Diagnostic Role of Exome Sequencing in Immune Deficiency Disorders

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Exome sequencing of immunodeficient newborns

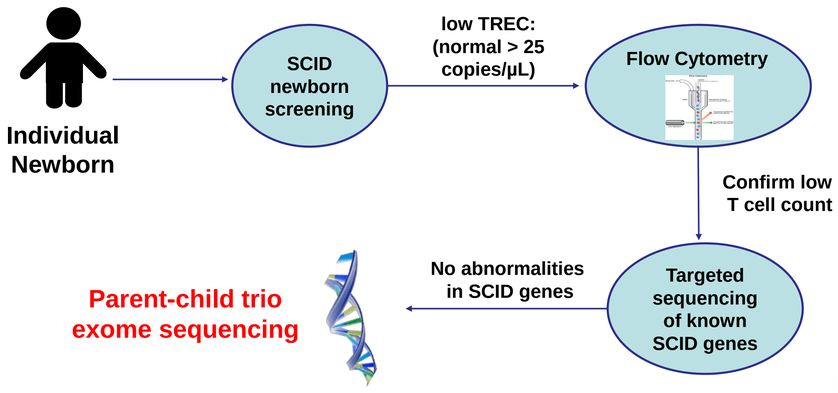
We discuss the use of exome sequencing for the diagnosis of newborns with severe immune diseases of uncertain underlying causes. Many were identified from population based newborn screening for severe combined immundeficiencies (SCID), that revealed low or absent T cell receptor excision circles (TRECs), a biomarker for T-cell lymphocyte production. However, the low TREC can be observed in several other rare non-SCID immune disorders as well.

Genome sequencing can potentially resolve the genetic basis of these disorders through precise characterization of molecular defects in an individual newborn's DNA. To interpret genomic variant data, we developed an analysis protocol whose distinctive features enabled solving numerous clinical cases. It integrates variant annotation, variant filtering, and gene prioritization to prioritize millions of called variants to a manageable shortlist of possible causative variants.

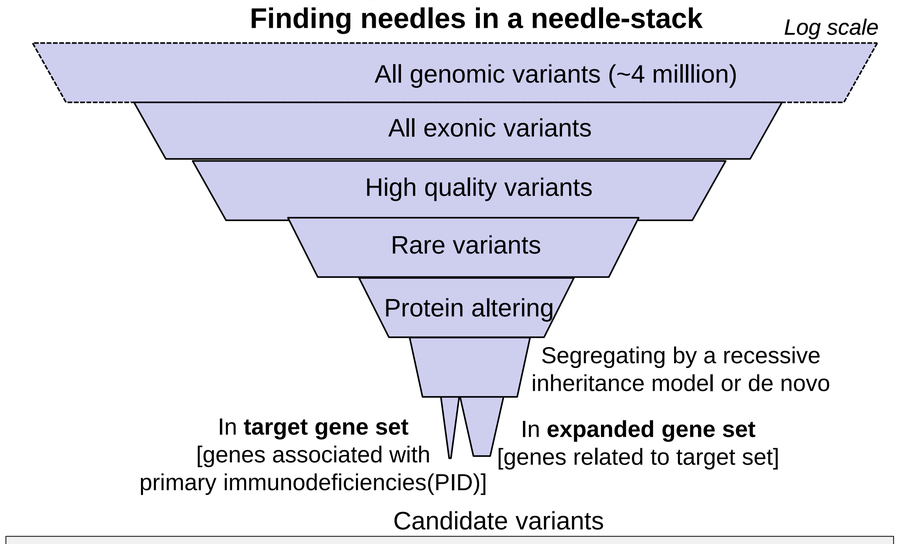
We applied the protocol to exome sequences from several newborn patients with undiagnosed primary immune disorders. These case studies highlight unique features of the protocol that facilitate clinical diagnosis with the help of deep sequencing.

Exome sequencing performed when newborn screening and clinical evaluation fail to identify cause of immunodeficiency

Newborn screening for severe combined immunodeficiency (SCID) uses quantification of **T cell receptor excision circles (TREC)** which correlate with the number of circulating T cells



Locating the causative variants: Finding needles in a needle-stack

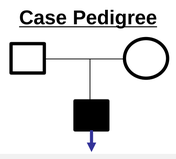


Prioritization
The candidate genes/variants are prioritized based on interactions and disease phenotypes shared with target gene set as well as inheritance pattern and predicted deleteriousness.

ILLUSTRATIVE CASE STUDIES

A likely novel SCID gene is identified based on putative haploinsufficiency annotations

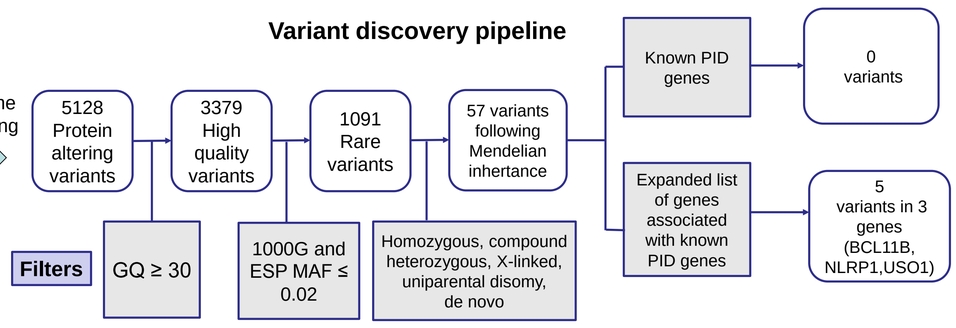
Case Pedigree



Patient with undetectable TRECs and absent corpus callosum

Trio exome sequencing

Variant discovery pipeline



Challenge
No homozygous or compound heterozygous mutations in SCID or SCID-related genes

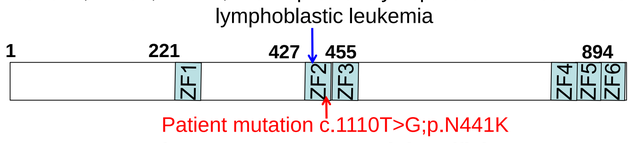
Gene prioritization

		Candidate Genes		
		BCL11B	NLRP1	USO1
Gene Prioritization	**Protein interactions & pathways shared with target genes** (BIOGRID, MINT, HPRD, Pathway Commons)	✓	✗	✓
	Disease phenotypes shared with target genes & proband (MGI, HPO, GAD, OMIM, NHGRI-GWAS)	✓	✗	✗
Variant Prioritization	**Mendelian inheritance** homozygous, compound heterozygous, X-linked, haploinsufficient	✓	✓	✓
	Predicted deleteriousness population frequency, conservation, structural impact, etc.	✓	✓	✗

- We found **BCL11B interactions with genes involved in T cell activation** and phenotype annotations associated with low T cells.
- BCL11B is listed by our pipeline as a putative **haploinsufficient** gene based on literature database annotation, leading to the hypothesis that a single copy of the mutant gene can likely cause disease

***De novo* heterozygous missense mutation in BCL11B gene**

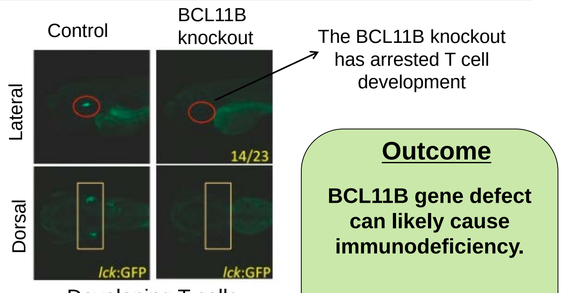
C432Y,H445Y,R447H,E452K previously implicated in T cell acute lymphoblastic leukemia



Rare Mutation
Absent in 1000 genomes, UW Exome Sequencing Project, and Exome Aggregation Consortium

Haploinsufficient Gene
Bcl11b$^{+/-}$ genotype provides susceptibility to γ-ray-induced mouse thymic lymphomas[3]

Zebrafish model validates BCL11B immunodeficiency

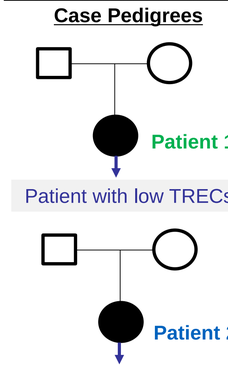


Developing T cells transgenically marked by GFP

Outcome
BCL11B gene defect can likely cause immunodeficiency.
First report of SCID caused by heterozygous variant

Exome sequencing reveals newborn T lymphopenia as a characteristic in Ataxia Telangiectasia

Two unrelated newborn patients
Case Pedigrees

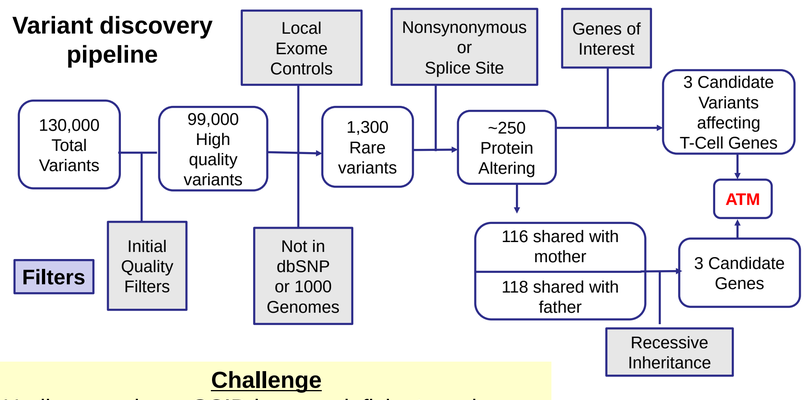


Patient with low TRECs

Patient with low TRECs

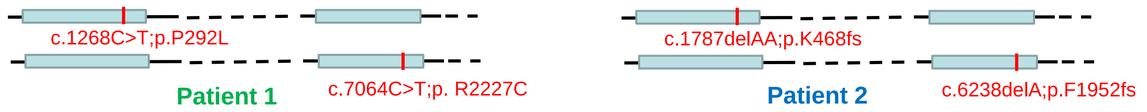
Trio exome sequencing

Variant discovery pipeline



Challenge
Undiagnosed non-SCID immunodeficient newborns

Deleterious mutations located in the ataxia telangiectasia (AT) mutated (ATM) gene[2]



AT: A Neurodegenerative and Immune Disorder

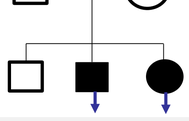
Characteristics
- Ataxia, motor symptoms with a cerebellar focus
- Typically diagnosed between ages 2-11 years
- Patient 1: Wobbly gait reported at 14-16 months
- Variable T and B lymphocytopenia

Prognosis
- Progressive, irreversible
- 10-15% lymphoma by early adulthood
- Median age of death: 22 years
- Experimental treatments in animal models

Outcome
T lymphocytopenia in newborns can be a feature of AT as revealed by TREC screening and exome sequencing. The children's diseases wouldn't have been detected otherwise for years.
Early detection of AT helps avoid diagnostic odessey, including avoidance of live vaccines and irradiation, which should not be administered to AT patients.Early diagnosis of AT can also help genetic counseling when considering future pregnancies

A novel human autoimmune syndrome is discovered due to combined hypomorphic and activating mutations in ZAP70

Case Pedigree



- Two siblings affected with early onset bullous pemphigoid, a chronic autoimmune skin disease, and uncontrollable colitis and proteinuria
- Both underwent bone marrow transplantation which resolved their pre-transplant autoimmunity

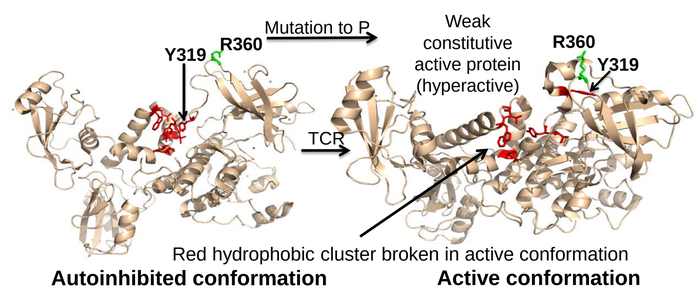
Trio exome sequencing

Two novel, missense mutations in the zeta-chain (TCR) associated protein kinase gene (ZAP70)

NM_001079.3
Maternal chromosome c.574C>T;p.R192W
Paternal chromosome c.1097G>C;p.R360P

Immunological assessments confirm combination of variants lead to defective T cell activation

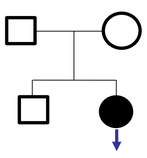
Challenge
Null mutations in ZAP70 previously implicated in profound immunodeficiency. However, inconsistent with patient autoimmune symptoms



Outcome
Heterozygous combination of a hypomorphic and a hyperactive *ZAP70* variant leads to inappropriate T cell activation, adding a new mechanism to the existing phenotype repertoire of ZAP70

SCID causing gene is identified despite inconsistency of the reference genome

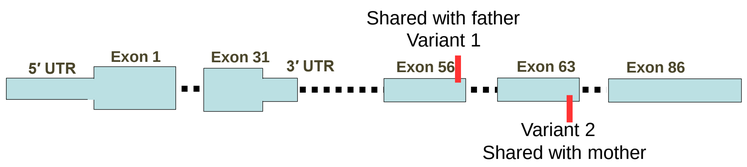
Case Pedigree



Patient with severe influenza pneumonia and diagnosed as having SCID, though of unknown etiology.

Trio exome sequencing

Initial analysis
Compound heterozygous mutations found in the 3′ UTR region of a known PID gene, PRKDC



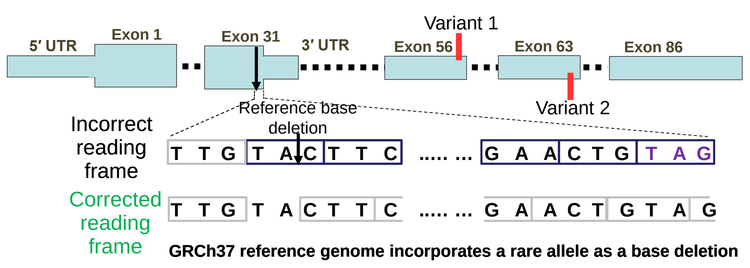
Typically, the UTR region variants are not considered strong disease-causing candidates

Challenge
Existing methods unable to correctly annotate the PRKDC gene variants

Our annotation tool, Varant[1], flagged the *PRKDC* gene with CDS inconsistency (CDS non multiple of 3) and correctly identified the non-synonymous variants.

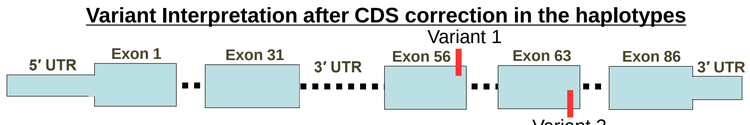
PRKDC gene is involved in DNA double-strand break repair

- Encodes **DNA-PK$_{cs}$**, a serine/threonine-protein kinase that acts as a molecular sensor for DNA damage.
- Involved in DNA non-homologous end joining (NHEJ) required for double-strand break (DSB) repair and V(D)J recombination.
- PRKDC knockout in mice observed to have SCID



GRCh37 reference genome incorporates a rare allele as a base deletion

Variant Interpretation after CDS correction in the haplotypes



Varant flagged the *PRKDC* gene with **CDS inconsistency** annotation.

Variant-1: **A → G, ATG → GTG, p.Met1592Val, MAF = 0.00008** — Non-Synonymous Mutation

Variant-2: **C → T, CGC → TGC, p.Arg2915Cys, MAF = 0.00033** — Non-Synonymous Mutation

Outcome
Discovered PRKDC despite genome issues.Parents learned their recurrence risks for future children and were alerted of child's radiation sensitivity

Conclusion

- Exome sequencing makes definitive diagnosis of several rare immune related disorders with uncharacteristic symptoms with success rate of ~50% in cases with trios
- T Cell Excision Circles (TREC) screening successfully identifies non-SCID T lymphopenic disorders that may not otherwise be diagnosed until later in life.
- Testing of individual genes is avoided which can be costly, time-intensive and unhelpful
- Early detection provides information to offer prompt appropriate treatment and guidance, family genetic counseling, and avoidance of the diagnostic odyssey.

References

1. http://compbio.berkeley.edu/proj/varant/
2. Mallott J, et al. 2013. *J Clin Immunol* 33:540–549. DOI: 10.1007/s10875-012-9846-*1*
3. Kamimura K, et al. 2007. *Biochem Biophys Res Commun*.355:538-542. DOI: 10.1016/j.bbrc.2007.02.003

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