Genomics and Genetic Evaluation of Immunodeficiencies

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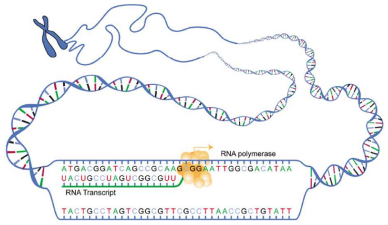
Disclosure

No conflicts of interest relative to this presentation

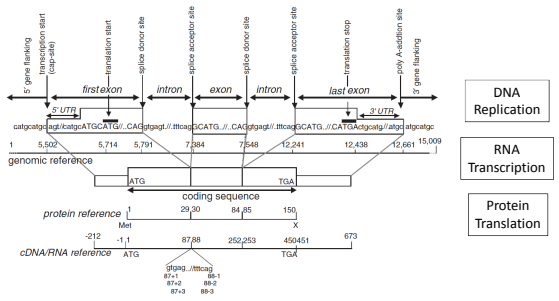
Learning Objectives

- Identify the basic elements of a gene structure, DNA replication, RNA transcription and protein translation
- Recognize the different types of DNA mutations, inheritance, penetrance and expressivity patterns
- Define different methods and approaches for DNA variant (mutation) analysis
- Distinguish the advantages and the limitations of next generation sequencing (NGS) in establishing the genetic basis of primary immunodeficiencies

Genetic Organization



Genetic Organization



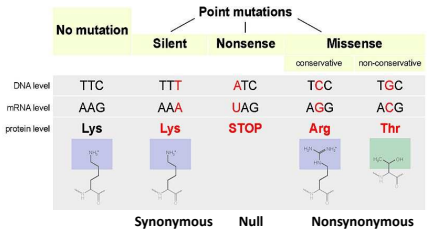
Mutation/Polymorphism/Variant

A mutation is defined as a permanent change in the nucleotide sequence, while a polymorphism is defined as a variant with a frequency above 1%. However, the terms "mutation" and "polymorphism", which have been used widely, often lead to confusion due to incorrect assumptions of pathogenic and benign effects respectively. Thus, it is recommended that both terms be replaced by the term "variant" with the following modifiers: (1) pathogenic, (2) likely pathogenic, (3) uncertain significance, (4) likely benign, or (5) benign. While these modifiers may not address all human phenotypes, they comprise a five-tier system of classification for variants relevant to Mendelian disease as addressed in this guidance. It is recommended that all assertions of pathogenicity (including "likely pathogenic") be reported with respect to a condition and inheritance pattern (*e.g.* c.1521_1523delCTT (p.Phe508del), pathogenic, cystic fibrosis, autosomal recessive).

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. PMID: 25741868

Genetic Variation Analysis

Nomenclature



Single base substitutions/point mutations represent ~70% of disease causing changes

Genetic Variation Analysis

"Sentence" format

Wild Type Sequence	one two big zoo
Missense	one two bit zoo
Nonsense	one two
Frame shift	one twz obi gzo
Insertion (in frame)	one two and big zoo
Deletion (in frame)	one two zoo
Duplication (in frame)	one two two big zoo

Genetic Variation Analysis

Nomenclature

Alternative (Muller's morphs)	Description
Amorph	Complete loss of function
Hypomorph	Partial loss of function
Hypermorph	Dominant gain of function
Neomorph	Novel function
Antimorph	Dominant negative function

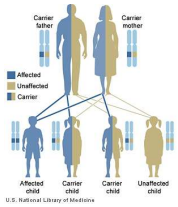
Genetic Lab Report

Typically report single nucleotide substitutions using the five tier system:

- Variant pathogenic - prior report of disease association
- Variant likely to be pathogenic, likely to be benign or benign based on "*in silico*" evaluation (computer programs that predict the impact of the change, the conservation of the invovled nucleotide, etc)
- Variant of unknown significance (VUS) which is the most difficult to utilize clinically since it leaves the potential of a causal relationship to disease unknown

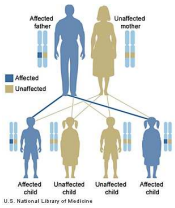
Inheritance Patterns

Autosomal Recessive



In nonconsanguinous families diseased individuals typically have compound heterozygous variants while in consanguinous families disease is usually associated with homozygous variants

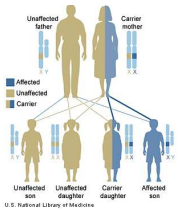
Autosomal Dominant



One allele with the disease causing variant results in disease (*i.e.* there is one abnormal & one normal allele)

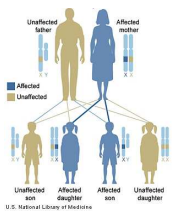
Inheritance Patterns

X-linked Recessive



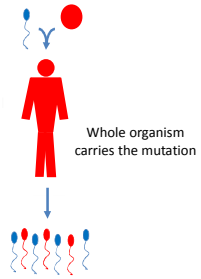
Disease is only seen in males unless there is altered X chromosome inactivation or an XO female (Turner syndrome)

X-linked Dominant

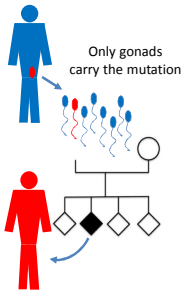


Inheritance Patterns

Germline mutations



Gonadal mosaicism



Penetrance and Expressivity

Penetrance

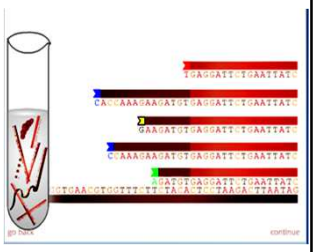
All-or-None phenomenon
"Black/White"

Expressivity

Severity of a phenotype
"Shades of gray"

Genetic Analysis: Sanger Method

- Gene specific testing using a chain termination method
- PCR to amplify desired genomic region
- Second PCR with normal (deoxy) plus modified (dideoxy) labeled nucleotides that terminate the PCR extension
- Generates different sized fragments of DNA
- Gel electrophoresis applied to separate these fragments for analysis

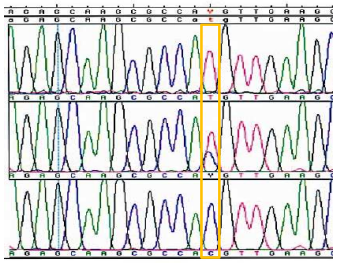


Genetic Analysis: Sanger Method

Control

Carrier

Patient



X-linked SCID due to a single nucleotide substitution

Next Generation Sequencing (NGS)/ Massively Parallel Sequencing

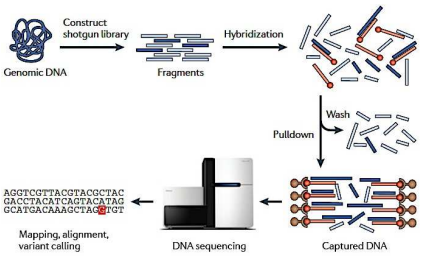
Targeted: select panel of genes (vary in number based on the target objective)

Exome: ~3x 10^7 coding bp (~1% of genome)
~20,000 protein-coding genes
predicted to detect ~85% of disease causing mutations

Genome: ~3x 10^9 coding & noncoding bp

Genetic Analysis

Next-Gen/Massively Parallel sequencing



Mutation Analysis in PIDs

Classification by Phenotype or Syndrome

- Clinical*: Severe Combined Immunodeficiency, velo-cardio-facial, Cartilage-hair hypoplasia, ...*
- Immunologic*: Agammaglobulinemia with no B cells, Hyper IgM, Hyper IgE, …*
- ID susceptibility*: Susceptibility to Mycobacterial Diseases, to Herpes simplex virus, CMC, …*

…multiple gene defects could be associated with each phenotype/syndrome (genetic heterogeneity)

frontiers in IMMUNOLOGY

Stoddard et al. METHODS ARTICLE published: 03 November 2014 doi: 10.3389/fimmu.2014.00531

Targeted NGS: a cost-effective approach to molecular diagnosis of PIDs

Rationale/Cost calculation

Phenotype	Genes (amplicons)	Total amplicons	Sanger sequencing for all listed genes	Targeted NGS (HaloPlex/Ion Torrent PGM)
DOCK8 deficiency	*DOCK8 (62)*	62	$620	$580
Mendelian susceptibility to mycobacterial disease (MSMD)	*IFNGR1 (8), IFNRG2 (8), IL12RB1 (17), IL12B (7), ISG15 (3), STAT1 (21), IRF8 (16), GATA2 (15), CYBB (20)*	115	$1,150	
Immunodeficiency with hyper-IgM (HIGM)	*CD40LG (8), CD40 (12), ICOS (5), PIK3CD (18), AICDA (5), NFKBIA (7)*	55	$550	
Autoimmune lymphoproliferative syndrome (ALPS) and ALPS-like diseases	*FAS (9), FASL (4), CASP8 (10), CASP10 (11), NRAS (4), KRAS (5), CTLA4 (8), PIK3CD (18)*	69	$690	
Severe combined immunodeficiency (SCID)	*CD3D (6), CD3E (11), DCLRE1C (29), ORAI1 (6), ADA (12), IL2RG (8), IL7R (14), NHEJ1 (6), PTPRC (40), RAG1 (7), RAG2 (4), JAK3 (18), CORO1A (14),PRKCD (22), AK2 (18)*	199	$1,990	

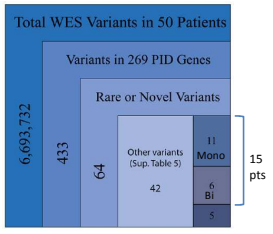
Validation

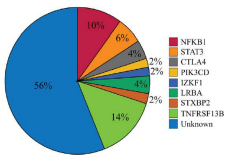
True positives	52	True negatives	59012
False negatives	1	False positives	2
False negative rate	1.9	False positive rate	0.003
Sensitivity (exonic)	98.113	Specificity	99.997

-120 PID pts tested
-173 gene targeted capture
-18 pts diagnosed
15% efficacy

Genetic Diagnosis Using Whole Exome Sequencing in Common Variable Immunodeficiency

Maffucci et al.
Frontiers in Immunology
published: 13 June 2016
doi: 10.3389/fimmu.2016.00220





- 50 CVID patients tested
- WES: 269 gene targeted analysis
- 15 pts diagnosed

22/50 = **30% efficacy** (excluding TACI mutations) in a set of **selected CVID patients** using targeted analysis

Rapid molecular diagnostics of severe primary immunodeficiency determined by using targeted next-generation sequencing

YU ET AL
J ALLERGY CLIN IMMUNOL
■■■ 2016

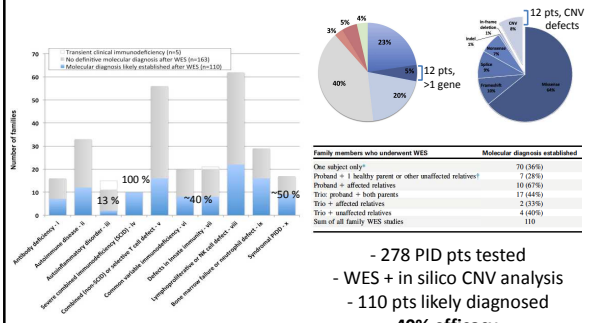
NGS panel	Comprehensive SCID panel: 46 genes
Genes included	T−B+ SCID: *CD3D, CD3E, CORO1A, IL2RG, IL7R, JAK3, PTPRC* T−B− SCID: *ADA, AK2, DCLRE1C, LIG4, NHEJ1, PRKDC, RAG1, RAG2* Others: *AP3B1, CASP8, CD3G, CD8A, CHD7, CITA, DOCK8, FOXN1, FOXP3, IKZF1, ITK, LCK, LYST, ORAI1, PNP, PRF1, RFX5, RFXANK, RFXAP, RMRP, SH2D1A, STAT5B, STIM1, STX11, STXBP2, TAP1, TBX1, TTC7A, UNC13D, XIAP, ZAP70*

Patient ID	Age	Sex	Clinical presentations	Gene	Deleterious variants*	Zygosity	Inheritance
P1	1 mo	M	Family history consistent with X-linked SCID	*IL2RG*	c.505C>T (p.Q169*)	Hemizygous	X-linked
P2	5 wk	M	Family history	*IL2RG*	c.285_286dupTA (p.N96Ifs*52)	Hemizygous	X-linked
P3	2 wk	M	CHARGE syndrome, absent TRECs on NBS	*CHD7*	c.7282C>T (p.R2428*)	Heterozygous	AD
P4	3 wk	F	SCID	*RAG1*	c.1648G>A (p.D55IN)	Heterozygous	AR
					c.2924G>A (p.R975Q)	Heterozygous	
P5	3 wk	M	Absent TRECs on NBS	*IL7R*	c.354C>A (pC118*)	Homozygous	AR
P6	5.5 mo	M	Low TRECs on NBS, severe lymphopenia	*FOXN1*	c.958C>T (p.R320W)	Heterozygous	AR
					c.1556T>A (p.L519Q)	Heterozygous	
P7	3 mo	M	Absent TRECs on NBS	*FOXN1*	c.1275_1278delACTC (p.L426Ifs*123)	Heterozygous	AR
				CHD7	c.7538G>A (p.R2513Q)†	Heterozygous	AD
P8	6 wk	M	Undetectable TREC, diarrhea/rash	*IL2RG*	c.202G>A (p.E68K)	93%, Hemizygous	X-linked
P9	4 wk	M	SCID	*IL2RG*	c.173C>G (p.P58R)	Hemizygous	X-linked
P10	3 wk	M	Absent TRECs on NBS	*RMRP*	n.128G>C	Homozygous	AR
					n.*22-C	Homozygous	
P11	6 mo	M	Low TRECs on NBS	*ATM*	**c.2284_2285delCT (p.Y762Vfs*2)**	Heterozygous	AR
					c.3802delG (p.V1268Ffs*5)	Heterozygous	
P12	14 mo	M	Absent T and B cells	*DCLRE1C*	Exon 1 to 4 deletion	Homozygous	AR
P13	5.5 mo	M	SCID, MHC class II deficiency	*CITA*	c.1464T>G (p.L498R),	Hemizygous	AR
					One copy loss of the whole gene	Homozygous	
P14	7 wk	F	Absent TRECs on NBS, Rash	*ADA*	**c.956_960delAAGAG (p.E319Gfs*3)**	Heterozygous	AR
					c.34-4257_36-1054dup (exon 2)	Heterozygous	

20 SCID+ pts tested
46 gene targeted capture
14 pts diagnosed
70% efficacy

Primary immunodeficiency diseases: Genomic approaches delineate heterogeneous Mendelian disorders

Stray-Pedersen et. al., JACI, 139, 2017, 232–245



Family members who underwent WES	Molecular diagnosis established
One subject only*	70 (36%)
Proband + 1 healthy parent or other unaffected relatives†	7 (28%)
Proband + affected relatives	10 (67%)
Trio: proband + both parents	17 (44%)
Trio + affected relatives	2 (33%)
Trio + unaffected relatives	4 (40%)
Sum of all family WES studies	110

- 278 PID pts tested
- WES + in silico CNV analysis
- 110 pts likely diagnosed
40% efficacy

Genetic Variation Analysis in PIDs

Next-Gen/Massively Parallel Sequencing

Diagnostic Success Rate

- Type of study: WGS > WES > Targeted
  - Type of phenotype: SCID > … Autoinflammatory
    - Type of family: 2 or more affected > singleton
      - Type of mutation: Exons > UTR, intron,...

but NO SINGLE MOLECULAR TOOL will solve all the diagnostic challenges, more importantly, DOES NOT REPLACE KNOWLEDGE OR COMMON SENSE

Case Study Applying NGS

Case Study: 6yo F, HIM syndrome

I have a very interesting case of a girl with HIM who has been tested for the known genes - all are normal

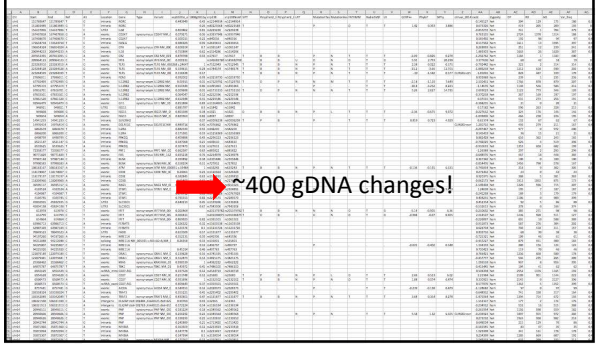
We do have a research protocol To evaluate patients with unknown PIDs, you will need to get informed consent and then forward a sample

Case Study: 6yo F, HIM syndrome

- History per referring MD:
- Denied consanguinity, healthy at birth, fully vaccinated (well tolerated)
- <1 yo: ear/respiratory infections
- 3 yo: bronchiectasis (*Pseudomonas*)
- 4 yo: LN, HSM, cytopenias (Plt, RBC, lymphocytes)
  - IgM 1966, IgG 26, IgA 40; Poor antibody response (Tet, Pneumo, Rubella, isohemagglutinins)
  - Bone marrow biopsy, no malignancy
  - Lymph node biopsy, many CD138 IgM+ (rare CD138 IgG+)
  - Mildly reduced mitogen proliferations (antigens NL)
  - Genetic testing for CD40, CD40L, ALPS was NI. HIV neg.
  - IVIG and Bactrim prophylaxis.

Case Study: 6yo F, HIM syndrome

Targeted NGS, 250 PID-causing genes



~400 gDNA changes!

Case Study: 6yo F, HIM syndrome

Targeted NGS, 250 PID-assoc genes

Exonic Harmful Rare

	A	B	C	D	E	F	G	H	I	J	K
1	Chr	Start	End	Ref	Alt	Location	Gene	Type	Variant	esp6500si	1000g2011
11	chr1	209964123	209964123	C	T	exonic	IRF6	nonsynonym	IRF6 NM_00	0.000231	
16	chr1	67792499	67792499	G	A	exonic	IL12RB2	nonsynonym	IL12RB2 NM_	0.00915	0.01
18	chr1	67816701	67816701	C	T	exonic	IL12RB2	nonsynonym	IL12RB2 NM_	0.009688	0.01
46	chr11	613978	613978	C	T	exonic	IRF7	nonsynonym	IRF7 NM_004	0.000464	
47	chr11	614799	614799	C	T	exonic	IRF7	nonsynonym	IRF7 NM_004	0.000411	
140	chr19	17945696	17945696	C	T	exonic	JAK3	nonsynonym	JAK3 NM_00	0.009457	0.0037
155	chr19	7708058	7708058	C	T	exonic	STXBP2	nonsynonym	STXBP2 NM_	0.011841	0.01
161	chr19	853384	853384	A	G	exonic	ELANE	nonsynonym	ELANE NM_001	972 c.347A>G p.N116S	
276	chr5	1294166	1294166	C	T	exonic	TERT	nonsynonym	TERT NM_00	0.021226	0.01
386											

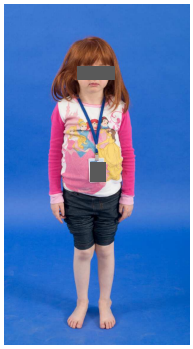
~400 changes → 9 potentially deleterious changes but none of them explain the pt's HIM phenotype!

Case Study: 6yo F, HIM syndrome

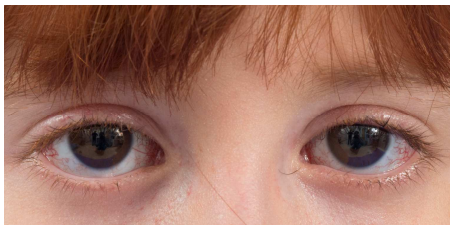
TARGETED NGS diagnostic approach was not conclusive, could we arrange to see the patient at NIH?

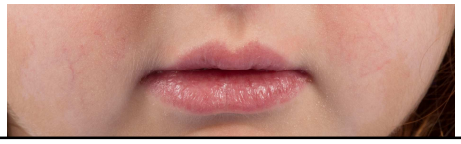
Case Study: 6yo F, HIM syndrome

- Physical Examination
- Short stature for age
- Tachypnea, crackles
- Abd: HSM, G tube
- Dental caries,
- Ankle bony outgrowth



Case Study: 6yo F, HIM syndrome





Case Study: 6yo F, HIM syndrome

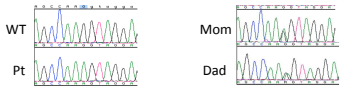
Targeted NGS, review of ATM data

			Reads	% Coverage	
791	chr11:10809	ATM	744	70	*
792	chr11:10809	ATM	196	100	
793	chr11:10810	ATM	636	100	
794	chr11:10811	ATM	390	95	*
795	chr11:10811	ATM	57	100	
796	chr11:10811	ATM	220	100	
797	chr11:10811	ATM	54	100	
798	chr11:10812	ATM	338	98	*
799	chr11:10812	ATM	404	96	*
800	chr11:10812	ATM	340	100	
801	chr11:10812	ATM	283	91	*
802	chr11:10812	ATM	453	100	

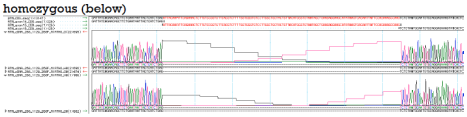
Not 100% covered during the targeted NGS

Case Study: 6yo F, HIM syndrome

Sanger sequencing test result(s):
ATM NM_000051 c.2250G>A heterozygous rare SNP (rs1137887, ExAC allele frequency 8.274e-06)



cDNA: *ATM* r.2125_2250del (exon skipping), p.I709_K750del (in-frame) homozygous (below)



Diagnosis: ataxia telangiectasia, increased IgM is seen in ~30% of ATM and absence of neurologic findings is seen very rarely but reported

Lesson From the 6 yo "HIM" Patient

- Careful physical exam and medical history is absolutely crucial in interpreting genomic data
- Phenotypic variability is a reality and must be taken into account when thinking about genomic evaluation
- NGS is a powerful technique but as with any lab test it is not infallible, careful data review may be necessary since interpretation criteria may exclude potentially relevant genes

Start with a Clinical Phenotype

- Evaluate the genotype
  - Test genomic DNA
  - Assess copy number (deletions/insertions)
  - Possible additional testing using cDNA
- Characterize the protein when possible: present vs decreased vs absent, if present is it altered in size (immunoblot)
- Undertake *ex vivo* functional testing (if possible) to validate the role of the defect in affecting immune function

Why Evaluate Genomic Changes in PIDs

- Provides opportunity to screen for at risk patients and provide family counseling
- Allows screening potential family donors
- Identifies potential therapeutic targets
- Increases understanding of a specific disorders, genetic mechanisms (penetrance, expressivity)
- Enhances opportunity to define potential contributions of environmental factors (epigenetics) on human disease

Thank You