

Unbiased targeted next-generation sequencing molecular approach for primary immunodeficiency diseases

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Background: Molecular genetics techniques are an essential diagnostic tool for primary immunodeficiency diseases (PIDs). The use of next-generation sequencing (NGS) provides a comprehensive way of concurrently screening a large number of PID genes. However, its validity and cost-effectiveness require verification.

Objectives: We sought to identify and overcome complications associated with the use of NGS in a comprehensive gene panel incorporating 162 PID genes. We aimed to ascertain the specificity, sensitivity, and clinical sensitivity of the gene panel and its utility as a diagnostic tool for PIDs.

Methods: A total of 162 PID genes were screened in 261 patients by using the Ion Torrent Proton NGS sequencing platform. Of the 261 patients, 122 had at least 1 known causal mutation at the onset of the study and were used to assess the specificity and sensitivity of the assay. The remaining samples were from unsolved cases that were biased toward more phenotypically and genotypically complicated cases.

Results: The assay was able to detect the mutation in 117 (96%) of 122 positive control subjects with known causal mutations. For the unsolved cases, our assay resulted in a molecular genetic

diagnosis for 35 of 139 patients. Interestingly, most of these cases represented atypical clinical presentations of known PIDs. **Conclusions:** The targeted NGS PID gene panel is a sensitive and cost-effective diagnostic tool that can be used as a first-line molecular assay in patients with PIDs. The assay is an alternative choice to the complex and costly candidate gene approach, particularly for patients with atypical presentation of known PID genes. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: Immunodeficiency, next-generation sequencing, targeted, genetic, primary immunodeficiency, mutation, variants, Saudi, diagnosis

Primary immunodeficiency diseases (PIDs) are a phenotypically and genetically heterogeneous group of more than 200 monogenic inherited disorders resulting in immune defects that predispose patients to infections, autoimmune diseases, lymphoproliferation, and malignancies.¹ Diagnostic workup for PIDs is a multistep process that involves taking a detailed personal and family history and the performance of complex laboratory assays. Molecular genetic testing became an essential diagnostic tool for PIDs because it often provides a definitive diagnosis, assists in genetic counseling, permits early prenatal diagnosis and carrier identification, establishes the diagnosis in atypical cases, provides genotype-phenotype correlation, and allows presymptomatic identification of affected patients with PIDs.²

The clinical and immunologic characteristics of affected patients guide selection of the candidate gene or genes to be tested. However, given the genetic and phenotypic heterogeneity of PIDs, candidate gene selection is not a straightforward task. One such example is Omenn syndrome, which can be caused by hypomorphic mutations in any of the severe combined immunodeficiency disease (SCID)-causing genes (*RAG1/2*, *Artemis*, *IL7RA*, *MRP*, *ADA*, *LIG4*, *IL2RG*, *JAK3*, and *AK2*).^{3,4} In addition, mutations in *RAG1/2* can present with SCID, Omenn syndrome, and combined immunodeficiency (CID) or autoimmunity.⁵⁻⁸

Sanger sequencing of multiple candidate genes is laborious, time-consuming, inefficient, and expensive. Next-generation sequencing (NGS) has been applied to comprehensively study several Mendelian monogenic disorders, as well as complex

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Supported by the National Science, Technology and Innovation Plan strategic technologies program in Saudi Arabia (KACST: 11-BIO-1441-20).

Disclosure of potential conflict of interest: H. Al-Mousa, L. El-Baik, O. Khier, A. Al-Otaibi, and S. Al-Hissi have received a grant from King Abdulaziz City for Science and Technology (grant no. KACST: 11-BIO-1441-20). The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication June 5, 2015; revised November 11, 2015; accepted for publication December 2, 2015.

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0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2015.12.1310>

Abbreviations used

CID:	Combined immunodeficiency
CNV:	Copy number variation
ePCR:	Emulsion PCR
HGMD:	Human Gene Mutation Database
indel:	Insertion/deletion
NGS:	Next-generation sequencing
PID:	Primary immunodeficiency disease
SCID:	Severe combined immunodeficiency disease
SNP:	Single nucleotide polymorphism
SNV:	Single nucleotide variant
WES:	Whole-exome sequencing
WGS:	Whole-genome sequencing

diseases.⁹⁻¹¹ Whole-exome sequencing (WES) with NGS can provide a rapid diagnosis and has now been adopted by several laboratories as the method of choice for the diagnosis of various PIDs.¹²⁻¹⁶ This is because of its speed and comprehensiveness, with the coding regions of the whole set of genes in the genome (the exome) able to be screened in 1 run. However, the clinical WES is still relatively expensive, and it yields so much information that it requires highly trained investigators and substantial validating sequencing for successful and timely interpretation. In addition, it is not yet clinically verified in terms of sensitivity and specificity over a large number of patients from different populations. A promising solution to overcome the complications associated with WES for the diagnosis of PIDs is the use of targeted NGS, in which a panel of selected genes of interest is screened to identify the causal variant. This approach reduces the cost, eases interpretative challenges, and speeds up diagnosis for the majority of PIDs.

The usefulness of a targeted NGS approach in PID diagnostics was recently demonstrated by 3 studies. Nijman et al¹³ used array-based and in-solution enrichment techniques combined with SOLiD sequencing (Applied Biosystems, Bleiswijk, The Netherlands) for mutation screening in 170 PID genes. They were able to accurately detect point mutations and exonic deletions in 41 patients with a known genetic diagnosis, which enabled the identification of causal mutations in 4 of 26 patients with undiagnosed PIDs.

Moens et al¹⁶ adopted a selector-based sequencing capture assay together with Illumina sequencing (Illumina, San Diego, Calif) to identify disease-causing mutations in 179 known PID genes. The usefulness of this assay for molecular diagnosis of PIDs was investigated by sequencing DNA from 33 patients, 18 of whom had at least 1 known causal mutation at the onset of the study. They were able to identify the disease-causing mutations in 60% of the investigated patients.

Stoddard et al¹⁵ performed HaloPlex custom target enrichment (Agilent Technologies) and NGS by using the Ion Torrent PGM (Thermo Fisher, Carlsbad, Calif) to screen 173 genes in 11 healthy control subjects, 13 patients with PIDs previously evaluated with either an identified mutation or single nucleotide polymorphism (SNP), and 120 patients with undiagnosed PIDs. A molecular diagnosis was reached for 18 of 120 patients who previously lacked a genetic diagnosis, including 9 who had atypical presentations and negative results in extensive previous genetic and functional studies.

In this study we have assessed an NGS-based multiplex assay that encompasses 162 PID genes using a larger number of patients

compared with previous studies to ultimately test whether an NGS-based genetic evaluation can be used as a sensitive and accurate routine diagnostic tool for mutation screening in patients with PIDs.

METHODS**Patient material**

A total of 261 patients suspected of having PIDs were recruited to the study. One hundred twenty-two patients with PIDs with known mutations were included to validate our high-throughput sequencing approach, as well as the data analysis. These 122 patients with various clinical diagnoses included 34 with SCID, 29 with hyper-IgE syndrome, 11 with hyper-IgM syndrome, 10 with Griscelli syndrome, 9 with chronic granulomatous disease, 7 with ataxia telangiectasia, 5 with agammaglobulinemia, 5 with Wiskott-Aldrich syndrome, 4 with bare lymphocyte syndrome, 2 with Chediak-Higashi syndrome, 1 with Mendelian susceptibility to mycobacterial disease, 1 with pneumococcal sepsis, 1 with autoimmune polyendocrinology syndrome, 1 with cartilage hair hypoplasia, and 1 with leukocyte adhesion defect type 1. Those 122 patients encompassed substantial allelic and genetic heterogeneity. Eighty-six had single nucleotide variants (SNVs), 24 had short insertions/deletions (indels), and 12 had large deletions (Table 1 and see Table E1 in this article's Online Repository at www.jacionline.org). Another 139 patients were suspected of having PIDs but without a confirmed genetic diagnosis. They represented various types of PIDs, including 27 cases of SCID, 21 cases of CID, 15 cases of chronic granulomatous disease, 14 cases of hyper-IgE syndrome, 11 cases of common variable immunodeficiency, 9 cases of ill-defined immunodeficiency, 5 cases of hyper-IgM syndrome, 5 cases of agammaglobulinemia, 5 cases of hypogammaglobulinemia, 4 cases of bare lymphocyte syndrome, 4 cases of ataxia telangiectasia like, 3 cases of autoimmune enteropathy, 3 cases of hemophagocytic lymphohistiocytosis, 2 cases of Omenn disease, 2 cases of autoimmune lymphoproliferative syndrome, 2 cases of autoinflammatory disease, 2 cases of chronic mucocutaneous candidiasis, 2 cases of Griscelli syndrome, 2 cases of lymphoproliferative disease, 1 case of neutropenia, and 1 case of Chediak-Higashi syndrome. These patients were recruited into the study to assess the ability of the assay to identify the underlying genetic defects (Table II). Seventy of the 139 patients had extensive previous genetic studies based on a candidate gene approach with no identified genetic defects. Detailed clinical and immunologic data and a list of genes that were Sanger sequenced are shown in Table E2 in this article's Online Repository at www.jacionline.org for patients with previous genetic studies and in Table E3 in this article's Online Repository at www.jacionline.org for new cases with no previous genetic studies. All research involving patient materials was approved by the Institutional Review Board at King Faisal Specialist Hospital & Research Center (RAC: 2080 025). Written consent was obtained from patients or their guardians and supported by the National Science, Technology and Innovation Plan program in the Kingdom of Saudi Arabia (KACST: 11-BIO-1441-20).

DNA extraction and Sanger sequencing

DNA was isolated from peripheral blood samples obtained by means of venipuncture from patients and parents, when available, by using the Gentra Puregene Blood Extraction Kit (Qiagen, Valencia, Calif). For the purpose of sequencing, PCR was performed, as described previously.¹⁷ Sequencing primers were designed to amplify the region of interest, which was sequenced, as previously described.¹⁸ Sequence data were aligned against the reference GenBank sequences and examined for variation. For novel mutation verification, anonymized 250 DNA samples derived from normal Saudi blood donors were sequenced to rule out potential novel Saudi normal SNPs.

Primer design and multiplexing assay development

One hundred sixty-two PID genes were used as a basis for the design and synthesis of a highly multiplexed gene panel with Ion AmpliSeq Designer software (Life Technologies, Carlsbad, Calif). Primer design was based on

TABLE I. Clinical diagnosis for patients with known causal mutations at the onset of the study

Diagnosis	Total	Picked by using targeted NGS	Picked by using CNV	Missed
SCID	34	31	2	1
Hyper-IgE syndrome	29	19	10	1
Griscelli syndrome	10	10		
Hyper-IgM syndrome	11	11		
Chronic granulomatous disease	9	9		
Ataxia telangiectasia	7	7		
Agammaglobulinemia	5	5		
Wiskott-Aldrich syndrome	5	4		1
Bare lymphocyte syndrome	4	3		1
Chediak-Higashi syndrome	2	2		
Mendelian susceptibility to mycobacterial disease	1	1		
Pneumococcal sepsis	1	1		
Autoimmune polyendocrinopathy syndrome	1	1		
Cartilage hair hypoplasia	1	1		
Leukocyte adhesion deficiency 1	1			1

generating amplicons with an average length of 200 bp, providing 90% minimum coverage of the coding sequence and, on average, 10-bp flanking regions of associated introns. Following an *in silico* design, coverage was assessed for compliance with design criteria, and manual processes were applied on a gene-by-gene basis to ensure adequate coverage and resolve factors, such as 3' SNPs that could affect primer efficiency. Primers were then synthesized and pooled into 2 multiplex reactions based on PCR compatibility, minimizing the likelihood of primer-primer interactions. After synthesis, primer pools were tested for coverage, recommended multiplexing, and other quality control metrics to ensure specifications were met.

Library preparation and NGS

Ten nanograms each of all DNA samples were used to obtain the Ion Proton AmpliSeq library. DNA was amplified by using gene panel Primer Pools, AmpliSeq HiFi mix (Thermo Fisher), and 17 amplification cycles. PCR pools for each sample were combined and subjected to primer digestion with FuPa reagent (Thermo Fisher). Pooled amplicons were then ligated with universal adapters. After purification, libraries were quantitated by using quantitative PCR and normalized to 100 pmol/L. Normalized libraries were barcoded (ligated with 42 different Ion Xpress Barcode adapters) and pooled in equal ratios for emulsion PCR (ePCR) on an Ion OneTouch System. After ePCR, templated Ion Sphere particles were enriched by using the Ion OneTouch ES. Both ePCR and enrichment procedures followed the manufacturer's instructions (Thermo Fisher). The template-positive Ion PI Ion Sphere particles were processed for sequencing on the Ion Proton instrument (Thermo Fisher).

Data processing and bioinformatics analysis

The data of each gene panel run were analyzed through 2 major workflows: the first was for detecting point mutations (SNPs and indels), and the second was for detecting copy number variations (CNVs; large segmental indels).

Workflow for detecting point mutations

The workflow for detecting point mutations was a multistep pipeline. In the first step of this pipeline, the quality of each read was verified, and regions of the reads with low quality (<20) were trimmed out. The runs with low yield after this quality check were repeated. In the second step the reads were aligned to the hg19 reference sequence. The alignment program in use was

TABLE II. Clinical diagnoses of patients with unknown causal mutations at the onset of the experiment

Diagnosis	Total	Solved by using targeted NGS	Solved by using CNVs	Unsolved
SCID	27	2	6	19
CID	21	10		10
Chronic granulomatous disease	15	1	1	13
Hyper-IgE syndrome	14		3	11
Common variable immunodeficiency disease	11			11
Ill-defined immunodeficiency	9			9
Hyper-IgM syndrome	5	3		2
Hypogammaglobulinemia	5	1		4
Agammaglobulinemia	5		1	4
Bare lymphocyte syndrome	4	3		1
Ataxia telangiectasia	4			4
Autoimmune enteropathy	3	1		2
Hemophagocytic lymphohistiocytosis	3			3
Omenn disease	2	1		1
Autoimmune lymphoproliferative syndrome	2			2
Autoinflammatory disease	2			2
Chronic mucocutaneous candidiasis disease	2		1	1
Griscelli syndrome	2			2
Lymphoproliferative disease	2			2
Chediak-Higashi syndrome	1	1		
Neutropenia	1			1

tMap distributed within the Ion Torrent Suite (Thermo Fisher; <https://github.com/iontorrent/TS>). The alignment step is limited only to the regions of the target genes to speed up the analysis. In the third step the aligned reads were processed for variant calling by using the Torrent Suite Variant Caller TVC program, which is tuned to Ion Torrent data and considers quality scores and signal information specific to the Ion Torrent technology. In the subsequent step the variants were annotated by using public and in-house databases. The public databases included those available from the ANNOVAR package and other commercial data sets, such as the Human Gene Mutation Database (HGMD).¹⁹ The in-house databases included collections of disease-causing and polymorphic variants published or identified by different Saudi research teams. In the final step of the pipeline, nonrelevant variants were filtered out based on their quality, functional characteristics, and frequency in our data sets. In the filtration step low-quality variants were filtered out; a variant is considered of low quality if the respective base quality score (which is a Phred-like score produced by the Torrent Suite base caller) is less than 100 (the threshold of 100 was selected to achieve 100% sensitivity over the control data). Intronic synonymous variants and those present in population databases (specifically those that are in the 1000 Genomes database with a minor allele frequency >1%) were filtered out. Furthermore, variants that were frequent (minor allele frequency >1%) in our in-house database were also filtered out.

After applying filtering criteria, all nonsense, frameshift, and canonical splice site variants were considered pathogenic. For interpretation and classification of remaining SNV variants, nucleotide and amino acid conservation and effects on protein sequence were analyzed with PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>). In cases in which the changes were in splice sites, Mutation Taster (<http://www.mutationtaster.org/>) was used to predict the effect on mRNA splicing.

This filtering step allowed us to reduce the initial number of detected variants from an average of 633 to 6. The key factor for this improvement over published work is the large number of samples that enabled us to accumulate information about systematic errors and common variants that are too frequent

to cause disease. The average number of variants after removing nonfunctional variants was reduced to 309. After removing common variants in the 1000 Genomes database, the number decreased to 111; after removing variants common in our in-house database, the number decreased further to 22. After removing the remaining low-quality variants, the number decreased to just 6. The number of potential disease-causing variants after the filtration steps could be further reduced when taking phenotypic information into account.

Workflow for detecting CNVs

Our analytic workflow for detecting CNVs detects segmental deletions by comparing normalized average amplicon coverage from all samples with that from individual samples. In this way we were able to detect deletions of individual amplicons. The first step in the workflow was to normalize the number of reads for each amplicon given the total number of reads within a particular run. Based on all runs of the given panel, the distribution read number per amplicon was then computed. For individual samples, amplicons with read numbers lying at less than the 5% tail of the histogram were considered homozygous deletions with a 95% level of confidence. Amplicons with read numbers above the 5% tail of the histogram and less than 1 SD from the average were considered candidates for heterozygous amplicon deletions. In this study we focused on homozygous deletions. All potential pathogenic CNVs were validated when possible by using long-range PCR for single-exon deletions with primers flanking the CNV “loss/deleted” exons. The CytoScan HD Array (Affymetrix, Santa Clara, Calif) was used to confirm large loss/deletion segments. The CytoScan HD array contains 2.6 million markers, of which 750,000 are genotype SNPs. The data were analyzed by using the Chromosome Analysis Suite, version Cyto3.0, against build 38 of the UCSC Genome Browser.

RESULTS

Target enrichment performance and gene coverage

Table E4 in this article's Online Repository at www.jacionline.org includes a list of the genes used in this panel and information regarding the design and sequencing performance. As shown in Table E4, primer design aimed at covering more than 99.5% of the coding region for all genes. The actual observed coverage of the targeted regions after running the panels over all samples was 96.51%. From the 162 genes, only 9 had coverage of less than 90%. Table E5 in this article's Online Repository at www.jacionline.org shows additional statistics about the sequencing quality, alignment, and overall coverage of target regions. Average coverage of 461× was achieved for samples sequenced by this study.

Sensitivity and specificity

Analytic sensitivity and specificity measure the accuracy of the approach over the control cases. The assay was able to correctly detect mutations (SNPs, indels, and CNVs) in 117 of 122 control samples with known causal mutations, achieving an overall sensitivity of 96%. Table E1 summarizes all the results related to the samples with known variants.

Accuracy of detecting known SNVs and short indels.

The assay detected 105 of 110 known point mutations, achieving an overall sensitivity for SNVs of 96%. The sensitivity for detecting SNPs was as high as 97% (83/86), and that for detecting indels (22/24) was 92%. The mutations that were missed because of low read depth at the variant position (3 SNVs and 2 short indels) are also listed in Table E1. The total number of variants believed to be true and sent to Sanger sequencing was 119; 14 of them turned out to be false-positive variants, leading to an overall specificity of 88.2%. Decomposing the specificity by

mutation type, the specificity for SNPs was 90% (83 confirmed of 92), and that for indels was 81% (22 confirmed of 27).

Accuracy of detecting known CNVs. Some types of PID can also be caused by large insertions or deletions. Therefore CNV analyses were carried out for each of the sequenced samples. Twelve of the 122 positive control subjects had known large deletions involving *DOCK8* in 10 patients and *DCLRE1C* in 2 patients. CNV analyses were able to detect all of these deletions, resulting in a sensitivity of 100% for detecting large homozygous deletions. For 7 of the 12 cases, the precision (positive predictive value) was 100% because these included relatively long homozygous segmental deletions. For the remaining 5 cases, the hit list included 4 to 5 regions, leading to a precision of about 22%, which makes the overall precision rate about 30%. This is expected with NGS sequencing because of the nonuniformity of coverage and poor performance of the primers at some regions. For specificity, we tested 20 samples that were predicated to have homozygous deletions through CNV analysis for confirmation by using the CytoScan SNP microarray and exon-specific amplification analysis. Only 9 cases were confirmed, yielding 45% specificity for homozygous deletions.

Clinical sensitivity

Mutation detection in patients with unknown disease-causing variants. There were 139 patients in our study for whom the disease-causing mutation was unknown. SNPs and other variants detected by using NGS were first filtered, as described above, to identify potential causal mutations. Lists of variants were then further prioritized based on phenotypic information and subjected to Sanger sequencing. Twenty-three mutations (approximately 17%) were considered potentially disease causing based on the patient's phenotype. All were further confirmed by using Sanger sequencing. A summary of the confirmed disease-causing variants in these samples is given in Table III.

In the next step CNV analyses were performed for those patients (116 patients) in whom no causal SNV or indel could be identified. Twelve potential CNVs (approximately 10%) were detected that involved *DCLRE1C* (n = 4), *DOCK8* (n = 3), *BTK* (n = 1), *NCF1* (n = 1), *CARD11* (n = 1), *IL2RG* (n = 1), and *PNP* (n = 1). Five of the suspected exonic deletions were confirmed by using the 2.7 M CytoScan SNP microarray (Affymetrix, Santa Clara, Calif) and 4 through failure of exons to amplify by using internally controlled multiplex PCR amplification (Table IV).

Genotype-phenotype correlations of the solved cases. The diagnostic efficiency of our targeted NGS PID panel was demonstrated by its ability to detect the underlying genetic defects in 35 of 139 patients. Three patients with bare lymphocyte syndrome were found to harbor mutations in *RFXANK* (P1032 and P1300) and *CIITA* (P1294). One patient with chronic granulomatous disease was found to have a mutation in *CYBB* (P1098). A patient with Chediak-Higashi syndrome had a mutation in *LYST* (P1310). Two patients with SCID and 1 with Omenn syndrome were found to have mutations in *RAG1* (P1208 and P1209) and *RAG2* (P1157). A patient with hyper-IgM syndrome had a mutation in *AICDA* (P910), and another patient with hypogammaglobulinemia had a mutation in *BTK* (P1129).

The targeted NGS PID panel was able to achieve a molecular diagnosis for several patients who had previous negative findings

TABLE III. Mutations detected in patients with unknown disease-causing variants

Patient ID	Disease	Gene	Mutation	Effect	Zygosity	Mutation type	Detected by using targeted NGS	Confirmed by using Sanger sequencing
1222	Autoimmune enteropathy	<i>LRBA</i>	NM_001199282:exon24:c.3985_3986delGA	p.D1329 YfsX18	Homozygous	Short indel	Yes	Yes
1294	Bare lymphocyte syndrome	<i>CIITA</i>	NM_000246:exon16:c.C3136G	p.L1046V	Homozygous	SNV	Yes	Yes
1300	Bare lymphocyte syndrome	<i>RFXANK</i>	NM_134440:exon4:c.268+1G>C	N/A	Homozygous	SNV	Yes	Yes
1032	Bare lymphocyte syndrome	<i>RFXANK</i>	NM_003721:exon3:c.7_10insCTTA	p.L3PfsX47	Homozygous	Short indel	Yes	Yes
1098	Chronic granulomatous disease	<i>CYBB</i>	NM_000397:exon13:c.1618delG	p.E540fs	Hemizygous	Short indel	Yes	Yes
1310	Chediak-Higashi syndrome	<i>LYST</i>	NM_000081:exon5:c.G1303T	p.E435X	Homozygous	SNV	Yes	Yes
1148	CID	<i>AK2</i>	NM_001625:exon6:c.C545A	p.A182D	Homozygous	SNV	Yes	Yes
1149	CID	<i>PTPRC</i>	NM_080921:exon29:c.T3062C	p.L1021S	Homozygous	SNV	Yes	Yes
1257	CID	<i>CD247</i>	NM_198053:exon2:c.58+8C>T	N/A	Homozygous	SNV	Yes	Yes
1115	CID	<i>DCLRE1C</i>	NM_022487:exon10:c.C614G	p.S205C	Homozygous	SNV	Yes	Yes
1019	CID	<i>AK2</i>	NM_001625:exon6:c.C545A	p.A182D	Homozygous	SNV	Yes	Yes
1030	CID	<i>AK2</i>	NM_001625:exon6:c.C545A	p.A182D	Homozygous	SNV	Yes	Yes
1312	CID	<i>ZAP70</i>	NM_001079:exon12:c.G1606A	p.G536S	Homozygous	SNV	Yes	Yes
1311	CID	<i>ZAP70</i>	NM_001079:exon12:c.G1606A	p.G536S	Homozygous	SNV	Yes	Yes
916	CID	<i>AK2</i>	NM_001625:exon6:c.C545A	p.A182D	Homozygous	SNV	Yes	Yes
1004	CID	<i>STAT1</i>	NM_007315: c.1220T>G	p.L407R	Homozygous	SNV	Yes	Yes
1231	Hyper-IgM syndrome	<i>DNMT3B</i>	NM_001207056:exon18:c.G2029A	p.V677M	Homozygous	SNV	Yes	Yes
992	Hyper-IgM syndrome	<i>ATM</i>	NM_000051:exon63:c.9065delA	p.G3022fs	Homozygous	Short indel	Yes	Yes
910	Hyper-IgM syndrome	<i>AICDA</i>	NM_020661:exon2:c.152A>T	p.N51I	Homozygous	SNV	Yes	Yes
1129	Hypogammaglobulinemia	<i>BTX</i>	NM_000061:exon20:c.1909-9T>C	N/A	Hemizygous	SNV	Yes	Yes
1208	Omenn syndrome	<i>RAG1</i>	NM_000448:exon2:c.554delG	p.R185fs	Homozygous	Short indel	Yes	Yes
1157	SCID	<i>RAG2</i>	NM_000536:exon2:c.T184G	p.F62V	Homozygous	SNV	Yes	Yes
1209	SCID	<i>RAG1</i>	NM_000448:exon2:c.G2041T	p.E681X	Homozygous	SNV	Yes	Yes

despite extensive molecular studies. They represented atypical presentations of known PIDs that were unlikely to be solved by using the candidate gene approach. Details of those patients are presented in [Tables E2 and E3](#). Patients 1019 and 1030 are 2 siblings (8- and 7-year-old boys) referred with the diagnosis of hypogammaglobulinemia and found to have homozygous *AK2* mutations (c.C545A, p.A182D) that were predicted by PolyPhen-2 and Sorting Intolerant From Tolerant to be damaging. Neither patient had evidence of agranulopoiesis or severe T-cell dysfunction. Both of their parents were heterozygous for this mutation. Two other unrelated patients (P916 and P1148) were referred with the diagnosis of CID. Their detailed immunologic workup is shown in [Table E2](#). Both were found to harbor the same homozygous damaging mutation in *AK2* (c.C545A, p.A182D). Their parents were heterozygous for this mutation. Agranulopoiesis was absent in both patients. Although the 4 patients (from 3 families) with this *AK2* mutation have no clear family relationship, they were all descendants of the same tribe.

P1004, a 5-month-old girl with a history of disseminated BCGitis who was given a diagnosis of leaky SCID, was found to have a damaging mutation in *STAT1* (c.1220T>G, p.L407R). Both

parents were heterozygous. P1149 was a 5-year-old girl with hypogammaglobulinemia and a poor specific antibody response. She was found to have a damaging homozygous mutation in *PTPRC* (c.T3062C, p.L1021S). Both parents were heterozygous.

The 2 siblings (P1311 and P1312) were given a diagnosis of CID. Both were found to have a homozygous damaging *ZAP70* mutation (c.G1606A p.G536S) for which their parents were heterozygous. Classical severe CD8 deficiency, which is characteristic of defects in *ZAP70*, was absent in either sibling. Their detailed immunologic workups are shown in [Table E3](#). The same mutation was found previously in another patient (P1082) who had the typical CD8 deficiency.

P992 was given a diagnosis of hyper-IgM syndrome. Details of the immunologic workup are presented in [Table E3](#). The targeted NGS PID panel identified a homozygous nonsense *ATM* mutation (c.9065_9065delA, p.G3023AfsX10). The patient had no apparent ataxia or telangiectasia.

P1257 was given a diagnosis of CID and found to have a homozygous splice site mutation in *CD247* (c.58+8C>T). P1222, a patient with autoimmune enteropathy, hypothyroidism, uveitis, and arthritis, was found to have a homozygous nonsense mutation

TABLE IV. Large homozygous deletions predicted through CNV analysis

Patient ID	Disease	Gene	Confirmed by using CytoScan SNP microarray	Exon PCR amplification failure
1099	Agammaglobulinemia	<i>BTK</i>	No	No
1133	Chronic granulomatous disease	<i>NCF1</i>	No	No
1225	Chronic mucocutaneous candidiasis	<i>PNP</i>		Yes
1202	Hyper-IgE syndrome	<i>DOCK8</i>	Yes	
1252	Hyper-IgE syndrome	<i>DOCK8</i>	Yes	
1258	Hyper-IgE syndrome	<i>DOCK8</i>	Yes	
1214	SCID	<i>DCLRE1C</i>	Yes	
1286	SCID	<i>DCLRE1C</i>	Yes	
1142	SCID	<i>CARD11</i>	No	No
906	SCID	<i>DCLRE1C</i>		Yes
1028	SCID	<i>DCLRE1C</i>		Yes
1168	SCID	<i>IL2RG</i>		Yes

in *LRBA* (c.3985_3986delGA p.D1329YfsX18). P1231 (see Table E2), who was referred as a case of hyper-IgM syndrome, was found to have a novel homozygous missense mutation in *DNM3TB* (c.G2029A, p.V677M), which was predicted to be damaging. Retrospectively, the patient was reported to have hypertelorism, low-set ears, and chromosomal centromeric instability.

CNV analysis predicted large deletions on 6 patients with SCID, involving *DCLRE1C* in 4 patients (P1214, P1286, P906, and P1028), *IL2RG* in 1 patient (P1168), and *CARD11* in 1 patient (P1142); 5 of them were reconfirmed by using the 2.7 M CytoScan SNP microarray or failure of exon PCR amplification. Another 3 patients with hyper-IgE syndrome were predicted to have *DOCK8* deletions (P1202, P1252, and P1258) that were all confirmed by using microarray analysis. One patient (P1225) with chronic mucocutaneous candidiasis was suspected to have a deletion of *PNP*. Another 2 patients with agammaglobulinemia (P1099) and chronic granulomatous disease (P1133) were predicted to have large deletions involving *BTK* and *NCF1*, respectively, although neither could be confirmed by further analysis (Table IV).

Diagnostic efficiency

Targeted NGS, as applied in this study, achieved an overall clinical sensitivity of 25% (35/139 cases) for all patients with unknown genetic defects. A clinical sensitivity of 14% (10/70 cases) was observed within the cohort of the 70 patients who were biased toward more phenotypically and genotypically complicated cases and whose cases were unsolved through the classical conventional candidate gene Sanger sequencing approach. The sensitivity of the panel was higher for new cases with no previous genetic workup (25 [36%] of 69 cases). When considering the combined cohort of solved cases (Sanger-sequenced positive control subjects) and *de novo* or unsolved cases from this study, a molecular diagnosis was reached in 152 of 261 patients, yielding a clinical sensitivity of 58%.

Targeted NGS costs

The consumables cost for our targeted NGS method is approximately \$250 per sample based on multiplexing of

approximately 40 samples per run. Because samples within a single run are not limited to those for the PID panel alone, multiplexing at this level is manageable in a routine diagnostic setting. It is clear that targeted NGS is a cost-effective and more rapid alternative to Sanger sequencing in terms of reagent costs for all genes but in particular for large genes, such as *DOCK8*, and for genetically heterogeneous phenotypes (eg, Omenn syndrome). In addition, the labor required for classical Sanger sequencing is much greater than that for targeted NGS.

Novel variants not in HGMD

Our study identified 89 disease-causing variants, most of which were previously identified by means of Sanger sequencing and redetected by using the NGS PID panel; 44 of these variants had no HGMD records and are considered novel. Interestingly, 2 of the 44 have just been added in the 2015 release of HGMD. Lists of the variants and their HGMD records are shown in Table E6 in this article's Online Repository at www.jacionline.org.

DISCUSSION

Despite major advances in investigating patients presenting with symptoms and signs suggestive of PIDs, a significant fraction of patients still lack a definitive genetic diagnosis, a situation primarily attributed to the phenotypic and genotypic heterogeneity of PIDs. However, the development of high-throughput DNA-sequencing technologies has revolutionized the genetic approach to PIDs and might allow more efficient identification of the genetic basis of inherited immunologic disorders. More recently, NGS-based WES has been successfully used for molecular diagnosis of various Mendelian disorders, especially in patients with neurological phenotypes. In this context 25% of 250 random patients were found to have deleterious mutations.²⁰

The cost of clinical WES is still high, with a long turnaround time. The interpretation of resulting variants remains a big challenge that requires expert analysis because incidental findings not related to clinical indications pose challenging diagnostic and ethical dilemmas.²¹ Alternatively, a focused and clinically driven gene panel-based analysis reduces many of the complexities associated with WES or whole-genome sequencing (WGS). One key difficulty in the design of these panels is defining the appropriate list of genes for a given phenotype because of the remarkable variability in clinical presentations. Indeed, gene panels developed by different laboratories for the same indication are seldom identical, and the discrepancy can be significant. If a panel is designed to focus on certain PID phenotypes, there is a high probability that atypical presentations will be missed. An ideal PID gene panel will include all known PID genes that allow a relaxed clinical indication, such as recurrent infections or immune dysregulation, to trigger testing for PID genes. This list can be easily revised on a regular basis to include newly discovered genes.

Unbiased targeted NGS provides significant opportunities for screening and diagnosing PIDs. We have shown in this study that our recently developed panel that contains 162 PID-related genes was capable of resolving the mutation status for 96% of 122 patients with known genetic defects tested in a single assay. The main reason for missing some mutations was low read depth. Of 162 genes in our panel, only 2 showed inadequate coverage

TABLE V. Comparison of targeted NGS in PID studies

	Stoddard et al ¹⁵	Nijman et al ¹³	Moens et al ¹⁶	Our assay
Technology	Ion Torrent PGM	SOLiD	Illumina Genome Analyzer IIx	Ion Torrent Proton
Enrichment	HaloPlex	Array-based and in-solution-based capture	Selector-based target	AmpliSeq
Genes	173	170	179	162
Total PID cases	133	67	33	261
PID control subjects	13	41	18	122
PID new (test/not yet solved) cases	120	26	15	139
Solved cases	18/120 (15%)	4/26 (15%)	6/15 (40%)	35/139 (25%)
Analytic sensitivity (over control [overall])		40/43 (93%)	20/24 (83%)	117/122 (96%)
Analytic sensitivity (over control [SNV])	98% (exonic)	21/22 (95%)	11/14 (79%)	83/86 (98%)
Analytic sensitivity (over control [indel])		11/13 (85%)	7/8 (87%)	22/24 (92%)
CNV	ND	Done	Done	Done
Sensitivity (large deletions)	ND	8/8 (100%)	2/2 (100%)	12/12 (100%)
Specificity	ND*		18/22 (82%)	88% (90% for SNPs and 81% for indels)
Filtration quality:	NA	15-25	16.5	6
Average no. of variants remaining after filtration per file before expert evaluation				
Cost per sample (consumable)	580 USD	NA	200 USD	250 USD
Coverage (total coverage of targeted bases)	89%	95.2%	88% at 20×	96.5%
Problematic genes	2	9	2	2

NA, Not applicable; USD, US dollars.

*The specificity measure of Stoddard et al¹⁵ is not defined in terms of variants sent to Sanger sequencing in which some of them turned out to be true and others turned out to be false. Rather, it is computed by dividing the number of false-positive results by the whole number of bases in the whole set (59,012 bases), leading to almost 100%, which is not practical. Therefore we dropped their measure from the table.

consistent with that reported by other targeted NGS panels.^{13,16} Table V^{13,15,16} shows a comparison of the performance of our assay against the published targeted NGS PID panels in regard to technology, sensitivity, specificity, cost, and gene coverage. Our study included a larger number of patients than most, with various PID phenotypes and underlying genetic defects, and confirmed the utility of the assay in relation to obtaining a genetic diagnosis for PIDs. However, most of the patients enrolled in this study come from a consanguineous population, and thus our study was efficient in detecting homozygous mutations/deletions. This approach is expected to perform well in detecting compound heterozygous mutations, even though we did not test its performance under those circumstances. Additionally, we did not extensively test our approach for detecting missense mutations in autosomal dominant disorders other than *STAT3* or *FAS*. Thus the success rate of this approach in finding causative PID mutations might be lower when applied to outbred populations.

The results in this study illustrate the effectiveness of the developed assay for detecting point mutations and CNVs in known PID genes. The relatively low mutation detection rate in our cohort for patients compared with positive control subjects might be considered a major limitation. However, currently, our panel covers only 162 of 240 gene reported by the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency published in 2014.¹ We also cannot rule out the possibility that some of the cases in our study represent a novel genetic entity because our cohort included many atypical cases. The negative cases from our study are likely to be enriched in those that might result in discovery of novel genetic causes of PIDs.

The diagnostic efficiency of the assay is clearly demonstrated by our ability to obtain a genetic diagnosis in patients with atypical presentations of known PIDs. For example, 4 patients with *AK2* defects presented with variable clinical features that include CID and hypogammaglobulinemia without agranulopoiesis and were unlikely to be screened for *AK2* mutations based on current clinical data and the candidate gene approach. Similarly, 2 siblings were found to harbor a *ZAP70* mutation without CD8 deficiency.

Nonetheless, the identification of disease-causing genetic defects in patients with PIDs is challenging. Assay design and optimization are crucial for balanced and adequate coverage across all targeted genes. Filtering out of noise or other sequencing artifacts and the need for manual curation of results are essential. Inadequate coverage of some coding and flanking regions of PID genes leading to false-negative results required consideration when drawing conclusions for cases with negative findings. Limitations in the design of the PID panel to amplicons encompassing the coding and flanking regions of genes, particularly intron-exon boundaries, must be recognized. Similarly, inability to cover pathological variants in introns, promoter and regulatory, or to detect somatic mosaicism are limitations, although for the latter, the higher coverage offered by gene panel sequencing is an advantage. Detection of large deletions and in particular insertions requires novel statistical approaches for reliable detection and poses greater challenges when heteroallelic. In our study we were able to efficiently identify homozygous deletions based on read numbers in relation to normalized samples used for comparison. Because we are using target-based sequencing, CNV analysis might carry a higher false-positive detection rate because of nonuniformity of coverage and poor performance of the primers at some regions, especially for the heterozygous calls.

Chromosomal rearrangements cannot be identified by using current library construction and data analysis applied to gene panels, including those used in this study.

Pathogenicity of detected variants in the absence of functional studies is heavily dependent on filtration against databases recording human variation, including the Exome Variant Server, 1000 Genomes database, dbSNP, and others. Establishment and inclusion of ethnically specific variant databases arising out of national programs are likely to be a rich source of information to differentiate pathogenic changes from rare and often population-specific variants. Similarly, databases of known pathological variants, such as HGMD, are also a vital resource for interpretation of data from NGS assays, including those limited to targeted gene panels.²²

The targeted NGS PID panel is an efficient screening tool that can be used as a first step to screen for the presence or absence of pathogenic mutations. The use of this panel allowed identification of possible pathogenic mutations in genes that were unlikely to be screened by using the classical candidate gene approach. All identified mutations require confirmation by means of Sanger sequencing and genotype-phenotype correlation. Rare or novel variants require careful interpretation with *in silico* tools, such as PolyPhen-2 or Sorting Intolerant From Tolerant.^{23,24} All novel variants, especially missense mutations in PID genes with atypical clinical presentations, will require functional and experimental validation to prove that they are actually deleterious. Functional assays will always remain the benchmark for causal association with a disease phenotype, irrespective of the molecular approach used.

The data available in regard to the underlying molecular genetic defects of various PIDs among Arab populations are still limited. The large number of variants we identified in the course of this study represents an unprecedented resource for the Arab variome (nearly all patients in this study were of Arab ethnicity). In this study we have added 44 novel disease alleles from a total of 89 identified. This will be invaluable to the interpretation of clinical molecular genetic tests on PID genes in Arab patients because it will help address the uncertainty surrounding the identification of many Arab-specific or Arab-enriched rare variants.

The current assay is cost-effective and has a rapid turnaround time. The bioinformatic analyses and lists of potential candidate mutations generated is much shorter compared with WES or WGS. Interpretation of resulting data is simpler, with a lower likelihood of incidental findings compared with WES and WGS. Therefore we believe that the current assay can be used as a first-line molecular genetic screen for diagnosis in patients with suspected PIDs. WES or WGS can be applied to further work up unsolved cases.

We thank the patients and their families for their participation. All NGS experiments and analyses were performed as part of the Saudi Human Genome Program (SHGP) at KACST and King Faisal Specialist Hospital & Research Center.

Clinical implications: The targeted NGS PID gene panel is a sensitive and cost-effective diagnostic tool that can be used as a first-line molecular assay, particularly for patients with atypical presentation of PIDs.

REFERENCES

1. Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, Cunningham-Rundles C, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. *Front Immunol* 2014;5:162.
2. Notarangelo LD, Sorensen R. Is it necessary to identify molecular defects in primary immunodeficiency disease? *J Allergy Clin Immunol* 2008;122:1069-73.
3. Villa A, Notarangelo LD, Roifman CM. Omenn syndrome: inflammation in leaky severe combined immunodeficiency. *J Allergy Clin Immunol* 2008;122:1082-6.
4. Santagata S, Villa A, Sobacchi C, Cortes P, Vezzoni P. The genetic and biochemical basis of Omenn syndrome. *Immunol Rev* 2000;178:64-74.
5. Villa A, Sobacchi C, Notarangelo LD, Bozzi F, Abinun M, Abrahamsen TG, et al. V(D)J recombination defects in lymphocytes due to RAG mutations: severe immunodeficiency with a spectrum of clinical presentations. *Blood* 2001;97:81-8.
6. Villa A, Sobacchi C, Vezzoni P. Recombination activating gene and its defects. *Curr Opin Allergy Clin Immunol* 2001;1:491-5.
7. Sobacchi C, Marrella V, Rucci F, Vezzoni P, Villa A. RAG-dependent primary immunodeficiencies. *Hum Mutat* 2006;27:1174-84.
8. de Villartay JP, Lim A, Al-Mousa H, Dupont S, Dechanet-Merville J, Coumou-Gatbois E, et al. A novel immunodeficiency associated with hypomorphic RAG1 mutations and CMV infection. *J Clin Invest* 2005;115:3291-9.
9. Navin N, Hicks J. Future medical applications of single-cell sequencing in cancer. *Genome Med* 2011;3:31.
10. Fokstuen S, Makrythanasis P, Nikolaev S, Santoni F, Robyr D, Munoz A, et al. Multiplex targeted high-throughput sequencing for Mendelian cardiac disorders. *Clin Genet* 2014;85:365-70.
11. Guilmatre A, Highnam G, Borel C, Mittelman D, Sharp AJ. Rapid multiplexed genotyping of simple tandem repeats using capture and high-throughput sequencing. *Hum Mutat* 2013;34:1304-11.
12. Chou J, Ohsumi TK, Geha RS. Use of whole exome and genome sequencing in the identification of genetic causes of primary immunodeficiencies. *Curr Opin Allergy Clin Immunol* 2012;12:623-8.
13. Nijman IJ, van Montfrans JM, Hoogstraal M, Boes ML, van de Corput L, Renner ED, et al. Targeted next-generation sequencing: a novel diagnostic tool for primary immunodeficiencies. *J Allergy Clin Immunol* 2014;133:529-34.
14. Ghosh S, Krux F, Binder V, Gombert M, Niehues T, Feyen O, et al. Array-based sequence capture and next-generation sequencing for the identification of primary immunodeficiencies. *Scand J Immunol* 2012;75:350-4.
15. Stoddard JL, Niemela JE, Fleisher TA, Rosenzweig SD. Targeted NGS: a cost-effective approach to molecular diagnosis of PIDs. *Front Immunol* 2014;5:531.
16. Moens LN, Falk-Sorqvist E, Asplund AC, Bernatowska E, Smith CI, Nilsson M. Diagnostics of primary immunodeficiency diseases: a sequencing capture approach. *PLoS One* 2014;9:e114901.
17. Routier FH, Hounsell EF, Rudd PM, Takahashi N, Bond A, Hay FC, et al. Quantitation of the oligosaccharides of human serum IgG from patients with rheumatoid arthritis: a critical evaluation of different methods. *J Immunol Methods* 1998;213:113-30.
18. Alsum Z, Hawwari A, Alsmadi O, Al-Hissi S, Borrero E, Abu-Staitieh A, et al. Clinical, immunological and molecular characterization of DOCK8 and DOCK8-like deficient patients: single center experience of twenty-five patients. *J Clin Immunol* 2013;33:55-67.
19. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010;38:e164.
20. Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med* 2013;369:1502-11.
21. Evans BJ. Minimizing liability risks under the ACMG recommendations for reporting incidental findings in clinical exome and genome sequencing. *Genet Med* 2013;15:915-20.
22. Weiss MM, Van der Zwaag B, Jongbloed JD, Vogel MJ, Bruggenwirth HT, Lekanne Deprez RH, et al. Best practice guidelines for the use of next-generation sequencing applications in genome diagnostics: a national collaborative study of Dutch genome diagnostic laboratories. *Hum Mutat* 2013;34:1313-21.
23. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009;4:1073-81.
24. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7:248-9.

Supplementary Table 1: PID patients with known causal mutations for Assay validation

Patients	Disease	Gene	Mutation	Effect	Zygosity	Mutation type	Detected by T-NGS	Detected by CNV	Comment
1176	Agammaglobulinemia	<i>BTK</i>	NM_000061:exon8:c.C763T	p.R255X	Hemizygous	SNV	YES		
1177	Agammaglobulinemia	<i>BTK</i>	NM_000061:exon8:c.C763T	p.R255X	Hemizygous	SNV	YES		
1178	Agammaglobulinemia	<i>BTK</i>	NM_000061:exon8:c.C763T	p.R255X	Hemizygous	SNV	YES		
1014	Agammaglobulinemia	<i>BTK</i>	NM_000061:exon2:c.C82T	p.R28C	Hemizygous	SNV	YES		
984	Agammaglobulinemia	<i>BTK</i>	NM_000061:exon8:c.763C>T	p.R255X	Hemizygous	SNV	YES		
1139	Ataxia telangiectasia	<i>ATM</i>	NM_000051:exon63:c.9065delA	p.E3022fs	Homozygous	Short Indel	YES		
1193	Ataxia telangiectasia	<i>ATM</i>	NM_000051:exon5:c.381delA	p.T127fs	Homozygous	Short Indel	YES		
986	Ataxia telangiectasia	<i>ATM</i>	NM_000051:exon61:c.8833_8834delCT	L2945VfsX10	Homozygous	Short Indel	YES		
1093	Ataxia telangiectasia	<i>ATM</i>	Splice:NM_000051:exon42:c.6198+1G>A	N/A	Homozygous	SNV	YES		
931	Ataxia telangiectasia	<i>ATM</i>	NM_000051:exon8:c.902_903insT	p.A302CfsX3	Homozygous	Short Indel	YES		
988	Ataxia telangiectasia	<i>ATM</i>	NM_000051:exon5:c.381delA	p.T127fs	Homozygous	Short Indel	YES		
1005	Ataxia telangiectasia	<i>ATM</i>	NM_000051:exon5:c.381delA	p.T127fs	Homozygous	Short Indel	YES		
1116	Autoimmune polyendocrinopathy syndrome	<i>AIRE</i>	NM_000383:exon2:c.C274T	p.R92W	Homozygous	SNV	YES		
1213	Bare Lymphocyte Syndrome	<i>RFXA</i> <i>NK</i>	NM_003721:exon6:c.A362T	p.D121V	Homozygous	SNV	YES		

1224	Bare Lymphocyte Syndrome	RFXA NK	NM_003721:exon6:c.A362T	p.D121V	Homozygous	SNV	YES	
1246	Bare Lymphocyte Syndrome	RFXA NK	NM_003721:c.269+1delGinsTCAC	N/A	Homozygous	Short Indel	NO	low read depth at the variant position
1215	Bare Lymphocyte Syndrome	RFXA NK	Splice: NM_134440:exon4:c.268+1G>C	N/A	Homozygous	Short Indel	YES	
1200	Cartilage Hair hypoplasia	RMRP	ncRNA; C.7 A>G	N/A	Homozygous	SNV	YES	
958	Chronic Granulomatous Disease	CYBA	splice: NM_000101:exon6:c.369+1G>T	N/A	Homozygous	SNV	YES	
1276	Chronic Granulomatous Disease	CYBB	NM_000397:exon6:c.G618A	p.W206X	Hemizygous	SNV	YES	
1095	Chronic Granulomatous Disease	CYBB	NM_000397:exon6:c.G618A	p.W206X	Hemizygous	SNV	YES	
955	Chronic Granulomatous Disease	CYBB	NM_000397:exon5:c.C388T	p.R130X	Hemizygous	SNV	YES	
960	Chronic Granulomatous Disease	CYBB	NM_000397:exon13:c.1618delG	E540fs	Hemizygous	Short Indel	YES	
1283	Chronic Granulomatous Disease	CYBB	NM_000397:exon6:c.G618A	p.W206X	Hemizygous	SNV	YES	
879	Chronic Granulomatous Disease	CYBB	NM_000397:exon10:c.1217T>A	p.L406X	Hemizygous	SNV	YES	
1002	Chronic Granulomatous Disease	CYBB	NM_000397:exon13:c.1618delG	p.E540fs	Hemizygous	Short Indel	YES	
966	Chronic Granulomatous Disease	NCF2	NM_000433:exon2:c.C229T	p.R77X	Homozygous	SNV	YES	
1230	Chediak-Higashi Syndrome	LYST	NM_000081:exon23:c.G6694T	p.G2232X	Homozygous	SNV	YES	

1316	Chediak-Higashi Syndrome	<i>LYST</i>	Splice: NM_000081:exon37:c.9044+1G>T	N/A	Homozygous	SNV	YES	
1188	Griscelli Syndrome	<i>MLPH</i>	NM_001042467:exon2:c.G104A	p.R35Q	Homozygous	SNV	YES	
1189	Griscelli Syndrome	<i>MLPH</i>	NM_001042467:exon2:c.G104A	p.R35Q	Homozygous	SNV	YES	
1044	Griscelli Syndrome	<i>MLPH</i>	NM_001042467: exon8: c.986delC	p.S329fs	Homozygous	Short Indel	YES	
1040	Griscelli Syndrome	<i>RAB27A</i>	NM_004580:exon6:c.C598T	p.R200X	Homozygous	SNV	YES	
1043	Griscelli Syndrome	<i>RAB27A</i>	NM_004580.4:c.148_149delinsC	p. R50QfsX35	Homozygous	Short Indel	YES	
1120	Griscelli Syndrome	<i>RAB27A</i>	NM_004580:exon5:c.A400C	p.K134Q	Homozygous	SNV	YES	
1135	Griscelli Syndrome	<i>RAB27A</i>	NM_004580:exon2:c.53_54delCT	p.S18WfsX15	Homozygous	Short Indel	YES	
882	Griscelli Syndrome	<i>RAB27A</i>	NM_004580:exon6:c.598C>T	p.R200X	Homozygous	SNV	YES	
892	Griscelli Syndrome	<i>RAB27A</i>	NM_004580:exon6:c.514C>T	Q172X	Homozygous	SNV	YES	
1037	Griscelli Syndrome	<i>RAB27A</i>	NM_004580:exon6:c.598C>T	p.R200X	Homozygous	SNV	YES	
897	Hyper IgE Syndrome	<i>DOCK8</i>	DOCK8 deletion	N/A	Homozygous	Exonic Deletion	YES	
1155	Hyper IgE Syndrome	<i>DOCK8</i>	Splice: NM_203447:exon7:c.827+6T>C	N/A	Homozygous	SNV	YES	
1163	Hyper IgE Syndrome	<i>DOCK8</i>	NM_203447:exon8:c.850_851delCT	p.L284RfsX24	Homozygous	Short Indel	YES	
1199	Hyper IgE Syndrome	<i>DOCK8</i>	DOCK8 deletion	N/A	Homozygous	Exonic Deletion	YES	

1269	Hyper IgE Syndrome	DOCK8	NM_203447:exon44:c.T5625G	p.Y1875X	Homozygous	SNV	YES	
1036	Hyper IgE Syndrome	DOCK8	NM_203447:exon44:c.T5625G	p.Y1875X	Homozygous	SNV	YES	
1045	Hyper IgE Syndrome	DOCK8	NM_203447:exon40:c.C5132A	p.S1711X	Homozygous	SNV	YES	
1054	Hyper IgE Syndrome	DOCK8	NM_203447:exon44:c.T5625G	p.Y1875X	Homozygous	SNV	YES	
1056	Hyper IgE Syndrome	DOCK8	NM_203447:exon44:c.T5625G	p.Y1875X	Homozygous	SNV	YES	
1094	Hyper IgE Syndrome	DOCK8	DOCK8 deletion	N/A	Homozygous	Exonic Deletion	YES	
1101	Hyper IgE Syndrome	DOCK8	Splice: NM_203447:c.4626+5G>A	N/A	Homozygous	SNV	YES	
1114	Hyper IgE Syndrome	DOCK8	NM_203447:exon40:c.C5132A	p.S1711X	Homozygous	SNV	YES	
1122	Hyper IgE Syndrome	DOCK8	NM_203447: c.4626+5 G>A	N/A	Homozygous	SNV	YES	
1296	Hyper IgE Syndrome	DOCK8	NM_203447:exon44:c.T5625G	p.Y1875X	Homozygous	SNV	YES	
994	Hyper IgE Syndrome	DOCK8	NM_203447:exon40:c.C5132A	p.S1711X	Homozygous	SNV	YES	
1006	Hyper IgE Syndrome	DOCK8	NM_203447:exon44:c.T5625G	p.Y1875X	Homozygous	SNV	YES	
1096	Hyper IgE Syndrome	DOCK8	NM_203447:exon44:c.T5625G	p.Y1875X	Homozygous	SNV	YES	
1198	Hyper IgE Syndrome	DOCK8	DOCK8 deletion	N/A	Homozygous	Exonic Deletion	YES	
1274	Hyper IgE Syndrome	DOCK8	DOCK8 deletion	N/A	Homozygous	Exonic Deletion	YES	
1123	Hyper IgE Syndrome	DOCK8	DOCK8 deletion	N/A	Homozygous	Exonic Deletion	YES	

1126	Hyper IgE Syndrome	<i>DOCK8</i>	DOCK8 deletion	N/A	Homozygous	Exonic Deletion	YES	
1239	Hyper IgE Syndrome	<i>DOCK8</i>	DOCK8 deletion	N/A	Homozygous	Exonic Deletion	YES	
1254	Hyper IgE Syndrome	<i>DOCK8</i>	DOCK8 deletion	N/A	Homozygous	Exonic Deletion	YES	
1186	Hyper IgE Syndrome	<i>DOCK8</i>	DOCK8 deletion	N/A	Homozygous	Exonic Deletion	YES	
1111	Hyper IgE Syndrome	<i>STAT3</i>	NM_003150:exon13:c.G1145A	p.R382Q	Heterozygous	SNV	YES	
1013	Hyper IgE Syndrome	<i>STAT3</i>	NM_003150:exon13: c.C1144T	p.R382W	Heterozygous	SNV	YES	
1015	Hyper IgE Syndrome	<i>STAT3</i>	NM_003150:exon20:c.G1868T	p.W623L	Heterozygous	SNV	YES	
907	Hyper IgE Syndrome	<i>STAT3</i>	NM_003150:exon13:c.C1144T	p.R382W	Heterozygous	SNV	NO	low read depth at the variant position
1012	Hyper IgE Syndrome	<i>STAT3</i>	NM_003150:exon13:c.C1144T	p.R382W	Heterozygous	SNV	YES	
1089	Hyper IgM Syndrome	<i>AICDA</i>	NM_020661:exon3:c.G169A	p.V57M	Homozygous	SNV	YES	
1088	Hyper IgM Syndrome	<i>AICDA</i>	NM_020661:exon3:c.G169A	p.V57M	Homozygous	SNV	YES	
1090	Hyper IgM Syndrome	<i>AICDA</i>	NM_020661:exon3:c.G169A	p.V57M	Homozygous	SNV	YES	
1171	Hyper IgM Syndrome	<i>CD40</i>	Splice: NM_001250:exon3:c.256+2T>C	N/A	Homozygous	SNV	YES	
1194	Hyper IgM Syndrome	<i>CD40</i>	uc002xrf.1:exon3:c.C170T	p.T57M	Homozygous	SNV	YES	
1071	Hyper IgM Syndrome	<i>CD40</i>	NM_152854:exon3:c.T247C	p.C83R	Homozygous	SNV	YES	

1072	Hyper IgM Syndrome	CD40	NM_152854:exon3:c.T247C	p.C83R	Homozygous	SNV	YES	
889	Hyper IgM Syndrome	CD40	Splice: NM_001250:exon3:c.256+2T>C	N/A	Homozygous	SNV	YES	
894	Hyper IgM Syndrome	CD40	Splice: NM_001250:exon3:c.256+2T>C	N/A	Homozygous	SNV	YES	
1137	Hyper IgM Syndrome	CD40L G	NM_000074:exon5:c.A506G	p.Y169C	Hemizygous	SNV	YES	
1104	Hyper IgM Syndrome	CD40L G	NM_000074:exon5:c.719_720delAT	p.N240SfsX3	Hemizygous	Short Indel	YES	
1314	Pneumococcal sepsis	IRAK4	NM_001145258:exon5:c.451_451delT	p.S151LfsX13	Homozygous	Short Indel	YES	
1240	Leukocyte Adhesion deficiency-1	ITGB2	NM_000211: c.897+1 G>A	N/A	Homozygous	SNV	NO	low read depth at the variant position
1260	Microcephaly with Immunodeficiency	NHEJ1	Splice: NM_024782:exon4:c.390+1G>C	N/A	Homozygous	SNV	YES	
1058	Microcephaly with Immunodeficiency	NHEJ1	Splice: NM_024782:exon4:c.390+1G>C	N/A	Homozygous	SNV	YES	
1128	Mendellian Susceptibility to mycobacterial disease	IFNGR 2	NM_005534:exon5:c.C705A	p.Y235X	Homozygous	SNV	YES	
1110	Omenn Syndrome	RAG2	NM_000536:exon2:c.C1332G	p.I444M	Homozygous	SNV	YES	
1134	Severe Combined Immunodeficiency Disease	ADA	NM_000022:exon7:c.G646A	p.G216R	Homozygous	SNV	YES	
1242	Severe Combined Immunodeficiency Disease	ADA	NM_000022: c.385G>A	p.V129M	Homozygous	SNV	NO	low read depth at the variant position
1309	Severe Combined Immunodeficiency Disease	ADA	NM_000022:exon5:c.G385A	p.V129M	Homozygous	SNV	YES	
1026	Severe Combined Immunodeficiency	ADA	Splice: NM_000022:exon13:c.1079-15T>A	N/A	Homozygous	SNV	YES	

Disease								
878	Severe Combined Immunodeficiency Disease	ADA	NM_000022:exon10:c.955_959delGAAGA	p.E319GfsX3A	Homozygous	Short Indel	YES	
932	Severe Combined Immunodeficiency Disease	DCLR E1C	NM_022487:exon5:c.G58A	p.G20R	Homozygous		YES	
886	Severe Combined Immunodeficiency Disease	DCLR E1C	DCLRE1C deletion	N/A	Homozygous	Exonic Deletion	YES	
1041	Severe Combined Immunodeficiency Disease	DCLR E1C	DCLRE1C deletion	N/A	Homozygous	Exonic Deletion	YES	
1158	Severe Combined Immunodeficiency Disease	IL7R	NM_002185:exon5:c.T636G	p.Y212X	Homozygous	SNV	YES	
1076	Severe Combined Immunodeficiency Disease	IL7R	NM_002185:exon8:c.885delT	p.N295fs	Homozygous	Short Indel	YES	
1103	Severe Combined Immunodeficiency Disease	JAK3	NM_000215:exon3:c.G308A	p.R103H	Homozygous	SNV	YES	
979	Severe Combined Immunodeficiency Disease	JAK3	NM_000215:exon7:c.C913T	p.Q305X	Homozygous	SNV	YES	
1007	Severe Combined Immunodeficiency Disease	JAK3	NM_000215:exon9:c.1207C>T	p.R403C	Homozygous	SNV	YES	
912	Severe Combined Immunodeficiency Disease	PNP	NM_000270:exon5:c.487T>C:	p.S163P	Homozygous	SNV	YES	
1010	Severe Combined Immunodeficiency Disease	PNP	Splice: NM_000270:exon2:c.12-1G>C	N/A	Homozygous	SNV	YES	
1145	Severe Combined Immunodeficiency Disease	RAG1	NM_000448:exon2:c.A1438G	p.S480G	Homozygous	SNV	YES	

1150	Severe Combined Immunodeficiency Disease	RAG1	NM_000448:exon2:c.T1201C	p.S401P	Homozygous	SNV	YES		
1151	Severe Combined Immunodeficiency Disease	RAG1	NM_000448:exon2:c.T1201C	p.S401P	Homozygous	SNV	YES		
1201	Severe Combined Immunodeficiency Disease	RAG1	NM_000448:exon2:exon1:c.554delG	p.R185fs	Homozygous	Short Indel	YES		
1205	Severe Combined Immunodeficiency Disease	RAG1	NM_000448:exon2:exon1:c.554delG	p.R185fs	Homozygous	Short Indel	YES		
1121	Severe Combined Immunodeficiency Disease	RAG1	NM_000448:exon2:c.G2210A	p.R737H	Homozygous	SNV	YES		
1301	Severe Combined Immunodeficiency Disease	RAG1	NM_000448:exon2:c.C1180T	p.R394W	Homozygous	SNV	YES		
1302	Severe Combined Immunodeficiency Disease	RAG1	NM_000448:exon2:exon1:c.554delG	p.R185fs	Homozygous	Short Indel	YES		
1192	Severe Combined Immunodeficiency Disease	RAG2	NM_000536:exon2:c.C442T	p.R148X	Homozygous	SNV	YES		
1048	Severe Combined Immunodeficiency Disease	RAG2	NM_000536:exon2:c.1403_1406delATC T	p.H468RfsX16	Homozygous	Short Indel	YES		
1106	Severe Combined Immunodeficiency Disease	RAG2	NM_000536:exon2:c.374_375delCA	p.T125RfsX10	Homozygous	Short Indel	YES		
943	Severe Combined Immunodeficiency Disease	RAG2	NM_000536:exon2:c.G686C	p.R229P	Homozygous	SNV	YES		
1022	Severe Combined Immunodeficiency Disease	RAG2	NM_000536:exon2:c.C1332G	p.I444M	Homozygous	SNV	YES		
1029	Severe Combined Immunodeficiency Disease	RAG2	NM_000536:exon2:c.T547C	p.F183L	Homozygous	SNV	YES		

1082	Severe Combined Immunodeficiency Disease	ZAP70	NM_001079:exon12:c.G1606A	p.G536S	Homozygous	SNV	YES	
999	Severe Combined Immunodeficiency Disease	ZAP70	NM_001079:exon5:c.569G>C	p.R190T	Homozygous	SNV	YES	
999	Severe Combined Immunodeficiency Disease	ZAP70	NM_001079:exon5:c.569G>C	p.R190T	Homozygous	SNV	YES	
1140	Wiskott-Aldrich Syndrome	WAS	NM_000377:exon2:c.G257A	p.R86H	Hemizygous	SNV	YES	
1159	Wiskott-Aldrich Syndrome	WAS	NM_000377:c.1297_1298InsG	A433GfsX62	Hemizygous	Short Indel	NO	low read depth at the variant position
1210	Wiskott-Aldrich Syndrome	WAS	NM_000377:exon1:c.G91A	p.E31K	Hemizygous	SNV	YES	
1112	Wiskott-Aldrich Syndrome	WAS	NM_000377:exon2:c.G257A	p.R86H	Hemizygous	SNV	YES	
1119	Wiskott-Aldrich Syndrome	WAS	NM_000377:exon4:c.G397A	p.E133K	Hemizygous	SNV	YES	

Supplementary table2 : Clinical, immunological data and list of genes Sanger sequenced for patients with previous genetic work up

Patient	Clinical and immunological data	Working Diagnosis	Pervious genetic work up	T-NGS / CNV result
915	4 years old male with partial albinism, grey silvery hair, CMV induced hemophagocyticlymphohistiocytosis, Pigment clumps in hair shafts, no giant leukocytes inclusion bodies.	Griscelli Disease	<i>RAB27A</i>	
916	20 years old female with history of recurrent chest infections, bronchiectasis, inflammatory bowel disease, psoriasis, CMV infection and poor weight gain. WBC, absolute neutrophils and lymphocytes counts were normal, IgG: 4.5, IgA: 1.57, IgM: < 0.18 g/l. CD3: 800-4000, CD4: 500-1300, CD8: 300-2400, CD19: 150-350, CD16/56: 50-250 / mm ³ , CD3+CD45RA+ 20%, PHA: 33000-111000 CPM, No maternal cell engraftment	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C, ICOS, CR2, CD19, CD81</i>	AK2 c.C545A p.A182D
927	30 years old female, with history of recurrent sinopulmonary infections since early childhood. CD3 2170, CD4 1201, CD8 901, CD19 < 23 /mm ³ , PHA 44390 CPM, IgG 1.5, IgA <0.25, IgM <0.17 g/l.	Agammaglobulinemia	<i>BTK, BLNK</i>	
935	3 years old male, with history of recurrent chest infections, persistent CMV infections, autoimmune hemolytic anemia, CD3 1583, CD4 950, CD8 361, CD19 728, CD56 558 /mm ³ , IgG 2.8, IgA 4.9, IgM 0.7 g/l, IgE 24990KU/L, PHA 24699 CPM.	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C</i>	
936	2 years old female, with recurrent chest infections, chronic diarrhea, CMV Infection and autoimmune hemolytic anemia. CD3 4098, CD4 2466, CD19 1760, CD56 503 /mm ³ , PHA 27914 CPM, IgG 12.3, IgA 3.8, IgM 0.8 g/l, IgE 8370KU/L.	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C</i>	
938	1 month old female, diagnosed as SCID on new born screening because of family history of SCID. CD3 4, CD4 <1, CD8 4, CD19 875, CD56 899 /mm ³ , IgG 7.3, IgA ,0.25, IgM 0.83 g/l, PHA 224 CPM.	Severe Combined Immunodeficiency Disease	<i>RAG1, RAG2, DCLRE1C, IL7R, JAK3 and CD3E, CD3G, CD247</i>	
946	5 years old male, with history of recurrent chest infections and lymphadenitis. 99% of the patient's cells failed to reduce DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	<i>CYBB, CYBA, NCF1, NCF2</i>	

947	15 years old female, with history of recurrent chest infections and deep seated abscesses. 99% of the patient's cells failed to reduce DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	<i>CYBA, NCF1, NCF2</i>	
948	10 years old male, with history of recurrent chest infections, lymphadenitis and skin abscesses. 99% of the patient's cells failed to reduce DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	<i>CYBB, CYBA, NCF1, NCF2</i>	
949	16 years old female, with history of recurrent chest infections, lymphadenitis and inflammatory bowel diseases. 99% of the patient's cells failed to reduce DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	<i>CYBA, NCF1, NCF2</i>	
956	18 years old male, with history of recurrent chest infections, lymphadenitis and skin abscesses. Family history of CGD, 99% of the patient's cells failed to reduce DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	<i>CYBB, CYBA, NCF1, NCF2</i>	
957	20 years old female, with history of recurrent chest infections, lymphadenitis and skin abscesses. 99% of the patient's cells failed to reduce DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	<i>CYBA, NCF1, NCF2</i>	
959	14 years old male, with history of chest infections, progressive ataxia, telangiectasia and Hodgkin's lymphoma. IgG 6.1, IgA 0.59, IgM 0.51 g/l, Alpha-fetoprotein (AFP) level is elevated high.	Ataxia Telangiectasia	<i>ATM</i>	
962	9 years old male, with history of recurrent chest infections, bronchiectasis and failure to thrive. CD3 734, CD4 98, CD8 587, CD19 815, CD56 82 /mm ³ , PHA 5295 CPM, IgG 12.8, IgA 0.7, IgM 0.7 g/l, CD3+ CD45RA+ 24%, CD4+ CD45RA+ 3%.	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C</i>	
969	8years old female, with history of recurrent lymphadenitis and skin abscesses. 99% of the patient's cells failed to reduce DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	<i>CYBA, NCF1, NCF2</i>	
973	5 years old male, with history of recurrent chest infections, deep-seated abscesses and inflammatory bowel disease, 99% of the patient's cells failed to reduce DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	<i>CYBB, CYBA, NCF1, NCF2</i>	
978	19 years old male, with history of recurrent chest infections and deep-seated abscesses, 99% of the patient's cells failed to reduce DHR-123 post PMA stimulation. Only 19% of patient's granulocytes reduced the NBT dye.	Chronic Granulomatous Disease	<i>CYBB, CYBA, NCF1, NCF2</i>	

983	8 months old female, with chronic diarrhea, failure to thrive and recurrent chest infections. CD3 1, CD4 1, CD8 <1, CD19 <1, CD56 563 /mm3, IgG <1.6 IgA <0.25, IgM <0.18 g/l, PHA 358 CPM	Severe Combined Immunodeficiency Disease	<i>RAG1, RAG2, DCLRE1C</i>	
990	2 years old female, with recurrent sinopulmonary infections, chronic diarrhea, eczema and autoimmune hemolytic anemia. CD3 973, CD4 98, CD8 872, CD19 70, CD56 108 /mm3, PHA 39169 CPM, IgG 18, IgA 7.2, IgM 0.29 g/l, 19% maternal lymphocytes engraftment.	Severe Combined Immunodeficiency Disease	<i>RAG1, RAG2, DCLRE1C</i>	
993	7 years old female, with history of recurrent sinopulmonary infections, eczema and family history of 4 children died because of chronic diarrhea and recurrent infections. CD3 1103, CD4 122, CD8 524, CD19 25, CD56 770 /mm3, PHA 4358 CPM, IgG 9.4, IgA 0.36, IgM 0.62 g/l, ADA normal, no maternal T-cell engraftment.	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C</i>	
996	17 years old female with recurrent chest infections and chronic immune thrombocytopenic purpura, CD3 1739, CD4 1112, CD8 548, CD19 364, CD56 121 / mm3, IgG 4.6, IgA<0.25, IgM 0.82 g/l, PHA 19159 CPM, intact CD40 and CD40L expression.	Common Variable Immunodeficiency Disease	<i>RAG1, RAG2, DCLRE1C ,ADA</i>	
997	8 months old boy with recurrent chest infections, chronic diarrhea, failure to thrive, hepatosplenomegaly, lymphadenopathy and T-cell lymphoma, CD3 1405, CD4 754, CD8 612, CD19 <1, CD56 27 /mm3 , IgG 3.8, IgA 0.61, IgM 1.26 g/l, PHA 79855 CPM	Ill-defined immunodeficiency	<i>RAG1, RAG2, DCLRE1C, ADA</i>	
1004	5 months old female with history of disseminated BCGitis and secondary hemophagocytosis, IgG: 9, IgA: 1.24, IgM: 0.5 g/l, CD3: 4170, CD4: 1600, CD8: 1300, CD19: 205, CD16/56: 141 /mm3, PHA: 3047 CPM. No maternal cell engraftment	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C</i>	STAT1 c.1220T>G p.L407R
1017	5 months old male withchronicdiarrhea, failure to thrive and recurrent chest infections. CD3 1, CD4 1, CD8 <1, CD19 <1, CD56 1289 /mm3, IgG 1.8, IgA <0.25, IgM <0.18 g/l, PHA 1211 CPM	Severe Combined Immunodeficiency Disease	<i>RAG1, RAG2, DCLRE1C, CD247</i>	
1019	8 years old male (brother of P1030) with history of recurrent chest infections. normal WBC and differential, IgG: 4.3, IgA: < 0.25, IgM: <0.18 g/l, CD3: 4200 , CD4: 2700 , CD8: 1300 , CD19: 1800 , CD16/56: 540 /mm3, CD3+CD45RA+ 44%, PHA: 49000 -73000 CPM, No maternal cell engraftment, No agranulopoeisis	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C, BTK</i>	AK2 c.C545A p.A182D

1021	8 month old female, with infantile osteopertosis, developmental delay, recurrent infections, leukocytosis and ectodermal dysplasia. IgG 7.6, IgA <0.25, IgM 0.28 g/l, CD3 14748, CD19 16436, CD56 1789 / mm3, PHA 50947 CPM.	Ill-defined immunodeficiency	<i>RAG1, RAG2, JAK3, DCLRE1C, FERMT3</i>	
1030	7 years old male (brother of P1019) with history of recurrent chest infections, normal WBC and differential, IgG: 1.6, IgA: < 0.25, IgM: < 0.18 g/l, CD3: 3400, CD4: 2100, CD8: 800, CD19: 1200, CD16/56: 600 /mm3, PHA: 50000- 185000 CPM, No maternal cell engraftment, No agranulopoeisis	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C, BTK</i>	AK2 c.C545A p.A182D
1035	12 years old female with severe eczema and recurrent ear and chest infections. CD3 2429, CD4 1280, CD8 458, CD19 725, CD56 425 / mm3, IgG 11.6, IgA 1.14, IgM 0.56 g/l, IgE 10040KU/L, PHA 80447 CPM	Hyper-IgE syndrome	<i>DOCK8, STAT3</i>	
1039	1 month old male with family history of SCID diagnosed on new born screening. CD3 1, CD4 1, CD8 <1, CD19 <1, CD56 2009 /mm3, IgG 6.4, IgA <0.25, IgM 0.31 g/l, PHA 2331 CPM	Severe Combined Immunodeficiency Disease	<i>RAG1, RAG2, DCLRE1C</i>	
1047	9 years male with recurrent chest infections and autoimmune thrombocytopenia. CD3 2263, CD4 1405, CD8 698, CD19 1088, CD56 350, IgG 1.5, IgA <0.25, IgM <0.18, PHA 69585 CPM.	Common Variable Immunodeficiency Disease	<i>ICOS, RAG1, RAG2, DCLRE1C</i>	
1050	2 years old female with CMV induced hemohagocyticlymphohistiocytosis	Hemohagocyticlymphohistiocytosis	<i>STX11, UNC13D, PRF1, STXBP2, RAB27A</i>	
1066	4 month old female, with chronic diarrhea, persistent chest infections, and recurrent skin abscesses. CD3 3429, CD4 1925, CD8 1289, CD19 1832, CD56 478 /mm3, CD3+ CD45RA+ 13%, CD4+ CD45 RA+<1%, PHA 32392 CPM, IgG 4.4, IgA <0.25, IgM <0.18 g/l, no maternal T-cell engraftment.	Severe Combined Immunodeficiency Disease	<i>RAG1, RAG2, DCLRE1C</i>	
1073	19 years old male, with history of recurrent infections, progressive ataxia and normal Alpha-fetoprotein (AFP)	Ataxia Telangiectasia	<i>ATM</i>	
1079	7 years old female, sickle cell disease and history of recurrent chest infections, CD3 360, CD4 120, CD8 156, CD19 321, CD56 430 /mm3, CD3+CD45RA+ <1%, CD4+CD45RA+ <1%, no maternal T-cell engraftment, PHA 13391 CPM, IgG 20.6, IgA .96, IgM 5.15 g/l, poor	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C, PNP, ADA</i>	

	specific antibody response to protein antigen.			
1084	6 months old male, persistent fever disseminated BCGitis, chronic diarrhea, 99% of the patient's cells failed to reduce DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	<i>CYBB, CYBA, NCF1, NCF2</i>	
1086	4 years female, Tetralogy of Fallot, recurrent chest infections, CD3 916, CD4 501 CD8 347, CD19 825, CD56 616 / mm ³ , IgG 5.8, IgA 0.8, IgM 0.92 g/l, PHA 14030 CPM	Ill-defined immunodeficiency	<i>RAG1, RAG2, DCLRE1C, JAK3</i>	
1087	3 years old male, insulin-dependent diabetes mellitus (IDDM), short stature, epiphyseal dysplasia, recurrent chest infections and failure to thrive. CD3 1877 , CD4 1038, CD8 548, CD19 66, CD56 276 / mm ³ , IgG 3.1, IgA <0.25, IgM 0.21 g/l, poor specific antibody response to protein antigen, PHA 260148 CPM	Ill-defined immunodeficiency	<i>BTK, FOXP3</i>	
1097	4 years old male, with history of sinopulmonary infections, diarrhea and persistent CMV infections. CD3 2473, CD4 1076, CD8 1042, CD19 107, CD56 616 /mm ³ , PHA 51542 CPM, IgG 5, IgA 0.65, IgM 0.37 g/l.	Severe Combined Immunodeficiency Disease	<i>RAG1, RAG2, DCLRE1C, PNP</i>	
1113	8 years old male with severe atopic dermatitis, food allergy and recurrent chest infections, Normal immunological work up, IgE 8340KU/L.	Hyper-IgE syndrome	<i>DOCK8</i>	
1136	5 years old male, with severe atopic dermatitis, food allergy, recurrent skin abscesses. CD3 3974, CD4 2209, CD8 1610, CD19 1153, CD56 386 /mm ³ , IgG 8.9, IgA 0.86, IgM 0.98 g/l, IgE 32458KU/L, PHA 100640 CPM.	Hyper-IgE syndrome	<i>DOCK8, STAT3</i>	
1138	22 years old female, with progressive ataxia since the age of 10 yrs, no telangiectasia, normal Alpha-fetoprotein (AFP).	Ataxia telangiectasia	<i>ATM</i>	
1141	5 years old female, with history of persistent mucocutaneous candidiasis, failure to thrive and recurrent chest infections, CD3 2888, CD4 695, CD8 1338, CD19 1041, CD56 197 / mm ³ , PHA 127523 CPM, IgG 21.6, IgA 1.6, IgM 2.7 g/l.	Chronic mucocutaneous candidiasis disease	<i>CARD9, CANDN1, CLEC7A, IL17RA, IL17F, STAT1, TRAF3IP2, AIRE, RAG1 & RAG2</i>	
1142	8 month old female, with persistent chest infections, and mucocutaneous candidiasis, and chronic diarrhea. CD3 8, CD4 4, CD8 4,	Severe Combined Immunodeficiency Disease	<i>IL7R, CD3E, CD3G, CD247</i>	CARD11 Deletion

	CD19 1678, CD56 1595 /mm3, IgG 2.8, IgA 0.33, IgM 0.31 g/l, PHA 3162 CPM.			
1147	3 years old female, with recurrent chest infections, chronic diarrhea, failure to thrive, recurrent vasculitis and arthritis and recurrent autoimmune thrombocytopenia, CD3 482, CD4 364, CD8 51, CD19, 344, CD56 100 /mm3, CD3+CD45RA+ 39%, CD4+CD45RA+ 31%, PHA 117160 CPM, IgG 7.8, IgA 1.28, IgM 0.8 g/l, IgE 4250KU/L.	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C, WASP, DOCK8, WIP,FOXP3, ZAP70</i>	
1148	18 years old female with history of recurrent chest infections, bronchiectasis,, poor weight gain and recurrent fever , Normal WBC and differential. IgG: 3.4, IgA: 0.92 , IgM: < 0.18 g/l, CD3: 3475 , CD4: 2080 , CD8: 1105 , CD19: 670 , CD16/56: 47 / mm3, PHA: 33000 -127000 CPM	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C, IL2RG, CD79A, CD79B, BTK</i>	AK2 c.C545A p.A182D
1149	5 years old female with history of recurrent chest infections, IgG: 1.6, IgA: < 0.25 , IgM: 0.5 g/l, Poor antibody response, CD3: 2275 , CD4: 1600 , CD8: 558 , CD19: 700 , CD16/56: 170 /mm3, PHA: 80000-214000 CPM, Normal CD40 and CD40L expression upon activation	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C, BLNK</i>	PTPRC c.T3062C p.L1021S
1164	7 years old male, with severe atopic dermatitis, food allergy, recurrent skin abscesses and oral candidiasis. CD3 1164, CD4 671, CD8 466, CD19 251, CD56 476 / mm3, PHA 88441 CPM, IgG 29.5, IgA <0.25, IgM 3.66 g/l, IgE 4031KU/L.	Hyper-IgE syndrome	<i>DOCK8</i>	
1170	3 years old male with history of recurrent skin abscess at age of 1 year, CD3 193, CD4 127, CD8 60, CD19 1816, CD56 3514 / mm3 , IgG 10.2, IgA 0.89, IgM 1.68 g/l, PHA 209305 CPM.	Ill-defined immunodeficiency	<i>RAG1, RAG2, DCLRE1C</i>	
1174	3 yrs old male, with severe atopic dermatitis, food allergy, recurrent skin abscesses, intermittent neutropenia. CD3 4974, CD4 3409, CD8 1410, CD19 1563, CD56 275 / mm3, IgG 7.2, IgA 0.49, IgM 0.71 g/l, IgE 22400KU/L, PHA 120840.	Hyper-IgE syndrome	<i>DOCK8, STAT3</i>	
1179	9 month old male, with history of recurrent chest infections, chronic diarrhea, failure to thrive. CD3 127, CD4 75, CD8 35, CD19 302, CD56 569 /mm3, PHA 3592 CPM, IgG 15.4, IgA 6.27, IgM 0.23 g/l.	Severe Combined Immunodeficiency Disease	<i>RAG1, RAG2, DCLRE1C, IL7R</i>	
1191	13 years old male, with severe atopic dermatitis, food allergy and recurrent chest infections. CD3 1537, CD4 834, CD8 615, CD19 352,	Hyper-IgE syndrome	<i>DOCK8</i>	

	CD56 55 / mm3, PHA 64180 CPM. IgG 17.4, IgA 1.9, IgM 1.37 g/l, IgE15050KU/L.			
1203	5 years old male, with history of sinopulmonary infections, persistent lymphadenopathy. CD3 2145, CD4 744, CD8 1277, CD19 243, CD56 315 /mm3, CD3+CD45RA+ 14%, CD4+CD45RA+ 1%, IgG <0.3, IgA <0.25, IgM 13.5 g/l, PHA 20035 CPM, Intact expression of CD40 and CD40L.	Hyper IgM syndrome	<i>RAG1, RAG2, DCLRE1C, AICDA, CD40, CD40LG</i>	
1212	9 years old female, with recurrent sinopulmonary infections, progressive ataxia, normal alpha feto-protein, normal immunological work up	Ataxia Telangiectasia	<i>ATM</i>	
1222	17 years old male with history of chronic diarrhea, failure to thrive, autoimmune enteropathy, hypothyroidism, uveitis and arthritis	Autoimmune enteropathy	<i>AIRE, FOXP3</i>	LRBA c.3985_3986delGA p.D1329YfsX18
1225	7 years old male, with persistent severe mucocutaneous candidiasis and recurrent skin abscesses. CD3 2799, CD4 1277, CD8 1318, CD19 546, CD56 292 /mm3, IgG 20.3, IgA 1.7, IgM 0.89 g/l, PHA 37799 CPM.	Chronic mucocutaneous disease	<i>AIRE</i>	PNP Deletion
1228	13 Years male, with chronic diarrhea, failure to thrive, Insulin-dependent diabetes mellitus, CD3 1061 , CD4 704, CD8 332, CD19 254, CD56 163 /mm3 , IgG 10.7 IgA 2.04 IgM 0.69 g/l, PHA 49830 CPM	Autoimmune enteropathy	<i>FOXP3</i>	
1233	8years old female, with severe atopic dermatitis and recurrent sinopulmonary infections. CD3 3387, CD4 1811, CD8 1296, CD19 970, CD56 930 / mm3, PHA 177486 CPM, IgG 15.6, IgA 1.8, IgM 0.87 g/l, IgE 78562KU/L.	Hyper-IgE syndrome	<i>DOCK8, STAT3</i>	
1235	15 years old male with persistent arthritis, skin rash, diarrhea, Insulin-dependent diabetes mellitus. CD3 2245 , CD4 1220, CD8 936, CD19 957, CD56 513 / mm3 , IgG 22.5 IgA <0.25 IgM 1.56 g/l	Autoinflammatory disease	<i>FOXP3, TNFRSF13B</i>	
1245	8 years old girl with recurrent chest infections, chronic diarrhea and failure to thrive, CD3 623, CD4 358, CD4 240, CD19 106, CD56 164 /mm3, CD3+CD45RA+ 12%, CD4+CD45RA+ 3%, PHA 35760 CPM, IgG 9.7, IgA 0.65, IgM 1 g/l.	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C</i>	

1251	11 years old female, with severe autoimmune hemolytic anemia and thrombocytopenia. With 8% double negative T-cells.	Autoimmune lymphoproliferative syndrome	<i>FAS, FASLG, CASP8, CASP10</i>	
1257	12 months old male with history of recurrent chest infections and poor weight gain since age of 6 months, family history of a sibling who died at age of 3 months with severe chest infection, IgG: 1.7, IgA: <0.25, IgM: < 0.18 g/l, CD3: 2975, CD4: 1700, CD8: 1245, CD19: 160, CD16/56: 141 /mm3, CD3+CD45RA+ 43%, PHA: 2523 -9718 CPM, No maternal cell engraftment.	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C</i>	CD247 c.58+8C>T
1259	19 years old male, with history recurrent sinopulmonary infections, bronchiectasis, CD3 3147, CD4 726, CD8 2455, CD19 <35, CD56 277 /mm3, IgG <1.5, IgA <.025, IgM <.018 g/l, PHA 100325 CPM.	Agammaglobulinemia	<i>BTK, BLNK</i>	
1262	6 month old male, with history of chronic diarrhea, failure to thrive, persistent CMV pneumonitis. CD3 54, CD4 52, CD8 <1, CD19 8, CD56 881 /mm3, PHA 343 CPM, IgG <1.5, IgA <0.25, IgM <0.17 g/l.	Severe Combined Immunodeficiency Disease	<i>RAG1, RAG2, DCLRE1C</i>	
1264	5 years old male with recurrent sinopulmonary infections, autoimmune thrombocytopenia and neutropenia, CD3 2176, CD4 1339, CD8 733, CD19 1282, CD56 400 /mm3, CD3+CD45RA+ 31%, CD4+CD45RA+ 16%, PHA 73786 CPM, IgG <1.5, IgA <0.25, IgM <0.18 g/l, no maternal T-cell engraftment.	Common Variable Immunodeficiency Disease	<i>ICOS, TNFRSF13B, DCLRE1C</i>	
1266	11 years female with history of eczema, recurrent skin abscesses, family history of a sibling with similar illness, CD3 1839, CD4 1191, CD8 547, CD19 367, CD56 333 /mm3 , IgG 15.3, IgA 2.87, IgM 2.57 g/l, PHA 4570 CPM.	Ill-defined immunodeficiency	<i>RAG1, RAG2, DCLRE1C, ADA, DOCK8</i>	
1280	9 years old female with severe atopic dermatitis, food allergy, recurrent skin abscesses. CD3 3077, CD4 1597, CD8 1273, CD19 546, CD56 453 /mm3, PHA 128661 CPM, IgG 15.2, IgA 2.07, IgM 1.7 g/l, IgE 42560KU/L.	Hyper-IgE syndrome	<i>DOCK8</i>	
1281	9 years old male with severe atopic dermatitis, food allergy, recurrent skin abscesses.. CD3 2974, CD4 2009, CD8 1813, CD19 583, CD56 395 /mm3, IgG 11.8, IgA 1.53, IgM 0.98 g/l, IgE 45289KU/L, PHA 98886 CPM.	Hyper-IgE syndrome	<i>DOCK8</i>	
1313	5 years old male with chronic diarrhea, failure to thrive and chronic mucocutaneous candidiasis. CD3 423, CD4 210, CD8 121, CD19 1145, CD56 741 /mm3, CD3+CD45RA+ 2%, CD4+CD45RA+ 3%, no maternal T-	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C</i>	

	cell engraftment, PHA 40024 CPM, IgG 3.3, IgA 0.58, IgM 2.38 g/l, intact expression of CD40 and CD40L upon activation.			
1317	8 years old female with chronic severe diarrhea, recurrent chest infections and family history of a sibling with similar illness. CD3 652, CD4 442, CD8 163, CD19 396, CD56 81 /mm3, CD3+CD45RA+ 52%, CD4+CD45RA+ 15%, PHA 81213 CPM, IgG <1.4, IgA <0.25, IgM <0.17 g/l.	Combined Immunodeficiency	<i>RAG1, RAG2, DCLRE1C</i>	
1229	5 years old female, with severe atopic dermatitis, recurrent sinopulmonary infections and chronic diarrhea. CD3 4317, CD4 2311, CD8 1786, CD19 1170, CD56 1030 / mm3, PHA 327486 CPM, IgG 17.4, IgA 1.3, IgM 0.94 g/l, IgE 69060KU/L.	Hyper-IgE syndrome	<i>DOCK8</i>	

WBC: White blood cell count, CMV: cytomegalovirus, EBV: Epstein Barr virus, PHA: Phytohemagglutinin T cell activation, SCID: Severe combined immunodeficiency disease.

Normal reference values: CD3 3100-4800 per mm3, CD4 2200-3300 per mm3, CD8 1100-1700 per mm3, CD19 1100-1900 per mm3, CD16+56+ 300-700 per mm3, IgG 2.5-9.1 g/L, IgA 0.2-1.2 g/L, IgM 0.2-1.5 g/L, IgE8-250 KU/Land PHA 94935-171149 CPM.

Supplementary table3 : Clinical and immunological data for patients with no previous genetic work up

Patient	Clinical and immunological data	Working Diagnosis	T-NGS /CNV result
888	17 years old male, with severe atopic dermatitis, recurrent chest infections and bronchiectasis. CD3 2646, CD4 1040, CD8 1575, CD19 378, CD56 95 /mm3, PHA 49337 CPM, IgG 27, IgA 1.7, IgM 0.77 g/l, IgE 34770KU/L, poor specific antibody response to protein antigen.	Hyper-IgE syndrome	
896	3 years old male, with severe chronic diarrhea and failure to thrive, CD3 1699, CD4 1225, CD8 514, CD19 <20, CD56 198 /mm3, IgG 14.2, IgA <0.25, IgM 0.34 g/l, PHA 84234 CPM.	Autoimmune enteropathy	
906	1 year old male, with chronic diarrhea, failure to thrive and recurrent chest infections. CD3 <1, CD4 <1, CD8 <1, CD19 <1, CD56 348 /mm3, IgG 10.1, IgA <0.25, IgM <0.17 g/l.	Severe Combined Immunodeficiency Disease	<i>DCLRE1C</i> Deletion
910	27 years old female, with recurrent chest infections. CD3 4334, CD4 1397, CD8 2697, CD19 385, CD56 98 /mm3, IgG <1.4, IgA <0.25, IgM 20.3 g/l, PHA 66435 CPM, intact expression of CD40 and CD40L upon activation.	Hyper IgM syndrome	<i>AICDA</i> c.152A>T p.N51I
937	9 months old female, with recurrent chest infections, chronic diarrhea, failure to thrive. CD3 <1, CD4 <1, CD8 <1, CD19 <1, CD56 356 /mm3, IgG 1.6, IGA <0.25, IgM <0.18 g/l.	Severe Combined Immunodeficiency Disease	
939	16 years old male, with recurrent chest infections, retinitis pigmentosa, CD3 2485, CD4 1038, CD8 1227, CD19 440, CD56 189 /mm3, IgG 2.1, IgA 0.47, IgM 0.18 g/l, poor antibody response to polysaccharides antigen, PHA 47036 CPM.	Hypogammaglobulinemia	
944	15 years old female, with severe autoimmune hemolytic anemia, thrombocytopenia and generalized lymphadenopathy. 13% double negative T-cells.	Autoimmune lymphoproliferative syndrome	
953	34 years old male, with history of Hodgkin's lymphoma and recurrent chest infections. CD3 4471, CD4 291, CD8 4142, CD19, 38, CD56 62 /mm3, IgG <1.5, IgA <0.25, IgM <0.18 g/l, PHA 4818 CPM.	Lymphoproliferative disease	

964	1-month old female, with family history of Chronic Granulomatous Disease, 14% NBT dye reduction by the patients granulocytes as compared to two normal controls	Chronic Granulomatous Disease	
970	5 months old male with recurrent chest infections and chronic diarrhea, CD3 <1, CD4<1, CD8<1, CD19 <1, CD56 780 /mm3, IgG <1.5, IgA <0.25, IgM <0.18 g/l, PHA 358 CPM.	Severe Combined Immunodeficiency Disease	
972	8 years old female with recurrent chest infections, family history of Chronic Granulomatous Disease, and 99% of the patient's cells failed to reduce DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	
975	25 years old female, with recurrent chest infections, bronchiectasis, CD3 1547, CD4 794, CD8 733, CD19 143, CD56 244 /mm3, PHA 39976 CPM, IgG 2.1, IgA <0.23, IgM 2.22 g/l, intact expression of CD40 and CD40L upon activation.	Common Variable Immunodeficiency Disease	
976	23 years old male, with recurrent chest infections, bronchiectasis and auto-immune thrombocytopenia. CD3 3243, CD4 736, CD8 2466, CD19 147, CD56 37 /mm3, PHA 32016 CPM, IgG 3.1, IgA <0.23, IgM 0.24 g/l, intact expression of CD40 and CD40L upon activation.	Common Variable Immunodeficiency Disease	
991	18-month male, with global development delay, recurrent chest infections, CD3 633, CD4 336, CD8 196, CD19 223, CD56 338 /mm3, IgG 5.9, IgA 1.4, IgM 0.48 g/l, PHA 189665 CPM.	Ill-defined immunodeficiency	
992	7 years old male with history of recurrent chest infections, chronic diarrhea, failure to thrive, pancytopenia,hepatosplenomegaly, chronic EBV infection, brachial artery aneurysm, recurrent vasculitis, stroke and seizure disorder. IgG: < 1.4, IgA: < 0.25 , IgM: 24.4 g/l, Normal CD40 and CD40L expression upon activation, CD3: 3655 , CD4: 619, CD8: 2400, CD19: 95, CD16/56: 851 /mm3, PHA: 2190 CPM	Hyper IgM syndrome	ATM c.9065delA p.G3022fs
998	5-month old female, with recurrent chest infections, chronic diarrhea, failure to thrive. CD3 <1, CD4 <1, CD8 <1, CD19 <1, CD56 41 /mm3, IgG 3.6, IGA <0.25, IgM <0.18 g/l.	Severe Combined Immunodeficiency Disease	
1018	33 years old male, with history of recurrent chest infections, lymphadenitis and skin abscesses. Family history of CGD, 99% of the patient's cells failed to	Chronic Granulomatous Disease	

	reduce DHR-123 post PMA stimulation.		
1020	11 years old female, with recurrent chest infections, bronchiectasis and family history of hypogammaglobulinemia. CD3 6048, CD4 5011, CD8 950, CD19 2160, CD56 259 /mm3, IgG 1.7, IgA <0.25, IgM <0.17 g/l, poor specific antibody response, intact expression of CD40 and CD40L upon activation.	Common Variable Immunodeficiency Disease	
1023	10 months old male, with chronic diarrhea and severe unresolved pneumonitis. CD3 <1, CD4 <1, CD8 <1, CD19 598, CD56 97 /mm3, PHA 4260 CPM, IgG 1.6, IgA <0.25, IgM <0.18 g/l.	Severe Combined Immunodeficiency Disease	
1028	10-month old male, with chronic diarrhea, failure to thrive and disseminated BCGitis. CD3 252, CD4 202, CD8 49, CD19 <1, CD56 377 /mm3, PHA 3646 CPM, IgG <1.5, IgA <0.125, IgM <0.18 g/l.	Severe Combined Immunodeficiency Disease	<i>DCLRE1C</i> Deletion
1031	22 years old female, with recurrent chest infections, and bronchiectasis, CD3 3750, CD4 2868, CD8 794, CD19 221, CD56 /397 mm3, IgG 1.5, IgA <0.25, IgM <0.18 g/l, PHA 68549 CPM, intact expressions of CD40 and CD40 L upon activation.	Hypogammaglobulinemia	
1032	16 years old male, with recurrent chest infections and bronchiectasis. CD3 2336, CD4 440, CD8 1456, CD19 247, CD56 /110 mm3, Total MHC class II <1%, and MHC class II CD19 <1%, PHA 44568 CPM, IgG 6.3, IgA <0.25, IgM 0.44 g/l.	Bare Lymphocyte Syndrome	<i>RFXANK</i> c.7_10insCTTA p.L3PfsX47
1046	8 years old female, with recurrent chest infections, bronchiectasis and autoimmune thrombocytopenia. CD3 2435, CD4 736, CD8 1605, CD19 127, CD56 137 /mm3, PHA 48685 CPM, IgG 1.8 IgA <0.23, IgM 0.24 g/l, intact expression of CD40 and CD40L upon activation.	Common Variable Immunodeficiency Disease	
1049	5 years old male, with recurrent chest infections, CD3 1333, CD4 1125, CD8 265, CD19 251, CD56 /292 mm3, IgG 1.5, IgA <0.45, IgM <0.16 g/l, PHA 277427 CPM.	Hypogammaglobulinemia	
1057	4-month old male, with chronic diarrhea and severe unresolved pneumonitis. CD3 <1, CD4 <1, CD8 <1, CD19 <1, CD56 97 /mm3, PHA 3152 CPM, IgG 2.7, IgA <0.25, IgM <0.18 g/l.	Severe Combined Immunodeficiency Disease	
1061	2 years old female, with recurrent chest infections and disseminated varicella infection, chronic diarrhea. CD3 493, CD4 170, CD8 314, CD19 108, CD56 260 /mm3, PHA 4194 CPM, IgG 4.7, IgA 1.5, IgM 0.30 g/l, poor specific antibody response to protein antigen.	Severe Combined Immunodeficiency Disease	

1062	4-month old female, with chronic diarrhea, severe persistent CMV pneumonitis and eczema. CD3 1914, CD4 362, CD8 1523, CD19 2473, CD56 173 /mm3, Total MHC class II <1%, and MHC class II CD19 <1%, PHA 20378 CPM, IgG 4.6, IgA <0.25, IgM <0.18 g/l. No maternal t-cell engraftment.	Bare Lymphocyte Syndrome	
1067	16 years old male, with recurrent chest infections, neutropenia, IgG 1.5, IgA <0.25, IgM 6.4 g/l. Lack of expression of CD40L on the service of T-helper cells post PMA stimulation.	Hyper IgM syndrome	
1091	1 year old male, with fulminant EBV hepatitis, generalized lymphadenopathy and family history of a sibling with similar illness. CD3 1997, CD4 1081, CD8 831, CD19 969, CD56 518 /mm3, PHA 133915 CPM, IgG 19, IgA 2.66, IgM 3.85 g/l.	Lymphoproliferative disease	
1098	2-month old male, with family history of chronic granulomatous disease, Oxidative burst assay showed that 97% of the patient's cells failed to reduced DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	<i>CYBB</i> c.1618delG p.E540fs
1099	13 years old male, with recurrent chest infections and rheumatoid arthritis. CD3 2126, CD4 696, CD8 1241, CD19 <1, CD56 185, IgG 1.8, IgA <0.25, IgM <0.18 g/l, IgE<2, PHA 92527 CPM.	Agammaglobulinemia	<i>BTK</i> Deletion
1115	13 years old female, with recurrent chest infections, bronchiectasis and hypothyroidism. CD3 2964, CD4 1323, CD8 1429, CD19 2117, CD56 159 /mm3, PHA 3598 CPM, IgG <1.4, IgA <0.25, IgM <0.17 g/l.	Combined Immunodeficiency	<i>DCLRE1C</i> c.C614G p.S205C
1129	17 years old male, with recurrent sinopulmonary infections. CD35736, CD4 433, CD8 2922, CD19 121, CD56 232 /mm3, CD3+CD45RA+ 59% , CD4+CD45RA+ 3%, IgG 2.8, IgA 0.9, IgM 0.29 g/l, poor antibody response to protein antigen, PHA 45116 CPM.	Hypogammaglobulinemia	<i>BTK</i> c.1909-9T>C
1133	4 years old male, with history recurrent lymphadenitis, oxidative burst assay showed that 99% of the patient's cells failed to reduced DHR-123 post PMA stimulation.	Chronic Granulomatous Disease	<i>NCF1</i> Deletion
1153	9 years old female, with history of persistent fever, subcutaneous nodular rash, vasculitis, intermittent arthritis, hepatosplenomegaly, normal ANA rheumatoid factor, CD3 762, CD4 431, CD8 240, CD19 506, CD56 97 /mm3, PHA 134068 CPM, IgG 5.6, IgA 0.9, IgM <0.18 g/l.	Autoinflammatory disease	
1157	4-month old male, with chronic diarrhea, failure to thrive, and severe respiratory syncytial virus infection. CD3 <1, CD4 <1, CD8 <1, CD19 <1, CD56	Severe Combined Immunodeficiency Disease	<i>RAG2</i> c.T184G

	28 /mm ³ , PHA 3152, IgG 2.7, IgA <0.25, IgM <0.18 g/l.		p.F62V
1168	12 years old male, with brain aspergillus fungal infection, normal oxidative burst assay. CD3 1049, CD4 626, CD8 292, CD19 658, CD56 125 /mm ³ , PHA 12761 CPM, IgG 3.4, IgA 0.94, IgM 0.73 g/l.	Ill-defined immunodeficiency	<i>IL2RG</i> Deletion
1173	13-month old male, with partial albinism, gray silvery hair, history of persistent fever, pancytopenia and CMV infection. No giant leukocytes inclusion bodies, hair microscopy showed irregular melanine pigments.	Griscelli syndrome	
1175	4 years old female, with generalized lymphadenopathy, hematosplenomegaly, pancytopenia and persistent EBV infections. CD3 5609, CD4 3251, CD8 2336, CD19 106, CD56 40 /mm ³ , IgG 6.5, IgA <0.25, IgM 0.24 g/l, PHA 20516 CPM.	Hemophagocytic lymphohistiocytosis	
1202	9 years old male, with severe atopic dermatitis, food allergy, recurrent chest infections and oral candidiasis. CD3 1164, CD4 671, CD8 466, CD19 251, CD56 476 /mm ³ , PHA 48451 CPM, IgG 29.5, IgA <0.25, IgM 3.66 g/l, IgE 48831 KU/L.	Hyper-IgE syndrome	<i>DOCK8</i> Deletion
1206	15 years old female, with recurrent chest infections, chronic diarrhea, bronchiectasis, failure to thrive, and persistent CMV infection. CD3 3309, CD4 1655, CD8 1504, CD19 1504, CD56 150 /mm ³ , PHA 12659 CPM, IgG 3.6, IgA <0.23, IgM <0.28 g/l.	Combined Immunodeficiency	
1208	3-month old female, with exfoliative dermatitis, lymphadenitis, chronic diarrhea and BCGitis. CD3 545, CD4 258, CD8 183, CD19 <1, CD56 203 /mm ³ , PHA 19660, IgG 6.2, IgA <0.25, IgM <0.18 g/l.	Omenn disease	<i>RAG1</i> c.554delG p.R185fs
1209	3-month old female, with recurrent chest infections and chronic diarrhea. CD3 2, CD4 <1, CD8 <1, CD19 <1, CD56 390 /mm ³ , PHA 571, IgG 7.4, IgA 0.25, IgM <0.18.	Severe Combined Immunodeficiency Disease	<i>RAG1</i> c.G2041T p.E681X
1211	8 months male, with recurrent subcutaneous abscesses, chronic diarrhea and persistent oral thrust, multi focal osteomyelitis, neutropenia. IgG <1.5, IgA <0.25, IgM 0.23 g/l, CD3 7382, CD4 4494, CD8 2135, CD19 2966, CD56 601 /mm ³ , PHA 223198 CPM, intact expression of CD40 and CD40L, normal oxidative burst assay and normal CD18 and CD11 expression.	Ill-defined immunodeficiency	
1214	11-month old male, with recurrent chest infections, chronic diarrhea and BCGitis. CD3 109, CD4 6, CD8 86, CD19 <1, CD56 670/mm ³ , IgG 11.2, IgA <0.25, IgM <0.18 g/l.	Severe Combined Immunodeficiency Disease	<i>DCLRE1C</i> Deletion

1231	8 Years old female with history of recurrent chest infection, hypertelorism, low set ears, IgG: 1.7, IgA: < 0.25, IgM: 1.54 g/l, PHA: 190889 CPM, intact expression of CD40 and CD40L upon activation	Hyper IgM syndrome	<i>DNMT3B</i> c.G2029A p.V677M
1236	4 years old male, with history of persistent fever, hepatosplenomegaly and pancytopenia, elevated ferritin, low fibrinogen and bone marrow hemophagocytosis	Hemophagocytic lymphohistiocytosis	
1238	7 years old male, with severe atopic dermatitis, recurrent chest infections. Eosinophilia, CD3 2356, CD4 1040, CD8 1278, CD19 452, CD56 185/mm ³ , PHA 56953 CPM, IgG 14, IgA 1.9, IgM 0.87 g/l, IgE 44580 KU/L.	Hyper-IgE syndrome	
1243	4 weeks female with family history of agammaglobulinemia, diagnosed on new born screening. CD3 2235, CD4 1604, CD8 461, CD19 <1, CD56 220 /mm ³ , IgG 612, IgA <0.25 IgM < 0.18 g/l, PHA 243575 CPM.	Agammaglobulinemia	
1244	14-month old female, with chronic diarrhea, failure to thrive, recurrent chest infections and BCGitis, CD3 28, CD4 <1, CD8 <1, CD19 5185, CD56 <1 /mm ³ , PHA 1807 CPM, IgG 5.5, IgA <0.25, IgM 0.25 g/l.	Severe Combined Immunodeficiency Disease	
1252	5 years old female, with severe atopic dermatitis, recurrent chest infections and chronic diarrhea. CD3 1394, CD4 540, CD8 796, CD19 351, CD56 398 /mm ³ , PHA 35680 CPM, IgG 5.6, IgA <0.25, IgM 0.82 g/l, IgE 69456 KU/L.	Hyper-IgE syndrome	<i>DOCK8</i> Deletion
1255	17 years old female, with recurrent chest infections and bronchiectasis. CD3 1152, CD4 686, CD8 412, CD19 137, CD56 82 /mm ³ , PHA 110214 CPM, IgG 3.4, IgA 4.34, IgM 0.46 g/l, poor specific antibody response to polysaccharides antigen.	Common Variable Immunodeficiency Disease	
1256	6 years old male, with recurrent chest infection, type-1 diabetes mellitus, hypothyroidism, growth hormone deficiency and alopecia totalis. CD3 5186, CD4 3332, CD8 1839, CD19 67, CD56 825 /mm ³ , CD3+CD45RA+ 58%, CD4+CD45RA+ 41%, PHA 100466 CPM, IgG 1.8, IgA <0.25, IgM <0.18 g/l.	Common Variable Immunodeficiency Disease	
1258	7 years old male, with severe atopic dermatitis, food allergy and recurrent chest infections. CD3 2184, CD4 1071, CD8 966, CD19 346, CD56 259 /mm ³ , PHA 35985 CPM, IgG 8.9 IgA <0.25, IgM 0.9 g/l, IgE 49785 KU/L.	Hyper-IgE syndrome	<i>DOCK8</i> Deletion
1265	8 years old male, with recurrent chest infections. CD3 4446, CD4 2246, CD8 1965, CD19 1968, CD56 259 /mm ³ , PHA 140434 CPM, IgG 4.2, IgA 0.97, IgM 0.88 g/l, poor specific antibody to protein antigen.	Common Variable Immunodeficiency Disease	
1270	5 years old male, with recurrent chest infections, family history of	Hypogammaglobulinemia	

	hypogammaglobulinemia. CD3 1910, CD4 1088, CD8 671, CD19, 302, CD56 413 /mm3, IgG <1.5, IgA <0.25, IgM <0.18 g/l, PHA 109595 CPM. Intact expressions of CD40 and CD40L upon activation.		
1271	10-month old female, with exfoliative dermatitis, chronic diarrhea and persistent CMV pneumonitis. CD3 7382, CD4 4494, CD8 2277, CD19 871, CD56 293 /mm3, PHA 18589 CPM, IgG 2.4, IgA <0.25, IgM 0.18 g/l.	Omenn's disease	
1286	1-month old male, with family history of SCID. CD3 <1, CD4 <1, CD8 <1, CD19 <1, CD56 389 /mm3, IgG 4.5, IgA <0.25, IgM <0.18 g/l.	Severe Combined Immunodeficiency Disease	<i>DCLRE1C</i> Deletion
1291	8 years old male, with chronic diarrhea, recurrent chest infections and failure to thrive. CD3 343, CD4 144, CD8 138, CD19 0, CD56 16 /mm3, CD3+CD45RA+ 7%, CD4+CD45RA+ 1%, PHA 469 CPM, IgG 10.7, IgA 0.38, IgM <0.18 g/l.	Severe Combined Immunodeficiency Disease	
1294	13-month old male, with chronic diarrhea, failure to thrive and persistent CMV , pneumonitis and retinitis, CD3 3079, CD4 370, CD8 2621, CD19 1196, CD56 168 /mm3, Total MHC class II <1%, and MHC class II/CD19 <1%, PHA 23892 CPM, IgG 2.3, IgA <0.25, IgM 0.4 g/l.	Bare Lymphocyte Syndrome	<i>CIITA</i> c.C3136G p.L1046V
1297	1 month old male, with family history of SCID. CD3 <1, CD4 <1, CD8 <1, CD19 <1, CD56 241 /mm3, IgG 5.6, IgA <0.25, IgM <0.18 g/l.	Severe Combined Immunodeficiency Disease	
1299	16-month old male, with recurrent chest infections, chronic diarrhea and eczema. CD3 679, CD4 679, CD8 0, CD19 15, CD56 159 /mm3, PHA 7533 CPM, IgG 5.2, IgA 2.51, IgM 0.23 g/l.	Severe Combined Immunodeficiency Disease	
1300	4 years old male, with chronic diarrhea, recurrent chest infections, failure to thrive. CD3 5294, CD4 512, CD8 3643, CD19 228, CD56 114 /mm3, Total MHC class II <1%, and MHC class II/CD19 <1%, PHA 14316 CPM, IgG 4.9, IgA 3.45, IgM 0.2 g/l.	Bare Lymphocyte Syndrome	<i>RFXANK</i> c.268+1G>C
1303	10 years old male, with cyclic neutropenia. CD3 5102, CD4 4376, CD8 928, CD19 995, CD56 332 /mm3, PHA 185476 CPM, IgG 11.9, IgA <0.25, IgM 0.59 g/l.	Neutropenia	
1304	17 years female with recurrent sinopulmonary infections, CD3 1137, CD4 655, CD8 494, CD19 12, CD56 115 /mm3, IgG <1.5, IgA <0.23, IgM <0.18 g/l, PHA 192026.	Agammaglobulinemia	
1308	10 years old female, with recurrent chest infections, autoimmune	Common Variable	

	thrombocytopenia, sclerosing cholangitis, suspected sarcoidosis. CD3 2614, CD4 1496, CD8 953, CD19 92, CD56 131 /mm ³ , CD3+CD45RA+ 19%, CD4+CD45RA+ 5%, PHA 42547 CPM, IgG 1.8, IgA <0.25, IgM 0.38 g/l, intact expression of CD40 and CD40L upon activation.	Immunodeficiency Disease	
1310	3 years old male, with partial albinism, gray silvery hair, and EBV induced hemohagocytic syndrome. Blood film showed leukocytes inclusion bodies; hair microscopy showed irregular melanine pigments distribution.	Chediak-Hegashi Syndrome	<i>LYST</i> c.G1303T p.E435X
1311	7 years old girl (sister of Patient 1312) with history of recurrent chest infections, chronic diarrhea, poor weight and persistent CMV / EBV infections. Normal WBC, differential, Normal IgG, IgA, IgM, CD3: 2230 , CD4: 940 , CD8: 1230 , CD19: 450 , CD16/56: 112 /mm ³ , PHA: 4000 CPM, No maternal cell engraftment	Combined Immunodeficiency	<i>ZAP70</i> c.G1606A p.G536S
1312	9 years old girl (sister of Patient 1311) with history of recurrent chest infections, chronic diarrhea, poor weight and persistent CMV / EBV infections. Normal WBC, differential, Normal IgG, IgA, IgM, CD3: 1790 , CD4: 720 , CD8: 1005 , CD19: 322 , CD16/56: 68 /mm ³ , CD3+CD45RA+ 20%, PHA: 388 CPM, No maternal cell engraftment	Combined Immunodeficiency	<i>ZAP70</i> c.G1606A p.G536S

WBC: White blood cell count, CMV: cytomegalovirus, EBV: Epstein Barr virus, PHA: Phytohemagglutinin T cell activation, SCID: Severe combined immunodeficiency disease.

Normal reference values: CD3 3100-4800 per mm³, CD4 2200-3300 per mm³, CD8 1100-1700 per mm³, CD19 1100-1900 per mm³, CD16+56+ 300-700 per mm³, IgG 2.5-9.1 g/L, IgA 0.2-1.2 g/L, IgM 0.2-1.5 g/L, IgE8-250 KU/L and PHA 94935-171149 CPM.

Supplementary table 4: PID panel genes list, design and sequencing performance

Genes	Amplicons	Total Bases	Covered Bases	Missed Bases	Design Coverage	Average Coverage	Final Coverage	Num Exons	No Exons with 100% Coverage	No Exon Cov >0.95	>0.9	>0.8	>0.7	>0.5	>0.2	<0.2	0
ACP5	9	978	978	0	100.00%	99.63%	99.63%	4	4	0	0	0	0	0	0	0	0
ADA	13	1092	1059	33	97.00%	99.39%	96.41%	12	11	0	0	0	0	0	0	0	1
AICDA	6	597	597	0	100.00%	99.55%	99.55%	5	5	0	0	0	0	0	0	0	0
AIRE	21	1638	1627	11	99.30%	92.43%	91.78%	14	13	0	1	0	0	0	0	0	0
AK2	10	992	992	0	100.00%	99.54%	99.54%	9	9	0	0	0	0	0	0	0	0
ATM	105	9206	9185	21	99.80%	99.64%	99.44%	67	66	0	1	0	0	0	0	0	0
BLM	37	4254	4254	0	100.00%	99.61%	99.61%	21	21	0	0	0	0	0	0	0	0
BLNK	18	1528	1513	15	99.00%	99.54%	98.55%	19	18	0	0	1	0	0	0	0	0
BTK	24	2015	2015	0	100.00%	98.91%	98.91%	23	23	0	0	0	0	0	0	0	0
C1QA	5	738	738	0	100.00%	99.62%	99.62%	2	2	0	0	0	0	0	0	0	0
C1QB	5	762	762	0	100.00%	99.62%	99.62%	2	2	0	0	0	0	0	0	0	0
C1QC	5	738	738	0	100.00%	99.87%	99.87%	2	2	0	0	0	0	0	0	0	0
C1R	16	1886	1886	0	100.00%	99.65%	99.65%	9	9	0	0	0	0	0	0	0	0
C1S	17	2067	2067	0	100.00%	99.47%	99.47%	11	11	0	0	0	0	0	0	0	0
C2	23	2332	2332	0	100.00%	99.64%	99.64%	19	19	0	0	0	0	0	0	0	0
C3	52	4992	4992	0	100.00%	98.71%	98.71%	41	41	0	0	0	0	0	0	0	0
C4B,C4A	102	10750	10750	0	100.00%	94.78%	94.78%	84	84	0	0	0	0	0	0	0	0
C4B,C4A	102	10470	10470	0	100.00%	94.78%	94.78%	82	82	0	0	0	0	0	0	0	0
C5	57	5031	5022	9	99.80%	99.59%	99.39%	41	40	1	0	0	0	0	0	0	0
C6	25	2805	2805	0	100.00%	99.58%	99.58%	17	17	0	0	0	0	0	0	0	0
C7	24	2532	2532	0	100.00%	99.52%	99.52%	18	18	0	0	0	0	0	0	0	0
C8A	15	1755	1755	0	100.00%	99.61%	99.61%	11	11	0	0	0	0	0	0	0	0
C8B	16	1776	1776	0	100.00%	99.43%	99.43%	12	12	0	0	0	0	0	0	0	0
C9	16	1680	1680	0	100.00%	99.66%	99.66%	11	11	0	0	0	0	0	0	0	0
CARD9	20	1656	1656	0	100.00%	94.90%	94.90%	14	14	0	0	0	0	0	0	0	0
CASP10	16	1821	1799	22	98.80%	99.59%	98.39%	11	10	1	0	0	0	0	0	0	0
CASP8	13	2131	2128	3	99.90%	99.48%	99.38%	12	11	1	0	0	0	0	0	0	0
CCBE1	15	1221	1221	0	100.00%	99.61%	99.61%	11	11	0	0	0	0	0	0	0	0
CD19	17	1767	1767	0	100.00%	99.56%	99.56%	15	15	0	0	0	0	0	0	0	0
CD247	8	528	528	0	100.00%	99.48%	99.48%	9	9	0	0	0	0	0	0	0	0
CD3D	6	516	516	0	100.00%	99.52%	99.52%	5	5	0	0	0	0	0	0	0	0

CD3E	9	624	624	0	100.00%	99.45%	99.45%	8	8	0	0	0	0	0	0	0	0
CD3G	7	549	549	0	100.00%	99.55%	99.55%	6	6	0	0	0	0	0	0	0	0
CD40	11	862	826	36	95.80%	99.40%	95.23%	10	8	0	0	0	0	0	2	0	0
CD40LG	7	793	793	0	100.00%	99.37%	99.37%	6	6	0	0	0	0	0	0	0	0
CD46	17	1329	1329	0	100.00%	99.62%	99.62%	15	15	0	0	0	0	0	0	0	0
CD59	4	387	387	0	100.00%	99.47%	99.47%	3	3	0	0	0	0	0	0	0	0
CD79A	6	867	867	0	100.00%	88.83%	88.83%	6	6	0	0	0	0	0	0	0	0
CD79B	8	744	744	0	100.00%	99.59%	99.59%	7	7	0	0	0	0	0	0	0	0
CD81	11	711	685	26	96.30%	96.58%	93.00%	8	7	0	0	0	0	1	0	0	0
CD8A	8	708	708	0	100.00%	88.46%	88.46%	6	6	0	0	0	0	0	0	0	0
CFD	10	762	762	0	100.00%	93.98%	93.98%	5	5	0	0	0	0	0	0	0	0
CFH	41	3717	3703	14	99.60%	99.59%	99.20%	24	23	0	0	0	0	0	0	0	1
CFHR1	10	993	982	11	98.90%	89.40%	88.41%	6	5	0	0	1	0	0	0	0	0
CFI	20	1752	1752	0	100.00%	99.66%	99.66%	13	13	0	0	0	0	0	0	0	0
CFP	11	1410	1410	0	100.00%	96.36%	96.36%	9	9	0	0	0	0	0	0	0	0
CIITA	30	3393	3393	0	100.00%	99.15%	99.15%	19	19	0	0	0	0	0	0	0	0
CLEC7A	7	854	854	0	100.00%	99.58%	99.58%	8	8	0	0	0	0	0	0	0	0
CR2	26	3279	3279	0	100.00%	99.51%	99.51%	19	19	0	0	0	0	0	0	0	0
CTLA4	5	740	740	0	100.00%	99.58%	99.58%	5	5	0	0	0	0	0	0	0	0
CXCR4	7	2130	2130	0	100.00%	99.74%	99.74%	3	3	0	0	0	0	0	0	0	0
CYBA	8	588	588	0	100.00%	96.89%	96.89%	6	6	0	0	0	0	0	0	0	0
CYBB	20	1762	1762	0	100.00%	97.67%	97.67%	20	20	0	0	0	0	0	0	0	0
DCLRE1C	19	2105	2045	60	97.10%	97.65%	94.81%	17	14	1	0	0	0	0	0	0	2
DNMT3B	25	2740	2740	0	100.00%	99.54%	99.54%	24	24	0	0	0	0	0	0	0	0
DOCK8	56	6428	6413	15	99.80%	99.10%	98.90%	49	48	0	0	1	0	0	0	0	0
ELANE	8	804	804	0	100.00%	97.24%	97.24%	5	5	0	0	0	0	0	0	0	0
FADD	4	627	627	0	100.00%	89.64%	89.64%	2	2	0	0	0	0	0	0	0	0
FAS	13	1020	1020	0	100.00%	99.64%	99.64%	10	10	0	0	0	0	0	0	0	0
FASLG	10	853	853	0	100.00%	99.35%	99.35%	5	5	0	0	0	0	0	0	0	0
FCGR3B	7	1115	1115	0	100.00%	99.48%	99.48%	8	8	0	0	0	0	0	0	0	0
FCN3	9	900	900	0	100.00%	99.55%	99.55%	8	8	0	0	0	0	0	0	0	0
FERMT3	21	2129	2026	103	95.20%	98.74%	94.00%	15	14	0	0	0	0	0	0	0	1
FOXN1	14	1947	1947	0	100.00%	99.59%	99.59%	8	8	0	0	0	0	0	0	0	0
FOXP3	13	1296	1296	0	100.00%	99.52%	99.52%	11	11	0	0	0	0	0	0	0	0
G6PC3	10	1041	1041	0	100.00%	99.64%	99.64%	6	6	0	0	0	0	0	0	0	0
GATA2	12	1527	1527	0	100.00%	98.70%	98.70%	6	6	0	0	0	0	0	0	0	0

GFI1	11	1269	1269	0	100.00%	95.91%	95.91%	6	6	0	0	0	0	0	0	0
GPI	18	2189	2185	4	99.80%	98.40%	98.20%	17	16	0	1	0	0	0	0	0
HAX1	9	959	959	0	100.00%	99.67%	99.67%	8	8	0	0	0	0	0	0	0
ICOS	7	600	600	0	100.00%	99.49%	99.49%	5	5	0	0	0	0	0	0	0
IFNG	5	501	501	0	100.00%	99.73%	99.73%	4	4	0	0	0	0	0	0	0
IFNGR1	13	1470	1461	9	99.40%	99.03%	98.44%	7	6	0	0	1	0	0	0	0
IFNGR2	9	1014	1014	0	100.00%	89.74%	89.74%	7	7	0	0	0	0	0	0	0
IGHG2	8	1132	1111	21	98.10%	95.48%	93.67%	4	2	2	0	0	0	0	0	0
IGHM	11	1975	1975	0	100.00%	99.77%	99.77%	1	1	0	0	0	0	0	0	0
IGLL1	5	691	691	0	100.00%	99.66%	99.66%	4	4	0	0	0	0	0	0	0
IKBKG	12	1651	1651	0	100.00%	98.60%	98.60%	11	11	0	0	0	0	0	0	0
IL10RA	14	1737	1737	0	100.00%	99.62%	99.62%	7	7	0	0	0	0	0	0	0
IL10RB	8	985	985	0	100.00%	94.49%	94.49%	8	8	0	0	0	0	0	0	0
IL12B	9	987	987	0	100.00%	99.70%	99.70%	6	6	0	0	0	0	0	0	0
IL12RB1	22	2114	2023	91	95.70%	99.55%	95.27%	18	17	0	0	0	0	0	1	0
IL17F	5	492	492	0	100.00%	99.71%	99.71%	3	3	0	0	0	0	0	0	0
IL17RA	23	2601	2585	16	99.40%	99.08%	98.49%	13	12	1	0	0	0	0	0	0
IL1RN	8	673	673	0	100.00%	99.54%	99.54%	8	8	0	0	0	0	0	0	0
IL21R	14	1732	1732	0	100.00%	99.56%	99.56%	10	10	0	0	0	0	0	0	0
IL2RA	10	819	819	0	100.00%	99.57%	99.57%	8	8	0	0	0	0	0	0	0
IL2RG	11	1117	1117	0	100.00%	99.59%	99.59%	9	9	0	0	0	0	0	0	0
IL7R	12	1387	1387	0	100.00%	99.52%	99.52%	9	9	0	0	0	0	0	0	0
IRAK4	15	1501	1501	0	100.00%	99.56%	99.56%	12	12	0	0	0	0	0	0	0
IRF8	10	1281	1277	4	99.70%	99.47%	99.17%	8	7	1	0	0	0	0	0	0
ITCH	27	2719	2585	134	95.10%	99.53%	94.65%	25	23	0	1	0	0	0	0	1
ITGB2	26	2310	2310	0	100.00%	99.59%	99.59%	15	15	0	0	0	0	0	0	0
ITK	19	1863	1863	0	100.00%	99.47%	99.47%	17	17	0	0	0	0	0	0	0
JAK3	30	3375	3288	87	97.40%	97.97%	95.42%	23	21	1	0	0	1	0	0	0
LAMTOR2	4	378	378	0	100.00%	98.86%	98.86%	4	4	0	0	0	0	0	0	0
LIG1	30	2760	2756	4	99.90%	99.51%	99.41%	27	26	1	0	0	0	0	0	0
LIG4	15	2736	2736	0	100.00%	99.76%	99.76%	1	1	0	0	0	0	0	0	0
LPIN2	24	2691	2691	0	100.00%	99.51%	99.51%	19	19	0	0	0	0	0	0	0
LRBA	89	8757	8757	0	100.00%	99.61%	99.61%	58	58	0	0	0	0	0	0	0
LRRC8A	14	2433	2433	0	100.00%	99.75%	99.75%	2	2	0	0	0	0	0	0	0
LYST	96	11406	11406	0	100.00%	99.63%	99.63%	51	51	0	0	0	0	0	0	0
MAGT1	14	1104	1104	0	100.00%	99.58%	99.58%	10	10	0	0	0	0	0	0	0

MASP2	16	2075	2075	0	100.00%	98.09%	98.09%	12	12	0	0	0	0	0	0	0	0
MBL2	5	747	747	0	100.00%	99.47%	99.47%	4	4	0	0	0	0	0	0	0	0
MEFV	21	2565	2526	39	98.50%	98.85%	97.37%	13	12	0	1	0	0	0	0	0	0
MLPH	18	1803	1803	0	100.00%	99.55%	99.55%	15	15	0	0	0	0	0	0	0	0
MS4A1	8	894	894	0	100.00%	99.57%	99.57%	6	6	0	0	0	0	0	0	0	0
MVK	11	1191	1191	0	100.00%	99.54%	99.54%	10	10	0	0	0	0	0	0	0	0
MYD88	8	1156	1156	0	100.00%	99.72%	99.72%	7	7	0	0	0	0	0	0	0	0
MYO5A	54	5568	5559	9	99.80%	99.19%	98.99%	41	40	0	1	0	0	0	0	0	0
NBN	23	2265	2265	0	100.00%	99.55%	99.55%	16	16	0	0	0	0	0	0	0	0
NCF1	11	1173	1045	128	89.10%	97.85%	87.18%	11	9	0	0	0	0	0	0	0	2
NCF2	18	1581	1581	0	100.00%	99.54%	99.54%	15	15	0	0	0	0	0	0	0	0
NCF4	12	1440	1440	0	100.00%	99.48%	99.48%	11	11	0	0	0	0	0	0	0	0
NFKBIA	10	954	954	0	100.00%	93.45%	93.45%	6	6	0	0	0	0	0	0	0	0
NHEJ1	8	900	900	0	100.00%	99.56%	99.56%	7	7	0	0	0	0	0	0	0	0
NLRP12	19	3360	3359	1	100.00%	99.64%	99.64%	11	10	1	0	0	0	0	0	0	0
NLRP3	20	3388	3388	0	100.00%	99.67%	99.67%	10	10	0	0	0	0	0	0	0	0
NOD2	24	3123	3123	0	100.00%	99.67%	99.67%	12	12	0	0	0	0	0	0	0	0
NRAS	5	570	570	0	100.00%	99.69%	99.69%	4	4	0	0	0	0	0	0	0	0
ORAI1	6	912	905	7	99.20%	86.81%	86.11%	2	1	1	0	0	0	0	0	0	0
PIGA	12	1468	1468	0	100.00%	95.95%	95.95%	6	6	0	0	0	0	0	0	0	0
PLCG2	37	3798	3798	0	100.00%	99.25%	99.25%	32	32	0	0	0	0	0	0	0	0
PNP	9	870	870	0	100.00%	99.56%	99.56%	6	6	0	0	0	0	0	0	0	0
PRF1	11	1668	1668	0	100.00%	99.77%	99.77%	2	2	0	0	0	0	0	0	0	0
PSTPIP1	17	1251	1251	0	100.00%	99.40%	99.40%	15	15	0	0	0	0	0	0	0	0
PTPRC	43	4085	3889	196	95.20%	99.60%	94.82%	33	29	1	0	1	1	0	0	0	1
RAB27A	6	666	666	0	100.00%	99.54%	99.54%	5	5	0	0	0	0	0	0	0	0
RAC2	6	579	579	0	100.00%	99.30%	99.30%	6	6	0	0	0	0	0	0	0	0
RAG1	16	3132	3132	0	100.00%	99.73%	99.73%	1	1	0	0	0	0	0	0	0	0
RAG2	8	1584	1584	0	100.00%	99.73%	99.73%	1	1	0	0	0	0	0	0	0	0
RFX5	15	1851	1851	0	100.00%	99.54%	99.54%	9	9	0	0	0	0	0	0	0	0
RFXANK	8	864	864	0	100.00%	98.53%	98.53%	9	9	0	0	0	0	0	0	0	0
RFXAP	8	819	819	0	100.00%	93.87%	93.87%	3	3	0	0	0	0	0	0	0	0
RMRP	2	268	268	0	100.00%	99.40%	99.40%	1	1	0	0	0	0	0	0	0	0
SH2D1A	4	523	523	0	100.00%	99.46%	99.46%	5	5	0	0	0	0	0	0	0	0
SLC37A4	14	1518	1518	0	100.00%	99.62%	99.62%	11	11	0	0	0	0	0	0	0	0
SMARCAL1	24	2865	2865	0	100.00%	99.58%	99.58%	16	16	0	0	0	0	0	0	0	0

SP110	23	2367	2356	11	99.50%	99.43%	98.94%	21	19	2	0	0	0	0	0	0	0
STAT1	26	2333	2333	0	100.00%	99.54%	99.54%	24	24	0	0	0	0	0	0	0	0
STAT3	26	2548	2464	84	96.70%	99.28%	96.01%	25	24	0	0	0	0	0	0	0	1
STAT5B	20	2364	2302	62	97.40%	99.50%	96.91%	18	17	0	0	0	0	1	0	0	0
STIM1	20	2525	2525	0	100.00%	99.53%	99.53%	15	15	0	0	0	0	0	0	0	0
STK4	12	1464	1464	0	100.00%	99.47%	99.47%	11	11	0	0	0	0	0	0	0	0
STX11	5	864	864	0	100.00%	99.76%	99.76%	1	1	0	0	0	0	0	0	0	0
STXBP2	22	1964	1964	0	100.00%	98.51%	98.51%	21	21	0	0	0	0	0	0	0	0
TAP1	18	2427	2379	48	98.00%	99.55%	97.56%	11	10	0	1	0	0	0	0	0	0
TAP2	20	2142	2142	0	100.00%	99.71%	99.71%	12	12	0	0	0	0	0	0	0	0
TAPBP	14	1562	1562	0	100.00%	99.51%	99.51%	9	9	0	0	0	0	0	0	0	0
TAZ	15	879	879	0	100.00%	95.65%	95.65%	11	11	0	0	0	0	0	0	0	0
TBX1	18	1786	1771	15	99.20%	79.47%	78.83%	10	9	1	0	0	0	0	0	0	0
TERC	3	451	451	0	100.00%	98.60%	98.60%	1	1	0	0	0	0	0	0	0	0
TNFRSF13B	8	882	882	0	100.00%	99.53%	99.53%	5	5	0	0	0	0	0	0	0	0
TNFRSF13C	5	555	555	0	100.00%	49.54%	49.54%	3	3	0	0	0	0	0	0	0	0
TNFRSF1A	18	1368	1330	38	97.20%	98.69%	95.93%	10	9	0	0	1	0	0	0	0	0
TYK2	31	3564	3436	128	96.40%	99.14%	95.57%	23	22	0	0	0	0	0	0	0	1
UNG	9	1254	1254	0	100.00%	99.20%	99.20%	8	8	0	0	0	0	0	0	0	0
VPS13B	110	12524	12468	56	99.60%	98.91%	98.51%	64	60	0	3	1	0	0	0	0	0
WAS	15	1509	1509	0	100.00%	95.97%	95.97%	12	12	0	0	0	0	0	0	0	0
XIAP	13	1494	1494	0	100.00%	99.52%	99.52%	6	6	0	0	0	0	0	0	0	0
ZAP70	18	2021	2021	0	100.00%	98.35%	98.35%	13	13	0	0	0	0	0	0	0	0
		Average:			99.49%	97.02%	96.51%										

Supplementary table 5:Run and alignment quality of NGS runs

Quality Metrics after Sequencing and Before Read Mapping

Metric	Result
Bases in all amplicons (Target Bases)	1015569
Number of Bases at Q0	590,800,000
Number of Bases at Q17	556,300,000
Mean Read Length at Q0	167.9
Mean Read Length at Q17	157.8
Number of Reads at Q0	3,488,807
Number of Reads at Q17	3,488,807
Average Depth (Q0)	582
Average Depth (Q17)	548

Quality Metrics after Read Mapping

Metric	Result
Number of mapped reads	3427739
Percent reads on target	81.78%
Average reads per amplicon	547.92
Percent base reads on target (i.e., percent of the bases mapped to the target regions)	81.62%
Average percent coverage of the target regions	97.02%
Average Depth (Average base coverage after Alignment)	461

Supplementary table 6 - Lists of variants and their HGMD records.

gene	start	end	reference	Alt	zygosity	Ref Gene splice info	dbSNP	1000G	SGP 737	SGP DG5	HGMD2014
ADA	43251481	43251481	T	G	hom	ADA:NM_000022:exon8:c.385G>A;p.V129M		0	13	0	0
ADA	43249674	43249678	CTCTT	0	hom	ADA:NM_000022:exon10:c.956_960del;p.E319fs (c.955_959delGAAGA;p.E319GfsX3)		0	0	0	Info=CLASS=DM;MUT=ALT;ID=CD930882;Ref=CCTCTT;Alt=C;Start=43249673;End=43249678;HET=.;MAF=.;dbSNP=.;Disease=Adenosine deaminase deficiency]
ADA		43251680	C	T	hom	ADA:NM_000022:exon7:c.G646A:p.G216R	rs121908723	0	1	0	Info=CLASS=DM;MUT=ALT;ID=CM910007;Ref=C;Alt=T;Start=43251680;End=43251680;HET=.;MAF=.;dbSNP=rs121908723;Disease=Adenosine deaminase deficiency]
ADA	43248503	43248503	A	T	hom	ADA: Splice: NM_000022:exon13:c.1079-15T>A		0	1	0	Info=CLASS=DM;MUT=ALT;ID=CS025995;Ref=A;Alt=T;Start=43248503;End=43248503;HET=.;MAF=.;dbSNP=rs387906268;Disease=Immunodeficiency, severe combined]
AICDA	8759465	8759465	T	A	hom	AICDA:NM_020661:exon2:c.152A>T;p.N51I		0	0	0	0
AICDA	8758069	8758069	C	T	hom	AICDA:NM_020661:exon3:c.G169A;p.V57M;AICDA:NM_020661:exon3:c.G169A;p.V57M	::	0	4	0	0
AIRE	45706581	45706581	C	T	hom	AIRE:NM_000383:exon2:c.C274T:p.R92W	rs140630532	0	1	0	Info=CLASS=DM;MUT=ALT;ID=CM088348;Ref=C;Alt=T;Start=45706581;End=45706581;HET=.;MAF=.;dbSNP=rs140630532;Disease=APEC ED]
AK2	33478957	33478957	G	T	hom	AK2:NM_001199199:exon6:c.521C>A;p.A174D,AK2:NM_001625:exon6:c.545C>A;p.A182D,AK2:NM_013411:exon6:c.545C>A;p.A182D;AK2:NM_001199199:exon6:c.C521A;p.A174D,AK2:NM_001625:exon6:c.C545A;p.A182D,AK2:NM_013411:exon6:c.C545A;p.A182D;AK2:NM_001199199:exon6:c.C521A;p.A174D,AK2:NM_001625:exon6:c.C545A;p.A182D,AK2:NM_013411:exon6:c.C545A;p.A182D;AK2:N		0	3	0	0

						M_001199199:exon6:c.C521A:p.A174D,AK2:NM_001625:exon6:c.C545A:p.A182D,AK2:NM_013411:exon6:c.C545A:p.A182D					
ATM	108225582	108225583	CT	0	hom	ATM:NM_000051:exon61:c.8831_8832del:p.2944_2944del		0	0	0	Info=CLASS=DM;MUT=ALT;ID=CD961818;Ref=ACT;Alt=A;Start=108225581;End=108225583;HET=.;MAF=.;dbSNP=.;Disease=Ataxia telangiectasia
ATM	108186841	108186841	G	A	hom	ATM: Splice: NM_000051:exon42:c.6198+1G>A		0	1	0	Info=CLASS=DM;MUT=ALT;ID=CS982079;Ref=G;Alt=A;Start=108186841;End=108186841;HET=.;MAF=.;dbSNP=.;Disease=Ataxia telangiectasia
ATM	108236129	108236129	A	0	hom	ATM:NM_000051:exon63:c.9065delA:p.E3022fs;ATM:NM_000051:exon63:c.9065delA:p.E3022fs	rs56253573	0	1	0	Info=CLASS=DM;MUT=ALT;ID=CI030356;Ref=A;Alt=AG;Start=108236127;End=108236127;HET=.;MAF=.;dbSNP=.;Disease=Ataxia telangiectasia
ATM	108117691	108117691	0	T	hom	ATM:NM_000051:exon8:c.903dupT:p.G301fs		0	0	0	Info=CLASS=DM;MUT=ALT;ID=CM0910482;Ref=G;Alt=A;Start=108117691;End=108117691;HET=.;MAF=.;dbSNP=rs202208861;Disease=Breast cancer, susceptibility to
ATM	108106446	108106446	A	0	hom	ATM:NM_000051:exon5:c.381delA:p.T127fs;ATM:NM_000051:exon5:c.381delA:p.T127fs;ATM:NM_000051:exon5:c.381delA:p.T127fs	::	0	1	0	Info=CLASS=DM;MUT=ALT;ID=CD991593;Ref=CA;Alt=C;Start=108106445;End=108106446;HET=.;MAF=.;dbSNP=.;Disease=Ataxia telangiectasia
BTK	100604953	100604953	A	G	hom	BTK: Splice: NM_000061:exon20:c.1909-9T>C		0	1	1	0
BTK	100630191	100630191	G	A	hom	BTK:NM_000061:exon2:c.C82T:p.R28C		0	1	0	Info=CLASS=DM;MUT=ALT;ID=CM040689;Ref=G;Alt=T;Start=100630191;End=100630191;HET=.;MAF=.;dbSNP=.;Disease=Agammaglobulin aemia Info=CLASS=DM;MUT=ALT;ID=CM980262;Ref=G;Alt=A;Start=100630191;End=100630191;HET=.;MAF=.;dbSNP=.;Disease=Agammaglobulinaemia
BTK	100615569	100615569	G	A	hom	BTK:NM_000061:exon8:c.763C>T:p.R255X,BTK:NM_001287344:ex	rs128621193;rs12862119	0	4	0	Info=CLASS=DM;MUT=ALT;ID=CM940188;Ref=G;Alt=A;

						on8:c.865C>T;p.R289X,BTK:NM_001287345:exon9:c.763C>T;p.R255X;BTK:NM_000061:exon8:c.C763T;p.R255X;BTK:NM_000061:exon8:c.C763T;p.R255X	3;rs128621193;rs128621193				Start=100615569;End=100615569;HET=.;MAF=.;dbSNP=rs128621193;Disease=Aga mmaglobulinaemia]
CD247	167487637	167487637	G	A	hom	CD247: Splice: NM_198053:exon2:c.58+8C>T	rs201494226	0	11	3	0
CD40	44750911	44750911	C	T	hom	CD40:NM_001250:exon3:c.C170T;p.T57M,CD40:NM_152854:exon3:c.C170T;p.T57M		0	2	0	Info=CLASS=DM;MUT=ALT;ID=CM125023;Ref=C;Alt=T;Start=44750911;End=44750911;HET=.;MAF=.;dbSNP=.;Disease=CD40 deficiency]
CD40	44750988	44750988	T	C	hom	CD40:NM_001250:exon3:c.T247C;p.C83R,CD40:NM_152854:exon3:c.T247C;p.C83R;CD40:NM_001250:exon3:c.T247C;p.C83R,CD40:NM_152854:exon3:c.T247C;p.C83R	rs28931586	0	2	0	Info=CLASS=DM;MUT=ALT;ID=CM013796;Ref=T;Alt=C;Start=44750988;End=44750988;HET=.;MAF=.;dbSNP=rs28931586;Disease=Hyper-IgM syndrome]
CD40	44750999	44750999	T	C	hom	CD40: Splice: NM_001250:exon3:c.256+2T>C	::	0	3	0	Info=CLASS=DM;MUT=ALT;ID=CS125024;Ref=C;Alt=C;Start=44750999;End=44750999;HET=.;MAF=.;dbSNP=.;Disease=CD40 deficiency]
CD40LG	135741507	135741508	AT	G	hom	CD40LG:NM_000074:exon5:c.719_720del;p.240_240del		0	1	0	0
CD40LG	135741294	135741294	A	G	hom	CD40LG:NM_000074:exon5:c.A506G;p.Y169C		0	1	0	Info=CLASS=DM;MUT=ALT;ID=CM147296;Ref=A;Alt=G;Start=135741294;End=135741294;HET=.;MAF=.;dbSNP=.;Disease=Hyper-IgM syndrome]
CIITA	11012370	11012370	C	G	hom	CIITA:NM_000246:exon16:c.C3136G;p.L1046V		0	1	0	0
CYBA	88712523	88712523	C	A	hom	CYBA: NM_000101:exon6:c.369+1G>T		0			Info=CLASS=DM;MUT=ALT;ID=CS081888;Ref=C;Alt=T;Start=88712523;End=88712523;HET=0;MAF=0;dbSNP=0;Disease=Chronic granulomatous disease Info=CLASS=DM;MUT=ALT;ID=CS961535;Ref=C;Alt=G;Start=88712523;End=88712523;HET=0;MAF=0;dbSNP=0;Disease=Chronic granulomatous disease
CYBB	37664324	37664324	T	A	hom	CYBB:NM_000397:exon10:c.1217T>A;p.L406X		0	0	0	0
CYBB	37652968	37652968	C	T	hom	CYBB:NM_000397:exon5:c.C388T;p.R130X		0	1	0	Info=CLASS=DM;MUT=ALT;ID=CM960439;Ref=C;Alt=T;

											Start=37652968;End=37652968;HET=.;MAF=.;dbSNP=.;Disease=Chronic granulomatous disease Info=CLASS=DM;MUT=ALT;ID=CI012025;Ref=C;Alt=CT;Start=37652968;End=37652968;HET=.;MAF=.;dbSNP=.;Disease=Chronic granulomatous disease
CYBB	37655338	37655338	G	A	hom	CYBB:NM_000397:exon6:c.G618A:p.W206X;CYBB:NM_000397:exon6:c.G618A:p.W206X	;	0	4	0	Info=CLASS=DM;MUT=ALT;ID=CM084947;Ref=G;Alt=A;Start=37655338;End=37655338;HET=.;MAF=.;dbSNP=.;Disease=Chronic granulomatous disease
CYBB	37670075	37670075	G	O	hom	CYBB:NM_000397:exon13:c.1618delG;p.E540fs;CYBB:NM_000397:exon13:c.1618delG;p.E540fs;CYBB:NM_000397:exon13:c.1618delG;p.E540fs	::	0	2	0	Info=CLASS=DM;MUT=ALT;ID=CD097718;Ref=TG;Alt=T;Start=37670074;End=37670075;HET=.;MAF=.;dbSNP=.;Disease=Chronic granulomatous disease
DCLRE1C	14977523	14977523	C	T	hom	DCLRE1C:NM_022487:exon5:c.G58A:p.G20R,DCLRE1C:NM_001033855:exon6:c.G403A:p.G135R,DCLRE1C:NM_001033857:exon7:c.G43A:p.G15R,DCLRE1C:NM_001033858:exon8:c.G43A:p.G15R		0	2	0	0
DCLRE1C	14950482	14950482	T	A	hom	DCLRE1C:NM_022487:exon10:c.C614G;p.S205C		0	17	0	0
DNMT3B	31395653	31395653	G	A	hom	DNMT3B:NM_001207056:exon18:c.G2029A:p.V677M,DNMT3B:NM_001207055:exon19:c.G2131A:p.V711M,DNMT3B:NM_175849:exon20:c.G2257A:p.V753M,DNMT3B:NM_175848:exon22:c.G2446A:p.V816M,DNMT3B:NM_175850:exon22:c.G2482A:p.V828M,DNMT3B:NM_006892:exon23:c.G2506A:p.V836M		0	2	0	Info=CLASS=DM;MUT=ALT;ID=CM108877;Ref=G;Alt=A;Start=31395653;End=31395653;HET=.;MAF=.;dbSNP=.;Disease=Immunodeficiency, centromeric instability and facial anomalies syndrome
DOCK8	421052	421052	G	O	hom	DOCK8:NM_001190458:exon30:c.3827delG;p.G1276fs,DOCK8:NM_001193536:exon31:c.3923delG;p.G1308fs,DOCK8:NM_203447:exon32:c.4127delG;p.G1376fs		0	2	0	0
DOCK8	325693	325694	CT	O	hom	DOCK8:NM_001190458:exon7:c.646_647del;p.216_216del,DOCK8:NM_001193536:exon7:c.646_647del;p.216_216del,DOCK8:NM_203447:exon8:c.850_851del;p.284_284del		0	1	0	Info=CLASS=DM;MUT=ALT;ID=CD134253;Ref=CCT;Alt=C;Start=325692;End=325694;HET=.;MAF=.;dbSNP=.;Disease=Immunodeficiency,

4del						combined]					
DOCK8	317134	317134	T	C	hom	DOCK8: Splice: NM_203447:exon7:c.827+6T>C	0	2	0	Info=CLASS=DM;MUT=ALT; ID=CS128300;Ref=T;Alt=C; Start=317134;End=317134; HET=.;MAF=.;dbSNP=.;Disease=Hyper-IgE syndrome, autosomal recessive]	
DOCK8	304643	304643	G	A	hom	DOCK8: Splice: NM_203447:c.4626+5G>A					
DOCK8	439297	439297	C	A	hom	DOCK8:NM_001190458:exon38:c.C4832A:p.S1611X,DOCK8:NM_001193536:exon39:c.C4928A:p.S1643X,DOCK8:NM_203447:exon40:c.C5132A:p.S1711X;DOCK8:NM_001190458:exon38:c.C4832A:p.S1611X,DOCK8:NM_001193536:exon39:c.C4928A:p.S1643X,DOCK8:NM_203447:exon40:c.C5132A:p.S1711X;DOCK8:NM_001190458:exon38:c.C4832A:p.S1611X,DOCK8:NM_001193536:exon39:c.C4928A:p.S1643X,DOCK8:NM_203447:exon40:c.C5132A:p.S1711X	::	0	2	0	Info=CLASS=DM;MUT=ALT; ID=CM108405;Ref=C;Alt=A; Start=439297;End=439297; HET=.;MAF=.;dbSNP=.;Disease=Hyper-IgE syndrome, autosomal recessive]
DOCK8	446414	446414	T	G	hom	DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43: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775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y1875X;DOCK8:NM_001190458:exon42:c.T5325G:p.Y1775X,DOCK8:NM_001193536:exon43:c.T5421G:p.Y1807X,DOCK8:NM_203447:exon44:c.T5625G:p.Y					

c.T5625G:p.Y1875X											
IFNGR2	34804627	34804627	C	A	hom	IFNGR2:NM_005534:exon5:c.C705A:p.Y235X	0	1	0	Info=CLASS=DM;MUT=ALT;ID=CM131736;Ref=C;Alt=A;Start=34804627;End=34804627;HET=.;MAF=.;dbSNP=.;Disease=Mycobacterial infection	
IL7R	35876093	35876093	T	O	hom	IL7R:NM_002185:exon8:c.885delT:p.N295fs	0	1	0	0	
IL7R	35873680	35873680	T	G	hom	IL7R:NM_002185:exon5:c.T636G:p.Y212X	0	1	0	0	
IRAK4	44171539	44171539	T	O	hom	IRAK4:NM_001145258:exon5:c.451delT:p.S151fs,IRAK4:NM_001145257:exon6:c.451delT:p.S151fs,IRAK4:NM_001145256:exon7:c.451delT:p.S151fs,IRAK4:NM_016123:exon7:c.823delT:p.S275fs,IRAK4:NM_001114182:exon8:c.823delT:p.S275fs	0	1	0	0	
ITGB2	46320234	46320234	C	T	hom	ITGB2: splice: NM_000211:c.897+1 G>A					Info=CLASS=DM;MUT=ALT;ID=CS920765;Ref=C;Alt=T;Start=46320234;End=46320234;HET=.;MAF=.;dbSNP=rs201752283;Disease=Leukocyte adhesion deficiency
JAK3	17951086	17951086	G	A	hom	JAK3:NM_000215:exon9:c.1207C>T:p.R403C	0	0	0	0	
JAK3	17954586	17954586	C	T	hom	JAK3:NM_000215:exon3:c.G308A:p.R103H	0	1	0	0	
JAK3	17952520	17952520	G	A	hom	JAK3:NM_000215:exon7:c.C913T:p.Q305X	0	1	0	0	
LRBA	151771894	151771895	TC	O	hom	LRBA:NM_001199282:exon24:c.3985_3986del:p.1329_1329del,LRBA:NM_006726:exon24:c.3985_3986del:p.1329_1329del	0	1	0	0	
LYST	235972815	235972815	C	A	hom	LYST:NM_000081:exon5:c.G1303T:p.E435X	0	1	0	0	
LYST	235922459	235922459	C	A	hom	LYST:NM_000081:exon23:c.G6694T:p.G2232X	0	1	0	0	
LYST	235894146	235894146	C	A	hom	LYST: Splice: NM_000081:exon37:c.9044+1G>T	0	2	0	0	
MLPH	238436125	238436125	C	O	hom	MLPH:NM_001042467:exon8:c.986delC:p.S329fs,MLPH:NM_024101:exon8:c.986delC:p.S329fs	0	3	1	0	
MLPH	238402173	238402173	G	A	hom	MLPH:NM_001042467:exon2:c.G104A:p.R35Q,MLPH:NM_024101:exon2:c.G104A:p.R35Q;MLPH:NM_001042467:exon2:c.G104A:p.R35Q,MLPH:NM_024101:exon2:c.G104A:p.R35Q	0	2	0	0	

NCF2	183556058	183556058	G	A	hom	NCF2:NM_000433:exon2:c.C229T:p.R77X,NCF2:NM_001190789:exon2:c.C229T:p.R77X,NCF2:NM_001190794:exon2:c.C229T:p.R77X,NCF2:NM_001127651:exon3:c.C229T:p.R77X		0	1	0	Info=CLASS=DM;MUT=ALT;ID=CM095679;Ref=G;Alt=A;Start=183556058;End=183556058;HET=.;MAF=.;dbSNP=.;Disease=Chronic granulomatous disease
NHEJ1	220022193	220022193	C	G	hom	NHEJ1: Splice: NM_024782:exon4:c.390+1G>C	;	0	3	0	0
PNP	20940466	20940466	G	C	hom	PNP: Splice: NM_000270:exon2:c.12-1G>C		0	0	0	0
PNP	20943246	20943246	T	C	hom	PNP:NM_000270:exon5:c.487T>C:p.S163P		0	0	0	Info=CLASS=DM;MUT=ALT;ID=CM095474;Ref=T;Alt=C;Start=20943246;End=20943246;HET=.;MAF=.;dbSNP=.;Disease=Nucleoside phosphorylase deficiency
PTPRC	198723433	198723433	T	C	hom	PTPRC:NM_080921:exon29:c.T3062C:p.L1021S,PTPRC:NM_002838:exon32:c.T3545C:p.L1182S	rs114970039	0.0014	5	5	0
RAB27A	55497857	55497857	G	A	hom	RAB27A:NM_004580:exon6:c.514C>T:p.Q172X,RAB27A:NM_183234:exon7:c.514C>T:p.Q172X,RAB27A:NM_183235:exon7:c.514C>T:p.Q172X,RAB27A:NM_183236:exon7:c.514C>T:p.Q172X		0	0	0	0
RAB27A	55526984	55526984	0	C	hom	RAB27A:NM_004580.4:c.148_149delinsC					Info=CLASS=DM;MUT=ALT;ID=CD001540;Ref=TC;Alt=T;Start=55526983;End=55526983;HET=0;MAF=0;dbSNP=.;Disease=Griscelli syndrome
RAB27A	55527079	55527080	AG	0	hom	RAB27A:NM_004580:exon2:c.53_54del:p.18_18del,RAB27A:NM_183234:exon3:c.53_54del:p.18_18del,RAB27A:NM_183235:exon3:c.53_54del:p.18_18del,RAB27A:NM_183236:exon3:c.53_54del:p.18_18del		0	1	0	Info=CLASS=DM;MUT=ALT;ID=CD030348;Ref=CAG;Alt=C;Start=55527078;End=55527080;HET=.;MAF=.;dbSNP=.;Disease=Griscelli syndrome
RAB27A	55516154	55516154	T	G	hom	RAB27A:NM_004580:exon5:c.A400C:p.K134Q,RAB27A:NM_183234:exon6:c.A400C:p.K134Q,RAB27A:NM_183235:exon6:c.A400C:p.K134Q,RAB27A:NM_183236:exon6:c.A400C:p.K134Q		0	1	0	Info=CLASS=DM;MUT=ALT;ID=CM086898;Ref=T;Alt=C;Start=55516154;End=55516154;HET=.;MAF=.;dbSNP=.;Disease=Griscelli syndrome
RAB27A	55497773	55497773	G	A	hom	RAB27A:NM_004580:exon6:c.598C>T:p.R200X,RAB27A:NM_183234:exon7:c.598C>T:p.R200X,RAB27A:NM_183235:exon7:c.598C>T:p.R200X,RAB27A:NM_183236:exon7:c.598C>T:p.R200X,RAB27A:NM_183236:exon7:c.598C>T:p.R200X	::	0	1	0	Info=CLASS=DM;MUT=ALT;ID=CM073287;Ref=G;Alt=A;Start=55497773;End=55497773;HET=.;MAF=.;dbSNP=.;Disease=Griscelli syndrome

						M_004580:exon6:c.598C>T:p.R200X,RAB27A:NM_183234:exon7:c.598C>T:p.R200X,RAB27A:NM_183235:exon7:c.598C>T:p.R200X,RAB27A:NM_183236:exon7:c.598C>T:p.R200X;RAB27A:NM_004580:exon6:c.C598T:p.R200X,RAB27A:NM_183234:exon7:c.C598T:p.R200X,RAB27A:NM_183235:exon7:c.C598T:p.R200X,RAB27A:NM_183236:exon7:c.C598T:p.R200X					
RAG1	36596895	36596895	G	T	hom	RAG1:NM_000448:exon2:c.G2041T:p.E681X	0	1	0	0	
RAG1	36596034	36596034	C	T	hom	RAG1:NM_000448:exon2:c.C1180T:p.R394W	0	2	0	Info=CLASS=DM;MUT=ALT;ID=CM065417;Ref=C;Alt=T;Start=36596034;End=36596034;HET=.;MAF=.;dbSNP=.;Disease=Omenn syndrome	
RAG1	36596292	36596292	A	G	hom	RAG1:NM_000448:exon2:c.A1438G:p.S480G	0	1	0	Info=CLASS=DM;MUT=ALT;ID=CM147136;Ref=A;Alt=G;Start=36596292;End=36596292;HET=.;MAF=.;dbSNP=.;Disease=Immunodeficiency, severe combined	
RAG1	36597064	36597064	G	A	hom	RAG1:NM_000448:exon2:c.G2210A:p.R737H	rs104894286	0	1	0	Info=CLASS=DM;MUT=ALT;ID=CM981696;Ref=G;Alt=A;Start=36597064;End=36597064;HET=.;MAF=0.0002/1;dbSNP=rs104894286;Disease=Omenn syndrome Info=CLASS=DM;MUT=ALT;ID=CM981696;Ref=G;Alt=A;Start=36597064;End=36597064;HET=.;MAF=0.0002/1;dbSNP=rs104894286;Disease=Omenn syndrome Info=CLASS=DM;MUT=ALT;ID=CM981696;Ref=G;Alt=A;Start=36597064;End=36597064;HET=.;MAF=0.0002/1;dbSNP=rs104894286;Disease=Omenn syndrome
RAG1	36596055	36596055	T	C	hom	RAG1:NM_000448:exon2:c.T1201C:p.S401P;RAG1:NM_000448:exon2:c.T1201C:p.S401P	rs199474682	0	2	0	Info=CLASS=DM;MUT=ALT;ID=CM010068;Ref=T;Alt=C;Start=36596055;End=36596055;HET=.;MAF=.;dbSNP=rs199474682;Disease=Omenn syndrome
RAG1	36595408	36595408	G	G	hom	RAG1:NM_000448:exon2:c.554del	:::	0	4	0	0

						G:p.R185fs;RAG1:NM_000448:exon2:c.554delG;p.R185fs;RAG1:NM_000448:exon2:c.554delG;p.R185fs;RAG1:NM_000448:exon2:c.554delG;p.R185fs					
RAG2	36615344	36615345	TG	0	hom	RAG2:NM_000536:exon2:c.374_375del;p.125_125del,RAG2:NM_001243785:exon3:c.374_375del;p.125_125del,RAG2:NM_001243786:exon3:c.374_375del;p.125_125del	0	1	0	0	
RAG2	36614313	36614316	AGAT	0	hom	RAG2:NM_000536:exon2:c.1403_1406del;p.468_469del,RAG2:NM_001243785:exon3:c.1403_1406del;p.468_469del,RAG2:NM_001243786:exon3:c.1403_1406del;p.468_469del	0	1	0	0	
RAG2	36615535	36615535	A	C	hom	RAG2:NM_000536:exon2:c.T184G;p.F62V,RAG2:NM_001243785:exon3:c.T184G;p.F62V,RAG2:NM_001243786:exon3:c.T184G;p.F62V	0	2	0	0	
RAG2	36615172	36615172	A	G	hom	RAG2:NM_000536:exon2:c.T547C;p.F183L,RAG2:NM_001243785:exon3:c.T547C;p.F183L,RAG2:NM_001243786:exon3:c.T547C;p.F183L	0	1	0	0	
RAG2	36615277	36615277	G	A	hom	RAG2:NM_000536:exon2:c.C442T;p.R148X,RAG2:NM_001243785:exon3:c.C442T;p.R148X,RAG2:NM_001243786:exon3:c.C442T;p.R148X	0	1	0		Info=CLASS=DM;MUT=ALT;ID=CM010085;Ref=G;Alt=A;Start=36615277;End=36615277;HET=.;MAF=.;dbSNP=.;Disease=Omenn syndrome
RAG2	36615033	36615033	C	G	hom	RAG2:NM_000536:exon2:c.G686C;p.R229P,RAG2:NM_001243785:exon3:c.G686C;p.R229P,RAG2:NM_001243786:exon3:c.G686C;p.R229P	0	1	0		Info=CLASS=DM;MUT=ALT;ID=CM961219;Ref=C;Alt=T;Start=36615033;End=36615033;HET=.;MAF=.;dbSNP=r s121917894;Disease=Immunodeficiency, severe combined, B cell -ve
RAG2	36614387	36614387	G	C	hom	RAG2:NM_000536:exon2:c.C1332G;p.I444M,RAG2:NM_001243785:exon3:c.C1332G;p.I444M,RAG2:NM_001243786:exon3:c.C1332G;p.I444M,RAG2:NM_000536:exon2:c.C1332G;p.I444M,RAG2:NM_001243785:exon3:c.C1332G;p.I444M,RAG2:NM_001243786:exon3:c.C1332G;p.I444M	0	2	0		Info=CLASS=DM;MUT=ALT;ID=CM098872;Ref=G;Alt=C;Start=36614387;End=36614387;HET=.;MAF=.;dbSNP=.;Disease=Omenn syndrome
RFXANK	19304761	19304761	0	CTTA	hom	RFXANK:NM_003721:exon3:c.6_7insCTTA:p.E2fs,RFXANK:NM_134440:exon3:c.6_7insCTTA:p.E2fs	0	1	0	0	

RFXANK	19307854	19307854		TCAC	hom	RFXANK: Splice: NM_003721:c.269+1delGinsTCAC					
RFXANK	19307856	19307856	G	C	hom	RFXANK: Splice: NM_134440:exon4:c.268+1G>C	;	0	2	0	0
RFXANK	19308354	19308354	A	T	hom	RFXANK:N _M _134440:exon5:c.A2 93T:p.D98V,RFXANK:N _M _003721 :exon6:c.A362T;p.D121V;RFXANK :N _M _134440:exon5:c.A293T;p.D9 8V,RFXANK:N _M _003721:exon6:c. A362T;p.D121V	rs104894709	0	2	0	Info=CLASS=DM;MUT=ALT; ID=CM033005;Ref=A;Alt=T; Start=19308354;End=19308 354;HET=.;MAF=.;dbSNP=r s104894709;Disease=MHC class II deficiency
RMRP	3651204	3651204	G	C	hom	RMRP: ncRNA; C.7 A>G		0	3	0	0
STAT1	191851581	19185158 1	A	O	hom	STAT1:N _M _007315: c.1220T>G;p.L407R		0	0	0	0
STAT3	40475042	40475042	C	A	het	STAT3:N _M _003150:exon20:c.G18 68T:p.W623L,STAT3:N _M _139276: exon20:c.G1868T;p.W623L,STAT 3:N _M _213662:exon20:c.G1868T;p .W623L		0	1	0	0
STAT3	40481660	40481660	C	T	het	STAT3:N _M _003150:exon13:c.G11 45A:p.R382Q,STAT3:N _M _139276: exon13:c.G1145A:p.R382Q,STAT 3:N _M _213662:exon13:c.G1145A:p .R382Q	rs113994136	0	2	0	Info=CLASS=DM;MUT=ALT; ID=CM074585;Ref=C;Alt=T; Start=40481660;End=40481 660;HET=.;MAF=.;dbSNP=r s113994136;Disease=Hyper- IgE syndrome Info=CLASS=DM; ;MUT=ALT;ID=CM074585;R ef=C;Alt=T;Start=40481660; End=40481660;HET=.;MAF =.;dbSNP=rs113994136;Dis ease=Hyper-IgE syndrome Info=CLASS=DM; MUT=ALT;ID=CM074585;R ef=C;Alt=T;Start=40481660; End=40481660;HET=.;MAF =.;dbSNP=rs113994136;Dis ease=Hyper-IgE syndrome Info=CLASS=DM; MUT=ALT;ID=CM074585;R ef=C;Alt=T;Start=40481660; End=40481660;HET=.;MAF =.;dbSNP=rs113994136;Dis ease=Hyper-IgE syndrome Info=CLASS=DM; MUT=ALT;ID=CM074585;R ef=C;Alt=T;Start=40481660; End=40481660;HET=.;MAF =.;dbSNP=rs113994136;Dis ease=Hyper-IgE syndrome Info=CLASS=DM; MUT=ALT;ID=CM074585;R

syndrome Info=CLASS=DM; MUT=ALT;ID=CM941405;Ref=G;Alt=A;Start=48542796;End=48542796;HET=.;MAF=.;dbSNP=rs132630268;Disease=Wiskott-Aldrich syndrome Info=CLASS=DM; MUT=ALT;ID=CM941405;Ref=G;Alt=A;Start=48542796;End=48542796;HET=.;MAF=.;dbSNP=rs132630268;Disease=Wiskott-Aldrich syndrome										
ZAP70	98349351	98349351	G	C	hom	ZAP70:NM_001079:exon5:c.569G>C:p.R190T	0	1	0	0
ZAP70	98354343	98354343	G	A	hom	ZAP70:NM_207519:exon4:c.G685A:p.G229S,ZAP70:NM_001079:exon12:c.G1606A:p.G536S;ZAP70:NM_207519:exon4:c.G685A:p.G229S,ZAP70:NM_001079:exon12:c.G1606A:p.G536S;ZAP70:NM_207519:exon4:c.G685A:p.G229S,ZAP70:NM_001079:exon12:c.G1606A:p.G536S	0	3	0	0