



American College of
Medical Genetics
Medical Genetics: Translating Genes Into Health

Emerging Tests and Technologies in Newborn Screening

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The Main Drivers of Change in Newborn Screening

- ◆ **Evolution and expansion driven by:**
 - Families
 - New technology
 - Has implications for many of the criteria by which conditions are assessed for NBS (cost, candidate conditions, time of screening)
- ◆ State based decision-making in US
- ◆ National advisory committees now developing in the US

ACMG 2



Technologies are Applicable to All Newborn Screening Program Components

- ◆ **Screening and testing technologies**
 - Detection systems (target or signal)
 - Testing platforms (e.g., MS/MS, IEF/HPLC, arrays)
 - Result analysis
- ◆ **Information technologies**
 - In laboratories (data management)
 - Between laboratories and providers
- ◆ **Therapeutics coming fast**
 - Enzyme replacement
 - Chaperones
 - Molecularly targeted treatments

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Emerging Screening Laboratory Technologies

- ◆ **Open testing systems**
 - Provide broad information due to analyte relationships to multiple conditions
 - MS/MS profiling
 - Some proteomic arrays
 - Functional phenotype tests
 - DNA sequencing
- ◆ **Directed (intentional) testing systems**
 - Targeted enzyme assays
 - DNA and expression arrays
 - Some proteomic arrays
- ◆ **Hybrids**
 - SRM MS/MS

ACMG 4



Issues Based on Analytical Targets

- ◆ **Functional assays**
 - Detect products of pathways
 - Accumulated products
 - Missing products
 - Enzymology to show loss of activity
 - Physiological tests
- ◆ **Pathway components**
 - Detect critical components of a pathway
 - DNA
- ◆ **Intermediates**
 - RNA expression assays
 - Enzyme assays for partial loss of activity

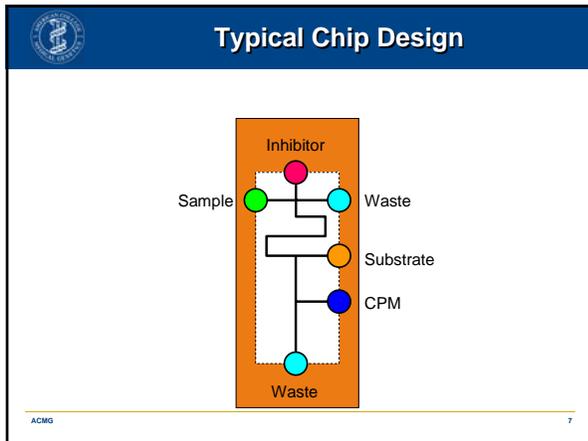
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Technologies Now and in the Near Future

- ◆ **Integrating molecular diagnostics**
- ◆ **Physiologic tests**
- ◆ **Robotics**
- ◆ **Nanotechnology:** in which nano- (or micro-) chips are utilized as the assay platform. Able to accommodate all existing technologies
 - Potential applications:
 - Everything
 - Potential costs
 - Higher than labs in the short term due to QA issues
 - Location:
 - Nursery

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- ### Candidate Conditions for Expansion of Uniform Panel
- ALD
 - CDG type Ib
 - CMV
 - DMD
 - G6PD
 - Fabry disease
 - FHC
 - Fragile X
 - HIV
 - Krabbe disease
 - Pompe disease
 - SCID
 - SLO
 - SMA
 - Toxoplasmosis
 - Wilson disease
- ACMG 8

Newborn Screening Card

= unlimited blood supply?

ACMG 9

Mutations in genes required for T-cell development: IL7R, CD45, IL2RG, JAK3, RAG1, RAG2, ARTEMIS, and ADA and severe combined immunodeficiency: HuGE review

Lisa Kalman, PhD¹, Mary Lou Lindgren, MD², Lisa Kobrynski, MD³, Robert Vogt, PhD⁴, Harry Hanson, PhD⁵, Jolyn Tenkin Howard, MSPH⁶, and Rebecca Buckley, MD⁷

Gene	Locus	Gene product/function	Presence of			Mode of inheritance ^a	No. unique mutations identified	OMIM No.	References
			T-cell	B-cell	NK-cell				
IL7R	3p13	IL7 receptor. Needed for T-cell development. Activates JAK3 kinase	-	+	+	AR	5	146661	2,3
CD45	1q11-q12	Protein tyrosine phosphatase. Regulates Src kinases required for T-cell and B-cell antigen receptor signal transduction	-	+	+	AR	3	151469	5-8
IL2RG	Xq13.1	gamma-4 chain of IL2, 4, 7, 8, 15 cytokine receptors. Needed to activate SACS for intracellular signaling	-	+	-	XLR	169	30830	9, 96
JAK3	19p13.1	Tyrosine Kinase. Needed for differentiation of hematopoietic cells	-	+	-	AR	27	600173	14,15,97
RAG1	13p13	DNA recombinase. RAG1/RAG2 mediate DNA recombination during B-cell and T-cell development	-	-	+	AR	44	179435	16,98
RAG2	13p13	DNA recombinase. RAG1/RAG2 mediate DNA recombination during B-cell and T-cell development	-	-	+	AR	18	179436	17,99
ARTEMIS	8p9	Involved in DNA repair during V(D)J recombination	-	-	+	AR	9	605888	18,19,100
ADA	20q13.11	Part of the purine salvage and methylation pathways. Needed for removal of toxic metabolites (e.g. ATP, 5-adenosyl homocysteine) that inhibit lymphoid cells.	-	-	-	AR	54	102790	23-25

^aMode of inheritance: AR, autosomal recessive; XLR, X-linked recessive.

ACMG 10

- ### Newborn Screening for Severe Combined Immunodeficiency Syndrome (SCID)
- ◆ Detection of profound T-cell lymphocytopenia
 - ◆ Measure T-cell specific proteins on DBS
 - ◆ Measure T-cell receptor excision circles (TRECs) on DBS
 - ◆ Secondary targeted mutation testing
- ACMG 11

Basic and clinical immunology

Development of population-based newborn screening for severe combined immunodeficiency

Ken Chan, MS,^{1,2} and Jeanne M. Paik, MD,¹ Bethesda, MD, and New Haven, Conn

Background: Severe combined immunodeficiency (SCID) is a treatable, inherited lack of cellular and humoral immunity caused by diverse mutations in several different genes, and leading to death in infancy unless immune reconstitution is provided. Currently no population screening exists for SCID, but such diagnosis would improve outcome.

Objective: Because all patients with SCID make few or no T cells, we asked whether the absence of T-cell receptor excision circles (TRECs), DNA excision by newly formed T cells, could identify SCID regardless of genotype.

Methods: DNA isolated from dried blood spots was assayed by real-time PCR to quantify TRECs. Control PCR was performed on a segment of the beta-globin gene. After pilot studies with adults and cord blood control subjects, blood from SCID patients was tested on filters and analyzed, followed by screening of actual blood spots from the Maryland Newborn Screening Program. Finally, newborn blood spots were retested and tested from 1 infant after their diagnosis of SCID.

Results: In contrast to filters from the newborn screening program, which had a mean of 1026 TRECs in one 3-mm punch, samples from 13 infants with SCID had <50 TRECs. The newborn screening filter was not used from a state laboratory for one of these infants plus another infant who had died of SCID previously, although both samples had detectable beta-actin DNA, unlike the TRECs.

Conclusion: TRECs were sensitive to detect T-cell lymphocytes in newborn dried blood spots on filter infants with SCID who receive early, life-saving treatment. (J Allergy Clin Immunol 2006;118:1030-4.)

Key words: SCID, newborn screening, TREC, T-cell receptor excision circle, T-cell excision circle, newborn primary immunodeficiency, congenital, bone marrow transplant, dried blood spot.

Abbreviations used:
 BMT, Bone marrow transplant
 DBS, Dried blood spot
 HEMES, Hematopoietic Engraftment Supportive Materials
 TREC, T-cell receptor excision circle
 SCID, Severe combined immunodeficiency
 TCR, T-cell receptor
 TRECs, T-cell receptor excision circles

defects in both cellular and humoral immunity.¹⁻⁴ In infants with SCID due to infection in the first year of life, unless immunity is reconstituted by bone marrow transplantation (BMT),⁵ only one replacement,⁶ or, in some recent cases, gene therapy.^{7,8} There is no current screening program for SCID, which is caused by diverse mutations in any of at least 13 known genes and others not presently known, as summarized in Table 1.^{9,10}

Screening newborns for treatable genetic disorders allows early intervention.¹¹⁻¹⁶ After the introduction by Guthrie of filter paper spotted with heel-stick blood to screen for phenylketonuria (PKU),¹⁷ all states in the United States test newborns for PKU, hypothyroidism, and galactosemia.^{18,19} New tests use tandem mass spectrometry to detect many additional metabolic disorders²⁰⁻²³ and DNA analysis to diagnose specific mutations causing cystic fibrosis, hemoglobinopathies, and beta-globin deficiency.²⁴⁻²⁶ Screening programs are evolving to meet the growing number of conditions that can be identified and treated.^{27,28}

ACMG 12

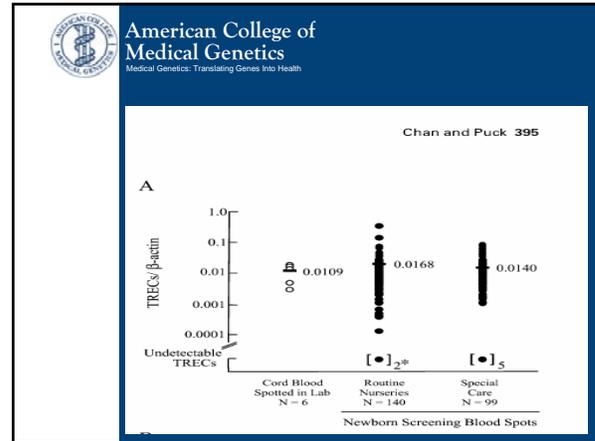
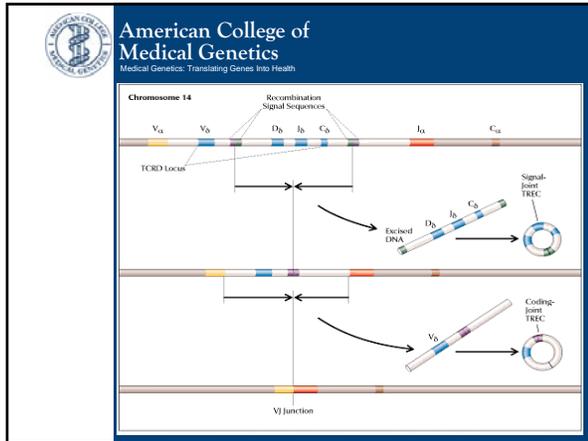
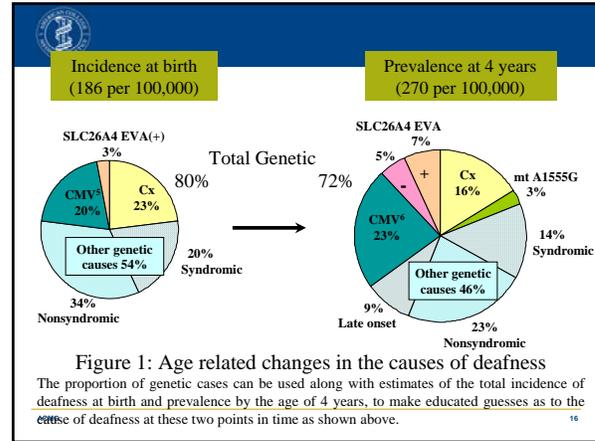


TABLE II. Clinical and molecular data and TREC analysis at time of diagnosis in 23 patients

Case no.	Age at diagnosis	Sex	Family history	Clinical history	Total lymphocytes	T cells	B cells	AK cells	SCD genotype	TRECβ actin	PCR from blood spot ^a
140	2.4	M	-	Healthy	463	13	136	48	g2RG	---	---
126	1 wk	M	-	Healthy	Not available	0	444	3	gAK7	---	---
220	2 wk	M	-	27-wk gestation, respiratory distress	220	24	448	NA	g2RG	---	---
140	1 mo	M	-	Healthy	461	9	363	13	g2RG	---	---
139	1 mo	F	-	Thrush, pneumonia, abscesses, FTTII (lost to post-RMF control)	1788	0	3072	0	g2RG	---	---
344	7 mo	M	-	Diarrhea, thrush, Pneumocystis jirovecii	2700	0	2724	0	g2RG	---	---
303A	4 mo	M	-	FTT, chronic upper respiratory infections, rash	486	80	96	107	Not g2RG or gAK7	---	---
303B	4 mo	M	-	FTT, chronic upper respiratory infections, rash	423	204	146	130	Not g2RG or gAK7	---	---
365	4 mo	M	-	Diarrhea, FTTII	907	0	609	130	Not g2RG	---	---
318.2	4 mo	M	-	Thrush (border of 30-1)	2103	22	3605	NA	g2RG	---	---
316	4 mo	M	-	Cytomegalovirus (transmitted to the child of infection and immune insufficiency)	393	16	371	0	g2RG	---	---
349	4 mo	M	-	FTT, malnutrition	1289	38	236	0	g2RG	---	---
343	6 mo	M	-	FTT, diarrhea, FFP	1088	108	600	0	g2RG	---	---
342	6 mo	F	-	FTT, diarrhea, FFP	1028	10	630	30	AK, not gAK7	Trace	---
352	6 mo	F	-	FTT, FFP	2360	93	3008	NA	AK	Trace	---
320	7 mo	M	-	FTT	1222	19	1057	0	g2RG with deletion	Trace	---
323	7 mo	M	-	FTT, absent milk and formula	376	48	389	0	SCD with deletion	Trace	---
355	7 mo	F	-	FTT (lost before RMT)	1360	28	1017	335	AK	Trace	---
354	7 mo	M	-	FTT, diarrhea, FFP	1080	0	921	0	Not g2RG	---	---
328	8 mo	M	-	FTT, diarrhea, FFP	739	24	670	14	gAK7	---	---
342	9 mo	M	-	FTT, diarrhea, malnutrition	2700	82	2336	140	g2RG	---	---
318.4	12 mo	M	-	FTT, diarrhea, CMV, bronchiolitis	869	268	420	0	g2RG	Trace	---
315	13 mo	M	-	Diarrhea, elevated IgE, HSV, Roseola	1000-2000	268-300 (+, 80%)	558	205	Not g2RG or gAK7	---	---



POTENTIAL AREAS FOR UNHS EXPANSION

- ◆ Improve diagnostic follow-up of etiology
- ◆ Identify infants at risk for late onset prelingual hearing loss
- ◆ Supplement audiologic with molecular screening (will detect >50% of infants at risk for late onset prelingual HL):
 - DFNB1 (CX 26 and CX 30 at locus)
 - SLC26A4
 - CMV
 - 12S rRNA

ACMG 17

Non-Invasive/Point of Care Monitoring and Testing

- ◆ Back to the bedside

ACMG 18

Nature of the Device

- On the unit
- Part of the bed
- Wearable
- Implantable
- Circulating

ACMG 19

Non-invasive - IR based

Bilicheck

ACMG 20

