.3)	0.1	6 (26.6)	1 (25.0)	1.0
Allergy, n (%)	2 (9.5)	0 (0.0)	0.07	0 (0.0)	0 (0.0)	1.0	2 (9.5)	0 (0.0)	0.07	0 (0.0)	0 (0.0)	1.0
Chronic diarrhea, n (%)	2 (33.3)	9 (60.0)	0.3	0 (0.0)	1 (25.0)	1.0	2 (33.3)	0 (0.0)	0.5	9 (60.0)	1 (25.0)	0.3
Lymphadenopathy, n (%)	1 (16.6)	2 (13.3)	1.0	2 (66.6)	2 (50.0)	1.0	1 (16.6)	2 (66.6)	0.2	2 (13.3)	2 (50.0)	0.1
FTT, n (%)	1 (16.6)	6 (40.0)	0.6	2 (66.6)	1 (25.0)	0.4	1 (16.6)	2 (66.6)	0.2	6 (40.0)	1 (25.0)	1.0
Neutropenia, n (%)	5 (83.3)	7 (46.6)	0.1	0 (0.0)	0 (0.0)	1.0	5 (83.3)	0 (0.0)	0.04*	7 (46.6)	0 (0.0)	0.2
Leukocytes (cells/μL) (IQR)	21,750.0 (10,602.0-27,790)	10,860.0 (6,400.0-19,400.0)	0.2	9,300.0 (12,200.0-12,200.0)	9,200.0 (7,475.0-10,775.0)	0.1	2,1750.0 (10,602.0-27,790)	9,300.0 (12,200.0-12,200.0)	0.3	10,860.0 (6,400.0-19,400.0)	9,200.0 (7,475.0-10,775.0)	0.3
Lymphocytes (cells/μL) (IQR)	15,058.0 (7,562.8-19,500.0)	7,728.0 (3,828.0-12,616.0)	0.2	3,757.0 (2,958.0-4,557.0)	3,429.0 (2,110.0-4,365.0)	1.0	15,058.0 (7,562.8-19,500.0)	3,757.0 (2,958.0-4,557.0)	0.1	7,728.0 (3,828.0-12,616.0)	3,429.0 (2,110.0-4,365.0)	0.07
Neutrophils (cells/μL) (IQR)	1,868.0 (764.0-5,535.0)	1,836.0 (956.5-4,900.0)	1.0	8,506.5 (3,441.0-13,572.0)	5,371.0 (3,571.0-6,616.7)	0.6	1,868.0 (764.0-5,535.0)	8,506.5	0.1	1,836.0 (956.5-4,900.0)	5,371.0 (3,571.0-6,616.7)	0.03*
Hemoglobin (g/dL) (IQR)	11.0 (8.7-11.7)	12.0 (11.0-13.0)	0.2	11.1 (11.0-12.0)	11.5 (11.0-12.0)	0.7	11.0 (8.7-11.7)	11.1 (11.0-12.0)	0.3	12.0 (11.0-13.0)	11.5 (11.0-12.0)	0.6
Platelets (10³/μL) (IQR)	271.0 (97.0-411.0)	362.0 (280.0-3,838.0)	0.1	245.0 (170.0-263.0)	232.0 (194.0-270.0)	0.5	271.0 (97.0-411.0)	245.0 (170.0-263.0)	0.6	362.0 (280.0-3838.0)	232.0 (194.0-270.0)	0.1
Total CD3 (cells/mm³) (IQR)	13,367.0 (4,409.4-14,459.0)	3,247.4 (1,415.8-6,323.6)	0.09	2,677.8 (2,484.7-2,870.9)	2,434.3 (1,864.8-3,668.8)	0.5	13,367.0 (4,409.4-14,459.0)	2,677.8 (2,484.7-2,870.9)	0.2	3,247.4 (1,415.8-6,323.6)	2,677.8 (2,484.7-2,870.9)	0.6
Total CD4 (cells/mm³) (IQR)	7,242.7 (2,232.9-8,608.6)	3,552.0 (612.4-4,324.2)	0.1	719.5 (709.9-729.1)	999.0 (928.8-165.8)	0.08	7242.7 (2,232.9-8,608.6)	719.5 (709.9-729.1)	0.2	3,552.0 (612.4-4,324.2)	999.0 (928.8-165.8)	0.2
Total CD8 (cells/mm³) (IQR)	2,970.4 (1,349.0-4,666.00)	1,434.9 (777.7-2,400.2)	0.1	1,954.7 (1,868.4-2,041.0)	765.9 (650.1-987.7)	0.08	2,970.4 (1,349.0-4,666.00)	1,954.7 (1,868.4-2,041.0)	0.2	1,434.9 (777.7-2,400.2)	765.9 (650.1-987.7)	0.1
Total CD19 (cells/mm³) (IQR)	1,963.5 (785.7-4,027.8)	1,071.0 (109.6-6,060.6)	0.2	739.1 (384.5-109.3)	811.4 (499.5-371.5)	0.5	1,963.5 (785.7-4,027.8)	739.1 (384.5-109.3)	0.2	1,071.0 (109.6-6,060.6)	811.4 (499.5-371.5)	0.7
IgG (mg/dL) (IQR)	45.0 (0.0-151.2)	76.0 (17.0-206.0)	0.3	10.0 (4.0-100.0)	10.5 (4.0-146.7)	1.0	45.0 (0.0-151.2)	10.0	0.7	76.0 (17.0-206.0)	10.5 (4.0-146.7)	0.2
IgA (mg/dL) (IQR)	11.0 (0.0-63.5)	8.0 (4.0-22.0)	0.6	9.0	6.5 (4.2-14.0)	0.7	11.0 (0.0-63.5)	9.0	0.6	8.0 (4.0-22.0)	6.5 (4.2-14.0)	0.6
IgM (mg/dL) (IQR)	155.0 (79.2-265.5)	206.0 (80.0-360.0)	0.4	420.0	1,214.5 (496.5-1,832.0)	0.4	155.0 (79.2-265.5)	420.0	0.4	206.0 (80.0-360.0)	1,214.5 (496.5-1,832.0)	0.01*

FTT, Failure to thrive; *IQR*, interquartile range.
*Statistical significance set at *P* < .05.

TABLE E12. Comparison of involved organs in patients with severe and mild *CD40L* and *AICDA* mutations

Parameter	Severe *CD40L* mutations	Mild *CD40L* mutations	*P* value	Severe *AICDA* mutations	Mild *AICDA* mutations	*P* value	Severe *CD40L* mutations	Severe *AICDA* mutations	*P* value	Mild *CD40L* mutations	Mild *AICDA* mutations	*P* value
Upper RTIs, n (%)	5 (83.3)	12 (80.0)	1.0	2 (66.6)	4 (100)	0.4	5 (83.3)	2 (66.6)	1.0	12 (80.0)	4 (100)	1.0
Lower RTIs, n (%)	5 (83.3)	11 (73.3)	1.0	2 (66.6)	2 (50.0)	1.0	5 (83.3)	2 (66.6)	1.0	11 (73.3)	2 (50.0)	0.5
Ophthalmologic, n (%)	2 (33.3)	1 (6.6)	0.1	0 (0.0)	1 (25.0)	1.0	2 (33.3)	0 (0.0)	0.5	1 (6.6)	1 (25.0)	0.3
Urinary tract, n (%)	1 (16.6)	4 (26.6)	1.0	0 (0.0)	1 (25.0)	1.0	1 (16.6)	0 (0.0)	1.0	4 (26.6)	1 (25.0)	1.0
Hematologic, n (%)	5 (83.3)	11 (73.3)	1.0	3 (100.0)	1 (25.0)	0.1	5 (83.3)	3 (100.0)	1.0	11 (73.3)	1 (25.0)	0.1
Gastrointestinal, n (%)	3 (50.0)	9 (60.0)	1.0	0 (0.0)	1 (25.0)	1.0	3 (50.0)	0 (0.0)	0.4	9 (60.0)	1 (25.0)	0.3
Rheumatologic, n (%)	2 (33.3)	3 (20.0)	0.5	0 (0.0)	1 (25.0)	1.0	2 (33.3)	0 (0.0)	0.5	3 (20.0)	1 (25.0)	1.0
Dermatologic, n (%)	3 (50.0)	3 (20.0)	0.2	0 (0.0)	0 (0.0)	1.0	3 (50.0)	0 (0.0)	0.4	3 (20.0)	0 (0.0)	1.0
Multiple sites, n (%)	6 (100.0)	12 (80.0)	0.5	3 (100.0)	2 (50.0)	0.4	6 (100.0)	3 (100.0)	1.0	12 (80.0)	2 (50.0)	0.2