“The Future of Pediatric Diagnostics is Now”

Patrice M. Milos, Ph.D.
President and CEO, Claritas Genomics
August 21st, 2013

CLARITAS GENOMICS

BioConference Live Presentation

Presentation Overview

- Why is Pediatric Genetics So Important Today?
- The Exponential Increase In Technologies
- Our Growing Knowledge of Human Disease Genetics
- The Evolving Use of Genetic Testing
- Translating Our Knowledge for Pediatric Healthcare
- The Key Differentiators of Claritas Genomics

Our Knowledge of Disease Genetics Is Rapidly Expanding

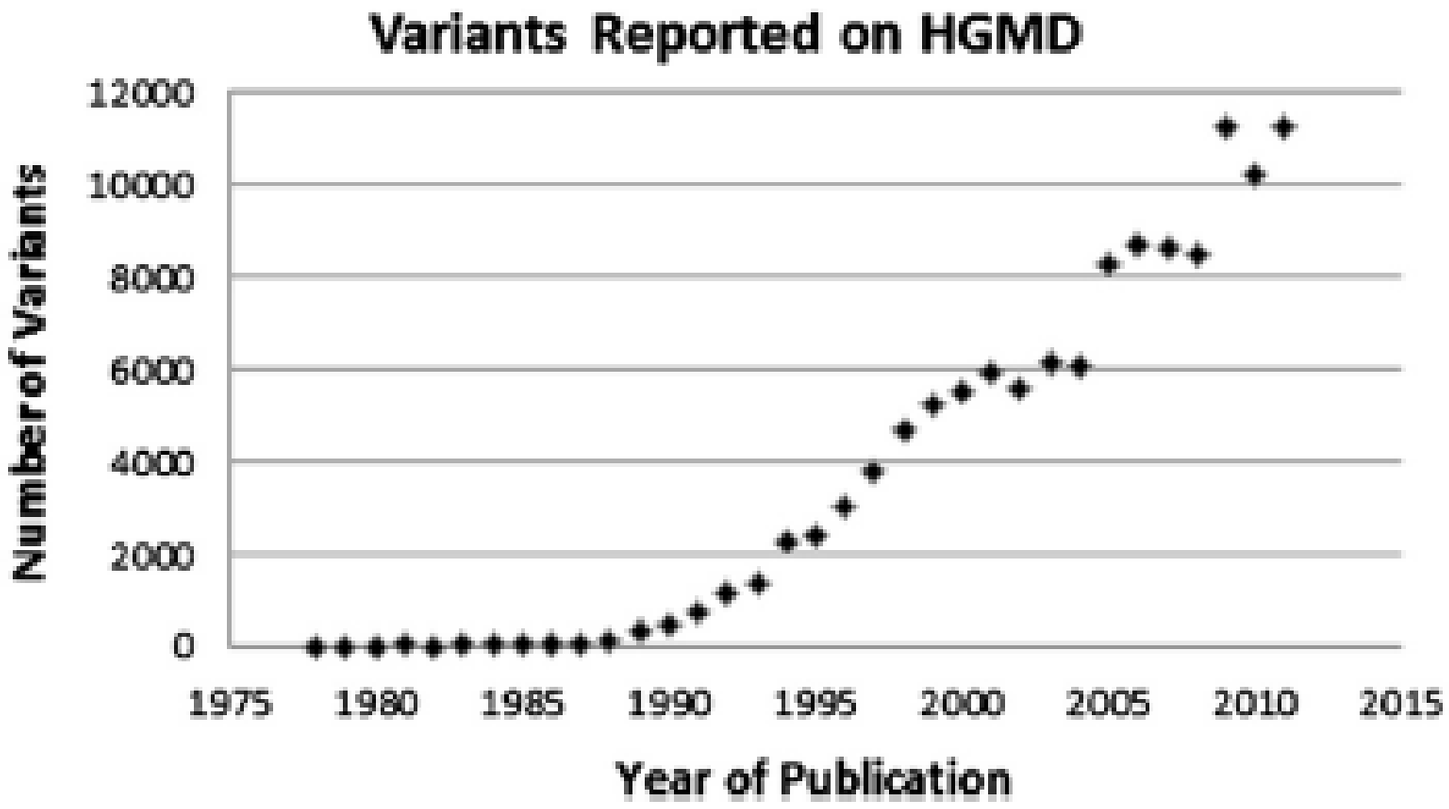


Figure 1. Total human disease variants reported on Human Gene Mutation Database (HGMD) according to the year of publication.

Canadian Journal of Cardiology (2013) 29:934-939

Gene Therapy to Correct Wiskott-Aldrich Syndrome

Sciencexpress Research Articles

Lentiviral Hematopoietic Stem Cell Gene Therapy in Patients with Wiskott-Aldrich Syndrome

Alessandro Aiuti,[1,2,3,4]* Luca Biasco,[1]† Samantha Scaramuzza,[1]† Francesca Ferrua,[2,3,5] Maria Pia Cicalese,[2,3] Cristina Baricordi,[1] Francesca Dionisio,[1] Andrea Calabria,[1] Stefania Giannelli,[1] Maria Carmina Castiello,[1,5] Marita Bosticardo,[1] Costanza Evangelio,[2,3] Andrea Assanelli,[3,6] Miriam Casiraghi,[2] Sara Di Nunzio,[2] Luciano Callegaro,[2] Claudia Benati,[7] Paolo Rizzardi,[7] Danilo Pellin,[8] Clelia Di Serio,[8] Manfred Schmidt,[9] Christof Von Kalle,[9] Jason Gardner,[10] Nalini Mehta,[11] Victor Neduva,[11] David J. Dow,[11] Anne Galy,[12] Roberto Miniero,[13] Andrea Finocchi,[4] Ayse Metin,[14] Pinaki Banerjee,[15] Jordan Orange,[15] Stefania Galimberti,[16] Maria Grazia Valsecchi,[16] Alessandra Biffi,[1,2,3] Eugenio Montini,[1] Anna Villa,[1,17] Fabio Ciceri,[3,6] Maria Grazia Roncarolo,[1,2,3,5‡] Luigi Naldini[1,5‡]

Wiskott-Aldrich syndrome (WAS) - Inherited immunodeficiency caused by mutations in the gene encoding WASP, a protein regulating the cytoskeleton

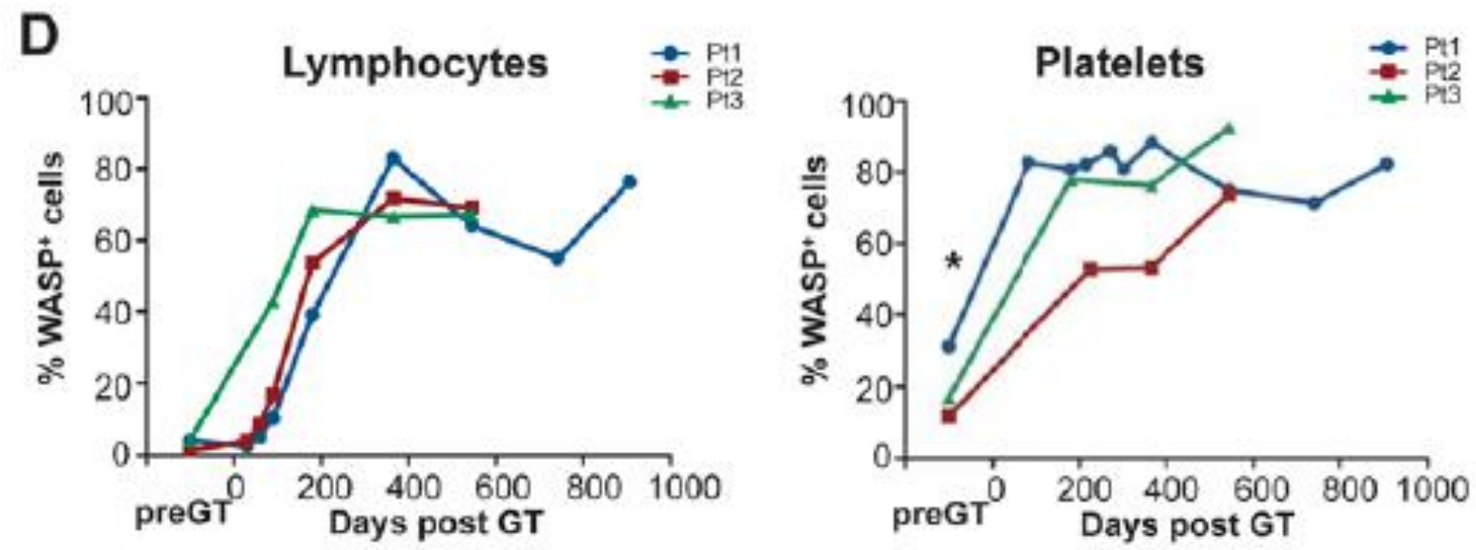
Science. 2013 Jul 11. [Epub ahead of print]

Correction of Genetic Deficiency for >20 Months

	Patient 1	Patient 2	Patient 3
WAS mutation	Exon 10: C>T 995 (R321X)	IVS10del11nt	37C>T (R13X)
WASP expression	<5%	<5%	<5%

Following stem cell transduction with WT WAS

Vector copies/genome	1.9 (BM)-1.4 (MPB)	2.4	2.8
Transduction efficiency (CFC)	92% (BM)–88% (MPB)	97%	100%
Follow-up (mo)	32	23	20



Gene Therapy for Metachromatic Leukodystrophy

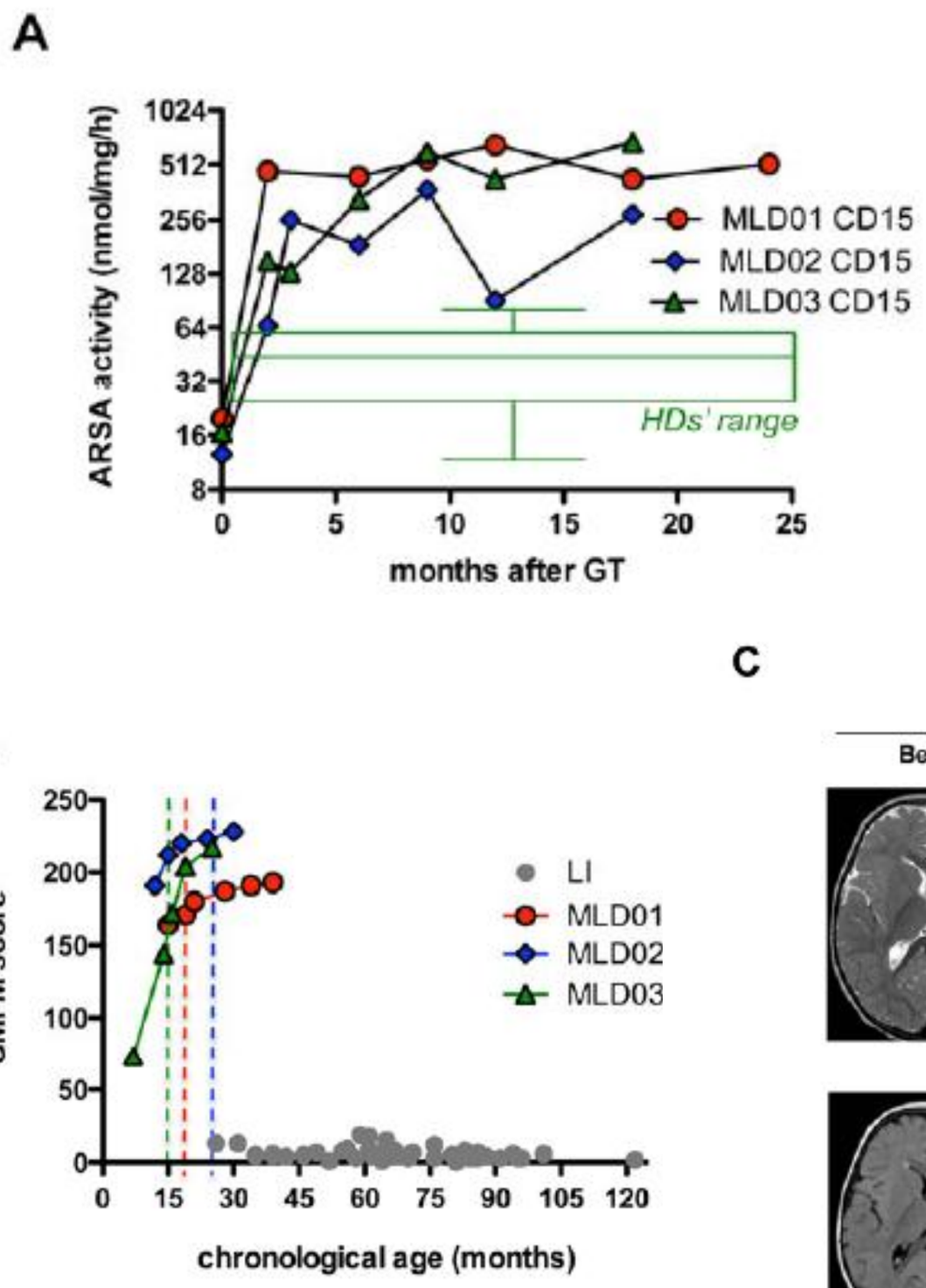
Sciencexpress Research Articles

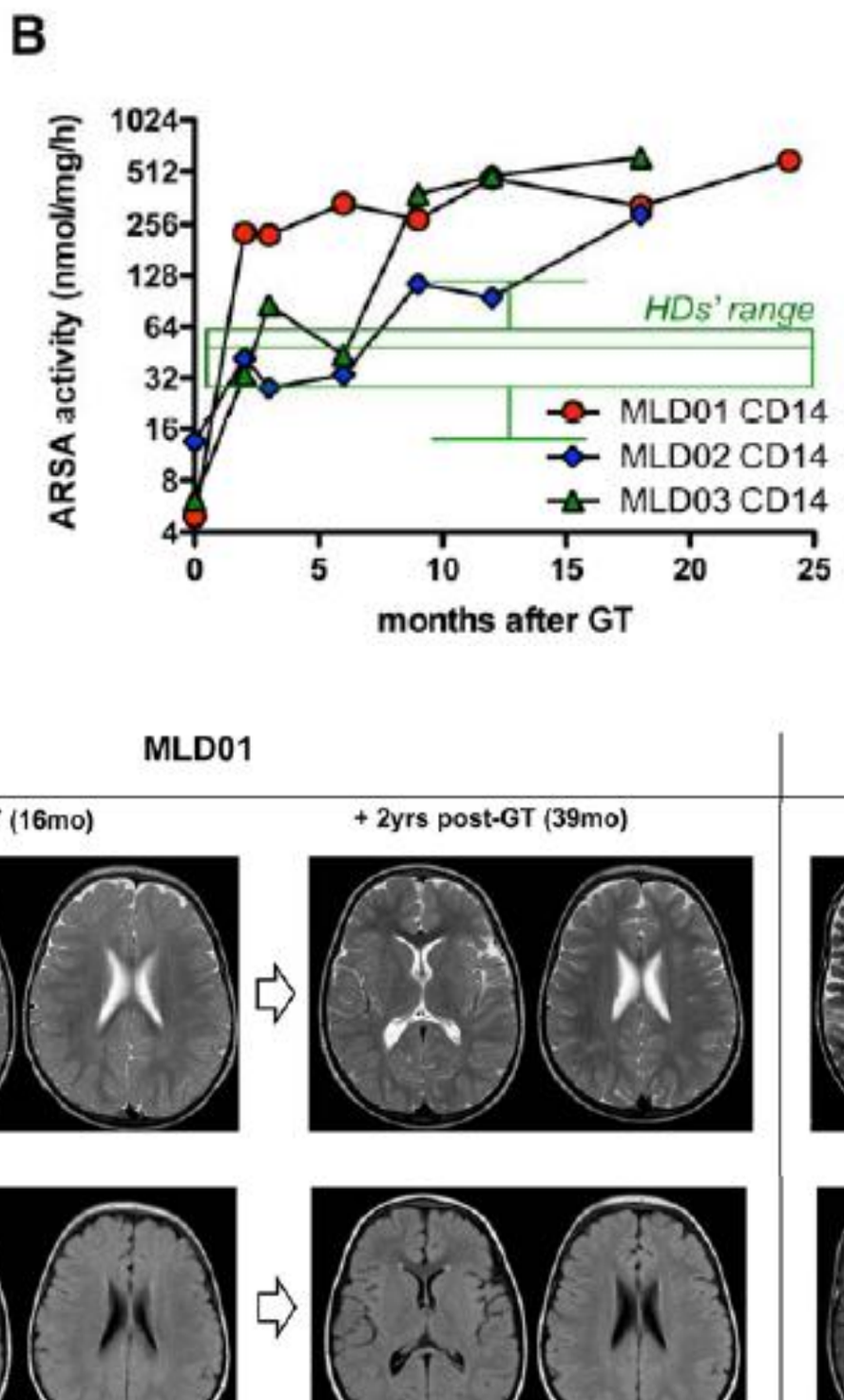
Lentiviral Hematopoietic Stem Cell Gene Therapy Benefits Metachromatic Leukodystrophy

Alessandra Biffi,[1,2,3]*§ Eugenio Montini,[1]* Laura Lorioli,[1,2,3,4] Martina Cesani,[1] Francesca Fumagalli,[2,4,5] Tiziana Plati,[1] Cristina Baldoli,[6] Sabata Martino,[7] Andrea Calabria,[1] Sabrina Canale,[2] Fabrizio Benedicenti,[1] Giuliana Vallanti,[8] Luca Biasco,[1] Simone Leo,[9] Nabil Kabbara,[10] Gianluigi Zanetti,[9] William B. Rizzo,[11] Nalini A. L. Mehta,[12] Maria Pia Cicalese,[2,3] Miriam Casiraghi,[2] Jaap J. Boelens,[13] Ubaldo Del Carro,[5] David J. Dow,[12] Manfred Schmidt,[14] Andrea Assanelli,[3,15] Victor Neduva,[12] Clelia Di Serio,[4] Elia Stupka,[16] Jason Gardner,[17] Christof von Kalle,[14] Claudio Bordignon,[4,8] Fabio Ciceri,[3,15] Attilio Rovelli,[18] Maria Grazia Roncarolo,[1,2,3,4] Alessandro Aiuti,[1,2,3,19] Maria Sessa,[2,5] Luigi Naldini[1,4]§

Metachromatic leukodystrophy (MLD) - Inherited lysosomal storage disease caused by arylsulfatase A (ARSA) deficiency

Genetic Correction of Leukodystrophy





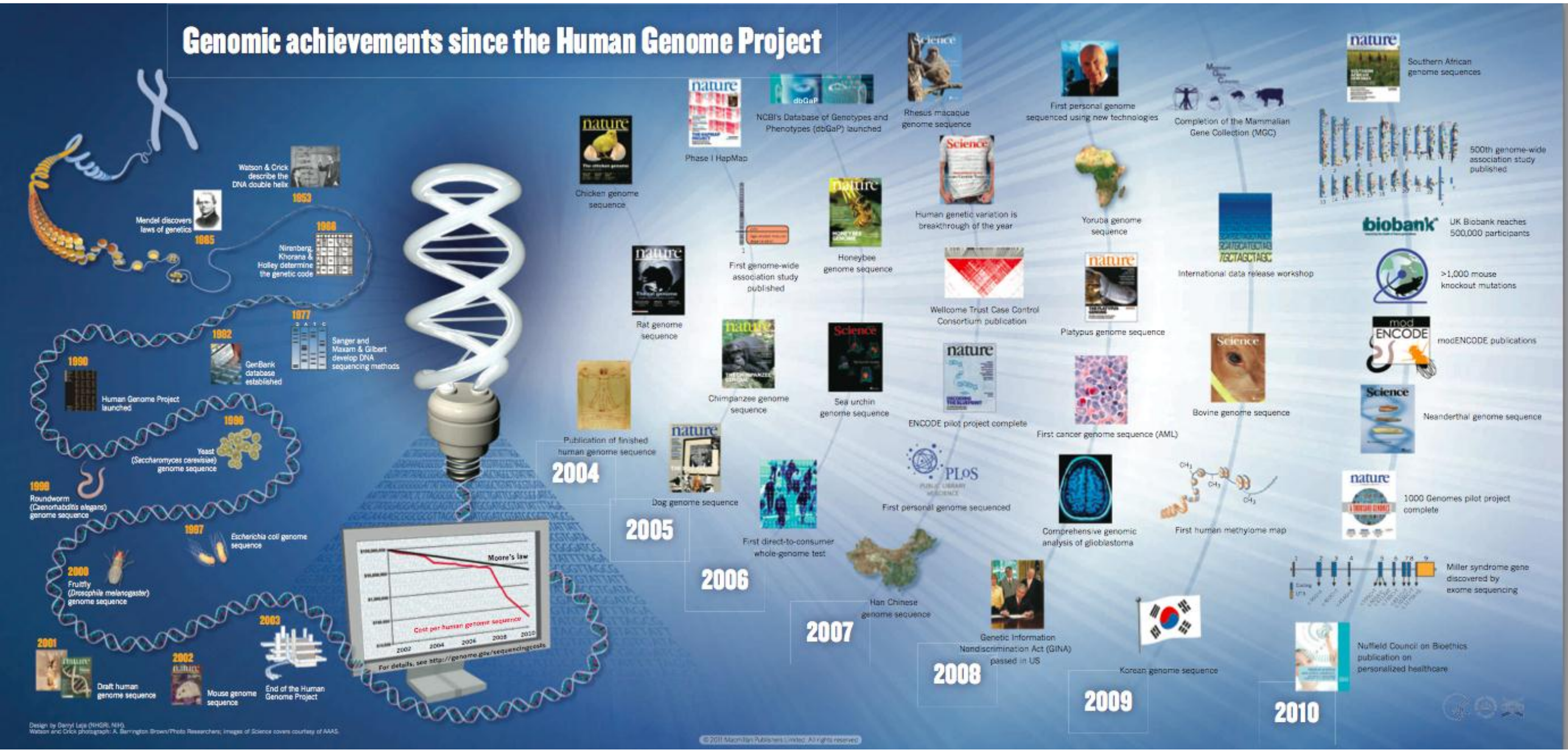
Our Ability to Translation Genomics Knowledge

PERSPECTIVE

doi:10.1038/nature09764

Charting a course for genomic medicine from base pairs to bedside

Eric D. Green[1], Mark S. Guyer[1] & National Human Genome Research Institute*



Extensive Sequencing to Diagnose Rare Disorders

Sir Archibald Garrod, around 1910.

Inborn Errors of Metabolism

Second Edition

Archibald E. Garrod

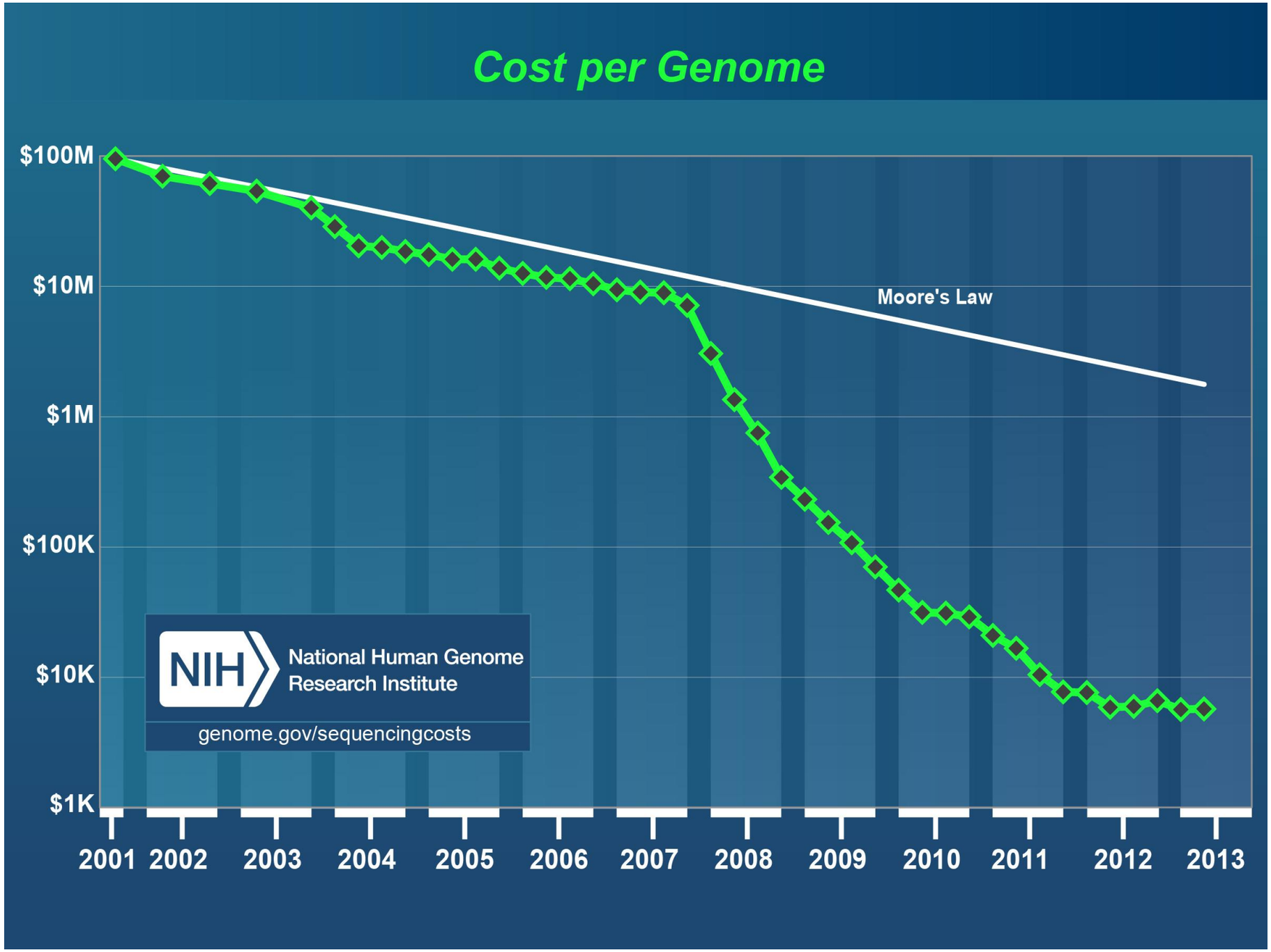
Henry Frowde and Hodder & Stoughton
The Lancet Building
London

1923

1902 – disorder in the metabolism of phenylalanine and tyrosine

- Disorder of joints and heart valves with curious darkening of urine
- Treatment with dietary restriction

Technology Advancements Have Been Staggering



Sequencing the ‘Mendelianome’

Sci Transl Med 12 January 2011:
Vol. 3, Issue 65, p. 65ra4
DOI: 10.1126/scitranslmed.3001756

RESEARCH ARTICLE

HUMAN GENOMICS

Carrier Testing for Severe Childhood Recessive Diseases by Next-Generation Sequencing

Callum J. Bell[1,*], Darrell L. Dinwiddie[1,2,*], Neil A. Miller[1,2], Shannon L. Hateley[1], Elena E. Ganusova[1], Joann Mudge[1], Ray J. Langley[1], Lu Zhang[3], Clarence C. Lee[4], Faye D. Schilkey[1], Vrunda Sheth[4], Jimmy E. Woodward[1], Heather E. Peckham[4], Gary P. Schroth[3], Ryan W. Kim[1] and Stephen F. Kingsmore[1,2,†]

- Exome sequencing for known genes:

  -- Sequence an exome

  -- Interpret a ‘region of interest’

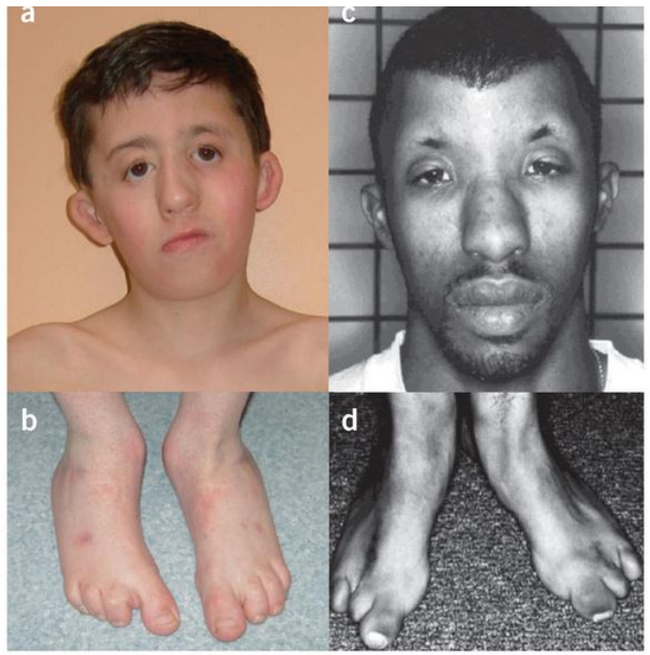
Sequencing to Diagnose Rare Disorders

Exome sequencing identifies the cause of a mendelian disorder

Sarah B Ng, Kati J Buckingham, Choli Lee, Abigail W Bigham, Holly K Tabor, Karin M Dent, Chad D Huff, Paul T Shannon, Ethylin Wang Jabs, Deborah A Nickerson, Jay Shendure & Michael J Bamshad

Affiliations | Contributions | Corresponding authors

Nature Genetics **42**, 30–35 (2010) | doi:10.1038/ng.499
Received 02 October 2009 | Accepted 09 November 2009 | Published online 13 November 2009 | 22 November 2009



(**a**,**b**) A 9-year-old boy with Miller syndrome caused by mutations in *DHODH*. Facial anomalies (**a**) include cupped ears, coloboma of the lower eyelids, prominent nose, micrognathia and absence of the fifth digits of the feet (**b**). (**c**,**d**) A 26-year-old man with methotrexate embryopathy. Note the cupped ears, hypertelorism, sparse eyebrows and prominent nose (**c**) accompanied by absence of the fourth and fifth digits of the feet (**d**). **c** and **d** are reprinted with permission from ref. 30.

Genomic Sequencing in Patient Care

Ng, Shendure: Miller syndrome, 4 cases

Exome sequenced reveals causal mutations in DHODH

Lifton: Undiagnosed congenital chloride diarrhea (consanguinous)

Exome seq reveals homozygous SLC23A chloride ion transporter mutation

Return diagnosis of CLD (gi) not suspected Bartter syndrome (renal)

Worthey, Dimmock: 4-year old, severe unusual IBD

Exome seq reveals XIAP mutation (at a highly conserved aa)

proimmune disregulation opt for bone marrow transplant over chemo

Mardis, Wilson: acute myelocytic leukemia but not classical translocation

Genome sequencing (1 week + analysis) reveals PML-RARA translocation

Directs ATRA (all trans retinoic acid) treatment decision

Choi (Nephrology); Lupski (Neuro); Worthey (GI);....

Baylor, Wisconsin, Partners, Ambry, GeneDx, others launched or announced

Adopted from Benjamin Neale (Analytic and Translational Genetics Unit, MGH)

Translating Discoveries to Patients

Genetic Testing

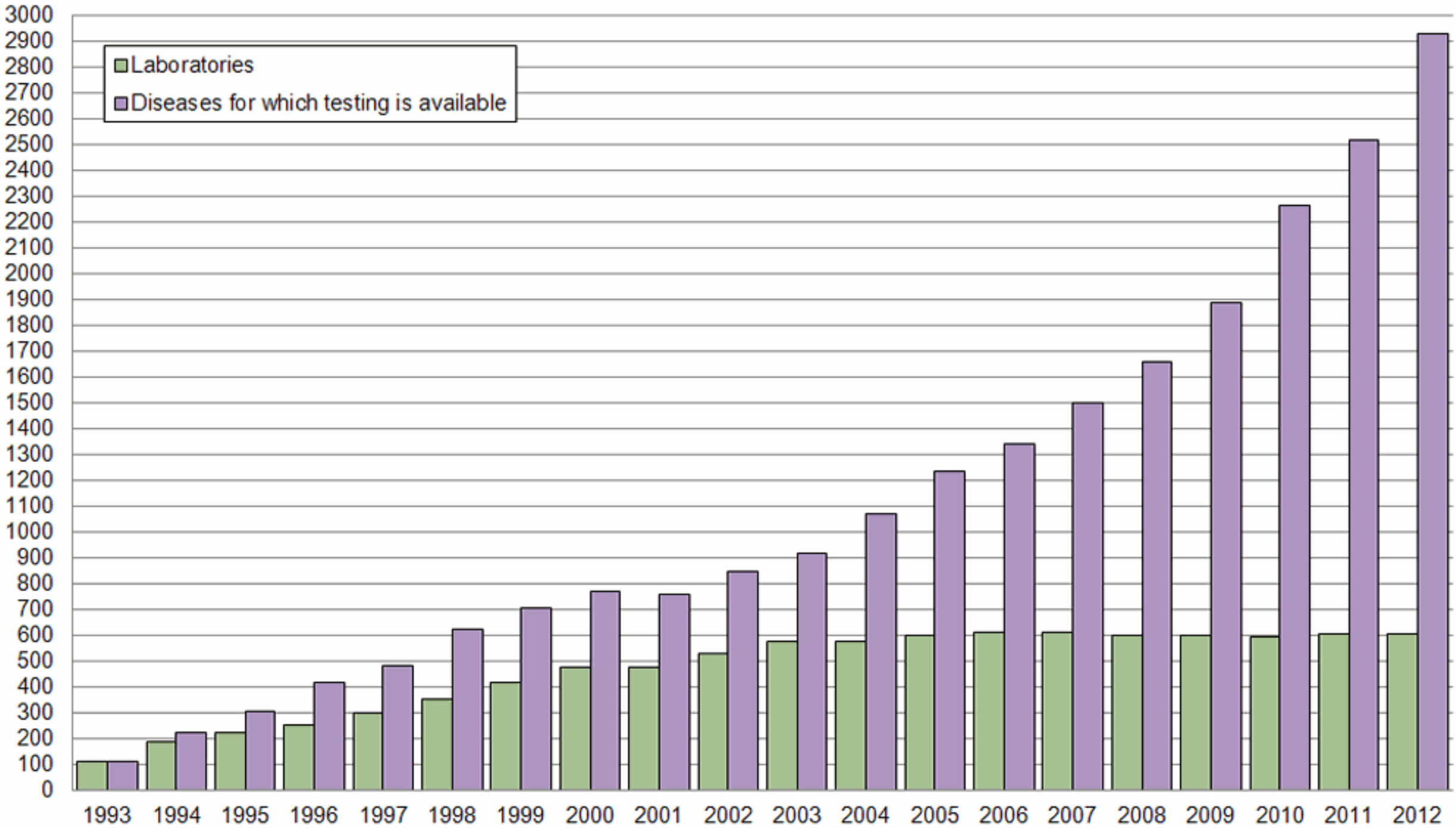
AMA Classifies Genetic Testing Today

Diagnostic Testing: Determine whether an individual has a genetic disease. Current estimates suggest more than 4000 diseases are caused by single gene mutations.

Predictive Medicine: Determine whether an individual has an increased risk for a particular disease.

Pharmacogenomics: Classify subtle variations in an individual's genetic makeup to determine whether a drug is suitable for a particular patient, and if so, what would be the safest and most effective dose.

Opportunity to Use Molecular Tests Increasing



Data source: GeneTests database (2012)/www.genetests.org

The Availability and Use of Genetic Testing

There are >1200 genetic tests available to physicians to aid in the diagnosis and therapy for >1000 different diseases.

Genetic testing is performed for the following reasons:

- conformational diagnosis of a symptomatic individual
- pre-symptomatic testing for estimating risk developing disease
- pre-symptomatic testing for predicting disease
- prenatal diagnostic screening
- newborn screening
- pre-implantation genetic diagnosis
- carrier screening
- forensic testing
- paternal testing

Costs and Complexity of Tests Increasing

- Some Sanger tests are $15,000
- Even single gene tests can be $5,990
- A clinical exome can be $7,500
- Clinical genome $12,000
- Getting an answer may not be likely. In some cases rate of getting a successful answer is 25%

Context: Cost pressures in the health care system

An Opportunity to Build A Pediatric Network

Claritas Genomics – Who Are We?

- Clinical pediatric genetic diagnostic laboratory
- Best quality services, from pre-order to post-report
- Test for any condition
- Close partnership with clinical and genetic expertise from pediatric hospitals for test development and interpretation
- Single gene tests to gene panels to exomes
- Proprietary copy number variation with special emphasis on autism spectrum disorders
- Optimizing Life Technologies' Ion Proton platform

Building a Network Through Our Services

Claritas: an interface enabling our community to address the challenges and opportunities of clinical use of NGS:

- *Complexity of measurement*
- *Complexity of interpretation*
- *Clinical utility*
- *Appropriate utilization*
- *Reimbursement*
- *Result reporting*
- *Support services and explanation*
- *Discovery*

Right question
Right test
Right result

Claritas Management Team and Key Members

Patrice Milos, PhD — Chief Executive Officer
- Senior executive with 18+ years experience in genomics and medicine
- Pharma Experience, CSO/R&D at Helicos BioSciences

Mary Ellen Cortizas, JD — Chief Operating Officer
>25 years experience in Clinical Laboratory Management and Operations
- Recognized leader among Clinical Lab peers
- Co-founder Claritas Genomics

Nurjana Bachman, PhD — Chief Business Officer
- Completed >100 transactions over 8 years as BD director
- Specialty in corporate collaborations
- Co-founder Claritas Genomics

Peter Park, PhD — HMS, BCH — Associate Professor Informatics Program
- Key consultant, medical informatics leader and visionary
- Co-founder Claritas Genomics

Timothy Yu, MD PhD — HMS, BCH — Assistant Professor Neurology
- Key consultant, medical informatics leader and visionary
- Co-founder Claritas Genomics

David Margulies, MD — HMS, CBMI Ex. — Ex. Director Gene Partnership
- Medical and Information Systems Entrepreneur
- Founder of Correlagen Dx, CERNER, CareInsight
- Co-founder Claritas Genomics

Claritas Board of Directors

Robert Higgins
General Partner,
Highland Capital Partners

David Margulies, MD
Executive Director,
Gene Partnership, BCH

Ronnie Andrews
President, Medical Sciences
Life Technologies

Patrice Milos, Ph.D.
CEO, Claritas

Erik Halvorsen, PhD
Executive Director, Technology &
Innovation Development Office
(BCH Observer)

Mark Gardner
VP & GM Advanced Genomic
Systems (Life Tech Observer)

Claritas Genomics - Funding

- Arose out of Boston Children's Hospital (>10y)
- Investment from Life Technologies
- Investment from Large Computing Company
- Building Partnerships with Major Pediatric Hospitals in the US
- Working to Partner with Country Health Systems

What Does The Pediatric Community Want?

Patients	Fast, reliable answer
Clinicians	Support, streamlined process
Hospitals	One stop shopping, best value, integrated reporting
Payers	Utility, value
Researchers and health systems	Large numbers of well-characterized samples, scalability to enable discovery

What Do Pediatric Hospitals Want?

Pediatric Hospitals

Cost-effective measurements (sequencing or otherwise)

Reliable interpretation

Partners who could enable us and our peers to build knowledge and advance medical genomics for patients

Meeting Partners' Needs

Partner	*Need*	*Claritas Service*
Patients	Fast, reliable answer	Genetic testing with consistent interpretation
Clinicians	Support, streamlined process	Pairing test with clinical need, easy ordering and billing
Hospitals	One stop shopping, best value, integrated reporting	Advice navigating testing landscape and consolidating send-out
Payers	Utility, value	Right test, strong clinical justification
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Fast, Reliable Answers

- Test menu – over 110 tests currently offered
  - *Single gene - Now*
  - *NGS Panel for DMD – Available Now*
  - *Proprietary CNV Array – Optimized for Autism*
  - *NGS exome-based tests – Fall 2013*
  - *Genomes – 2014*
- Assays arising from the clinic, developed for clinicians
- CLIA facility, using all technology platforms
- Goals:

  Affordability, efficiency, analytic automation, scalability

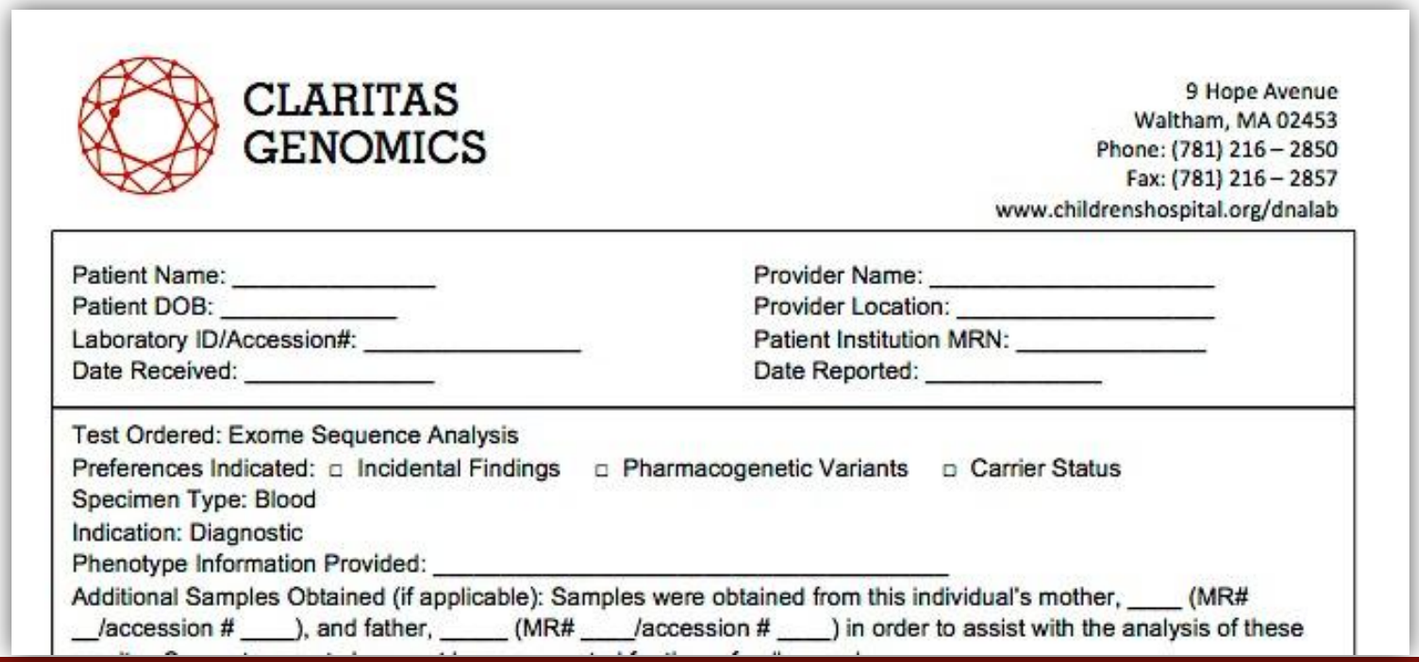
Interpretation – Providing Clinical Context

Bioinformatics:

- Experts from Boston Children's, Harvard, Life Technologies
- Ensures consistent, scalable interpretation

Clinical practitioners:

- Expert genetic counselors, medical directors



CLARITAS GENOMICS

9 Hope Avenue
Waltham, MA 02453
Phone: (781) 216 – 2850
Fax: (781) 216 – 2857
www.childrenshospital.org/dnalab

Patient Name: _____
Patient DOB: _____
Laboratory ID/Accession#: _____
Date Received: _____

Provider Name: _____
Provider Location: _____
Patient Institution MRN: _____
Date Reported: _____

Test Ordered: Exome Sequence Analysis
Preferences Indicated: ☐ Incidental Findings ☐ Pharmacogenetic Variants ☐ Carrier Status
Specimen Type: Blood
Indication: Diagnostic
Phenotype Information Provided: _____
Additional Samples Obtained (if applicable): Samples were obtained from this individual's mother, ____ (MR# __/accession # ____), and father, _____ (MR# ____/accession # ____) in order to assist with the analysis of these

Claritas Diagnostic Testing

Exome and genome testing, simplified

Sample → Processing → Analysis → Reporting

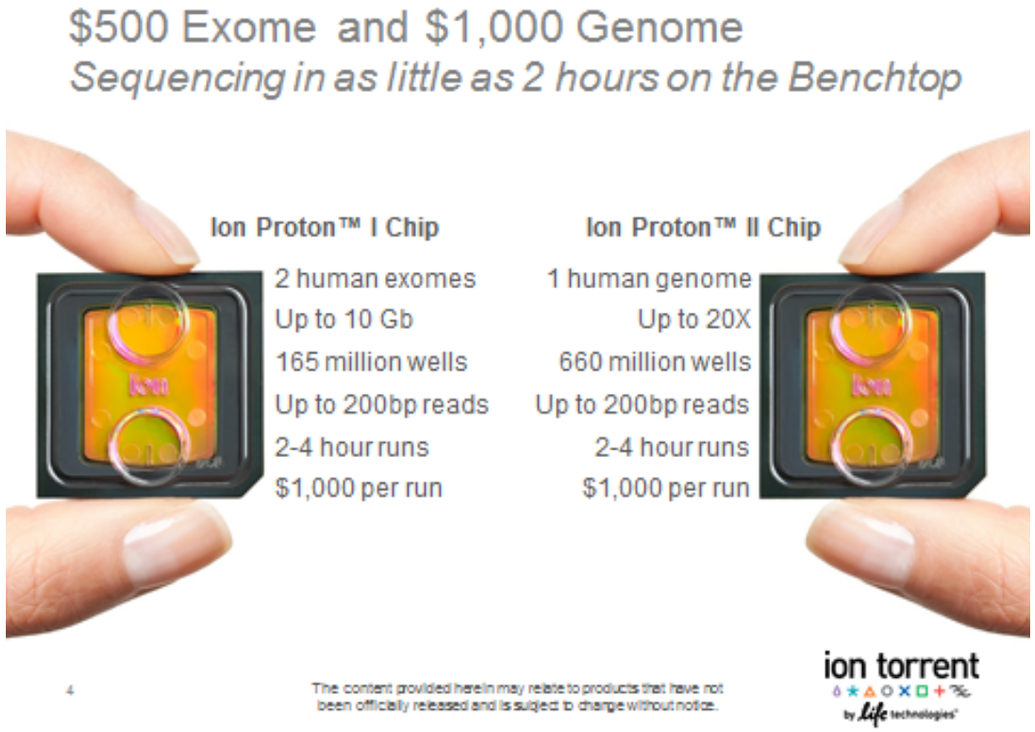
Claritas Diagnostic Testing

Exome and genome testing

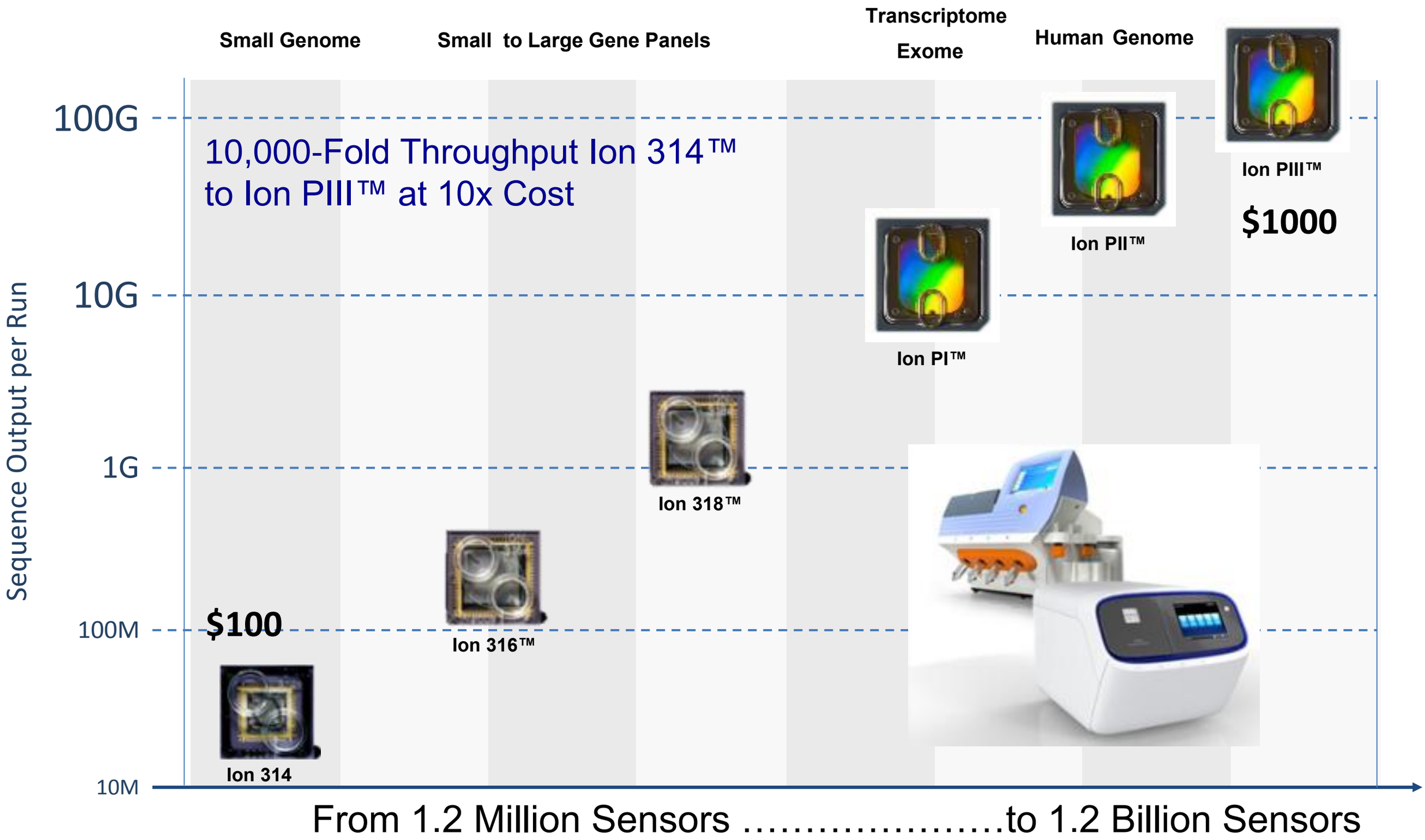
Sample → Processing → Analysis → Reporting

Can come from anywhere

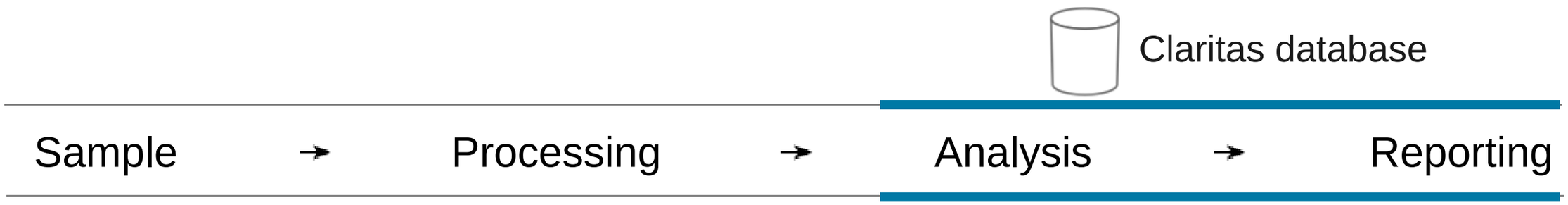
All platforms, optimizing to Life Technologies



Optimizing to Life's Ion Proton Platform



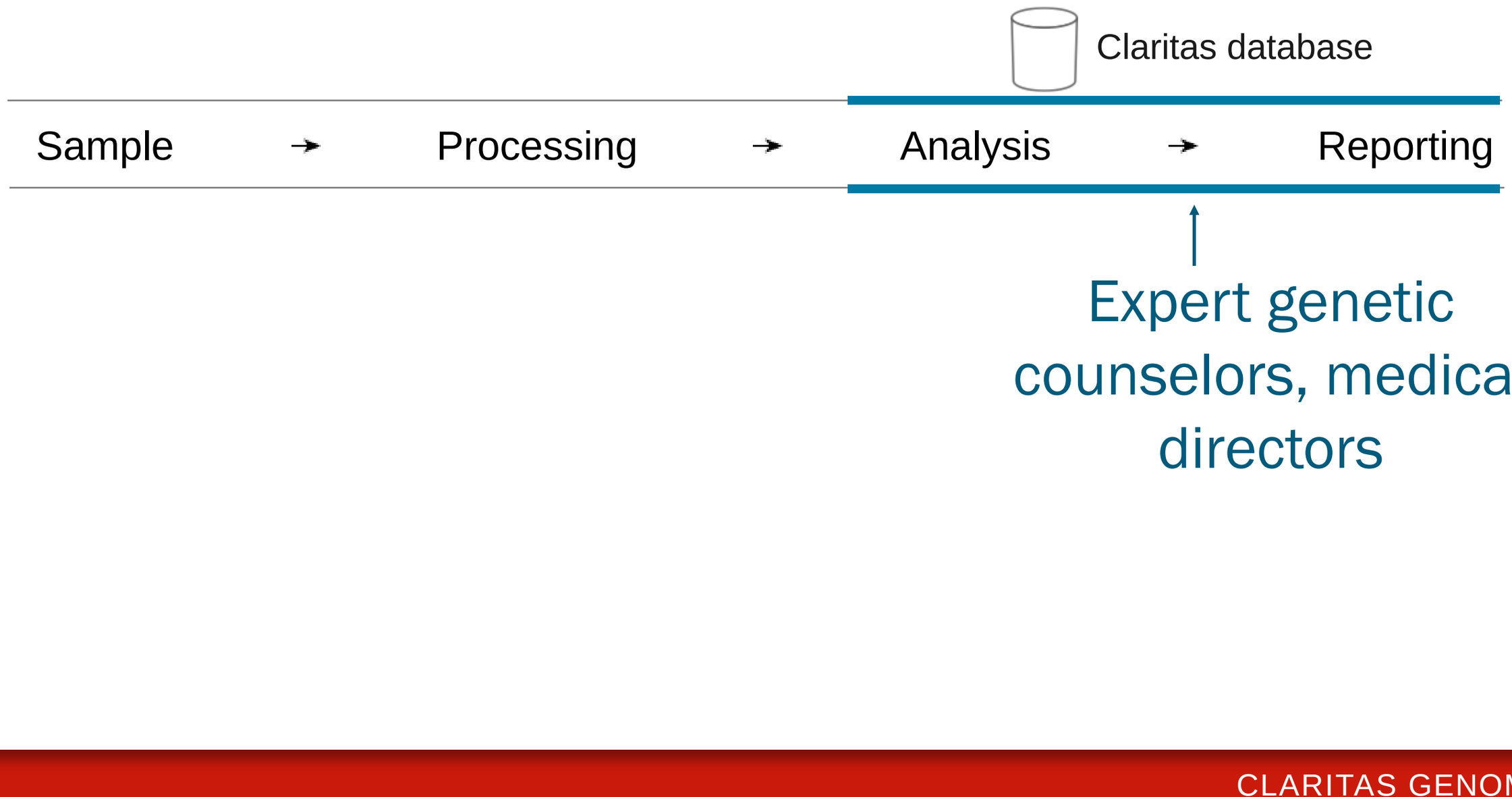
Claritas Diagnostic Testing



Software

Process signal, align → Score / Filter / Annotate → Report

Claritas Diagnostic Testing



Support And A Streamlined Process

Support:

- Help navigating the test menu when doctors have patients with complex presentation and family history
- Long term relationship – support post report
- Re-interpretation services with incorporation of new knowledge

Streamlined:

- Ability to order and integrate into hospital systems (EMR, billing, etc.)

Navigating the Menu

Test selection is tied to clinical presentation:

- *Takes away some of the guess work*
- *Upfront consultation to ensure that correct test is ordered*
- *Support justifying prior authorization*

Example – test selection transitioning…

…from this:

- 1p36 deletion syndrome
- 1q21.1 deletion/duplication
- 15q13.2-q13.3 deletion
- 16p11.2 deletion/duplication
- 17q12 deletion
- 17q21 microdeletion syndrome
- Achondroplasia
- Acute megakaryoblastic leukemia
- Alagille syndrome
- Angelman syndrome
- etc…

…to this:

Check all that apply:

- ✓ Failure to thrive
- ✓ Muscle weakness
- ✓ etc…

Meeting Partners' Needs

Partner	*Need*	*Claritas Service*
Patients	Fast, reliable answer	Genetic testing with consistent interpretation
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Researchers and health systems	Large numbers of well-characterized samples, scalability to enable discovery	Research, data network

One-Stop Shop, Best Value, Integration

- Hospital labs send millions of dollars to labs each year to run tests
- Individual institutions do not have the leverage to negotiate best prices
- Utilization guidelines based on evidence and best practices from the community

- Working with some of the largest providers of both testing services and integration processes to make a seamless experience

We can consolidate molecular send-outs through a streamlined system

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Strong Justification of Value

- Test menu arises from clinical needs
- Emphasis on ordering the right test for the symptoms
- Increasing chance of meaningful result
- Less wasteful testing
- Utilization analysis informative for ultimate cost-savings

Examples: Claritas Products Array Based Measurements

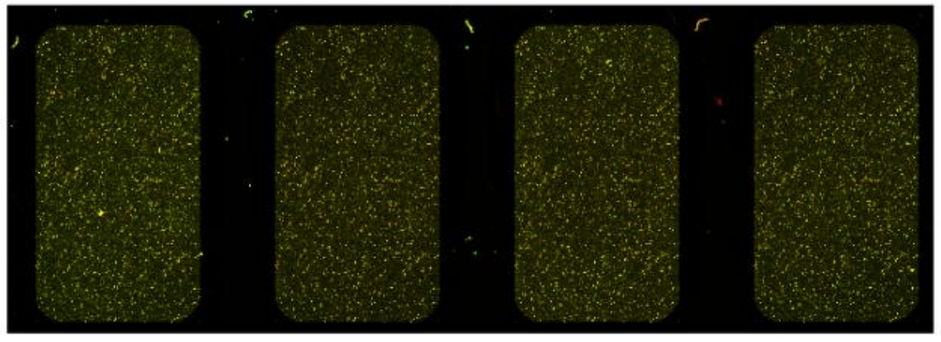
Copy Number Variations in Autism Spectrum Disorder

Claritas Product Utilizes Our Experience To Date

- >10,000 pediatric patients examined
- Majority of patients were phenotypically characterized as having developmental delays including autism
- Allowed customization of the ClaritasGenomicsChip to maximize detection of copy number events
- Data comparison to demonstrate distinct differences to other market products
- World-class physicians helping to inform interpretation of the data

Unique Features of Claritas

CHIPs use Agilent 4X180K CNV+SNP array platform.
CHIPs cover the whole human genome



Probe numbers

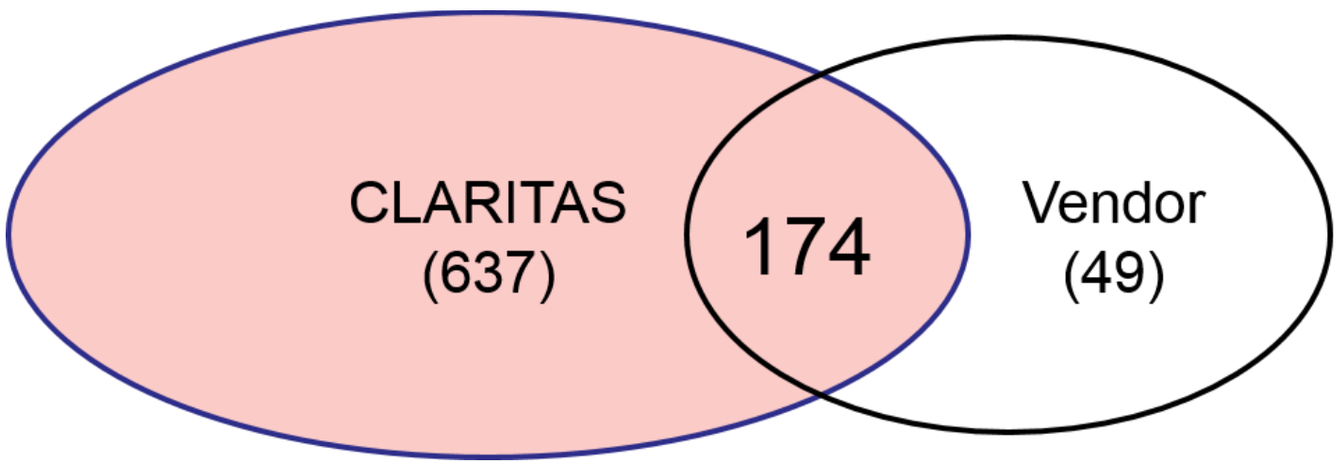
CHIP	CNV probe #	SNP probe #	AOH sensitivity
Claritas	150K	30K	5-10Mb
Vendor	120K	60K	7-10Mb*

*>10Mb is recommended clinical reporting cutoff for AOH
Claritas product provides SNP depth to allow such reporting across the genome

Enhanced Coverage in Clinical Relevant Genes

All known genomic imbalance disorders are covered on the chips, as well as important chromosome landmarks: telomeres and centromeres

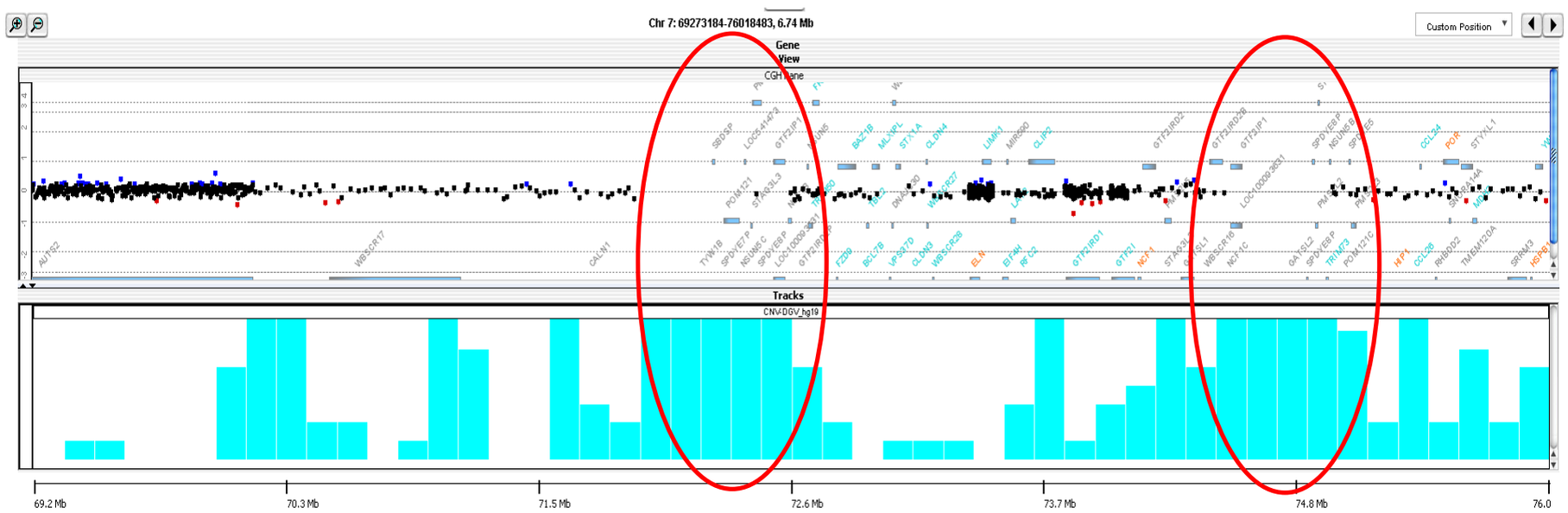
Much more clinically relevant genes have enhanced probe coverage on ClaritasCHIP(811 vs. 228, 174 genes overlap) to ensure CNV detection



Unique Design Features

ClaritasGenomicsCHIP

1. 30K SNP probes are sufficient to detect any AOH >7Mb. 30K more probes are allocated for copy number variant detection. Based on Claritas prior experience with previous 244 CNV array.
2. Enhanced probe coverage for genes known to be associated with developmental delay, intellectual disability and autism spectrum disorders, as well as for genes of haploinsufficiency.
3. Fewer probes are placed in clinically irrelevant copy number polymorphic regions (as shown in the image below).



Probe distribution

Copy number polymorphism track

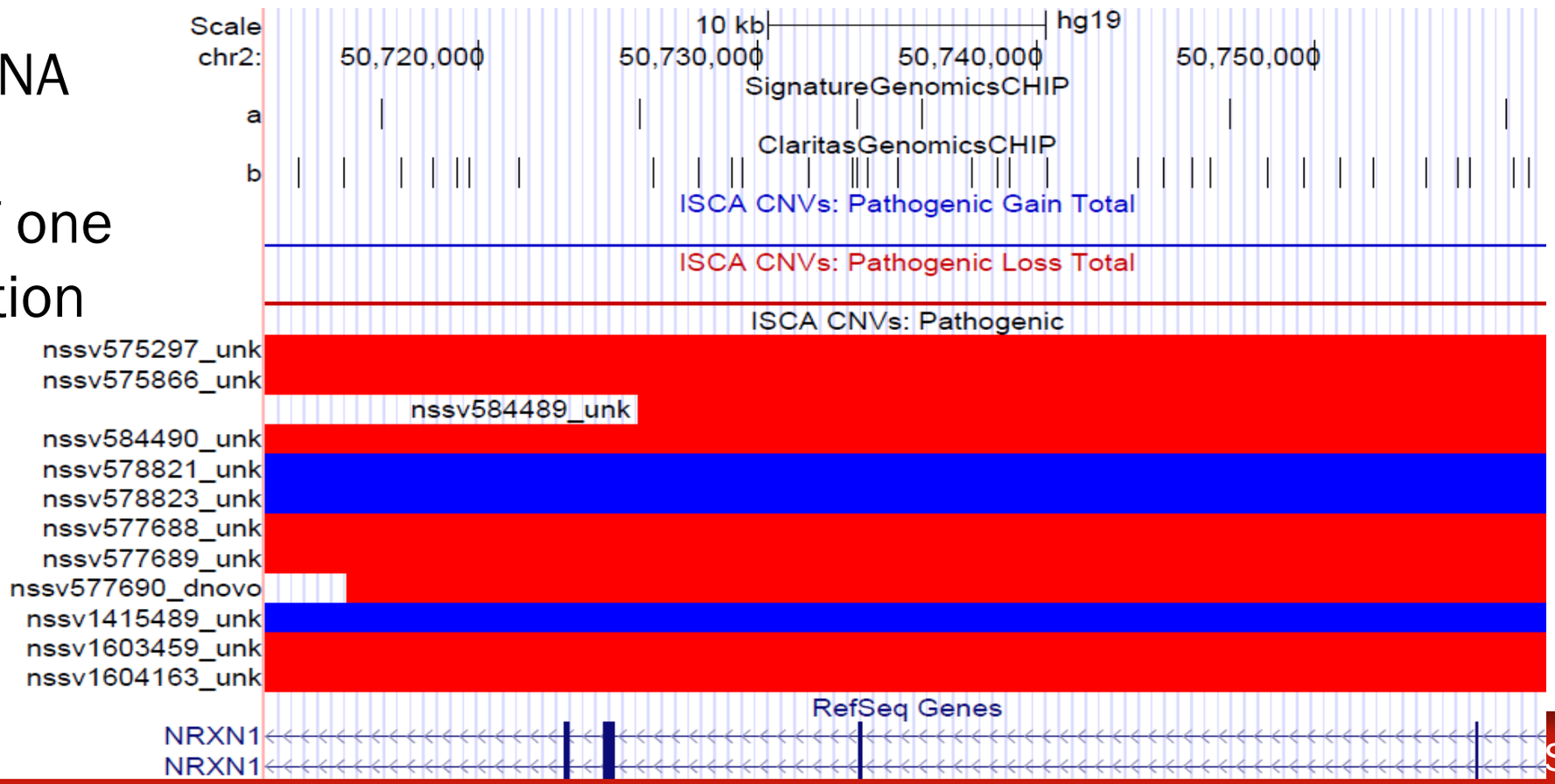
The *NRXN1* locus: Depth of Coverage

The whole gene probe density comparison

A small pathogenic CNV is covered by 33 probes by ClaritasCHIP

Patient DNA

Example of one exon deletion



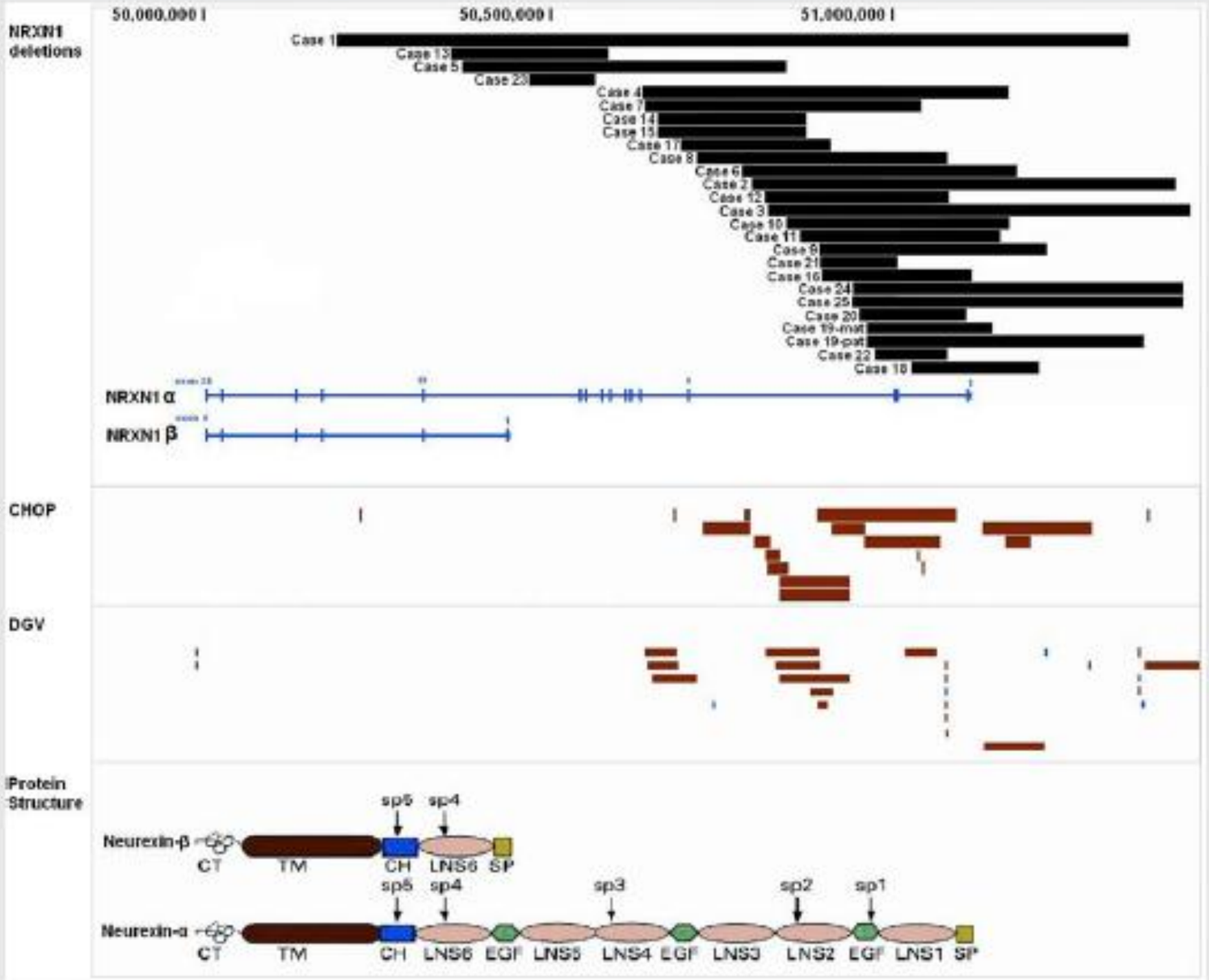
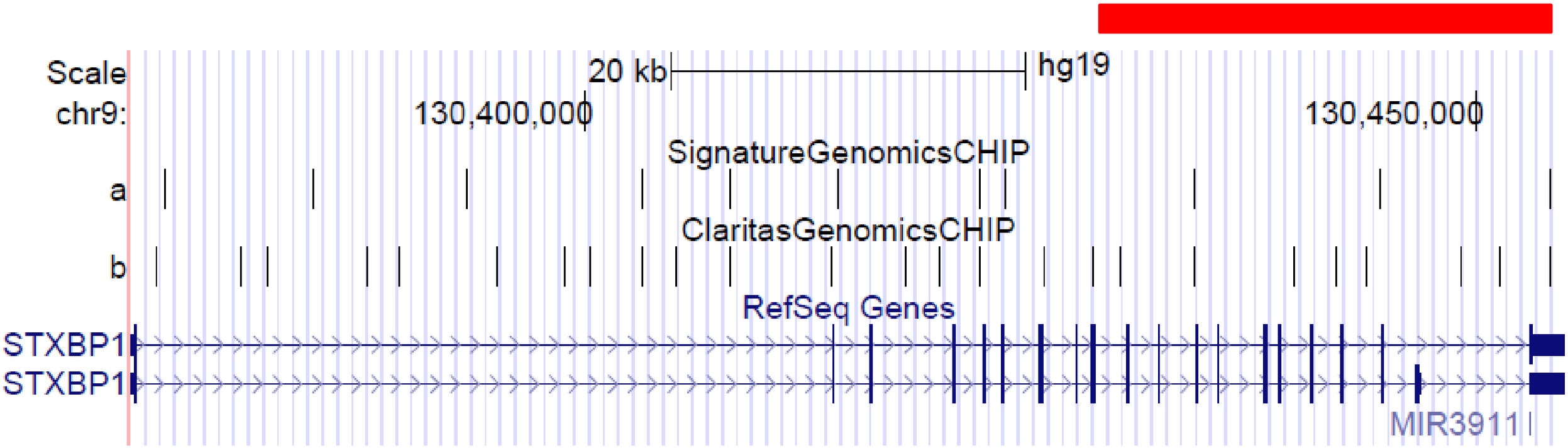


FIG. 1. *NRXN1* exonic deletions identified in 25 individuals (cases 1–25). Upper panel: Black bars indicate the deleted region in each case, with cases ordered according to the start genomic position. Two bars are shown for case 19 with two unique heterozygous deletions inherited from each parent (note, the overlap between these two bars marks the homozygously deleted region in the proband). All breakpoints appear to be unique; there was no evidence of clustering at low copy repeats at the resolution of the microarray analyses. The *NRXN1* coding region is shown in blue; exons are depicted by vertical lines. Middle panels: CNV data from the Childrens Hospital of Philadelphia (CHOP; deletions are shown in red) and Database of Genomic Variants (DGV; deletions are shown in red and duplications in blue). Lower panel: schemes of the protein structure of the α-Neurexin and β-Neurexin isoforms (CH, carbohydrate binding region; CT cytoplasmic tail; EGF epidermal growth factor-like domains; LNS 1–6 laminin, neurexin, sex hormone binding domains 1–6; SP signal peptide; TM, transmembrane region).

The *STXBP1* locus: Depth of Coverage

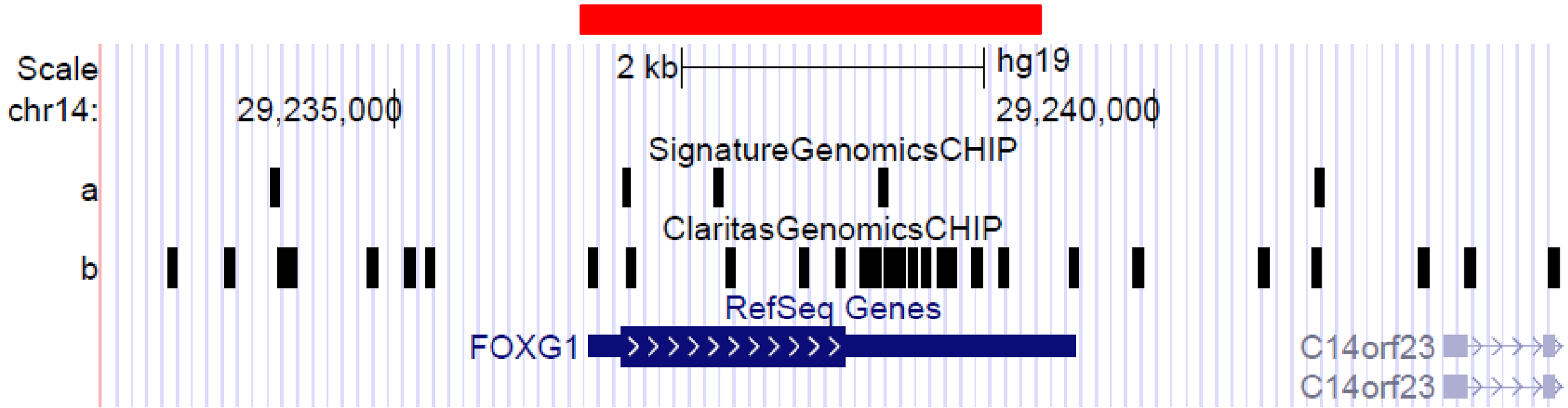
Requirement for 5 contiguous probes to provide high level of confidence in CNV detection



CHIP	Total probe #	Interval (kb)	Probe density	Detection sensitivity (kb)
Claritas	25	80	0.3	16
Vendor	11	80	0.14	36

Any exonic deletions less than 36kb in size (as the red bar) will be captured by Claritas Array

The *FOXG1* locus: Density and Spacing



CHIP	Total probe #	Interval (kb)	Probe density	Detection sensitivity (kb)
Claritas	16	3.2	5	1
Vendor	3	3.2	0.94	5.3

A whole FOXG1 gene deletion (red bar) will be captured by Claritas Array

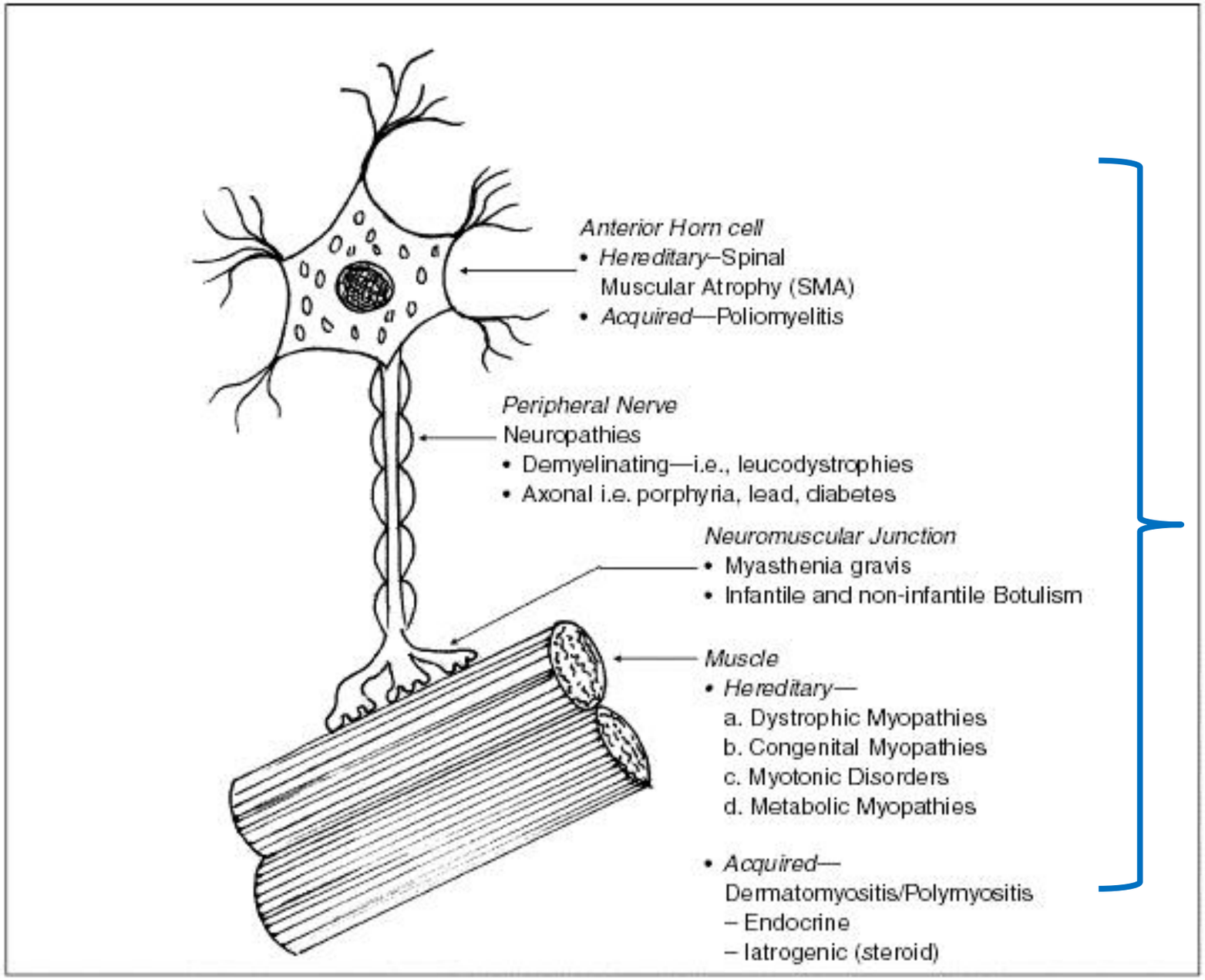
Examples: Claritas Products Next-Generation Sequencing Panel

Sequencing Genes for Neuromuscular Disorders

Background on Neuromuscular Disorders Panel

- Group of disorders involving muscles and/or their direct nerve system control.
- Neuromuscular disorders occur in ~1.5M Americans annually at any age
- Some 40 percent of them are under age 18
- All neuromuscular diseases are progressive in nature, and all result in muscle weakness and fatigue, many of which dramatically alter the quality of life for both patients and their families
- An active area in rare disease therapeutics

NMD Genetic Disorders



http://www.ncbi.nlm.nih.gov/books/NBK27251/figure/A13449/?report=objectonly

Clinical Indications – Phenotype Driven

NMD panel testing in Children

Common presenting symptoms may include:

- Infantile floppiness or hypotonia
- Delay in walking, abnormal gait, frequent falling, difficulty climbing steps, inability to hop (delayed motor milestones)
- Feeding and respiratory difficulties
- Abnormal gait characteristics (Waddling gait, lordotic posture, difficulty rising from floor (Gowers' sign))
- Muscle cramps or stiffness, prominence of calves
- Elevated creatinine kinase (CK) level

10 NMD Gene Panel Using Next-Generation Sequencing

Developed by physicians and subject matter experts at BCH

BMC Genetics

BioMed Central

Methodology article

Open Access

Automated DNA mutation detection using universal conditions direct sequencing: application to ten muscular dystrophy genes

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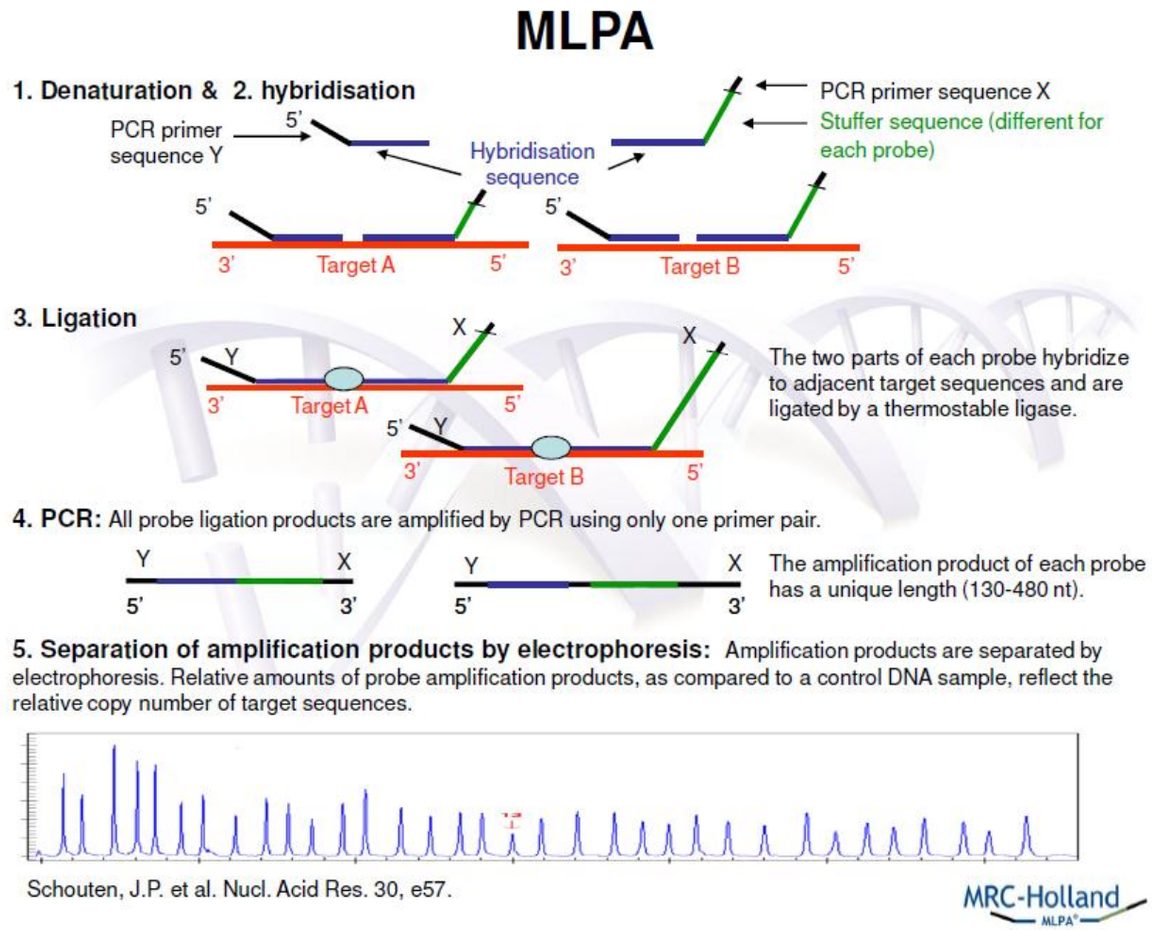
Received: 15 April 2009
Accepted: 18 October 2009

MLPA: Exonic Deletion/Duplication

Approximately 60% of DMD and BMD cases are caused by large deletions (one exon or greater) or large duplications in the DMD gene

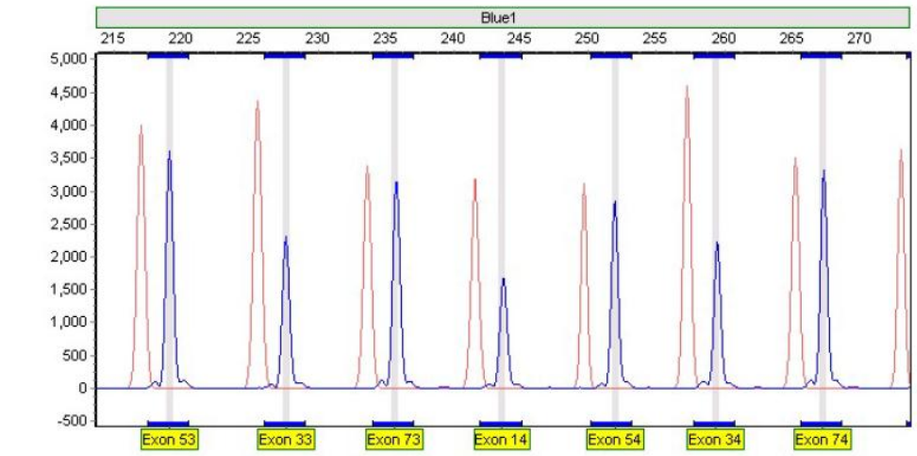


MLPA is used initially to assess exonic CNV

DMD exon 8-34 heterozygous deletion detected

MLPA Analysis Report - SoftGenetics	
Software: GeneMarker V1.85	**Analysis Type:** MLPA
Project: Untitled	**Compare Type:** MLPA Ratio
Technician:	**Normalization By:** Internal Control Probe Normalization (Adjusted)
Report Time: 08/03/2011 - 12:39:27	**Quantification By:** Peak Height
Panel: DMD p035-A2_012910	**Classification:** Loss < 0.75 <= Equivalent <= 1.30 < Gain
Control: Synthetic Control Sample	**Report Value Type:** Peak Ratio
Synthetic Used: DMD_1030600712A-f-p035-nml_2011-08-03_018.fsa /// DMD_L10027456-f-p035-nml_2011-08-03_017.fsa	

[redacted]

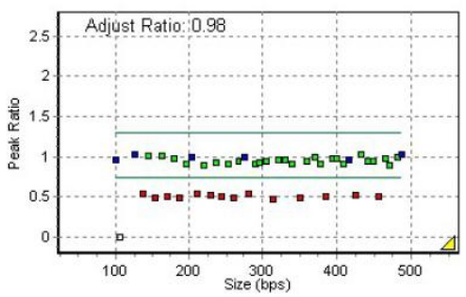


[redacted]

Machine: Childrens37301-16107-029
Run Time: 08/03/2011 - 11:44:57 -> 08/03/2011 - 12:19:11

set up: 08/02/11
complete: 08/03/11 HL

Conclusion	heterozygous out of frame del. exons 8-34	
	Date	Initial
Authorization 1	8/4/11	VL
Authorization 2	8/5/11	YS

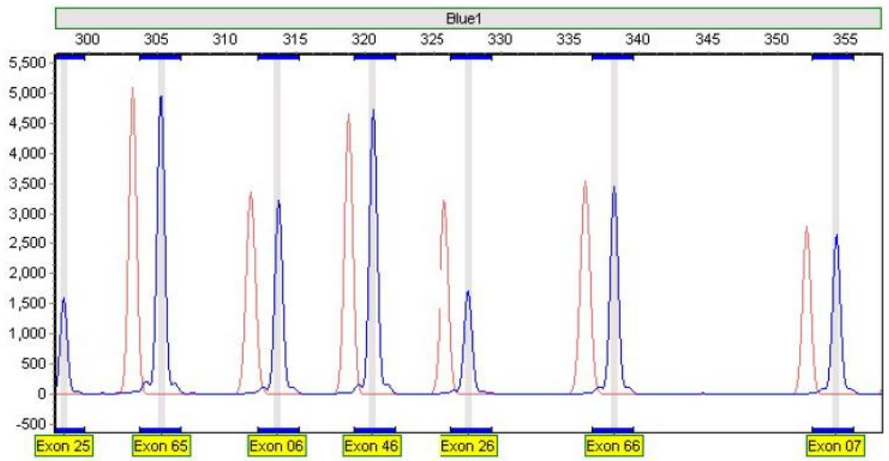


8/4/11--Abnormal--AS

	Probe Name	Bin Size	DMD_112010147 8A
1	DP427C	480.6	0.996
2	Exon 11	136.5	0.536
3	Exon 12	170.5	0.510
4	Exon 13	210.3	0.540
5	Exon 14	243.6	0.508
6	Exon 15	281.0	0.536
7	Exon 16	313.2	0.479
8	Exon 17	350.6	0.488
9	Exon 18	385.4	0.506
10	Exon 19	424.4	0.524
11	Exon 20	456.7	0.516
12	Exon 31	154.1	0.488
13	Exon 32	186.9	0.497
14	Exon 33	227.6	0.524
15	Exon 34	259.3	0.484
16	Exon 35	295.7	0.929
17	Exon 36	330.3	0.957
18	Exon 37	370.1	0.989
19	Exon 38	399.5	0.986
20	Exon 39	440.8	0.949
21	Exon 40	471.2	0.888
22	Exon 51	144.8	1.009
23	Exon 52	179.0	0.987
24	Exon 53	219.0	0.903
25	Exon 54	251.7	0.918
26	Exon 55	290.2	0.906
27	Exon 56	321.4	0.959
28	Exon 57	360.3	0.944
29	Exon 58	393.3	0.981
30	Exon 59	432.0	1.030
31	Exon 60	465.0	0.983
32	Exon 71	163.1	1.021
33	Exon 72	195.7	0.904
34	Exon 73	235.6	0.930
35	Exon 74	267.2	0.943
36	Exon 75	305.1	0.941
37	Exon 76	339.0	0.915
38	Exon 77	377.7	0.908
39	Exon 78	408.6	0.917
40	Exon 79	449.2	0.938
41	**X fragment**	101.4	0.962
42	**Xp22**	203.1	1.001
43	**Xq11.2**	126.8	1.023
44	**Xq13**	416.4	0.963
45	**Xq28**	275.0	0.991
46	**Xq28.**	486.4	1.023
47	**Y fragment**	105.8	-1

MLPA Analysis Report - SoftGenetics	
Software: GeneMarker V1.85	**Analysis Type:** MLPA
Project: Untitled	**Compare Type:** MLPA Ratio
Technician:	**Normalization By:** Internal Control Probe Normalization (Adjusted)
Report Time: 08/03/2011 - 12:38:09	**Quantification By:** Peak Height
Panel: DMD p034-A2_031710	**Classification:** Loss < 0.75 <= Equivalent <= 1.30 < Gain
Control: Synthetic Control Sample	**Report Value Type:** Peak Ratio
Synthetic Used: DMD_1030600712A-f-p034-nml_2011-08-03_011.fsa /// DMD_L10027456-f-p034-nml_2011-08-03_010.fsa	

DMD_[redacted]

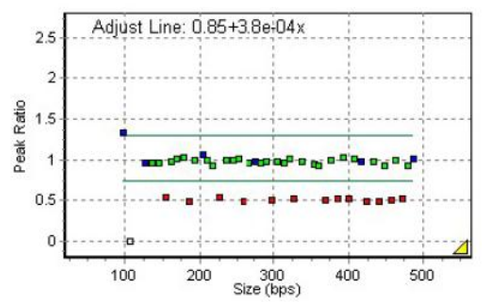


[redacted]

Machine: Childrens37301-16107-029
Run Time: 08/03/2011 - 11:44:57 -> 08/03/2011 - 12:19:11

set up: 08/02/11
complete: 08/03/11 HL

Conclusion	heterozygous out of frame del. exons 8-34	
	Date	Initial
Authorization 1	8/4/11	VL
Authorization 2	8/5/11	YS



8/4/11--Abnormal--AS

	Probe Name	Bin Size	DMD_112010147 8A
1	Exom 70	480.2	0.932
2	Exon 01	136.1	0.958
3	Exon 02	169.0	1.007
4	Exon 03	210.1	0.997
5	Exon 04	244.1	0.997
6	Exon 05	282.2	0.958
7	Exon 06	313.8	0.958
8	Exon 07	354.1	0.946
9	Exon 08	385.2	0.528
10	Exon 09	424.5	0.499
11	Exon 10	457.1	0.510
12	Exon 21	153.8	0.539
13	Exon 22	185.6	0.499
14	Exon 23	225.3	0.538
15	Exon 24	259.3	0.488
16	Exon 25	298.2	0.512
17	Exon 26	327.8	0.530
18	Exon 27	369.9	0.509
19	Exon 28	401.2	0.522
20	Exon 29	441.0	0.492
21	Exon 30	473.1	0.523
22	Exon 41	144.2	0.959
23	Exon 42	178.4	1.030
24	Exon 43	217.5	0.931
25	Exon 44	251.6	1.006
26	Exon 45	289.9	0.985
27	Exon 46	320.8	1.019
28	Exon 47	360.4	0.928
29	Exon 48	393.6	1.023
30	Exon 49	432.8	0.980
31	Exon 50	463.3	1.002
32	Exon 61	161.6	0.979
33	Exon 62	193.5	1.003
34	Exon 63	236.0	0.990
35	Exon 64	266.5	0.959
36	Exon 65	305.2	0.973
37	Exon 66	338.1	0.973
38	Exon 67	377.3	0.998
39	Exon 68	408.1	1.006
40	Exon 69	448.7	0.937
41	**X fragment**	97.8	1.329
42	**Xp22**	203.2	1.068
43	**Xq11.2**	126.9	0.965
44	**Xq13**	416.5	0.985
45	**Xq28**	486.5	1.009
46	**Xq28.**	275.0	0.973
47	**Y fragment**	105.9	-1

Panel: DMD and Limb-girdle Dystrophy Genes

Gene	Locus	Coordinate (hg19)	Amino Acid
CAPN3	15q15.1-q21.1	42651698..42704515	734
CAV3	3p25	8775486..8788451	151
DMD	Xp21.2	31137345..33357726	3685
FKRP	19q13.32	47249303..47261832	495
LMNA	1q21.2-q21.3	156084461..156109878	572
SGCA	17q21	48243366..48253293	178
SGCB	4q12	52886861..52904485	318
SGCD	5q33-q34	155753767..156194799	290
SGCG	13q12	23755060..23899304	291
TRIM32	9q33.1	119449581..119463579	653

Covers 110kb Gene Sequence

Amplicon Generation: A Robust Automation Process

- Regions of interest
  - Coding exons
  - Regulatory and intronic regions with reported pathogenic mutations
- Primers
- Thermocycle programs
  - Uniqueness
  - Robustness

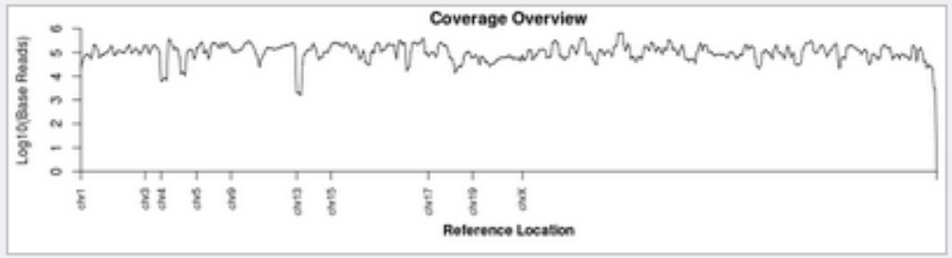
- Genomic DNA QC
- Amplicon QC
  - Completeness
  - Evenness
  - cleanness

235 amplicons in three 96-wells plates at one condition (TD-PCR) on Hamilton

Coverage Performance

Coverage Analysis Report

R_2013_07_09_18_08_48_user_WG1-170_Auto_user_WG1-170_442



Number of mapped reads	719,063
Percent reads on target	87.51%
Average base coverage depth	2,938
Uniformity of base coverage	93.91%

Amplicon Read Coverage		Target Base Coverage	
Number of amplicons	147	Bases in target regions	25,812
Percent assigned amplicon reads	44.87%	Percent base reads on target	48.93%
Average reads per amplicon	2,195	Average base coverage depth	2,938
Uniformity of amplicon coverage	93.20%	Uniformity of base coverage	93.91%
Amplicons with at least 1 read	99.32%	Target base coverage at 1x	99.81%
Amplicons with at least 20 reads	99.32%	Target base coverage at 20x	99.81%
Amplicons with at least 100 reads	97.96%	Target base coverage at 100x	98.66%
Amplicons with at least 500 reads	93.20%	Target base coverage at 500x	95.15%
Amplicons with no strand bias	97.96%	Target bases with no strand bias	95.79%
Amplicons reading end-to-end	14.97%		

Initial Validation Studies

Number of Runs/Samples to Validate Sensitivity:

Control and Mutation Positive Samples - 11 runs

Multiple Runs – Same Samples

Multiple Samples – Confirm Variants and Mutations

Mutation Confirmation: Total of 41 mutations

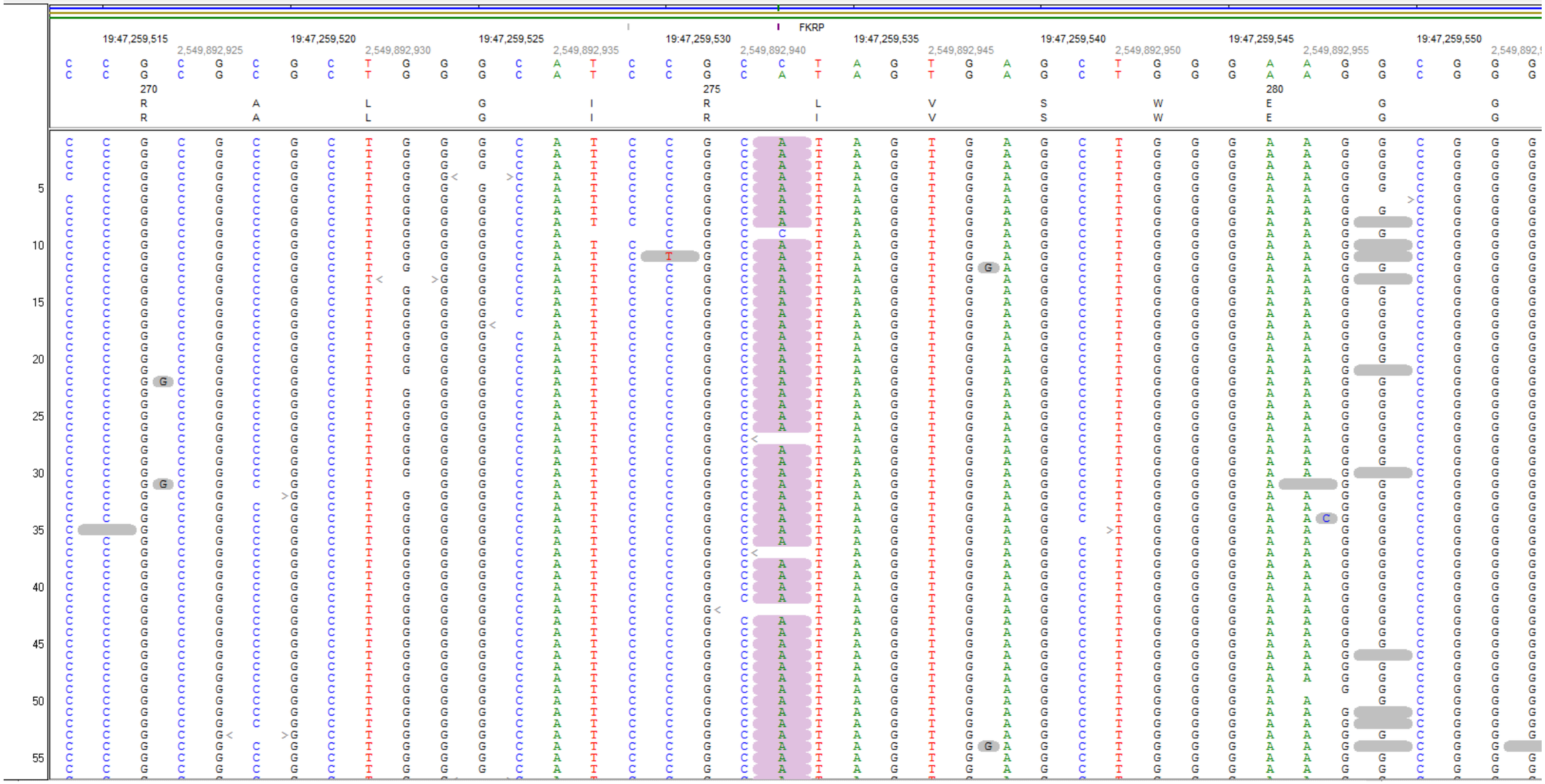
Mutation Missed: None*

*Note: SampleA8_run75 and Sample5_run86, the Sanger result was heterozygous c.2168+13T>C (rs228373) but the NGS result supported “homozygous”. Re-sequencing via Sanger confirmed that it was a homozygous.

Repeat rate: minimal

Detection of FKRP Homozygous Mutation

c.826C>A/p.276L>I

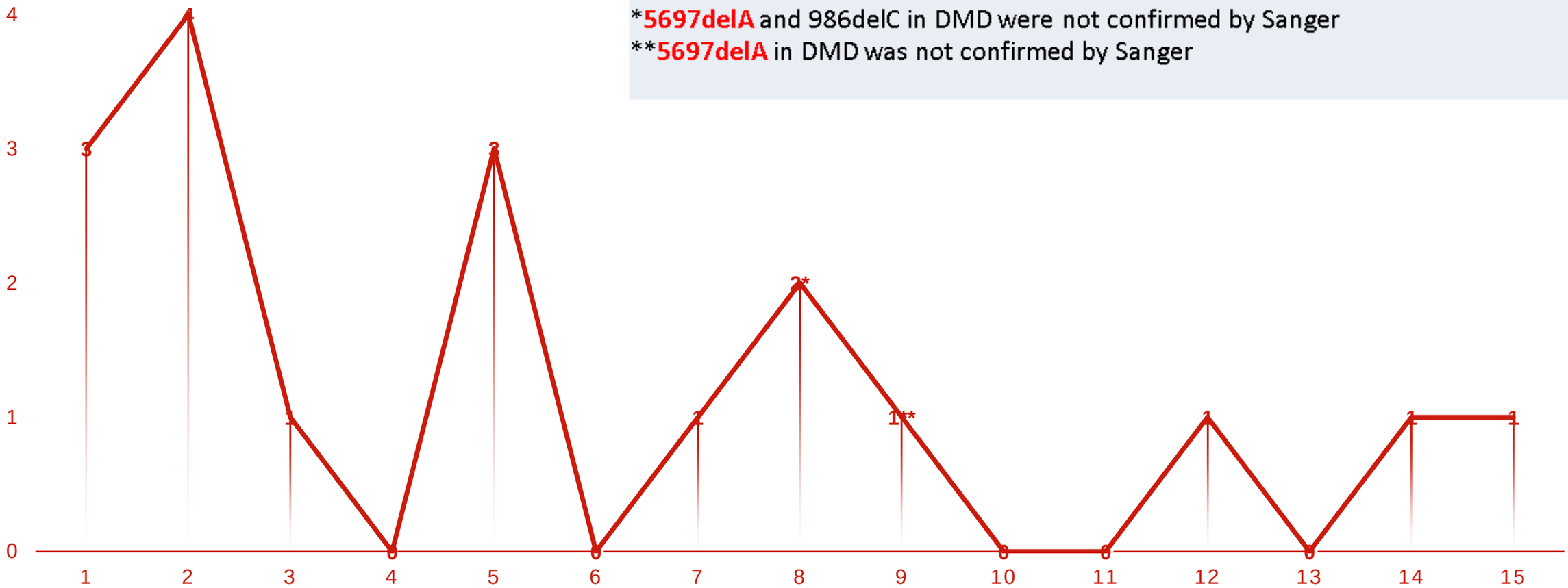


The variant is reported in dbSNP but pathogenic in homozygous condition

Sanger Confirmation of Variants

VARIANTS CONFIRMED BY SANGER FOR NMD PANEL
CLASSIFICATION OF VARIANTS: LIKELY BENIGN, VARIANT OF UNKNOWN SIGNIFICANCE, LIKELY PATHOGENIC AND PATHOGENIC

Average 1.2 variant/sample for the 10 gene NMD panel (18/15)
*5697delA and 986delC in DMD were not confirmed by Sanger
**5697delA in DMD was not confirmed by Sanger



Clinical Interpretation and Reporting

- Check clinical significance of identified SNP in the ROI
- Curate and query Claritas mutation database
- Informatics analysis of novel variants
- Categorization and scoring of variants
- Reporting template generation
- Client Services issues report

Summary of the First Eleven Clinical Samples

NMD Next-gen Panel

One patient: Multi-exon deletion in DMD (should be picked up by MLPA- recommend MLPA test first)

Three patients with Pathogenic or Likely Pathogenic variants: FKRP, DMD and CAPN3 genes

Two patients: Variants of unknown significance

Five patients: No variant result (no variant reported)

Meeting Partners' Needs

Partner	*Need*	*Claritas Service*
Patients	Fast, reliable answer	Genetic testing with consistent interpretation
Clinicians	Support, streamlined process	Pairing test with clinical need, easy ordering and billing
Hospitals	One stop shopping, best value, integrated reporting	Advice navigating testing landscape and consolidating send-out
Payers	Utility, value	Right test, strong clinical justification
Researchers and health systems	Large numbers of well-characterized samples, scalability to enable discovery	Research, data network

For Researchers: Data Network

- High quality data in
- Scale, automation via bioinformatics
- Ability to query by a variety of aspects
- Leverage
- Enable discovery in genetic diseases in a new way – with the goal of helping patients

Large Health Systems and Pharma/Biotech can also benefit from this infrastructure

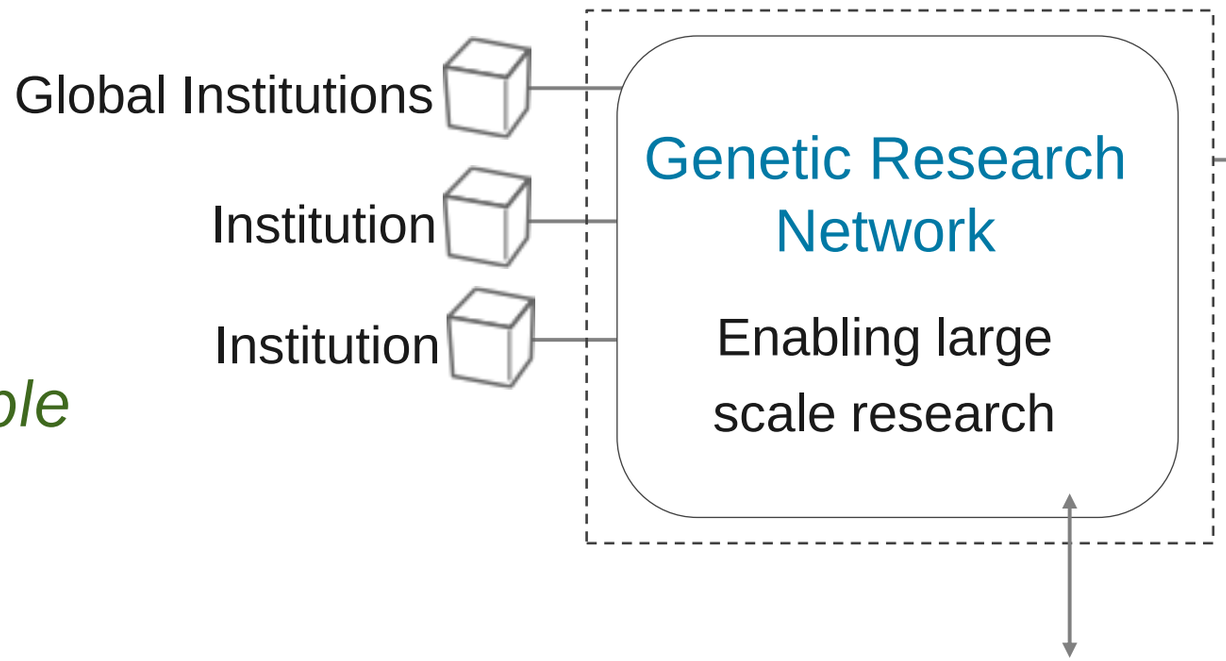
For Researchers: Data Network

- Compliant with: HIPAA, consent, IRB
- Sharing of information while protecting publication rights
- Contributors own their data

- Structured data input, well characterized samples
- Good representation of samples with rare conditions in the dataset
- Systems/software to query
- Great for population and clinical studies

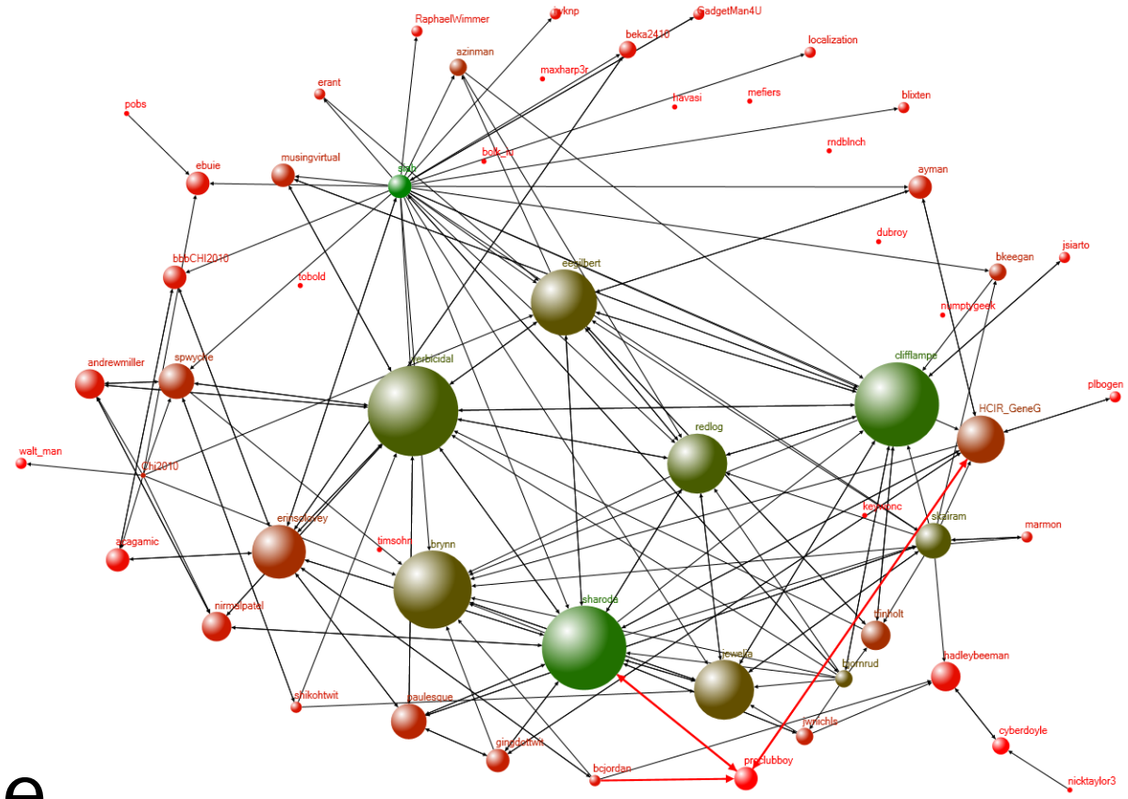
Claritas Data Network

Data management and analysis partnerships possible



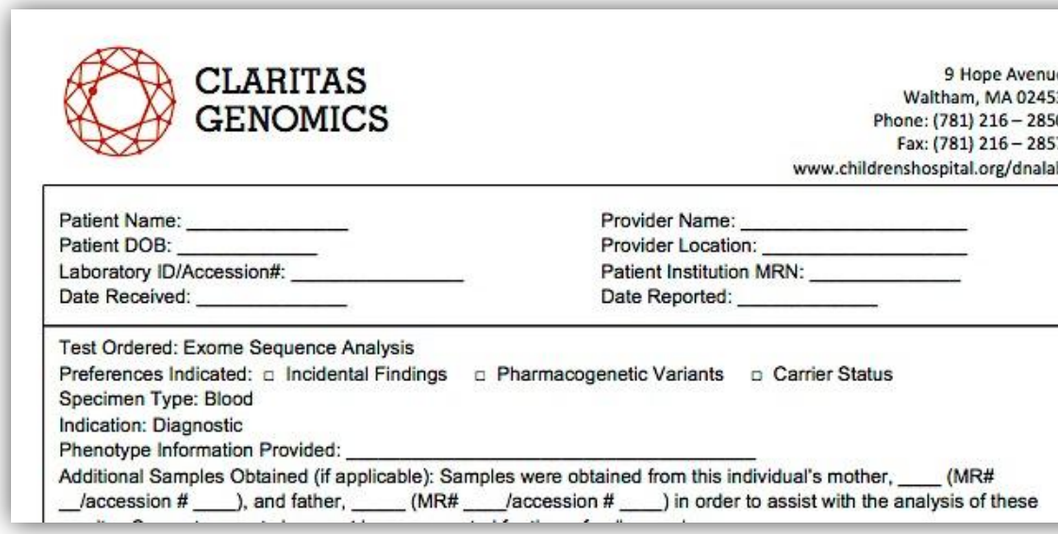
Claritas: Enabling a Clinical and Research Network

- Break down barriers between organizations
- Work together to address the challenges in pediatric genetic diagnostics
- Consolidate and lower costs while maintaining access to best services



Network facilitates scalability and sustainability.

Ultimately good for patients.



CLARITAS GENOMICS

9 Hope Avenue
Waltham, MA 0245
Phone: (781) 216 – 285
Fax: (781) 216 – 285
www.childrenshospital.org/dnalab

Patient Name: _____ Provider Name: _____
Patient DOB: _____ Provider Location: _____
Laboratory ID/Accession#: _____ Patient Institution MRN: _____
Date Received: _____ Date Reported: _____

Test Ordered: Exome Sequence Analysis
Preferences Indicated: ☐ Incidental Findings ☐ Pharmacogenetic Variants ☐ Carrier Status
Specimen Type: Blood
Indication: Diagnostic
Phenotype Information Provided: _____
Additional Samples Obtained (if applicable): Samples were obtained from this individual's mother, ____ (MR# __/accession # ____), and father, _____ (MR# ____/accession # ____) in order to assist with the analysis of these

Partner	*Need*	*Claritas Service*
Patients	Fast, reliable answer	Genetic testing with consistent interpretation
Clinicians	Support, streamlined process	Pairing test with clinical need, easy ordering and billing
Hospitals	One stop shopping, best value, integrated reporting	Advice navigating testing landscape and consolidating send-out
Payers	Utility, value	Right test, strong clinical justification
Researchers and health systems	Large numbers of well-characterized samples, scalability to enable discovery	Research, data network

Building a Network Through Our Services

Claritas: an interface enabling our partners to address the challenges and opportunities of clinical use of Next Generation Sequencing

- *Complexity of measurement*
- *Complexity of interpretation*
- *Clinical utility*
- *Appropriate utilization*
- *Reimbursement*
- *Result reporting*
- *Support services and explanation*
- *Discovery*

Right question
Right test
Right result

CLARITAS
GENOMICS

Using genomics to enhance pediatric health

BioConference Live Presentation