Diagnostic Yield and Therapeutic Consequences of Targeted Next-Generation Sequencing in Sporadic Primary Immunodeficiency

Georgios Sogkas a, b, Natalia Dubrowinskaja a, b, Katharina Schütz c, Lars Steinbrück d, Jasper Götting d, Nicolaus Schwerk c, Ulrich Baumann c, Bodo Grimbacher b, e, f, Torsten Witte a, b, Reinhold E. Schmidt a, b, Faranaz Atschekzei a, b

a Department of Rheumatology and Immunology, Hannover Medical School, Hannover, Germany; b Hannover Medical School, Cluster of Excellence RESIST (EXC 2155), Hanover, Germany; c Department of Pediatric Pneumology, Allergy and Neonatology, Hannover Medical School, Hannover, Germany; d Institut of Virology Hannover Medical School, Hannover, Germany; e Institute for Immunodeficiency, Center for Chronic Immunodeficiency (CCI), Medical Center, Faculty of Medicine, Albert-Ludwigs-University of Freiburg, Freiburg im Breisgau, Germany; f DZIF – German Center for Infection Research, Satellite Center Freiburg, Braunschweig, Germany

Abstract

Introduction: Primary immunodeficiencies (PIDs) are a heterogeneous group of disorders characterized by increased susceptibility to infections, immune dysregulation, and/or malignancy. Genetic studies, especially during the last decade, led to a better understanding of the pathogenesis of primary immunodeficiencies and contributed to their classification into distinct monogenic disorders falling under one of the >430 currently known inborn errors of immunity (IEI). The growing availability of molecular genetic testing resulted in the increasing identification of patients with IEI. Here, we evaluated the diagnostic yield and the clinical consequences of targeted next-generation sequencing (tNGS) in a cohort of 294 primary immunodeficiency patients, primarily consisting of cases with sporadic primary antibody deficiency. Method: We have custom designed a tNGS panel to sequence a cohort of PID patients. Agilent’s HaloPlex Target Enrichment System for Illumina was used for DNA target enrichment. Results: tNGS identified a definite or predicted pathogenic variant in 15.3% of patients. The highest diagnostic rate was observed among patients with combined immunodeficiency or immune dysregulation, for whom genetic diagnosis may affect therapeutic decision-making. Conclusion: Next-generation sequencing has changed diagnostic assignment and paved the way for targeted therapeutic intervention with agents directed at reverting the disease-causing molecular abnormality or its pathophysiological consequences. Therefore, such targeted therapies and identifying the genetic basis of PID can be essential for patients with manifested immune dysregulation as conventional immunomodulatory regimens may exert an immunosuppressive effect, aggravating their immunodeficiency or may only inadequately control autoimmune or lymphoproliferative manifestations.
Introduction

Primary immunodeficiencies (PIDs) or inborn errors of immunity (IEI) are heterogeneous disorders caused by a deficiency or a defect in one or more components of the immune system [1, 2]. So far, pathogenic variants in >430 genes have been reported to cause PID. However, the strictly monogenic aetiology of PID seems questionable when considering the incomplete penetrance and variable expressivity of genetic variants reported as disease-causing [3]. Variable expressivity, including variable age of onset and a broad spectrum of manifestations, ranging from “infections-only” to disease complicated with immune dysregulation and/or malignancy, makes a timely diagnosis of PID difficult, which leads to an increased rate of morbidity and mortality [4]. The clinical diagnosis of PID most commonly falls under one of the primary antibody deficiencies (PADs), especially common variable immunodeficiency (CVID) [5, 6]. In the last decade, the increasing availability of next-generation sequencing (NGS) technologies led to identifying the involvement of new genes in PADs. It aided the characterization of the molecular pathways involved in human B cell development and function [2, 7, 8]. The most common symptomatic PAD, CVID [7], is still considered a largely polygenic disorder, though according to more recent reports, the proportion of monogenic forms has increased, exceeding 20% of cases [8–10].

Genetic testing is currently routinely performed in most medical centres treating PID patients, resulting in an increasing number of monogenic IEI. Consequently, several studies of cohorts of patients with the same IEI that evaluated their phenotypes and outcomes have been published over the last couple of years, for example, identifying an increased risk of malignancies or association of particular IEI with systemic rheumatic or interstitial lung disease may alter diagnostic and follow-up procedures [11–13]. Further, in case of particular monogenic defects, individualized therapies with agents tailored to revert consequences of the disease-underlying genetic defect can be considered. For example, JAK-inhibitors may help treat immune dysregulation in the context of STAT3 or STAT1 gain-of-function [14], PI3Kδ-specific inhibition is effective against lymphoproliferative manifestation of the activated PI3Kδ syndrome [15], and abatacept may be effective in treating enteropathy and autoimmunity in patients with CTLA4 insufficiency or LRBA deficiency [16, 17].

Overall, timely molecular diagnosis and setting-specific decisions may reduce the harms associated with PIDs and favour a better disease outcome. Hence, we employed a targeted NGS (tNGS) approach to identify the genetic background of immunodeficiency in a cohort of 294 patients, most of whom were sporadic cases of a PAD, aiming at increasing the diagnostic yield and optimizing patients’ medical care.

Materials and Methods

Study Cohort

This single-centre study included a total of 294 patients with a PID, visiting the immunology outpatient clinics of either the Department of Paediatrics (N = 28) or the Department of Rheumatology and Immunology (N = 266) of the Hannover Medical School. The patients were clinically diagnosed with PID according to the European Society for Immunodeficiencies criteria (online suppl. Table 1; see www.karger.com/doi/10.1159/000519199 for all online suppl. material) was created with the help of Agilent’s SureDesign application. Blood samples were collected in the immunology outpatient clinics of Paediatrics and the Department of Rheumatology and Immunology of the Hannover Medical School. Genomic DNA was isolated from the whole peripheral lymphocyte immunophenotyping, patient’s clinical history of infections, bronchiectasis (computed tomography-confirmed), autoimmune cytopenias, such as autoimmune haemolytic anaemia, idiopathic thrombocytopenic purpura, organ-specific autoimmunity (including vitiligo, psoriasis, insulin-dependent diabetes mellitus, autoimmune thyroiditis, primary thyroid failure, atrophic gastritis, and rheumatic disease including rheumatoid arthritis, Sjögren’s syndrome, systemic lupus erythematosus, and seronegative arthritis) diagnosed according to the American College of Rheumatology [ACR]/European League Against Rheumatism [EULAR] classification criteria), benign lymphoproliferation, granulomatous disease, enteropathy, and malignancies. Benign lymphoproliferation was defined as splenomegaly (≥11 cm on palpation or ultrasound, including the previous splenectomy of an enlarged spleen) and/or persistent lymphadenopathy (on palpation, ultrasound, computed tomography, or magnetic resonance scan). Granulomatous disease was defined as at least 1 biopsy-proven unexplained granuloma, excluding Crohn’s disease-associated granulomas. Enteropathy included all cases of biopsy-proven inflammatory bowel disease (ulcerative colitis and Crohn’s disease) and intestinal hyperlymphocytosis (lymphocytic infiltration of the interepithelial mucous, the lamina propria and/or the submucosa). Malignancies included haematological and all other forms of cancer. All patients and their parents, in the case of paediatric patients, signed an informed consent form.

Targeted NGS

A customized panel of genes associated with PID (online suppl. Table 1; see www.karger.com/doi/10.1159/000519199 for all online suppl. material) was created with the help of Agilent’s web-based SureDesign application. Blood samples were collected in the immunology outpatient clinics of Paediatrics and the Department of Rheumatology and Immunology of the Hannover Medical School. Genomic DNA was isolated from the whole peripheral blood using a QIAamp DNA Blood Midi Kit (Qiagen) and quantified by a Qubit dsDNA BR Assay Kit (ThermoFisher). DNA target enrichment was performed using the Agilent’s HaloPlex Target Enrichment System for Illumina Sequencing, following the manufacturer’s instructions (Agilent’s user manual) and as already de-
scribed [19]. In brief, DNA was fragmented using a restriction enzyme master mix prepared following the kit’s protocol, and digestion was validated by gel electrophoresis. DNA fragments were hybridized to the HaloPlex probe capture library by adding the Hybridization Master Mix and Indexing Primer Cassettes. After an incubation step, the hybridized DNA fragments were captured using HaloPlex Magnetic Beads, that is, a biotin-streptavidin system and washed. In order to create circular fragments, the ends were ligated by adding the kit’s ligation master mix. Subsequently, the target libraries were amplified by polymerase chain reaction. Finally, the amplified target libraries were purified with the help of AMPure XP beads and washed in ethanol. Target enrichment was validated using an Agilent TapeStation. Samples were pooled in equimolar amounts. The libraries were subjected to denaturation by adding NaOH and diluted to a final concentration of 8–12 pM. Sequencing was performed on an Illumina MiSeq system using an Illumina v2 reagent kit, following the manufacturer’s protocol. Data analysis was performed with the help of Agilent’s SureCall software. Most likely disease-causing candidates were confirmed by Sanger sequencing. Familial segregation was examined when samples were available.

## Variants Filtering Procedure

FastQ files were aligned to the human reference genome (UCSC hg19, GRCh37) and analysed using Agilent Technologies – SureCall NGS software. Variants were selected according to criteria at the variant level, including allele frequency (AF), variant annotation, and potential functional effect. Using databases of variants (e.g., dbSNP, 1000 Genomes Project, Exome Aggregation Consortium, and Genome Aggregation Database) and disease-causing variants (HGMD and OMIM), we selected all rare or private variants with an AF of <1%. Furthermore, we kept nonsense variants, variants affecting the splice site, frameshift, in-frame indels, start or stop codon changes, as well as missense variants that were predicted to be deleterious by having a combined annotation-dependent depletion (CADD) score >15 and a mutation significance cut-off score below the CADD score. Also, variants were tested by MutationTaster [20], Provean, and Human Splicing Finder [21] for the pathogenicity.

## Statistical Analysis

For statistical calculation, we used GraphPad prism 5.00 (GraphPad, La Jolla, San Diego, CA, USA). Descriptive statistics are reported as the median and interquartile range in continuous variables and as counts and percentages for dichotomous variables. The \( \chi^2 \) test compared categorical variables. Non-categorical variables were compared with the Mann-Whitney test. All comparisons were 2-tailed, and \( p < 0.05 \) was considered significant.

## Results

### Clinical Characterization of PID Patients

Patients’ demographic data and characteristics, including the clinical diagnosis and manifestations of PID, are summarized in Table 1. Most of the 294 PID patients who underwent tNGS displayed a PAD (263/294, 89.5%), more commonly diagnosed as CVID (227/294, 77.2%).

CVID was more commonly diagnosed in adult patients, whereas combined immunodeficiencies (CIDs) were more common among patients receiving the first diagnosis of PID in childhood (online suppl. Table 2). The vast majority of studied patients had a history of recurrent infections (278/294, 94.6%), which in most cases (170/278, 61.2%) was accompanied by at least 1 non-infectious PID-associated comorbidity. Non-infectious comorbidities were more common manifestations of immune dysregulation, including autoimmunity, lymphoproliferative disorders, and asthma.

### Table 1. Characteristics of studied patients (\( N = 294 \))

| Characteristic                              | N (%)          |
|---------------------------------------------|----------------|
| Median age at diagnosis of PID \(^1\) (IQR), years | 30 (18–42.3) |
| Age group at diagnosis of PID               |                |
| 0–4 years                                   | 14 (5.1)       |
| 5–18 years                                  | 62 (22.5)      |
| >18 years                                   | 200 (72.5)     |
| Male sex                                    | 128 (43.5)     |
| Consanguinity \(^2\)                        | 7 (2.4)        |
| Familial history of PID                     | 34 (11.6)      |
| Clinical diagnosis of PID                   |                |
| CVID                                        | 227 (77.2)     |
| CID                                         | 29 (9.8)       |
| Unclassified antibody deficiency            | 21 (7.1)       |
| Isolated IgG subclass deficiency            | 7 (2.4)        |
| SPAD                                        | 4 (1.4)        |
| Agammaglobulinemia                          | 3 (1)          |
| Other                                       | 3 (1)          |
| Clinical manifestations of PID              |                |
| Recurrent respiratory tract infections       | 278 (94.6)     |
| Bronchiectasis                              | 60 (20.4)      |
| “Infections only” disease                   | 108 (36.7)     |
| Lymphadenopathy                             | 44 (15)        |
| Splenomegaly                                | 74 (25.2)      |
| Enteropathy                                 | 27 (9.2)       |
| ILD                                         | 20 (6.8)       |
| Organ-specific autoimmunity                 | 58 (19.7)      |
| Autoimmune cytopenia\(^3\)                  | 44 (15)        |
| Atopic disease\(^4\)                        | 48 (16.3)      |
| Granulomatous disease                       | 25 (8.5)       |
| Lymphoma                                    | 5 (1.7)        |
| Other malignancy                            | 24 (8.2)       |

\(^1\) CID, combined immunodeficiency; CVID, common variable immunodeficiency; ILD, interstitial lung disease; IQR, interquartile range; no, number; PID, primary immunodeficiency disorder; SPAD, specific antibody deficiency; ALHA, autoimmune haemolytic anaemia; ITP, immune thrombocytopenic purpura. Analysis based on 276/294 studied patients with the known year of diagnosis. \(^2\) Analysis included 3 patients from the same consanguineous family. \(^3\) ALHA and/or ITP. \(^4\) Atopic dermatitis and/or allergic rhinitis and/or asthma.
tive, and atopic disease. Among 294 patients, 96 (32.7%) had at least 1 autoimmune disease, and 13 (4.4%) displayed polyautoimmunity (i.e., >1 autoimmune disease). Consistent with previous studies [22, 23], idiopathic thrombocytopenic purpura was the most common autoimmune manifestation in this cohort (36/294, 12.2%), followed by autoimmune haemolytic anaemia (17/294, 5.8%) (online suppl. Fig. 1). Twenty-eight out of 294 (9.5%) patients displayed a malignant disease. Among those, 8/28 had a history of skin cancer in the form of basal cell carcinoma, 4/28 patients had B cell non-Hodgkin lymphoma, 4/28 patients had gastric adenocarcinoma, and 4/26 patients had colorectal adenocarcinoma. PID-associated clinical manifestation was similar in patients with a paediatric first diagnosis of PID (<18 years) and those diagnosed in the adulthood (online suppl. Table 2).

**Identified Variants**

Considering the AF and CADD and mutation significance cut-off scores of each identified variant, as described above, we identified 45/294 (15.3%) patients to harbour at least 1 predicted disease-causing variant in a PID-associated gene (Fig. 1). The majority of those (23/45) harboured a genetic variant, which has been previously reported as pathogenic (Table 2). In particular, we found 37 monoallelic variants in 35/294 (11.9%) patients. Among those, more common ones were variants in NFKB1 (7/37 monoallelic variants in 9/294 patients), followed by variants in STAT3 (5/37 monoallelic variants in 6/294 patients). Other PID genes with monoallelic variants included CTLA4, GATA2, NFKB2, STAT1, IRELBP2, NFKBIA, TCF3, PIK3R1, SOCS1, RELA, MYH9, and VAV1 (Fig. 2). Six biallelic variants were detected in 8/294 (2.7%) patients. Those included variants in IGLL1, LRBA, CARMIL2, DCLRE1C, and PIK3CD (Table 2) and were identified mainly in patients stemming from consanguineous families (5/8, 62.5%). One patient with CID had compound heterozygous mutations in RAG1, and a patient with X-linked agammaglobulinemia harboured a hemizygous mutation in BTK. Six novel variants of uncertain significance recognized in 5 PID-candidate genes, that is, VAV2, SEC61A2, PIK3R4, and USP8, were identified in 4/294 patients. An additional patient had 2 new variants in RELB with a yet unknown allelic phase. Finally, 21/294 (7.1%) patients had at least 1 monoallelic variant in TNFRSF13B (online suppl. Table 2), the gene encoding the transmembrane activator and calcium-modulating cyclophilin ligand interactor, which is a disease-predisposing gene [57, 58]. Phenotypes of all patients identified to harbour pathogenic or likely pathogenic variants are summarized in online suppl. Table 3.

**Factors Affecting the Diagnostic Yield of tNGS**

Overall, genetic testing utilizing tNGS identified a likely genetic diagnosis in 15.3% of studied patients. The diagnostic yield of tNGS could be different in distinct patient subgroups. We therefore evaluated the effect of fac-
| Pat.ID | Gene | Diagnosis | Transcript ID | Transcript variant | Protein variant | dbSNP-ID | gnomAD frequency | CADD score | PolyPhen2 Pred | SIFT Pred | Variant Ref. | Gene Ref. |
|--------|------|-----------|---------------|------------------|----------------|----------|-----------------|-----------|----------------|----------|-----------|-----------|
| **Biallelic variants**               |     |           |               |                  |               |          |                 |           |                |          |            |           |
| P.204 | IGLL1 | Agammaglobulinaemia | NM_020070.4 | c.[258_258del]; [258_258del] | p.[[Gln88Asnfs*7]; [[Gln88Asnfs*7]] | RS533238876 | 0.0009198 | 22 | NA | NA | 24 |
| P.71  | LRBA  | CVID      | LRG_1324      | c.[767_767+9del]; | LRG_1324p1:p.? | NA | NA | NA | 25 |
| P.219 | CARMIL2 | CID | NC_00016.10 | c.[467-1G>A]; [467-1G>A] | p.? | 26 | NA | 26 |
| P.289 | CARMIL2 | CID | NC_00016.10 | c.[467-1G>A]; [467-1G>A] | p.? | 26 | NA | 26 |
| P.208 | PIK3CD | CVID | NM_005026.5 | c.[1654_1654delG]; [1654_1654delG] | p.[Val552Serfs*26]; [Val552Serfs*26] | 24 | NA | 28 |
| P.273 | DCLRE1C (ARTEMIS) | Atypical SCID (CID) | LRG_54t1 | c.[464+1G>A]; [464+1G>A] | p.? | 30 |
| P.280 | DCLRE1C (ARTEMIS) | Atypical SCID (CID) | LRG_54t1 | c.[464+1G>A]; [464+1G>A] | p.? | | |
| **Monoallelic variants identified** |     |           |               |                  |               |          |                 |           |                |          |            |           |
| P.114 | NFKB1 | CVID | NM_003998.4 | c.[1012_1012delT]; [1012=] | p.[Ser338Leufs*94]; [[Ser338=]] | 35 | NA | NA | 19, 32 |
| P.183 | NFKB1 | CVID | NM_003998.4 | c.[1012_1012delT]; [1012=] | p.[Ser338Leufs*94]; [[Ser338=]] | 35 | NA | NA | 19, 32 |
| P.122 | NFKB1 | CVID | NM_003998.4 | c.[904dupT]; [904=] | p.[p.Ser302Phefs*7]; [[Ser302]] | 34 | NA | NA | 19, 32 |
| P.150 | NFKB1 | CVID | NM_003998.4 | c.[1726dupA]; [1726] | p.[Ile576Asnfs*6]; [[Ile576=]] | 35 | NA | NA | 19, 32 |
| P.200 | NFKB1 | CVID | NM_003998.4 | c.[875_875delG]; [875=] | p.[Gly366Aspfs*140]; [[Gly366=]] | 19, 32 |
| P.210 | NFKB1 | CVID | NM_003998.4 | c.[470G>C]; [470=] | p.[Arg157Pro]; [[Arg157=]] | 33 | Probably damaging | D |
| P.232 | NFKB1 | CVID | NM_003998.4 | c.[691C>T]; [691=] | p.[p.Arg231Cys]; [[Arg231=]] | 0.00004949 | 24 |
| P.233 | NFKB1 | CVID | NM_003998.4 | c.[691C>T]; [691=] | p.[Arg231Cys]; [[Arg231=]] | 0.00004949 | 8, 33 |
| P.254 | NFKB1 | Unclassified antibody deficiency | LRG_1316t1 | c.[731-3C>G]; [731=] | LRG_1316p1:p.|= | 17 | NA | NA | |
| P.26  | NFKB2 | CVID | NM_001077494.3 | c.[1097G>A]; [1097=] | p.[Gly366Asp]; [[Gly366=]] | 19 | NA | NA | 38 |
| P.181 | NFKB2 | CVID | NM_001077494.3 | c.[2557C>T]; [2557=] | p.[Val218Asla]; [[Val218=]] | 0.00002845 | 33 |
| P.236 | NFKBIA | CID | NM_020529.3 | c.[653T>C]; [653=] | p.[Val218Asla]; [[Val218=]] | 25 | NA | NA | 33 |
| P.154 | STAT3 | CVID | NM_003150.4 | c.[653T>C]; [653=] | p.[Val218Asla]; [[Val218=]] | 27 | NA | NA | 33 |

Table 2. Definite and predicted pathogenic genetic variants identified through tNGS in a cohort of (N = 294) PID patients.
| PatID | Gene | Diagnosis | Transcript ID | Transcript variant | Protein variant | dbSNP-ID frequency | CADD score | PolyPhen2_Pred | SIFT_Pred | Variant Ref. |
|-------|------|-----------|---------------|-------------------|---------------|------------------|------------|----------------|-----------|--------------|
| P.278 | STAT3 | CID | NM_003150.4 | c.[1276T>C]; [1276=] | p.([Cys426Arg]); [Cys426=] | 22 | Benign | T | 38 |
| P.282 | STAT3 | CVID | NM_003150.4 | c.[859T>G]; [859=] | p.([Leu287Val]); [Leu287=] | 20 | Probably damaging | D | 39 |
| P.265 | STAT3 | CID | NM_003150.4 | c.[0.2144C>T]; [2144=] | p.([Thr715Met]); [Thr715=] | 21 | Possibly damaging | T | 39–40 |
| P.227 | STAT3 | Unclassified antibody deficiency | HIES | NM_003150.4 | c.[0.1907C>T]; [1907=] | p.([Ser636Phe]); [Ser636=] | 24 | NA | NA | 12 |
| P.246 | STAT3 | CID | NM_003150.4 | c.[1198C>G]; [1198=] | p.([Leu400Val]); [Leu400=] | 27 | Probably damaging | D | 45, 14 |
| P.56 | CTLA4 | CVID | NM_005214.5 | c.[2T>C]; [2=] | p.([Val40Met]); [Val40=] | 25 | Possibly damaging | D | 41 |
| P.165 | CTLA4 | CID | NM_005214.5 | c.[118G>A]; [118=] | p.([Val40Met]); [Val40=] | 25 | Possibly damaging | D | 42, 43 |
| P.215 | CTLA4 | CVID | NM_005214.5 | c.[118G>A]; [118=] | p.([Val40Met]); [Val40=] | 25 | Possibly damaging | D | 44 |
| P.263 | IRF2BP2 | CVID | NM_001077397.1 | c.[1597T>G]; [1597=] | p.([Cys533Gly]); [Cys533=] | 24 | NA | NA | 47–48 |
| P.159 | IRF2BP2 | CVID | NM_001077397.1 | c.[1597T>G]; [1597=] | p.([Cys533Gly]); [Cys533=] | 24 | NA | NA | 48 |
| P.257 | RELA | CVID | NM_001145138.2 | c.[622G>A]; [622=] | p.([Glu208Lys]); [Glu208=] | 34 | Possibly damaging | D | 45 |
| P.257 | GATA2 | CVID | NM_001145662.1 | c.[1343C>A]; [1343=] | p.([Ser448Trp]); [Ser448=] | 25 | Possibly damaging | D | 46 |
| P.101 | STAT1 | CID | NM_007315.4 | c.[1198C>G]; [1198=] | p.([Leu400Val]); [Leu400=] | 27 | Possibly damaging | D | 47–48 |
| P.262 | STAT1 | CID | NM_007315.4 | c.[800C>T]; [800=] | p.([Ala267Val]); [Ala267=] | 45 | NA | NA | 48 |
| P.164 | TCF3 | CVID | NM_001136139.4 | c.[1604T>C]; [1604=] | p.([Leu535Pro]); [Leu535=] | 22 | NA | NA | 49 |
| P.166 | TCF3 | CVID | NM_001136139.4 | c.[1660C>T]; [1660=] | p.([Arg545Trp]); [Arg545=] | 17 | NA | NA | 50 |
| P.270 | MYH9 | CVID | NM_002473.6 | c.[3838G>A]; [3838=] | p.([Val1280Met]); [Val1280=] | 23 | NA | NA | 51 |
| P.253 | VAV1 | CVID | NM_001258206.2 | c.[29G>T]; [29=] | p.([Trp10Leu]); [Trp10=] | 29 | NA | NA | 52 |
| P.253 | VAV2 | CVID | NM_003371.4 | c.[2327C>A]; [2327=] | p.([Pro776His]); [Pro776=] | 27 | NA | NA | 53 |
| P.294 | GATA2 | Unclassified antibody deficiency | CID | NM_001145662.1 | c.[481C>G]; [481=] | p.([Pro161Ala]); [Pro161=] | 26 | NA | NA | 54 |
| P.267 | SEC61A2 | CID | NM_001142628.1 | c.[718C>T]; [718=] | p.([Arg240Cys]); [Arg240=] | 29 | NA | NA | 55 |
| P.63 | PIK3R1 | CVID | NC_000005.9 | c.[635-11T>A]; [635=] | p.([Trp10Cys]); [Trp10=] | 16 | NA | NA | 56 |
| P.63 | GATA2 | CVID | NM_001145662.1 | c.[30G>C]; [30=] | p.([Trp10Cys]); [Trp10=] | 27 | NA | NA | 57 |
| P.246 | STAT3 | CID | NM_003150.4 | c.[0.1907C>T]; [1907=] | p.([Ser636Phe]); [Ser636=] | 24 | NA | NA | 58 |

**Table 2 (continued)**
### Table 2 (continued)

| Pat.ID | Gene | Diagnosis | Transcript ID | Transcript variant | Protein variant | dbSNP-ID | gnomAD frequency | CADD score | PolyPhen2-Pred | SIFT-Pred | Variant<sup>a</sup> Gene<sup>b</sup> |
|--------|------|-----------|---------------|-------------------|-----------------|----------|-----------------|------------|--------------|----------|----------------|
| P.127  | PIK3R4 | Unclassified antibody deficiency <br>CVID | NM_014602.3 | c.[1039C>T]; [1039=] | p.[Arg347Trp]; ([Arg347]) | 0.0013 | 32 | Possibly damaging | T |
| P.124  | SOCS1 | CVID | NM_003745.2 | c.[147-153del]; [147=] | p.[Pro50Thrfs*33]; ([Pro50=]) | 0.0001096 | 14 | NA | NA | 50 |
| P.135  | PIK3R1 | CVID | NM_181504.4 | c.[18G>T]; [18=] | p.[Trp6Cys]; ([Trp6=]) | 0.00023 | 16 | Probably damaging | D | 49 |
| P.182  | PIK3R4 | CVID | NM_014602.3 | c.[1039C>T]; [1039=] | p.[Arg347Trp]; ([Arg347]) | 0.001376 | 32 | Possibly damaging | T |
| P.264  | REL | Unclassified antibody deficiency <br>CVID | NM_001291746.2 | c.[395T>C]; [395=] | p.[Val132Ala]; ([Val132=]) | 0.000376 | 24 | Possibly damaging | NA | 43 |
| P.266  | PIK3R4 | CVID | NM_014602.3 | c.[3341A>G]; [3341=] | p.[Tyr1114Cys]; ([Tyr1114=]) | RS372167716 | 0.000048 | 28 | Possibly damaging | T |

**X-linked variants**

| Pat.ID | Gene | Diagnosis | Transcript ID | Transcript variant | Protein variant | dbSNP-ID | gnomAD frequency | CADD score | PolyPhen2-Pred | SIFT-Pred | Variant<sup>a</sup> Gene<sup>b</sup> |
|--------|------|-----------|---------------|-------------------|-----------------|----------|-----------------|------------|--------------|----------|----------------|
| P.279  | BTK | Agammaglobulinemia | NM_000061.3 | c.[427C>T]; [D] | p.His143Tyr | 26 | NA | 51 |

**Compound heterozygous variants**

| Pat.ID | Gene | Diagnosis | Transcript ID | Transcript variant | Protein variant | dbSNP-ID | gnomAD frequency | CADD score | PolyPhen2-Pred | SIFT-Pred | Variant<sup>a</sup> Gene<sup>b</sup> |
|--------|------|-----------|---------------|-------------------|-----------------|----------|-----------------|------------|--------------|----------|----------------|
| P.64   | RAG1 | CID | NM_000448.3 | c.[1431del; 1123C>G]; [1420C>T] | p.[Phe478Serfs*14; His375Asp]; ([Arg474Cys]) | NA, 25,29 | NA | 54 |

**Uncertain allele phase**

| Pat.ID | Gene | Diagnosis | Transcript ID | Transcript variant | Protein variant | dbSNP-ID | gnomAD frequency | CADD score | PolyPhen2-Pred | SIFT-Pred | Variant<sup>a</sup> Gene<sup>b</sup> |
|--------|------|-----------|---------------|-------------------|-----------------|----------|-----------------|------------|--------------|----------|----------------|
| P.108  | USP8 | CVID | NM_001128610.3 | c.1888A>T <br>(1888_1890del) | p.[Lys630*]; ([Lys630del]) | 41 | NA | T | 55 |
| P.182  | RELB | CVID | NM_006509.4 | c.1291G>C <br>(Glu431Gln) | p.[Glu527Lys] | 31, 17 | Possibly damaging | T | 56 |

CADD, combined annotation-dependent depletion; CID, combined immunodeficiency; CVID, common variable immunodeficiency; D, deleterious; F, female; freq., frequency; gnomAD, genome aggregation database; M, male; MSC, mutation significance cut-off; NA, not available; Pat. ID, patient identification number; pred., prediction; ref., reference; ref. seq., reference sequence; SCID, severe combined immunodeficiency; T, tolerated; tNGS, targeted next-generation sequencing. *Previous report on the pathogenicity of the identified variant. **Previous report on the involvement of the mutated gene in primary immunodeficiency.
tors such as consanguinity, family history of PID, age at diagnosis of PID, the clinical diagnosis of PID, and its clinical manifestations on the diagnostic rate of tNGS. As expected, consanguinity or familial history of PID led to a considerably higher diagnostic rate (Table 3). In particular, in the subgroup of patients with consanguinity or a family history of PID (37/294), the diagnostic performance of tNGS would be 48.6% (18/37). In contrast, excluding those patients would result in reduction of the diagnostic yield to 10.5% (27/257). Regarding the clinical diagnosis of PID, the highest diagnostic rate has been identified in the subgroup of patients with CID. Among evaluated clinical manifestations, a predicted disease-causing variant was more commonly found among the subgroup of patients with enteropathy and organ-specific autoimmunity.

**Therapeutic Consequences of Genetic Diagnosis through tNGS**

Genetic testing through tNGS had a higher diagnostic rate among patients with immune dysregulation, manifesting as organ-specific autoimmunity or enteropathy, identifying among else, monoallelic gain-of-function variants in STAT1 and STAT3, CTLA4 variants leading to CTLA4 insufficiency, PIK3R1 variants, likely resulting in hyperactivation of PI3Kδ, and biallelic LRBA variants, likely causing LRBA deficiency. As discussed above, targeted therapies can be considered for those genetic diagnoses, especially for treating manifestations of immune dysregulation, such as autoimmune and/or lymphoproliferative disease.

Based on genetic diagnosis, already 2 patients diagnosed with STAT3 gain-of-function are treated with baricitinib, due to their progressive interstitial lung disease (P.227, P.265). A paediatric patient harbouring a STAT3 gain-of-function variant (P.278) developed severe ILD, which led to aggressive immunosuppressive treatment with glucocorticosteroids and hydroxychloroquine mycophenolate mofetil, intravenous immunoglobulins, and etanercept, which were all ineffective in controlling its progressive course. Consequently, this child underwent bilateral lung transplantation at the age of 14 years and died 12 months later due to treatment-resistant chronic allograft dysfunction. The genetic analysis by tNGS has been only performed post-mortem and revealed a mutation in STAT3 (c.1276T>C, p.C426R), whose gain-of-function effect has been confirmed [38]. Timely genetic diagnosis, in that case, would have led to employing treatment with a JAK-inhibitor or tocilizumab, which appears more effective in treating immune dysregulation in the context of STAT3 gain-of-function syndrome. Similar to the STAT3 gain-of-function patients, a patient with STAT1 gain-of-function (P.101) displayed steroid-refractory autoimmune haemolytic anaemia and lymphopenia, which were successfully controlled after introducing treatment with baricitinib. Among identified patients with a CTLA4 mutation, 2 displayed a therapy-refractory enteropathy (P.165, P.215) and will be offered an abatacept treatment.

Besides targeted treatment of immune dysregulation, genetic diagnosis may lead to timely haematopoietic stem cell transplantation (HSCT), especially in patients with CIDs. Among tested patients, we identified a patient with RAG1 deficiency (P.64). This patient was diagnosed with progressive multifocal leukoencephalopathy, which had a lethal outcome. Considering the link between RAG1 deficiency and SCID or CID [52, 53], earlier genetic diagnosis in that patient could have led to a timely consideration of HSCT. Similar is the case of the patient diagnosed with IκBα gain-of-function, who currently suffers from recurrent infections, including pneumonia, and already has severe bilateral bronchiectasis (P.236) [34]. Given the lethal outcome of this IEI, timely genetic diagnosis at the age of
the first diagnosis might have led to HSCT before the currently existing severe lung damage.

In summary, in 4/294 (1.4%) patients, genetic diagnosis led to the introduction of a targeted therapy, tailored to revert the consequences of the identified genetic defect. However, considering patients with retrospective, post-mortem diagnosis, or currently treatment-refractory immune dysregulation, the fact that some of the identified predicted pathogenic variants, such as those in LRBA or PIK3R1, have not been functionally validated, genetic diagnosis through tNGS could affect the follow-up and/or result in therapeutic consequence in substantially higher number of tested patients.

### Discussion/Conclusion

tNGS has been employed in the present study to investigate the genetic basis of immunodeficiency in a cohort of patients who mainly had sporadic PID, falling under a PAD. This sequencing approach revealed a likely pathogenic genetic variant in approximately 15% of tested patients. This diagnostic rate was relatively low, though similar to the one reported in some previous genetic studies, employing WES or WGS on cohorts of patients with sporadic immunodeficiency [9, 50, 59]. The diagnostic rate of tNGS was significantly higher in the subgroup of patients with a familial history of PID or those with an early onset of disease. CID or immunodeficiency complicated with enteropathy or organ-specific autoimmunity were additional factors associating with a significantly higher diagnostic rate. The overall low diagnostic rate of tNGS in the present study may be explained through the dependence of the diagnostic performance of genetic testing and tNGS on factors such as consanguinity or family history with PID [60], which were present in a minority of tested patients. Further, the limited diagnostic yield of tNGS may reflect the polygenic origin of PID and the pathogenic role of additional yet unidentified genetic and/or epigenetic modifiers [3]. Given the increasing number of genes linked to IEI, additional disease-causing genes may be identified in the near future. For this reason and as the gene panel tested in the present study did not include all currently known PID genes, employing a

### Table 3. Factors associating with the identification of predicted disease-causing genetic variants

| Variable                                      | Predicted pathogenic variant (N = 45) | No pathogenic variant (N = 249) | OR (95% CI)         | p value* |
|------------------------------------------------|---------------------------------------|---------------------------------|---------------------|----------|
| Familial history of PID                        | 16                                    | 18                              | 7.08 (3.26–15.39)   | <0.0001*** |
| Consanguinity                                 | 5†                                    | 2                               | 5.44 (2.90–82.33)   | 0.0011*** |
| Age group at diagnosis²                       |                                       |                                 |                     |          |
| 0–4 years                                      | 7                                     | 7                               | 5.9 (1.96–17.76)    | 0.0028**  |
| 5–18 years                                     | 14                                    | 48                              | 1.72 (0.85–3.49)    | 0.17 (ns) |
| >18 years                                      | 23                                    | 177                             | 0.32 (0.17–0.62)    | 0.0009*** |
| CVID                                           | 25                                    | 202                             | 0.29 (0.15–0.57)    | 0.0004*** |
| CID                                            | 13                                    | 16                              | 5.92 (2.61–13.43)   | <0.0001*** |
| Unclassified antibody deficiency               | 4                                     | 17                              | 1.33 (0.43–4.16)    | 0.54 (ns) |
| Infections only disease                       | 14                                    | 94                              | 0.74 (0.38–1.47)    | 0.5 (ns)  |
| Lymphadenopathy                                | 8                                     | 36                              | 1.28 (0.55–2.97)    | 0.65 (ns) |
| Splenomegaly                                   | 10                                    | 64                              | 0.83 (0.39–1.76)    | 0.71 (ns) |
| Enteropathy                                    | 10                                    | 17                              | 3.90 (1.65–9.20)    | 0.003**  |
| ILD                                            | 4                                     | 16                              | 1.42 (0.45–4.47)    | 0.52 (ns) |
| Organ-specific autoimmunity                    | 20                                    | 38                              | 4.86 (2.46–9.61)    | <0.0001*** |
| Autoimmune cytopenia²                          | 9                                     | 35                              | 1.85 (0.86–3.98)    | 0.13 (ns) |
| Atopic disease                                 | 9                                     | 39                              | 1.35 (0.60–3.02)    | 0.51 (ns) |
| Granulomatous disease                         | 3                                     | 22                              | 0.74 (0.21–2.57)    | 0.78 (ns) |
| Malignancy                                     | 4                                     | 24                              | 0.92 (0.30–2.78)    | 1 (ns)    |

CI, confidence interval; CID, combined immunodeficiency; CVID, common variable immunodeficiency; ILD, interstitial lung disease; ns, not significant; OR, odds ratio; PID, primary immunodeficiency disorder; SPAD, specific antibody deficiency. * p < 0.05. ** p < 0.01. *** p < 0.001. † Including 3 patients from the same family. Analysis based on 276/294 studied patients with the known year of diagnosis. Autoimmune hemolytic anemia (AIHA) and/or immune thrombocytopenic purpura (ITP). Atopic dermatitis and/or allergic rhinitis and/or asthma.
broader sequencing approach, such as WES, may have resulted in a higher diagnostic rate.

Overall, 3.1% of tested PID patients harboured a predicted pathogenic NFKB1 variant, which was the most common genetic defect among tested patients. This finding is in line with previous studies [61, 62], which identified heterozygous loss-of-function variants in NFKB1 as the most common genetic defect in patients with CVID. In compliance with previous studies of large cohorts of PID patients with NFKB1 mutations, this subset of patients displayed variable immunodeficiency, ranging from infections-only antibody deficiency to CID and marked immune dysregulation [19]. Characterization of phenotypes of patients with immunodeficiency linked to heterozygous NFKB1 mutations revealed a relatively high prevalence of autoimmune and lymphoproliferative manifestations [19, 32, 61]. Together with the recent identification of NFKB1 mutations in patients with sheer rheumatic disease and secondary hypogammaglobulinaemia [63], the latter leads to the assumption that NF-κB1-related disease is primarily a condition of immune dysregulation rather than mere immunodeficiency [32]. The observation of progressive hypogammaglobulinaemia in currently asymptomatic mutation carriers and the likely concurrent progressive course of immunodeficiency [61] may lead to a later disease onset in some currently asymptomatic mutation carriers; immunodeficiency might be precipitated from the introduction of immunomodulatory regimens for their autoimmune manifestations [63]. The aforementioned finding emphasizes the importance of early molecular diagnosis of NF-κB1-related disease and the need to develop targeted NF-κB pathway-based therapeutic intervention.

The increasing availability of sequencing technologies, especially of NGS, has improved our understanding of the pathogenesis of PID and paved the way for targeted therapeutic intervention with agents directed at reverting the disease-causing molecular abnormality or its pathophysiological consequences [64, 65]. Therefore, such targeted therapies and identifying the genetic basis of PID can be essential for patients with manifest immune dysregulation as conventional immunomodulatory regimens may exert an immunosuppressive effect, aggravating their immunodeficiency or may only inadequately control autoimmune or lymphoproliferative manifestations. Already employed individualized therapeutic approaches in the field of IEI include mTOR- or PI3Kδ-specific inhibitors in patients with mutations in PIK3CD, PTEN, or PIK3R1 causing activated PI3Kδ syndrome [29, 49]; abatacept (CTLA4-Ig) in patients with LRBA deficiency; CTLA4 insufficiency or DEF6 deficiency [16, 17, 66]; jakinibs in patient with STAT1 or STAT3 gain-of-function [14]; and the CXCR4 antagonist plerixafor in patients with warts, hypogammaglobulinaemia, immunodeficiency, and myelokathexis syndrome [67]. Despite the lack of randomized controlled clinical trials, evidence from case reports and case series suggests both the excellent tolerability and efficacy of such targeted therapies. Together with their focused biological effect, it makes them attractive, especially when compared with conventional immunomodulatory regimens. Here, molecular diagnosis through tNGS resulted in treatment with baricitinib in a subgroup of patients with gain-of-function mutations in STAT1 or STAT3. Further, post-mortem identification of therapeutically relevant mutations in 3 studied patients, who directly or indirectly died of treatment-refractory immune dysregulation, highlights the importance of early genetic diagnosis, especially in patients with the autoimmune or lymphoproliferative disease as this would have resulted in timely consideration of targeted therapy. In the current study, the rate of patients who received targeted therapies based on genetic diagnosis was low and may underestimate the clinical significance of genetic diagnosis. The main reasons for the lack of a therapeutic consequence were delayed genetic testing, including cases with post-mortem diagnosis, and the lack of functional validation of some of the identified variants. Further, genetic diagnosis should lead to genetic counseling of family members and family cascade testing, which can lead to genetic diagnosis in additional patients and may expand the clinical consequences of genetic testing.

Generally, in comparison to whole-exome (WES) or whole-genome sequencing (WGS), the targeted sequencing gene panel has an increased sequencing depth and allows rapid, cost-effective, and simultaneous sequencing of multiplexed samples in 1 sequencing run [68]. However, given the growing number of genes linked to IEI, it may require >1 gene panel for the genetic work-up PID. Despite testing for variations in genes most commonly linked to IEI or currently considered therapeutically relevant, the latter limitation of tNGS may underestimate the prevalence of monogenic disease in the studied cohort as discussed above. For this purpose and based on a recommendation of the IUIS experts committee, the best way to identify a disease-associated genetic variation in a new PID patient might consist of a tNGS followed by WES or WGS for unresolved cases [43]. However, given the currently dropping prices of WES and WGS, this suggestion may need to be revised. An additional limitation of our study is the in silico evaluation of the pathogenicity of identified variants, which may overestimate the prevalence of PID-related variants.
Even in the case of a definite molecular diagnosis, for most patients harbouring an IEI-causing mutation, the disease does not follow Mendelian inheritance. The identified pathogenic variant is commonly associated with incomplete penetrance variable expressivity, rendering disease course and therapeutic choice difficult. Therefore, precision medicine, whose scope is to be served through routine genetic testing, may be further facilitated by identifying an additional germline or somatic genetic modification as well as epigenetic factors and identifying the role of environmental factors [69–71]. A better understanding of those factors could provide additional insight into disease pathogenesis’s fundamental processes, offering a better perspective towards genetic counselling and precision medicine.

In conclusion, the employed Haloplex tNGS-approach is a time- and cost-effective sequencing method, providing a unique diagnostic tool for identifying known or novel variants in PID-associated genes. This approach had a high diagnostic rate, especially within the subgroup of patients with CID or immune dysregulation, for whom genetic diagnosis may affect therapeutic decision-making. However, given the increasing number of genes associated with IEI, which at the moment amount to >430, a panel-sequencing approach is ineffective in integrating all disease-relevant genetic loci, highlighting the importance of sequential sequencing, where negative-targeted NGS may be followed by WES or WGS. The latter may be especially relevant for patients who, in addition to antibody deficiency, display cellular immunodeficiency or those with complications such as autoimmunity, lymphoproliferation, or malignant disease as identifying the genetic cause of disease may affect follow-up procedures or therapeutic decision-making.

**Acknowledgements**

We thank all nurses, physicians, and documentation personnel of the outpatient clinics of the Department of Rheumatology and Immunology and the Department of Paediatrics of the Hannover Medical School for collecting blood samples, informing the patients about the study, and documenting patients’ medications.

**Statement of Ethics**

The institutional medical Ethical Committee at the Hannover Medical School approved the study (ethics approval number: Nr.8875_BO_K_2020). Written consent of all study participants or their parents or guardians was obtained. The authors have no ethical conflicts to disclose.

**Conflict of Interest Statement**

The authors have no other relevant affiliations or financial participation with any organization or entity with financial interest.

**Funding Sources**

This project was supported by the Deutsche Forschungsgemeinschaft (German Research Foundation) under Germany’s Excellence Strategy – EXC 2155 “RESIST” – Project ID 390874282 and the German Federal Ministry of Education and Research (BMBF) through a grant to the German Auto-Immunity Network, Grant code 01GM1910E Hannover. Georgios Sogkas receives funding from the Young Academy Clinician/Scientist program of the Hannover Medical School, Germany and the Rosemarie-Germscheid foundation. All the authors and this project are supported by the German Center for Infection Research (DZIF TTU 07.801).

**Author Contributions**

Research design was conceived by F.A., G.S., B.G., and R. E. S.; sample collection and analyses were performed by F. A., N. D., G. S., and U.B.; data analysis was performed by F.A. and G.S.; writing and contributing to writing of the manuscript was performed by F. A., G.S., and all the authors.

**Data Availability Statement**

The data used to support the findings of this study are available from the corresponding author upon request.

**References**

1. Al-Herz W, Notarangelo LD. Classification of primary immunodeficiency disorders: one fits-all does not help anymore. Clin Immunol. 2012 Jul;144(1):24–3.
2. Bousfiha A, Jeddane I, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W, et al. The 2017 IUIS phenotypic classification for primary immunodeficiencies. J Clin Immunol. 2018 Jan; 38(1):129–43.
3. Gruber C, Bogunovic D. Incomplete penetrance in primary immunodeficiency: a skeleton in the closet. Hum Genet. 2020 Jun; 139(6–7):745–57.
4. Christiansen M, Offer sen R, Jensen JMB, Petersen MS, Larsen CS, Mogensen TH. Identification of novel genetic variants in CVID patients with autoimmunity, autoinflammation, or malignancy. Front Immunol. 2020 Jan 27;10:3022.
5. El-Helou SM, Biegener AK, Bode S, Ehl SR, Hegg M, Maccari ME, et al. The German national registry of primary immunodeficiencies (2012–2017). Front Immunol. 2019 Jul 19;10:1272.
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 Jan-Feb;4(1):38-59.

Bogaert DJ, Dullaers M, Lambrecht BN, Vermaelen KY, De Baere E, Haerynck F. Genes associated with common variable immunodeficiency: one diagnosis to rule them all? J Med Genet. 2016 Sep;53(9):575-90.

de Valles-Ibanez G, Esteve-Sole A, Piquer M, Gonzalez-Navarro EA, Hernandez-Rodriguez J, Laayouni H, et al. Evaluating the genetics of common variable immunodeficiency: monogenic model and beyond. Front Immunol. 2018 May 14;9:636.

Cooper MA, Castro-Vicente V, arkwright PD, Fabre A, Marchal S, Barlogis V, Mari B, Bartram C, Hartog J, Maccari ME, Schwab C, et al. Therapeutic resistance to targeted PI3Kδ syndrome – targeted therapy for the activated PI3Kδ syndrome. Front Immunol. 2018 Nov;9:2012.

D. MutationTaster2: mutation prediction for interpretation of germline mutations. Nucleic Acids Res. 2009 May;37(11):3426-31.

Deval S, Dexpert L, Hamroun D, Lalande M, Collod-Bèroud G, Clastres M, Bèroud C. Human splicing finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res. 2009 May;37(11):3426-31.

Drake CM, Schuler PG, Schuelke M, Seelow D. MutationTaster2: mutation prediction for interpretation of germline mutations. Nucleic Acids Res. 2009 May;37(11):3426-31.

Flanagan SE, Haapaniemi E, Sogkas G, Dubrowinskaja N, Adriawan IR, Ringshausen FC, Moshous D, Pannetier C, Chasseval Rd Rd, Dubois C, van Hagen PM, Holland S, et al. Effective “activation of the NF-κB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet. 2013 Nov 7;93(5):812-24.

Flanagan SE, Haapaniemi E, Sogkas G, Dubrowinskaja N, Adriawan IR, Ringshausen FC, Moshous D, Pannetier C, Chasseval Rd Rd, Dubois C, van Hagen PM, Holland S, et al. Effective “activation of the NF-κB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet. 2013 Nov 7;93(5):812-24.

Flanagan SE, Haapaniemi E, Sogkas G, Dubrowinskaja N, Adriawan IR, Ringshausen FC, Moshous D, Pannetier C, Chasseval Rd Rd, Dubois C, van Hagen PM, Holland S, et al. Effective “activation of the NF-κB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet. 2013 Nov 7;93(5):812-24.

Flanagan SE, Haapaniemi E, Sogkas G, Dubrowinskaja N, Adriawan IR, Ringshausen FC, Moshous D, Pannetier C, Chasseval Rd Rd, Dubois C, van Hagen PM, Holland S, et al. Effective “activation of the NF-κB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet. 2013 Nov 7;93(5):812-24.

Flanagan SE, Haapaniemi E, Sogkas G, Dubrowinskaja N, Adriawan IR, Ringshausen FC, Moshous D, Pannetier C, Chasseval Rd Rd, Dubois C, van Hagen PM, Holland S, et al. Effective “activation of the NF-κB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet. 2013 Nov 7;93(5):812-24.

Flanagan SE, Haapaniemi E, Sogkas G, Dubrowinskaja N, Adriawan IR, Ringshausen FC, Moshous D, Pannetier C, Chasseval Rd Rd, Dubois C, van Hagen PM, Holland S, et al. Effective “activation of the NF-κB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet. 2013 Nov 7;93(5):812-24.

Flanagan SE, Haapaniemi E, Sogkas G, Dubrowinskaja N, Adriawan IR, Ringshausen FC, Moshous D, Pannetier C, Chasseval Rd Rd, Dubois C, van Hagen PM, Holland S, et al. Effective “activation of the NF-κB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet. 2013 Nov 7;93(5):812-24.

Flanagan SE, Haapaniemi E, Sogkas G, Dubrowinskaja N, Adriawan IR, Ringshausen FC, Moshous D, Pannetier C, Chasseval Rd Rd, Dubois C, van Hagen PM, Holland S, et al. Effective “activation of the NF-κB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet. 2013 Nov 7;93(5):812-24.

Flanagan SE, Haapaniemi E, Sogkas G, Dubrowinskaja N, Adriawan IR, Ringshausen FC, Moshous D, Pannetier C, Chasseval Rd Rd, Dubois C, van Hagen PM, Holland S, et al. Effective “activation of the NF-κB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet. 2013 Nov 7;93(5):812-24.

Flanagan SE, Haapaniemi E, Sogkas G, Dubrowinskaja N, Adriawan IR, Ringshausen FC, Moshous D, Pannetier C, Chasseval Rd Rd, Dubois C, van Hagen PM, Holland S, et al. Effective “activation of the NF-κB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet. 2013 Nov 7;93(5):812-24.

Flanagan SE, Haapaniemi E, Sogkas G, Dubrowinskaja N, Adriawan IR, Ringshausen FC, Moshous D, Pannetier C, Chasseval Rd Rd, Dubois C, van Hagen PM, Holland S, et al. Effective “activation of the NF-κB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet. 2013 Nov 7;93(5):812-24.
Diagnostic Yield and Therapeutic Consequences of tNGS in PID

42 Keller MD, Pandey R, Li D, Glessner J, Tian L, Henrickson SE, et al. Mutation in IRF2BP2 is responsible for a familial form of common variable immunodeficiency disorder. J Allergy Clin Immunol. 2016 Aug;138(2):544–50.

43 Tangye SG, Al-Herz W, Boussifia A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol. 2020 Jan;40(1):24–64.

44 Collin M, Dickinson R, Bigley V. Haematopoietic and immune defects associated with GATA2 mutation. Br J Haematol. 2015 Apr;169(2):173–87.

45 van de Veerdonk FL, Plantinga TS, Hoischen A, van der Meché F, van der Wieren PH, et al. STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. N Engl J Med. 2011 Jul 7;365(1):54–61.

46 Boisson B, Wang YD, Bosompeem A, Ma CS, Lim A, Kochetkov T, et al. A recurrent dominant negative E47 mutation causes agammaglobulinemia and BCR(-) B cells. J Clin Invest. 2013 Nov;123(11):4781–5.

47 Heltzer ML, Orange JS. Impaired immunity in May–Hegglin anomaly due to MYH9 mutation. J Allergy Clin Immunol. 2006 Feb;117(2):291–2.

48 Orange JS. Natural killer cell deficiency. J Allergy Clin Immunol. 2013 Sep;132(3):515–25.

49 Nunes-Santos CJ, Uzel G, Rosenzweig SD. PI3K pathway defects leading to immunodeficiency and immune dysregulation. J Allergy Clin Immunol. 2019 May;143(5):1676–87.

50 Thaventhiran JED, Lango Allen H, Burren OS, Rae W, Greene D, Staples E, et al. Whole-genome sequencing of a sporadic primary immunodeficiency cohort. Nature. 2020 Jul;583(7814):90–5.

51 Hashimoto S, Tsukada S, Matsushita M, Miyawaki T, Niida Y, Yachie A, et al. Identification of Bruton’s tyrosine kinase (Btk) gene mutations and characterization of the derived proteins in 35 X-linked agammaglobulinemia families: a nationwide study of Btk deficiency in Japan. Blood. 1996 Jul 15;88(2):561–73.

52 Schroder C, Baerlecken NT, Pannicke U, Dork T, Witte T, Jacobs R, et al. Evaluation of RAG1 mutations in an adult with combined immunodeficiency and progressive multifocal leukoencephalopathy. Clin Immunol. 2017 Jun;179:1–7.

53 Lawless D, Geier CB, Farmer JR, Lango Allen H, Thwaites D, Atschezki F, et al. Prevalence and clinical challenges among adults with primary immunodeficiency and recombination-activating gene deficiency. J Allergy Clin Immunol. 2018 Jun;141(6):2303–6.

54 Matthews AG, Briggs CE, Yamanaka K, Small TN, Mooster JL, Bonilla FA, et al. Compound heterozygous mutation of RAG1 leading to Omenn syndrome. PLoS One. 2015 Apr 7;10(4):e0121489.

55 Dufner A, Kissner A, Niendorf S, Basters A, Reissig S, Schönlein A, et al. The ubiquitin-specific protease USP8 is critical for the development and homeostasis of T cells. Nat Immunol. 2015 Sep;16(9):950–60.

56 Sharfe N, Merico D, Karanxha A, Macdonald C, Dahi H, Ngan B, et al. The effects of RbB deficiency on lymphocyte development and function. J Autoimmun. 2015 Dec;65:90–100.

57 Castigl E, Wilson S, Garibyan L, Rachid R, Bonilla F, Schneider L, et al. Reexamining the role of TACI coding variants in common variable immunodeficiency and selective IgA deficiency. Nat Genet. 2007 Apr;39(4):430–1.

58 Romberg N, Chamberlain N, Saadoun D, Gentile M, Kimman T, Ng YS, et al. CVID-associated TACI mutations affect autoreactive B cell selection and activation. J Clin Invest. 2013 Oct;123(10):4283–93.

59 Gallo V, Dotta L, Giardino G, Cirllo E, Lougaris V, D’Assante R, et al. Diagnostics of primary immunodeficiencies through next-generation sequencing. Front Immunol. 2016 Nov 7;7:466.

60 Simon AJ, Golan AC, Lev A, Stauber T, Barel O, Somekh I, et al. Whole exome sequencing (WES) approach for diagnosing primary immunodeficiencies (PIDs) in a highly consanguineous community. Clin Immunol. 2020;214:108376.

61 Tujijenburp P, Lango Allen H, Burns SO, Greene D, Jansen MH, Staples E, et al. Loss-of-function nuclear factor kappaB subunit 1 (NFKB1) variants are the most common monogenic cause of common variable immunodeficiency in Europeans. J Allergy Clin Immunol. 2018 Oct;142(4):1285–96.

62 Maffucci P, Filion CA, Boisson B, Itani Y, Shang L, Casanova JL, et al. Genetic diagnosis using whole exome sequencing in common variable immunodeficiency. Front Immunol. 2016 Jun 13;7:220.

63 Sogkas G, Dubrowinskaja N, Adriaan JR, Anin M, Witte T, Schmidt RE, et al. High frequency of variants in genes associated with primary immunodeficiencies in patients with rheumatic diseases with secondary hypogammaglobulinemia. Ann Rheum Dis. 2020 Oct 12. annrheumdis-2020-218280. Epub ahead of print.

64 Notarangelo LD, Fleisher TA. Targeted strategies directed at the molecular defect: toward precision medicine for select primary immunodeficiency disorders. J Allergy Clin Immunol. 2017 Mar;139(3):715–23.

65 Delmonte OM, Notarangelo LD. Targeted therapy with biologicals and small molecules in primary immunodeficiencies. Med Princ Pract. 2020;29(2):101–12.

66 Serwas NK, Hoeger B, Ardy RC, Stulz SV, Sui Z, Memaran N, et al. Publisher correction: human DEF6 deficiency underlies an immunodeficiency syndrome with systemic autoimmunity and aberrant CTLA-4 homeostasis. Nat Commun. 2019 Oct 2;10(1):4555–019. 124545

67 Dale DC, Bolyard AA, Kelley ML, Westrup EC, Makaryan V, Aprikyan A, et al. The CXCR4 antagonist plerixafor is a potential therapy for myelokathexis, WHIM syndrome. Blood. 2011 Nov 3;118(18):4963–6.

68 Stoddard JL, Niemela JE, Fleisher TA, Rosenzweig SD. Targeted NGS: a cost-effective approach to molecular diagnosis of PIDs. Front Immunol. 2014 Nov 3;5:531.

69 Rodríguez-Cortez VC, Del Pino-Molina L, Rodríguez-Ubreva J, Ciudad L, Gomez-Cabrero D, Company C, et al. Monozygotic twins discordant for common variable immunodeficiency reveal impaired DNA demethylation during naive-to-memory B-cell transition. Nat Commun. 2015 Jun 17;6:7335.

70 Rae W. Indications to epigenetic dysfunction in the pathogenesis of common variable immunodeficiency. Arch Immunol Ther Exp. 2017 Apr;65(2):101–10.

71 Jorgensen SF, Fevang B, Aukrust P. Autoimmunity and Inflammation in CVID: a possible crosstalk between immune activation, gut microbiota, and epigenetic modifications. J Clin Immunol. 2019;39(1):30–6.