Expanded genetic testing for primary immunodeficiencies: Findings from a 207-gene next-generation sequencing panel

P. Nicolosi, PhD, J. Holle, MS, R. Truty, PhD, H. Yu, PhD, C. Hartshorne, MS, S. Martin, MD, PhD, B. Johnson, PhD

Invitae, San Francisco, CA

BACKGROUND

- Many primary immunodeficiencies (PIDs) share overlapping presentations, complicating the clinical diagnosis
- Expanded next-generation sequencing panels are valuable in facilitating the diagnosis of patients with PIDs due to their ability to test many genes at once
- We developed a 207-gene next-generation sequencing panel inclusive of copy number variation analysis for the clinical diagnostic testing of patients with PIDs (**Figure 1**).

Primary panel (207 genes)

ACD	ACP5	ACTB	ADA	ADA2	ADAM17	ADAR	AICDA
AIRE	AK2	AP3B1	ATM	B2M	BCL10	BLNK	BLOC1S6
BTK	CARD11	CARD14	CARD9	CASP10	CASP8	CD247	CD27
CD3D	CD3E	CD3G	CD40LG	CD79A	CD79B	CD8A	CEBPE
CHD7	CIITA	CLPB	COPA	CORO1A	CR2	CSF2RA	CSF3R
CTC1	CTLA4	CTPS1	CTSC	CXCR4	CYBA	CYBB	DCLRE1B
DCLRE1C	DKC1	DNMT3B	DOCK2	DOCK8	ELANE	EPG5	FADD
FAS	FASLG	FERMT3	FOXN1	FOXP3	FPR1	G6PC3	GATA2
GFI1	HAX1	ICOS	IFIH1	IFNGR1	IFNGR2	IGLL1	IKBKB
IL10	IL10RA	IL10RB	IL12B	IL12RB1	IL17F	IL17RA	IL17RC
IL1RN	IL21	IL21R	IL2RA	IL2RG	IL36RN	IL7R	IRAK4
IRF7	IRF8	ISG15	ITCH	ITGB2	ITK	JAGN1	JAK3
LAMTOR2	LCK	LIG4	LPIN2	LRBA	LYST	MAGT1	MALT1
MAP3K14	MEFV	MOGS	MVK	MYD88	NBN	NCF2	NCF4
NFAT5	NFKB2	NFKBIA	NHEJ1	NHP2	NLRC4	NLRP12	NLRP3
NOD2	NOP10	ORAI1	PARN	PGM3	PIK3CD	PIK3R1	PLCG2
PMS2	PNP	POLE	PRF1	PRKCD	PRKDC	PSMB8	PSTPIP1
PTPRC	RAB27A	RAC2	RAG1	RAG2	RBCK1	RFX5	RFXANK
RFXAP	RHOH	RMRP	RNASEH2A	RNASEH2B	RNASEH2C	RORC	RTEL1
SAMHD1	SEMA3E	SH2D1A	SH3BP2	SLC29A3	SLC35C1	SLC37A4	SLC7A7
SMARCAL1	SP110	SPINK5	STAT1	STAT2	STAT3	STAT5B	STIM1
STK4	STX11	STXBP2	TAP1	TAP2	TAPBP	TAZ	TBK1
TCN2	TERC	TERT	TICAM1	TINF2	TLR3	TMC6	TMC8
TMEM173	TNFRSF13B	TNFRSF13C	TNFRSF1A	TNFRSF4	TNFSF12	TPP2	TRAF3
TRAF3IP2	TREX1	TRNT1	TTC7A	TYK2	UNC13D	UNC93B1	UNG
VPS13B	VPS45	WAS	WIPF1	XIAP	ZAP70	ZBTB24	

Figure 1. Genes included on the 207-gene Invitae Primary Immunodeficiency Panel.

METHODS

- NGS testing was performed as previously described,[1] and variant interpretation was carried out based on an expansion of the ACMG guidelines.[2]
- De-identified results of patients tested between April, 2017 and August, 2018 were reviewed and categorized by variants identified and their classifications:
  - Negative: no reportable variants identified
  - Positive: Pathogenic/Likely Pathogenic variants (P/LP)
  - Uncertain: Variants of Unknown Significance (VUS)
  - Of note, increased risk alleles, such as common *NOD2* alleles associated with Crohn's disease, were excluded.
- Positive results were further categorized as follows:

Likely genetic diagnosis	Carrier status	Heterozygous results
1 heterozygous P/LP allele in AD or XL gene	1 heterozygous allele in AR gene	1 heterozygous allele in a gene with AR and AD inheritance
1 homozygous P/LP allele in AR gene	1 heterozygous allele in XL gene in female	
1 hemizygous P/LP allele in XL gene in male		
2 heterozygous P/LP alleles in AR genes		
1 heterozygous P/LP allele and 1 VUS in AR genes		

- Clinical actionability of results was estimated based on published recommendations for treatment with hematopoietic stem cell transplantation (HSCT).

RESULTS

- During the studied period, 1631 patients underwent genetic testing with the 207-gene PID panel.
- The average turnaround time from test requisition to return of results was 16 days.
- The vast majority (94%) of patients had variants identified in one or more genes tested **(Figure 2).**
- P/LP variants were identified in 23% of patients (n=368) **(Figure 2).**
- Multiple variants of uncertain significance were identified in most patients (**Figure 3**).

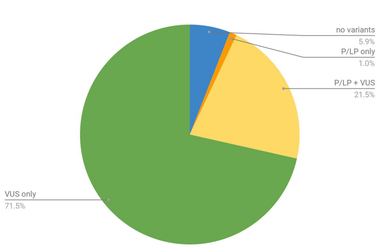

Figure 2. Total patients tested by result type.

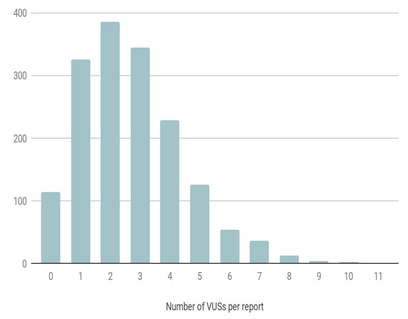

Figure 3. Number of variants of unknown significance per patient.

- CNVs made up 8% of P/LP variants identified (Figure 4).
- Of patients with pathogenic findings, 8% (31 patients) had pathogenic CNVs.
- The molecular diagnosis was dependent upon CNV analysis in 8 cases (**Table 1).**

Gene	Variant	Zygosity
ATM	Deletion (Exons 17-63) c.8486C>T (p.Pro2829Leu)	Heterozygous Heterozygous
CD40LG	Deletion (Exons 2-3)	Hemizygous
IRAK4	Deletion (Exons 10-12)	Homozygous
LRBA	Partial Deletion (Exon 14) c.787C>G (p.Leu263Val)	Heterozygous Heterozygous
SH2D1A	Deletion (Entire sequence)	Hemizygous
SH2D1A	Deletion (Entire sequence)	Hemizygous
XIAP	Deletion (Exons 4-5)	Hemizygous
XIAP	Deletion (Exons 2-5)	Hemizygous

Table 1. Eight cases in which CNV analysis facilitated the molecular diagnosis.

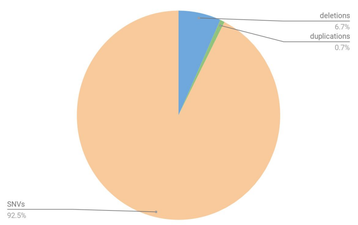

Figure 4. Pathogenic/Likely Pathogenic variants by variant type.

RESULTS

- 368 patients had positive findings (**Figure 5):**
  - 153 (9%) were heterozygous carriers of AR conditions
  - 135 (8%) were heterozygous for variants in genes with AR and AD inheritance patterns, in which the positive finding may or may not explain the patient's phenotype
  - 109 (7%) had likely genetic diagnoses
  - 28 patients had results in more than one category.

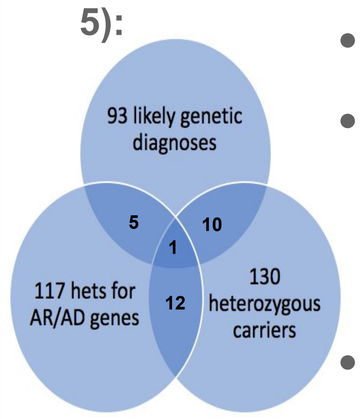

Figure 5. Clinical context of P/LP variants

- 74 patients (68% of patients with genetic diagnoses) were diagnosed with conditions for which there are published recommendations for consideration of HSCT (**Table 2**). Selected case studies are shown in **Table 3.**

BTK	CARD11	CD3D	CD40LG	CIITA	CTC1
CTPS1	CYBB	DCLRE1C	DKC1	DOCK8	ELANE
FOXP3	GATA2	IFNGR1	IL2RG	IRAK4	JAK3
LRBA	LYST	MVK	NFKB2	NHEJ1	PIK3CD
PNP	PRF1	PTPRC	RAG1	RFXANK	RMRP
SH2D1A	STK4	TACI	TTC7A	UNC13D	XIAP

Table 2. Published recommendations for consideration of HSCT were identified for 36 genes.

Genetic result	Clinical Indication for testing	Associated condition	HSCT considered	Source (PubMed)
CD40LG: p.Thr254Lys (hemi) RNASEH2B: p.Ala177Thr (het)	Male with suspected hyper IgM Syndrome. Myelodysplastic syndrome. Prolonged febrile neutropenia.	X-linked hyper-IgM syndrome Aicardi Goutieres syndrome 2 (carrier)	Yes N/A	PMID:16435016
DCLRE1C: p.Asp451Lysfs*11 (homo)	Hypogammaglobulinemia, common variable immunodeficiency, recurrent chest infection, ITP neutropenia	Severe combined immune deficiency	Yes	PMID:11806989
IFNGR1: c.373+2T>C (homo)	Lymphadenopathy	Mendelian susceptibility to mycobacterial disease	Yes	PMID:16715106
NFKB2: p.Arg853* (het)	Non-familial hypogammaglobulinemia	Common variable immunodeficiency	Yes	PMID:25595268
PIK3CD: p.Glu1021Lys (het)	Pancytopenia, splenomegaly, hypogammaglobulinemia, decrease in B lymphocytes	Activated PI3K-delta syndrome	Yes	PMID:27847301
RMRP, n.-5delins17 (het) RMRP, n.181G>C (het)	T positive, B positive, NK positive SCID; asymmetric IUGR; rhizomelia	Cartilage-hair hypoplasia-anauxetic dysplasia spectrum	Yes	PMID:20375313

Table 3. Case studies of six patients with genetic diagnoses in genes where treatment with HSCT may be considered.

CONCLUSIONS

- **Expanded next-generation sequencing panels offer an effective tool to aid in the rapid molecular diagnosis of patients with PIDs.**
- **Detection of copy number variations with multigene panels is critical, as CNVs represented 8% of pathogenic variants identified by our panel.**
- **In total, 23% of patients had positive findings, and a likely genetic diagnosis was made in 30% of these cases (7% of total cases). Importantly, 68% of conditions diagnosed are treatable with HSCT. Of note, the diagnostic yield observed here is likely lower than it would be in an unselected population of patients with PIDs; individuals with clear phenotypes may have been tested with single gene assays or targeted panel tests.**

References: 1. Lincoln SE, Kobayashi Y, Anderson MJ *et al*. A systematic comparison of traditional and multigene panel testing for hereditary breast and ovarian cancer genes in more than 1000 patients. *J Mol Diagn*. 2015;17(5):533-44.
2. Nykamp K, Anderson M, Powers M *et al*. Sherloc: a comprehensive refinement of the ACMG variant classification criteria. *Genet Med*. 2017;19(10):1105-17.

Disclosures: All of the authors are stockholders in and employees of Invitae.