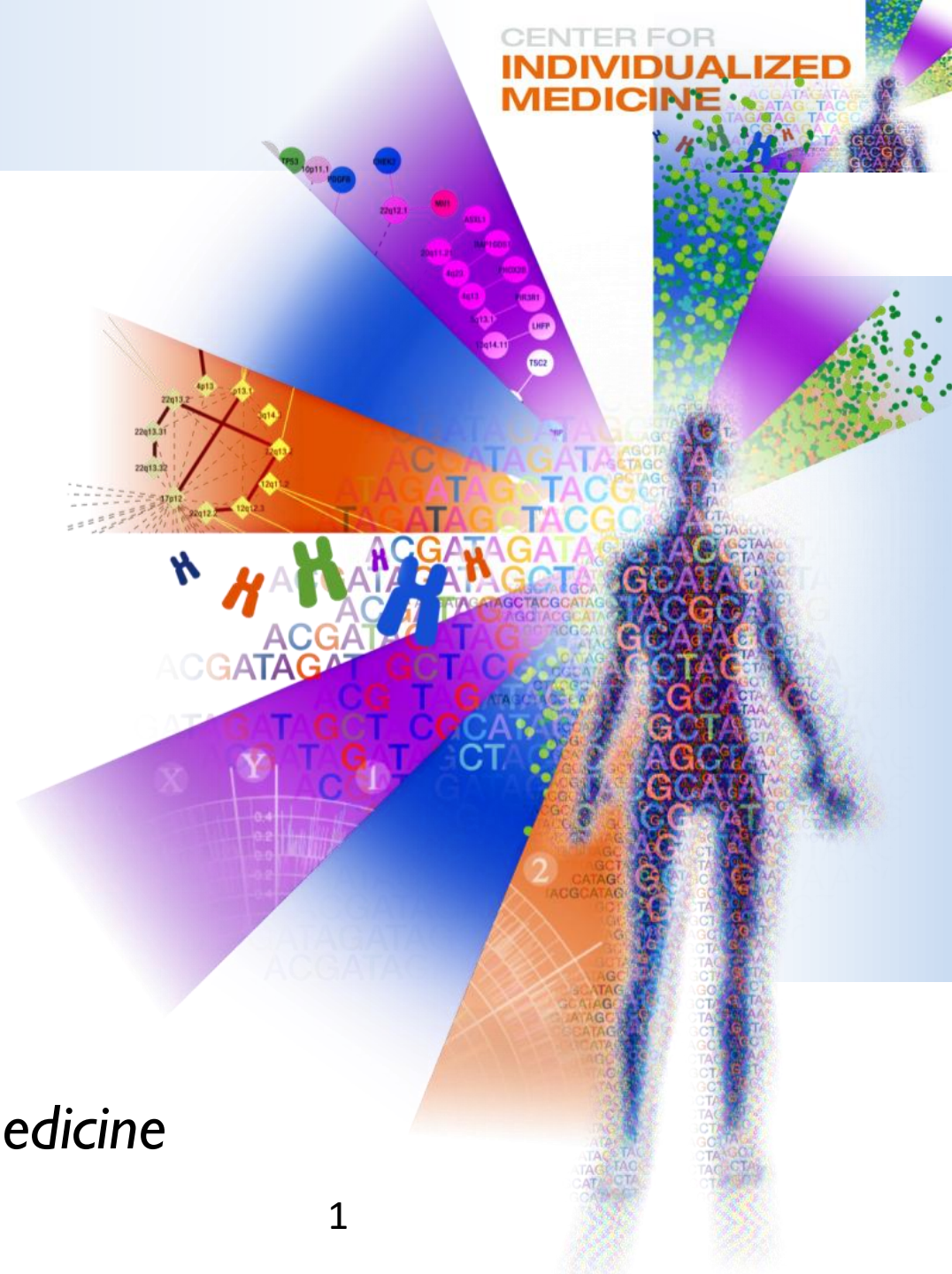
MAYO CLINIC

CENTER FOR INDIVIDUALIZED MEDICINE

Translational use of multifaceted RNA-Seq bioinformatics analysis in genetic disease investigation



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Associate Professor of Biomedical Informatics

Outline

- Discussing the role of RNA-sequencing in rare and undiagnosed disease
- Unit consists of four lectures:
  - Introduction to Rare and Undiagnosed Disease
  - Expression Analysis for Outlier Observations
  - Fusion analysis in RNA-sequencing data
  - Splicing analysis
- Lectures given by Gavin Oliver and Eric Klee

Introduction

- What is rare genetic disease?
- A common problem - when rare isn't rare
- Rare genetic disease diagnosis in the era of next-generation sequencing
- The promise of RNA-Seq in improving rare genetic disease diagnosis

Rare Genetic Disease

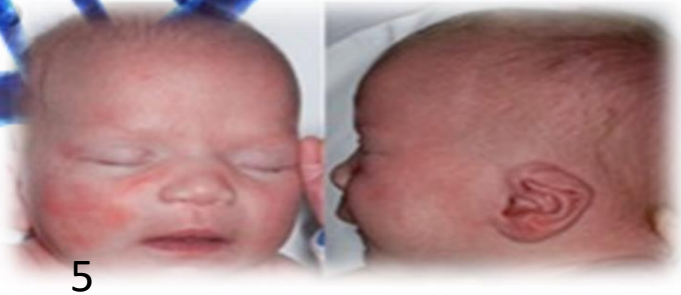
In the United States, a rare disease is defined as a condition that affects fewer than 200,000 people in the US. This definition was created by Congress in the Orphan Drug Act of 1983. There may be as many as 7,000 rare diseases. *rarediseases.info.nih.gov*

The European Union defines a disease or condition as rare if it affects fewer than 1 in 2,000 (1) people within the general population. Currently, there are over 6,000 (2) known rare diseases. *raredisease.org.uk*

80% have a genetic component

Rare Genomics Institute

Faces of Rare Genetic Disease

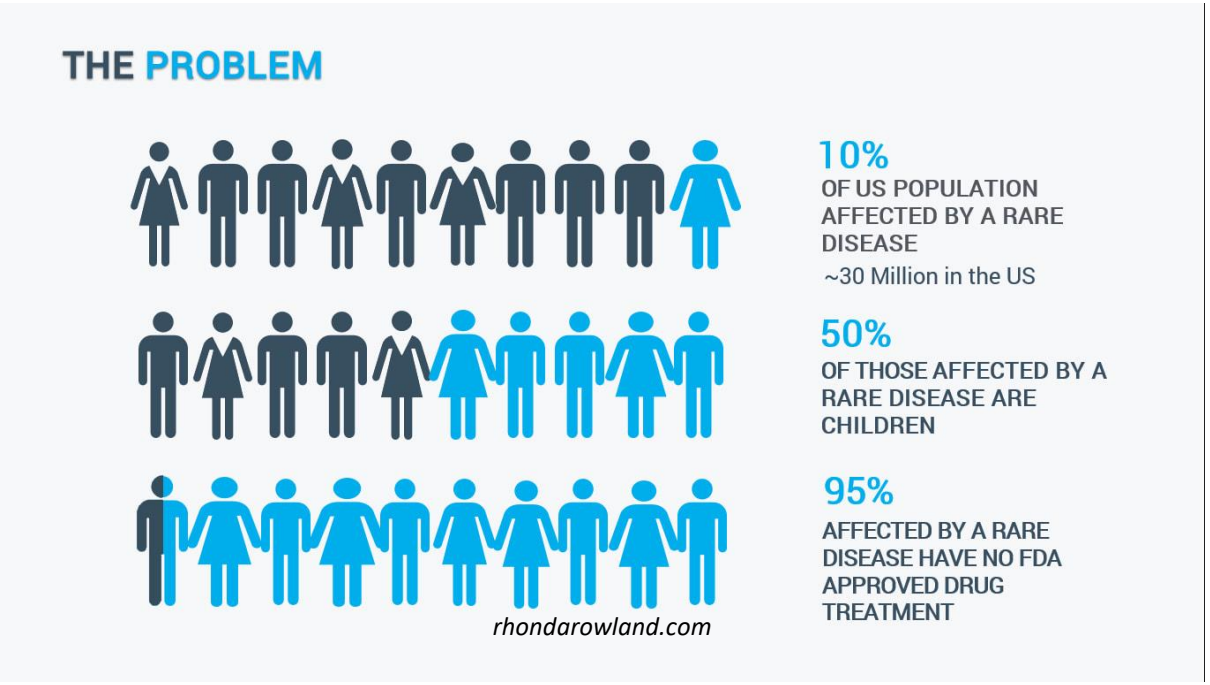


A common problem - when rare isn't rare

An estimated **300 million people** worldwide are affected by a rare disease *globalgenes.org*

1 in 10 Americans have a rare disease *raregenomics.org*

6% to 8% of the population of the European Union is affected by a rare disease *eurodis.org*

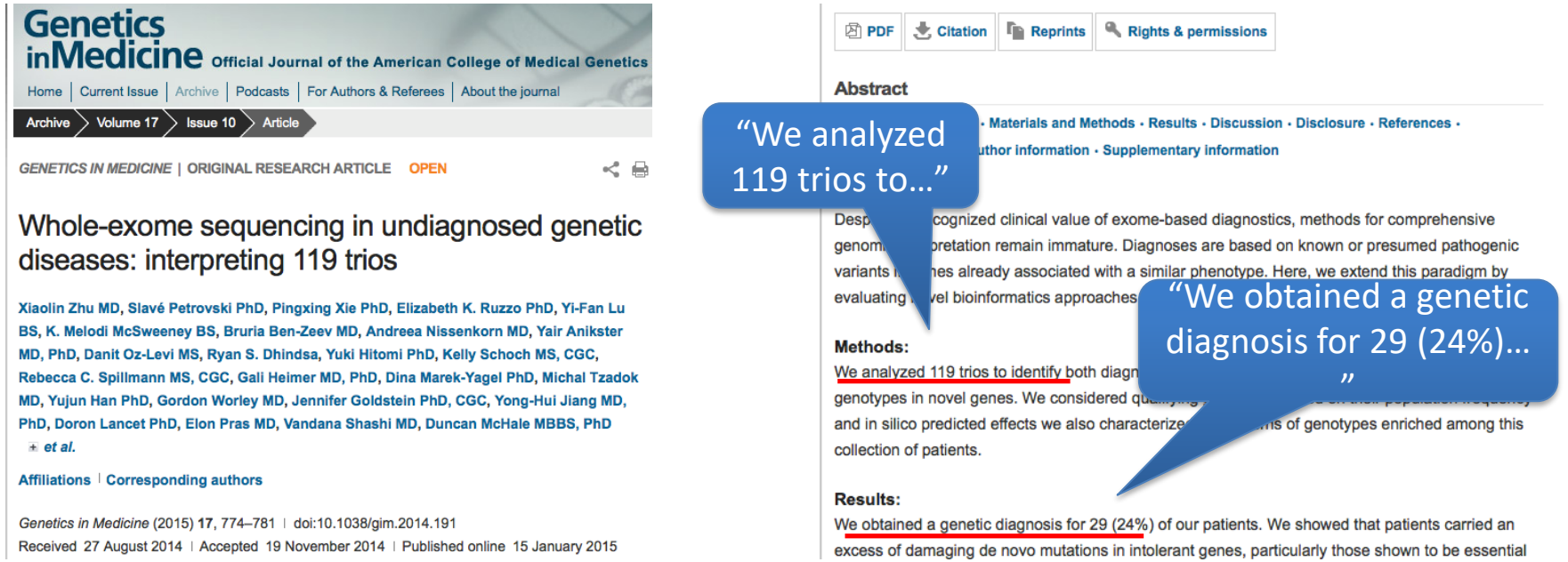


30% of patients with rare disease will not live to see their 5th birthday

Rare diseases are responsible for 35% of deaths in the first year of life

raregenomics.org

Proliferation of Exome Diagnostic Testing



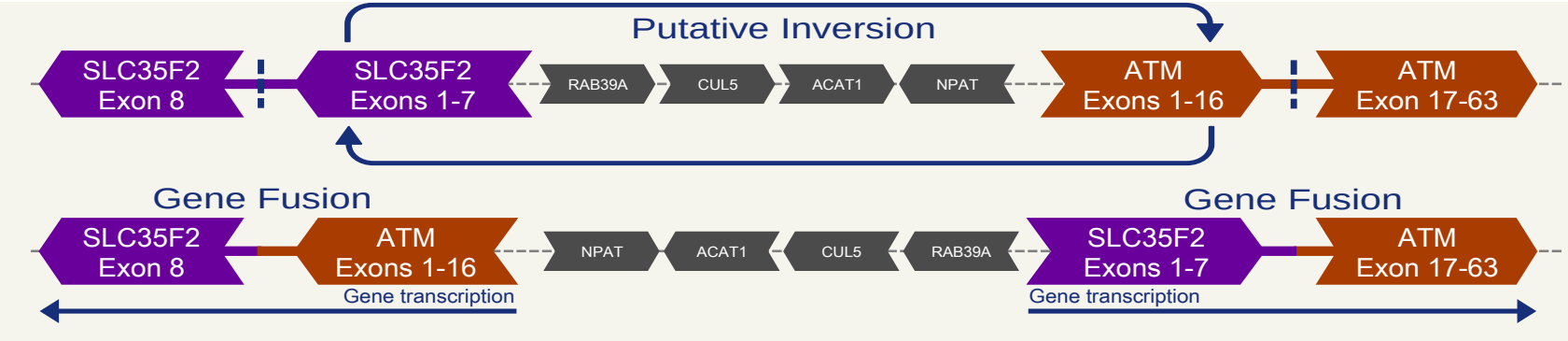
Clinical Exome Sequencing for Genetic Identification of Rare Mendelian Disorders - JAMA

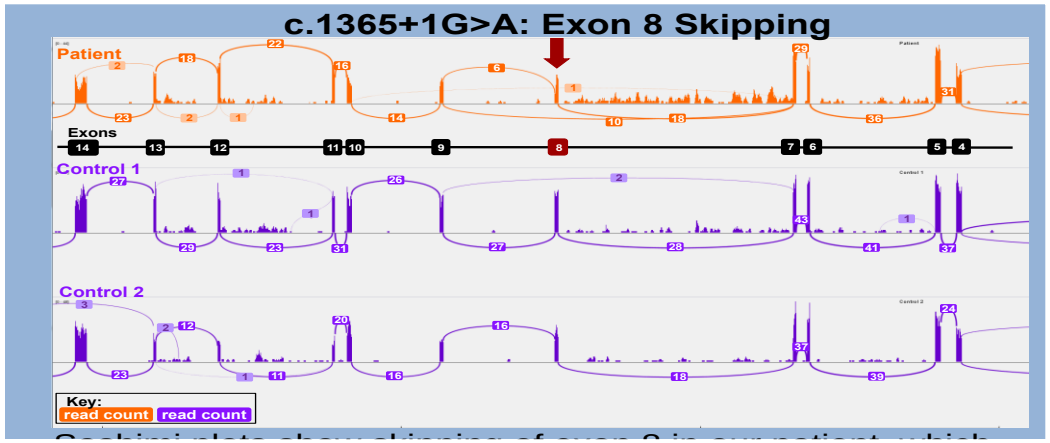
"Results—Of the 814 cases, the overall molecular diagnosis rate was 26%"

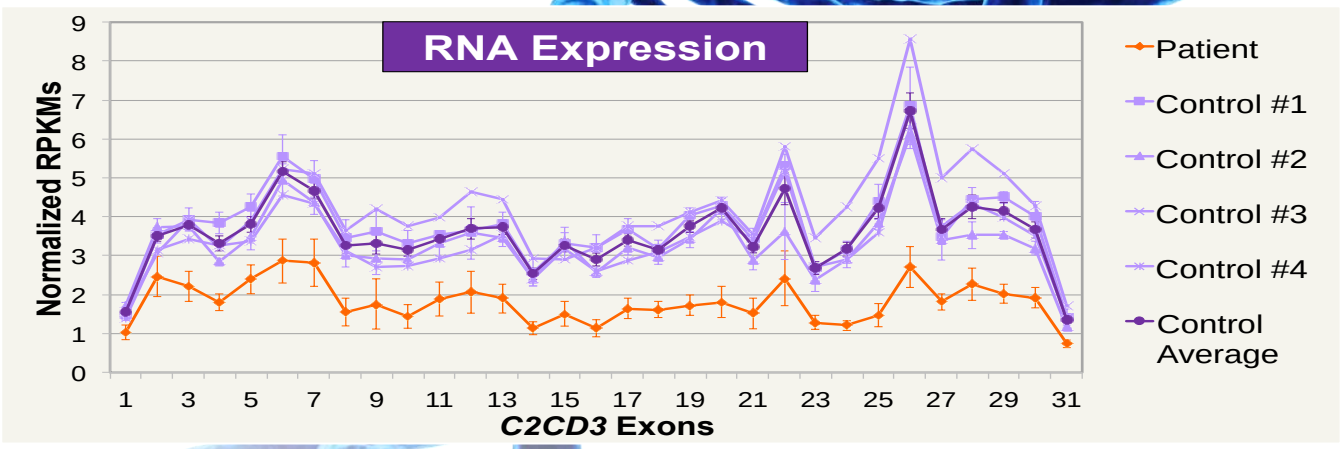
Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation – NEJM

"A molecular diagnosis was rendered for 2076 of 7374 patients (28.2%)"

RNA Sequencing to Improve Diagnostic Rate







Case Examples

1. Patient #1: GNPTAB – cryptic splicing
2. Patient #2: ATM – expressed fusion
3. Patient #3: CASK – outlier expression
4. Patient #4: SGSH – allele-specific expression

Patient Example Case #1

Age: 9y female

Reason for Referral: Mild global developmental delay, brain MRI abnormal joint contractures, slightly distinctive facial features

Clinical Testing reported compound heterozygous variants in MEGF10, which upon further review did not seem a good fit.

Requested raw data from the testing provider and reanalyzed

Label	ID	Meta	ID
Wide pubic symphysis	HP:0003183		
Vertebral hypoplasia	HP:0008417		
Short stature	HP:0004322		
Posterior scalloping of vertebral bodies	HP:0005121		
Platyspondyly	HP:0000926		
Periorbital fullness	HP:0000629		
Pectus carinatum	HP:0000768	Mild	HP:0012825
Narrow forehead	HP:0000341		
Mitral valve prolapse	HP:0001634	Mild	HP:0012825
Mitral regurgitation	HP:0001653	Mild	HP:0012825
Lumbar hyperlordosis	HP:0002938		
Intellectual disability	HP:0001249		
Hypoplastic distal radial epiphyses	HP:0006386		
Global developmental delay	HP:0001263	Mild	HP:0012825
Gastroesophageal reflux	HP:0002020		
Flexion contracture	HP:0001371		
Flattened humeral heads	HP:0003888		
Epicanthus	HP:0000286		
Dysarthria	HP:0001260		
Coarse facial features	HP:0000280	Mild	HP:0012825
Cafe-au-lait spot	HP:0000957		
Broad nasal tip	HP:0000455		
Beaking of vertebral bodies	HP:0004568		
Asymmetry of the ears	HP:0010722		
Aortic regurgitation	HP:0001659	Mild	HP:0012825
Abnormality of the skeletal system	HP:0000924		
Abnormality of the glenoid fossa	HP:0011912		

Candidate Splicing Variant Identified

GNPTAB **TWO HET c.3281_3282delGT, p.C1094fs* – mother is het, father is neg**

c.3434+639G>C, p.?splice – de novo, parents are neg

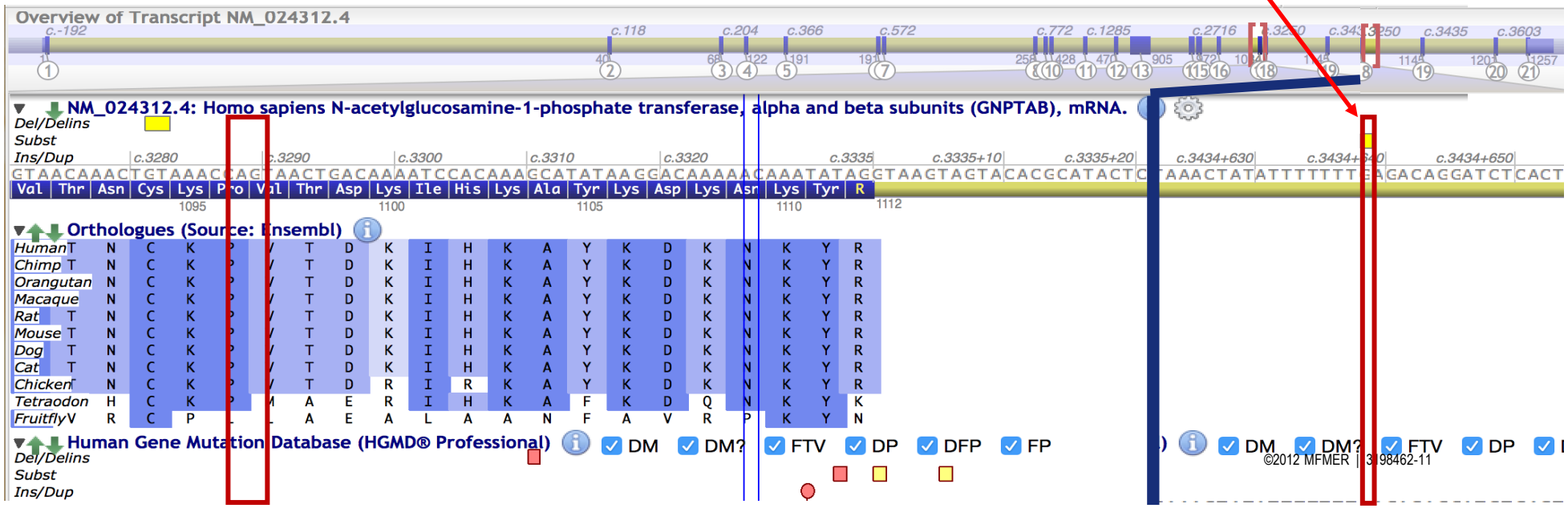
Disease: Mucolipidosis alpha/beta **AR** type II (MIM:252500) or III (MIM:252600)

Comments: Encodes two of three subunit types of the membrane-bound enzyme N-Acetylglucosamine-1-Phosphate Transferase Alpha And Beta Subunits. a heterohexameric complex composed of two alpha, two beta, and two gamma subunits. The encoded protein is proteolytically cleaved at the Lys928-Asp929 bond to yield mature alpha and beta polypeptides while the gamma subunits are the product of a distinct gene (GeneID 84572). In the Golgi apparatus, the heterohexameric complex **catalyzes the first step in the synthesis of mannose 6-phosphate (M6P) recognition markers on certain oligosaccharides of newly synthesized lysosomal enzymes**, which mediate vesicular transport of lysosomal enzymes to the endosomal/prelysosomal compartment.

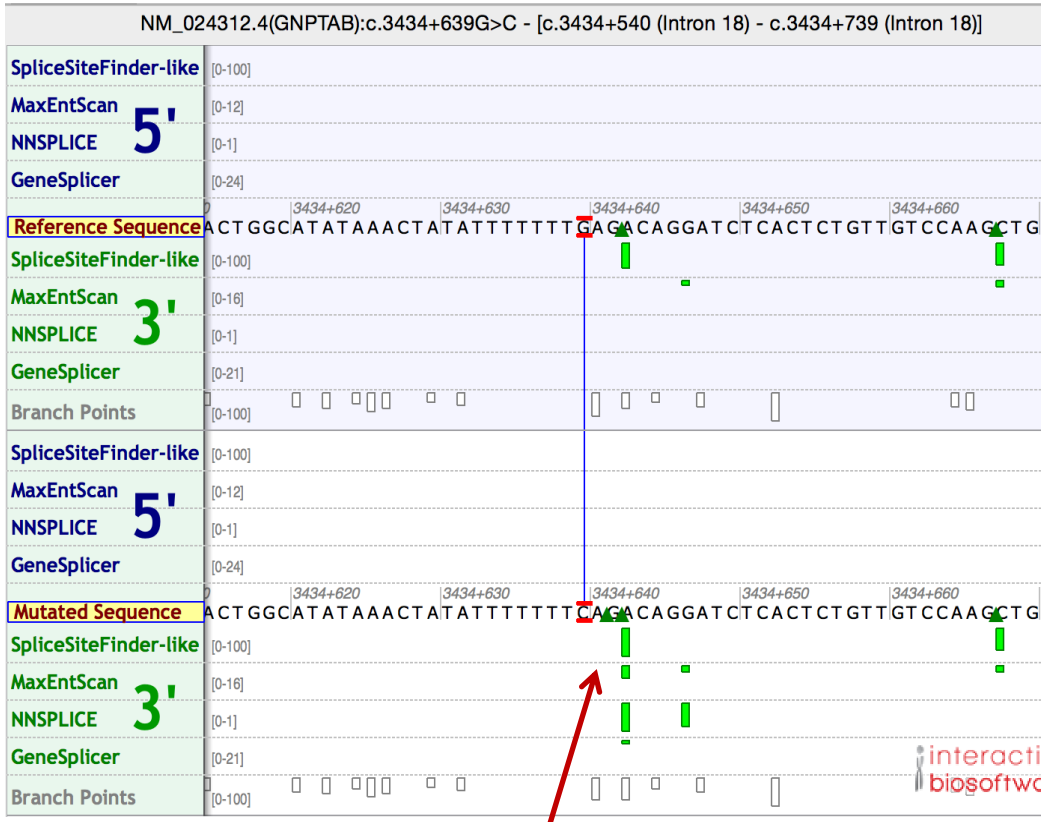
	c.3281_3282delGT, p.C1094fs* - mat	c.3434+639G>C, p.?splice – de novo
gnomAD:	NR	NR
In silico	NA	NA
Location:	Exon 17 of 21	Intron 18

A deep intronic variant was found in trans with a loss of function frame-shift variant.

If the intronic variant impacts gene splicing it may explain the patients phenotype



Splicing predictor identifies putative donor site



Patient variant creates spice acceptor

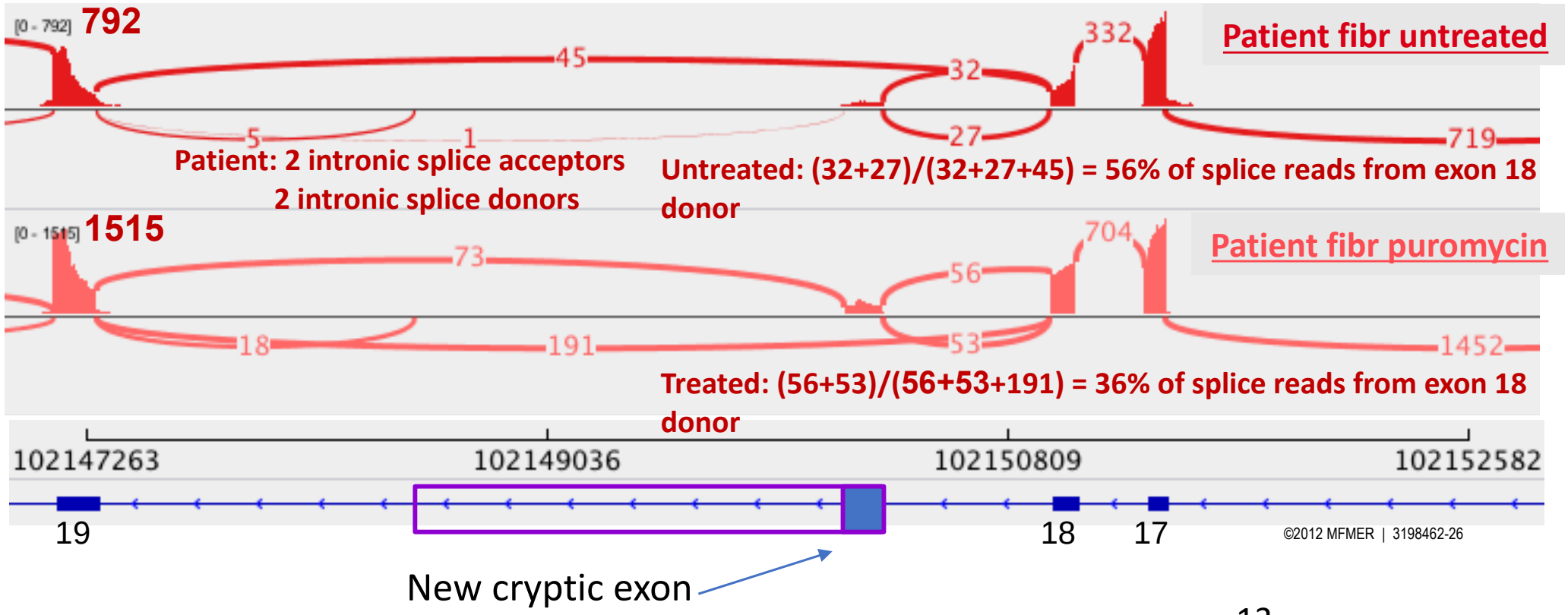
Cryptic splice donor

The intronic variant creates a predicted splice acceptor at c.3434+642 and there is a cryptic splice donor at 3434+1244

RNAseq confirms presence of a cryptic exon

***GNPTAB* TWO HET c.3281_3282delGT, p.C1094fs* – mother is het, father is neg**
c.3434+639G>C, p.?splice – de novo, parents are neg
Disease: Mucolipidosis alpha/beta **AR** type II (MIM:252500) or III (MIM:252600)

Read Depth



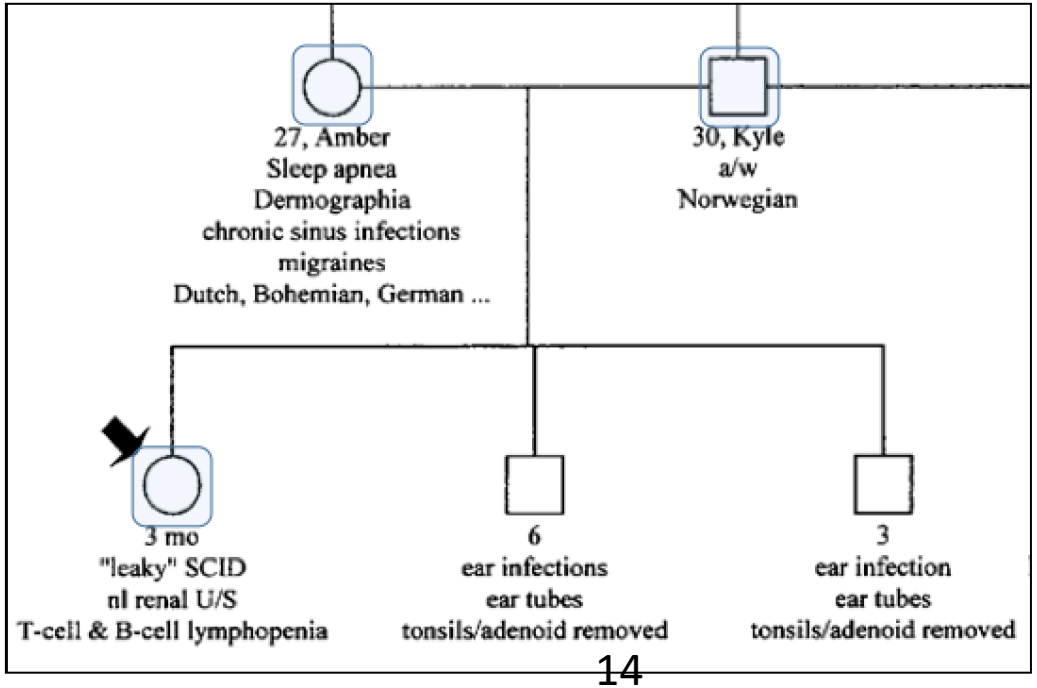
Patient Example Case #2

Patient Symptoms:

- Tested positive for SCID by newborn screening
- Evidence of radiation sensitivity (partial)
- B and T-cell lymphopenia (T-B-NK+)
- TCR vbeta spectratyping is suggestive of polyclonal gaussian repertoire which is most likely suggestive of a combined immune deficiency
- Thrombocytosis
- Some ataxia-related phenotype potentially manifesting recently
- Elevated AFP

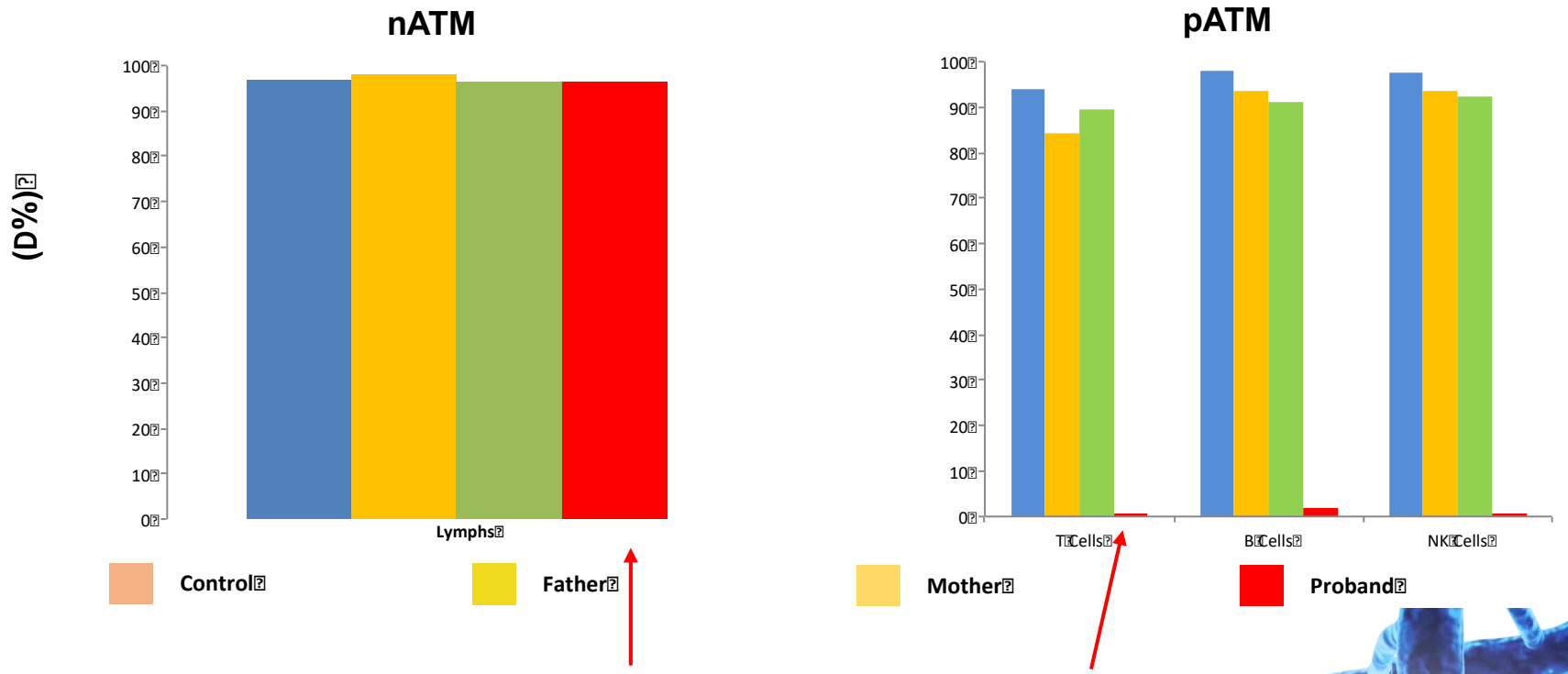
Single pathogenic variant in ATM (recessive condition) that would explain patient symptoms

Disease	Inher. pattern	Gene	Location	variant	inherited from
Ataxia Telagiectasia 208900	AR	ATM	chr11:108143540_108143542	c.3245_3247delinsTGAT p.His1082Leufs*14	Dad het



Protein testing to evaluate diagnosis

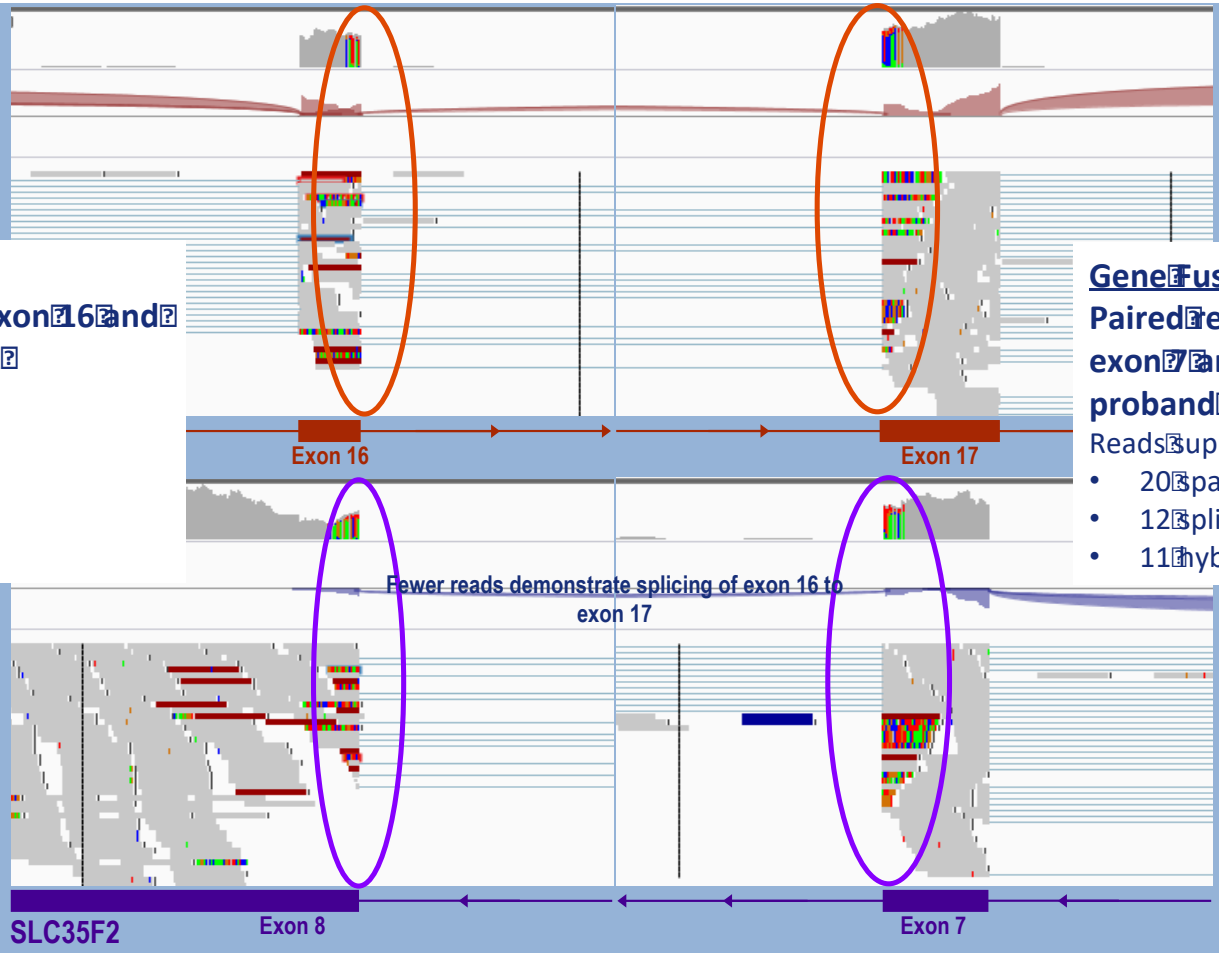
Protein studies in proband and parents showed that the ATM protein was present in all three individuals, but non-functional in the proband



Patient protein present, but not functional

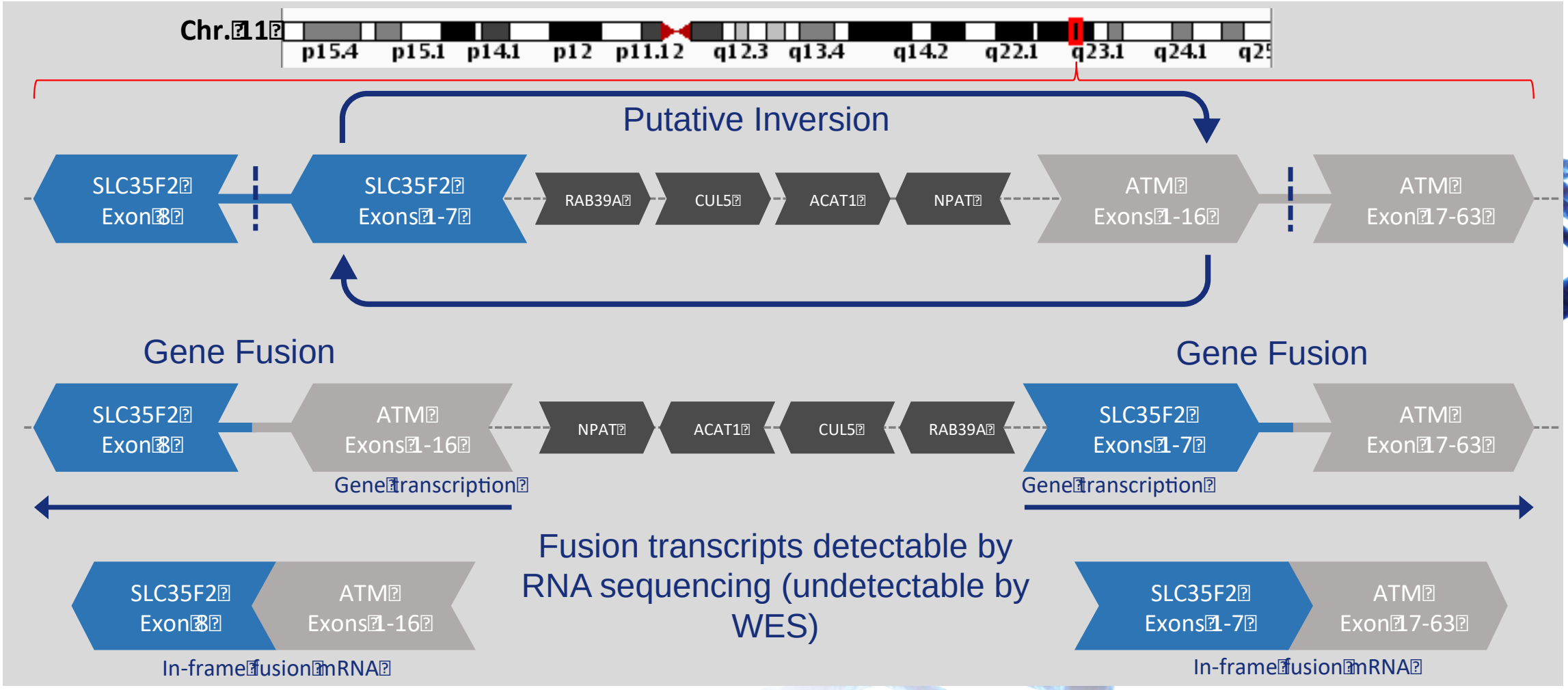
RNAseq identifies a gene fusion in ATM

Gene Fusion Analysis:
Paired reads mapping to ATM exon 16 and SLC35F2 exon 8 in proband RNA
Reads supporting fusion:
- 7 spanning reads
- 3 split reads
- 4 hybrid reads



Gene Fusion Analysis:
Paired reads mapping to SLC35F2 exon 7 and ATM exon 17 and in proband RNA
Reads supporting fusion:
- 20 spanning reads
- 12 split reads
- 11 hybrid reads

Fusion caused by DNA inversion

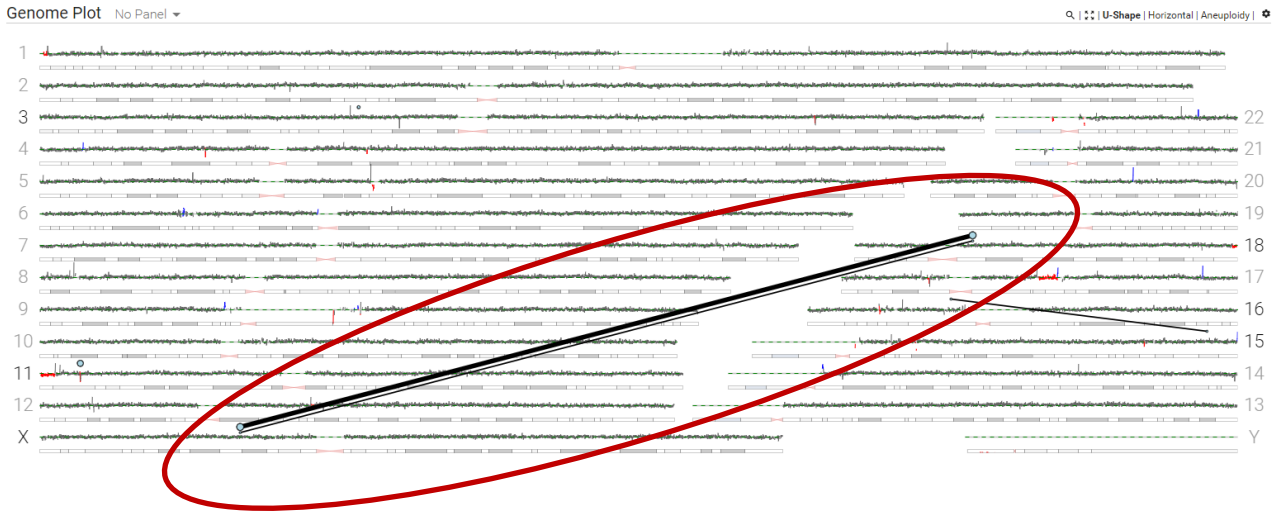


Patient Example Case #3

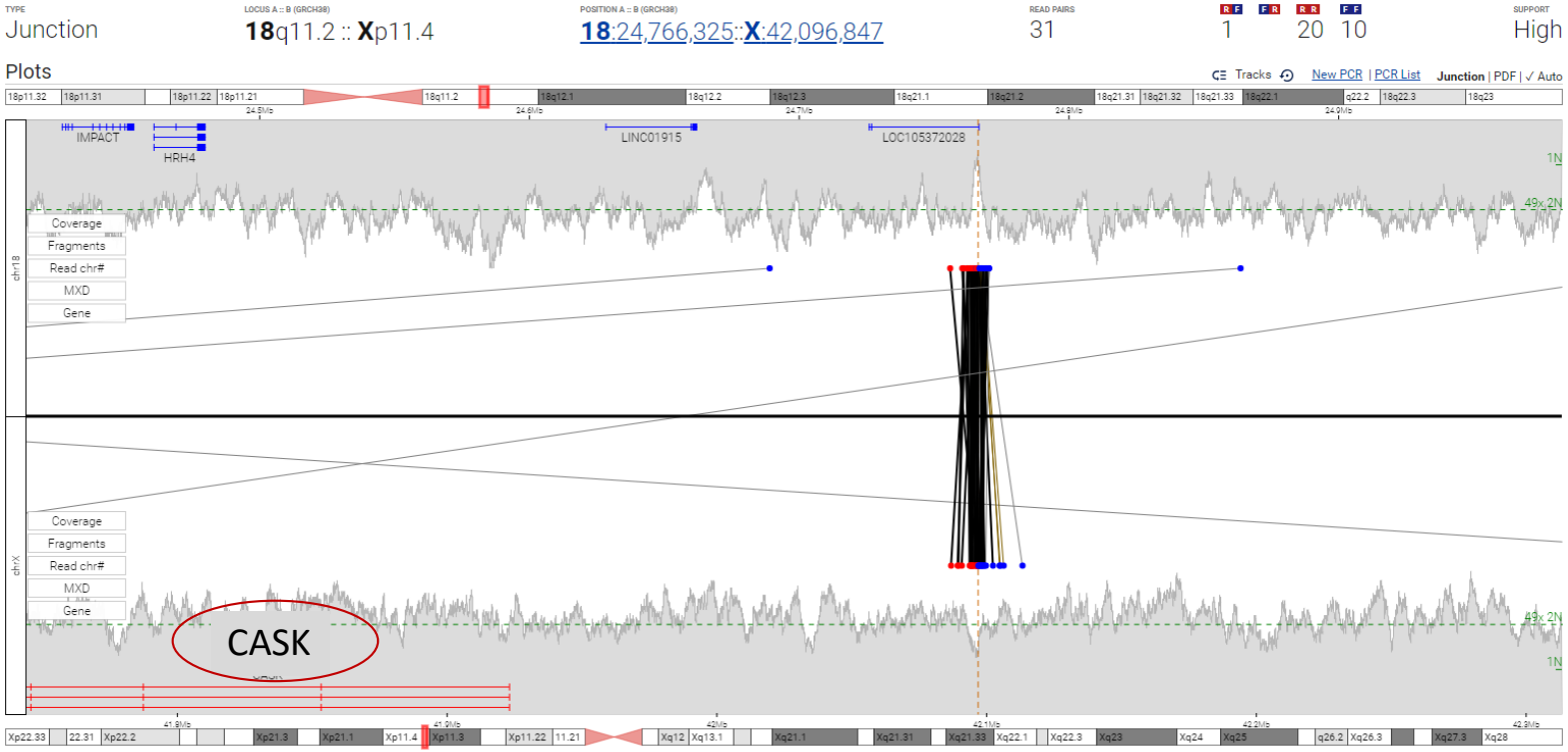
Patient Symptoms:

- 12 month old female
- GNAO1 related epileptic encephalopathy with cerebral and cerebellar atrophy
- corpus callosum dysgenesis
- left optic nerve hypoplasia
- Micropthalmia
- Cataract
- cleft soft palate
- ASD
- dysmorphic facies
- small size
- microcephaly

Mate-pair sequencing to investigate karyotype:
t(X;18)(p11.2;q11.2)



Mate-pair characterizes t(X;18)(p11.2;q11.2)



Chr18:

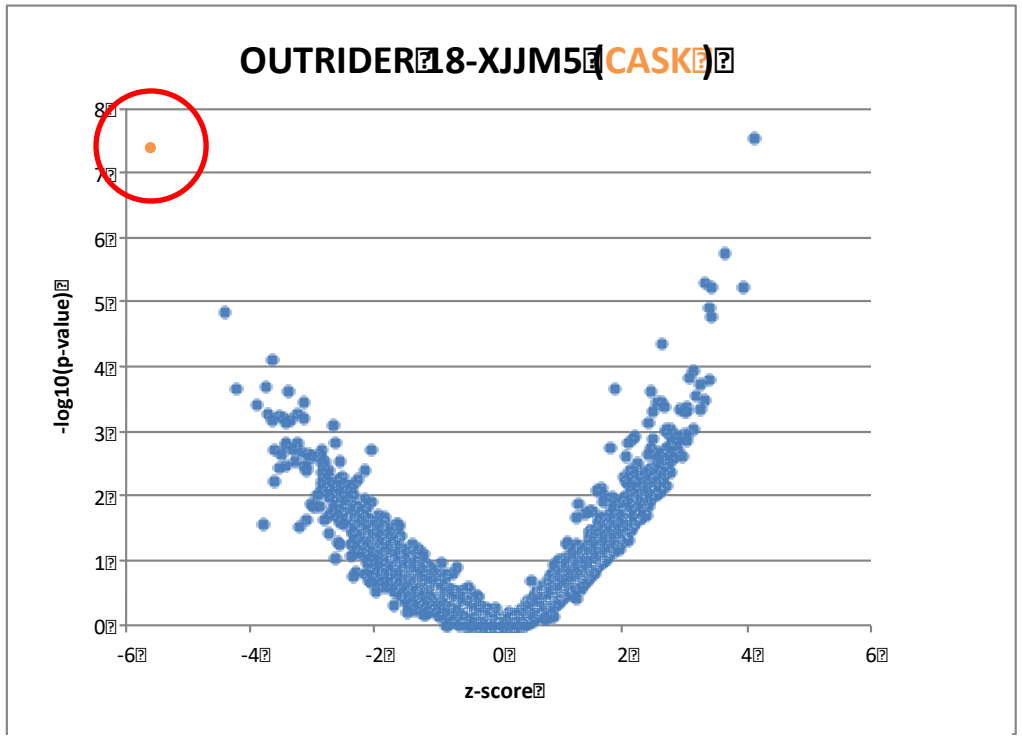
Disrupts LOC105372028 (non-coding RNA); haploinsufficiency not known to result in any abnormal phenotypes

ChrX:

No gene was disrupted at the Xp11.4 breakpoint, however CASK is located ~173 kb distal

CASK deficiencies are a strong phenotypic fit for the patient's symptoms

RNAseq Outlier Expression (CASK)



CASK:

p-value (unadjusted) = 4.08×10^{-8}

p-value (adjusted) = 0.0003

Z-score = -5.61

Log_2(fold change) = -0.99

Epigenetic Profiling

Hypothesis: epigenetic gene repression on chromosome 18 near the breakpoint that has been put upstream of CASK in this patient from the t(X;18) that is now causing repression of CASK expression.

Evaluate: H3K9me, H3K27me, H3K4me and H2K9Ac (activating mark) to see if these features are present near the breakpoints in blood lineage cells of unaffected individuals.

ChIP-seq experiment on the patient's blood sample to determine if there is a difference in the patient.

OUTRIDER: Brechtmann F, et al. Am J Hum Genet. 2018 Dec 6;103(6):907-917. PMID: 30503520

Patient Example Case #4

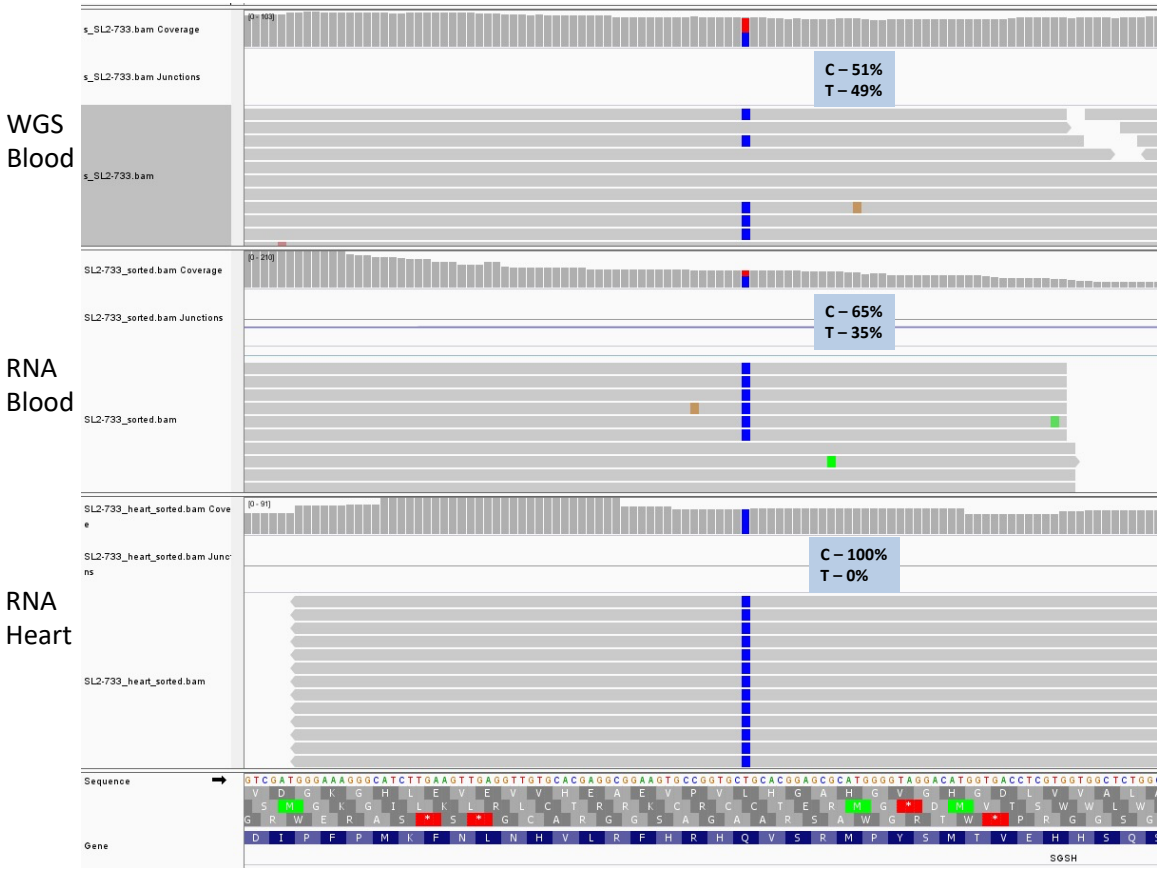
Phenotype

- Adult-onset hepatomegaly
- Hypertrophic cardiomyopathy
- Stroke (2014)
- A. Fib
- Non-dysmorphic

Glycosaminoglycans in Cerebrospinal Fluid:
GAGs in the CSF are 50-100 times higher than controls in others MPS with neurological compromise

Mucopolysaccharidosis type IIIA

- Mucopolysaccharidosis type III A (Sanfilippo syndrome type A; MPS IIIA) is characterized by psychomotor and speech delay, neurological regression, and behavioral disturbances.
- Somatic changes are usually milder than other MPSs, and include mild coarsening, mild dysostosis multiplex, and contractures. About half of patients have hepatomegaly but splenomegaly is infrequent.
- MPS IIIA is caused by biallelic pathogenic variants in *SGSH* resulting in a deficiency of sulfamidase, a lysosomal enzyme. The inability to degrade heparan sulfate leads to cellular accumulation of this glycosaminoglycan and increased excretion in urine.
- There is only one report of a patient with sulfamidase deficiency, increased excretion of heparan sulfate, and late-onset cardiomiopathy without neurological phenotype. However, focused genetic analysis failed to identify variants in *SGSH*. (Van Hove et al, 2003)



RNA Sequencing Reveals Allele-Specific Expression

ASE was noted initially in the blood, with a modest 65%-35% skew. Expression studies in a heart biopsy revealed complete skew 100%-0%.

Conclusions

Rare genetic disease can be individually rare, but collectively is quite common

NGS has transformed how rare genetic disease is tested, enabled considerably higher diagnostic rates and novel disease gene discovery

RNAseq can increase overall diagnostic yield in this patient population

RNAseq analysis is complex and involves looking at multiple event types

Questions