EMORY GENETICS LABORATORY

Defining actionable discoveries- Annotating Genomes and Reanalysis

A Laboratory Perspective (IOM Roundtable)

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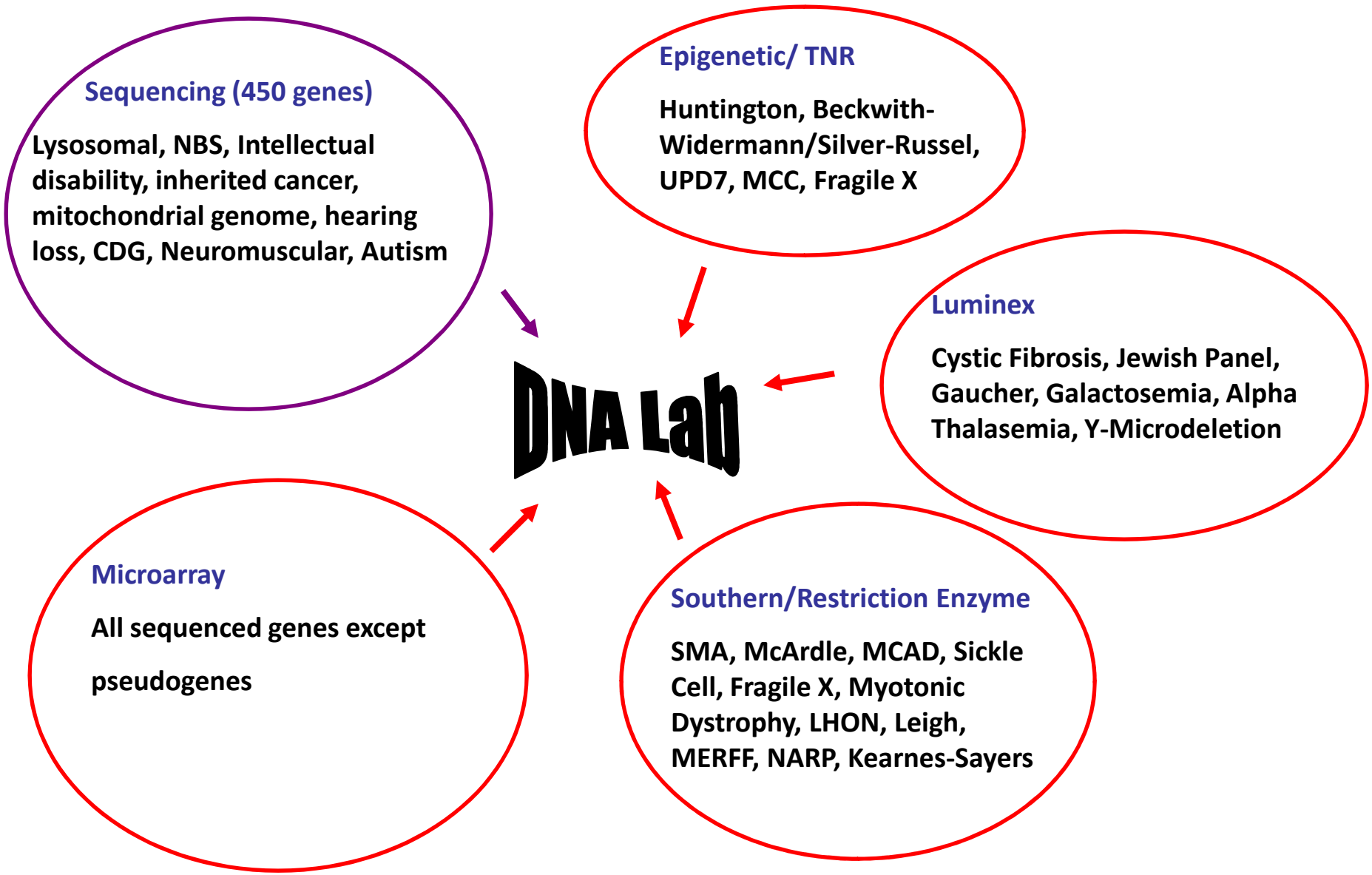
Associate Professor
Scientific Director, Emory Genetics Laboratory
Emory University

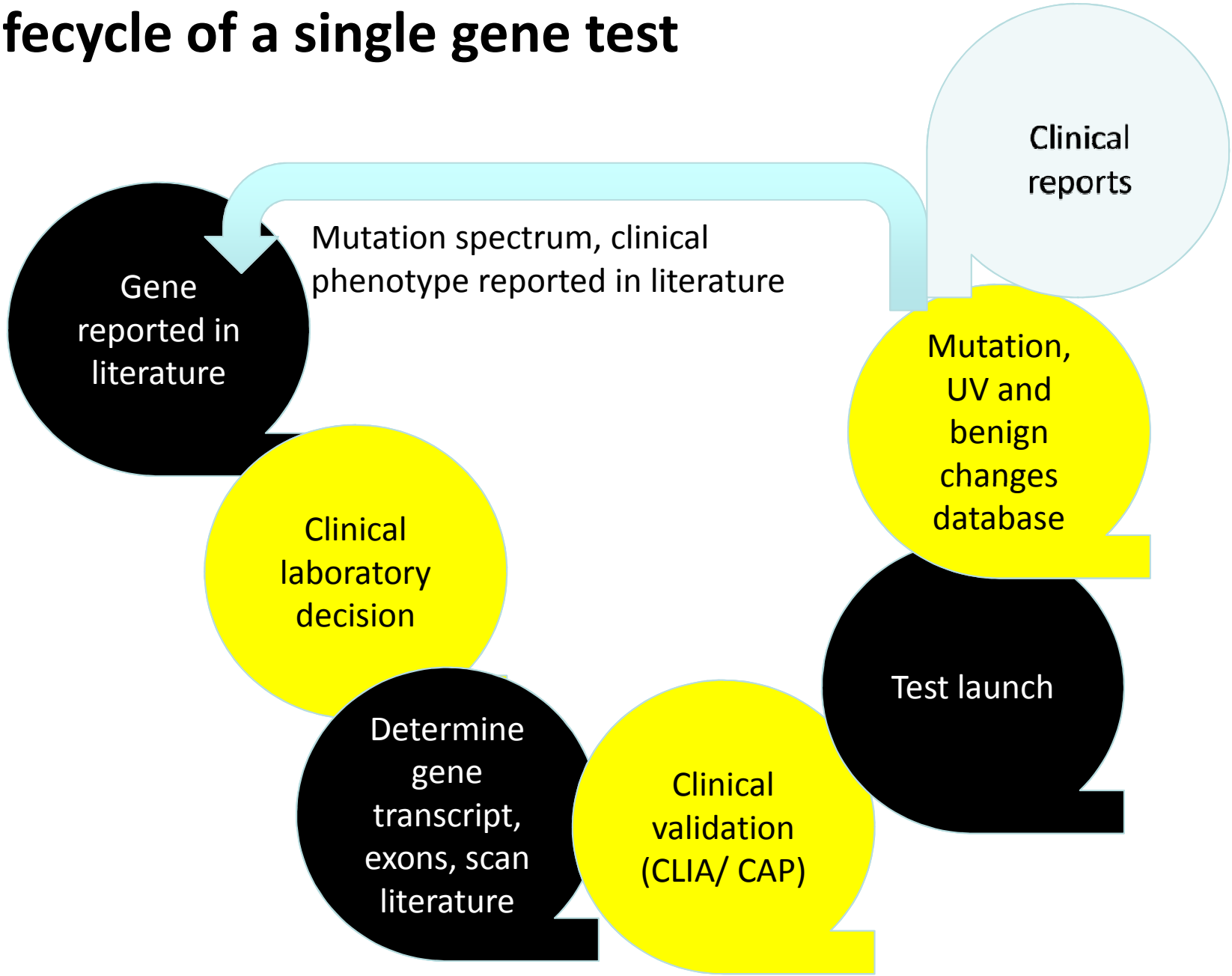
EMORY UNIVERSITY SCHOOL OF MEDICINE

Who are we?

- Department of Human Genetics, Emory University School of Medicine
- Not for profit clinical testing laboratory with a focus on rare genetic disorders
- Comprehensive laboratory
  - Biochemical, Cytogenetic and Molecular Genetics
  - Nutrition and clinical genetics
- Certified by CLIA, CAP, & NY state


Sequencing (450 genes)
Lysosomal, NBS, Intellectual disability, inherited cancer, mitochondrial genome, hearing loss, CDG, Neuromuscular, Autism
Epigenetic/ TNR
Huntington, Beckwith-Widermann/Silver-Russel, UPD7, MCC, Fragile X
Luminex
Cystic Fibrosis, Jewish Panel, Gaucher, Galactosemia, Alpha Thalasemia, Y-Microdeletion
DNA Lab
Microarray
All sequenced genes except pseudogenes
Southern/Restriction Enzyme
SMA, McArdle, MCAD, Sickle Cell, Fragile X, Myotonic Dystrophy, LHON, Leigh, MERFF, NARP, Kearnes-Sayers

Lifecycle of a single gene test



Single genes>>>>>> multi gene panels using next gen sequencing

Nextgen sequencing panels

XLID

- X-linked Intellectual disability
- 92 genes, 1500 amplicons, 898 kb
- Syndromic and non- syndromic
- Availability of positive controls
- Mainly males
- Hemizygous
- All types of genes: metabolic, ID

CDG

- Congenital Disorders of Glycosylation
- 25 genes, 288 amplicons, 101 kb
- Availability of positive controls
- Biochem test (first)

- Molecular confirmation

CMD

- Congenital Muscular Dystrophy
- 13 genes, 383 amplicons, 65 kb
- Genes well characterized
- Availability of positive controls
- Immunohistochemistry

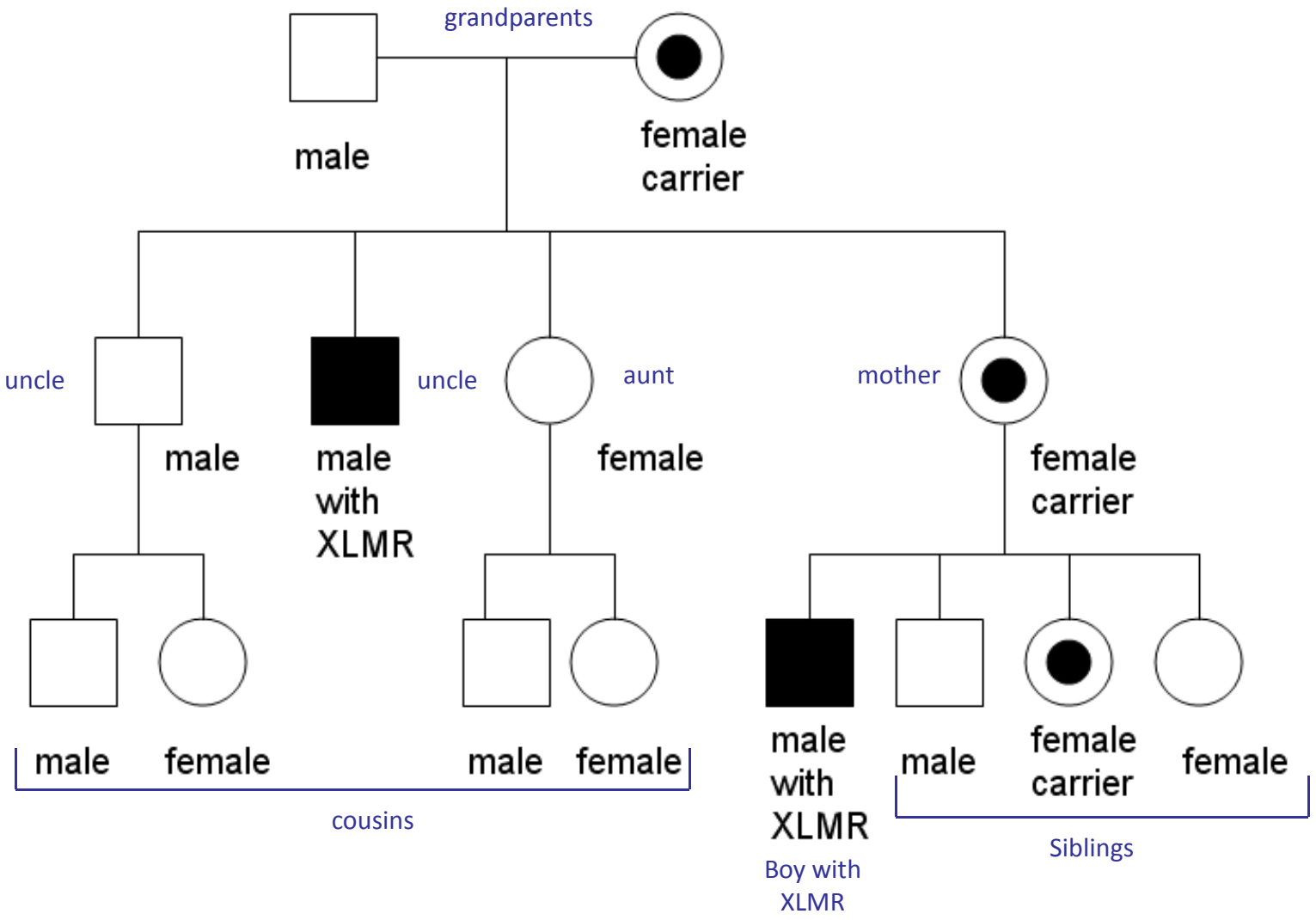
  - Painful muscle biopsy and no definitive results

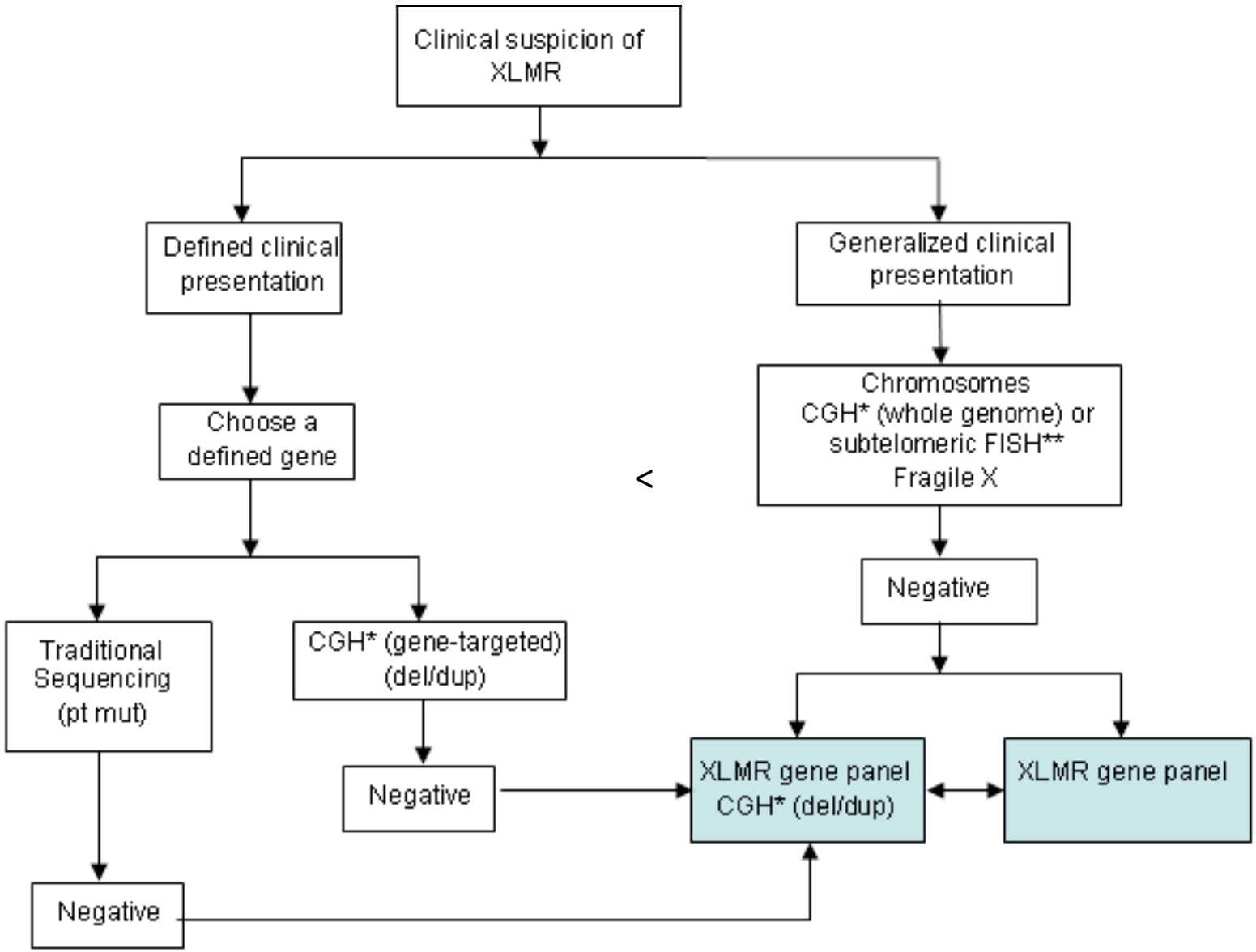
  - Secondary effect

X- linked intellectual disability (XLID)

Example

An example of a pedigree from a family with XLMR (ID)




Clinical suspicion of XLMR
Defined clinical presentation
Generalized clinical presentation
Choose a defined gene
Chromosomes CGH* (whole genome) or subtelomeric FISH** Fragile X
Traditional Sequencing (pt mut)
CGH* (gene-targeted) (del/dup)
Negative
Negative
XLMR gene panel CGH* (del/dup)
XLMR gene panel
Negative

Nature Genetics **41**, 510–512 (1 May 2009) | doi:10.1038/ng0509-510

X-cess of variants in XLMR

David L Nelson & Richard A Gibbs

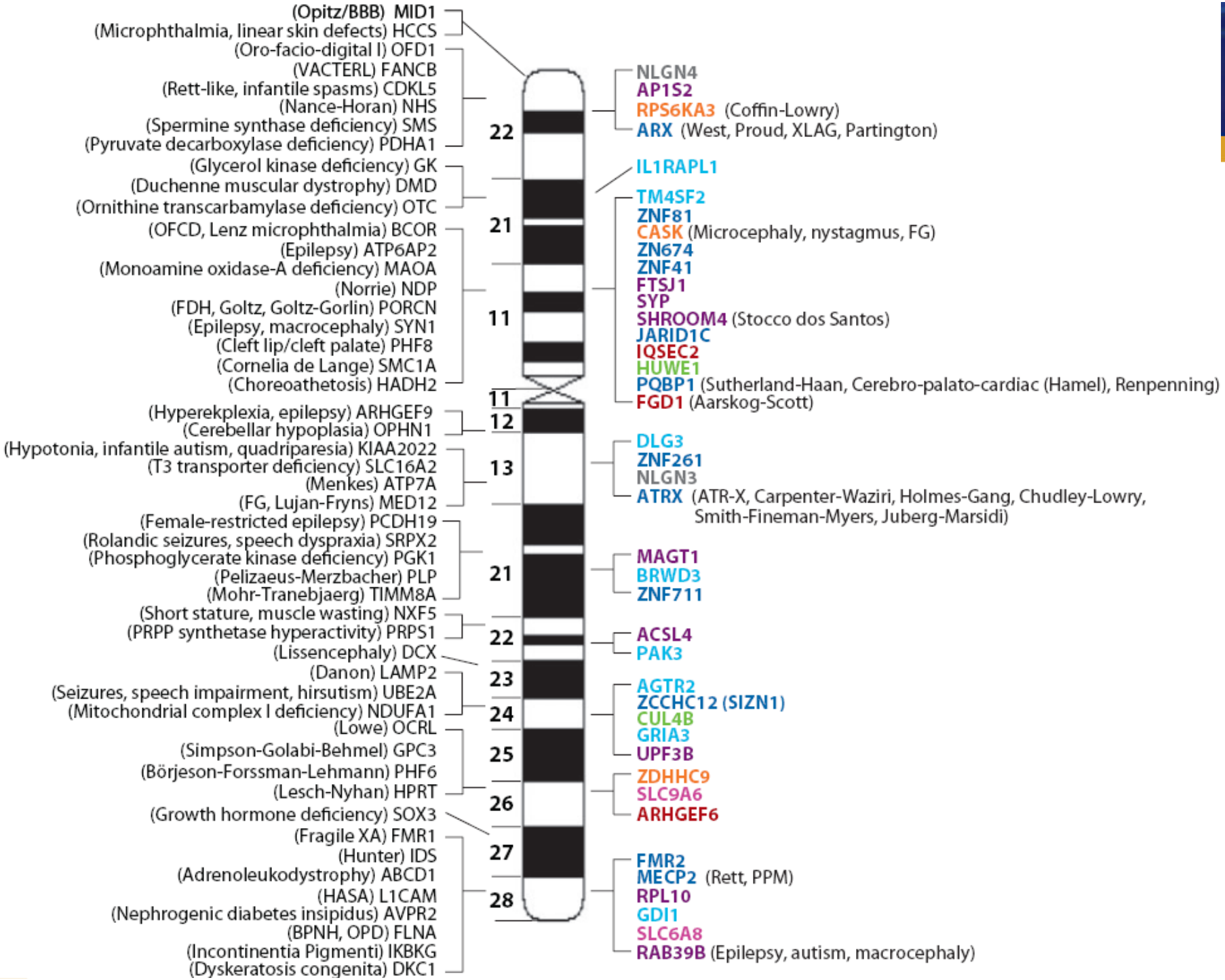
As DNA sequencing technology rapidly improves, the utility of sequencing is moving from the laboratory and becoming part of routine health care. Along this path, large-scale resequencing studies are essential to characterize the variation associated with human diseases.

doi:10.1038/nature09534

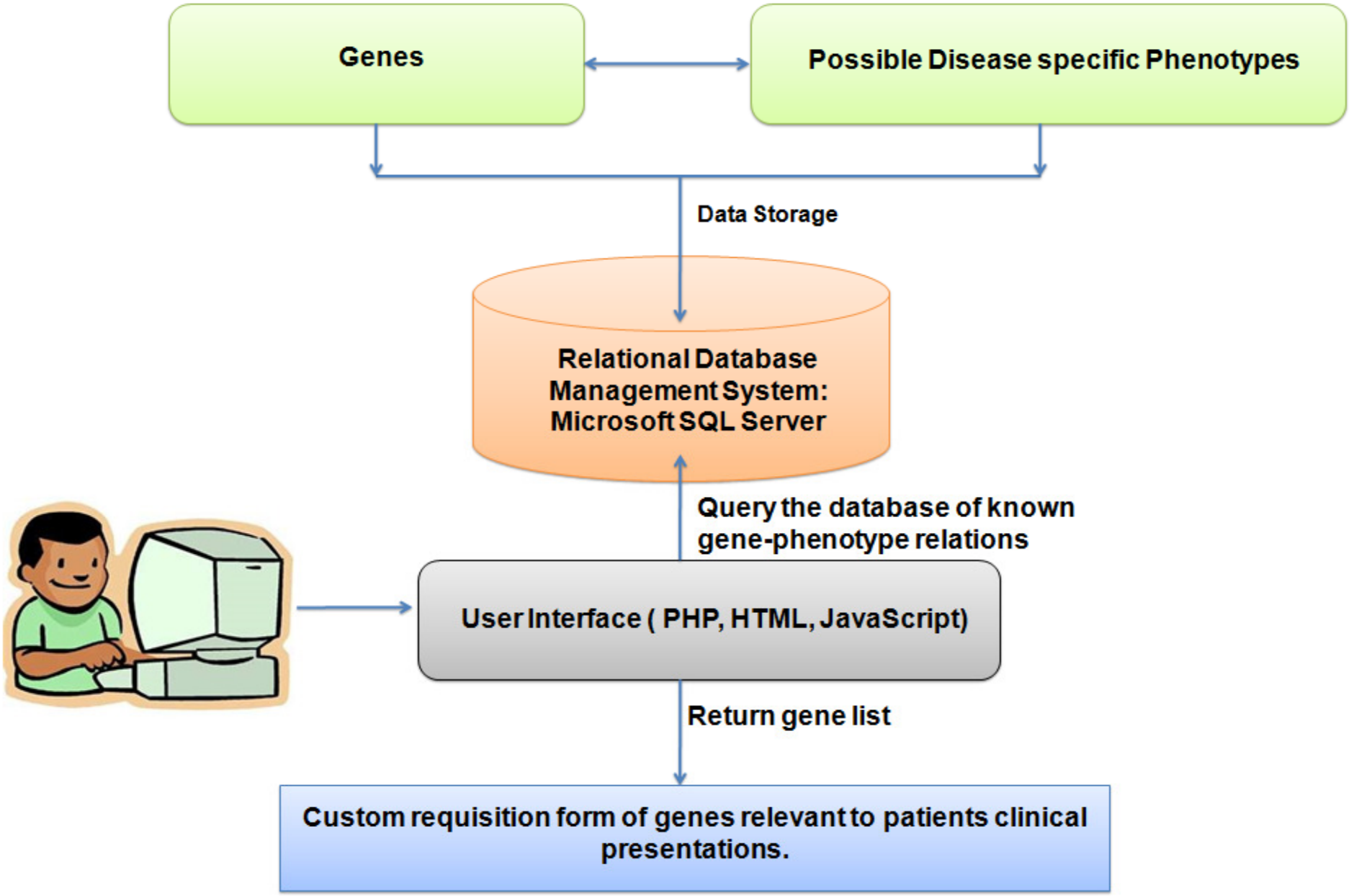
A map of human genome variation from population–scale sequencing

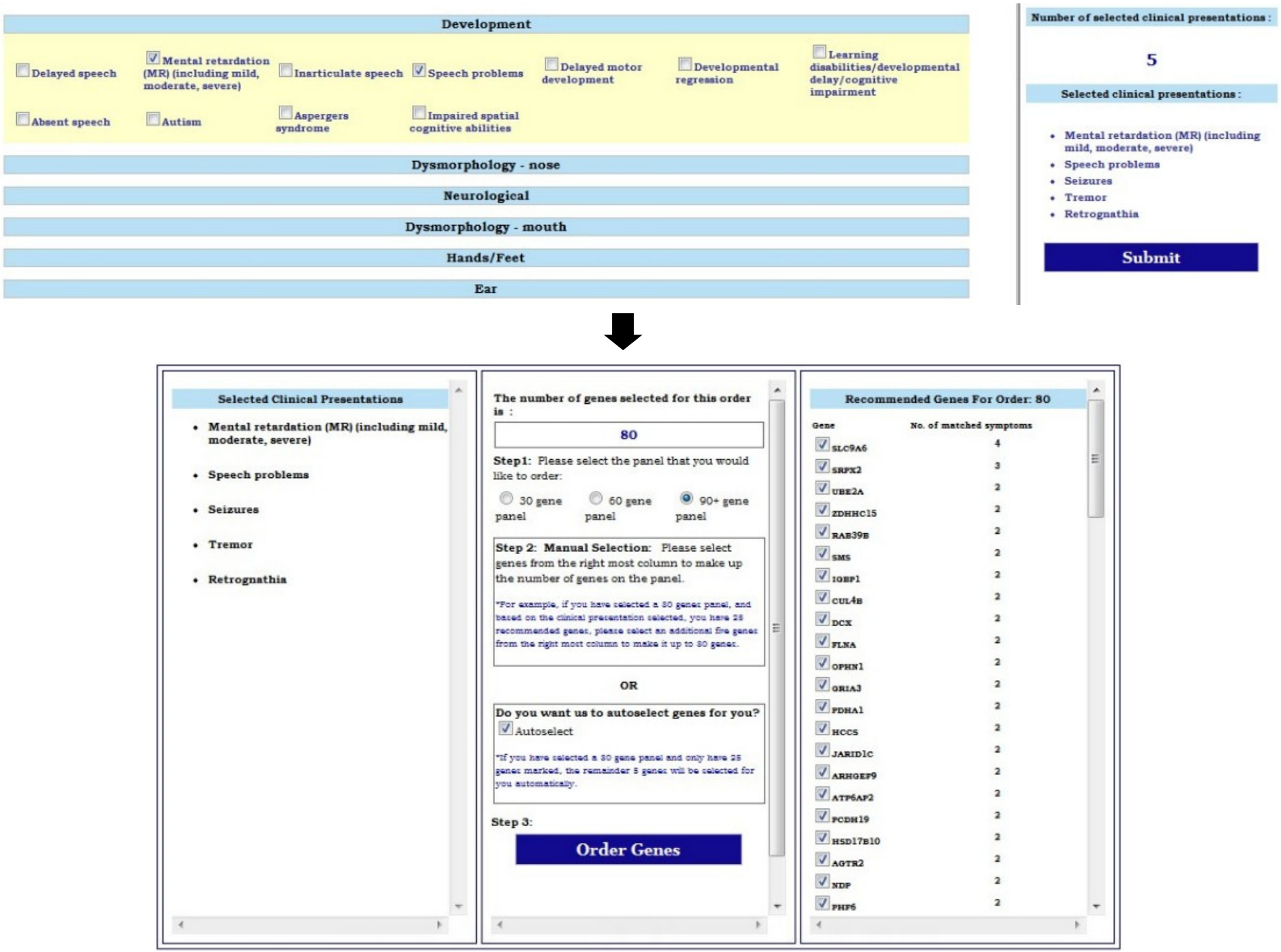
The 1000 Genomes Project Consortium*

The 1000 Genomes Project aims to provide a deep characterization of human genome sequence variation as a foundation for investigating the relationship between genotype and phenotype. Here we present results of the pilot phase of the project, designed to develop and compare different strategies for genome-wide sequencing with high-throughput platforms. We undertook three projects: low-coverage whole-genome sequencing of 179 individuals from four populations; high-coverage sequencing of two mother–father–child trios; and exon-targeted sequencing of 697 individuals from seven populations. We describe the location, allele frequency and local haplotype structure of approximately 15 million single nucleotide polymorphisms, 1 million short insertions and deletions, and 20,000 structural variants, most of which were previously undescribed. We show that, because we have catalogued the vast majority of common variation, over 95% of the currently accessible variants found in any individual are present in this data set. On average, each person is found to carry approximately 250 to 300 loss-of-function variants in annotated genes and 50 to 100 variants previously implicated in inherited disorders. We demonstrate how these results can be used to inform association and functional studies. From the two trios, we directly estimate the rate of *de novo* germline base substitution mutations to be approximately 10^{-8} per base pair per generation. We explore the data with regard to signatures of natural selection, and identify a marked reduction of genetic variation in the neighbourhood of genes, due to selection at linked sites. These methods and public data will support the next phase of human genetic research.


(Opitz/BBB) MID1
(Microphthalmia, linear skin defects) HCCS
(Oro-facio-digital I) OFD1
(VACTERL) FANCB
(Rett-like, infantile spasms) CDKL5
(Nance-Horan) NHS
(Spermine synthase deficiency) SMS
(Pyruvate decarboxylase deficiency) PDHA1
(Glycerol kinase deficiency) GK
(Duchenne muscular dystrophy) DMD
(Ornithine transcarbamylase deficiency) OTC
(OFCD, Lenz microphthalmia) BCOR
(Epilepsy) ATP6AP2
(Monoamine oxidase-A deficiency) MAOA
(Norrie) NDP
(FDH, Goltz, Goltz-Gorlin) PORCN
(Epilepsy, macrocephaly) SYN1
(Cleft lip/cleft palate) PHF8
(Cornelia de Lange) SMC1A
(Choreoathetosis) HADH2
(Hyperekplexia, epilepsy) ARHGEF9
(Cerebellar hypoplasia) OPHN1
(Hypotonia, infantile autism, quadriparesia) KIAA2022
(T3 transporter deficiency) SLC16A2
(Menkes) ATP7A
(FG, Lujan-Fryns) MED12
(Female-restricted epilepsy) PCDH19
(Rolandic seizures, speech dyspraxia) SRPX2
(Phosphoglycerate kinase deficiency) PGK1
(Pelizaeus-Merzbacher) PLP
(Mohr-Tranebjaerg) TIMM8A
(Short stature, muscle wasting) NXF5
(PRPP synthetase hyperactivity) PRPS1
(Lissencephaly) DCX
(Danon) LAMP2
(Seizures, speech impairment, hirsutism) UBE2A
(Mitochondrial complex I deficiency) NDUFA1
(Lowe) OCRL
(Simpson-Golabi-Behmel) GPC3
(Börjeson-Forssman-Lehmann) PHF6
(Lesch-Nyhan) HPRT
(Growth hormone deficiency) SOX3
(Fragile XA) FMR1
(Hunter) IDS
(Adrenoleukodystrophy) ABCD1
(HASA) L1CAM
(Nephrogenic diabetes insipidus) AVPR2
(BPNH, OPD) FLNA
(Incontinentia Pigmenti) IKBKG
(Dyskeratosis congenita) DKC1
22
21
11
11
12
13
21
22
23
24
25
26
27
28
NLGN4
AP1S2
RPS6KA3 (Coffin-Lowry)
ARX (West, Proud, XLAG, Partington)
IL1RAPL1
TM4SF2
ZNF81
CASK (Microcephaly, nystagmus, FG)
ZN674
ZNF41
FTSJ1
SYP
SHROOM4 (Stocco dos Santos)
JARID1C
IQSEC2
HUWE1
PQBP1 (Sutherland-Haan, Cerebro-palato-cardiac (Hamel), Renpenning)
FGD1 (Aarskog-Scott)
DLG3
ZNF261
NLGN3
ATRX (ATR-X, Carpenter-Waziri, Holmes-Gang, Chudley-Lowry,
Smith-Fineman-Myers, Juberg-Marsidi)
MAGT1
BRWD3
ZNF711
ACSL4
PAK3
AGTR2
ZCCHC12 (SIZN1)
CUL4B
GRIA3
UPF3B
ZDHHC9
SLC9A6
ARHGEF6
FMR2
MECP2 (Rett, PPM)
RPL10
GDI1
SLC6A8
RAB39B (Epilepsy, autism, macrocephaly)

Phenotype driven XLID gene selection tool




Development
Delayed speech
Mental retardation (MR) (including mild, moderate, severe)
Inarticulate speech
Speech problems
Delayed motor development
Developmental regression
Learning disabilities/developmental delay/cognitive impairment
Absent speech
Autism
Aspergers syndrome
Impaired spatial cognitive abilities
Dysmorphology - nose
Neurological
Dysmorphology - mouth
Hands/Feet
Ear
Number of selected clinical presentations :
5
Selected clinical presentations :
Mental retardation (MR) (including mild, moderate, severe)
Speech problems
Seizures
Tremor
Retrognathia
Submit
Selected Clinical Presentations
Mental retardation (MR) (including mild, moderate, severe)
Speech problems
Seizures
Tremor
Retrognathia
The number of genes selected for this order is :
80
Step1: Please select the panel that you would like to order:
30 gene panel
60 gene panel
90+ gene panel
Step 2: Manual Selection: Please select genes from the right most column to make up the number of genes on the panel.
*For example, if you have selected a 80 genes panel, and based on the clinical presentation selected, you have 28 recommended genes, please select an additional five genes from the right most column to make it up to 80 genes.
OR
Do you want us to autoselect genes for you?
Autoselect
*If you have selected a 80 gene panel and only have 28 genes marked, the remainder 5 genes will be selected for you automatically.
Step 3:
Order Genes
Recommended Genes For Order: 80
Gene
No. of matched symptoms
SLC9A6 4
SRPX2 3
UBE2A 2
ZDHHC15 2
RAB39B 2
SMS 2
IGBP1 2
CUL4B 2
DCX 2
FLNA 2
OPHN1 2
GRIA3 2
PDHA1 2
HCCS 2
JARID1C 2
ARHGEF9 2
ATP6AP2 2
PCDH19 2
HSD17B10 2
AGTR2 2
NDP 2
PHF6 2

EMORY GENETICS LABORATORY

X-LINKED INTELLECTUAL DISABILITY SEQUENCING PANEL

2165 North Decatur Road | Decatur, Georgia 30033 | Phone: 404-778-8499 or 800-366-1502 | Fax: 404-778-8559 | http://geneticslab.emory.edu

PATIENT INFORMATION

Last Name _____ First _____ MI____

DOB ____/____/____ Pt. ID/Med Rec# _____

Address _____

_____

City _____ State _____ Zip _____

Preferred Phone (____) _____ - _____

Other # where pt. can be reached (____) _____ - _____

Gender: ☐ Male ☐ Female ☐ Unknown ☐ Ambiguous

Parent name (if pt is a minor)/Spouse_____

Ethnicity of pt. (check all that apply): ☐ African-American ☐ Asian

☐ Caucasian/NW European ☐ E. Indian ☐ Hispanic ☐ Jewish-Ashkenazi

☐ Jewish-Sephardic ☐ Mediterranean ☐ Native American

☐ Native Hawaiian/Other Pacific Islander ☐ Other:_____

PHYSICIAN ORDERING TEST (NPI Required)

Name_____

NPI _____

Address _____

_____

City _____ State _____ Zip _____

Phone (____) _____ - _____Fax (____) _____ - _____

Email _____

Genetic Counselor _____

Phone (____) _____ - _____Fax (____) _____ - _____

Email_____

REFERRING HOSPITAL OR LABORATORY

Name_____

NPI _____

Address _____

_____

City _____ State _____ Zip _____

Phone (____) _____ - _____Fax (____) _____ - _____

Email _____

DUPLICATE REPORT TO

Name_____

Address _____

_____

City _____ State _____ Zip _____

Phone (____) _____ - _____Fax (____) _____ - _____

Email _____

SPECIMEN INFORMATION

Date Collected ____/____/____ Time ____:____ ☐ AM ☐ PM

SAMPLE TYPE

☐ Blood (B) ☐ Serum (S) ☐ Urine (U) ☐ Plasma (P) ☐ CSF ☐ DNA

☐ Amniotic Fluid (AF) ☐ POC ☐ Chorionic Villi (CV) ☐ Saliva (SLV)

☐ Fibroblast Cult (CF) ☐ Dried Blood Spot (DBS) ☐ Other

CLINICAL INFORMATION *REQUIRED*

Clinical findings/family history/test results (attach copies if appropriate)

Indication: ☐ Diagnostic ☐ Carrier

Is patient/spouse/family member pregnant?

☐ No ☐ Yes **Check if relevant:** ☐ Egg donor ☐ Sperm donor

LMP_____ EDD_____

Genotypes

Proband _____

Mother _____

Father _____

Diet

☐ Formula ☐ MCT Oil

☐ Breast Milk ☐ Hyperal

☐ Special _____

Medications

☐ Anticonvulsants ☐ Antidepressants ☐ Antibiotics ☐ HAF/TPN

☐ Carnitine ☐ Oral Contraceptives ☐ Other _____

Has Patient had any of the following?

☐ Transfusion ☐ Bone Marrow Transplant

BILLING INFORMATION *Required*

ICD9 Codes:

Please complete billing information on page 2 of this form.

FOR LAB USE ONLY

Recid____/____/____ Unboxed By

Sender:_____

TEMP SPECIMEN COLOR # TUBES

R C F PT.

R C F TES

R C F BIL

Pages PH

Accessioned by: Labeled by:

[ ] Facility Bill [ ] Insurance Bill [ ] Self Pay

INFORMATION FOR HEALTH CARE PROVIDERS AND PATIENTS

Emory Genetics Laboratory retains patient samples indefinitely for validation, educational purposes and/or research. For molecular cytogenet submitted clinical information and test results are also included in HIPAA-compliant, de-identified public databases as part of the National Inst diagnostic testing and our understanding of the relationships between genetic changes and clinical symptoms (for information about the molec consortium website at https://leca.genetics.emory.edu and for information about the molecular genetic database refer to the individual test des sample is maintained. Patients may request to withdraw consent for the storage of their sample and/or use of the data by: 1) calling the labora to speak with a laboratory genetic counselor or 2) visiting our website at http://www.geneticslab.emory.edu/opt-out.

[ ] Refusal for inclusion in these efforts may be indicated by checking this box. (If the box is not checked the data will be anonymiz

NEW YORK STATE PATIENTS ONLY

Physician acknowledges the implications of genetic testing have been discussed with the patient and informed consent from the patient/guard

Signature: Date:

EMORY GENETICS LABORATORY

X-LINKED INTELLECTUAL DISABILITY SEQUENCING PANEL

2165 North Decatur Road | Decatur, Georgia 30033 | Phone: 404-778-8499 or 800-366-1502 | Fax: 404-778-8559 | http://geneticslab.emory.edu

Patient Name: Last_____ First _____ MI _____

For sequential testing, indicate in the 'Order' column, the numerical order for testing to be processed. Example: (1) MCADD Mutation Panel, (2) MCADD Gene Sequencing indicates that (1) MCADD Mutation panel will be run first. If NEGATIVE, (2) MCADD Sequencing will be added.
Note: Call to discuss prenatal molecular genetic testing with the laboratory genetic counselor PRIOR to sending a prenatal sample. 5 ml maternal blood in an EDTA (purple top) tube MUST accompany a prenatal specimen.

The client ordered 90 Gene Panel

Order	MOLECULAR TESTING X-Linked Intellectual Disability	Spec
	OCRL	B
	PDHA1	B
	FTSJ1	B
	AFF2	B
	ARX	B
	ATP7A	B
	IL1RAPL1	B
	MED12	B
	ZDHHC9	B
	SYP	B
	ZNF711	B
	NLGN3	B
	OTC	B
	PGK1	B
	FGD1	B
	FMR1	B
	GDI1	B
	SHROOM4	B
	RPS6KA3	B
	SLC9A6	B
	SYN1	B
	TSPAN7	B
	ACSL4	B
	ARHGEF6	B
	CDKL5	B
	MBTPS2	B
	NLGN4X	B
	PLP1	B
	CUL4B	B
	DCX	B
	DKC1	B
	FANCB	B
	FLNA	B
	IGBP1	B
	ATRX	B
	KLF8	B
	RPL10	B
	MTM1	B
	DMD	B
	GRIA3	B
	LAMP2	B
	PQBP1	B
	PRPS1	B
	SMS	B
	UBE2A	B
	ZDHHC15	B
	RAB39B	B
	ABCD1	B
	OPHN1	B
	PAK3	B
	PHF8	B
	GK	B
	IDS	B
	KIAA2022	B
	MAOA	B
	ZNF81	B
	AGTR2	B
	NDP	B
	NXF5	B
	OFD1	B
	PHF6	B
	HSD17B10	B
	HPRT1	B
	BCOR	B
	MAGT1	B
	MECP2	B
	MID1	B
	SMC1A	B
	UPF3B	B
	ZNF674	B
	NHS	B
	NSDHL	B
	PCDH19	B
	HCCS	B
	JARID1C	B
	L1CAM	B
	SOX3	B
	GRPX2	B
	ARHGEF9	B
	ATP6AP2	B
	NDUFA1	B
	GPC3	B
	PORCN	B
	DLG3	B
	HUWE1	B
	SLC16A2	B
	TIMM8A	B
	SIZN1	B
	ZNF41	B
	AP1S2	B
	BRWD3	B
	CASK	B

CLINICAL FEATURES

Mental retardation (MR) (including mild, moderate, severe)

Speech problems

Seizures

Tremor

Retrognathia

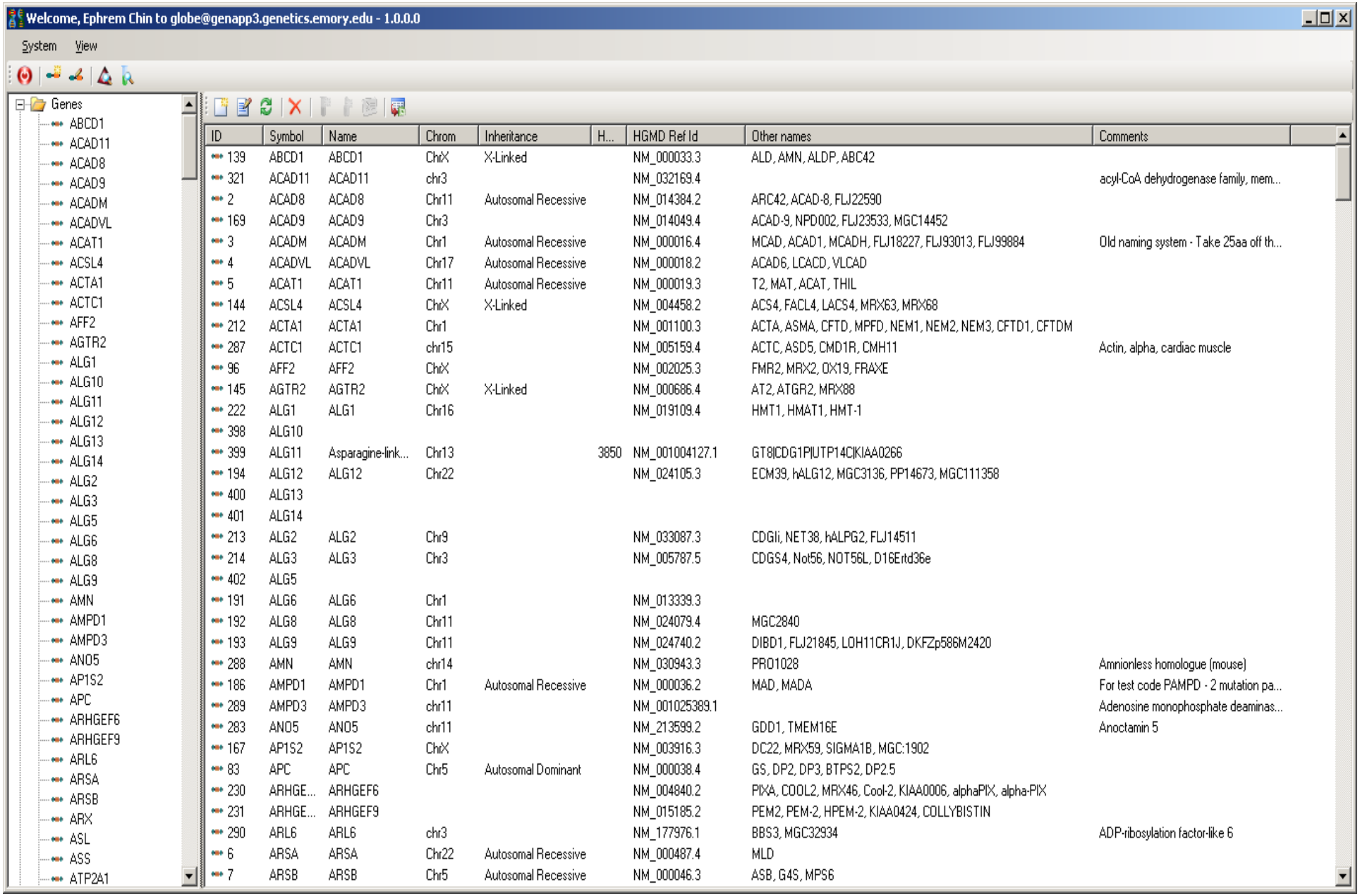
Specimen Codes: AF - Amniotic Fluid, B - Whole Blood, BM - Bone Marrow, DBS - Dried Blood Spot, CF - Cultured Fibroblasts, CSF - Spinal Fluid, CV - Chorionic Villi L - Leukocytes, M - Muscle, O - Other, P- Plasma, POC - Product of Conception, RBC - Red Blood Cells, S - Serum, SLV - Saliva, Slides - Slides, T - Tumor Tissue, U - Urine

Total variants detected using panels

Panel	Number of genes	Number of pseudogenes	Average variants found
XLID	92	3	31
CDG	25	1	20
CMD	13	0	39

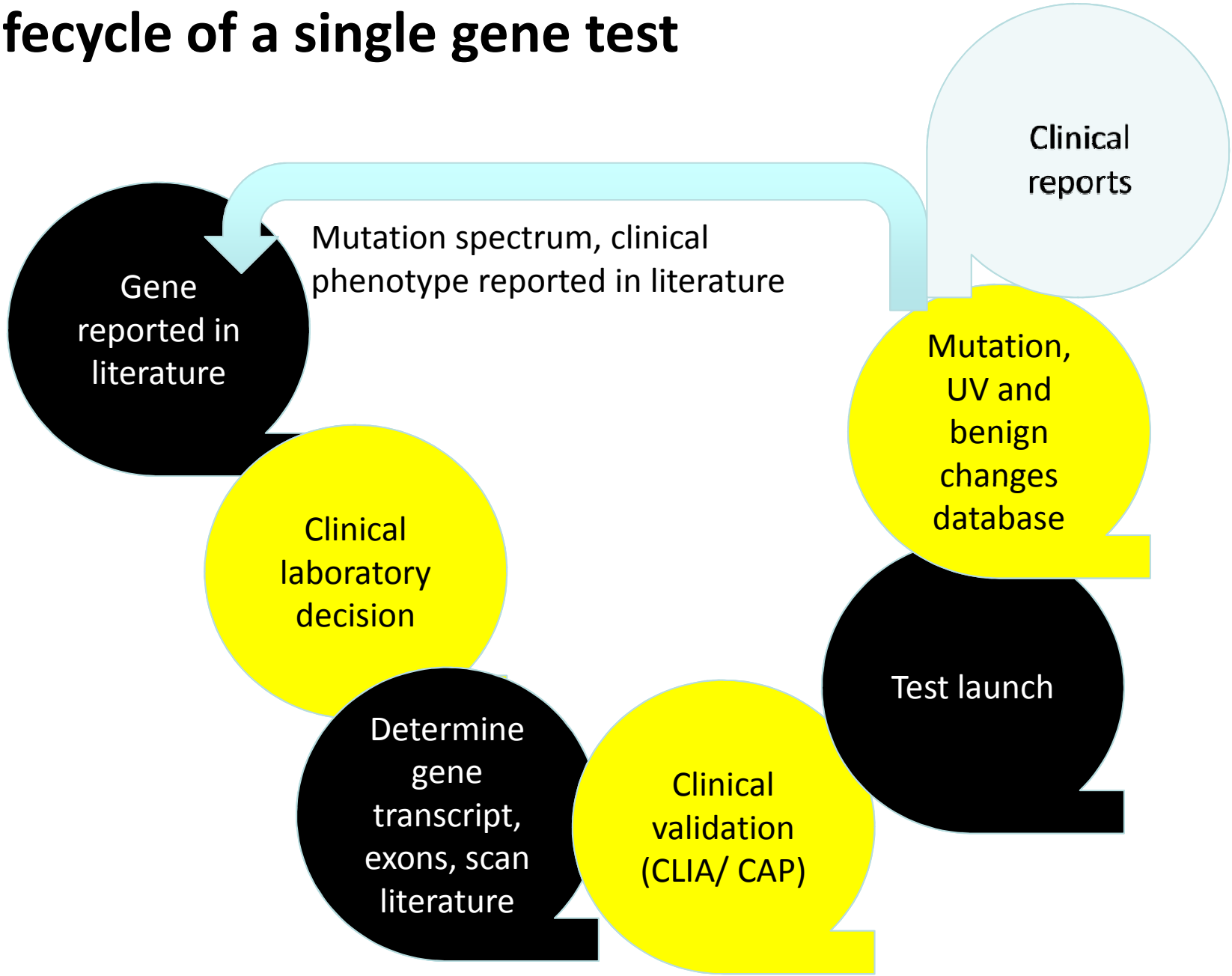
Internal Mutation database for continuous reclassification of variants

Mutation Database



ID	Symbol	Name	Chrom	Inheritance	H...	HGMD Ref Id	Other names	Comments
139	ABCD1	ABCD1	ChrX	X-Linked		NM_000033.3	ALD, AMN, ALDP, ABC42	
321	ACAD11	ACAD11	chr3			NM_032169.4		acyl-CoA dehydrogenase family, mem...
2	ACAD8	ACAD8	Chr11	Autosomal Recessive		NM_014384.2	ARC42, ACAD-8, FLJ22590	
169	ACAD9	ACAD9	Chr3			NM_014049.4	ACAD-9, NPD002, FLJ23533, MGC14452	
3	ACADM	ACADM	Chr1	Autosomal Recessive		NM_000016.4	MCAD, ACAD1, MCADH, FLJ18227, FLJ93013, FLJ99884	Old naming system - Take 25aa off th...
4	ACADVL	ACADVL	Chr17	Autosomal Recessive		NM_000018.2	ACAD6, LCACD, VLCAD	
5	ACAT1	ACAT1	Chr11	Autosomal Recessive		NM_000019.3	T2, MAT, ACAT, THIL	
144	ACSL4	ACSL4	ChrX	X-Linked		NM_004458.2	ACS4, FACL4, LACS4, MRX63, MRX68	
212	ACTA1	ACTA1	Chr1			NM_001100.3	ACTA, ASMA, CFTD, MPFD, NEM1, NEM2, NEM3, CFTD1, CFTDM	
287	ACTC1	ACTC1	chr15			NM_005159.4	ACTC, ASD5, CMD1R, CMH11	Actin, alpha, cardiac muscle
96	AFF2	AFF2	ChrX			NM_002025.3	FMR2, MRX2, OX19, FRAXE	
145	AGTR2	AGTR2	ChrX	X-Linked		NM_000686.4	AT2, ATGR2, MRX88	
222	ALG1	ALG1	Chr16			NM_019109.4	HMT1, HMAT1, HMT-1	
398	ALG10							
399	ALG11	Asparagine-link...	Chr13		3850	NM_001004127.1	GT8|CDG1P|UTP14C|KIAA0266	
194	ALG12	ALG12	Chr22			NM_024105.3	ECM39, hALG12, MGC3136, PP14673, MGC111358	
400	ALG13							
401	ALG14							
213	ALG2	ALG2	Chr9			NM_033087.3	CDGIi, NET38, hALPG2, FLJ14511	
214	ALG3	ALG3	Chr3			NM_005787.5	CDGS4, Not56, NOT56L, D16Ertd36e	
402	ALG5							
191	ALG6	ALG6	Chr1			NM_013339.3		
192	ALG8	ALG8	Chr11			NM_024079.4	MGC2840	
193	ALG9	ALG9	Chr11			NM_024740.2	DIBD1, FLJ21845, LOH11CR1J, DKFZp586M2420	
288	AMN	AMN	chr14			NM_030943.3	PRO1028	Amnionless homologue (mouse)
186	AMPD1	AMPD1	Chr1	Autosomal Recessive		NM_000036.2	MAD, MADA	For test code PAMPD - 2 mutation pa...
289	AMPD3	AMPD3	chr11			NM_001025389.1		Adenosine monophosphate deaminas...
283	ANO5	ANO5	chr11			NM_213599.2	GDD1, TMEM16E	Anoctamin 5
167	AP1S2	AP1S2	ChrX			NM_003916.3	DC22, MRX59, SIGMA1B, MGC:1902	
83	APC	APC	Chr5	Autosomal Dominant		NM_000038.4	GS, DP2, DP3, BTPS2, DP2.5	
230	ARHGE...	ARHGEF6				NM_004840.2	PIXA, COOL2, MRX46, Cool-2, KIAA0006, alphaPIX, alpha-PIX	
231	ARHGE...	ARHGEF9				NM_015185.2	PEM2, PEM-2, HPEM-2, KIAA0424, COLLYBISTIN	
290	ARL6	ARL6	chr3			NM_177976.1	BBS3, MGC32934	ADP-ribosylation factor-like 6
6	ARSA	ARSA	Chr22	Autosomal Recessive		NM_000487.4	MLD	
7	ARSB	ARSB	Chr5	Autosomal Recessive		NM_000046.3	ASB, G4S, MPS6	

Lifecycle of a single gene test



Genome web article – 25 May 2010

When it costs $1,000 to sequence a genome, why would you take a test that costs $5,000?

Callum Bell, program lead at the NCGR

Whole ex/ge “ome” analysis

Challenges

- Data interpretation- mapping, SNP and indel detection

- Who will do the analysis? Bioinformatics

- Time needed to do analysis

- Reclassification of VOUS

- Gene discovery in a clinical setting?
  - Identification of mutation (truncating vs. missense) in new gene of unknown function (e.g. biochemical)

- CLIA / CAP Certification

- Reagent cost vs. cost of the test

- How will the reports be written?

Future of Sequencing in a clinical laboratory

Strategy

- Use experience from single gene and panel sequencing

- Clinical testing (CLIA/CAP) based on known disease associated genes, positive controls available

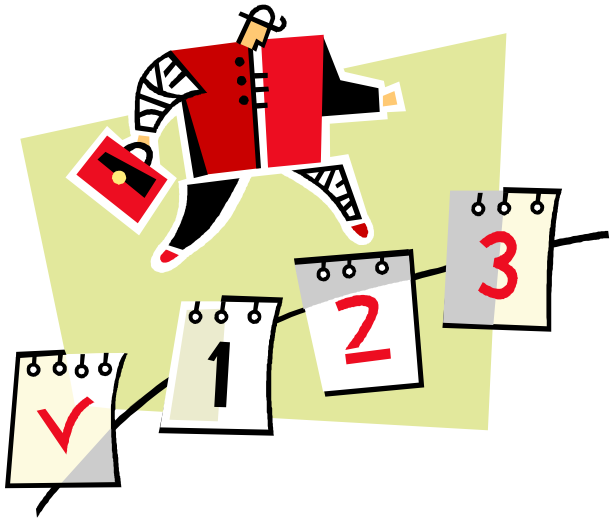
- Single gene (SS) >> Panels (SS or NGS) >> Exome >> Genome

- Logical interpretation of the data

- Exome / Genome analysis involves a TEAM
    - Clinical & Laboratory Geneticists, Genetic Counselor, and other healthcare specialist

- More appropriate for familial cases vs. isolated cases

- Clinical presentation



Sequencing panels

Exome

Genome

Databasing of sequence variation

- **Core laboratories**
- **Clinically curated variants**

Questions?