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Next Generation Sequencing Based Gene Identification Techniques as a Diagnostic Approach For Patients with the CVID Phenotype

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ABSTRACT

Objective: Common variable immunodeficiency (CVID) is a type of inborn errors of immunity (IEI) characterized by antibody deficiency, recurrent infections, autoimmunity, autoinflammation, lymphoproliferation, and malignancy, representing a broad phenotypic spectrum. Unlike other subgroups, only about 10% of CVID cases have a detectable genetic etiology, suggesting a complex inheritance pattern for this group. However, in cases with early onset, a positive family history and consanguinity with monogenic inheritance is considered likely. This study aimed to investigate the genetic etiology in patients diagnosed with CVID.

Materials and Methods: This study included 27 patients classified as CVID based on ESID diagnostic criteria, who were followed for antibody deficiency in our clinic between 2000 and 2017. Whole exome sequencing (WES) or gene panel sequencing was performed for each patient. For cases where standard analysis yielded no results, copy number variation (CNV) analysis was applied.

Results: The median age of diagnosis for patients included in the study was 11 years, with a consanguinity rate of 46%. Genetic factors were identified in 13 patients (48.1%). Whole exome sequencing was performed in 73% of the patients, while 37% underwent gene panel sequencing. In 8 cases with detected mutations, standard analysis was sufficient, but CNV analysis provided the result in 2 cases. In 2 patients, BTK mutations associated with X-linked agammaglobulinemia (XLA) were detected, and ICOS mutations were identified in another 2 patients. Heterozygous mutations in *NFKB1* and *NFKB2* were found in 2 patients without consanguinity or a family history. Mutations in *IGLL1* and *IGHM*, two known causes of autosomal recessive agammaglobulinemia, were found in 2 patients with early onset and positive family history. In one patient with EBV-associated lymphoma, an *ADA2* mutation was identified.

Conclusion: Although the efficacy of next-generation sequencing in CVID diagnosis is reported to be limited, we observed a higher prevalence of monogenic forms of CVID in our country, where consanguineous marriages are common, compared to the literature.

Keywords: Common variable immunodeficiency, next generation sequencing, whole exome sequencing

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INTRODUCTION

Common variable immune deficiency (CVID) is the most prevalent symptomatic group within inborn errors of immunity (IEI) with an estimated prevalence of 1 in 25,000 individuals (1-3). This disorder represents a heterogeneous spectrum of antibody deficiencies, the majority of which lack a defined genetic basis. Clinically, patients often present with recurrent sinopulmonary infections, autoimmune or inflammatory conditions, and neoplastic diseases. Due to its highly variable phenotype, CVID does not have a singular classification, and its diagnosis primarily relies on exclusion.

CVID is linked to a wide range of serious complications, including frequent infections, organ-specific immune-related disorders, immune dysregulation, and an increased risk of developing malignancies (4). According to diagnostic criteria established by the International Consensus (ICON) statement (4), the European Society for Immunodeficiencies (ESID) (5), and the International Union of Immunological Societies (IUIS) Expert Committee (6), key indicators of CVID include reduced serum IgG levels, often in combination with low IgM and/or IgA, poor vaccine-specific antibody responses, and the exclusion of alternative explanations for hypogammaglobulinemia. The ESID criteria further emphasize clinical aspects such as increased infection susceptibility, autoimmune disorders, granulomatous inflammation, polyclonal lymphoproliferation, and a family history of antibody deficiencies. In pediatric cases, CVID diagnosis takes into account age-related antibody deficiencies and a reduction in switched memory B cells, typically below 70% of the normal reference range for age. Notably, severe T cell impairments—such as decreased CD4+ T cell counts, abnormally low CD4+ T cell proportions for age, and lack of T cell proliferation—serve as exclusion factors for CVID (5). Patients with CVID display a broad spectrum of functional and phenotypic irregularities affecting both innate and adaptive immunity, highlighting that CVID is not a single disorder but a collection of conditions characterized by defective antibody production. The genetic diversity observed in individuals with CVID reflects the intricate biological mechanisms governing class switch recombination, B cell activation and signaling, migration, survival, and the maturation of memory B cells into long-lived plasma cells (7-10). For clinicians, an essential consideration is determining whether a patient's clinical presentation suggests an underlying genetic mutation and, if so, identifying the genes most likely responsible for the immune dysfunction.

The advent of whole exome sequencing (WES) and whole genome sequencing (WGS) has facilitated the identification of monogenic causes underlying the CVID phenotype in approximately 20%–30% of cases (7-10). These findings suggest that CVID is a complex condition that extends beyond classical Mendelian inheritance patterns. A monogenic cause is more likely in cases of early disease onset, a positive family history, or consanguinity (7-10). Here in this study, we report genetic analyses on 27 patients, originating mostly from consanguineous backgrounds, diagnosed with CVID according to ESID criteria, who were followed in two separate pediatric immunology centers in Ankara for antibody deficiency between 2000 and 2017.

MATERIALS and METHODS

Subjects were followed in the Pediatric Immunology department of Ankara University School of Medicine Department of Pediatric Immunology and Sami Ulus Research and Training Hospital between 2000 and 2017. The genetic analyses were carried out at Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases during October-December 2017. This study has been performed under the joint project of Immunologic and Genetic Characterization of Primary Immunodeficiency Syndromes, which was approved by the local ethical committee of Ankara University (04-181-56) and Research Center for Molecular Medicine of Austrian Academy of Sciences, and informed consent was obtained from all individuals and/or their legal guardians according to the Declaration of Helsinki.

Patients were diagnosed with CVID using ESID registry working criteria

Inclusion Criteria

1) At least one of the following:
   - Increased susceptibility to infections,
   - Autoimmunity,
   - Granulomatous disease
   - Unexplained polyclonal lymphoproliferation
   - Affected family member with antibody deficiency
2) Marked decrease of IgG and marked decrease of IgA with or without low IgM levels (measured at least twice; <2SD of the normal levels for their age);
3) At least one of the following:
   - Poor antibody response to vaccines (and/or absent isohaemagglutinins); i.e., absence of protective levels despite vaccination where defined

- Low switched memory B cells (<70% of age-related normal value)

4) Secondary causes of hypogammaglobulinaemia have been excluded
5) Diagnosis is established after the 4th year of life (but symptoms may be present before)
6) No evidence of profound T-cell deficiency, defined as 2 out of the following (y=year of life):
   - CD4 numbers/microliter: 2-6y <300, 6-12y <250, >12y <200
   - % Naive CD4: 2-6y <25%, 6-16y <20%, >16y <10%
   - Absent T cell proliferation

Exclusion criteria:

- Patients under 4 at the time for study
- Patients having T cell deficiency or dysfunction as defined above in the inclusion criteria
- Other known etiologies for primary or secondary antibody deficiencies.

We only diagnosed patients with CVID after the age of 4 but for some of them the symptoms were already present before that time and they were followed up until age 4 with primary antibody deficiency. Current ages of the patients are indicated in Table I. Immunologic laboratory investigations (Table II) and clinical histories were collected from the clinical record and selected manifestations of lymphoproliferative, autoinflammatory, and autoimmune complications were recorded. For purposes of the current study, these sometimes partly overlapping medical conditions are autoimmunity, bronchiectasis, cancer, lymphoma, and inflammatory bowel diseases.

Genetic Evaluation

The method has been described in detail previously in Kostel Bal et al. (11). A brief description is also added here. Genomic DNA (gDNA) was isolated from peripheral blood samples using the DNeasy Blood and Tissue Kit (Qiagen). Whole-exome sequencing (WES) involved library preparation and exome enrichment utilizing the Truseq Exome kit (Illumina) and Nextera flex v1.2, respectively, followed by paired-end sequencing on the Illumina HiSeq3000 system. From the obtained variant calls, nonsynonymous (nonsense, missense, small insertions and deletions) as well as splice-region variants (+/-8 bp from the intron/exon boundaries) were then filtered to exclude those with a minor allele frequency >0.01 in gnomAD (v2.1.1) (12).

An in-house database including sequencing data from >1200 individuals was used to further exclude recurrent variants with an allele frequency >0.01. The remaining variants were prioritized based on literature research and their Combined Annotation Dependent Depletion (CADD) pathogenicity prediction score in version GRCh37-v1.6 (13).

RESULTS

Demographic and Clinical Characteristics of Patients

We reviewed the clinical records of 27 patients from 26 unrelated families with a history of primary antibody deficiencies. Gender distribution was balanced (16 male, 11 female). Median age of disease manifestation was 5 (range: 6 months-23 years) and age of diagnosis was 11 (range: 6 months-53 years) years. All patients remained alive throughout the disease survey, receiving regular IVIG replacement and TMP-SMX prophylaxis. Two patients developed EBV-associated lymphoma and received rituximab as part of their chemotherapy scheme in addition to their regular IVIG replacement.

Genetic Characteristics

Genetic factors were identified in 13 patients (48.1%). Whole exome sequencing was performed in 73% of the patients, while 37% underwent gene panel sequencing. In 9 cases with detected mutations, standard analysis was sufficient, but CNV analysis provided the result in 2 cases. In 2 patients, *BTK* mutations associated with X-linked agammaglobulinemia (XLA) were detected. *ICOS* mutations were identified in another 2 patients. Heterozygous mutations in *NFKB1* and *NFKB2* were found in 2 patients without consanguinity or a family history. Autosomal recessive defects casing agammaglobulinemia are in fact reported very rarely; however, we detected *IGHM* and *IGLL1* mutations in a total of 4 patients. *IGLL1* defect, a known cause of autosomal recessive agammaglobulinemia, was identified in two siblings with early onset infections and positive family history. One of the patients having mutations in *IGHM* had the compound heterozygous constellation. Homozygous biallelic variant in *CD21* was deemed to be pathogenic in a patient with agammaglobulinemia. In one patient with EBV-associated lymphoma, a mutation in *ADA2* was identified. Surprisingly, we detected pathogenic mutation in the *CD40* gene in a patient having low immunoglobulin A and G levels and low switched memory B cells without lymphopenia.

Table I: The clinical and genetic characteristics of the patients

Patient	Age at first symptoms (year)	Age at diagnosis (year)	Current age (year)	Lymphoproli-feration	Autoimmunity	Infections	Consanguinity	Gender	Method	Mutations identified
P1	2.5	15	Deceased at the age 28	yes (splenomegaly)	no	HSV type 1 encephalitis, recurrent pneumonia	yes	M	panel	Heterozygous ***NFKB2*** **p.G849*fsTer14**
P2	0.5	0.5	16	no	no	Recurrent URTI and diarrhea	yes	M	WES	Homozygous ***IGLL1*** **p. G86*Ter**
P3	0.5	3.5	18	no	no	Recurrent URTI	yes	F	WES	Homozygous ***IGLL1*** **p. G86*Ter**
P4	5	13	34	no	no	Recurrent pneumonia and otitis	no	M	panel	Hemizygous ***BTK*** **p.R550L**
P5	3	7	28	yes (multiple LAP)	IBD, psoriasis	Recurrent diarrhea, tinea capitis	yes	F	WES	Homozygous ***ICOS*** **deletion of exon 2-3**
P6	10	11	31	yes (splenomegaly)	no	Recurrent pneumonia (RSV, Rhinovirus, influenza A+)	yes	F	WES	no
P7	1	11	33	yes (Hodgkin lymphoma)	ITP	Recurrent URTI	yes	F	WES	no
P8	1	3	33	no	no	Recurrent URTI	yes	M	panel	no
P9	1	1	18	no	no	Recurrent URTI	no	F	WES	Compound heterozygo-us variants in ***IGHM*** **p. Lys64GlnfsTer45** and **ENST00000637539.2 : c.649 + 1G > C**
P10	4	14	28	no	autoimmune hypothyroidism	Recurrent URTI	yes	F	WES	no
P11	14	16	25	no	no	Recurrent URTI	yes	M	panel	Homozygous ***CD21*** **p.T166A**
P12	0.5	2	10	no	no	Persistent giardiasis	yes	M	WES	Hemizygous ***BTK*** **p.EG301G**
P13	23	25	33	no	no	Recurrent diarrhea, giardiasis	no	M	WES	no
P14	10	53	Deceased at the age 60	yes (multiple systemic LAP)	ITP, AIHA	Recurrent URTI	no	F	panel	no
P15	2	5	13	no	no	Recurrent pneumonia and otitis	yes	F	WES	Homozygous ***IGHM*** **p.Lys64GlnfsTer45**

Table I continue

P16	7	10	21	no	no	Recurrent URTI	yes	F	WES	Heterozygous ***NFKB1*** **p.Cys119Arg**
P17	5	13	22	no	no	Recurrent pneumonia and otitis (Coronavirus 229E, RSV A/B, Adenovirus)	yes	M	panel	Homozygous ***CD40*** **p.Ter278AlanextTer34**
P18	5	10.5	25	no	no	Recurrent URTI, giardiasis	yes	M	WES	no
P19	14	14	30	no	no	Recurrent URTI and bronchiectasis	no	M	WES	no
P20	0.5	4	21	yes (splenomegaly)	autoimmune hypothyroidism, IBD, ITP	Recurrent pneumonia, sepsis, MIS-C (due to SARS-CoV2)	yes	M	WES	Homozygous ***ICOS*** **splice site**
P21	6	6	17	yes (multiple LAP)	no	Recurrent URTI and otitis	no	M	panel	no
P22	5	13	26	no	no	Recurrent URTI, giardiasis	no	M	panel	no
P23	43	45	50	no	no	Intracranial multiple fungal abscesses, recurrent pneumonia	yes	M	panel	no
P24	0.5	12	21	yes (splenomegaly)	no	Recurrent URTI and diarrhea	no	F	panel	no
P25	31	32	Deceased at the age of 34	yes (non-Hodgkin lymphoma)	no	Recurrent URTI	yes	F	WES	Homozygous ***ADA2*** **p.G48fs4***
P26	2	2	19	Yes (splenomegaly)	no	Recurrent URTI	no	M	WES	no
P27	18	18	29	Yes (multiple LAP)	no	URTI, ankylosing spondylitis, osteoporosis, FMF	yes	M	WES	no

AIHA: Autoimmune hemolytic anemia, **EBV:** Epstein-Barr Virus, **FMF:** Familial Mediterranean fever, **HSV:** Herpes simplex virus, **ITP:** Immune thrombocytopenic purpura, **LAP:** Lymphadenopathy, **MISC:** Multisystem Inflammatory Syndrome in Children, **RSV:** Respiratory syncytial virus, **URTI:** Upper respiratory tract infections, **WES:** Whole exome sequencing

Table II: Immunophenotyping of the patients

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23	P24	P25	P26	P27
WBC (6-17,5X10³/L)	9400	6980	6700	19900	10900	6400	7840	5400	9200	5600	4070	13100	15000	5830	10810	6200	5600	8600	5680	9900	7500	10700	21880	7690	7530	7340	12610
TNC (1-8X10³/L)	4200	3100	3090	18200	7900	4900	6870	3200	3700	3800	1870	6360	10100	2710	4660	2400	3000	5380	3880	2500	3900	6540	17800	5320	5510	4640	9250
TLC (2-17X10³/L)	3700	3230	3220	700	2000	1100	640	1700	4600	1100	1790	5320	2300	2420	5220	2900	2100	2500	1310	6700	2800	3130	1920	1620	1710	1980	2500
HEMOGLOBIN (10-14 G/DL)	12.4	10.5	11	15.8	11.9	11.9	12.1	10.5	10.0	9.7	15.9	13.1	15.6	12.9	12.1	13.8	12.9	10.5	14.9	10.5	12.7	13.0	13.8	11.4	10.5	13.8	13.5
PLATELET (150-450X 10⁹/L)	270	222	249	134	407	229	144	330	489	373	271	321	290	130	421	296	222	475	181	361	333	222	376	238	233	298	375
CD3 (%) (51-79%)	61	89	88	92	82	88	75	63	89	86	64	90	61	76	90	66	70	68	71	81	67	64	74	74	64	74	64
CD16+56+ (5-23%)	10	8	8	5	0.1	4	7	8	4	5	9	7	13	11	8	23	7	13	12	4	9	11	11	9	8	21	8
CD4 (31-54%)	28	40	45	56	48	57	36	40	53	24	34	52	28	26	47	35	37	28	37	49	30	25	38	31	34	21	36
CD8 (10-31%)	37	41	38	35	36	31	43	32	23	54	24	36	37	46	40	30	36	32	33	31	33	29	36	41	35	34	36
CD19 (14-44%)	19	1	2	1	8	5	5	5	1	5	20	1	19	7	1	9	21	18	13	14	16	20	11	14	25	14	18
CD20 (14-44%)	19	1	2	1	9	6	5	5	1	5	20	1	19	7	1	8	21	20	14	14	15	20	12	14	25	13	18
CD4+45RA+ (24-45%)	10	21	25	41	24	5	5	24	26	7	18	47	3	4	32	23	22	17	16	38	15	16	6	16	5	20	19

WBC: White blood cell, **TNC:** Total neutrophil count, **TLC:** Total lymphocyte count

Infectious Profile

Recurrent infections were the major symptomatology at the clinical presentation. Recurrent upper and lower bacterial respiratory tract infections were present in all of the patients as well as the viral infections cytomegalovirus (CMV), herpes simplex virus, SARSCoV2, RSV, and influenza A/B, which occurred in 26% of the patients (7/27). Parasites (*Giardia lamblia*; 4 patients) were also occasionally noted in patients having persistent diarrhea.

Clinical Phenotype

We next examined if subjects with autoinflammatory, autoimmune, lymphoproliferative, neoplastic, and/or gastrointestinal complications were more likely to have mutations in one or more of the genes identified in CVID, in contrast to others for whom a gene was not identified. (Table I). Various forms of autoimmunity were noted in 5 patients (18%) with no sex predominance. Lymphoproliferative symptoms were documented in 11 patients (45%), which is notably higher than the previous cohorts reported (7,9). Contrary to previous reports, we found no significant association between the presence of genetic mutations and lymphoproliferative or autoimmune manifestations ($p>0.05$).

DISCUSSION

The genetic investigation of CVID has garnered significant interest over the past two decades, as it is the most prevalent inborn error of immunity (IEI). Early research primarily focused on identifying the frequency of genetic mutations in patient cohorts. Subsequent studies characterized clinical phenotypes associated with specific gene mutations, such as *TACI* (14,15), *CTLA4* (16), *NFKB1* (17), *NFKB2* (18), *STAT3* (19), *PI3KCD* (20) and *LRBA* (21). These studies highlighted the infectious, autoimmune, and inflammatory features of these specific immune defects. In this study, we examined genetic data from our CVID cohort to assess whether specific clinical manifestations could help predict mutations in previously identified causal genes.

Among the 27 subjects analyzed, 13 (48.1%) had a detectable causative or associated genetic variant. Compared to other cohorts, this rate is relatively higher than from other countries but similar to the one from Turkey (22) reflecting the consanguineous background of our population (9,23). However, the majority of patients still lacked an identified genetic cause. Infections remained the most common complication among CVID patients, dividing them into an "infection-only" group and those with "additional non-infectious complications" (e.g., lymphoproliferation or autoimmunity). Lymphoproliferation was the most frequent non-infectious complication (45%), followed by autoimmunity (18%). Despite the previous literature data (7), we did not detect a significant enrichment of certain genetic pathologies within the patient cohort having autoimmunity and/or lymphoproliferation.

The most frequent mutations in our cohort involved the *ICOS*, *IGLL1*, and *NFKB* genes, which predominantly affect B cell function. Interestingly, no mutations were detected in genes like *CTLA4*, *STAT3*, or *LRBA*, which primarily impact T cell function. Unlike earlier studies (7-9,22,24), we strictly excluded patients with CD4 lymphopenia to focus on isolated B cell pathologies. Although this reduced the cohort size, it provided clearer insights into B cell-specific defects, which may have been underexplored previously. CVID subjects with currently identifiable gene variants, either associated with or causative of this immune defect, previously reported to have increased numbers of autoimmune manifestations, more significant respiratory disease and granulomatous changes in pathology (7). However, many patients without detectable genetic causes also shared similar clinical histories, suggesting potential contributions from metabolic, environmental, or epigenetic factors (25-28).

The diagnostic approach and criteria for CVIDs have been extensively debated within the field of clinical immunology. Recently, a combined clinical and genetic diagnostic strategy has been increasingly utilized in the management of CVID patients. However, due to the complexity and heterogeneity of the disease, genetic investigation remains a significant challenge. To address this, problem and standardize the clinical classification of our cohort we adopted the European Society for Immunodeficiencies (ESID) criteria, which do not include specific provisions for cases of agammaglobulinemia (5). Additionally, we aimed to exclude known clinical causes of low B-cell counts, particularly those identifiable via flow cytometry. Specifically, we excluded cases in which Bruton's tyrosine kinase (BTK) protein was found to be absent by flow cytometry. However, since all patients included in this study exhibited BTK protein expression, further genetic analysis was warranted. Through this approach, we identified three additional patients with *IGLL1* and *IGHM* mutations, consistent with previous studies (7,10) that have reported similar findings due to the diagnostic challenges associated with agammaglobulinemias.

Despite the relatively high prevalence of CVID among IEI, the rate of molecular genetic diagnosis remains low. Pathogenic variants are detected in only 2–10% of cases in non-consanguineous populations but can reach 54% in consanguineous populations (7,10). This disparity suggests that CVID may often result from multifactorial, digenic, or polygenic inheritance. Rare functional variants, somatic mutations, or epigenetic phenomena could also play a role in regulating B cell development and function (25, 26). While most CVID cases remain without a defined molecular cause, ongoing genetic discoveries continue to reveal the complexity of immunologic pathways essential for normal B cell development and memory maintenance. Further studies are likely to uncover digenic or polygenic causes, offering new insights into intersecting immune pathways. Understanding these genetic defects is critical for developing personalized treatments, monitoring comorbidities, and improving patient care, emphasizing the pivotal role of CVID research in advancing immunology.

Conflict of Interest

All the authors concur with the current version of the manuscript and declare no conflict of interest.

Author Contributions

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REFERENCES

1. Ameratunga R, Woon ST, Gillis D, Koopmans W, Steele R. New diagnostic criteria for common variable immune deficiency (CVID), which may assist with decisions to treat with intravenous or subcutaneous immunoglobulin. Clin Exp Immunol 2013;174(2):203-11.
2. Maffucci P, Filion CA, Boisson B, Itan Y, Shang L, Casanova JL, Cunningham-Rundles C. Genetic Diagnosis Using Whole Exome Sequencing in Common Variable Immunodeficiency. Front Immunol 2016;7:220.
3. Picard C, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. J Clin Immunol 2015;35(8):696-726.
4. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT, et al. International Consensus Document (ICON): Common Variable Immunodeficiency Disorders. J Allergy Clin Immunol Pract 2016;4(1):38-59.
5. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, et al; ESID Registry Working Party and collaborators. The European Society for Immunodeficiencies (ESID) Registry Working Definitions for the Clinical Diagnosis of Inborn Errors of Immunity. J Allergy Clin Immunol Pract 2019;7(6):1763-70.
6. Bousfiha A, Moundir A, Tangye SG, Picard C, Jeddane L, Al-Herz W, et al. The 2022 Update of IUIS Phenotypical Classification for Human Inborn Errors of Immunity. J Clin Immunol 2022;42(7):1508-20.
7. Cunningham-Rundles C, Casanova JL, Boisson B. Genetics and clinical phenotypes in common variable immunodeficiency. Front Genet 2024;14:1272912.
8. Lee TK, Gereige JD, Maglione PJ. State-of-the-art diagnostic evaluation of common variable immunodeficiency. Ann Allergy Asthma Immunol 2021;127(1):19-27.
9. Abolhassani H, Hammarström L, Cunningham-Rundles C. Current genetic landscape in common variable immune deficiency. Blood 2020;135(9):656-67.
10. Szczawińska-Popłonyk A, Ciesielska W, Konarczak M, Opanowski J, Orska A, Wróblewska J, et al. Immunogenetic Landscape in Pediatric Common Variable Immunodeficiency. Int J Mol Sci 2024;25(18):9999.
11. Bal SK, Islamoglu C, Haskologlu S, Karaman S, Jımenez-Heredıa R, Roca AS, et al. Extended Phenotype of VPS45 Defect with Additional Features of Combined Immunodeficiency and Neuromotor Developmental Delay Along with Severe Congenital Neutropenia. Asthma Allergy Immunol 2024;22:269-76.
12. McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, et al. The Ensembl Variant Effect Predictor. Genome Biol 2016;17(1):122.
13. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 2020;581(7809):434-43.
14. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 2014;46(3):310-5.
15. Salzer U, Bacchelli C, Buckridge S, Pan-Hammarström Q, Jennings S, Lougaris V. Relevance of biallelic versus monoallelic TNFRSF13B mutations in distinguishing disease-causing from risk-increasing TNFRSF13B variants in antibody deficiency syndromes. Blood 2009;113(9):1967-76.
16. Zhang L, Radigan L, Salzer U, Behrens TW, Grimbacher B, Diaz G, et al. Transmembrane activator and calcium-modulating cyclophilin ligand interactor mutations in common variable immunodeficiency: clinical and immunologic outcomes in heterozygotes. J Allergy Clin Immunol 2007;120(5):1178-85.

17. Schwab C, Gabrysch A, Olbrich P, Patiño V, Warnatz K, Wolff D, et al. Phenotype, penetrance, and treatment of 133 cytotoxic T-lymphocyte antigen 4-insufficient subjects. J Allergy Clin Immunol 2018;142(6):1932-46.
18. Lorenzini T, Fliegauf M, Klammer N, Frede N, Proietti M, Bulashevska A, et al. Characterization of the clinical and immunologic phenotype and management of 157 individuals with 56 distinct heterozygous NFKB1 mutations. J Allergy Clin Immunol 2020;146(4):901-11.
19. Klemann C, Camacho-Ordonez N, Yang L, Eskandarian Z, Rojas-Restrepo JL, Frede N, et al. Clinical and Immunological Phenotype of Patients With Primary Immunodeficiency Due to Damaging Mutations in NFKB2. Front Immunol 2019 Mar;10:297.
20. Fabre A, Marchal S, Barlogis V, Mari B, Barbry P, Rohrlich PS, et al. Clinical Aspects of STAT3 Gain-of-Function Germline Mutations: A Systematic Review. J Allergy Clin Immunol Pract 2019;7(6):1958-69.e9.
21. Jamee M, Moniri S, Zaki-Dizaji M, Olbrich P, Yazdani R, Jadidi-Niaragh F, et al. Clinical, Immunological, and Genetic Features in Patients with Activated PI3Kδ Syndrome (APDS): a Systematic Review. Clin Rev Allergy Immunol 2020;59(3):323-33.
22. Habibi S, Zaki-Dizaji M, Rafiemanesh H, Lo B, Jamee M, Gámez-Díaz L, et al. Clinical, Immunologic, and Molecular Spectrum of Patients with LPS-Responsive Beige-Like Anchor Protein Deficiency: A Systematic Review. J Allergy Clin Immunol Pract 2019;7(7):2379-86.e5.
23. Aygun A, Topyıldız E, Geyik M, Karaca NE, Durmaz A, Aksu G, et al. Current genetic defects in common variable immunodeficiency patients on the geography between Europe and Asia: a single-center experience. Immunol Res 2024;72(2):225-33.
24. Rojas-Restrepo J, Caballero-Oteyza A, Huebscher K, Haberstroh H, Fliegauf M, Keller B, et al. Establishing the Molecular Diagnoses in a Cohort of 291 Patients With Predominantly Antibody Deficiency by Targeted Next-Generation Sequencing: Experience From a Monocentric Study. Front Immunol 2021;12:786516.
25. Ramirez NJ, Posadas-Cantera S, Caballero-Oteyza A, Camacho-Ordonez N, Grimbacher B. There is no gene for CVID - novel monogenetic causes for primary antibody deficiency. Curr Opin Immunol 2021;72:176-85.
26. Del Pino-Molina L, Rodríguez-Ubreva J, Torres Canizales J, Coronel-Díaz M, Kulis M, Martín-Subero JI, et al. Impaired CpG Demethylation in Common Variable Immunodeficiency Associates With B Cell Phenotype and Proliferation Rate. Front Immunol 2019;10:878.
27. Rodríguez-Ubreva J, Arutyunyan A, Bonder MJ, Del Pino-Molina L, Clark SJ, de la Calle-Fabregat C, et al. Single-cell Atlas of common variable immunodeficiency shows germinal center-associated epigenetic dysregulation in B-cell responses. Nat Commun 2022;13(1):1779.
28. Jorgensen SF, Macpherson ME, Skarpengland T, Berge RK, Fevang B, Halvorsen B, et al. Disturbed lipid profile in common variable immunodeficiency - a pathogenic loop of inflammation and metabolic disturbances. Front Immunol 2023;14:1199727.