EMORY GENETICS LABORATORY

Evidence collection for standardizing genomic testing

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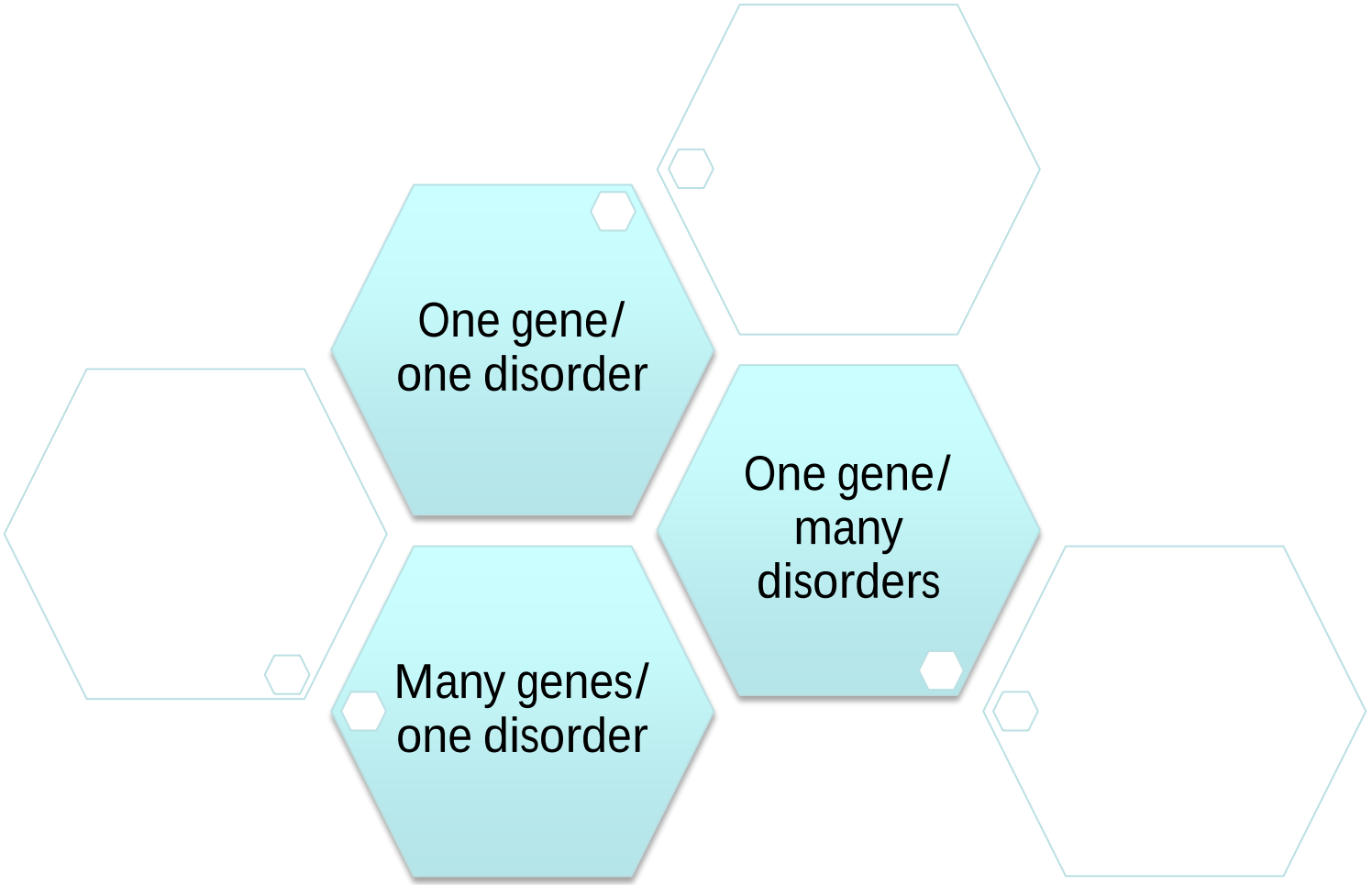
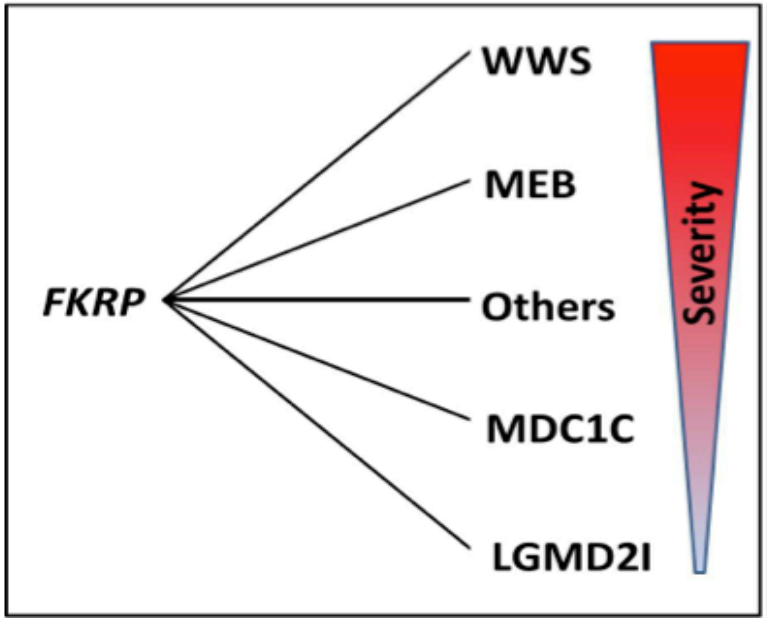
EMORY UNIVERSITY SCHOOL OF MEDICINE

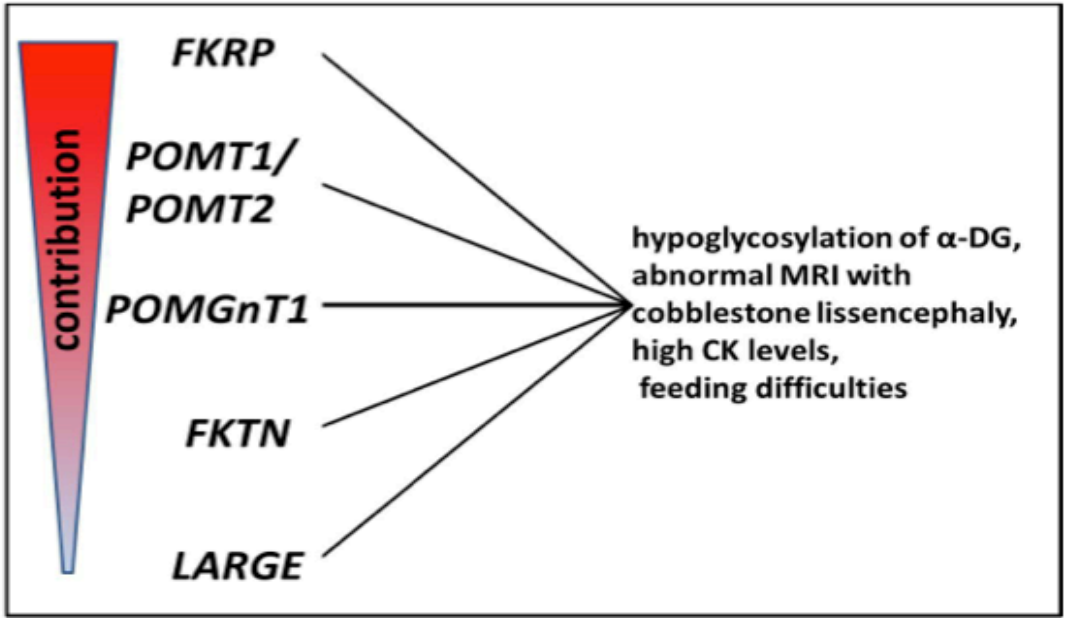
The growing complexity..



1 gene – varying phenotype



Similar phenotype – Many genes



CMD Form	Genes
Subtypes involving structural/extracellular matrix protein defects	*COL6A1, COL6A2, COL6A3, LAMA2, ITGA7, ITGA9,*
Subtypes involving glycosylation defects	*FKTN, LARGE, FKRP, POMT1, POMT2, POMGnT1, DPM2, DPM3, GTDC2, ISPD*
Subtypes involving nuclear envelop protein defects	*LMNA, SYNE1*
Rigid spine syndrome associated	*SEPN1, FHL1*
Mitochondrial	*CHKB*

Targeted mutation and Sequencing panels:

Clinically well defined cases

Technically complete:
Cover all exons of a gene

Covers entire mutation spectrum of the gene: Point mutation, indels, CNVs, deep intronic pathogenic variants

Exome
(Medical exome
VS
Research exome)

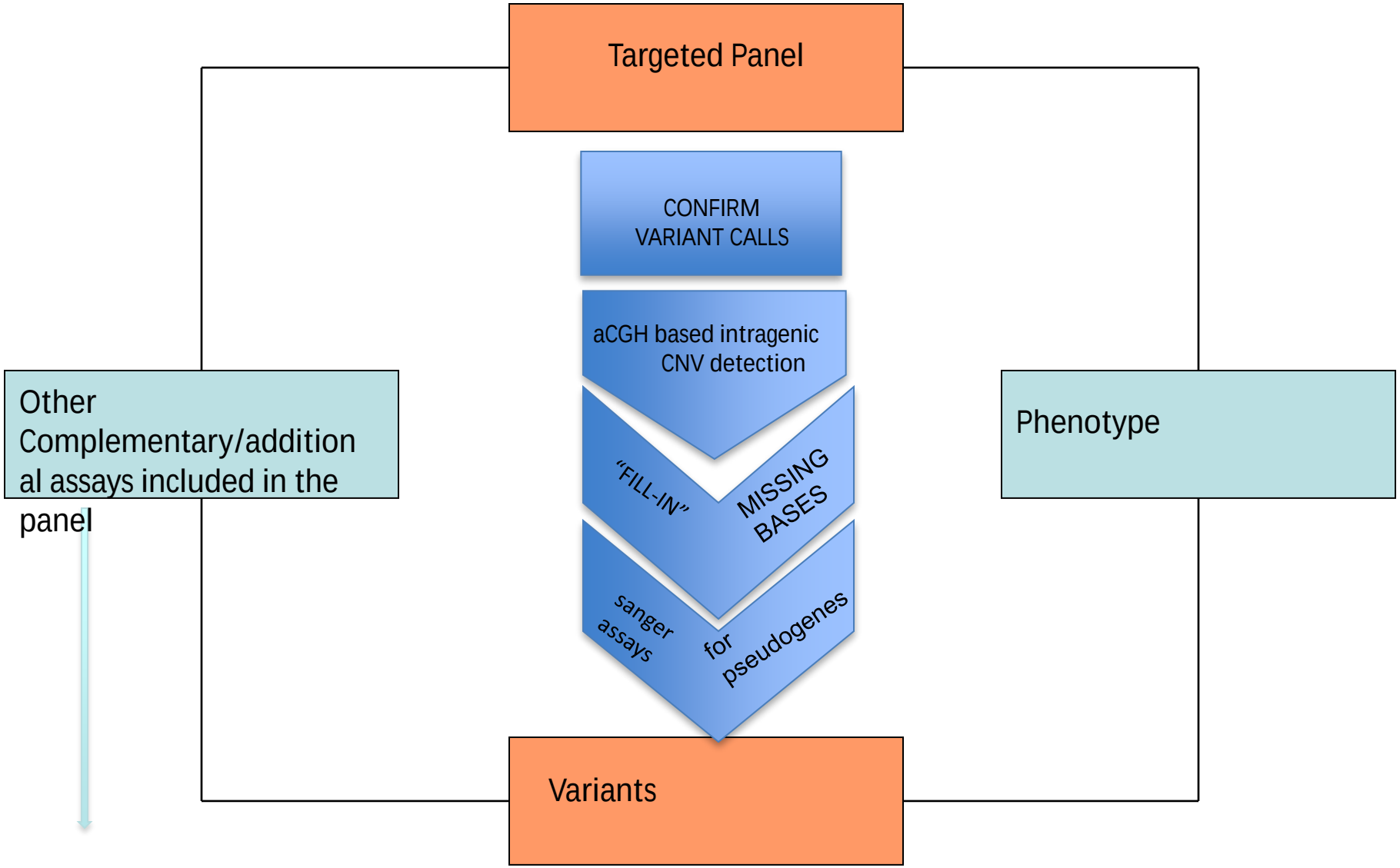
Complex/overlapping phenotypes or neg. for known genetic causes

<25% known clinically relevant genes

Technically incomplete: Does not cover all exons

Does not cover entire mutation spectrum of genes

Genome



XLID/Autism panel: FMR1, FMR2, Biochemical assays
Short stature panel: Russell Silver (H19/Lit1 methylation), UPD7
NMD panel: FKTN insertion assay

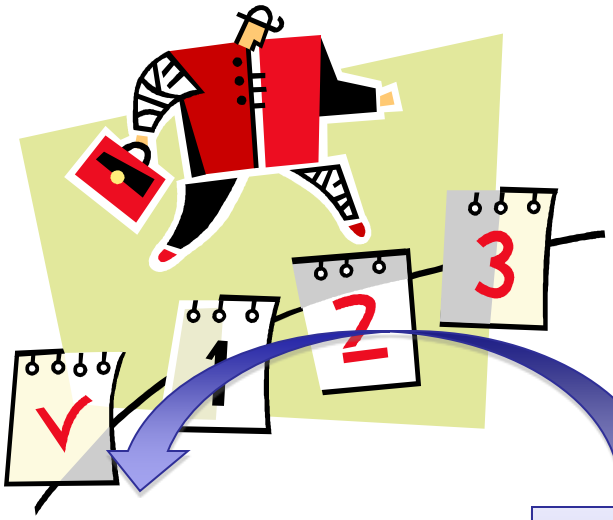
Chin et al, BMC Genetics, 2012, Askree et al, BMC Genetics, 2013, Valencia et al, J Mol Diag, 2013, Valencia et al, PLoS One, 2013

Diagnostic yields of the different NMD clinical tests

Clinical test ordered	No. of tests with diagnosis / No. of tests performed	Diagnostic yield (%)
CMD single gene testing	28/185	15
CMD *COL6A* sub panel	12/58	21
CMD comprehensive panel	26/75	35
LGMD single gene testing	82/312	19
LGMD comprehensive panel	18/61	30
NMD comprehensive panel	14/22	63

- Panel tests show consistently high clinical yield (> 5 fold) over single gene tests.
- LAMA2 and FKTN are major contributors to CMDs or at least have better diagnostic yield.
- Panel testing should be preferred over single gene testing for heterogeneous diseases like CMDs.

Ankala et al, Muscle and Nerve, 2012, Ankala et al, 2014, Lancet Neurol, submitted.



Sequencing panels

Exome (Medical exome <<<<>>>> Research exome)

Genome

EmExome Clinical Exome testing

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Exome

Complex/overlapping phenotypes or neg. for known genetic causes

<25% known clinically relevant genes

Technically incomplete: Does not cover all exons

Does not cover entire mutation spectrum of genes

GENE DISCOVERY: Collect evidence

Diagnost
- **New prese**
  gene
- **~30-40% yield**
- **Clinically interpretable**
- **Clinically Actionable**

markers

Targets known mutations

al exome

$$$$

Jones et al, AJHG, 2012, Ng et al AJHG, 2013, Enns et al, GIM, Accepted

High Level System Diagram

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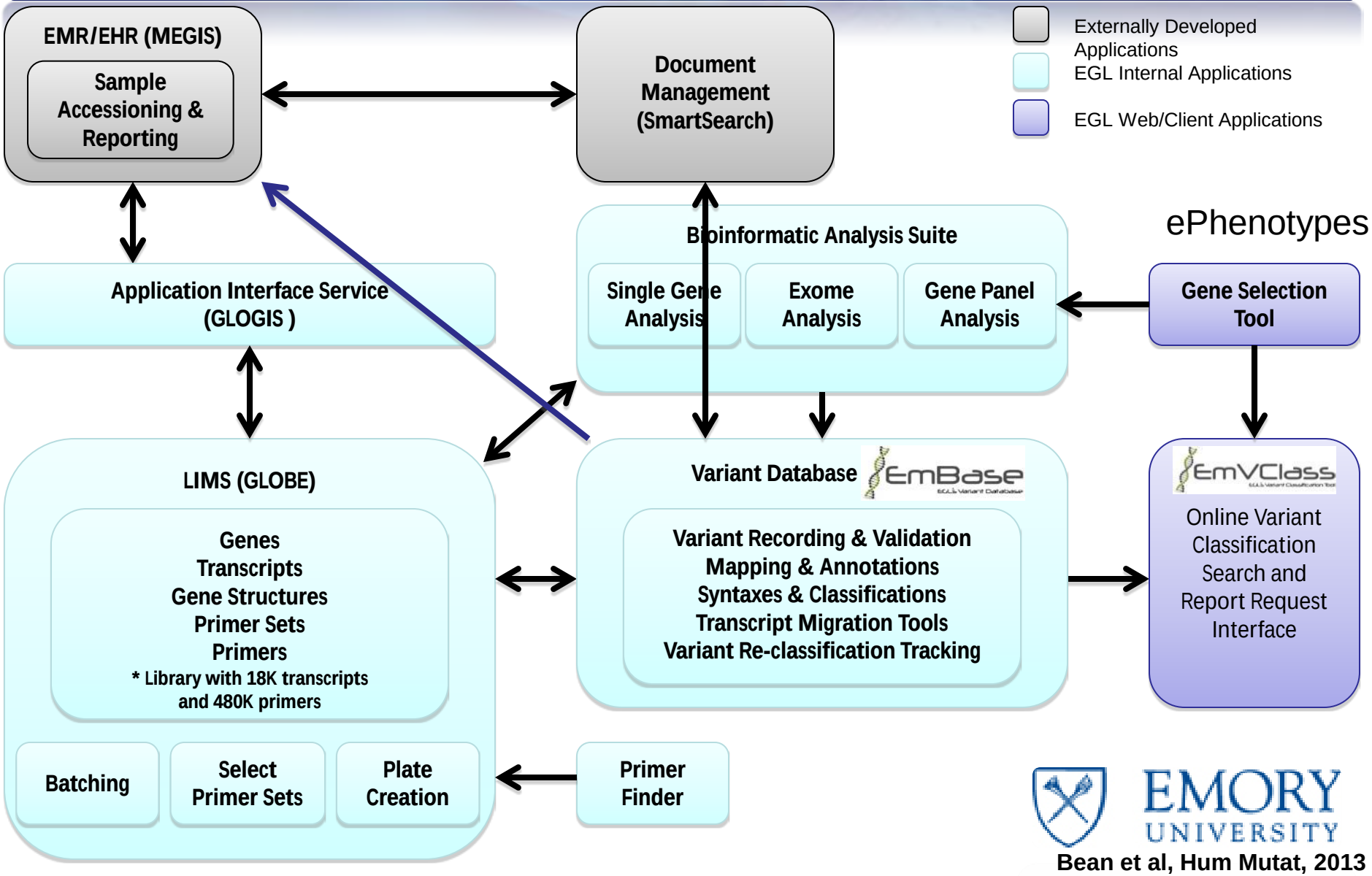

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Bean et al, Hum Mutat, 2013

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Client Employee

Email Address Password Login

Create an account | Forgot password?

Your partner in genetic healthcare

Home | Test Search | Test Ordering | Billing | Client Services | About EGL | Resources | Contact Us

EGL News Updates

- EGL Partners With Verinata to Offer Non-Invasive Prenatal Screening
- Emory Genetics Laboratory Expands Its International Footprint
- EGL Supports "Free the Data" Movement in Recent Publication

Coming Fall 2013

BRCA1 and *BRCA2* sequencing and deletion/duplication analysis

click here to learn more

SAVE TIME

Order Testing Online

click to learn more

EmExome: Whole Exome Sequencing

- ★ Three levels of service
- ★ Interactive variant classification tool
- ★ Sanger confirmation of clinically actionable changes

Click here to learn more about EGL's EmExome services

EmExome | Autism Disorders | Neuromuscular | Cancer | Next Generation Sequencing

QUICK LINKS

- Click Here to Learn About Recent Changes to Medicaid Coverage
- Requisition Forms
- Order Mailing Kits
- Request Insurance Verification
- Guide to Shipping Specimens
- Sample Submission Guidelines
- Prenatal Testing
- New Test Updates
- Discontinued Tests

DIRECTOR SPOTLIGHT

Dr. Christin Collins PhD, FACMG

Dr. Christin Collins is an Assistant Professor in the Department of Human Genetics and is a Molecular Laboratory Director at Emory Genetics Laboratory.

Click here to learn more about Dr. Collins' background

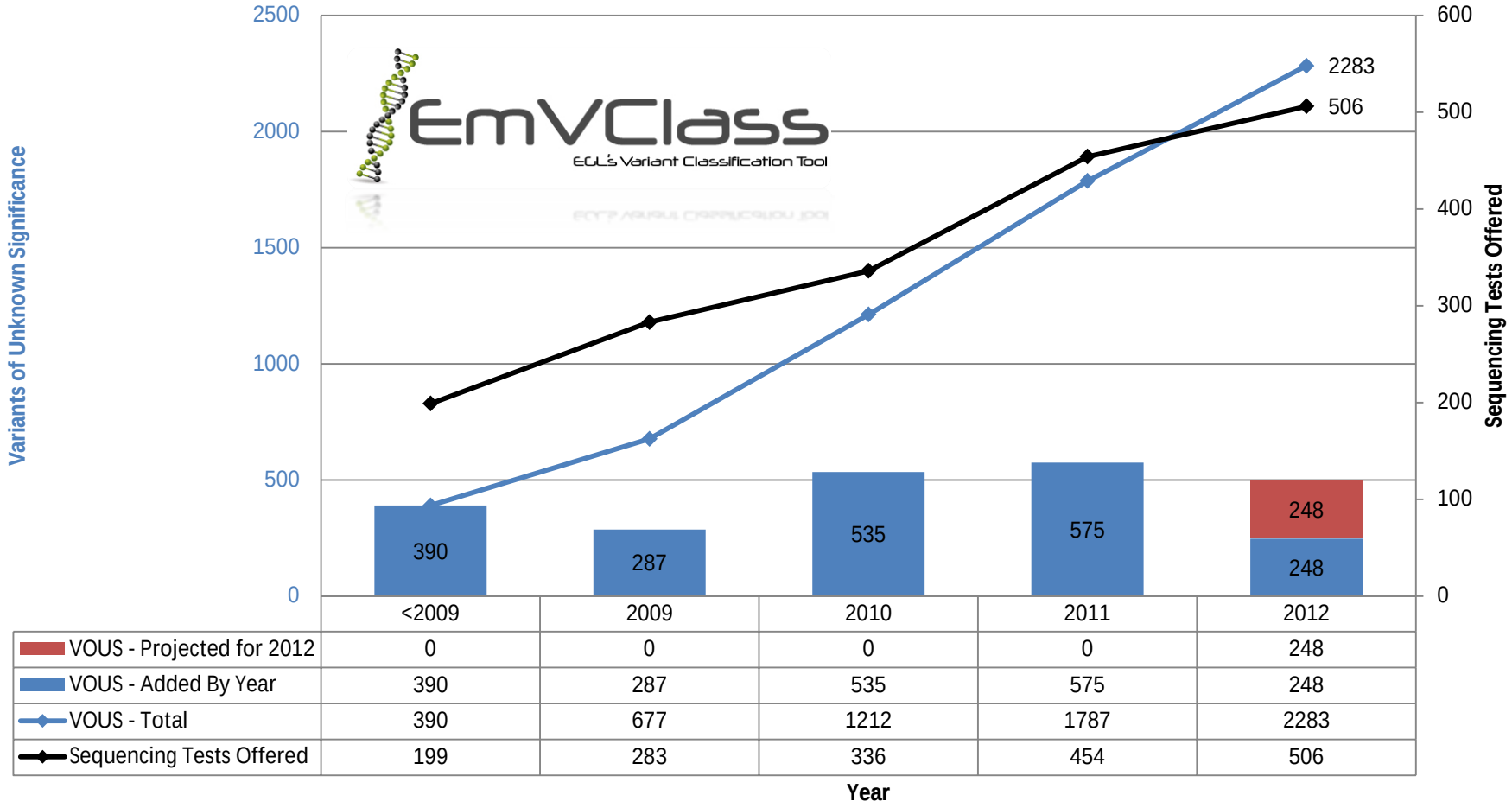
EmVClass

EGL's Variant Classification Catalog

Get up-to-the-date status updates on all variants that have been seen and analyzed by EGL

click here

Variants of Unknown Significance Added To EmBase by Year In Relation to Number of Sequencing Tests Offered



	<2009	2009	2010	2011	2012
VOUS - Projected for 2012	0	0	0	0	248
VOUS - Added By Year	390	287	535	575	248
VOUS - Total	390	677	1212	1787	2283
Sequencing Tests Offered	199	283	336	454	506

Year

Myriad detects 10-20 new missense variants each week despite testing >150,000 people for BRCA1/2 (Slide from W. Grody with data from B. Ward)

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Updating new knowledge on a variant

ACMG 2007 Guidelines: The testing laboratory should make an effort to contact physicians of previously tested patients in the event that new information changes the initial clinical interpretation of their sequence variant.

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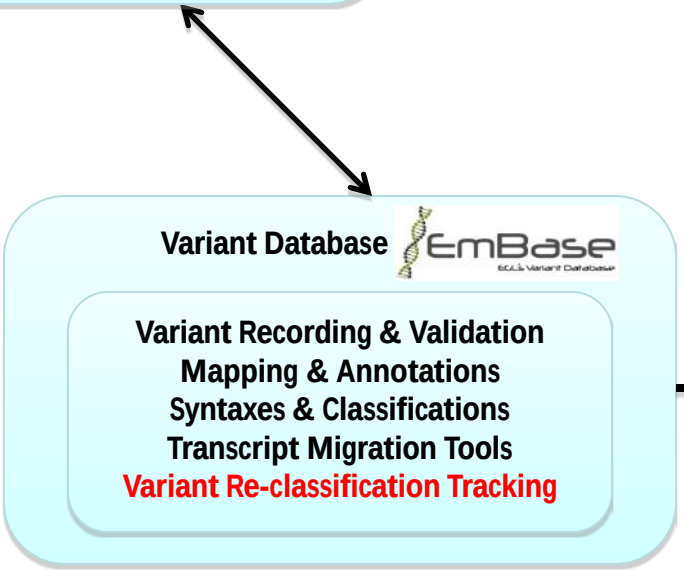
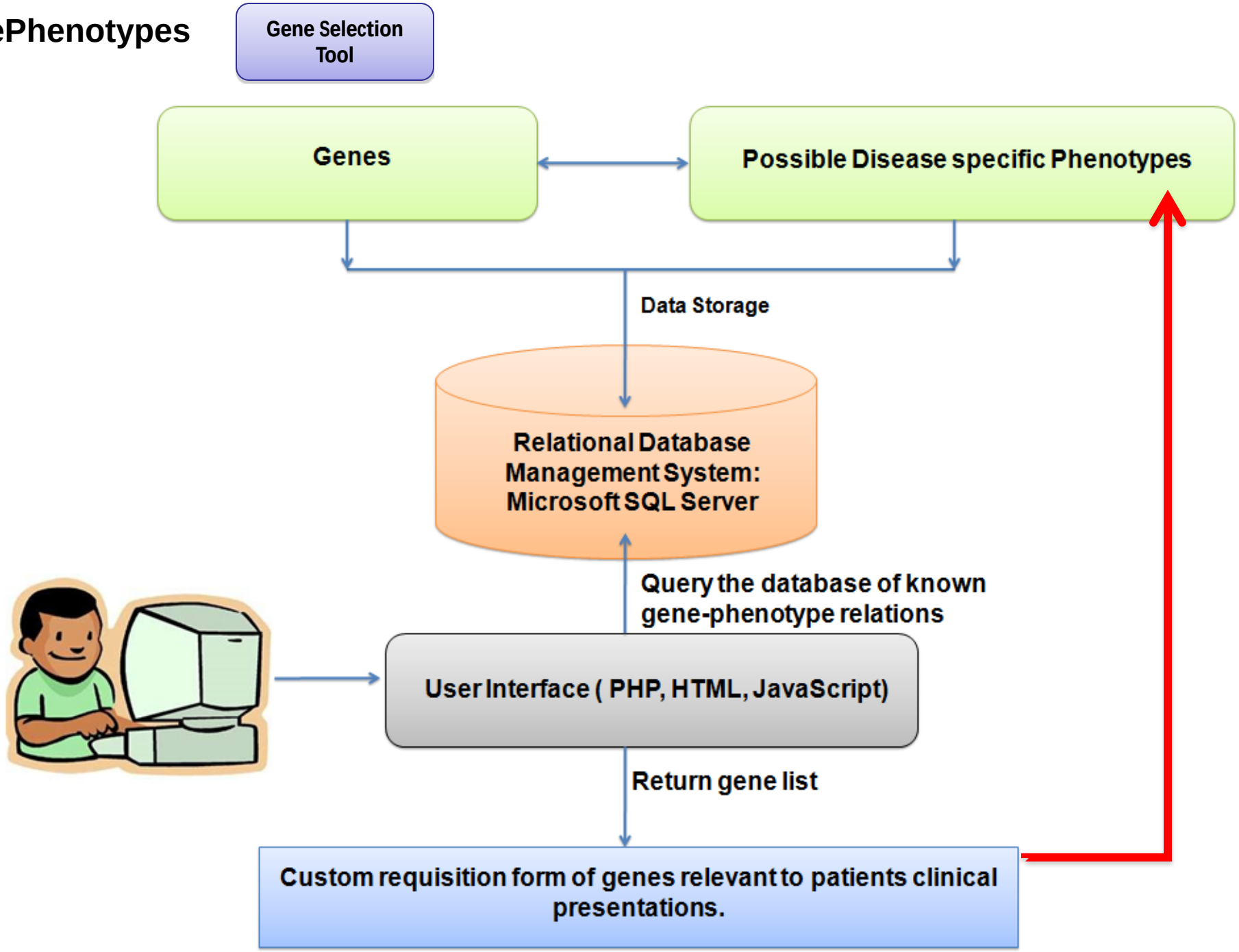
Reclassification Alert strategy

Variant reclassified-
- Scan database
- Identify affected patients
- Send alert
- Issue amended report

Variant check-
- Check EGL variants
- Check VOUS status in patient
- Request amended report



ePhenotypes



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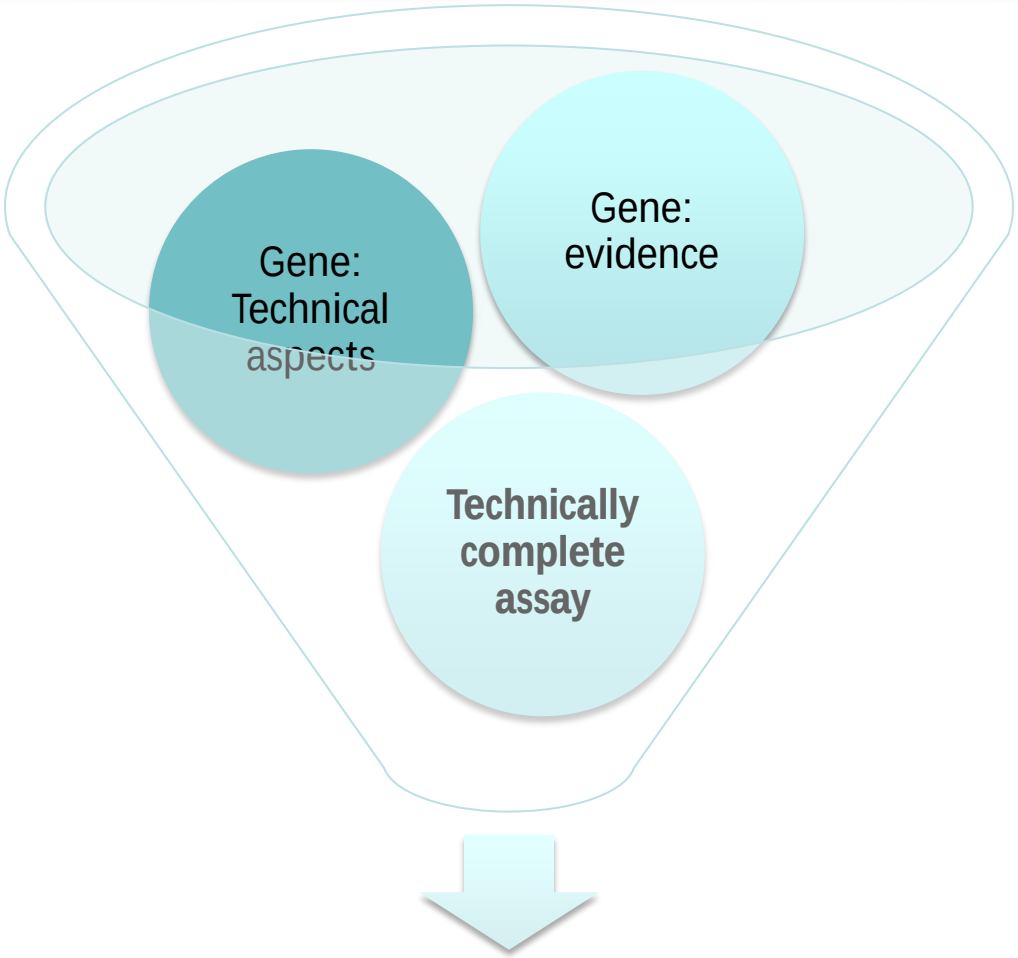
Finishing the exome.....



Medical Exome

just "because three is better than one”

CHOP

Emory

Harvard LMM

Medical Exome

A highly curated gene resource and a technically optimized assay to provide a stepping stone for standardizing interpretation of genetic variation to fulfill the promise of genomic medicine

THE MEDICAL EXOME PROJECT

FOUNDERS

- Emory Genetics Laboratory – *Madhuri Hegde*
- Children's Hospital of Philadelphia – *Avni Santani*
- Harvard/Partners Lab for Molecular Medicine – *Birgit Funke*



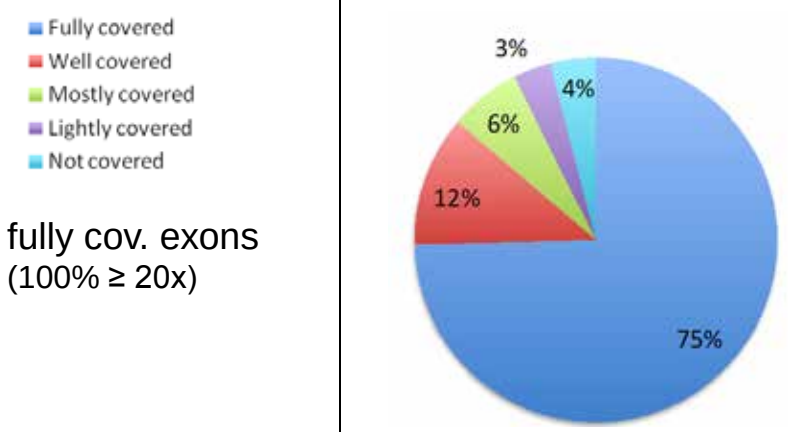
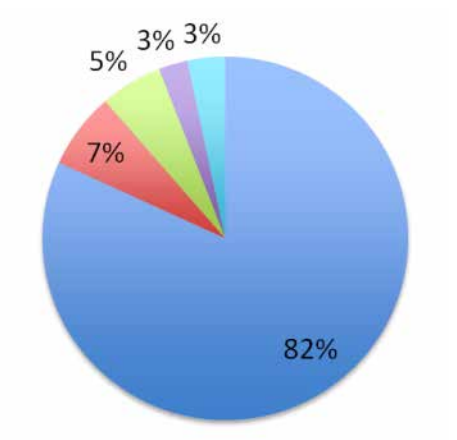
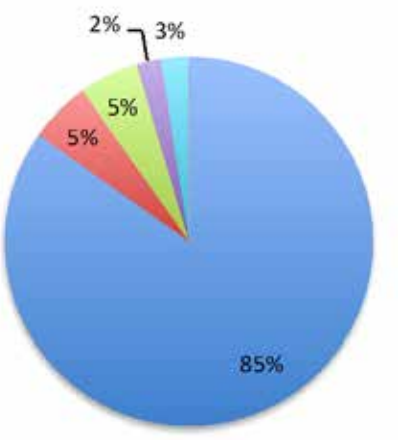
HELP STANDARDIZE MEDICAL EXOME SEQUENCING

- Develop a "medically enhanced exome" capture kit (all clinically significant genes adequately covered) + develop ancillary assays (pseudogenes, CNV detection, repeat regions, epigenetic assays)
- Define medically relevant genes + develop framework for iterative curation
- Support and integrate with evidence-based curation led community efforts
  - *Ledbetter/Martin/Mitchell/Nussbaum/Rehm* (U41)
  - *Berg/Evans/Ledbetter/Watson* (U01)
  - *Bustamante/Plon* (U01)
  - *ClinVar Database* (NCBI)

ClinGen

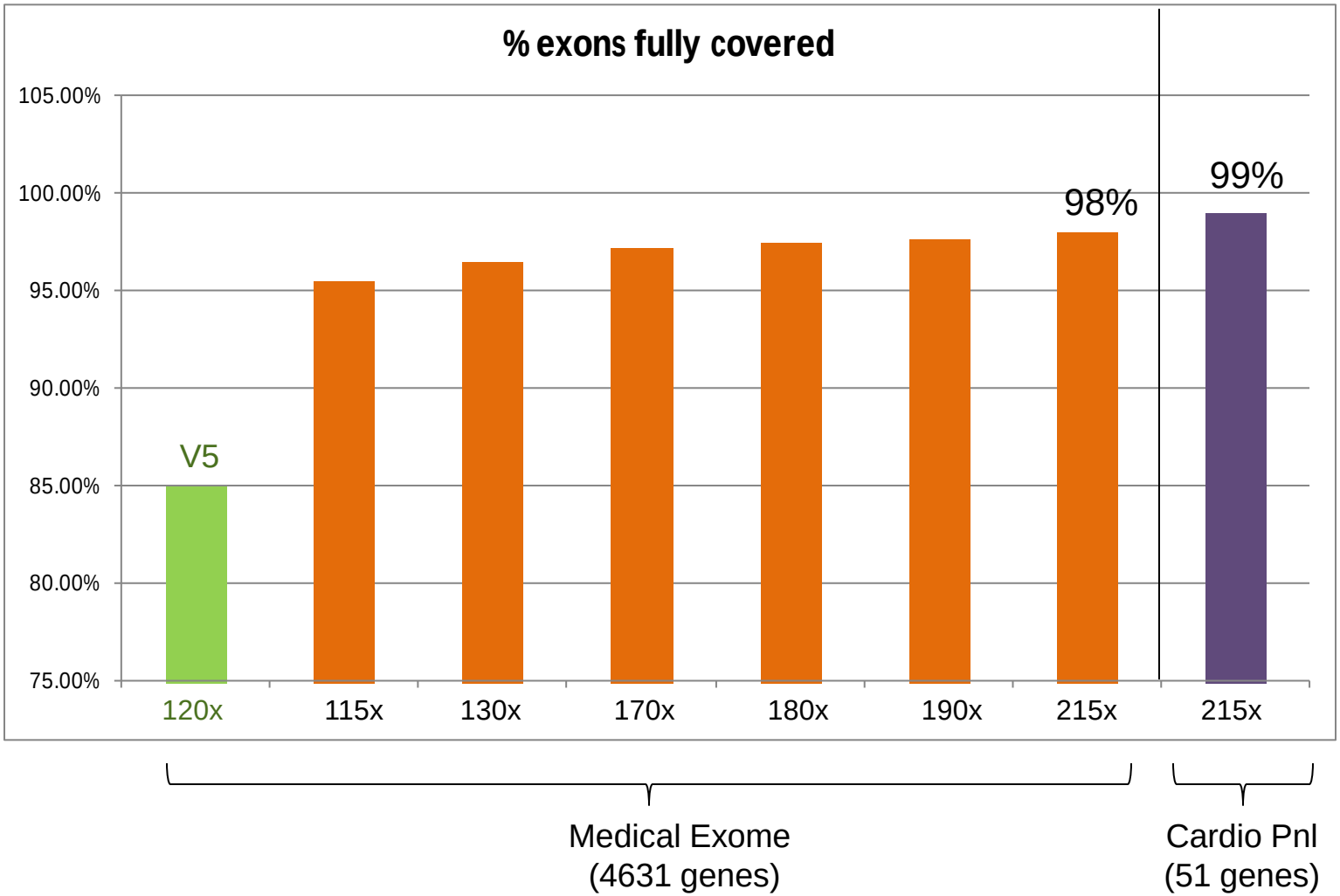
BASE LINE DATA (AGILENT V5)

HISEQ 2500 rapid run; 100x average cov (4-5 samples/ln)

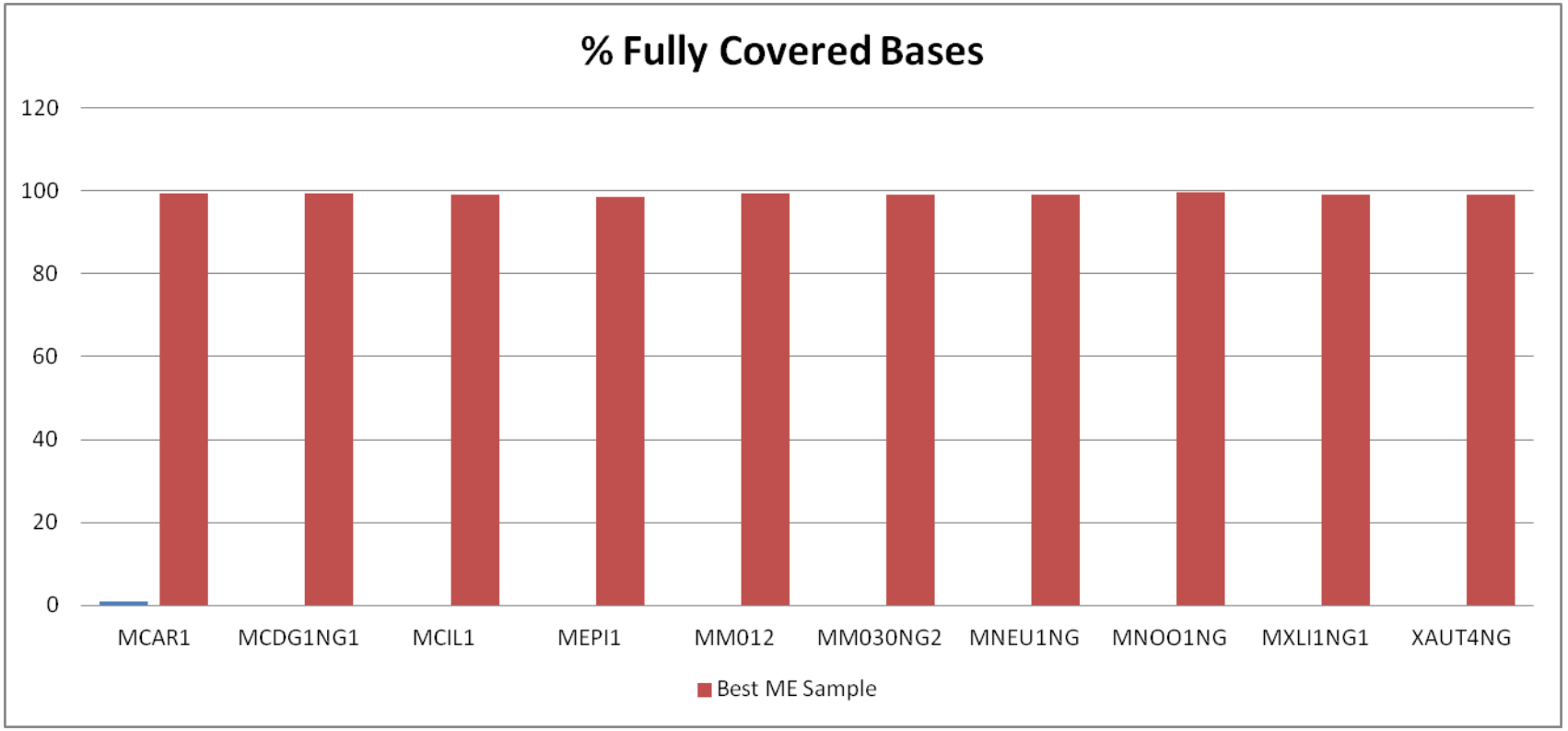
	Whole Exome (all genes)	Medically relevant genes (4631)	Cardiomyopathy Pnl (51)
bases < 20x	5.8% 2.3 Mb / 45 Mb	4.6% 326 Kb / 10.7 Mb	3.7% 9 Kb / 262 Kb
fully cov. exons (100% ≥ 20x)			

LMM Agilent v5-PLUS

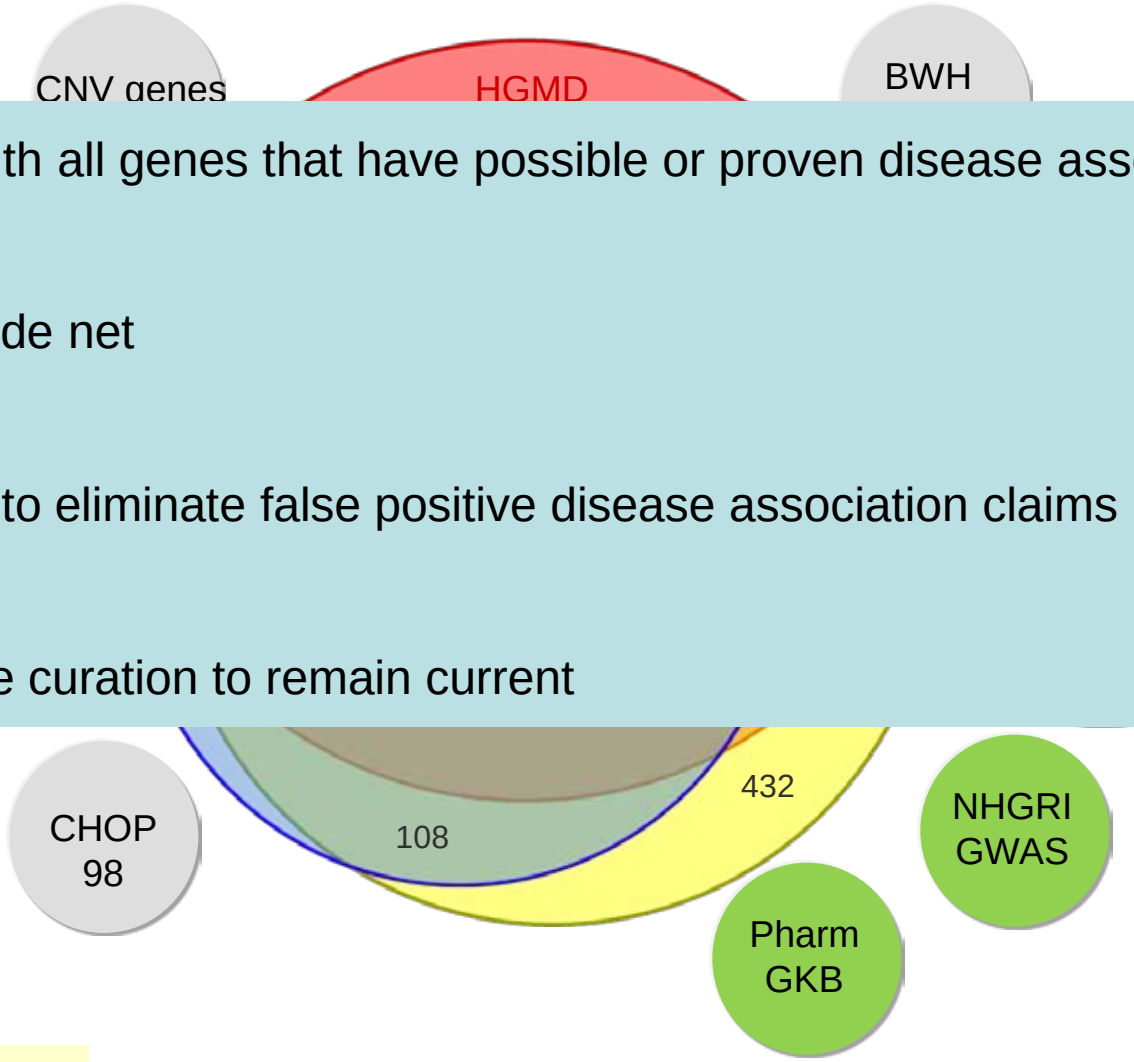
HISEQ 2500 rapid ; 4-5 samples/lane



Performance of Additional Gene Panels (EGL) (V5+ Exome data)



DEFINING THE MEDICAL EXOME



- Start with all genes that have possible or proven disease associations
- Cast wide net
- Curate to eliminate false positive disease association claims
- Iterative curation to remain current

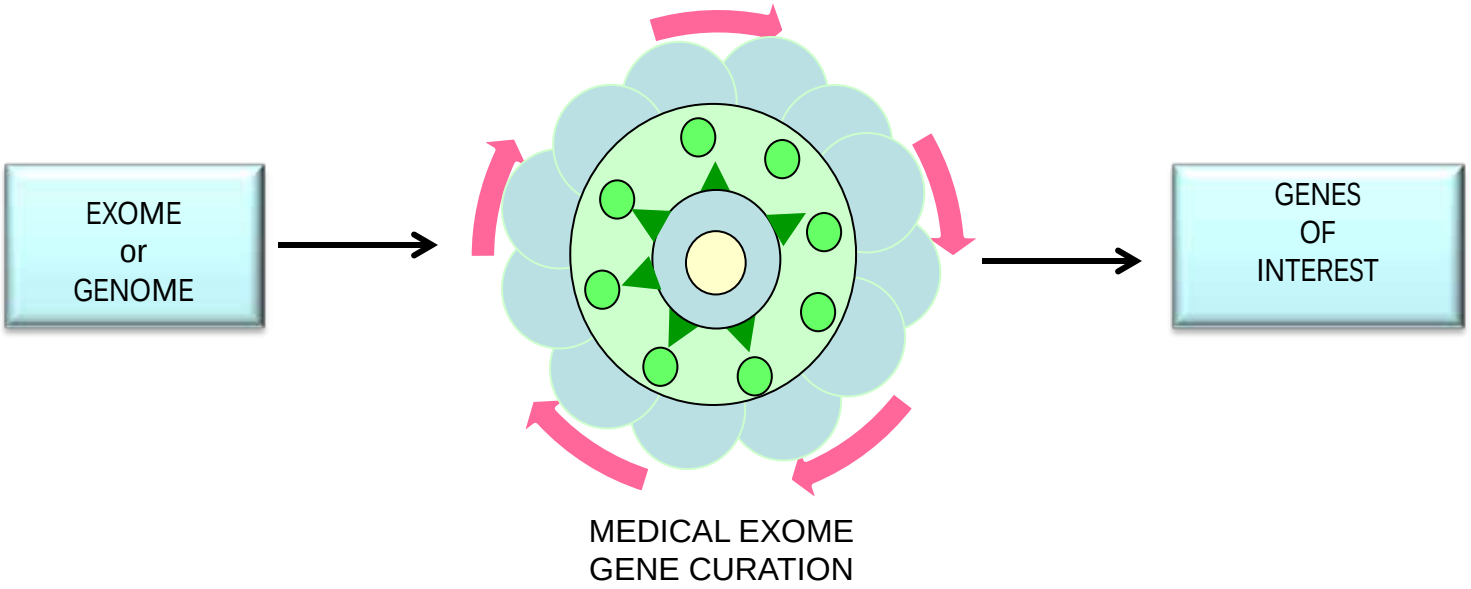
N = 4,371 genes

* OMIM - Morbid Map only

PROPOSED GENE CLASSIFICATION CRITERIA

EVIDENCE LEVEL	DESCRIPTION	CRITERIA
0	Gene of undetermined (no studies available) or unlikely significance	**Undetermined:** No reported evidence **Unlikely:** Evidence arguing against role in disease
1	Gene of "Uncertain Significance" (studies available, but insufficient to draw conclusions)	Single or few studies, variants, and families reported **AND** segregation not established **OR** No human studies reported but strong animal model data with relevance to human disease
2	Probably disease associated	Single or few studies, variants, and families reported **AND** limited segregation observed
3	Definitively established disease gene	Multiple studies, variants, and families reported **AND** significant segregation and/or strong functional evidence

PILOT CURATION



Many incorrect variant/gene and gene/disease associations

Pilot curation	MEDICALLY RELEVANT?			% NOT RELEVANT
	TOTAL	YES + MAYBE	NO	
PILOT GENE SET	535	429	106	20%

RELATED EFFORTS

PUBLSISHED STUDY	PMID	DESCRIPTION	# GENES	MED. EX. Not on published	NOT ON MED EX (V1)	
					not present	reviewed but excluded due to lack of evidence
Medical Exome v1	---	Unique genes with disease associations added and crossreferenced from 12 publically available databases and from curated gene lists from LMM, CHOP, and EGL	4631	---	---	---
ICCG	Rehm and Martin, pers. comm.	Curated dosage-sensitive genes only (as of 01/29/14: 215 haploinsufficient, 3 triplosensitive)	218	N/A	---	---
Boone et al. 2013	22878507	Identified all phenotypes in OMIM with associated disease gene and determined the inheritance pattern for each causal gene. Intersected with large aCGH results database.	1747	2884	1	---
Berg et al. 2012	22995991	Using the OMIM database, identified 2016 genes associated with Mendelian disorders and grouped them into "bins" based on clinical utility and validity	2016	2615	0	---
Solomon et al. 2013	23696674	Curated OMIM for conditions with established genetic causes, and cross-referenced entries with HGMD, PharmGKB, GeneTests, and published literature.	2616	2057	42	---
Koopman et al. 2012	22435372	Used OMIM, Reactome, Genecards, KEGG, UniProtKB, and MITOMAP to establish a comprehensive list of nuclear and mitochondrial genes associated with monogenic mitochondrial disorders	264	4368	1	---
Illumina TruSight One	N/A	Gene panel based on HGMD, OMIM, GeneTests, and a variety of custom panels (Illumina TruSight and other commercially available panels)	4812	635	128	688

JIRA - GENE CURATION DATABASE

- **JIRA – Freely available and centralized database of curated genes**
- **Pilot phase: Collaboration started with Deanna Church at NCBI, now managed by Melissa Landrum**
- **Expert driven gene curation - Capture evidence associating each gene to a disease**
- **Goal of the pilot phase – Mature curation interface and establish a robust workflow for gene curation**
- **Long term goals**
  - **Engage genetics community and invite open collaborations**
  - **Resource for clinical and research laboratories**
  - **Complement existing efforts such as ClinGen, ClinVar and ICCG**
  - **Support variant interpretation and improve patient care**

ANNOTATING GENES

SUMMARY SECTION

- Number of associated diseases with level 1(gene of uncertain significance)
- Number of associated diseases with level 2 (probably disease associated)
- Number of ass
- Medically acti
- ICCG haploin

A - Ontology to e

- OMIM disea
- Indicate if no

B - Basic gene fu

- Alternate nam
- Entrez ID
- Chromosome
- Cytogenetic b
- Genomic coo
- # unique exon
- # transcripts
- List of transcri
- Protein type (s
- Protein functio
- Known protei
- Summary of k
- Summary of n
- Expression – c
- Expression – a
- Pathways (?m
- Tolerance to v

- Summary of v
- Gene regions
- Imprinted gen
- Presence of ps

- Clinical Genor
- OMIM
- ClinVar
- GeneCards
- GeneReviews
- LSDBs
- UCSC, ENSEMBL

- Animal models with human disease phenotype (Y/N)
- Pharmacogenetic marker?

- HGMD
- LOVD
- Jackson Lab MGI database

D Classification by disease

- **Disease 1**
  - Mode of inheritance (multiple if appliccable)
  - Typical age at onset
  - Penetrance
  - Prevalence and expressivity
  - Mosaicism reported?
  - Ethnic/racial preponderance (e.g. Ashkenazi Jewish)
  - Medically actionable (Y/N) + free text
  - Molecular mechanism (gain of function, loss of function)
  - Variant spectrum (note if do novo mutations are common)
  - **Classificaton (disease 1)**
  - **Evidence (disease 1)**

Need to read papers….

PMID1

1. # subjects tested
2. # affecteds carrying a (potentially) significant variant
3. # of variants reported in this paper showing segregation – range 1
4. # of variants reported in this paper showing segregation – range 2
5. # of variants supported by in vitro fx data
6. # of variants supported by in vivo fx data
7. Other annotations TBD

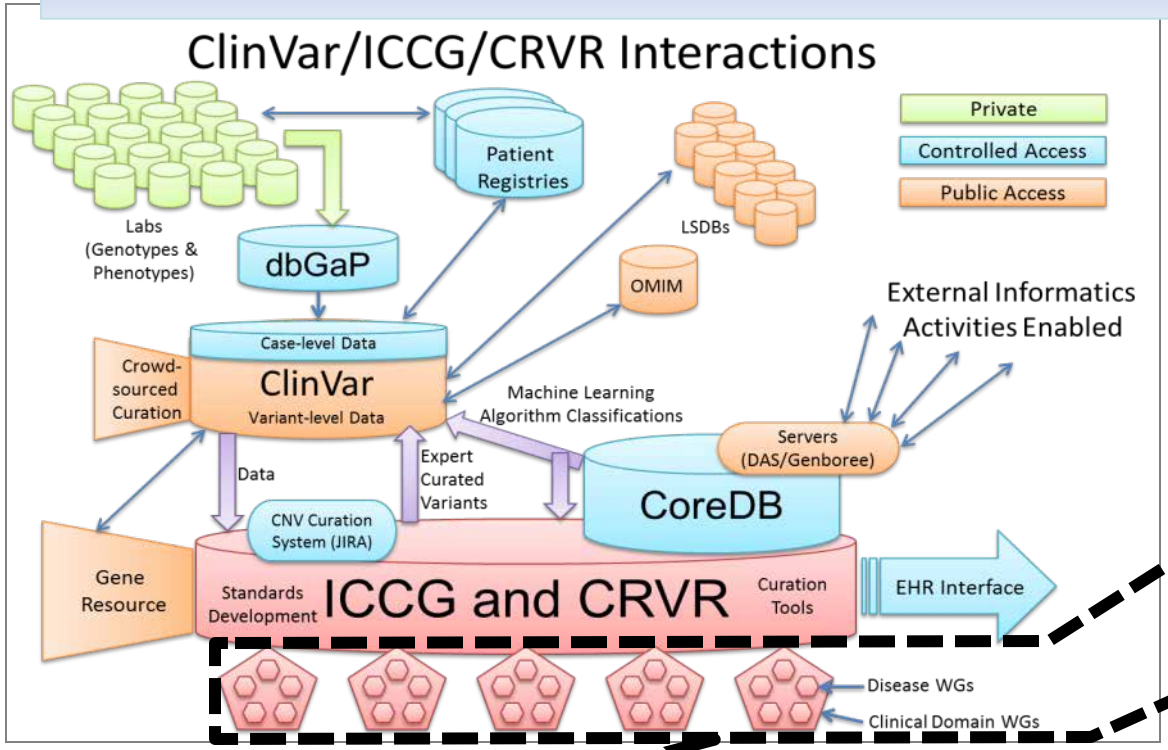
PMID2

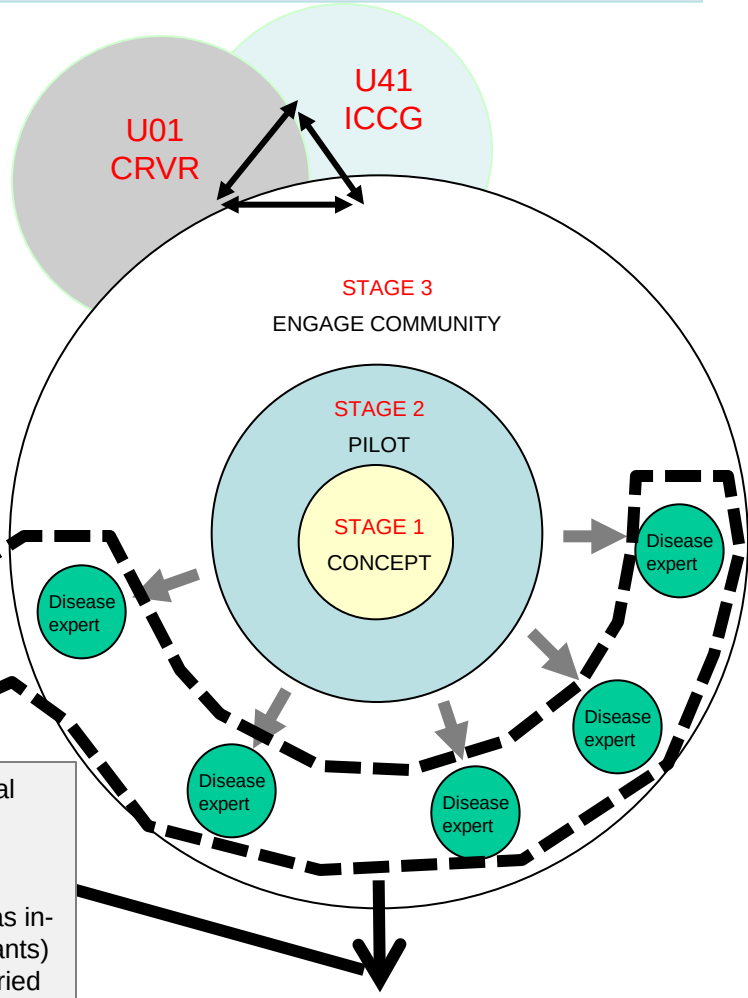
Same fileds as above

- **Disease 2**

Same fileds as above

POSSIBLE INTERACTIONS CLINGEN – MEDICAL EXOME PROJECT

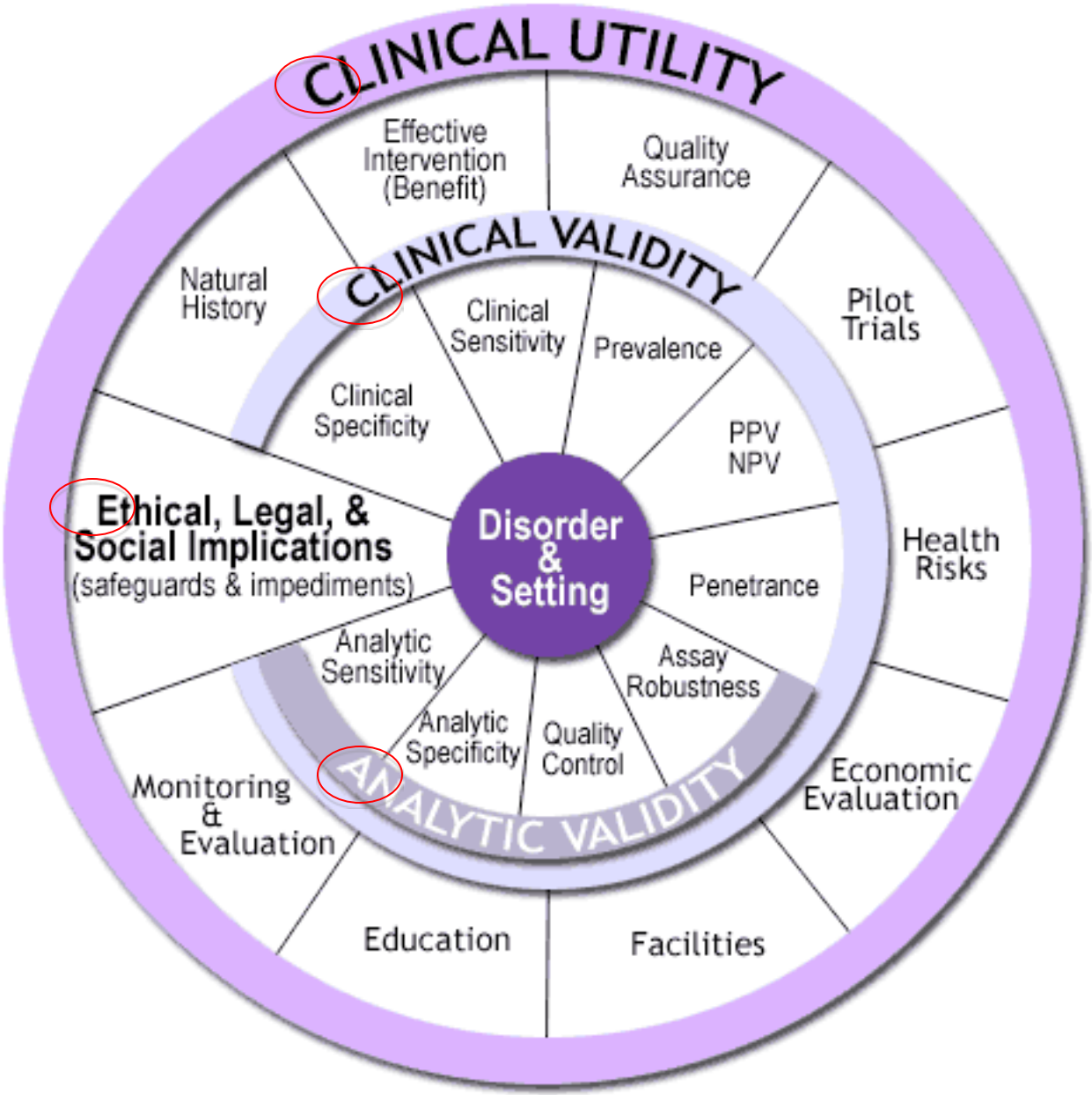




- Educate clinical domain workgroups
- Refine classification as in-depth (all variants) curation is carried out

MIN. ANTICIPATED CURATION TIME		BUDGETED CURATION TIME				
Medical Exome genes	4,631	**Type of Curator**	**#**	**Effort**	**hours/ FTE/yr (52 wks)**	**total hours/year**
Newly added genes over lifetime of grant	500	**Fellows** (this application)	3	80%	1,664	4,992
Total curation time in hours/gene (2 independent reviews)	5	**Fellows** (employed by participating labs)	8	10%	208	1,664
Administrative project coordination (20% effort x 52 weeks x 5 years)	2,080	**Addtl community volunteers** (years 2-5)	20	5%	104	2,080.00
Total curation hours expected (incl.10% safety margin)	**30,509**	Total available hours (years 2-5)				**32,864**

-Curate all genes <u>but not to completion (i.e.</u> assemble sufficient information to bin into
  -3: definitively disease associated
  -2:
  -1:
  -0: No evidence
-Develop gene classification rules
-Prioritize genes for in-depth curation by



http://www.cdc.gov/genomics/gtesting/ACCE/