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Pilot Study for the Implementation of a Multi-Gene SCID Panel in Newborn Screening

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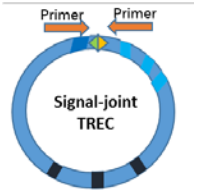
Current Testing Algorithm for SCID



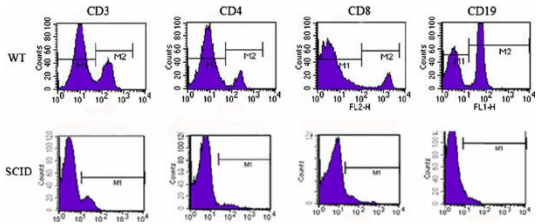
T-cell receptor excision circle (TREC) assay

< 125 Avg TRECs OR
2 samples <200 Avg TRECs

Refer to Specialist for Diagnostic Testing

Physician ordered tests

CBC, Flow cytometry, Mitogen studies



Molecular tests – gene panel

- Slow turn-around times
- Insurance denial
- Increased stress to families

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Potential Benefits of Molecular Testing by the NBS Program:

- Shortened Turn-Around Time (TAT)
- Faster diagnosis; phenotype prediction
- Earlier decision on best treatment options – better outcomes
- Cost savings to family and health care system
- Less stress for families

APHL/CDC Cooperative Agreement - Specific Aims

- Develop and validate a 2nd tier multi-gene immunodeficiency panel
- Evaluate Utility of Targeted NGS for SCID in a Consented Study
  - Identify causative gene?
  - Shortened time to diagnosis?
  - Earlier, targeted treatment?
- Provide CDC with quality control/reference samples

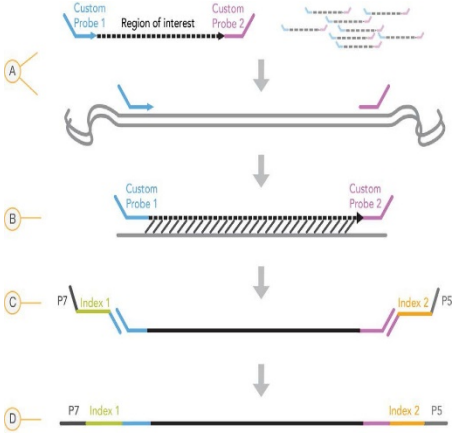
NYS NBS 39-gene SCID panel

Gene Selection:

- Commercial SCID panels
- CLSI guideline
- Literature search and case studies

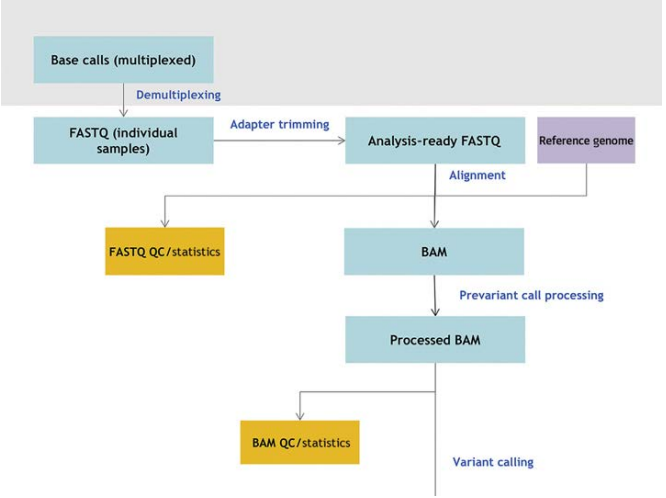
ADA	AK2	ATM	BLNK	BTK	CD3D	CD3E
CD3G	CD247	CD40LG	PTPRC	CHD7	CORO1A	DCLRE1C
DKC1	DOCK2	DOCK8	FOXN1	GATA2	IGHM	IL2RG
IL7R	JAK3	LIG4	MTHFD1	MTR	NHEJ1	NBN
PNP	PRKDC	RAC2	RAG1	RAG2	RMRP	SLC46A1
STAT5B	TBX1	WAS	ZAP70			

NGS Technology



Illumina TruSeq Custom Amplicon Panel

Illumina MiSeq



In-house Developed Bioinformatics Pipeline

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Validation

- NGS Sequence
  - 8 samples from infants diagnosed with SCID – genetic cause known
  - 13 additional samples from infants diagnosed with SCID or other immunodeficiency – genetic cause not reported to us
  - Reference Sample – NA12878 (NIST; GIAB) –published genotypes
- Sanger sequence
  - 8 samples Sanger sequenced for all 39 genes
    - 657 amplicons/sample = 5256 amplicons total
  - Sanger confirm all pathogenic/likely pathogenic/VOUS in remaining samples
- Assessed reproducibility through replicate testing within and between runs

Accuracy – Concordance with Commercial Diagnostic Lab

Sample	Gene	Variant (cDNA)	Variant (aa)	Zygosity	Agreement with Dx Lab
102	CHD7	c.434G>A	p.Trp145Ter	Het (dom)	Yes
105	ADA	c.301C>T	p.Arg101Trp	Hom	Yes
108	IL7R	c.83-2A>T	-	Het	Yes
	IL7R	c.353G>A	p.Cys118Tyr	Het	
110	IL2RG	c.982C>T	p.Arg328Ter	Hom	Yes
113	IL2RG	c.865C>T	p.Arg289Ter	Hom	Yes
115	ADA	c.301C>T	p.Arg101Trp	Hom	Yes
116	JAK3	c.2872G>T	p.Glu958Ter	Het	Yes
		c.1261delC	p.Leu421fs	Het	
120	RAG2	c.501A>C	p.Arg167Ser	Het	Yes
		c.283G>A	p.Gly95Arg	Het	

Validation Approved by NYS CLEP on 7/17/2018

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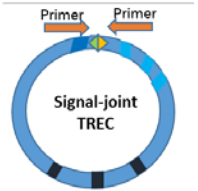
Proposed Algorithm for SCID NGS Consented Study



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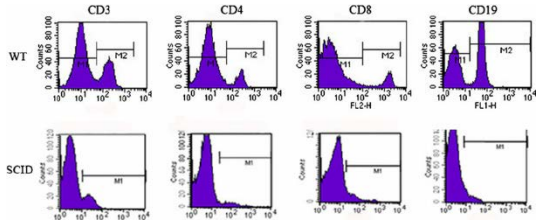

Immunodeficiency identified ; Obtain Informed Consent

39-gene SCID NGS panel

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Consented Study

- IRB approval - 8/10 NYS SCID Specialty Care Centers
- 3 Part Consent
  1) Consent to SCID NGS panel testing
  2) Consent to participation in Immunodeficiency Registry for Long-Term Follow-up
  3) Consent to provide a blood sample for CDC to develop quality assurance materials
  - ➢ Participants can consent to 1, 2, all 3, or none of the parts

Consented Study

- Since receiving approval to offer the SCID NGS test (7/17/2018):
  - 5 infants consented into the study

Consent Part	# Consented
Part I: Consent to SCID multi-gene NGS analysis	5
Part II: Consent to long term follow-up registry	4
Part III: Consent to blood sample for CDC QA materials	1

Case # 1

- Day 2 of life: TREC = 0
- Day 12 of life: TREC = 0
- Flow cytometry: Lymphopenia
- Day 18 of life: Consent for SCID NGS
- Variants detected:
  - *IL2RG* p.Cys182Ser (c.545G>C) – hemizygous – Likely Pathogenic
  - *MTHFD* p.Leu521= (c.1561T>C) – heterozygous – Uncertain Significance
- Diagnosis: X-linked SCID
- Day 88 of life: Stem cell transplant
- Last office visit: “Very well appearing happy child”; “Continues to do well overall”; “Stable engraftment”; followed monthly

Case # 2

- Day 2 of life: TREC = 30
- Day 7 of life: TREC = 2
- Flow cytometry: Lymphopenia
- Day 22 of life: Consent for SCID NGS
- Variant detected:
  - *DOCK8* p.Ala324Asp (c.971C>A) – heterozygous – Uncertain Significance
- Patient moved to NC – being followed at Duke
- SCID panel (86-gene) performed at a commercial diagnostic laboratory confirmed our results. Also identified heterozygous VOUS in *TAPBP* (not on our panel)
- Patient doing well, but continuing to be followed; low T-cell counts, functionally normal

Case # 3

- Day 21 of life: TREC = 190 (borderline – request repeat)
- Day 105 of life: TREC = 166 (borderline - referral)
- Day 115 of life: Consent for SCID NGS
- Flow cytometry: Not available at time of consent
- Variants detected:
  - NONE
- Day 135 of life: TREC = 398 (within acceptable limits)
- Case Closed as “No evidence of immune dysfunction”

Case # 4

- Day 2 of life: TREC = 164 (borderline – request repeat)
- Day 15 of life: TREC = 103 (referral)
- Day 29 of life: Consent for SCID NGS
- Flow cytometry: Not available at time of consent
- Variant detected:
  - *LIG4* p.Arg580Gln (c.1739G>A) – heterozygous – Uncertain Significance
- Day 30 of life: TREC = 1285 (within acceptable limits)
- Flow cytometry results: Normal
- Case Closed as “No evidence of immune dysfunction”

Case # 5

- Day 3 of life: TREC = 0
- Flow cytometry: “Unusual lymphocyte pattern” per physician
- Day 25 of life: Consent for SCID NGS
- Variants detected:
  - *RAG1* p.Cys176Phe (c.527G>T) – homozygous – Likely Pathogenic
  - *IL7R* p.Met10Val (c.28A>G) – heterozygous – Uncertain Significance
- Awaiting diagnosis and treatment plan from physician

Turn-Around Time (TAT)

Laboratory	SCID NGS Panel TAT (listed on lab website)
GeneDx	3 weeks
Invitae	10-21 days
Blueprint Genetics	4 weeks
Cincinnati Children's	42 days
Prevention Genetics	20-26 days
NYS Newborn Screening Lab	9 – 21 business days (average 14 days)

*Initial goal was to return NGS results in 7 calendar days

Barriers to Faster TAT

- NGS
  - Day 1:DNA extraction/quantification
  - Day 2 – 3: Library preps
  - Day 4: Sequencing (run time ~24 hours)
  - Day 5: Bioinformatic analysis of sequence data
- Sanger sequencing (starts Day 6)
  - Confirmation of Pathogenic, Likely Pathogenic, and VOUS
  - NGS low coverage regions (<20X coverage)
- Variant classification
  - Database searching
  - Literature review
  - ACMG criteria

Sanger Sequencing

# of Variants Identified per Sample	62 (50-78)
# of Variants Requiring Sanger Confirmation (Pathogenic, Likely Pathogenic or Uncertain Significance)	5 (2-10)
# of Regions with Low NGS Coverage (<20X) Requiring Sanger Sequencing	29 (28-30)

Exploring ways to decrease TAT for Sanger Sequencing:

- NextGen PCR instrument – decreases 2 hour amplification to 20 minutes
- Reagents for faster PCR amplicon clean-up

Summary

- A likely causative genetic variant was identified for 2 of 5 infants tested using a 39-gene SCID NGS panel; a VOUS was identified in a 3rd infant
- Turn-around time for NGS results was comparable or faster than the TAT listed by several commercial diagnostic labs
- Enrollment into the consented SCID NGS study has been slow
- A meeting with the Specialty Care Center Directors and participating families is planned for September 2019
  - Discuss experience with the consent process
  - Discuss benefits of identification of genetic cause of immunodeficiency
  - Discuss benefits of genetic testing being performed by Newborn Screening laboratory

Acknowledgments:

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