Molecular Diagnostics for Genetic Diseases

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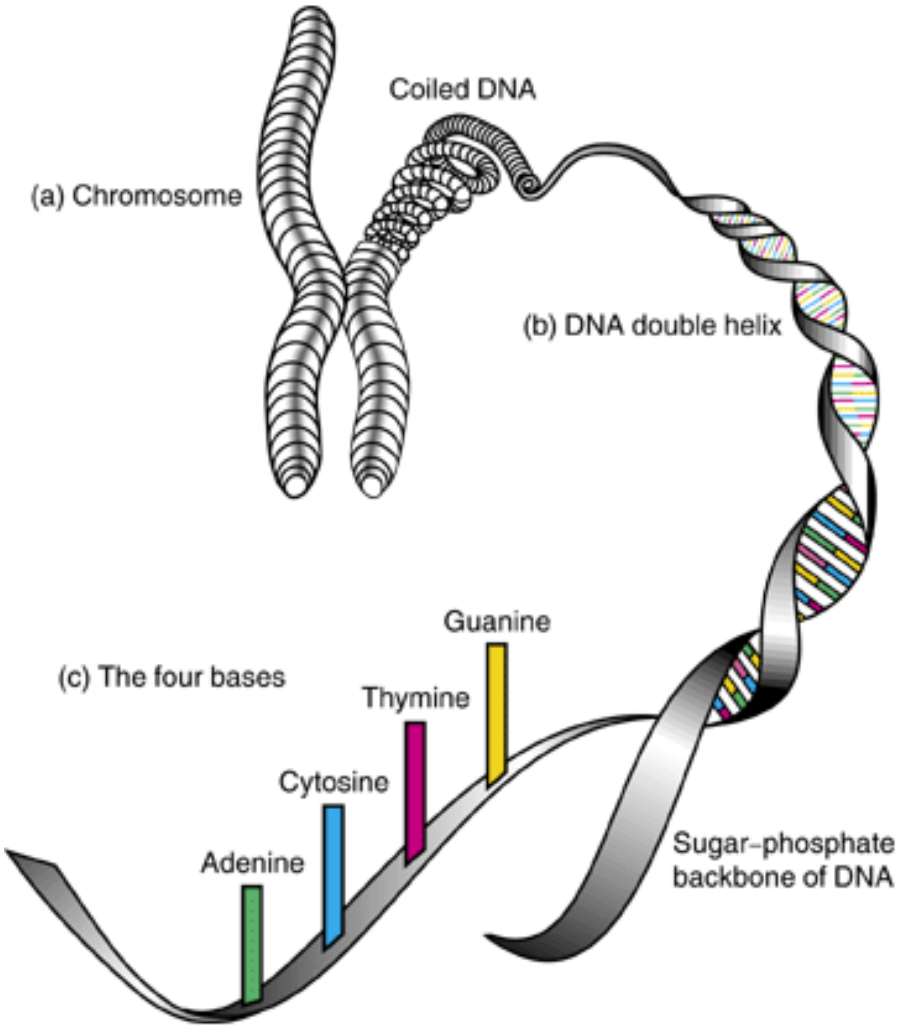

https://str.llnl.gov/str/Branscomb.html

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Growth of Clinical Genetics at Children's National

Huge increase in number of patients seen in the Division of Genetics and Metabolism

- 2009- 1,800
- 2016- 8,500

Correlates with large increase in new hires of ABMG Clinical Geneticists and CGCs

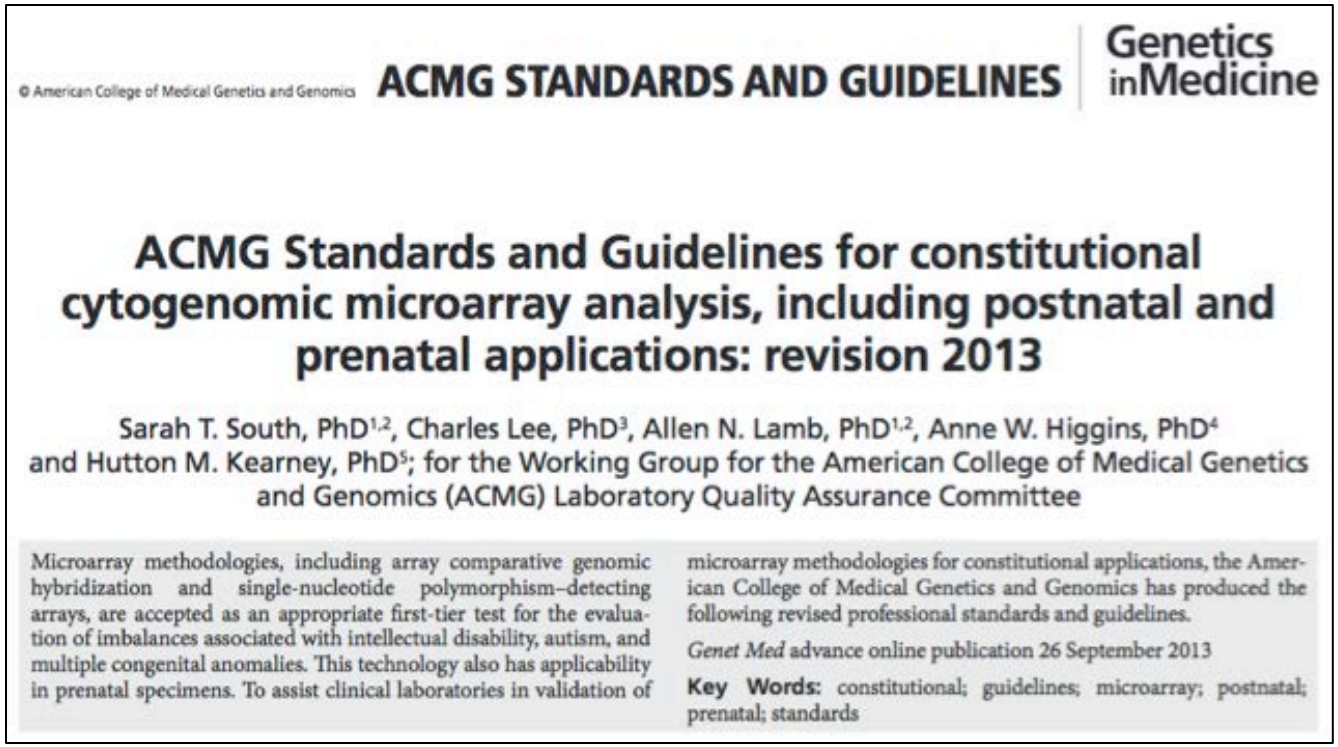
2009- 5 ABMG Clinical Geneticists and 5 CGCs

2016- 14 ABMG Clinical Geneticists and 18 CGCs

This resulted in an explosion of genetic testing at CN

Genetic Testing at Children's National

6/2013- Molecular Diagnostics Laboratory in Division of Laboratory Medicine goes live with Chromosomal Microarray



ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013

Sarah T. South, PhD[1,2], Charles Lee, PhD[3], Allen N. Lamb, PhD[1,2], Anne W. Higgins, PhD[4] and Hutton M. Kearney, PhD[5]; for the Working Group for the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee

Microarray methodologies, including array comparative genomic hybridization and single-nucleotide polymorphism–detecting arrays, are accepted as an appropriate first-tier test for the evaluation of imbalances associated with intellectual disability, autism, and multiple congenital anomalies. This technology also has applicability in prenatal specimens. To assist clinical laboratories in validation of microarray methodologies for constitutional applications, the American College of Medical Genetics and Genomics has produced the following revised professional standards and guidelines.

Genet Med advance online publication 26 September 2013

Key Words: constitutional; guidelines; microarray; postnatal; prenatal; standards

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Genetic Testing at Children's National

6/2013- Molecular Diagnostics Laboratory in Division of Laboratory Medicine goes live with Chromosomal Microarray

8/2014- Transitioned to first and only FDA cleared Chromosomal Microarray

Product News: FDA Clearance of Genetic Test for Developmental Delays and Intellectual Disabilities in Children

19 Mar 2014

CytoScan® Dx Assay represents a technology leap over traditional postnatal genetic tests and significantly improves diagnostic capability

Affymetrix, Inc. has announced that it has received 510(k) clearance from the U.S. Food and Drug Administration (FDA) to market its CytoScan® Dx Assay. This assay is intended for the postnatal detection of DNA copy number variants (CNV) in patients referred for chromosomal testing.

CytoScan Dx Assay is designed to help physicians diagnose children's developmental and intellectual disabilities more comprehensively by enabling a high-resolution genome-wide analysis of genetic aberrations. High resolution analysis can reveal small aberrations not readily seen using traditional techniques.

affymetrix
Biology for a better world

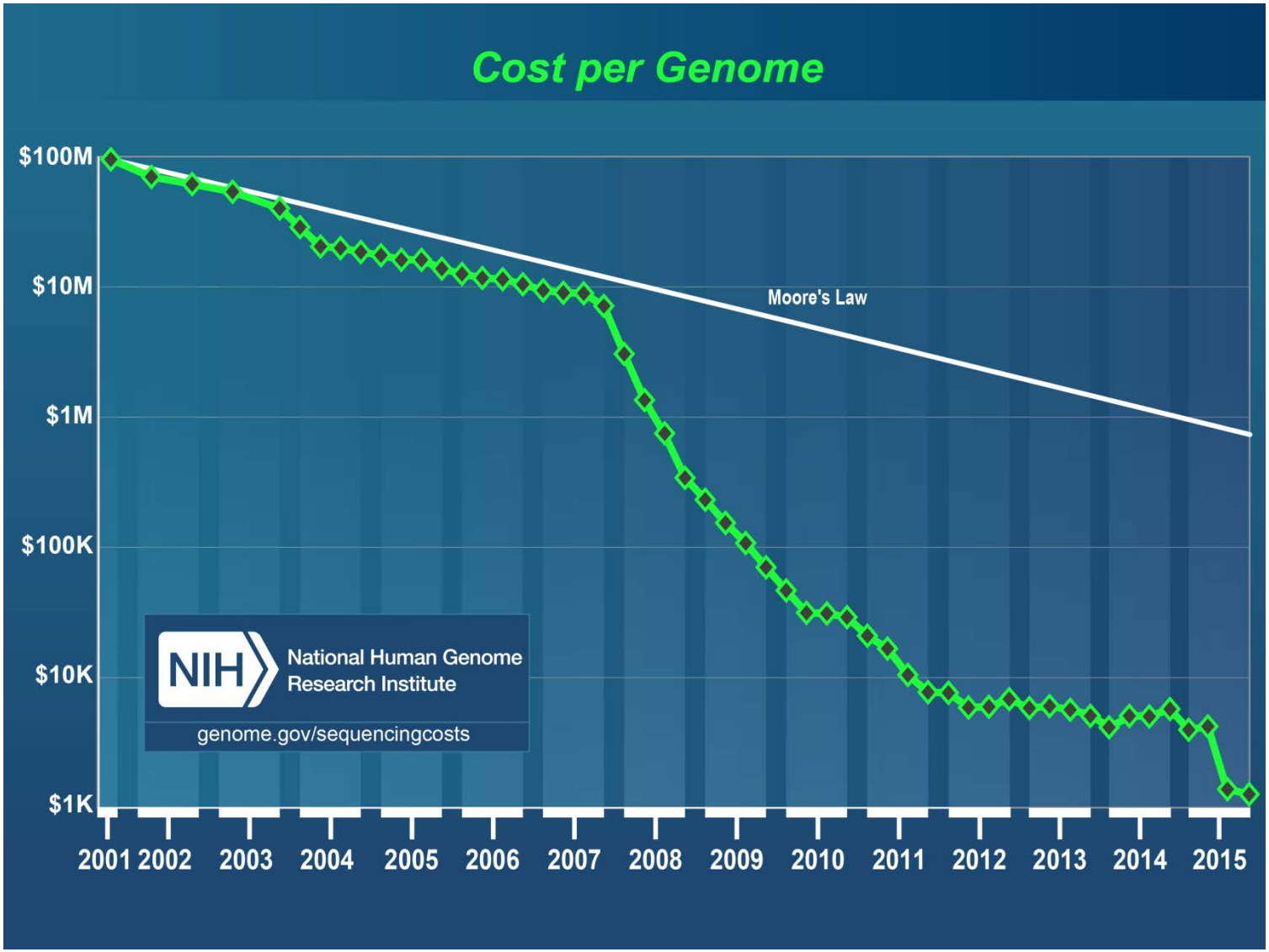
CytoScan® Dx Assay

To aid in the diagnosis of developmental delay and intellectual disability

Unrivaled performance. **Results that matter.**

IVD For *In Vitro* Diagnostic Use CE

Sequencing Advancements Driving Innovation



Prior Options for Clinical Sequencing

Single gene sequencing
Small gene panels
Large gene panels

Advantages

-Targeted to diagnose specific diseases or conditions

-Uncommon incidental findings

-Turn around time between 4-8 weeks

Disadvantages

-A negative result will lead to a completely new test needing to be ordered

-Patient will need to come back in for recollect and insurance will be billed again for a totally new test

-No customization of testing to specific patient

Whole Exome Sequencing
Whole Genome Sequencing

Advantages

-Comprehensive

-One test for all genes

-Unlikely to need an additional test ordered in immediate future

-Less money than ordering several other tests incrementally

Disadvantages

-Possibilities for many incidental findings

-Variants of uncertain significance will be higher

-Genes of uncertain significance will be reported

-Long turn around time 4-6 months

-Very expensive for a single test

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New Option for Sequencing Through CNMC

- 2/2015 Molecular Diagnostics Laboratory goes live with Clinical Personalized Sequencing Program
  - Using high throughput genomic sequencing we sequence all clinically relevant genes for every patient tested
  - We take a personalized approach to analyze and interpret only clinically relevant genes for that particular patient
    - Phenotype driven
    - Ability to leverage Chromosomal Microarray results to focus the analysis even further
  - If negative, additional genes to analyze can be added on, and since we have the data all we have to do is unmask the additional gene content

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Benefits to Patients, Physicians, and Lab

- Cost savings
  - Patient is billed less for initial test and any additional genes can be analyzed for a low cost
  - Laboratory can reduce interpretation costs and focus on variants in the genes that matter to the patient
- Shorter turn around time
  - Patient doesn't need to wait 4-6 months to get results
  - Currently 4-6 weeks
- Comprehensive
  - All clinically relevant genes are sequenced and additional genes can be analyzed for mutations rapidly by unmasking data
- Fewer incidental findings and variants of uncertain clinical significance
  - Patients analysis is customized to them, which yields less VUS results and no incidental findings
  - Laboratory spends less money on the interpretations and confirmations since the analysis is focused on clinically relevant genes

Ordering Process

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Ordering Process

- At present, we are really only accepting samples on patients in CNHS.
  - Hopefully this will change soon!

- Therefore, the best approach for our community pediatricians to have genetic testing done through CNHS is to refer your patient to the Genetics Department. This referral also includes the added benefits of:
  - Pre- AND post-test genetic counseling
  - Insurance authorization
    - Our lab offers the ability to extract and hold DNA at the time of the initial genetic visit. Therefore, when authorization is obtained, the family doesn't have to come back for a separate blood draw.

DIVISION OF LABORATORY MEDICINE
LABORATORY TEST REQUISITION # 5 (Molecular)

Requesting Physician: _____

*Requesting Physician Signature: _____; Pager #: _____

Service: _____

*Collection Date & Time: _____(date); _____(time)

*Collected by: _____ *Specimen Source: _____

***ICD10 DIAGNOSIS CODE(S) MANDATORY:**

PLACE PATIENT LABEL HERE

or provide at least 2 of the following:

Name:

DOB:

MRN:

Insurance company (required):

Insurance Authorization (circle one): ***NOT REQUIRED*** ***or*** ***GRANTED***

If authorization was granted, please provide authorization number and time frame:

	AANP	Aortic Aneurysm Panel
	ARVD	ARVD Panel
	BRUGA	Brugada Syndrome Panel
	CFTR	CFTR Sequencing
	PBMTNC	Chimerism - Post BMT Engraftment without Cell Selection
	PTCHD	Chimerism - Pre BMT (Donor)
	PTCHR	Chimerism - Pre BMT (Recipient)
	CMA	Chromosome Microarray
	CARP	Comprehensive Arrhythmia Panel
	ARCP	Comprehensive Arrhythmia & Cardiomyopathy Panel
	CCMP	Comprehensive Cardiomyopathy Panel
	CONTX	Comprehensive Connective Tissue Panel
	CEPIL	Comprehensive Epilepsy Panel
	CHLP	Comprehensive Hearing Loss Panel
	COMOI	Comprehensive OI Panel
	CONNX	Connexin Panel
	CPVTP	CPVT Panel
	CUTIS	Cutis Laxa Panel
	CFPCR	Cystic Fibrosis 60 Mutations
	DCMP	Dilated Cardiomyopathy
	DNEXT	DNA Extraction
	DOMOI	Dominant OI Panel
	ECTOP	Ectopia Lentis Panel
	EDSP	Ehlers Danlos Syndrome Panel
	FVL	Factor V Leiden Mutation Testing
	FBN1	FBN1 Sequencing
	FRAGX	Fragile X
	HCMP	Hypertrophic Cardiomyopathy Panel
	LVNCP	Left Ventricular NonCompaction Panel
	LOEYS	Loeys-Dietz Panel
	LONGQT	Long QT Syndrome
	NATP	NAT Panel
	NOONAN	Noonan Spectrum Disorders Panel
	FIIM	Prothrombin Gene Mutation (G20210A)
	PTEN	PTEN Sequencing
	RECOI	Recessive OI Panel
	SEPIL	STAT Epilepsy Panel
	STICK	Stickler Syndrome Panel
Personalized Sequencing Panels - **MUST** be discussed with Molecular Lab		
	PSPGL	Personalized Sequencing Panel by Gene List. Select either pre-built gene list or custom gene list in right column. If selecting a custom gene list, please either list genes, attach a gene list to this requisition, or e-mail the list to the LabMed Genetic Counselors.
	PSPP	Personalized Sequencing Panel by Phenotype. Clinical indication MUST be completed. Please allow 2-3 days for gene list to be generated.
Parental Testing		
		Chromosome Microarray Parental Testing must attach the child's test results or include the following information:
		Child's Name:
		Child's MRN:
		NGS Parental Testing must attach the child's test results or include the following information:
		Child's Name:
		Child's MRN:

Name of Primary Contact (physician, genetic counselor, nurse)

Number for Primary Contact

Clinical Indication and Family History

Personalized Sequencing Panel Pre-Built Gene Lists (Select below):

	Aplastic Anemia Panel
	Comprehensive Brain Malformation Panel
	Comprehensive Charcot-Marie-Tooth Panel
	Comprehensive NeuroMuscular Panel
	Congenital Disorders of Glycosylation Panel
	Congenital Muscular Dystrophy Panel
	Congenital Myasthenia syndrome Panel
	Congenital Myopathy Panel
	Congenital Disorders of Glycosylation Panel
	Febrile Seizure Panel
	Hemophagocytic Lymphohistiocytosis (HLH) Panel
	Intellectual Disability / Autism Spectrum Disorder Panel
	Microcephaly Panel
	Nuclear Mitochondrial Gene Panel
	Primary Ciliary Dyskinesia (PCD) Panel
	Rett / Angelman syndrome Panel
	X-linked Intellectual Disability Panel

Personalized Sequencing Panel Custom Gene List (write gene list below)

Ethnicity (check all that apply)

	African American
	Asian
	European Caucasian
	Hispanic
	Middle Eastern
	Native American
	Pacific Islander
	Undefined

***Required Field**

Questions/concerns can be addressed by contacting the Molecular Lab at x2631

Personalized Sequencing Panels (PSP)

PSP by Gene List

- Physician provides gene list
- Lab determines:
    - Is gene available for sequencing
    - Is gene well covered

PSP by Phenotype

- Physician provides a phenotype
- Lab generates a gene list using:
    - HPO
    - OMIM
    - PubMed
    - GeneTests
    - Other labs panels
    - Regions of homozygosity and/or CNVs (determined by chromosomal microarray)
- Lab determines:
    - Is gene available for sequencing
    - Is gene well covered

Personalized Sequencing Panels (PSP)

- Pricing based on number of genes
  - 1 gene
  - 2-5 genes
  - 6-15 genes
  - 16-50 genes
  - 51-100 genes
  - 101-150 genes
  - 151-200 genes
  - 201-250 genes
  - 251-300 genes
  - 301-500 genes

- If additional genes are requested, as long as the total # of genes stays within the same bracket a second analysis is available free of charge
- If the additional genes requested requires jumping to a different bracket (or if we have already done one free second analysis) then a minimal charge is associated with adding on additional genes that equals the difference between two brackets

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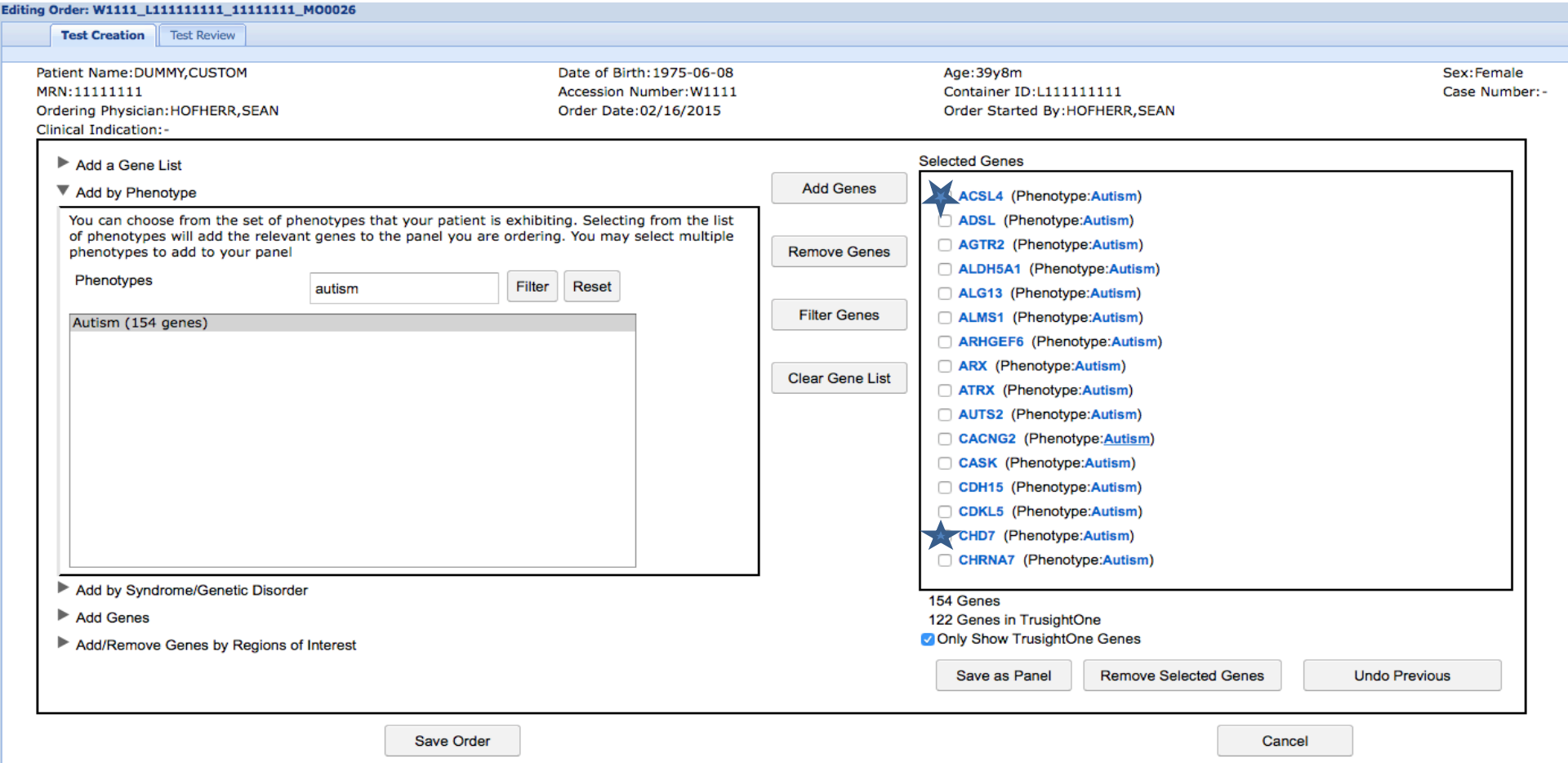
Genes associated with Autism



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ACSL4 vs *CHD7*

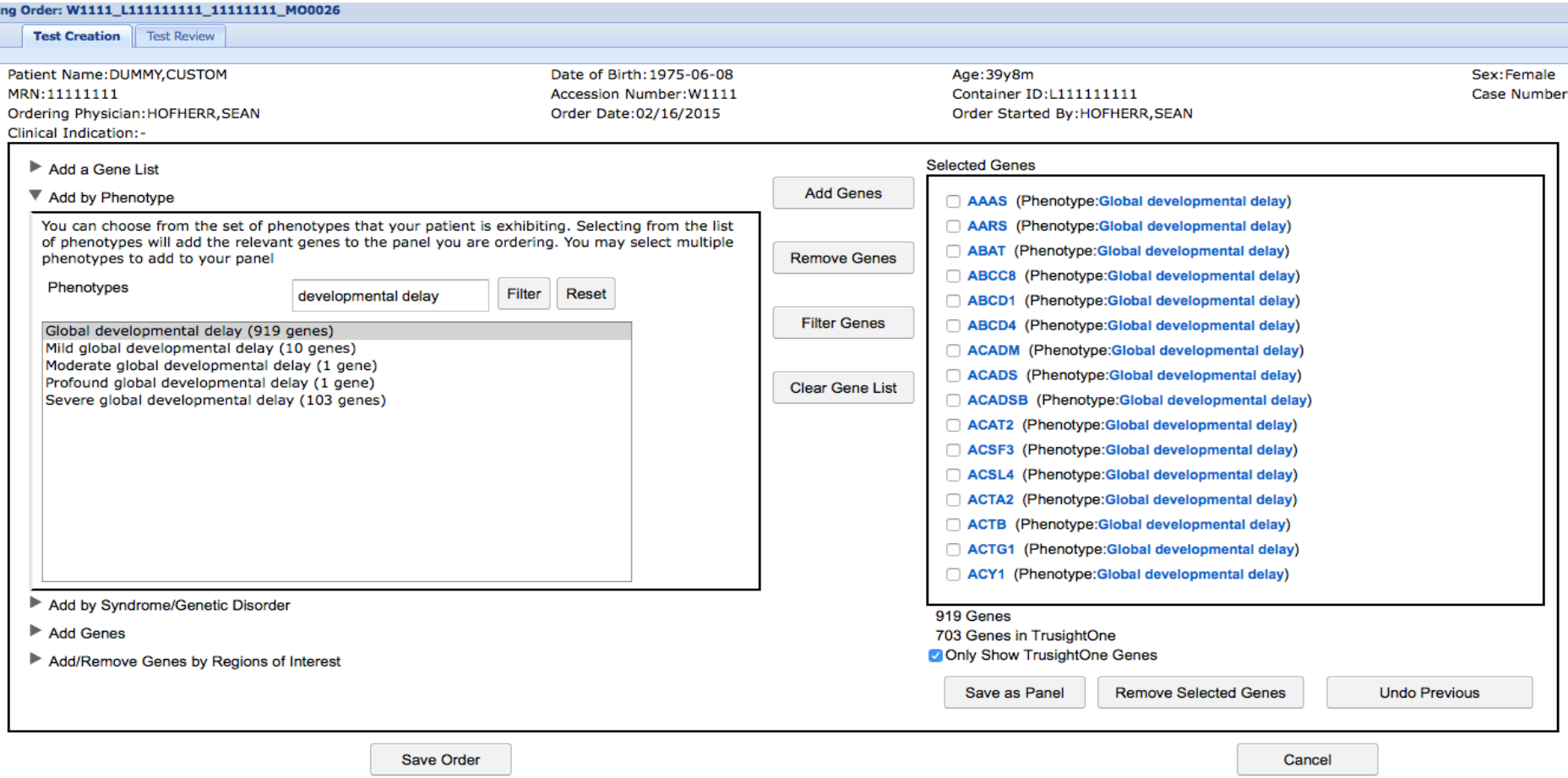
ACSL4 (X-linked Mental Retardation, type 68)

- X-linked Inheritance
- Phenotype: nonsyndromic, intellectual disability and autism in males
- Females have highly variable cognitive capacities, ranging from moderate mental retardation to normal intelligence depending on her lyonization.

CHD7 (CHARGE syndrome)

- Autosomal Dominant Inheritance
- Phenotype: ***c***oloboma, ***h***eart defects, choanal ***a***tresia, ***r***etarded growth and development, ***g***enital abnormalities, and ***e***ar anomalies
- Autism included in the phenotype, but certainly not the main feature

Genes associated with Developmental Delay



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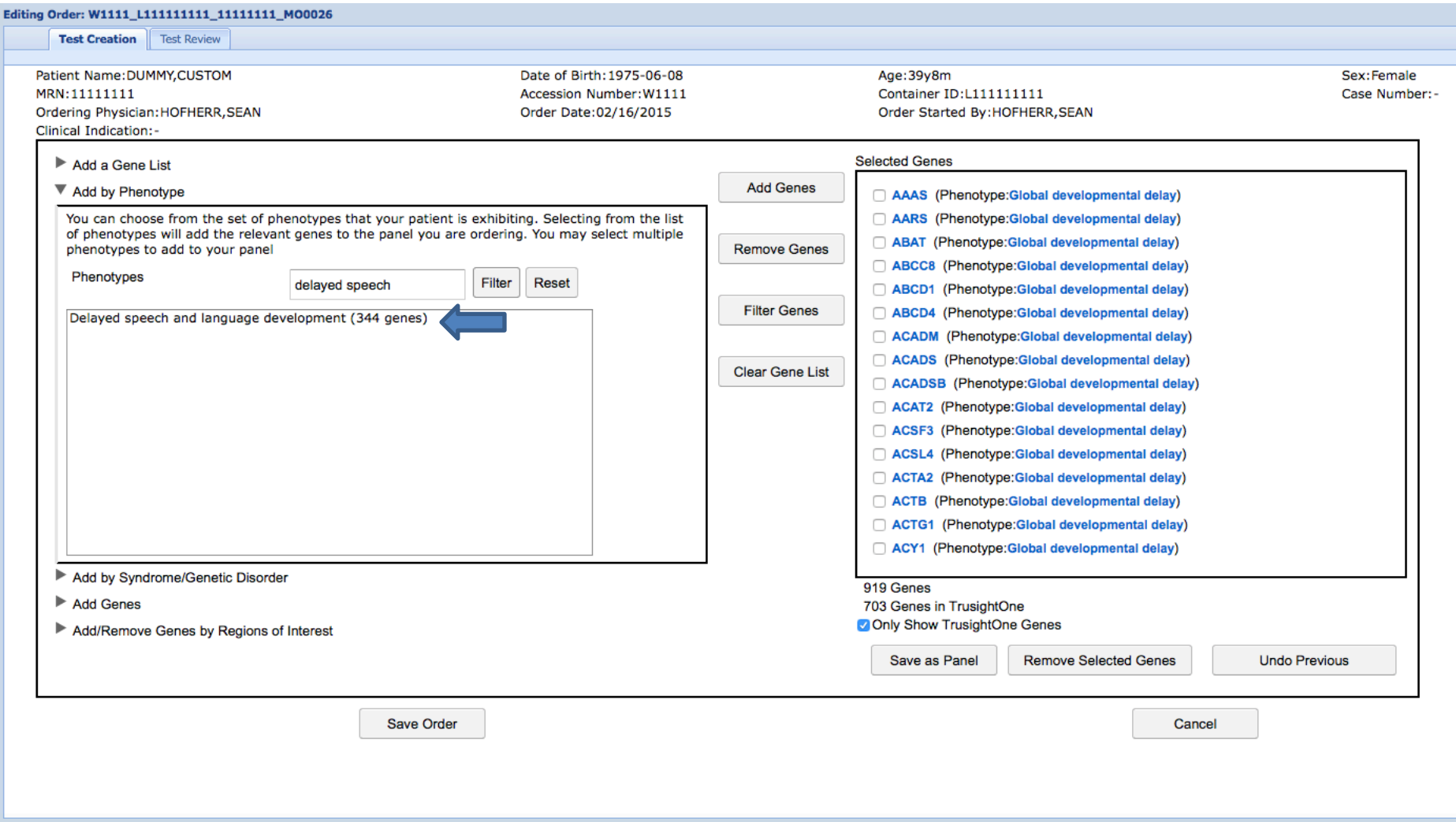
Genes associated with Speech Delay



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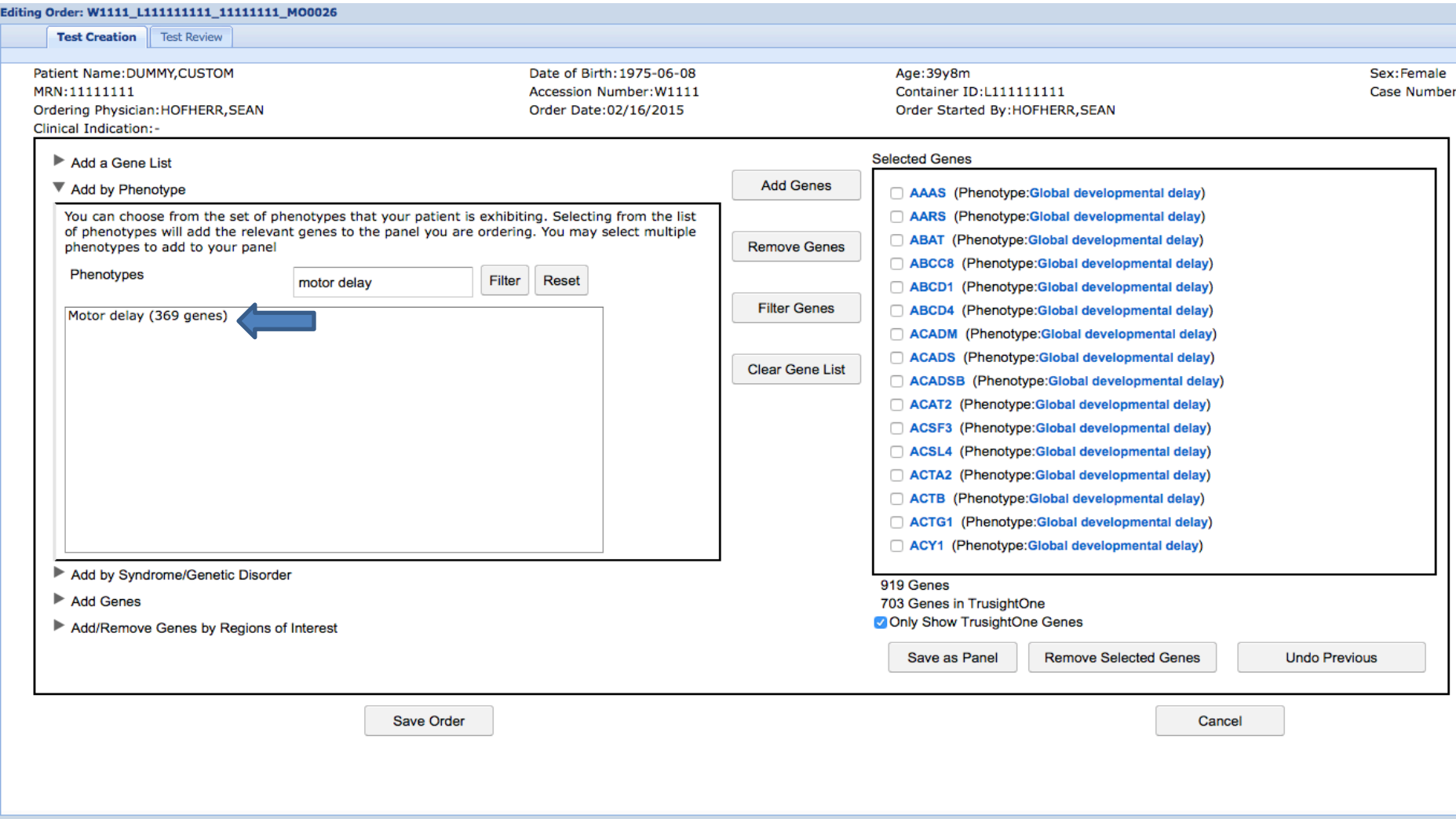
Genes associated with Motor Delay



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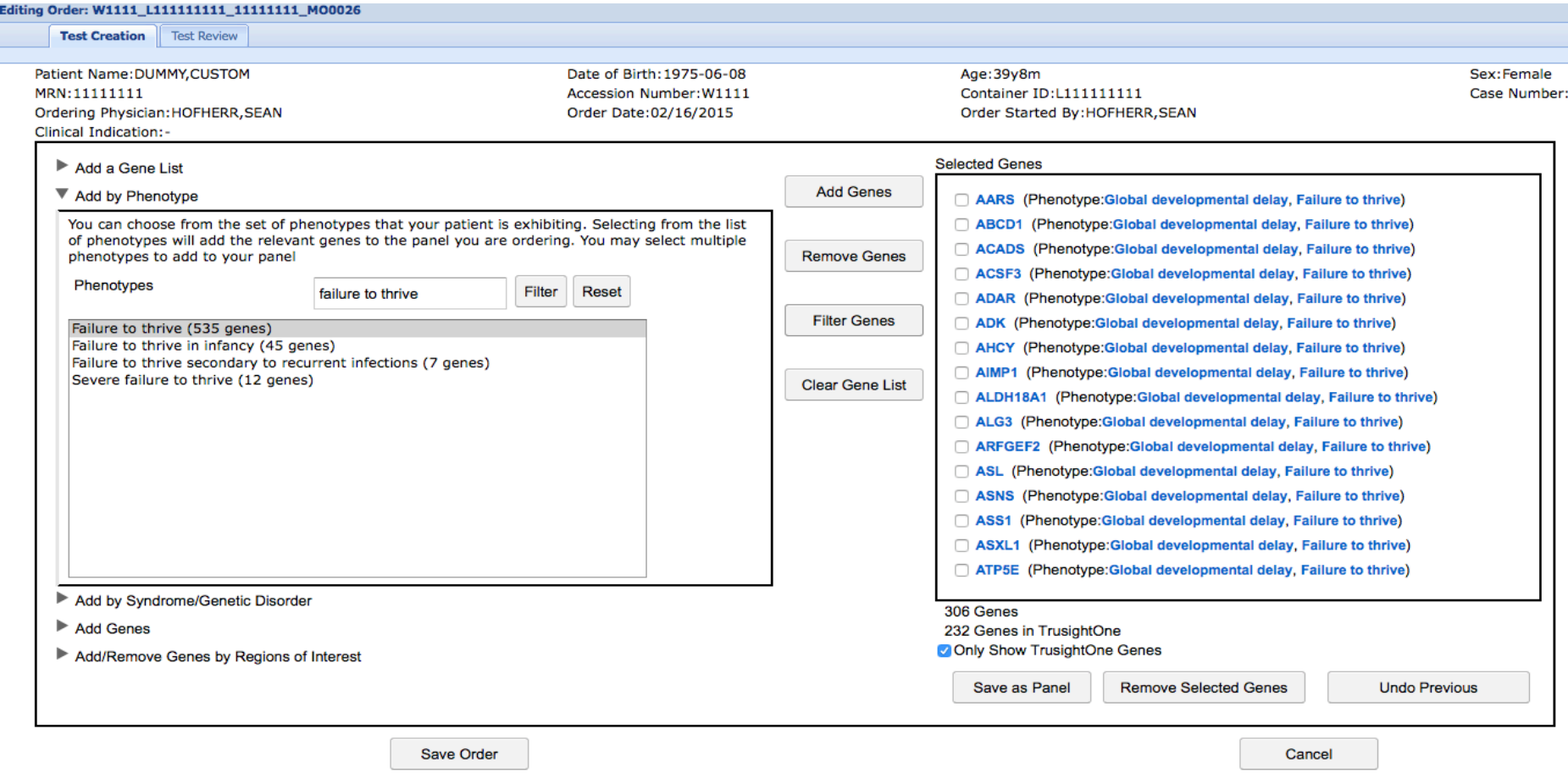
Child w/ developmental delay AND failure to thrive



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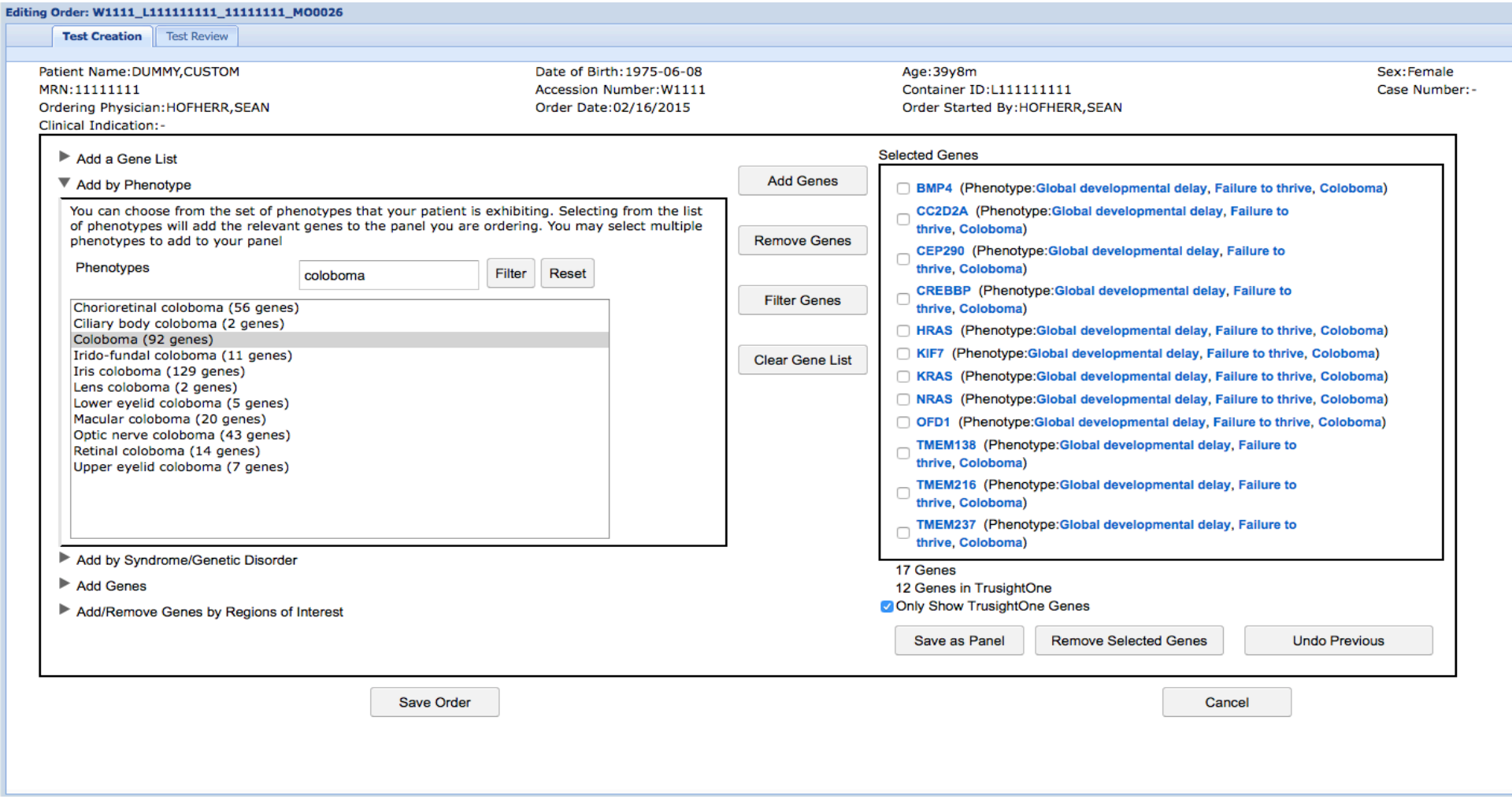
Child with developmental delay, failure to thrive AND coloboma



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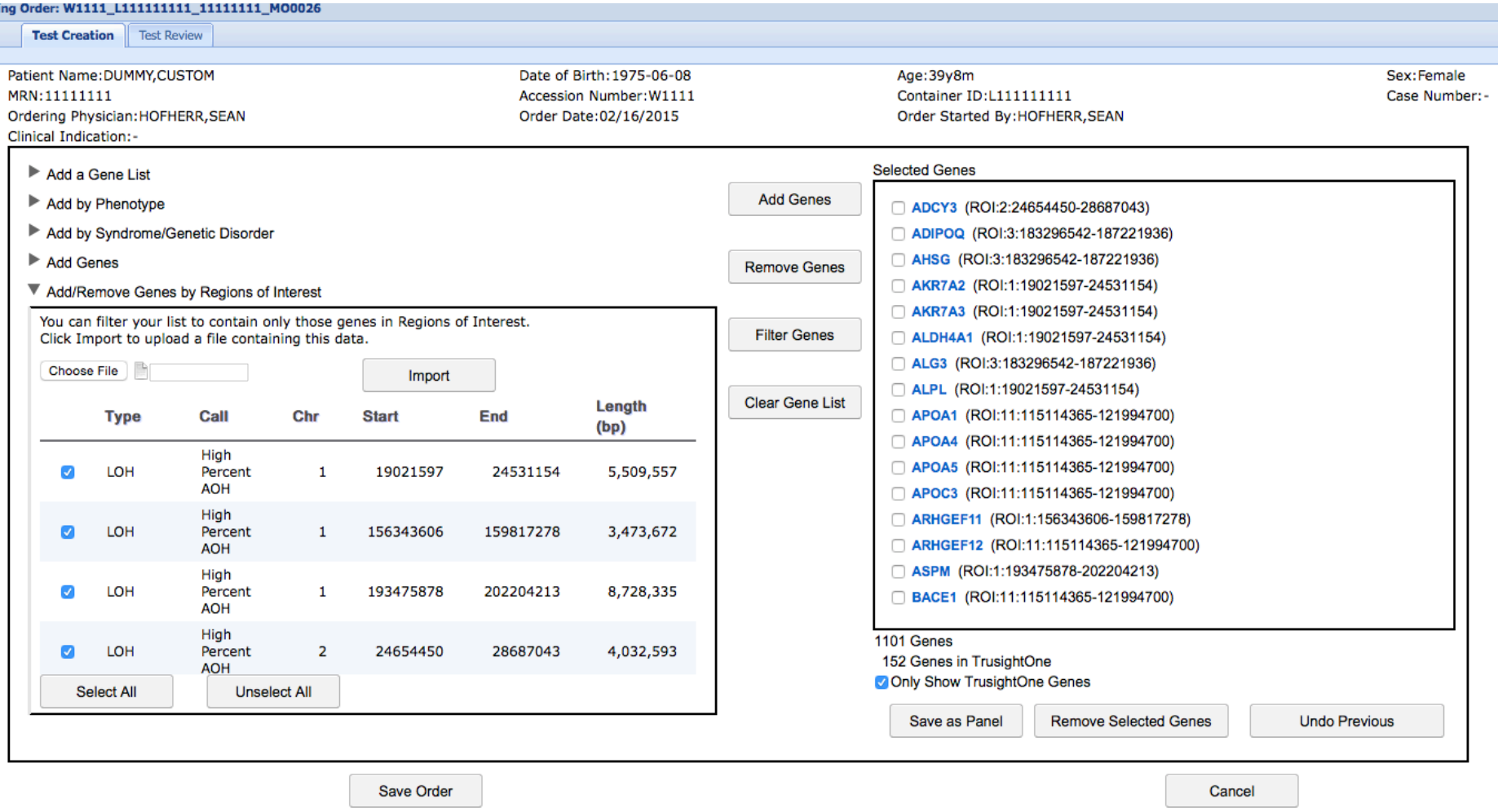
Child with AOH on CMA



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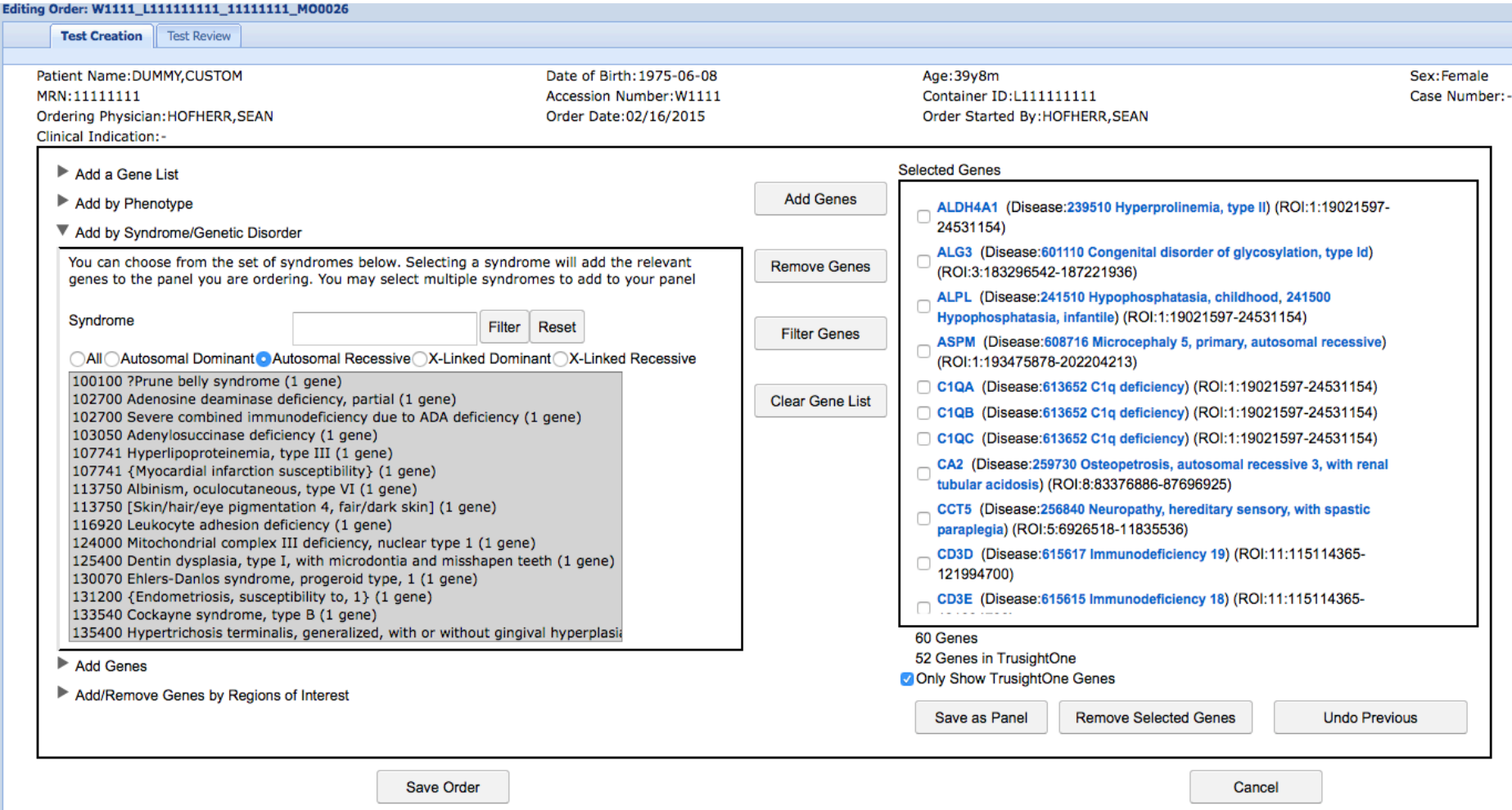
Child with AOH on CMA



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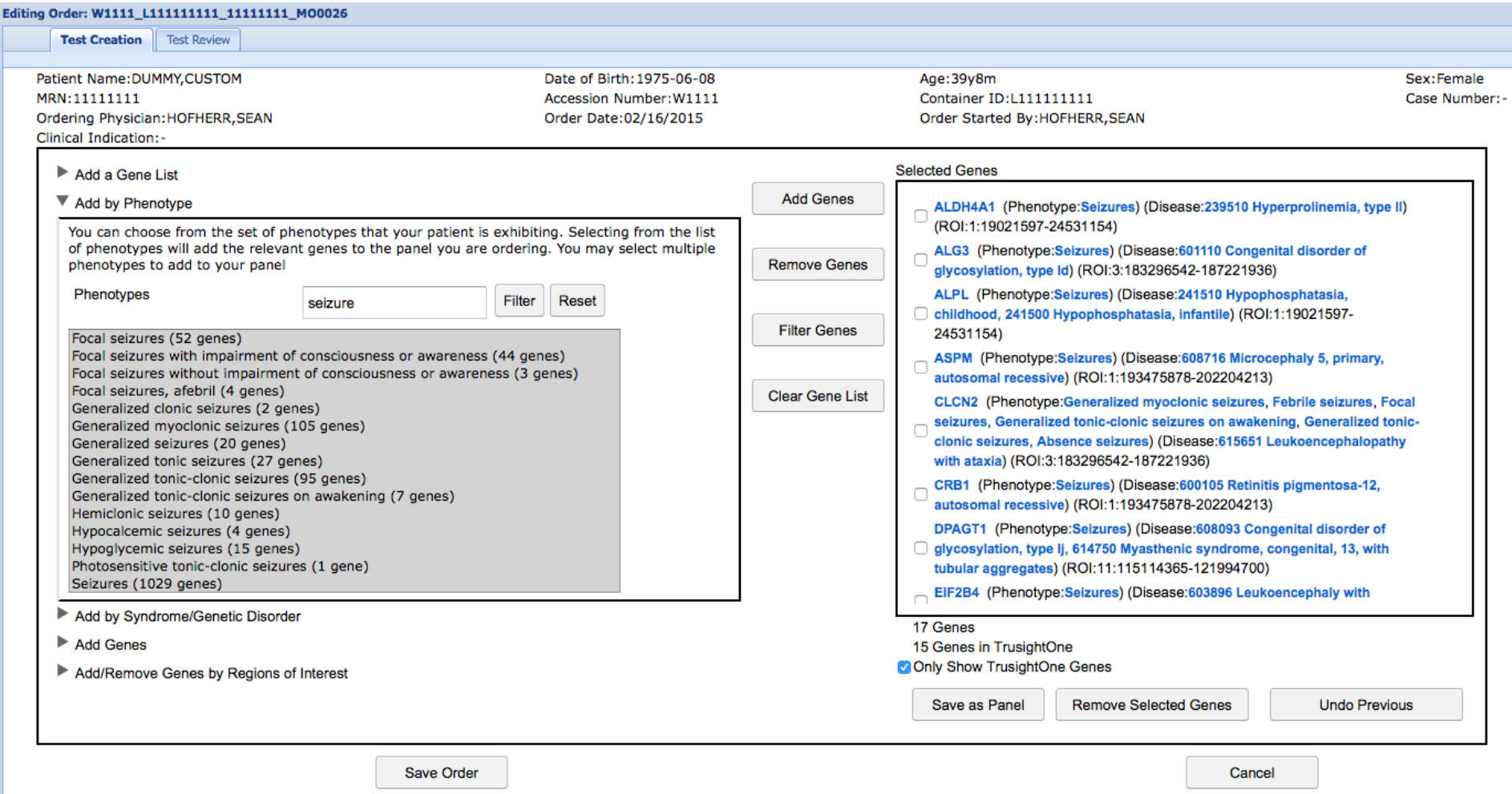
Child with AOH on CMA and a phenotype of seizures



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CMA case examples

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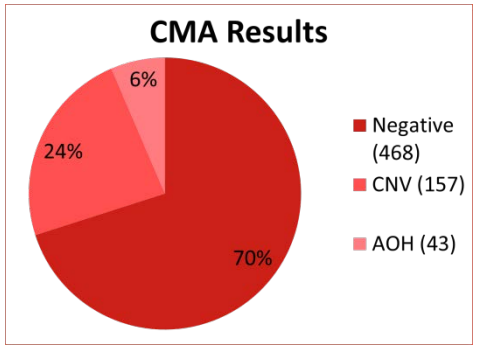
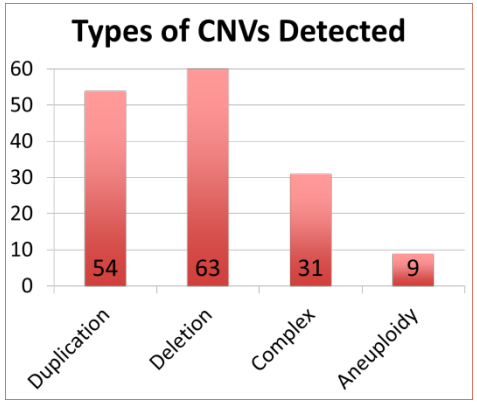
CytoScan®Dx Assay: Our 1st year experience





AOH & Identity by descent	N
1st degree relative	1
2nd degree relative	8
3rd degree relative	13
4th degree relative	13

AOH & UPD	N
Chr 7	2
Chr 14	4
Chr 15	2

Common CNVs Identified		N
1q21 del	ASD, microcephaly	2
1q21 dup	ASD, macrocephaly	2
3q13 del	Primrose	1
5p15 del	Cri du chat	1
5q35 del	Sotos	1
5q35 dup	Hunter McAlpine	1
7q11 del	Williams	5
15q11 del	PWAS	2
15q11 dup	PWAS	2
16p11 dup	ASD	1
16p13 dup	CREBBP	2
17p11 del	Smith Magenis	1
17p11 dup	Potocki Lupski	1
18p11 del	18p-	1
18p11 trip	Tetrasomy 18p	1
18q22 del	18q-	3
22q11 del	VCF	6
22q11 dup		4
22q13 del	Phelan McDermid	2

Inheritance of CNVs	N
De novo	15
Maternal	29
Paternal	11

Complex CNVs Identified	
1q44x4 1q44x3	Dup/Trip of 1q44
8p23.3p23.1x1 8p23.1p12x3	Derivative chromosome 8
15q13.2q13.3x1 16p11.2x1	15q13 AND 16p11 (220 kb, obesity) del syndromes
16p12.2x1 17p12x1	16p12 AND 17p12 (HNPP) del syndromes
22q11.1q11.21x4 22q11.21x3	Cat eye syndrome
Xq28x3 Xq28x4 Xq28x3	Complex X rearrangement

Aneuploidy	N
Trisomy 13	1
Trisomy 18	2
Trisomy 21	1
47,XXY	2
47,XYY	2
48,XXYY	1
Mosaic Trisomy 14	1
Mosaic Turner	1

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Is it 22q11?

Case Study #1

- 2.5 year old male
- Seen in clinic
- Developmental delay
- Limited speech
- Mild hypotonia

Case Study #2

- 1 day old female
- Seen in NICU
- Prenatal diagnosis of TOF
- No additional malformations

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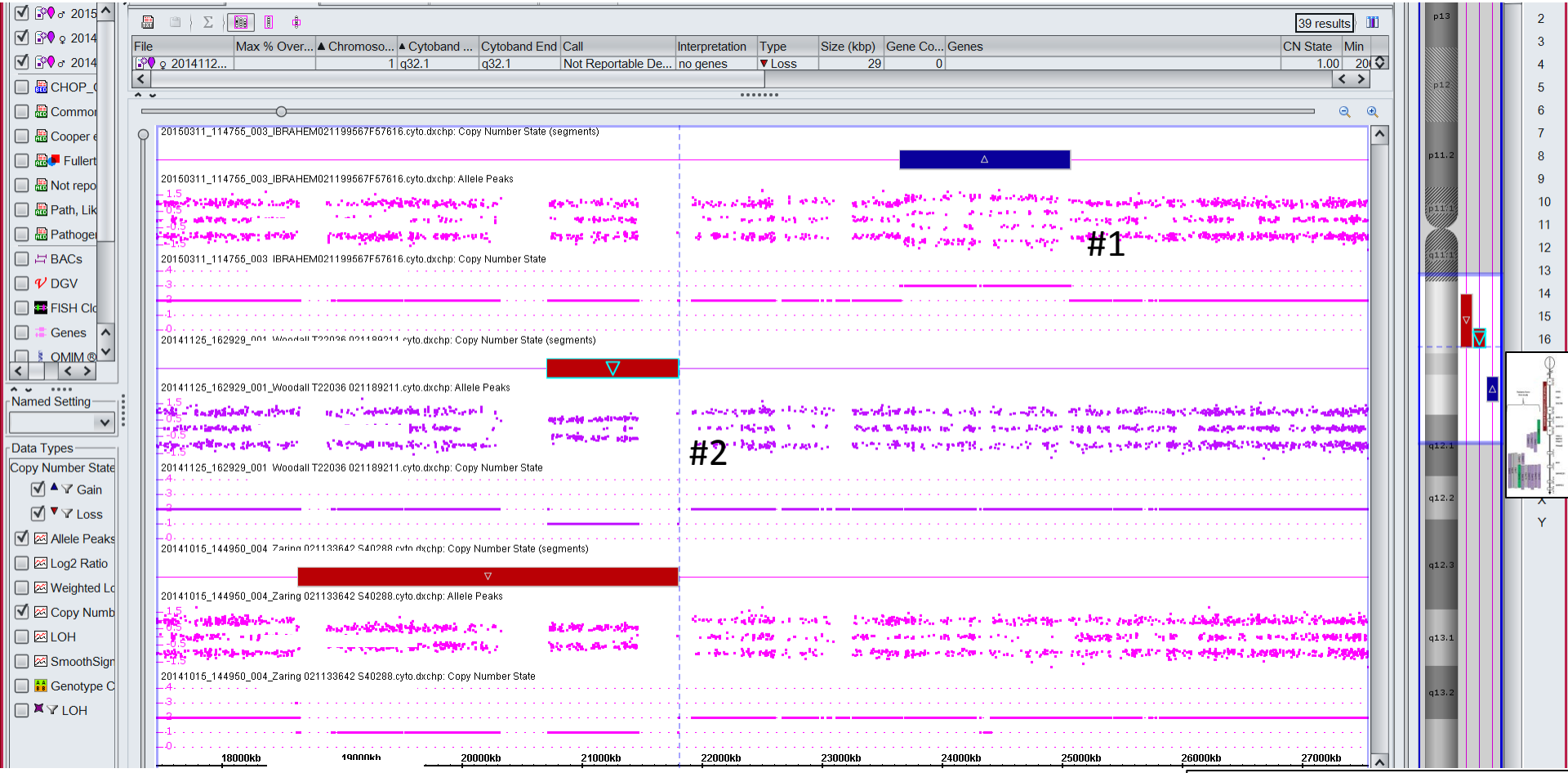
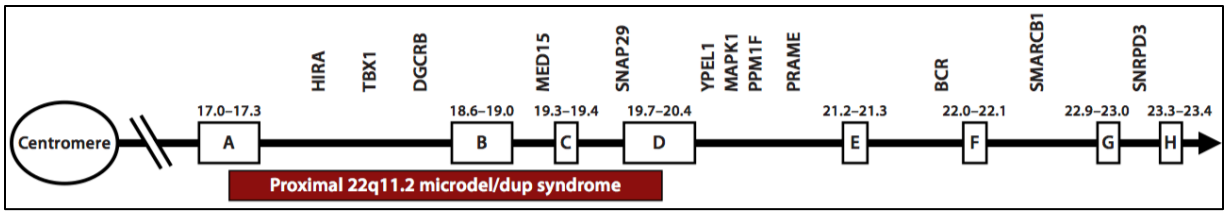
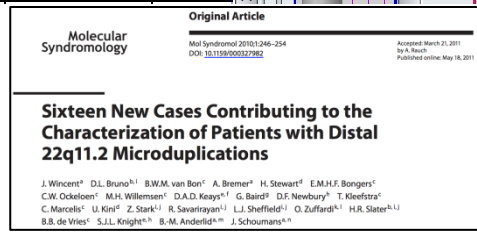
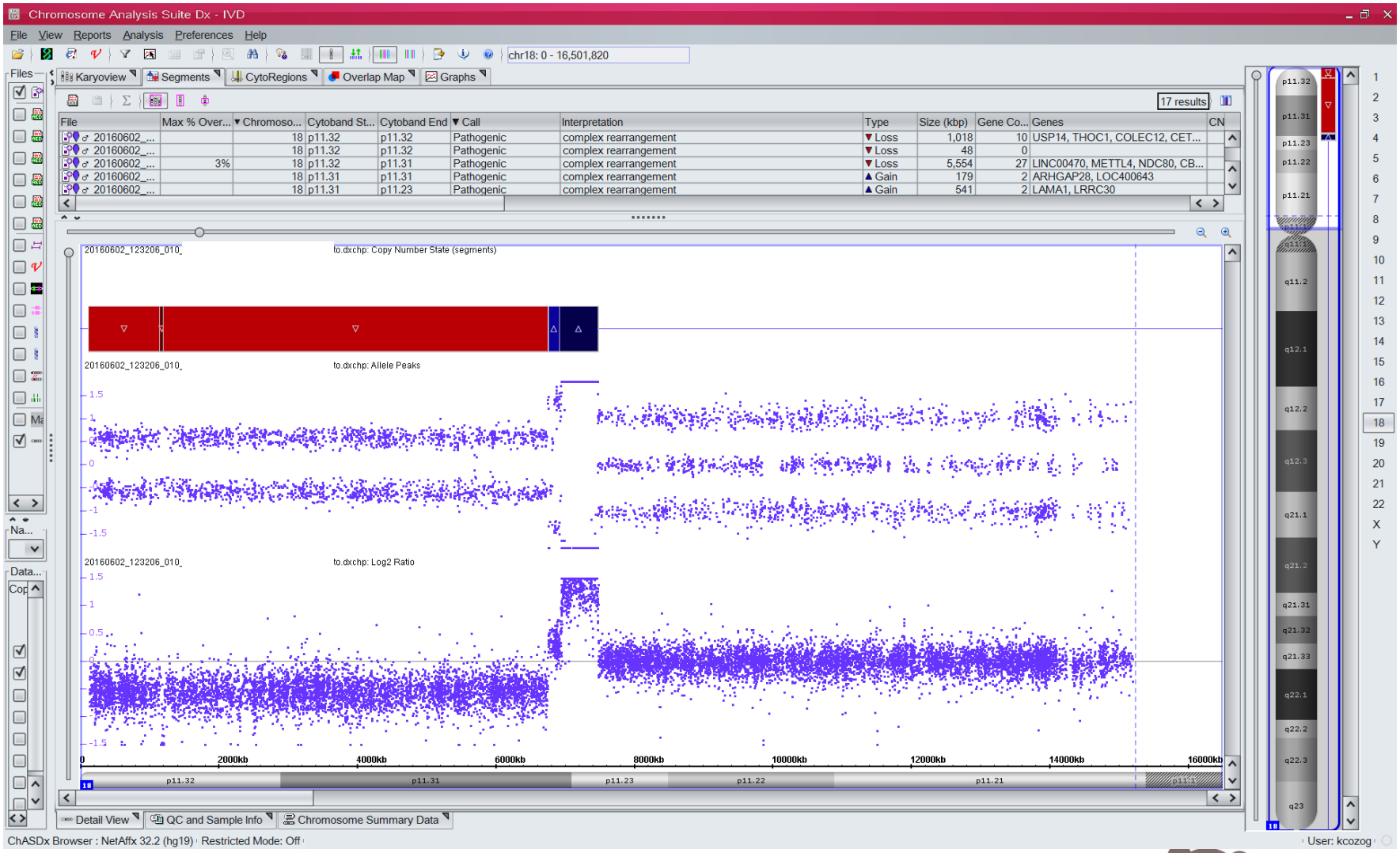
22q11 – Array is better than FISH







Array is better than FISH and Karyotype



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PWAS

Case Study #3

- 4 day old male
- Seen in NICU
- Profound hypotonia
- No major malformations

Case Study #4

- 16 month old male
- Seen in clinic
- Developmental delays
- Happy demeanor
- Wide-based gait

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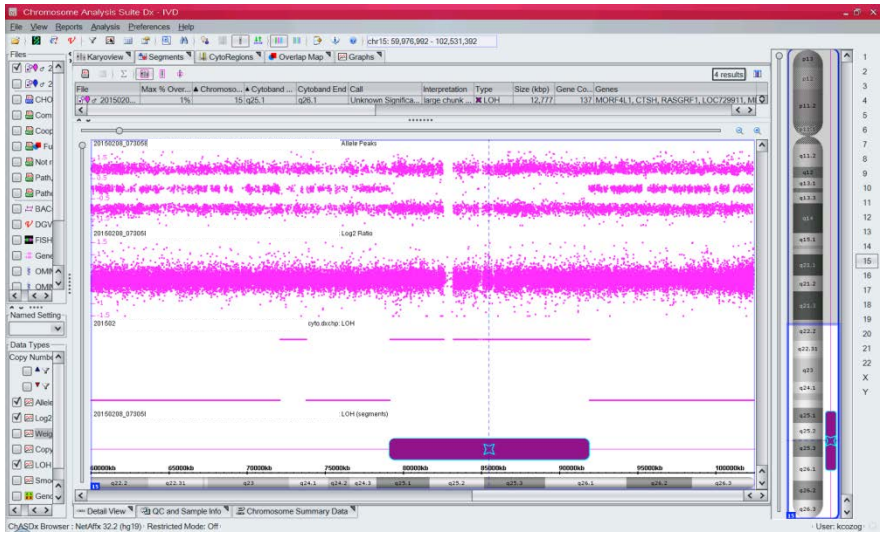
PWAS

Case Study #3

12.8Mb AOH at 15q25.1q26.1

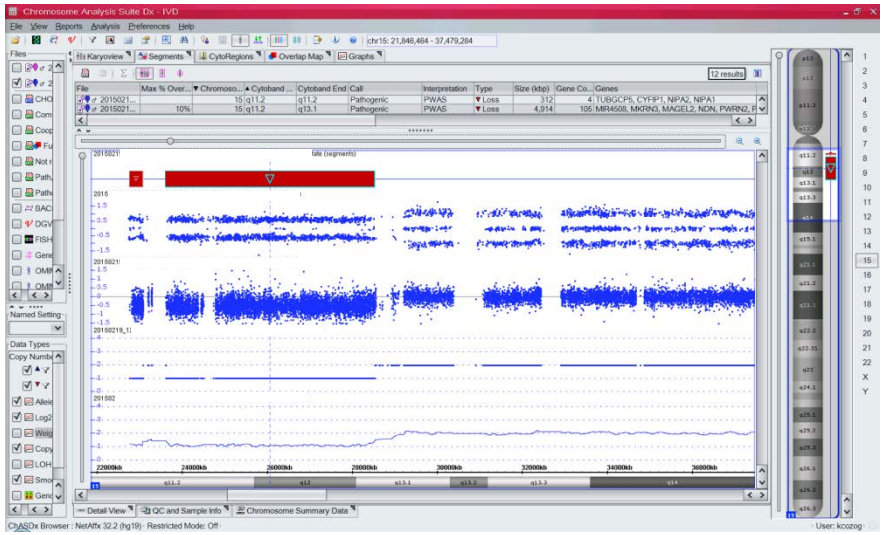
Concerning for UPD



F/U methylation studies confirm PWS

Case Study #4

5.8Mb deletion of 15q11.2q13.1



Phenotype consistent with AS

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NGS case examples

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Case Example #1

- 4-month-old boy with encephalopathy, myoclonus, spasticity, diffuse cerebral volume loss, and central apnea seen in Genetics clinic for the first time.
- "I think the most cost-effective approach will be to obtain a Personalized Gene Panel sequencing for genes that have previously been associated with the aforementioned conditions, mainly early myoclonic encephalopathy. This Gene Panel will include the following genes: *STXBP1, ARX, SLC25A22, PNKP, AMT, GLDC, GCSH, GLYCTK, ABAT, ATP7A, ADSL, ARHGEF9, GLRA1, GLRB, SLC6A5, SETBP1* and *GPHN.*"
- Insurance authorization was obtained and testing was sent:

RESULT SUMMARY: NEGATIVE. No clinically relevant alterations were identified in this patient.

CLINICAL INDICATION: 7 month old male with encephalopathy, myoclonus, spasticity, diffuse cerebral volume loss, and central apnea.

GENES ANALYZED*: ABAT, ADSL, AMT, ARHGEF9, ARX, ATP7A, GCSH, GLDC, GLRA1, GLRB, GLYCTK, GPHN, PNKP, SETBP1, SLC25A22, SLC6A5, STXBP1
*See Limitations section for information regarding areas of low coverage.

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Case Example #1

- Return visit to Genetics: 11-month-old boy with a history of polyhydramnios in-utero, microcephaly, encephalopathy, stimulation myoclonus/excessive startle reflex, spasticity, central apnea and trichomegaly.
- "PAA and UOA have been normal in the past, as was a PSP including several genes known to be associated with a similar phenotype. At this point, I would like to add a few genes to the already sent PSP. "
- A **FREE** second look was obtained:

RESULT SUMMARY: POSITIVE. Testing identified that this patient is compound heterozygous for two variants in TSEN54. One of these variants is a known pathogenic mutation. The other variant is a likely pathogenic deletion of two basepairs. Homozygous and compound heterozygous mutations in TSEN54 have been associated with multiple forms of pontocerebellar hypoplasia (MIM#610204, 277470, 225753).

CLINICAL INDICATION: A Personalized Sequencing Panel is requested on an 11 month old male with a history of encephalopathy, myoclonus, spasticity, central apnea, and trichomegaly.

GENES ANALYZED*: ALDH7A1, ASNS, ASXL1, B3GNT1, BOLA3, CDKL5, DAG1, FKRP, FKTN, GLRX5, HDAC8, ISPD, LARGE, LIAS, MECP2, NIPBL, PCDH19, PNPLA6, POMGNT1, POMT1, POMT2, RAD21, SCN1A, SEPSECS, SMC1A, SMC3, TSEN2, TSEN34, TSEN54
*See Limitations section for information regarding areas of low coverage.

RESULTS:

1) Pathogenic change in Gene TSEN54
Variant c.919G>T
p.A307S
Effect: Pathogenic

2) Likely Pathogenic change in Gene TSEN54
Variant c.670_671delAA
p.K224fs
Effect: Likely Pathogenic

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Benefits

This approach benefited this family in multiple aspects:

1. Provided patient with a diagnosis without a second 'stick.'
    - In general, babies don't like having their blood drawn.
2. Provided patient with a diagnosis at minimal cost.
    - Because of original gene list and tiered pricing structure, there was 'wiggle room' for the ordering physician to add on more genes without generating an additional cost to the family.
3. Provided the ordering physician with a diagnosis without having to interact with the insurance company again.
    - We all know how painful that experience can be!

Case Example #2

23y0 Female G1P0

U/S identified bilat enlarged and cystic kidneys

Seen at CNMC at 36 wk GA to discuss diagnosis, L&D plans, long-term prognosis

"At this point, the clinical picture seems consistent with autosomal recessive polycystic kidney disease."

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Case Example #2

Baby transferred to CNMC on DOL 2

- Respiratory Distress
- Hypoglycemia
- Minor dysmorphic features noted

Molecular genetic testing for PKD1, PKD2, HNF1B, PKHD1 obtained

Baby deteriorated on DOL3, was unable to be resuscitated and expired

NBS abnl for CPTII was called out the next day

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Case Example #2

CPT2 genes were added on to sample in lab

RESULT SUMMARY: POSITIVE ANALYSIS. Testing identified a homozygous known pathogenic mutation in CPT2. Homozygous and compound heterozygous mutations in CPT2 have been associated with many forms of CPTII deficiency, including lethal neonatal CPTII deficiency (MIM#608836).

CLINICAL INDICATION: Infant male with echogenic kidneys, history of prenatal oligohydramnios, hypoglycemia, hypothermia, respiratory distress, and an abnormal nbs.

GENES ANALYZED: CPT2, HNF1B, PKD1*, PKD2, PKHD1, SLC25A20 (Alias: CACT)
*See Limitations section for information regarding areas of low coverage.

RESULTS:

1) Pathogenic change in Gene CPT2
Variant c.680C>T
p.P227L
Effect: Pathogenic

INTERPRETATION:

Variant 1
CPT2
c.680C>T
p.P227L

This patient is homozygous for a missense substitution in CPT2. Homozygous and compound heterozygous mutations in CPT2 have been associated with many forms of CPTII deficiency, including lethal neonatal CPTII deficiency (MIM#608836). The detected change results in a single amino acid substitution (Pro>Leu) within exon 4. There is a moderate physicochemical difference resulting from the change of this highly conserved amino acid. This alteration has been reported before in databases of affected individuals and publications as a pathogenic mutation in patients with lethal neonatal CPTII deficiency.1,2 Based on the available information, this variant is pathogenic. (NM_000098.2)

Lethal Neonatal CPTII

Liver failure, hypoketotic **hypoglycemia**, cardiomyopathy, **respiratory distress**, and/or cardiac arrhythmias occur. Affected individuals have liver calcifications and **cystic dysplastic kidneys** [Vladutiu et al 2002b, Sigauke et al 2003].

Neuronal migration defects including cystic dysplasia of the basal ganglia have been reported [Pierce et al 1999].

Prognosis is poor. **Death occurs within days** to months.

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Benefits

This approach benefited this family in multiple aspects:

1. Provided patient with a diagnosis at minimal cost.
    - Sample was in the lab at time of abnormal NBS result which was after patient's demise and there was no additional charge to add on the CPT2 gene.
2. Provided the family with the correct recessive disorder.
    - Recurrence risk is same for ARPKD and CPTII = 25%.
    - But with proper diagnosis and molecular confirmation, future pregnancies can be tested.
3. Quick turn-around-time of process helped ensured family was aware of the actual diagnosis.
    - Results were disclosed with genetic counseling as part of the autopsy meeting with parents.

Case Example #3

- Newborn male born at an estimated 36-37 weeks to a G8P5→6 mom with no prenatal care.
- Apgars of 7^1, 9^5
- Developed respiratory distress and transferred to CNHS on an oscillator.
- Consult requested because of respiratory distress and concern for possible skeletal dysplasia

- PE: limited because of clinical state
    - Camptodactyly
    - Underrotated thumbs

Case Example #3

Consanguineous family

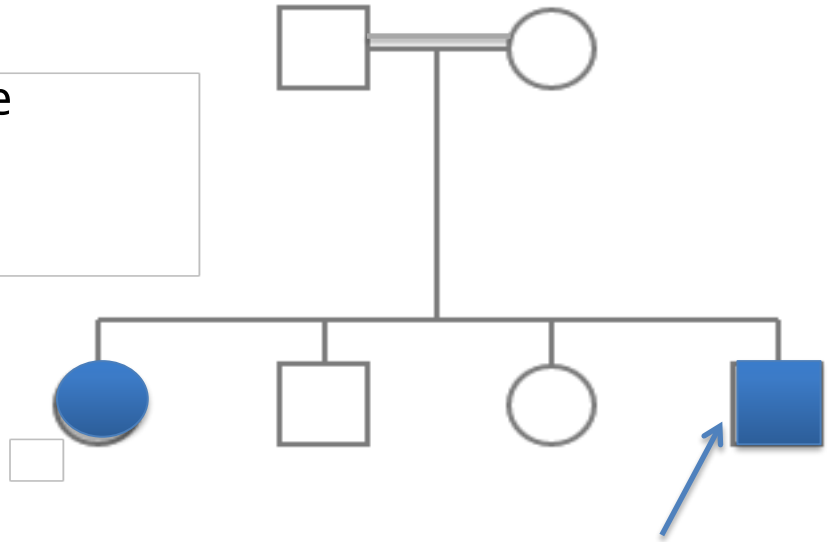
Parents: normal stature

2 siblings A&W with normal stature

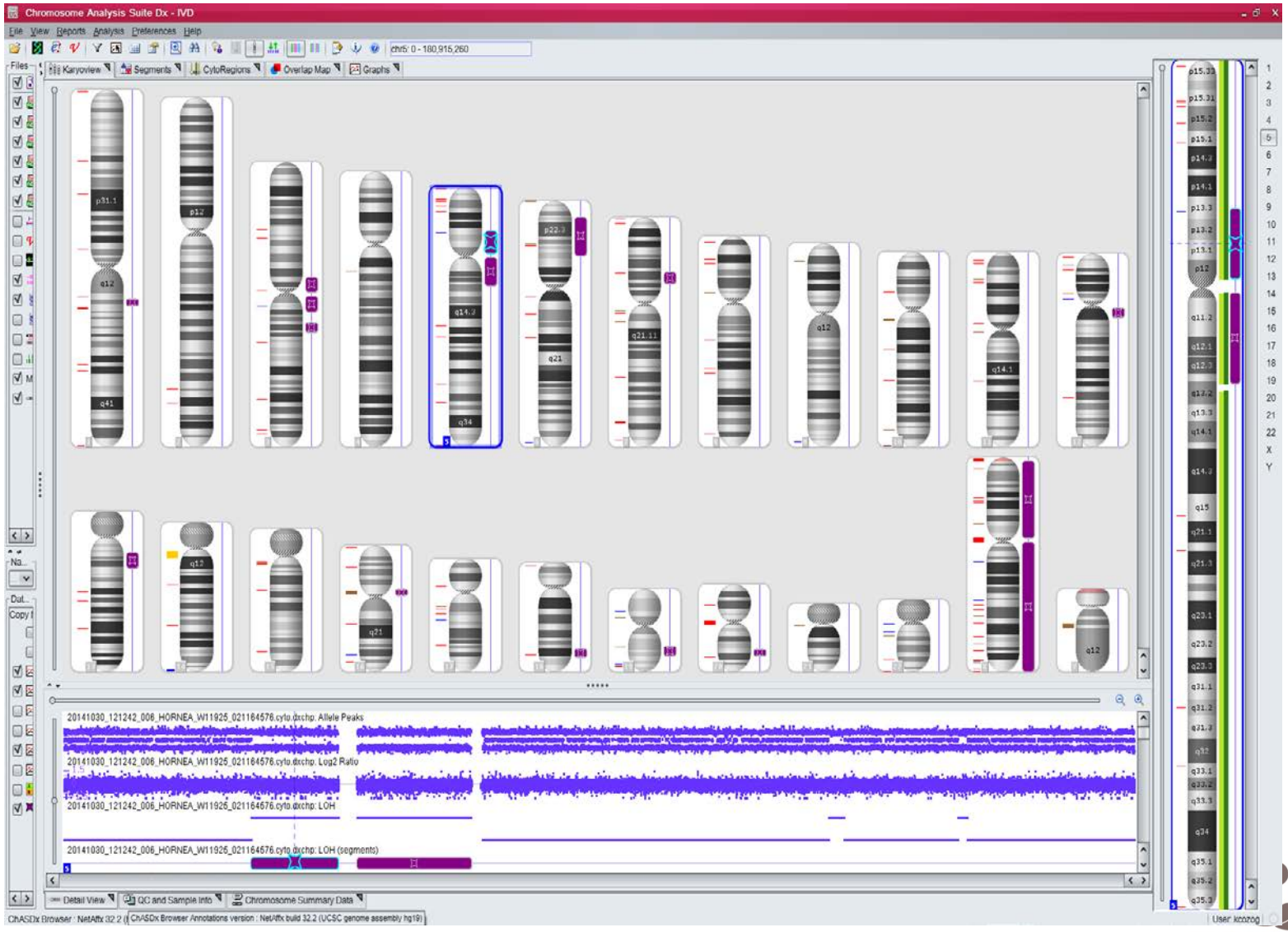
12 year-old sister with

- Short stature
- Broad joints
- Hip/knee dislocations
- Scoliosis
- Hypodontia
- Normal intelligence

- No prior genetic workup (and no active insurance to facilitate a workup)



CMA revealed 4.7% AOH



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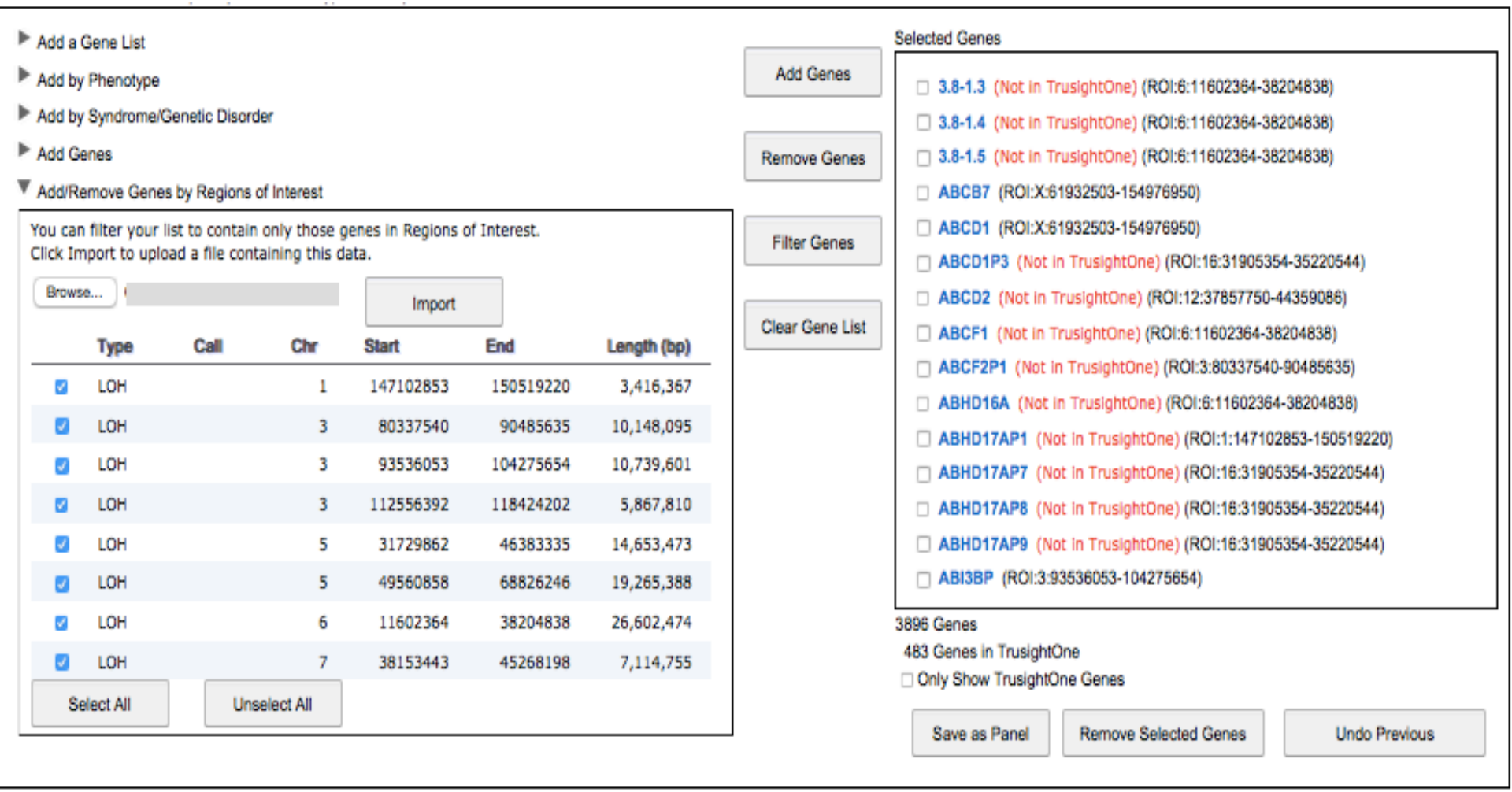
Total Genes in AOH



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AR Genes in AOH

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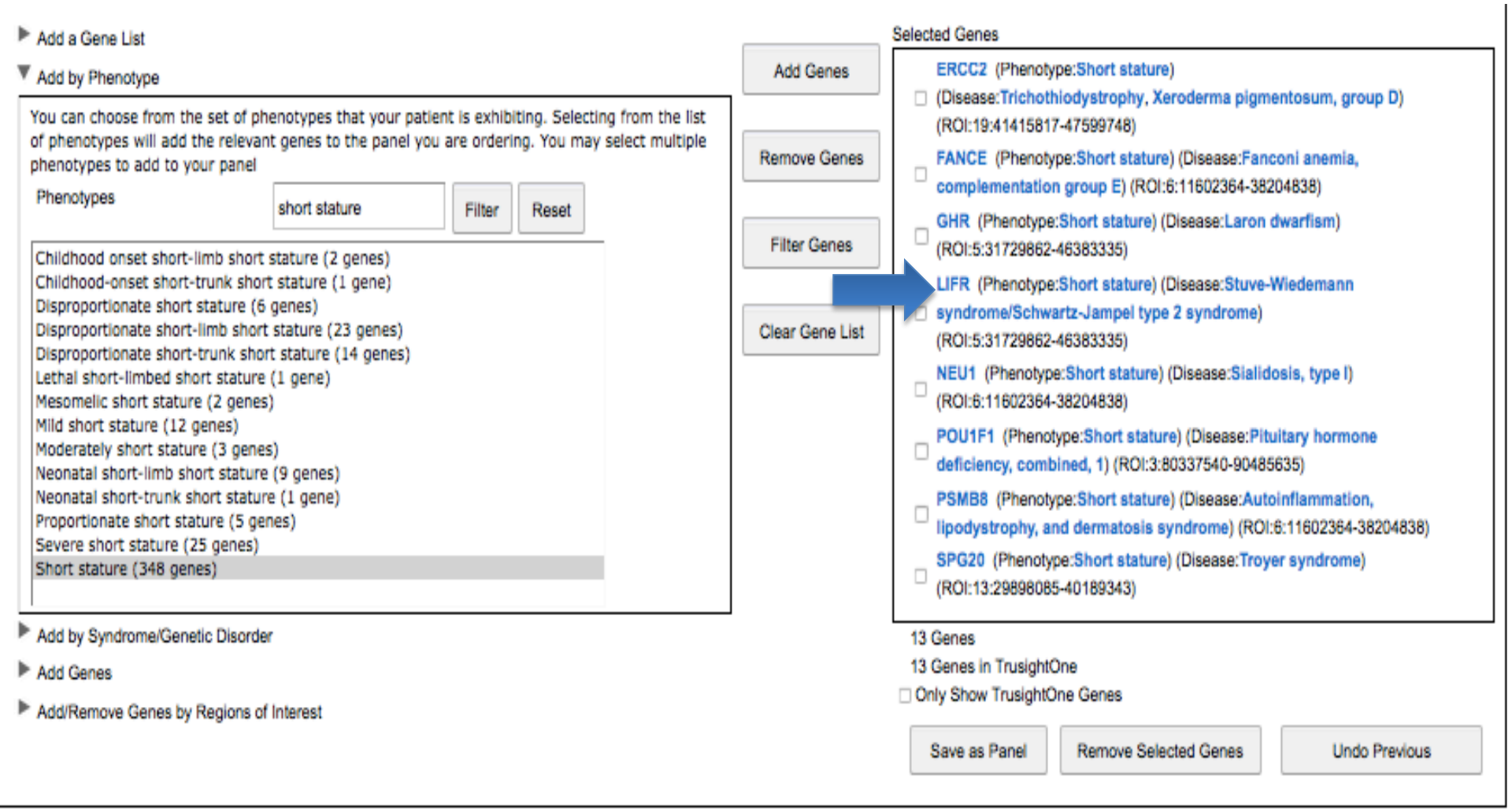
Clinically Relevant Genes in AOH



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Recommended Testing

- *LIFR* sequencing recommended
- Result: POSITIVE

Patient is homozygous for a known pathogenic mutation in exon 8 of *LIFR*. c.756dupT, which causes a frameshift

This is consistent with a diagnosis of Stuve-Wiedemann Syndrome. This is an autosomal recessive disorder characterized by skeletal changes, bowing of the lower limbs, severe osteoporosis and joint contractures, episodic hyperthermia, respiratory insufficiency and apnea, feeding problems and high mortality in early life. Those that survive tend to have normal intelligence.

Benefits

This approach benefited this family in multiple aspects:

1. Provided patient with a diagnosis at minimal cost.
    - Family refused to give an address; therefore, they were not eligible for Medicaid and were paying out-of-pocket for care.
2. Dictated medical care – concern for frequent respiratory events led to tracheostomy.
3. As patient's sister was without insurance, it provided her with a presumed diagnosis.
4. Quick turn-around-time of process helped family decision making (ordering of microarray to return of sequencing results was 6 weeks).

Case Example #4

Initial visit: 6 month old male with muscle weakness, hypotonia, and bilateral ptosis.

PE: myopathic face with positional plagiocephaly. Significant motor delay (head lag). Smiles and babbles.

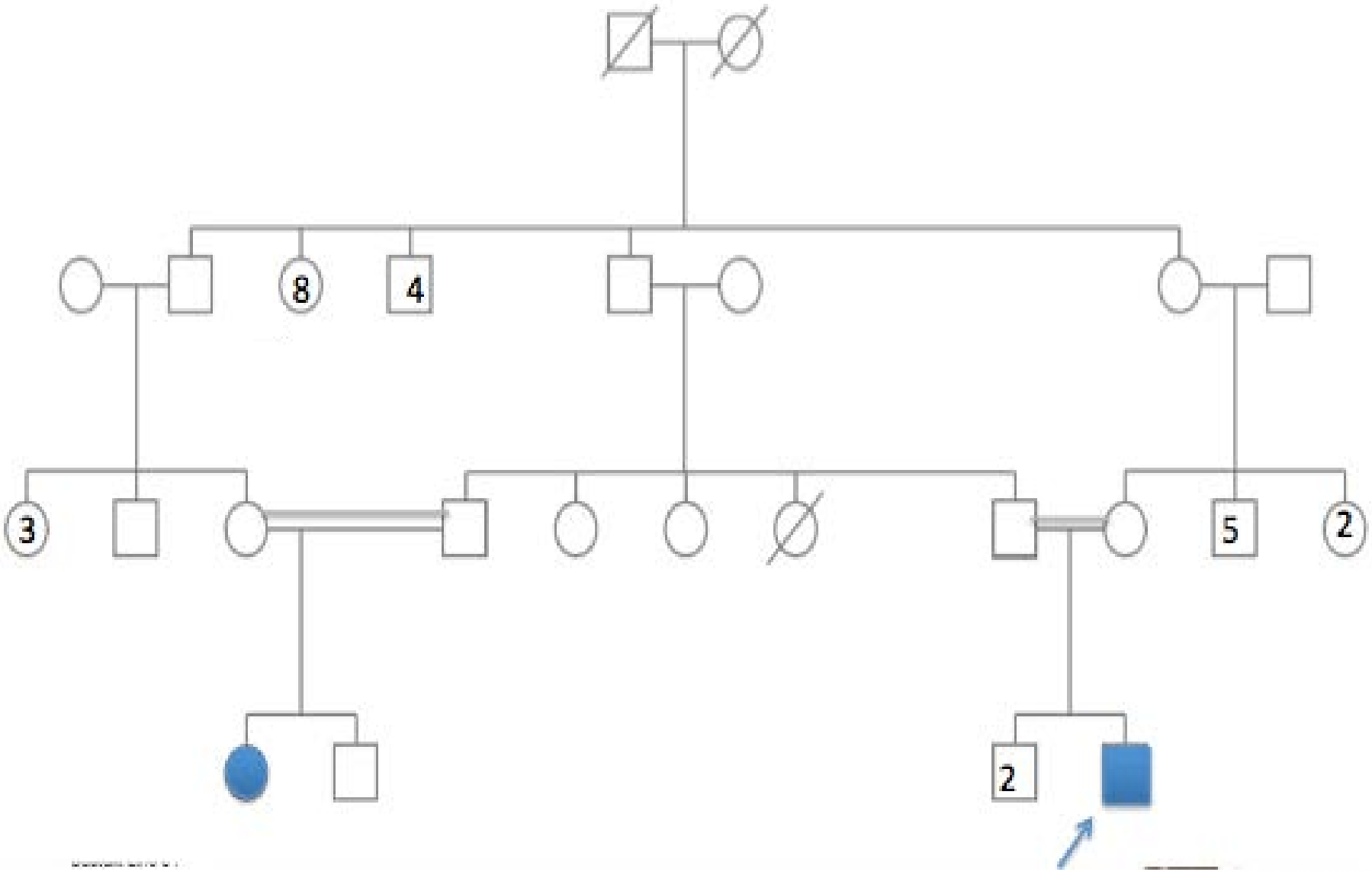
Reflexes: +1 symmetrically bilaterally on lower extremities. Unable to elicit reflexes on upper extremities.

Weight: 3rd percentile

Length: 50th percentile

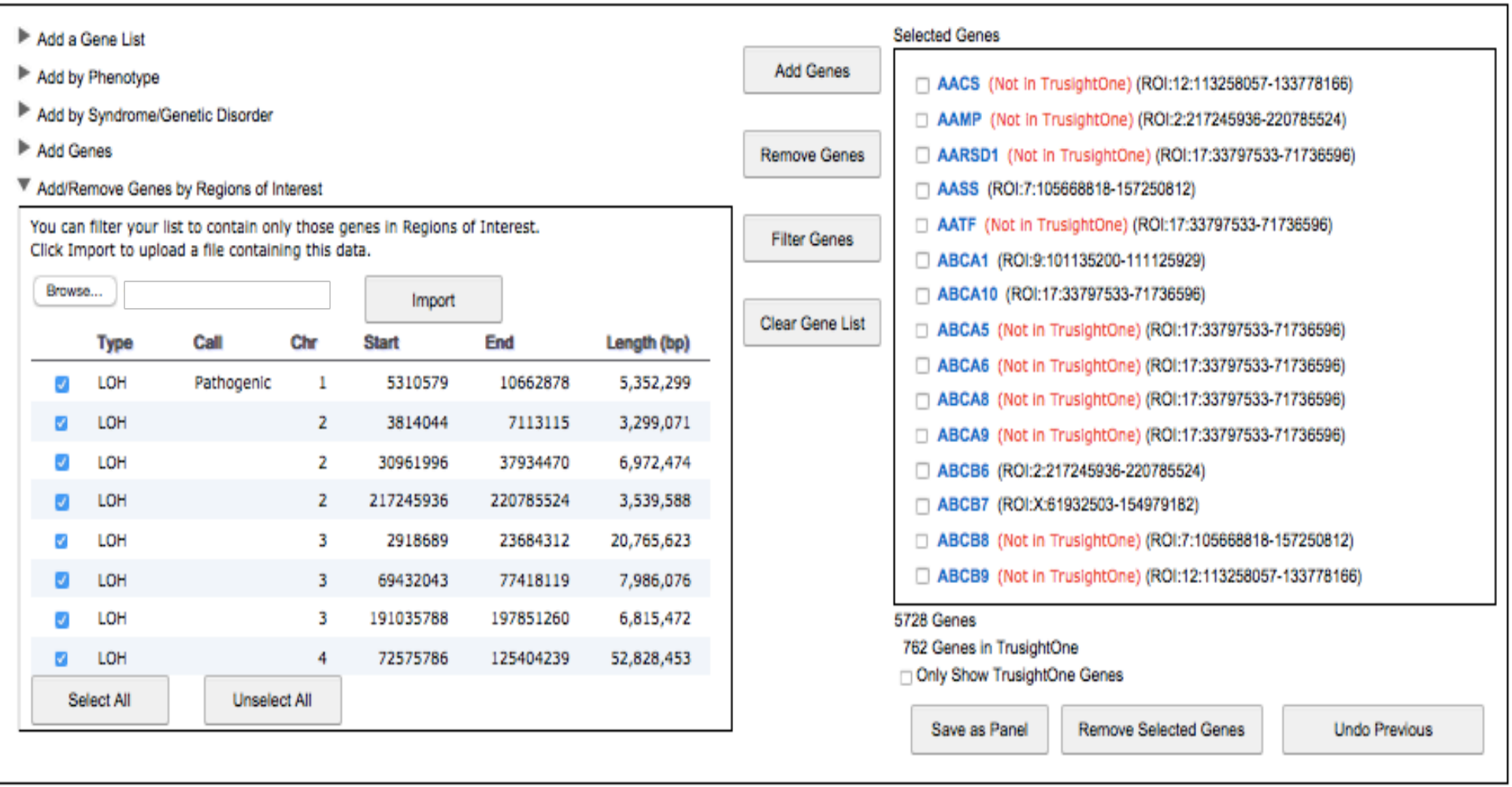
HC: 10th percentile

Case Example #4



Case Example #4

All Genes in AOH



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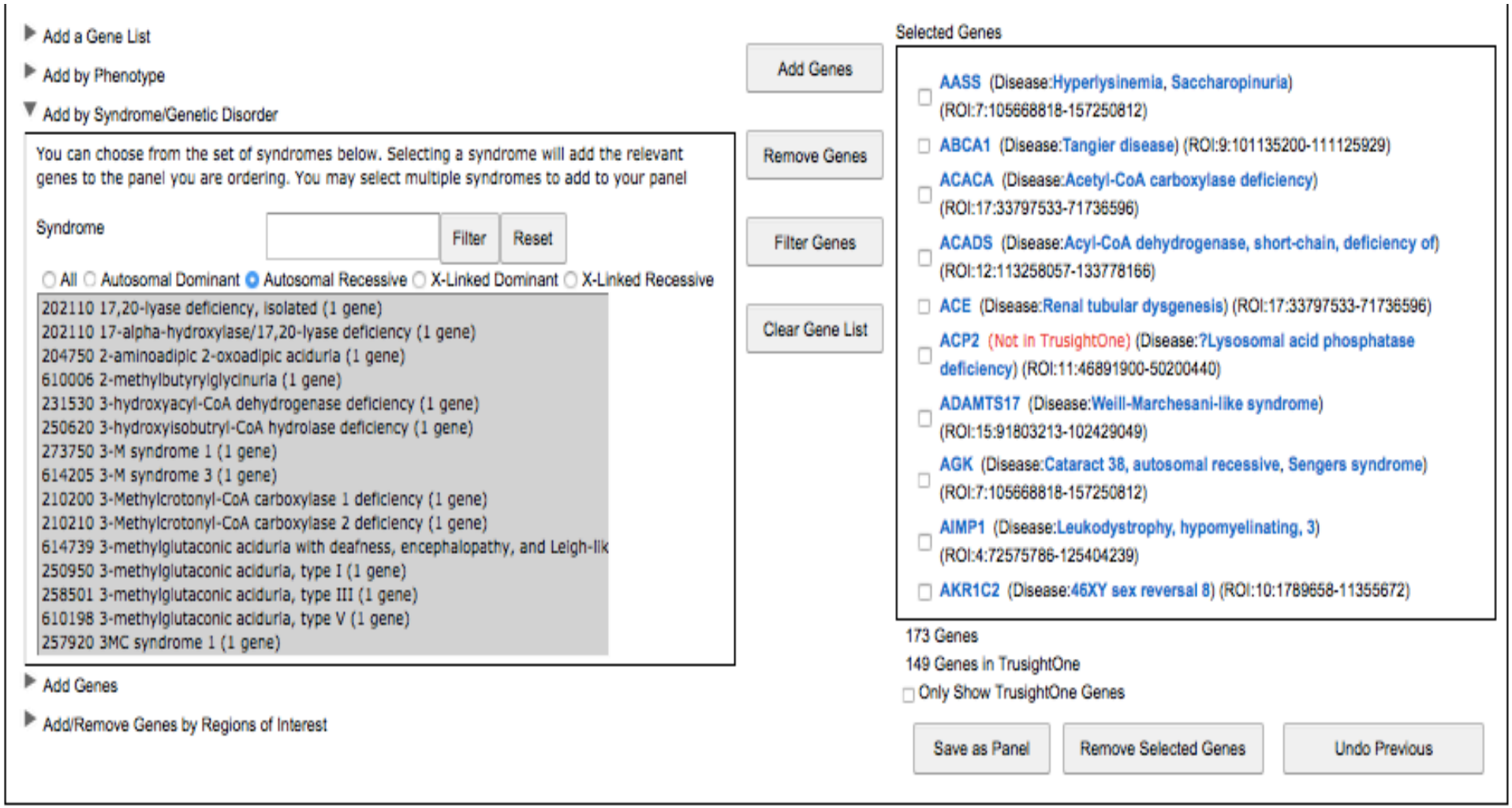
AR genes in AOH



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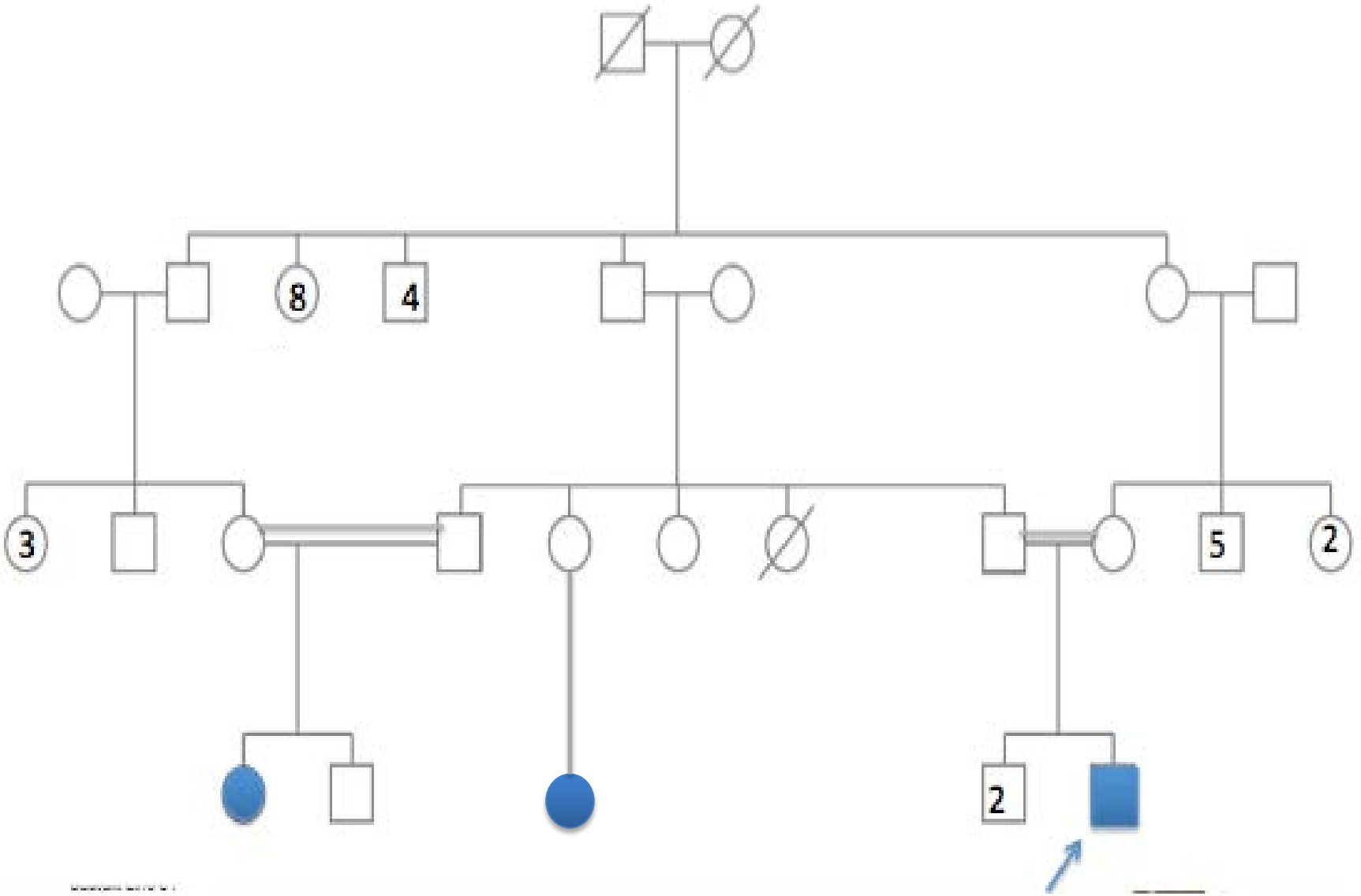
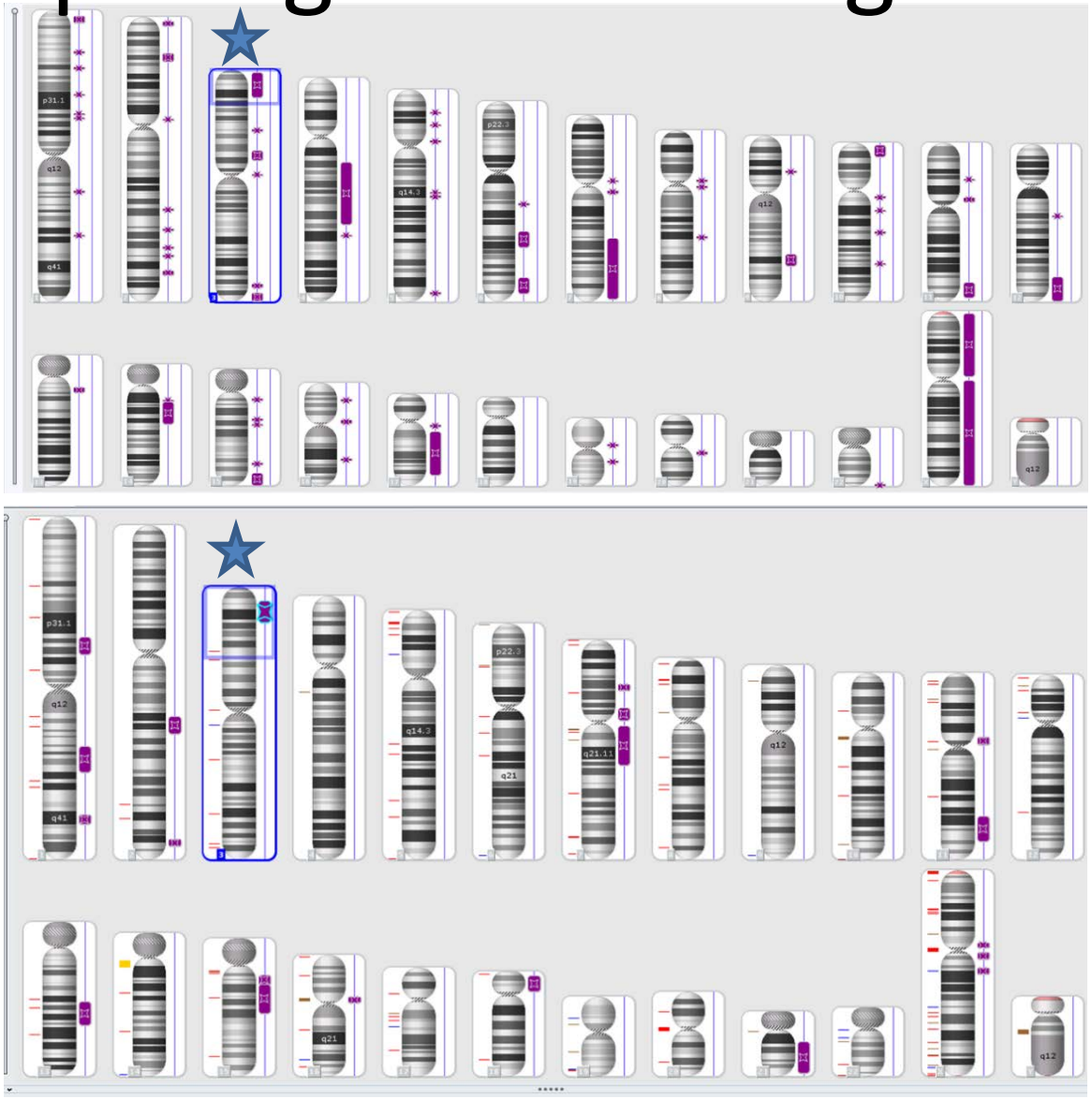
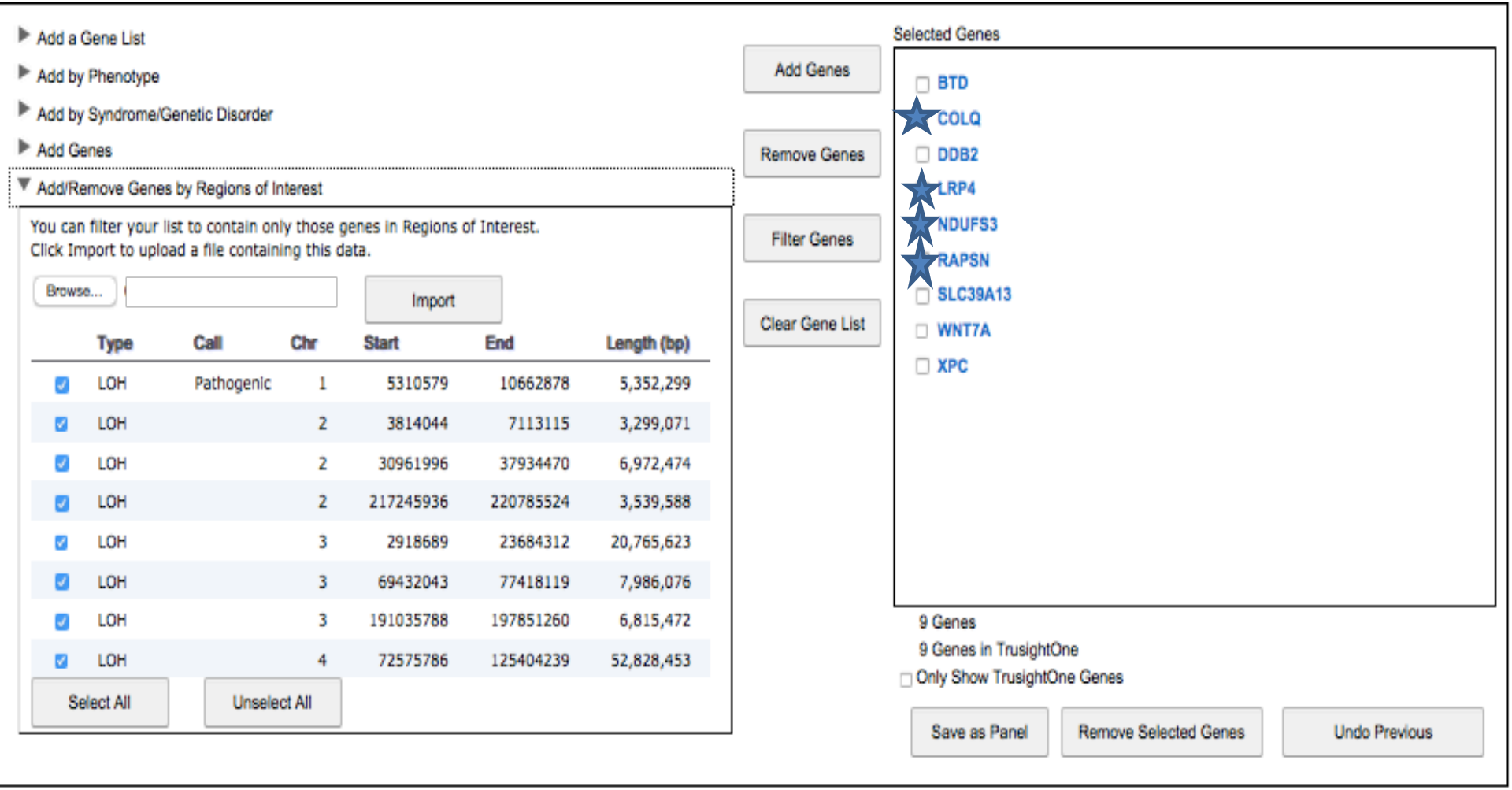
Surprise!



Comparing AOH amongst cousins



Common AR Genes



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RESULT SUMMARY: POSITIVE ANALYSIS. Testing identified a homozygous variant in COLQ. This finding is pathogenic and consistent with a diagnosis of Endplate Acetylcholinesterase Deficiency (MIM#603034).

CLINICAL INDICATION: 2 year old male with hypotonia, developmental delay, and muscle weakness

GENES ANALYZED: COLQ, LRP4, NDUFS3, RAPSN
*See Limitations section for information regarding areas of low coverage.

RESULTS:

1) Pathogenic change in Gene COLQ
Variant c.679C>T
p.R227*
Effect: Pathogenic

INTERPRETATION:

Variant 1
COLQ
c.679C>T
p.R227*

This patient is homozygous for nonsense substitution in COLQ. Homozygous and compound heterozygous alterations in COLQ have been associated with Endplate Acetylcholinesterase Deficiency (MIM#603034). The detected nonsense alteration in exon 12 interrupts the reading frame by introducing a premature stop codon. This alteration has been reported before in publications of individuals with Endplate Acetylcholinesterase Deficiency.1,2 Based on the available information at present, this variant is pathogenic. (transcript: NM_005677.3)

RECOMMENDATIONS: Clinical correlation between this result and the patient's phenotype is recommended. Genetic counseling is recommended to discuss the implications of this report.

Homozygous *COLQ* mutation

Mutations in *COLQ* cause a type of congenital myasthenic syndrome (CMS).

Phenotype: fatigue weakness of skeletal muscle with onset at or shortly after birth (or in early childhood)

Management: most individuals with CMS benefit from acetylcholine esterase inhibitors; however, those with *COLQ* mutations can range from no response to **detrimental** effects.

Benefits

This approach benefited this family in multiple ways:

1. Provided a presumed diagnosis for the patient's cousins.
    - Which would allow for cheaper confirmation testing – beneficial for the cousin whose insurance company had been denying genetic testing coverage.
2. Provided medical management recommendations not only for the patient, but his cousins.
3. Large, expensive genetic panels weren't necessary. All the testing was able to be completed for a lower price than the cost of most panels.

Questions/Concerns

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