Primary immunodeficiency diseases: Genomic approaches delineate heterogeneous Mendelian disorders

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Background: Primary immunodeficiency diseases (PIDDs) are clinically and genetically heterogeneous disorders thus far associated with mutations in more than 300 genes. The clinical phenotypes derived from distinct genotypes can overlap. Genetic etiology can be a prognostic indicator of disease severity and can influence treatment decisions.

Objective: We sought to investigate the ability of whole-exome screening methods to detect disease-causing variants in patients with PIDDs.

Methods: Patients with PIDDs from 278 families from 22 countries were investigated by using whole-exome sequencing. Computational copy number variant (CNV) prediction pipelines and an exome-tiling chromosomal microarray were also applied to identify intragenic CNVs. Analytic approaches initially focused on 475 known or candidate PIDD genes but were nonexclusive and further tailored based on clinical data, family history, and immunophenotyping.

Results: A likely molecular diagnosis was achieved in 110 (40%) unrelated probands. Clinical diagnosis was revised in about half (60/110) and management was directly altered in nearly a quarter (26/110) of families based on molecular findings. Twelve PIDD-causing CNVs were detected, including 7 smaller than 30 Kb that would not have been detected with conventional diagnostic CNV arrays.

Conclusion: This high-throughput genomic approach enabled detection of disease-related variants in unexpected genes; permitted detection of low-grade constitutional, somatic, and revertant mosaicism; and provided evidence of a mutational burden in mixed PIDD immunophenotypes. (J Allergy Clin Immunol 2017;139:232-45.)

Key words: *Primary immunodeficiency disease, whole-exome sequencing, copy number variants*

Abbreviations used

AD:	Autosomal dominant
ALPS:	Autoimmune lymphoproliferative syndrome
AR:	Autosomal recessive
BCM:	Baylor College of Medicine
BHCMG:	Baylor-Hopkins Center for Mendelian Genomics
CMA:	Chromosomal microarray analysis
CNV:	Copy number variant
CVID:	Common variable immunodeficiency
ExAC:	Exome Aggregation Consortium
HGMD:	Human Gene Mutation Database
HSCT:	Hematopoietic stem cell transplantation
MAF:	Minor allele frequency
MLPA:	Multiplex ligation-dependent probe amplification
OMIM:	Online Mendelian Inheritance in Man
PIDD:	Primary immunodeficiency disease
RIDDLE:	Radiosensitivity-immunodeficiency-dysmorphic features-learning difficulties
SCID:	Severe combined immunodeficiency
SNV:	Single nucleotide variant
WES:	Whole-exome sequencing
WGS:	Whole-genome sequencing

Primary immunodeficiency diseases (PIDDs) are clinically and genetically heterogeneous. The severe end of the clinical spectrum includes acutely life-threatening conditions. Milder disorders can present with frequent, severe, and/or unusual infections; with autoimmune, autoinflammatory, or lymphoproliferative phenomena; and with or without dysmorphic features.[1] Underdiagnosis and diagnostic delay can contribute to morbidity

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and mortality.[2-9] Identification of the molecular cause and disease mechanism or mechanisms might enable early protective interventions and potentially targeted or curative therapy.[10-17] Additionally, in patients with radiosensitive PIDDs, interventions such as radiographic imaging and the use of DNA-damaging radiomimetic drugs in pretreatment conditioning must be addressed.

Mutations in more than 300 genes are thus far known to cause PIDDs. Diagnostic molecular testing is further complicated by overlapping clinical phenotypes.[1] Genetic testing for PIDD-associated gene variants using iterative Sanger sequencing of single genes can be time-consuming and costly. Furthermore, analysis of very rare disease-related genes is not always available in diagnostic laboratories. Although the utility of targeted capture of a panel of known PIDD genes has been demonstrated, this method has limitations, such as the inability to detect new disease genes.[18-20] Whole-exome sequencing (WES) has the potential to provide rapid molecular diagnoses and improve diagnostic yield,[21,22] which is particularly useful in clinically and genetically heterogeneous disorders, such as PIDDs.

In an unbiased approach to disease gene discovery and molecular diagnosis in a large cohort (n = 278; Table I) with a broad range of PIDD phenotypes, we assayed all known relevant genes using WES. Study participants had been evaluated previously with conventional tools, including clinically available immunologic assays and genetic tests, but lacked a molecular diagnosis at the time of enrollment. WES and Sanger sequencing detected single nucleotide variants (SNVs) and small indels. Multiplex ligation-dependent probe amplification (MLPA),[23] chromosomal microarray analysis (CMA), and tailored bioinformatic analysis of WES data enabled detection of both intragenic and larger copy number variants (CNVs). Gene variant evaluation was performed according to recommended genetic guidelines, which include information concerning variant type; its relevance to phenotype, segregation, and predicted effect; and biological evidence for a definitive or potential functional effect.

METHODS

Clinical samples

Study centers included the Baylor-Hopkins Center for Mendelian Genomics (BHCMG) at Baylor College of Medicine (BCM) and the Center for Human Immunobiology, Division of Immunology Allergy and Rheumatology at Texas Children's Hospital, Houston, Texas, and Oslo University Hospital, Oslo, Norway. A total of 278 families with PIDD from 22 countries consecutively recruited from 2010 to 2015 participated. The 278 probands ranged in age from 7 weeks to 71 years, with a mean of 14.7 years. The 78 probands (47 male and 31 female probands) tested in Oslo ranged in age from 4 months to 71 years, with a mean of 18.4 years. At BHCMG, the 200 probands (114 male and 86 female probands) ranged in age from 7 weeks to 60 years, with a mean of 13.2 years.

Only clinically well-characterized affected subjects for whom appropriate conventional diagnostic investigations, including immunologic and clinically available genetic laboratory testing, had been previously performed were included. The diagnostic assessment was performed according to the European Society for Immunodeficiencies Registry diagnosis criteria (http://esid.org/Working-Parties/Registry/Diagnosis-criteria)[24] and/or the joint American Academy of Allergy, Asthma & Immunology and American College of Allergy, Asthma & Immunology's "Practice parameter for the diagnosis and management of primary immunodeficiency."[25,26] Disease manifestations ranged from mild to severe. Based on the clinical and immunologic presentation at the time of inclusion, probands were assigned to one of 10 PIDD subgroups (Table I): (I) antibody deficiency (humoral deficiency that does not fulfill the diagnostic criteria for common variable immunodeficiency [CVID]); (II) autoimmune disease; (III) autoinflammatory disorder; (IV) severe combined immunodeficiency (SCID); (V) combined immunodeficiency (not SCID) and selective T-cell deficiency; (VI) CVID; (VII) defect in innate immunity, including mucocutaneous candidiasis, hyper-IgE syndrome, Mendelian susceptibility to mycobacterial disease, and complement deficiency; (VIII) lymphoproliferative disease, hemophagocytic lymphohistiocytosis, and natural killer cell deficiency; (IX) neutrophil defect or congenital condition with bone marrow failure, such as dyskeratosis congenita and Fanconi-like phenotype, anemia, and thrombocytopenia; and (X) immunoosseous dysplasia, chromosomal disorder, or other syndromal PIDD. Because of the lack of a molecular diagnosis at enrollment, rather than using the molecularly based International Union of Immunological Societies classification system,[27] these subgroups above were used because they were constructed based on the presenting phenotype and not the ultimate genetic diagnosis.

TABLE I. Total number of subjects and families undergoing WES testing at the 2 different study centers

	Clinical phenotypes* per family	BHCMG, Houston, Tex	Oslo, Norway	Total
I	Antibody deficiency	14	2	16
II	Autoimmune disease	27	5	33
III	Autoinflammatory disorder	7	8	15
IV	SCID	6	4	10
V	Combined or selective T-cell defect	40	16	56
VI	CVID	13	7	20
VII	Defect in innate immunity	14	7	21
VIII	Lymphoproliferative or NK cell defect	53	9	62
IX	Bone marrow failure or neutrophil defect	12	17	29
X	Syndromal PIDD	14	3	17
	Sum of WES tested families	200	78	278
	Total no. of subjects undergoing WES	400	83	483

NK, Natural killer.
*The PIDD subgroups are based on the proband's clinical presentation before WES.

WES

For affected subjects, WES was performed with genomic DNA extracted from whole blood before hematopoietic stem cell transplantation (HSCT). WES of 78 probands (Table I), 4 unaffected family members, and 1 additional affected family member was performed at the Department of Medical Genetics, Oslo University Hospital. WES of 196 unrelated probands, 177 unaffected family members, and 27 additional affected family members was performed in Houston at the BHCMG.[28] In 4 of the 200 families tested at the BHCMG (Table I), obligate carriers, but not probands, underwent WES. Nine of the samples were tested by using WES at both places but were assigned to the center where the first analysis was performed and confirmed concordance of sequencing standards.

At the BCM Human Genome Sequencing Center, exome capture was performed with the in-house developed BCM-HGSC Core design (52 Mb; Roche NimbleGen, Madison, Wis), as previously described thoroughly.[21,29,30] In Oslo exome capture was performed with the 50-Mb SureSelect Human All Exon kit v.5 (Agilent Technologies, Santa Clara, Calif). For 13 affected subjects from whom blood samples yielded a low DNA concentration (<10 ng/μL), exome capture was performed with the 45-Mb Nextera Rapid Capture Exome (Illumina, San Diego, Calif). The 3 different capture methods provide similar output.[31,32] Sample preparation was performed according to the manufacturer's recommendations. For both locations, the captured exome was sequenced on the Illumina HiSeq 2500 platform (Illumina), with 100- or 125-bp paired-end reads resulting in an average coverage of 100× and more than 90% of bases with greater than 20× coverage. Details for data processing of the exome sequence are provided in the Methods section in this article's Online Repository at www.jacionline.org. For both centers, the bioinformatics pipelines were applied based on the GATK best practices and ANNOVAR.[33]

Identification of potential PIDD-causing variants

Initially, rare variants were selected based on the Exome Sequencing Project, 1000 Genomes (October 2013), the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org), and in-house databases that include WES data from phenotypically well-characterized subjects. Details for variant evaluation are provided in the Methods section in this article's Online Repository. All affected subjects were initially screened for variants in a list of 475 known or candidate PIDD genes, designated PIDD(475), which was collated from gene lists available at the Resource of Asian Primary Immunodeficiency Disease (http://rapid.rcai.riken.jp/RAPID) and International Union of Immunological Societies (http://www.iuisonline.org/iuis/),[1] and supplemented with genes known to affect telomere length or cause Fanconi anemia. If no PIDD-causing variants were identified from the PIDD(475) list, complete exome data were investigated for potential PIDD-causing variants. In the subjects who received a primary molecular diagnosis based on the PIDD(475) list, the complete exome data were also reviewed for occurrence of additional and modifying gene variants. Referring clinicians and molecular geneticists collaborated to assess the relevance of detected variants to the phenotype. In the exome-wide investigations variants of interest were selected based on rarity, previously published cases with the same gene variants, and evaluation of possible genotype-phenotype correlation based on gene function, pathway, expression pattern, and results from model organisms. Additional support for variants of potential effect was sought by using computational prediction tools (PhyloP,[34] GERP,[35] SIFT,[36] PolyPhen-2,[37] LRT,[38] and MutationTaster[39]).

For potential disease-causing variants, the proband and available affected and unaffected family members underwent Sanger sequencing to confirm cosegregation of detected variants with disease phenotypes (see the Methods section in this article's Online Repository for details). More than 1000 individual DNA samples were collected from the 278 PIDD families during the study. Altogether, 400 samples underwent WES (Table I). The remaining were collected and stored for Sanger segregation testing, and in total, 367 samples were used for Sanger segregation testing from family members of the 110 probands with a likely or potential molecular diagnosis presented in this article.

The functional effect of the detected variants (see Table E1 in this article's Online Repository at www.jacionline.org) was evaluated based on criteria adapted from the guidelines recommended by the American College of Medical Genetics and Genomics (see the Methods section in this article's Online Repository).[40] The reported potential disease-causing variants were of class 5 (definitive pathological), class 4 (likely pathological) and class 3, but class 3 variants were only reported when the affected gene was consistent with the phenotype or parts of the phenotype in the affected subject. When possible, further supporting data were generated through functional studies, such as cDNA sequencing, to confirm aberrant splicing with RNA extracted from PaxGene blood samples. The reported potential disease-causing variants were not present in a homozygous state, and allele frequency was less than 0.0001 in the heterozygous/hemizygous state (for dominant/X-linked inheritance, respectively) in the ExAC database (as of March 2016), with the exception of modifying variants with known functional effect. Some candidate genes belonged to an established potential PIDD gene list.[41] Additional lines of evidence included biological validation consistent with known clinically acceptable measures of a disease gene's function or at least in part with guidance proposed for unique discoveries in single cases,[42] the presence of PIDD gene protein homologues with relevant expression patterns belonging to the PIDD gene interactome, and segregation with identical immunophenotypes in several subjects within the same family, other families, or both. Given the residual uncertainty, we refer to the potentially causal linkage to disease as "likely." For otherwise previously unreported genes and pending complete biological validation,[41,42] these genes are referred to in this article as "potentially novel" to allow consideration of overall diagnostic yield for the appropriate fulfillment of established criteria.

CNVs

Two methods were used to identify CNVs: computational CNV prediction and microarray CNV detection. CNV prediction was initially performed for the probands. Microarray CNV detection was applied in selected cases in which no disease-causing variants were detected after exome sequencing or when CNV prediction data indicated the presence of a relevant CNV (see the Methods section in this article's Online Repository for details). One third of probands had been evaluated through a diagnostic CNV microarray before inclusion by using 180K array comparative genomic hybridization (Agilent Technologies) or SNP Array 6.0 (Affymetrix, Santa Clara, Calif), which did not provide a definitive diagnosis by itself.

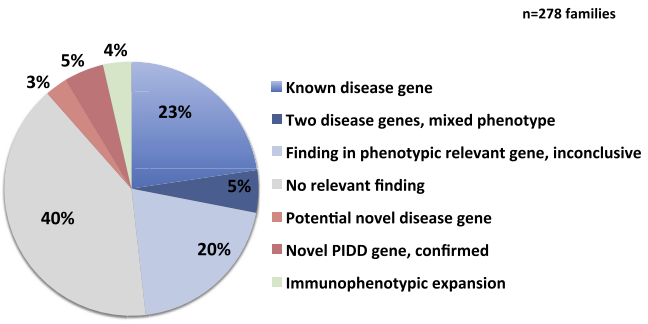

FIG 1. Status of molecular diagnosis after WES of 278 families with PIDDs. Findings are grouped in categories based on whether the detected disease-causing variants (1) affect known disease genes; (2) are located in more than 1 gene contributing to the assumed mixed phenotype (PIDD or non-PIDD) in the subjects; (3) affect a confirmed novel disease gene; (4) affect a potentially novel PIDD gene; (5) lead to immunophenotypic expansion, an immune phenotype observed in the affected subject modified from that which is historically characteristic of the particular gene; (6) affect a phenotypic relevant gene that was not presumably the major disease etiology or only 1 deleterious variant in a disease relevant AR gene (in total 57 cases); or (7) do not identify a molecular explanation by using the technologies applied here (111 cases).

RESULTS

In 278 PIDD families who underwent WES, a likely molecular diagnosis that explains all or part of the phenotype (PIDD and non-PIDD) was established in 110 (40% = 3 + 5 + 4 + 23 + 5; Fig 1 and Table II). Diagnostic yield varied by PIDD subgroup (Fig 2). The highest diagnostic rate was in patients with SCID (100%), followed by those with bone marrow failure (55%) and syndromal PIDD (53%). The lowest diagnostic rate was in the autoinflammatory subgroup (13%), even when transient disease was excluded (18%).

Genetic spectrum of disease-associated variants

In the 110 probands 148 different disease-associated, potentially causative or contributing variants were identified in 88 genes (Fig 3 and see Table E1). Forty-six variants had previously been reported as disease causing in the Human Gene Mutation Database (HGDM; http://www.hgmd.cf.ac.uk/), and another 6 were located in the same nucleotide position or induced a change in the same codon as a previously reported HGMD variant (see Table E1). In addition, 36 other variants met the criteria for American College of Medical Genetics and Genomics class 5 (pathogenic) and 10 variants for class 4 (likely pathogenic), and the rest were classified as variants of unknown significance (ie, class 3). Altogether, 98 (66%) variants were either reported before and/or likely or certain to be disease causing. The 148 detected variants included 136 SNVs: 95 missense, 13 frameshift, 11 nonsense, 14 splice variants, 2 indels, and 1 in-frame deletion (Fig 4). Many of these variants likely

TABLE II. Number of family members undergoing WES and detection rate

Family members who underwent WES	Total no. of families	Molecular diagnosis established	Total detection rate
One subject only*	192	70 (36%)	36%
Proband + 1 healthy parent or other unaffected relatives†	25	7 (28%)	43% (17/40)
Proband + affected relatives	15	10 (67%)	
Trio: proband + both parents	39	17 (44%)	42% (23/55)
Trio + affected relatives	6	2 (33%)	
Trio + unaffected relatives	10	4 (40%)	
Sum of all family WES studies	287	110	40%

*Including mother of proband 90.1 (see Table E1).
†Including both parents of proband 95.1 (see Table E1).

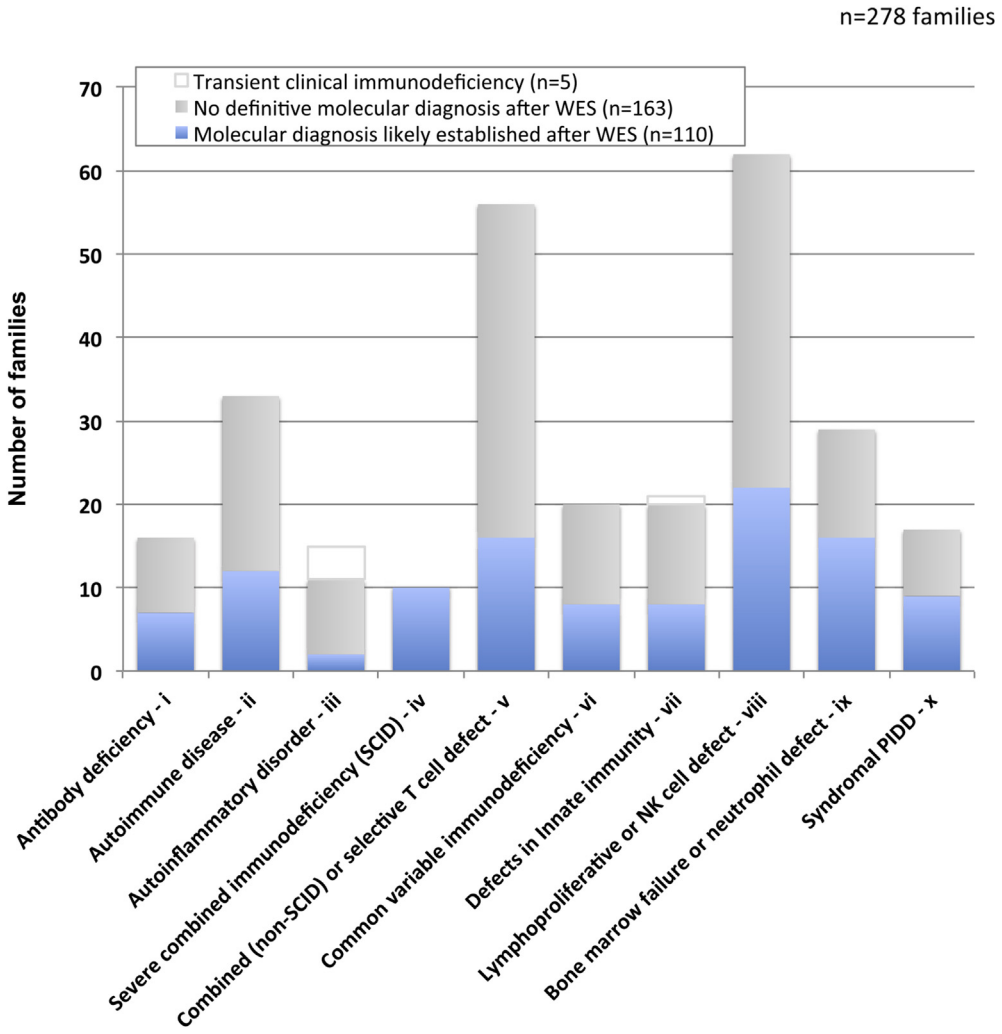

FIG 2. Diagnostic yield in the PIDD cohort (n = 278 families) by subgroup distribution based on the proband's diagnosis before WES plus CNV testing.

explain the PIDD immunophenotype, and some might also explain non-PIDD features of the clinical phenotype.

PIDD-causing CNVs were detected in 12 (11%) of 110 families. The CNVs constituted 12 (8%) of the 148 likely disease-causing variants (Fig 4) and involved 11 genes: *DKC1* (MIM: 305000), *DOCK8* (twice; MIM: 243700), *FANCA* (MIM: 227650), *IKZF1* (MIM: 603023), *IL7R* (MIM: 608971), *MAGT1* (MIM: 300853), *MYB* (MIM: 189990), *NCF1* (MIM: 233700), *PGM3* (MIM: 615816), *SMARCAL1* (MIM: 242900), and *TERC* (Telomerase RNA component; MIM: 127550; Table III). Homozygosity and hemizygosity for CNVs, as well as compound heterozygosity for a CNV and an SNV, were observed in 12 cases (Table III), 3 of which have been published previously.[43-45] Disease-causing CNVs ranged in size from 1.6 Kb to 3.4 Mb, averaging 0.45 Mb, with a median of 19.7 Kb. Seven (58%) CNVs were less than 30 Kb in size and would not have been detected by using standard diagnostic microarrays (Table III). The WES data were screened for CNVs by using computational prediction.[43]

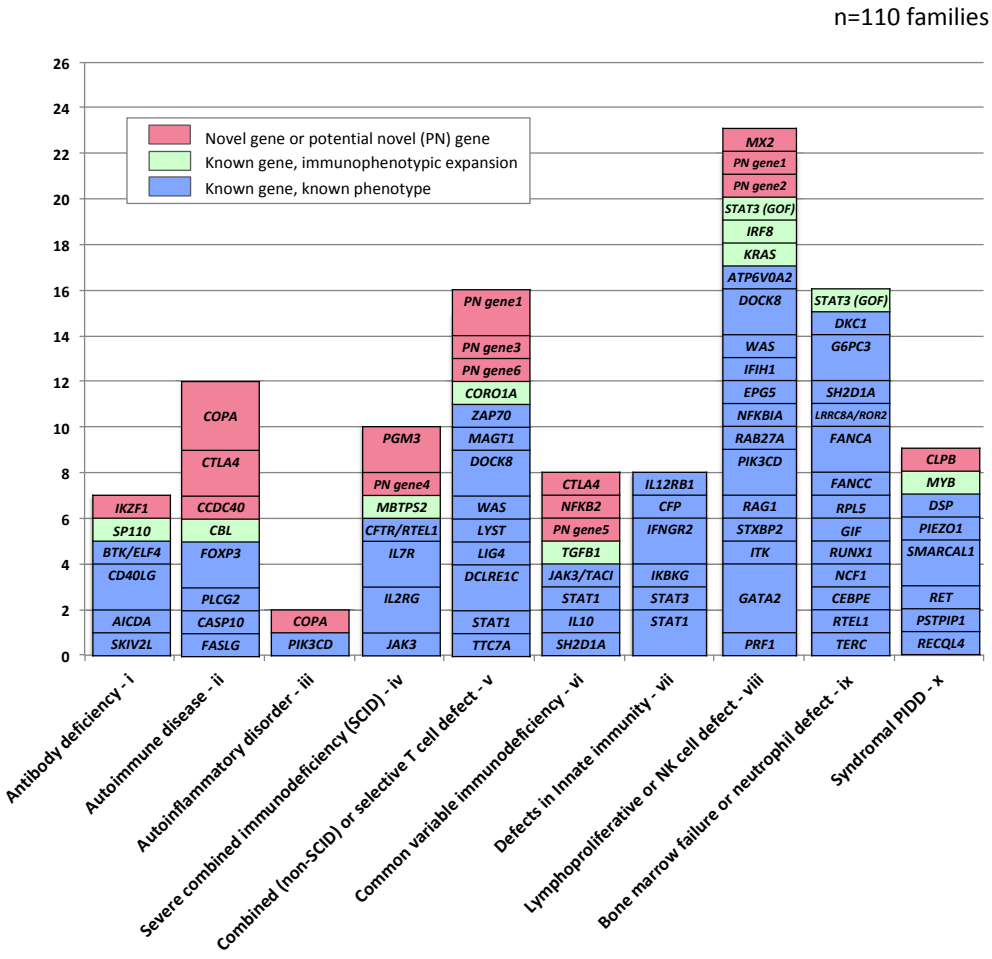

FIG 3. Distribution of the main likely disease genes in 111 families with established molecular diagnosis after WES plus CNV testing by PIDD subgroup before testing.

CNVs predicted as potentially disease associated were confirmed either by using an exon-tiling CNV microarray, MLPA, or PCR-based breakpoint analysis. Disease-causing CNVs in *PGM3*,[45] *FANCA*, *SMARCAL1*, *NCF1*, *TERC*, *MAGT1*, and *IKZF1*[46] were detected by using computational prediction (see Figs E1-E3 in this article's Online Repository at www.jacionline.org) and in *IL7R*, *MYB*, and *DKC1* by using exon-wise CMA (see Fig E1). The 2 homozygous *DOCK8* deletions were first noted on a single nucleotide polymorphism array used for WES quality control and subsequently visualized by using HMZDelFinder (see Fig E4 in this article's Online Repository at www.jacionline.org). The CNVs in *NCF1*[47] and *DKC1* were confirmed by using MLPA.

The *DKC1* duplication was detected by using CMA in samples from the family reported in 1970 by Hoyeraal et al,[48] the first report of dyskeratosis congenita, which was subsequently designated Hoyeraal-Hreidarsson syndrome (MIM: 305000). Our bioinformatic CNV tools were unable to detect duplication of this X-linked gene, even in retrospect. PCR located the duplication within the gene. Although it is unclear how the duplication affects gene function, the detected CNV is the likely cause of the disease in the family because *DKC1* is the only X-linked gene known to cause the specific phenotype. All 3 female carriers had a 100% skewed X-chromosome inactivation pattern. cDNA sequencing of 1 carrier failed to identify an aberrant *DKC1* product, which is in keeping with inactivation of the mutant allele.

Mendelian patterns in patients with PIDDs

Among the hereditability of variants observed (n = 126), autosomal recessive (AR) inheritance was most frequent (n = 57 [45%]), followed by autosomal dominant (AD; n = 49 [38%], excluding 1 case of somatic mosaicism) and X-linked inheritance (n = 20 [16%], Fig 4 and see Table E1).[1] Where parental samples were available, the likely disease-causing variants with dominant inheritance were *de novo* in 14 probands (see Table E1). This corresponds to a *de novo* mutation rate for AD mutations of at least 29%. Reduced penetrance or parental mosaicism was present in 9 families. Two variants in X-linked genes occurred *de novo* in affected male subjects (see Table E1). In 4 families obligate carriers were sequenced because affected probands were deceased or had undergone HSCT (families 90, 93, 95, and 111; see Table E1). A molecular diagnosis was established in 3 of these families, with variants in *DKC1*, *FANCC* (MIM: 227645), or *IL2RG* (MIM: 300400). In a single family 2 subjects suspected to have identical disease in fact had different PIDDs: one caused by a homozygous SNV in *RAG1* (subject 81.5 [MIM: 601457]) and the other by a homozygous CNV in *DOCK8* (proband 81.1). In another family 2 affected cousins with neutropenia and a congenital heart defect were suspected

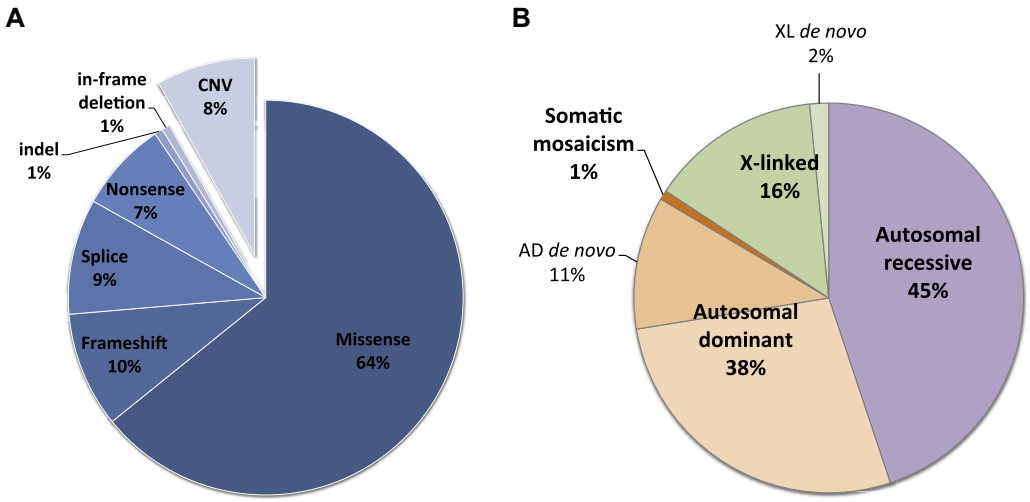

FIG 4. Disease-associated variants and inheritance patterns observed among the 110 families in the PIDD cohort in which a likely molecular diagnosis was established. **A,** Spectrum of disease-associated variants detected in the PIDD cohort. In total, 148 different variants were identified among the 110 families in which a likely molecular diagnosis was established. The SNVs are classified based on assumed effect on protein. The indel led to a frameshift and stop codon, and the in-frame deletion was previously reported to be disease causing through altered protein function. With the exception of the *DKC1* duplication, all 12 CNVs are deletions. All CNVs are assumed to result in loss of function. **B,** Inheritance patterns observed among the 110 families in the PIDD cohort in which a likely molecular diagnosis was established. The mosaic variant in *KRAS* is only compatible with life in the somatic mosaic state, and thus a Mendelian inheritance pattern is not applicable.

TABLE III. Disease-causing CNVs detected in the PIDD cohort

Sex	Age (y)	Diagnosis before WES (PIDD subgroup)	PIDD gene involved	Inheritance pattern for disease	Mutation type	Size of CNV	State	Affected family members
Male	1	Hoyeraal-Hreidarsson syndrome, X-linked (IX)	*DKC1*	XL	Duplication	14 kb	Hemi	3
Male	NA	Combined immunodeficiency (V)	*DOCK8*	AR	Deletion	355 kb	Hom	2
Female	5	HLH and NK cell defect (VIII)	*DOCK8*	AR	Deletion	84 kb	Hom	1
Female	34	Fanconi anemia, mild (IX)	*FANCA*	AR	Deletion	22-24.6 kb	Het	2
Male	30	Agammaglobulinemia (I)	*IKZF1*	AD	Deletion	15 kb	Het	1
Female	2	SCID, later debut (IV)	*IL7R*	AR	Deletion	224 kb	Het	1
Male	16	Immunodeficiency, X-linked, extensive warts (V)	*MAGT1*	XL	Deletion	16 kb	Hemi	2
Female	8	Immunodeficiency, progressive bone marrow failure; short stature, dysmorphic facial features (X)	*MYB*	AD	Deletion	3.4 Mb	Het	1
Female	71	Chronic granulomatous disease (IX)	*NCF1*	AR	Deletion	15 kb	Hom	1
Male	6	T- and B-cell deficiency and neutropenia (IV)	*PGM3*	AR	Deletion	1.24 Mb	Het	3
Female	4	Immuno-osseous dysplasia (X)	*SMARCAL1*	AR	Deletion	1.6 kb	Hom	1
Male	49	Dyskeratosis congenita, progressive bone marrow failure, short telomere lengths (IX)	*TERC*	AD	Deletion	3 kb	Het	1

Hemi, Hemizygous; *Het*, heterozygous; *HLH*, hemophagocytic lymphohistiocytosis; *Hom*, homozygous; *NA*, not available; *NK*, natural killer.

to have identical mutations but in fact did not (family 91, see Table E1 and Fig E5 in this article's Online Repository at www.jacionline.org).

A potential mutational burden

More than 1 gene can contribute to the PIDD phenotype in a given family. The presence of a dual molecular diagnosis is considered likely when diagnostic criteria for both genes can be fulfilled by the single patient. In our cohort this was observed in 12 (11%) families with a likely molecular diagnosis. For example, in one family 3 deceased male subjects (proband 93.1, see Fig E5 and Table E1) had immunodeficiency, leukemia, lymphoma, anemia, and solid tumors. The affected subjects shared a *SH2D1A* variant c.80G>A, p.Gly27Asp (NM_002351) causing X-linked lymphoproliferative syndrome (MIM: 308240). An additional variant (c.989T>C, p.Ile330Thr; NM_001018113) in *FANCB* (MIM: 300515), the gene for X-linked Fanconi anemia that maps 2.5 Mb from *SH2D1A*, cosegregated and might have contributed to the complex phenotype (see Fig E5). As another example, in another family an adult female subject (proband 27.1, see Table E1) presented with combined immunodeficiency, low absolute lymphocyte counts, very low levels of $CD8^+$ T cells, a poor PHA mitogen proliferative response, disseminated human papillomavirus infection, recurrent respiratory tract infections, vaccine-induced varicella, and cryptococcal meningitis. WES

identified compound heterozygous disease-causing variants in *ZAP70* (p.Gly245Arg in the SH2 domain and p.Pro502Leu in the kinase domain). Hypomorphic variants in *ZAP70* have been reported in subjects with a later-onset immunodeficiency[49] and might explain this proband's clinical presentation, low $CD8^+$ T-cell counts, and combined immunodeficiency. In addition, she harbored a homozygous missense variant in *RNF168*, the gene associated with another PIDD, Radiosensitivity-immunodeficiency-dysmorphic features-learning difficulties (RIDDLE) syndrome (MIM: 611943). Radiation sensitivity analyses performed by using colony survival assay[50] of the proband's EBV-transformed lymphoblasts demonstrated a reduced survival fraction (8%) and exhibited increased radiosensitivity, a hallmark of RIDDLE syndrome and, to our knowledge, not observed in ZAP70-deficient subjects. Of interest, the patient also had human papillomavirus–associated extensive scalp squamous cell cancer. All variants cosegregated with the disease in the family (see Fig E5). Impaired function of both proteins might underlie the blended phenotype.[21,22] In 12 of the 110 families in which the proband's disorder was attributed to deleterious variants in a disease gene, variants in additional genes were considered to have a potentially modifying effect (Fig 1 and see Fig E5 and Table E1, marked with footnote "fp").

Clan genomics and founder mutations

In families with known consanguinity or from areas where consanguineous marriages are common, disease-causing homozygous variants were observed as expected (see Table E1: 5.1, 30.1, 31.1, 40.1, 64.1, 81.1 and 81.5, 85.1, 91.4, 98.1, 101.1, 108.1, 110.1, 114.1, 122.1, 123.1, and 124.1). As an unexpected finding, we were also able to use WES to detect genomic regions with the region of absence of heterozygosity in subjects from presumed outbred populations, potentially reflecting distant parental kinship (Table E1: 33.1, 33.4, 39.1, 56.1, 87.1, 100.1, and 105.1), consistent with the clan genomics hypothesis.[51]

Variable expressivity

Reduced penetrance or variable expressivity was observed in families for which probands (see Table E1: 10.1, 11.1, 14.1, 15.1, 20.1, 47.1, and 79.1) had a likely AD disease–causing variant in *CTLA4* (MIM: 123890), *PIK3CD* (MIM: 602839), *NFKB2* (MIM: 164012), *FASLG* (MIM: 134638), and *COPA* (MIM: 601924). Some heterozygous mutation carriers in these families appeared clinically unaffected.[52] For example, proband 20.1 presented with neutropenia, enteropathy, and joint disease and harbored a missense mutation in *FASLG*, causing autoimmune lymphoproliferative syndrome (ALPS). Sanger sequencing confirmed that the variant was inherited from the father, who had only minor symptoms and laboratory signs of disease, suggesting reduced expressivity or lack of environmental triggering. Splicing variants were also identified that might be incomplete and cause milder phenotypes (see Fig E6 in this article's Online Repository at www.jacionline.org). Milder disease could also be due to low-grade mosaicism, such as in the *PIK3CD* family (proband 67.1), with the ratios of variant to total reads being 37%, 54%, and 42% in the 3 affected siblings but only 15% in their mildly affected father (see Table E1: 67.1).

Mendelian exceptions: Somatic and revertant mosaicism

In 3 families affected subjects displayed a milder phenotype and had findings suggesting somatic revertant mosaicism. Their less severe phenotypes could be related to a reduced number of cells harboring mutant alleles (see Fig E7 in this article's Online Repository at www.jacionline.org). In the first family a 6-month-old boy (proband 59.1) presented with eczema, diarrhea, BCG vaccination–associated infection, recurrent severe staphylococcal infections, and eosinophilia but normal T-cell, B-cell, and natural killer cell numbers. A previously reported X-linked disease-causing *IKBKG* mutation (c.1167dupC, p.Glu390Argfs*5 [NM_001099857]) was identified; his unaffected mother was a heterozygous carrier (69/284 reads by using WES). Interestingly, the *IKBKG* mutant allele was observed in only 35% (55/159) of reads in the boy's DNA extracted from blood (see Table E1). This finding argues for revertant mosaicism, which could explain his relatively mild phenotype compared with classic *IKBKG*-associated immunodeficiency (MIM: 300291) in male subjects. Revertant mosaicism and milder phenotypes were also detected in 2 other families with disease-causing variants in *FANCA* (see Fig E7) and *IL7R*.[44]

Female subject 65.1 (see Table E1) serves as an example of somatic mosaicism with severe clinical consequences. She presented with recurrent infections in early childhood and subsequently had lymphadenopathy, hepatosplenomegaly, and ALPS. She died at 4 years of age with a clinical phenotype reminiscent of hemophagocytic lymphohistiocytosis. WES identified a *de novo* variant in exon 2 of *KRAS* in 15 of 110 reads (13.6%; c.37G>T, p.Gly13Cys [NM_004985]). Mosaicism in blood was confirmed by means of Sanger sequencing (see Fig E7). The child had no dysmorphic features and a normal head circumference/stature. The variant was likely confined to her hematopoietic cells. Mosaicism for the identical variant was previously reported in a child with ALPS-like disease.[53] The phenotype is now designated RAS-associated autoimmune leukoproliferative disorder (MIM: 614470).

Unexpected genomic and correlated clinical findings

We included affected subjects from all subgroups of PIDDs through an unbiased genomic approach. In addition to using the PIDD(475) gene list, we further examined other disease-related Online Mendelian Inheritance in Man (OMIM) genes and all detected variants in other genes in an effort to identify new PIDD genes. For 90 of 278 probands, variants in disease-causing genes with known, new, or expanded phenotypes were observed (see Table E1).[30,54,55] Expansion of the immunophenotypic spectrum was associated with 9 OMIM genes: *SP110* (MIM: 235550), *CBL* (MIM: 613563), *MBTPS2* (MIM: 308205), *CORO1A* (MIM: 615401), *STAT3* (MIM: 147060, 615952), *IRF8* (MIM: 614894), *KRAS*, *MYB*, and *TGFB1* (MIM: 131300; Fig 3). Unexpected rare congenital syndromes were also detected in our cohort. In 6 families variants were detected in OMIM genes that have not previously been reported in association with immunodeficiency or bone marrow failure. These variants might or might not explain the immunologic defect or perhaps only parts of the affected subject's phenotype. For example, this could be the case in proband 113.1, with pathologic *CFTR* variants and recurrent pulmonary infections. Some cases might represent

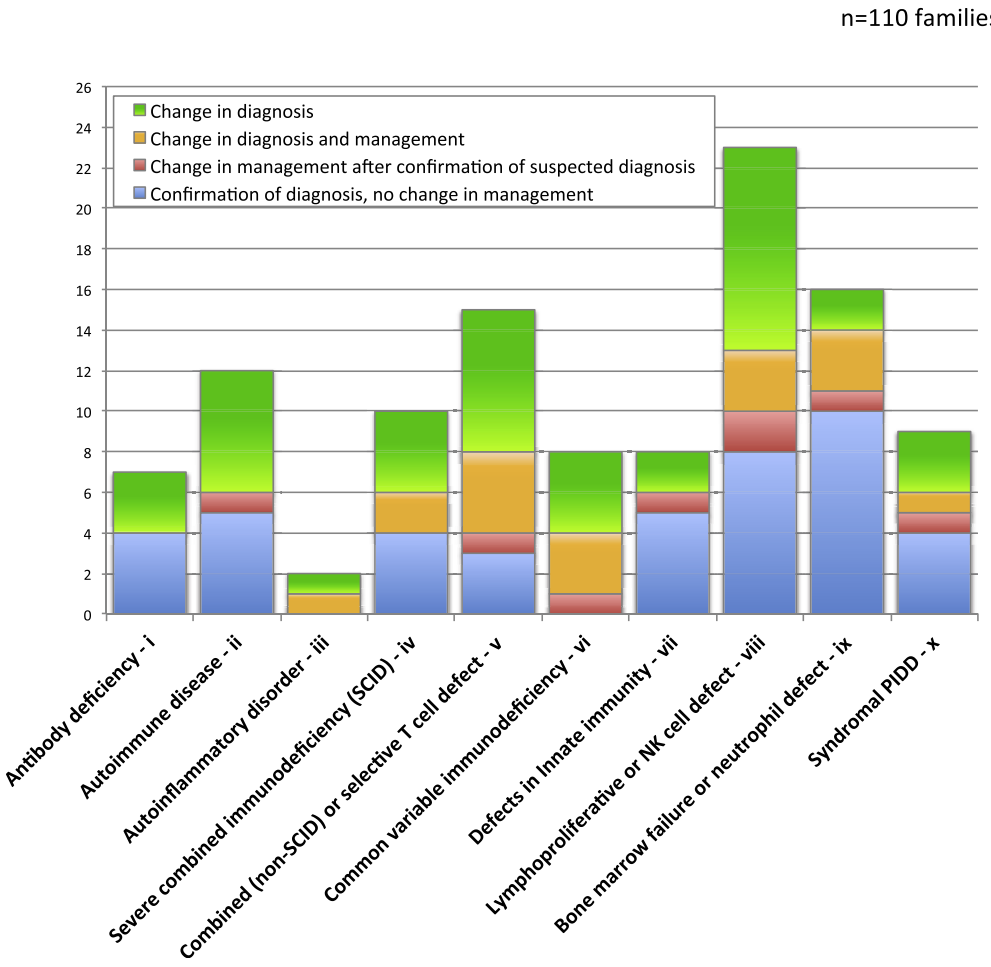

FIG 5. Alteration of specific diagnosis and change in management caused by molecular findings by using WES plus CNV testing. The distribution is shown by PIDD subgroup, which was based on clinical presentation at the time of inclusion.

multidisease burden, and others might represent true phenotypic expansions. Biological studies of relevant gene products in the immune system are in progress in such families. The insights derived from immunophenotypic expansion can be substantial and might relate to the specifics of individual mutations or broadened clinical presentations.

For example, proband 115.1 (see Table E1) presented with a SCID-like immunophenotype shortly after birth, as well as microcephaly, severe scaling erythrodermatitis, conjunctivitis, keratitis, and oligoclonal T cells, all features of Omenn syndrome, but with a normal IgE level and eosinophil count. He was ultimately found to have ichthyosis follicularis, alopecia, and photophobia (IFAP) syndrome caused by alteration of *MBTPS2*, which has been previously associated with immunodeficiency, although not of this severity (see Fig E8 in this article's Online Repository at www.jacionline.org).[56,57] Other unexpected variants, which explained all or parts of the probands' phenotypes, were identified in *RET* (MIM: 142623), *PLXNA1* (MIM: 601055), *ATP6V0A2* (MIM: 611716), *FBN1* (MIM: 154700), *MX2* (MIM: 147890), *CFTR* (MIM: 219700), *CCDC40* (MIM: 613808), and *CLPB* (MIM: 16271). None of these variants would have been identified if the samples were analyzed with a gene panel restricted to targeted capture of known PIDD genes only.

Change in diagnosis and management after WES

In 55% (60/110) of the families in whom a likely molecular diagnosis was identified by means of WES, the prior PIDD diagnosis based on clinical features and immunophenotyping was altered (Fig 5). Post-WES revision of diagnosis was observed across all PIDD subgroups. A likely etiological diagnosis was established in approximately half (range, 18% to 100%) of the affected subjects in each PIDD subgroup before WES. In 25% of cases (26 families and 32 affected subjects), WES findings directed a significant change in management (Fig 5). Ten subjects underwent HSCT, which was primarily guided by WES findings. In 4 additional subjects WES results supported a previous decision to treat with HSCT. Six probands had already undergone HSCT. For one of them, proband 115.1 with a molecular diagnosis of *MBTPS2*-IFAP syndrome, HSCT might not have been performed if the molecular diagnosis had been known.

Recent and new disease genes

Twenty-five percent of molecular findings were in genes reported as disease causing during the last 5 years: 5 in 2011, 4 in 2012, 7 in 2013, 3 in 2014, and 3 in 2015 (see Table E1). Eight were new disease genes (*CCDC40*,[58] *CLPB*,[59,60] *COPA*,[52]

CTLA4,[61,62] *IKZF1*,[46] *MX2*,[63,64] *NFKB2*,[65-67] and *PGM3*,[45,68,69]) at the time of detection and were either confirmed biologically by us or others. Six likely candidate PIDD genes were also identified in this study (listed as potentially novel, Fig 3). These are rare, damaging, conserved variants that fit biologically with the immunophenotype, but full biological confirmation to comply with proposed criteria[42] remains incomplete. This group of patients is included so as not to underestimate the potential effect of WES as applied to PIDDs. One example of a biologically confirmed new disease gene identified by means of WES comes from 2 families in our cohort with AR variants in *PGM3*. Functional studies detected reduced specific phosphoglucomutase enzymatic activity.[45] Additional PIDD-affected subjects with *PGM3* mutations were reported simultaneously,[68,69] and more than 10 families have been published since.[19,45,68-70] New and potentially novel PIDD genes were found in 21 families in our cohort (19%), and biological investigations to confirm these associations according to published standards[42] have been performed or are in progress.

In general, and as would be expected, every exome tested contained several rare exonic variants (minor allele frequency [MAF] <0.001 in 1000 Genomes data, in-house databases, Exome Sequencing Project, and ExAC) in PIDD genes and HGMD-reported variants with an MAF of less than 0.05. With the 148 likely causative variants excluded, the majority of the other rare variants were definitively not disease causing, but some represented single variants in AR trait genes in which disease genes were compatible with the affected subject's phenotype. These are included in Fig 1 among the 20% of families with findings in a phenotypically relevant gene, but findings are considered inconclusive, and molecular diagnoses are classified as unsolved.

DISCUSSION

Using genomic approaches, we identified the potential molecular basis for disease in 40% of 278 probands with various PIDD phenotypes of unknown etiology who had previously been investigated with conventional methods (although with some variation across the 24 different hospital centers contributing patients to this study). We found evidence for a mutational burden effect,[71] which might contribute to disease variability and underlie blended immunophenotypes, as well as somatic and revertant mosaicism. With no preset bioinformatic cutoff level for variant reads, we were able to detect mosaicism of less than 15% (*KRAS*, see Fig E7). Several disorders with significant nonimmunologic features, such as primarily skin or intestinal diseases, were diagnosed. Some cases might represent phenotypic expansion of a known disorder to include immunodeficiency. For AD traits, we noted reduced penetrance associated with variant alleles. For AR traits, we identified founder variants in outbred populations. Our approach resulted in expansion of the phenotypic spectrum associated with 9 previously known disease genes (Fig 3) and identification of 8 new PIDD genes previously reported by us (*PGM3*,[45] *COPA*,[52] and *IKZF1*[46]) or others (*CTLA4*,[61,62] *NFKB2*,[65-67] and *CLPB*[59,60]), new non-PIDD genes (*CCDC40*[58] and *MX2*[63,64]) reported by others and detected in our cohort as well and 6 potentially novel candidate genes. All of these new disease genes and potential novel candidate genes affected 21 probands (Fig 2 and see Table E1). We found PIDD-relevant variants in 88 different genes, 25% of which were first reported during the last 5 years (see Table E1). Twelve probands had presumably phenotypically relevant sequence alterations in more than 1 gene, which is compatible with a blended phenotype in 11%. Because this has not previously been reported for major PIDD cohort studies, variations in PIDD phenotypes might need to be revisited. Importantly, molecular findings resulted in a revised diagnosis in 60 families and altered management in 32 cases (Fig 5).

In contrast to a previous report,[22] we found an abundance of disease-causing CNVs. Disease-causing CNVs were detected in 11% of the families (Table III), half of which would not have been identified by using conventional chromosomal arrays. A broad spectrum of likely disease-related SNV types was observed, but the paucity of indels in our cohort deviates from the data reported for selected PIDD genes in HGMD. This finding might reflect characteristics of some immunodeficiency-related genes or perhaps challenges in algorithm detection of indels from WES data. However, the distribution of SNV types we report is similar to the results demonstrated by using WES in other heterogeneous Mendelian cohorts.[22]

X-linked disorders contribute to the male excess in population-based PIDD studies.[2] In our cohort with 58% molecularly undiagnosed male subjects, many subjects had already undergone testing for X-linked PIDD genes, such as *BTK*, *WAS*, *IL2RG*, *IPEX*, *DKC1*, *TAZ*, and *CD40L*. However, 16% of the potential causal variants in our cohort were located in these and other X-linked genes, including variants in *BTK* (c.141+11C>T; MIM: 300755) and *IL2RG* (c.924+5G>A) not detected by diagnostic Sanger sequencing. The same was true for the variant in *JAK3* (c.1695C>A; MIM: 600802) undetected by using Sanger sequencing prior to WES. The indels in *CTLA4* and *CD40LG* (MIM: 308230) were inadequately annotated on the initial WES data but visualized in Integrative Genomics Viewer and correctly classified by using Sanger sequencing. This emphasizes the ongoing value and importance of validation. The mosaic variants in *FANCA*, *IL7R*, and *KRAS* had not been detected without WES but were visualized by means of Sanger sequencing as small peaks on electropherograms, which could have been misinterpreted easily as an artifact.

Of the 364 genes in our PIDD(475) list with known inheritance patterns, 23 (6%) are associated with X-linked, 76 (21%) with AD, 247 (68%) with AR, and 18 (5%) with AD or AR inheritance. The distribution of inheritance patterns we observed mirrors the inheritance patterns documented for known PIDD genes, with slight skewing toward variants in AR genes and an increased trend toward variant discovery in AD genes. In our families with documented AD or AR inheritance, we identified disease-related variants in 38% and 45% of families, respectively. In cohorts with molecularly confirmed Mendelian disorders, including neurological and neurodevelopmental disorders associated with reduced reproductive fitness, the trend is reversed, with a 3:2 ratio of AD and AR disorders and more than 70% *de novo* variants in the AD genes.[22,72,73] In contrast, fewer than 30% of variants were proved to be *de novo* among the AD PIDD genes identified in this study. Even after including potential *de novo* variants in AD PIDD genes for whom parental samples were missing (*GATA2* in proband 88.1, *IKZF1* in 1.1, *RPL5* [MIM: 612561] in 99.1, *TERC* in 63.1, *STAT3* in 66.1, and *STAT1* [MIM: 614162] in 50.1, 60.1, and 37.1; see Table E1), the percentage (45%) was substantially lower than seen in patients with neurodevelopmental disorders.

Immunologically important genes are numerous and spread throughout the genome. Caution is required in attributing causality to variants identified by using WES because even rare inherited or *de novo* variants might not cause disease. Widespread use of WES in diagnostics and research reveals that even variants listed as deleterious in HGMD can be misclassified.[74] A case in point is the intronic *BTK* variant c.141+11C>T (NM_000061.2; see Table E1) detected in subjects without antibody deficiencies and reported frequently (160 hemizygotes) in healthy male subjects in the ExAC database. Another example is the affected male subject in our cohort with an X-linked, maternally inherited missense variant in *WAS* (exon 10 c.995T>C, NM_000377 [MIM: 301000]). This p.Val334Ala variant has previously been reported to be disease related (CM072118; HGMD) but has an MAF of 0.005. With 115 hemizygotes reported in ExAC, it seems unlikely that such a large number of affected male subjects with classic Wiskott-Aldrich syndrome or X-linked thrombocytopenia are included among the subjects in the ExAC database. Nonetheless, although the data argue that the variant might not be a primary cause of disease, we cannot exclude the possibility that the variant could have a milder or disease-modifying pathogenic effect. We did not identify any other disease-causing variants in this subject, and he is not included in the 110 families with a likely established molecular diagnosis.

Every exome contained several rare exonic variants in PIDD genes and HGMD-reported variants that were definitively not disease causing or only 1 variant in an AR trait gene that could theoretically contribute to the observed clinical phenotype. This general observation underscores the importance of evaluating a genetic variant in relationship to the clinical phenotype, especially when limited biological or functional evidence for causality exists.[75] Therefore optimal use of WES data in PIDD requires a dynamic iterative collaboration between clinicians, laboratory immunologists, molecular geneticists, and bioinformaticians. The standards for biological consideration of novel single-gene discoveries, as exemplified by PIDD, have been proposed and represent an important threshold to clear when evaluating candidate genes.[42] We have elected not to include the names of "potentially novel," otherwise unreported PIDD candidate genes in the present work in deference to these published criteria and their current status as not yet biologically confirmed. Investigations, collaborative evaluations, or both of these candidates are currently in progress to provide the necessary mutational impact testing and biological validation.

A few factors were considered that could potentially affect discovery rates. The ages of the probands among the families who received a likely or potential molecular diagnosis from our study were similar to the ages of those who did not. Thus clear Mendelian traits were observed across all age groups and not confined to the youngest children with PIDD (see Table E1). In Houston the overall discovery rate was 37.5% (75/200). Among the 200 families, 204 additional family members underwent WES, which might have improved the discovery rate (Table II). Mainly singleton cases underwent WES in Oslo; however, the overall discovery rate was still 44.9% (35/78). In general, across all subgroups, WES testing of more than 1 affected subject in the same family increased the diagnostic rate, as demonstrated in Table II and Table E1, and a larger proportion of potentially novel genes was discovered among those families. In 21 families more than 1 affected family member underwent WES as part of our study. Among all 110 families that received a likely or potential molecular diagnosis, 12 had more than 1 affected family member WES tested, and 5 of these had disease-related variants in novel PIDD genes. Formal WES trios (proband plus both parents) might have increased the discovery rate but not to the extent observed in other cohorts.[76-78] Because the numbers are small (Table II), however, definitive conclusions cannot be made. Finally, the fact that all patients were evaluated according to diagnostic criteria and conventional methods before inclusion, many of the more straightforward molecular diagnoses had been already made, and those patients did not contribute to this study. Thus if all presenting patients with PIDD were included, discovery rates in a PIDD cohort would have likely been higher.

PIDDs with severe and opportunistic infections represent distinct phenotypes with strong genetic components and simple Mendelian inheritance patterns, even in PIDD subgroups historically considered complex polygenic disorders, such as CVID.[79,80] Conversely, because of heterogeneity, known occurrence of genetic modifiers and susceptibility factors (eg, MBL deficiency [*MBL2*, MIM:614372]), reduced penetrance and variable expressivity between mutation carriers even within families, and strong influence by environmental factors (bacteria, viruses, fungi, nutrition status, and age), delineation of genetic causality in each subject can be challenging. Our 40% gene variant identification rate is high compared with that of other WES studies of various non-PIDD disease cohorts.[21,22] However, the rate is lower than for WES trios in cohorts with severe intellectual disability[76,77] and brain abnormalities.[81] Those conditions are characterized by highly penetrant gene variants, with skewing toward *de novo* deleterious variants in AD genes and perhaps less influenced by environmental factors, such as infection-triggered immune dysregulation. Further biological verification of causality will be needed for the variants classified as variants of unknown significance, and despite our effort taking the genome-wide approach, additional disease-causing variants can be discovered even among the 40% with an assumed molecular diagnosis.

For the 60% of families in this cohort who still lack a molecular diagnosis (Fig 1), further genomic studies are indicated in an effort to identify variants in noncaptured regions, including regulatory regions, low-grade mosaicism, small CNVs, and indels. Some of these families might also have polygenic PIDDs, which remains an active area of investigation. A stepwise approach for PIDD diagnostics with targeted next-generation sequencing,[20] followed as needed by WES, whole-genome sequencing (WGS), or both, has been previously proposed.[19] Our findings support such an approach, although use of WES, WGS, or both might ultimately become preferred to targeted multigene panel testing as technologies advance to provide results in a comparable timeframe. The need for rapid analysis is emphasized by the fact that acquisition of the proper diagnosis directly altered management of 25% of probands in our cohort. Targeted gene panel testing currently has advantages in terms of high coverage of genes of interest (including deep intronic regions), ease of data interpretation, and rapid turnaround time. Targeted capture of a limited number of PIDD genes can often deliver molecular diagnostic results within weeks or days at a lower cost, whereas gene hunting with inadequate coverage of regions of interest can highlight pitfalls associated with currently available WES and WGS.[18-20,82-85] WES, however, allows for

detection of variants in potentially novel gene candidates. It can uncover mutations in other genes, such as *CFTR*, that mimic PIDD. Our PIDD(475) gene list was an efficient means for detecting causative variants in known genes in 77 probands. We have also demonstrated the utility of WES combined with tools for intragenic CNV detection to identify small CNVs. It remains unknown whether targeted gene panels will prove more cost-effective than WES because various laboratory-based, insurance-related, and nationally implemented policies greatly influence cost analyses. WGS is not capture based, unlike targeted gene panel testing and WES, allowing for direct discovery of structural abnormalities, such as CNVs and inversions, and SNVs within nonexonic genomic regions, although currently at a substantially higher cost.[86]

Based on our data and a review of the literature, we suggest a 2-tiered approach. First is initial testing with a limited capture kit targeting (1) common PIDD-causing genes, (2) actionable gene variants, and (3) genes causing severe phenotypes and requiring a rapid molecular diagnosis, such as disorders characterized by low T-cell receptor excision circles on newborn screening for SCID[87] and lymphoproliferative disorders.[88] Optimal probe design of a targeted capture kit to ensure sufficient coverage of the targeted genes might enable replacement of Sanger sequencing by means of rapid testing on a bench-top next-generation sequencing platform.[20] Given substantial read depth of coverage, targeted gene test data might allow for detection of intragenic CNVs and low-grade mosaicism.

Second, if no PIDD-causing variants are identified by the above testing, we suggest proceeding to a genome-wide approach using either WES or WGS with a large, frequently updated PIDD gene candidate list, such as PIDD(475), that includes other extended immunologically PIDD-relevant genes as a bioinformatic filter in the initial variant screening.[41] Genome-wide data can be used to identify the absence of heterozygosity regions that can help guide the search for disease-causing variants in consanguineous families and in subjects from outbred but homogeneous populations.

Note in proof: Since the acceptance of this work, the gene PN1 (Fig 3) has been confirmed as *CARMIL2*, which is in press in *Molecular Genetics & Genomic Medicine* as Sorte HS, Osnes LTN, Fevang B, Aukrust P, Erichsen HC, Backe PH, et al: A potential founder variant in *CARMIL2/RLTPR* in three Norwegian families with warts, molluscum contagiosum, and T cell dysfunction.

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The URLs for data presented herein are as follows: 1000 Genomes Browser, http://browser.1000genomes.org/index.html; Arterosclerosis Risk In Communities (ARIC), http://www2.cscc.unc.edu/aric/; BHCMG, https://mendeliangenomics.org; Centers for Mendelian Genomics, http://www.mendelian.org; Baylor Miraca Genetics Laboratories, formerly known as the Medical Genetics Laboratories, BC, http://www.bcm.edu/geneticlabs/; dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/; Human Gene Mutation Database (HGMD), http://www.hgmd.cf.ac.uk/; HUGO Gene Nomenclature Committee (HGNC), http://www.genenames.org; Integrative Genomics Viewer, http://www.broadinstitute.org/igv/; NHLBI Exome Sequencing Project, http://evs.gs.washington.edu/EVS/; Online Mendelian Inheritance in Man (OMIM), http://www.omim.org; PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/; UCSC Genome Browser, http://genome.ucsc.edu/; clinical diagnosis criteria for the European Society for Immunodeficiencies Registry, http://esid.org/Working-Parties/Registry/Diagnosis-criteria.

Clinical implications: With a rate of likely molecular diagnosis of 40%, WES in tandem with CNV screening tools is effective for detecting a broad spectrum of disease-causing variants in patients with PIDDs.

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Update

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Corrigendum

In regards to the article in the January 2017 issue entitled "Primary immunodeficiency diseases: Genomic approaches delineate heterogeneous Mendelian disorders" (J Allergy Clin Immunol 2017;139:232-45), the authors have submitted a revised supplementary Table E1. In this revised table, the molecular details of patient 73.1 are being omitted. The authors state that this is being done because the group caring for this patient has undertaken an extensive molecular characterization of this patient and will be reporting their findings separately. In the interest of scientific integrity, the authors would like to avoid duplication of data. However, the research group agreed that the patient continues to be counted in the series (minus the molecular details) and thus the cumulative analyses and statistics in the paper are unchanged. Table E1 has been replaced online to reflect this change.