NGS: what method to apply in diagnostic setting (panels, WES and WGS) and where technology fails

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THE
HUMAN VARIOME
PROJECT
an NGO official partner of UNESCO
sharing data · reducing disease

4th Variant Effect Prediction Training Course
29-31 May 2019
Moscow, Russia

After this talk, you will:

- Know the technical considerations in selection of your diagnostic approach - panel, exome or genome sequencing

- Understand the considerations of coverage, duplicated regions and the range of mutational mechanisms detectable by NGS

- Know when and how diagnostic procedure can go wrong if a sub-optimal diagnostic approach is used

- Understand that knowledge of the genetic and mutational spectrum of diseases is essential prior to using NGS approaches

You are welcome to participate!

1. Open web browser on the phone and go to this address: **etc.ch/dWSw**

2. Once the question will appear, select your answer and click **Vote**

If it does not work

Qo. Does the voting work?

A. Yes

B. No

Voting link **etc.ch/dWSw**

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| https:// | directpoll.com/r?XDbzPBd3ixYqg8C5fs7H0DQfOB4WMbze4dJjuY1a8 |

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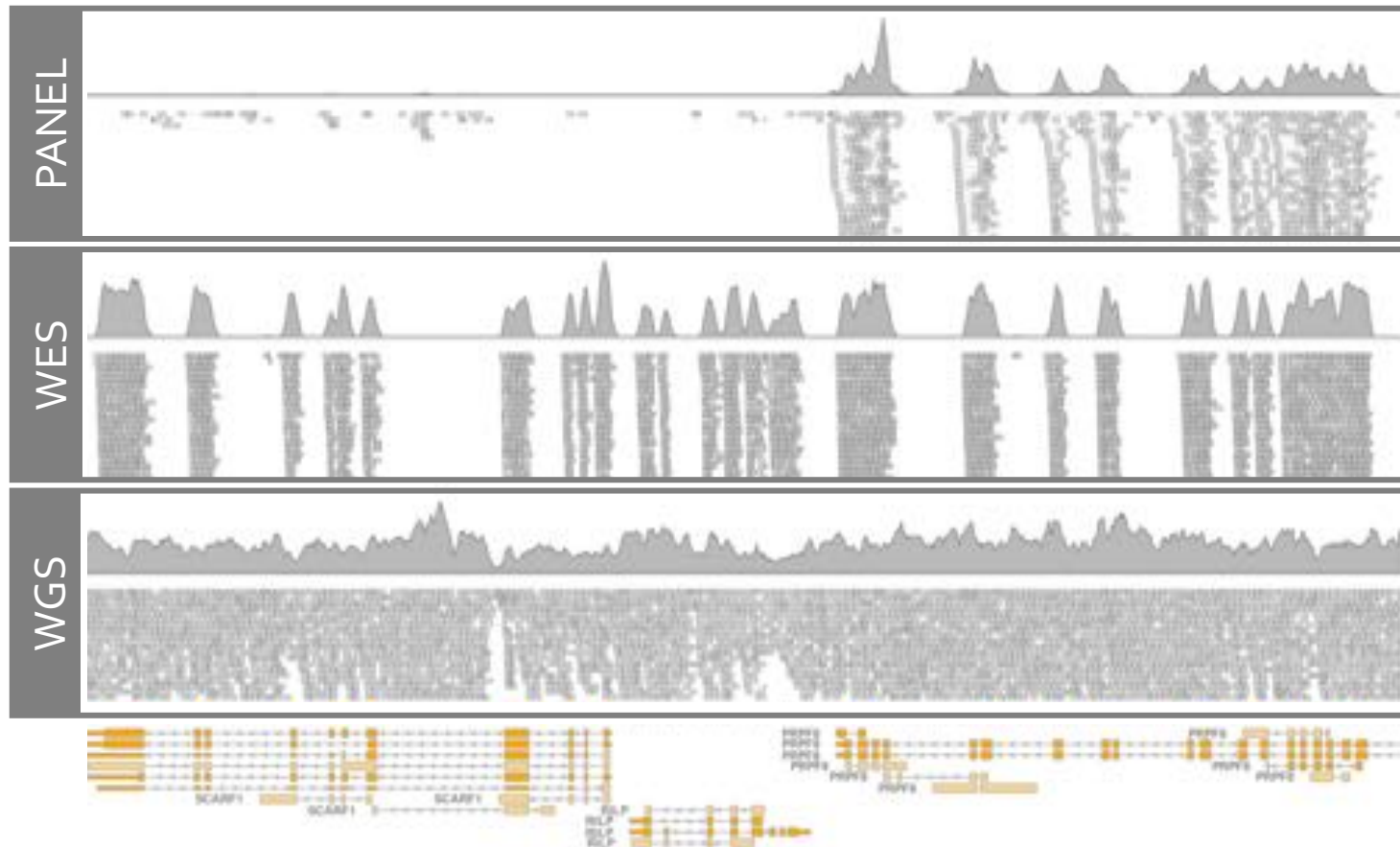
Voting link **etc.ch/dWSw**

Seeing the trees but not seeing the forest

Seeing the forest but not seeing the trees

The scope of NGS in disease diagnostics

NGS-based approaches

	PANEL	WES	WGS
Target size			
Target coverage			
Cost per sample			
Design effort			
Technical requirements			
Analytical difficulty			
Mutational spectrum			
Need for diagnostic hypothesis			
Complexity of interpretation			
Difficulty of genetic counselling			
Resource creation (population variability, etc.)			

Sequencing

Initial ivestment

Clinical utility

9
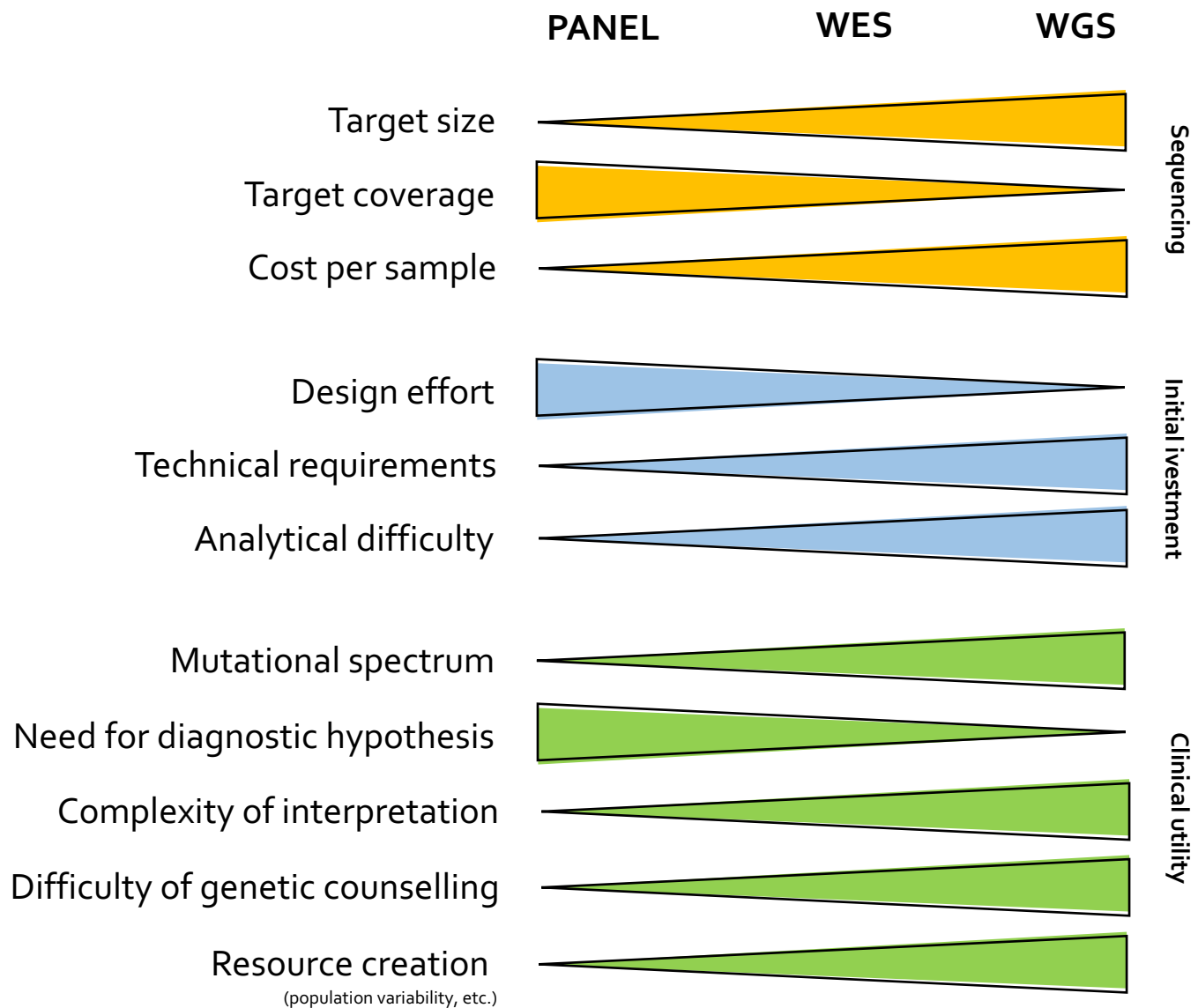
Diagnostic use scenarios

PANELS

Patients with well-defined diagnoses

Diseases associated with a single/few genes

Numerous patients with similar disease type
(ie. cardiomyopathy)

Assuring sufficient and customized coverage of regions of interest

Allows detection of mosaic/heteroplasmic variants

WES

Patients without a clear diagnostic hypothesis

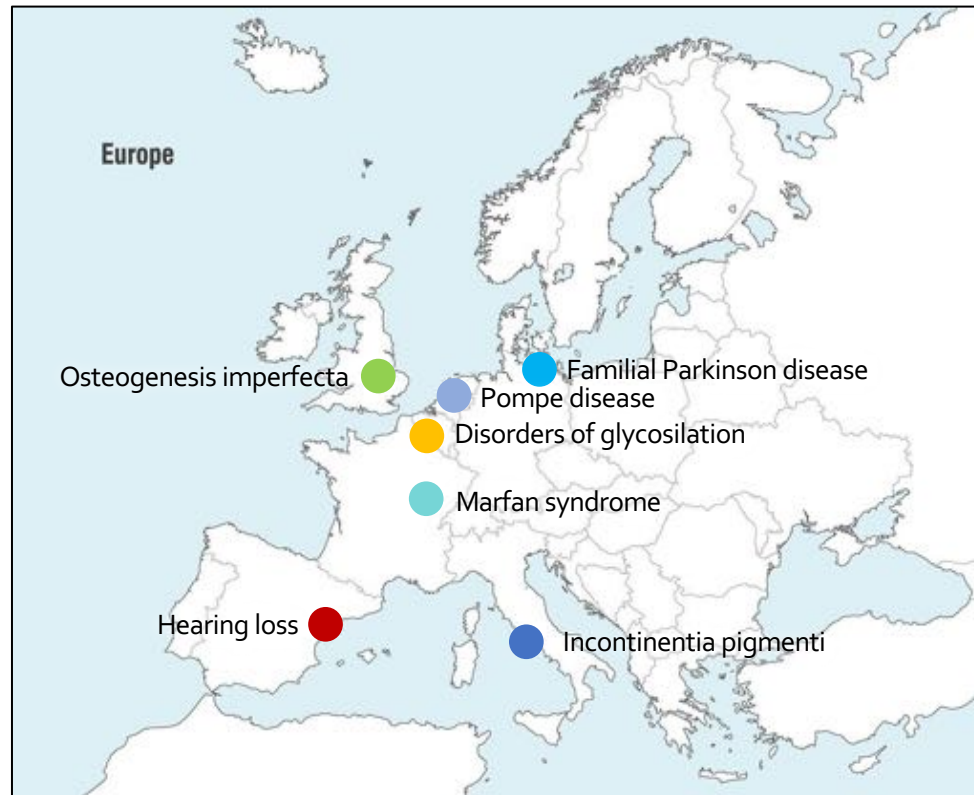Diseases with high genetic heterogeneity

Single test in institutions facing highly heterogeneous referrals

WGS

Patients with likely monogenic etiology and negative panel/exome

Single test in institutions facing highly heterogeneous referrals

Changing landscape of genetic testing

Osteogenesis imperfecta

Familial Parkinson disease
Pompe disease
Disorders of glycosilation
Marfan syndrome
Hearing loss
Incontinentia pigmenti

Genetic testing for specific conditions is concentrated in specialized in centres of expertise
(some examples on the map)

Changing landscape of genetic testing
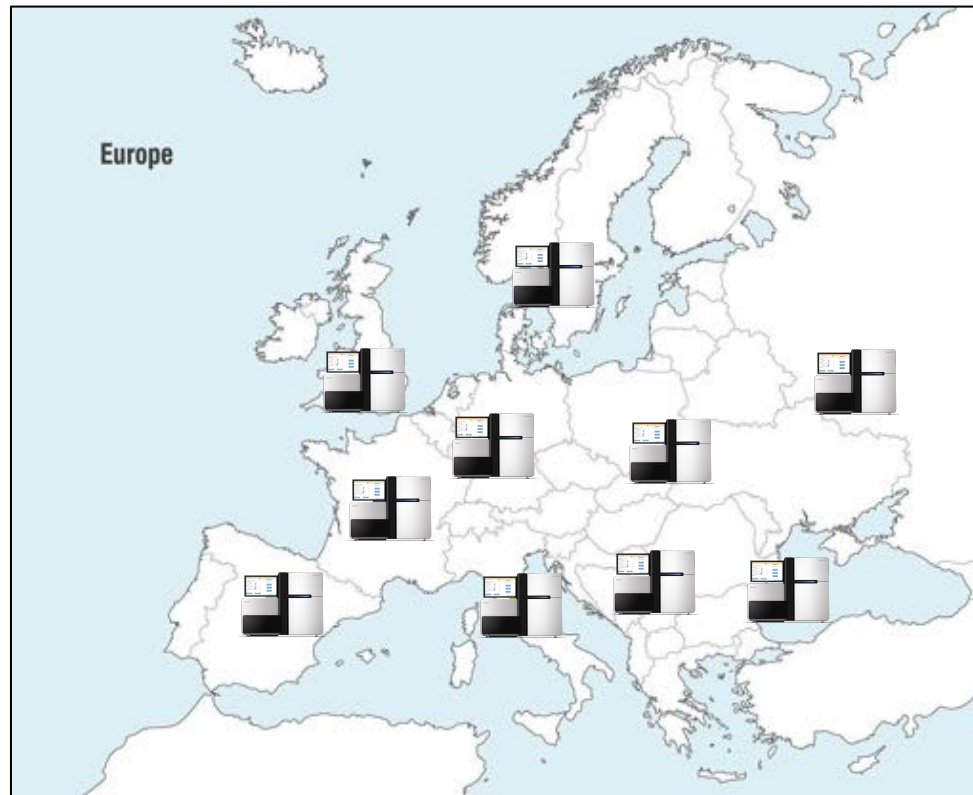
Next generation sequencing becomes widely available

12

Changing landscape of genetic testing
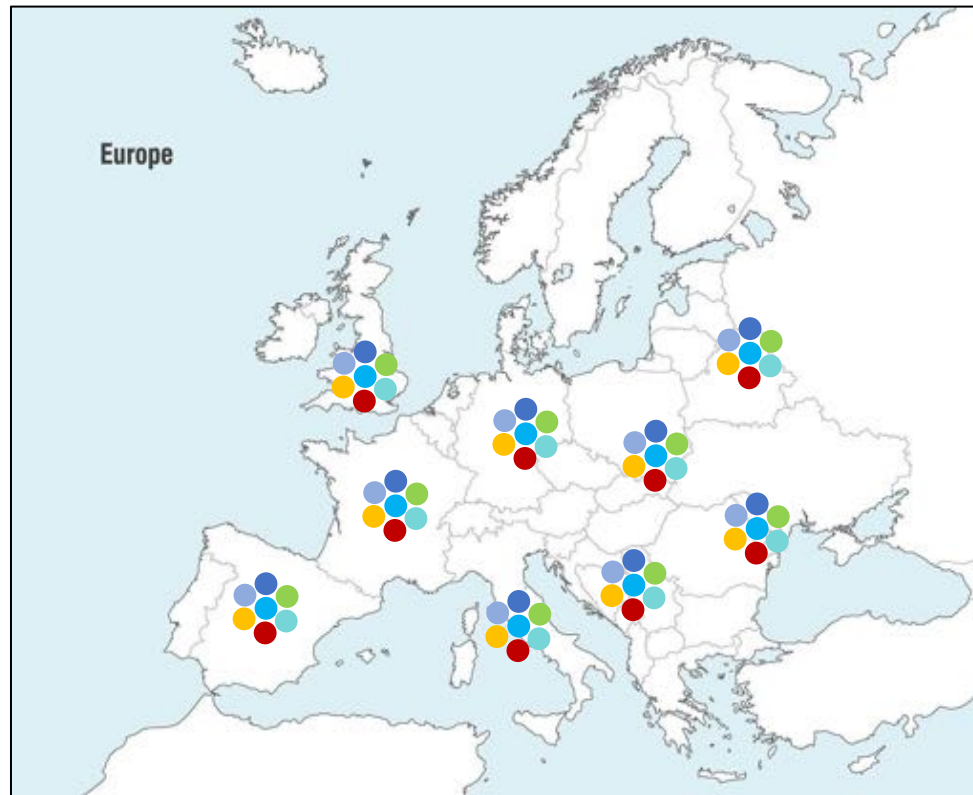
Using large panels, exome and genome sequencing every lab performs testing for almost every disorder

Considerations in selection of the NGS approach

Am I sequencing the region of interest?

Can I trust the results in my region of interest?

Can I detect the mutations associated with the referral condition?

How much can I trust the referral diagnosis?

Considerations in selection of the NGS approach

Am **I** sequencing the region of interest?

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Can I detect the mutations associated with the referral condition?

How much can I trust the referral diagnosis?

Case 1: Patient with blepharophimosis

- A 29-year old woman was referred to us for diagnostics of congenital anomaly of eye development – blepharophimosis.

- The patients mother and her maternal grandmother are also affected

Blepharophimosis is a narrowing of a palpebral fissure

https://webeye.ophth.uiowa.edu/eyeforum/cases-i/case114/BPES.html
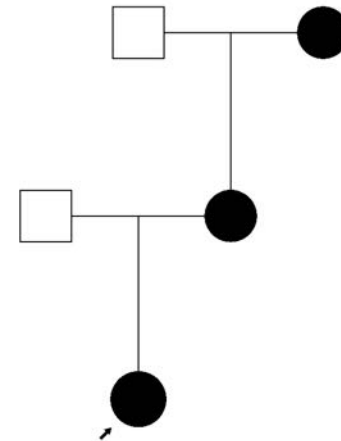
Case 1: Patient with blepharophimosis

- Whole exome sequencing
- The exome analysis was focused on 74 genes, the cause isolated/syndromic forms of blepharophimosis

Negative result!

Q1 - Patient with blepharophimosis: What to do next?

1. Include the parental samples (trio exome)

2. Check the coverage of the exome sequencing data

3. Perform whole genome sequencing

4. Perform a literature search to understand the genetic and mutational spectrum of blepharophimosis

5. Conclude the case as negative

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Asses the coverage of the exome sequencing data

1. Mean coverage: 69x

Is this information enough?

The significance of coverage in NGS

Reference TCAAAACTGTGAACGTAACACCAACGATCACGTCGATAGCGGGGGGCACAA
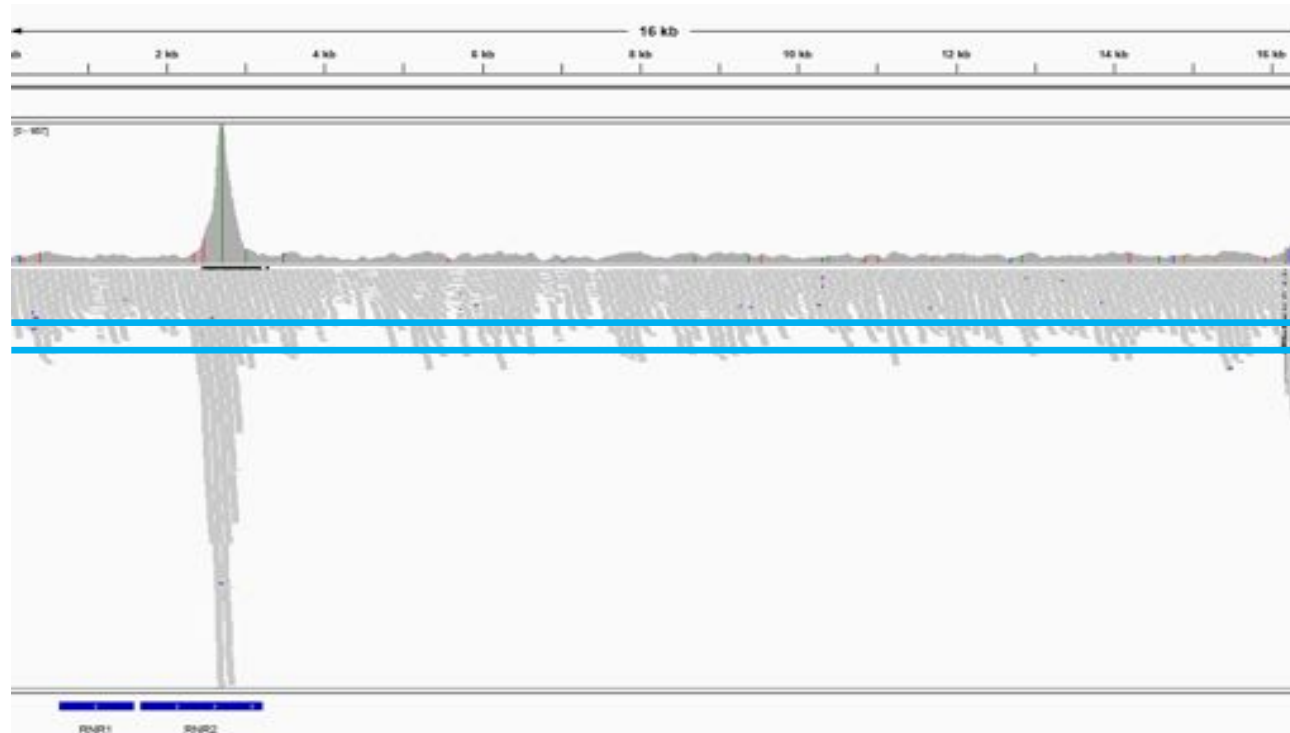
GTGAACGTAACACCAACG   ATAGCGGGGGGCACAA
AAACTGTGAACGTAACACCAAC   GATAGCGGGGGGCACA
ACTGTGAACGTAACACCAACGA   TAGCGGGGGGCACAA
GTGAACGTAACACCAACGATCA   ATAGCGGGGGGCACA
AACGTAACACCAACGATCAC   CGATAGCGGGGGGCA

Reads

TAACACCAACGATCA   GCGGGGGGCACAA
TGTGAACGTAACACCAAC   GATAGCGGGGGGCACA
TGAACGTAACACCAACGAT   ATAGCGGGGGGCACAA
GTGAACGTAACACCAACG   ATAGCGGGGGGCACA
CTGTGAACGTAACACCAAC   CGATAGCGGGGGGCAC

21

Base coverage

Reference	TCAAAACTGTGAACGTAACACCAACGATCACGTCGATAGCGGGGGCACAA
Reads	

```
                    GTGAACGTAACACCAACG         ATAGCGGGGGCACAA
               AAACTGTGAACGTAACACCAAC          GATAGCGGGGGCACA
                 ACTGTGAACGTAACACCAACGA           TAGCGGGGGCACAA
                    GTGAACGTAACACCAACGATCA        ATAGCGGGGGCACA
                      AACGTAACACCAACGATCAC      CGATAGCGGGGGCA
                         TAACACCAACGATCA              GCGGGGGCACAA
              TGTGAACGTAACACCAAC                GATAGCGGGGGCACA
                TGAACGTAACACCAACGAT               ATAGCGGGGGCACAA
                 GTGAACGTAACACCAACG               ATAGCGGGGGCACA
               CTGTGAACGTAACACCAAC            CGATAGCGGGGGCAC
```
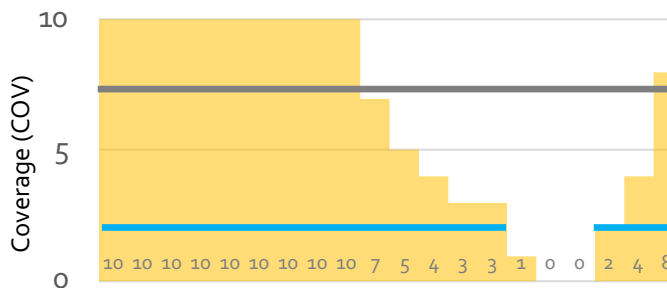
Coverage (COV)
Number of reads spanning a certain base after filtration

Mean coverage

Coverage (COV)

10

5

0

10 10 10 10 10 10 10 10 10 7 5 4 3 3 1 0 0 2 4 8

MEAN (COV)=6,4

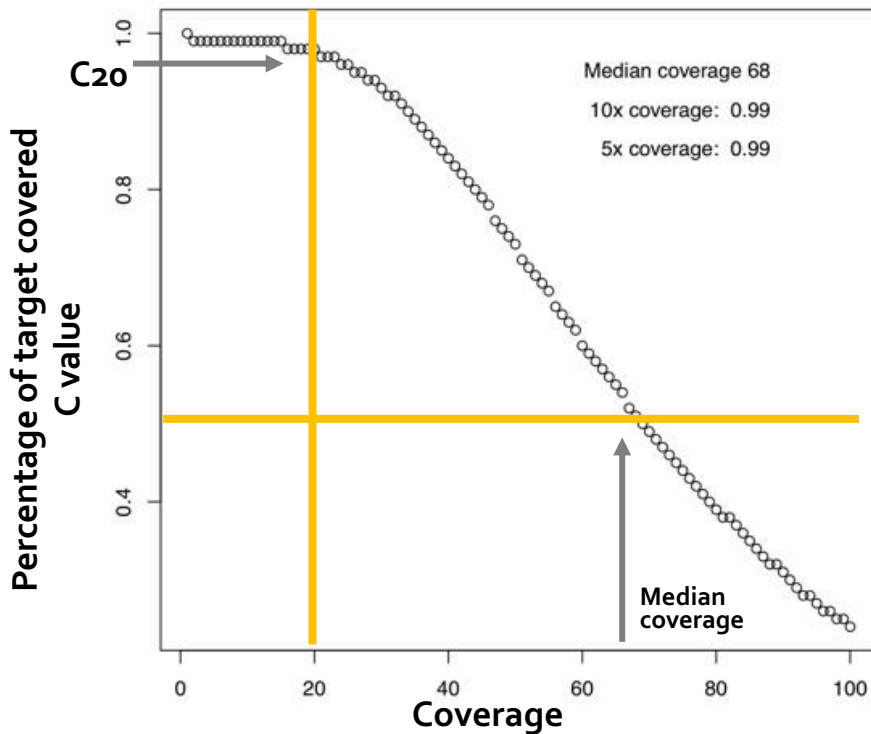**Reference** TCAAAACTGTGAACGTAACACCAACGATCACGTCGATAGCGGGGGCACAA

Reads
GTGAACGTAACACCAACG ATAGCGGGGGCACAA
AAACTGTGAACGTAACACCAAC GATAGCGGGGGCACA
ACTGTGAACGTAACACCAACGA TAGCGGGGGCACAA
GTGAACGTAACACCAACGATCA ATAGCGGGGGCACA
AACGTAACACCAACGATCAC CGATAGCGGGGGCA
TAACACCAACGATCA GCGGGGGCACAA
TGTGAACGTAACACCAAC GATAGCGGGGGCACA
TGAACGTAACACCAACGAT ATAGCGGGGGCACAA
GTGAACGTAACACCAACG ATAGCGGGGGCACA
CTGTGAACGTAACACCAAC CGATAGCGGGGGCAC

Region of interest

Mean coverage – MEAN (COV)
Mean of separate coverage values across a region of interest

Median is more robust for outliers in coverage

MEDIAN (COV)=50
MEAN (COV)=67

Only a minority of regions are covered at 67 or more thus mean does not give a realistic information of coverage!

Mitochondrial coverage profile with Agilent SureSelect All Exon v5
One high peak of coverage artificially inflates the mitochondrial average coverage, but does not affect the median coverage

Median coverage
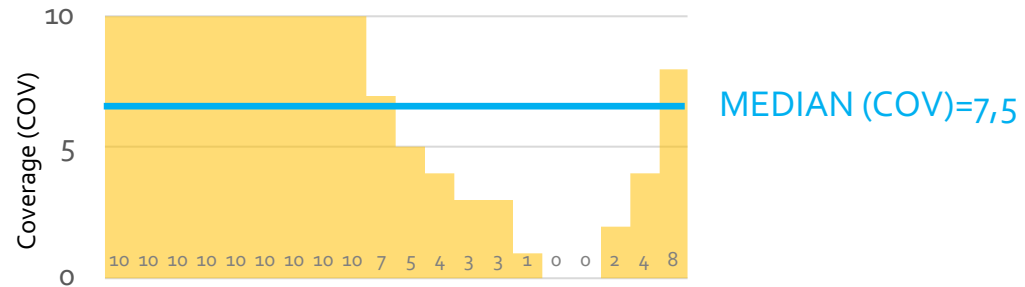
Coverage (COV)

10

5

0

MEDIAN (COV)=7,5

10 10 10 10 10 10 10 10 10 7 5 4 3 3 1 0 0 2 4 8

Reference TCAAAACTGTGAACGTAACACCAACGATCACGTCGATAGCGGGGGCACAA

Reads

GTGAACGTAACACCAACG   ATAGCGGGGGCACAA
AAACTGTGAACGTAACACCAAC   GATAGCGGGGGCACA
ACTGTGAACGTAACACCAACGA   TAGCGGGGGCACAA
GTGAACGTAACACCAACGATCA   ATAGCGGGGGCACA
AACGTAACACCAACGATCAC   CGATAGCGGGGGCA
TAACACCAACGATCA   GCGGGGGCACAA
TGTGAACGTAACACCAAC   GATAGCGGGGGCACA
TGAACGTAACACCAACGAT   ATAGCGGGGGCACAA
GTGAACGTAACACCAACG   ATAGCGGGGGCACA
CTGTGAACGTAACACCAAC   CGATAGCGGGGGCAC

Region of interest

Mean coverage – MEDIAN (COV)
Median of separate coverage values across a region of interest

Coverage uniformity

MEDIAN(COV)=7,5

UNIFORMITY=85%
85% of bases are covered at 0.2x MEDIAN COVERAGE

Coverage (COV)

10 10 10 10 10 10 10 10 10   7   5   4   3   3   1   0   0   2   4   8

Reference TCAAAACTGTGAACGTAACACCAACGATCACGTCGATAGCGGGGGCACAA

Reads

Region of interest

Coverage uniformity
Percentage of bases covered at minimum 0,2x median coverage

Percent of target covered at C or more

$C_{10}=45\%$

$C_5=50\%$

$C_1=90\%$

Coverage (COV)

10

5

0

10 10 10 10 10 10 10 10 10 7 5 4 3 3 1 0 0 2 4 8

Reference

TCAAAACTGTGAACGTAACACCAACGATCACGTCGATAGCGGGGGCACAA

Reads

GTGAACGTAACACCAACG   ATAGCGGGGGCACAA
AAACTGTGAACGTAACACCAAC   GATAGCGGGGGCACA
ACTGTGAACGTAACACCAACGA   TAGCGGGGGCACAA
GTGAACGTAACACCAACGATCA   ATAGCGGGGGCACA
AACGTAACACCAACGATCAC   CGATAGCGGGGGCA
TAACACCAACGATCA   GCGGGGGCACAA
TGTGAACGTAACACCAAC   GATAGCGGGGGCACA
TGAACGTAACACCAACGAT   ATAGCGGGGGCACAA
GTGAACGTAACACCAACG   ATAGCGGGGGCACA
CTGTGAACGTAACACCAAC   CGATAGCGGGGGCAC

Region of interest

C value
Percent of a target covered above a certain coverage threshold

27

Coverage characteristic

Profile of C values across various coverage thresholds

Coverage too low
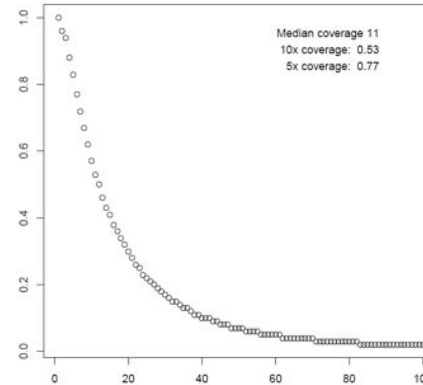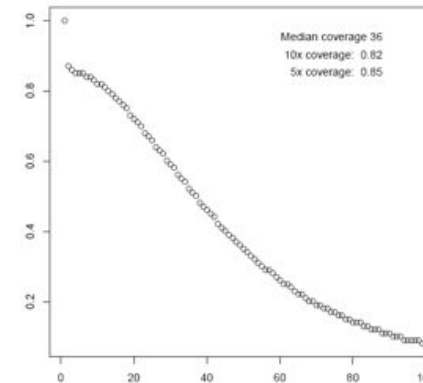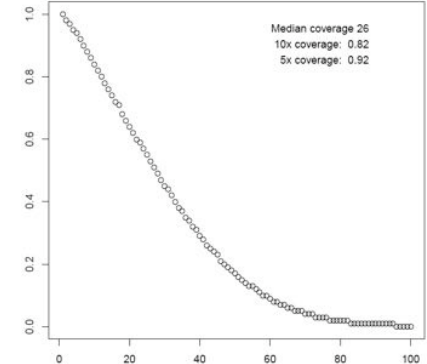
Some capture regions have no coverage

Inefficient capture
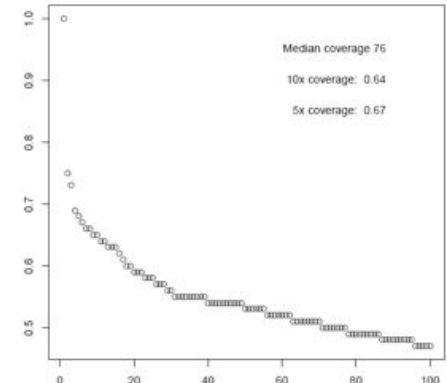
Considerable proportion of regions without any coverage

Case 1: Patient with blepharophimosis
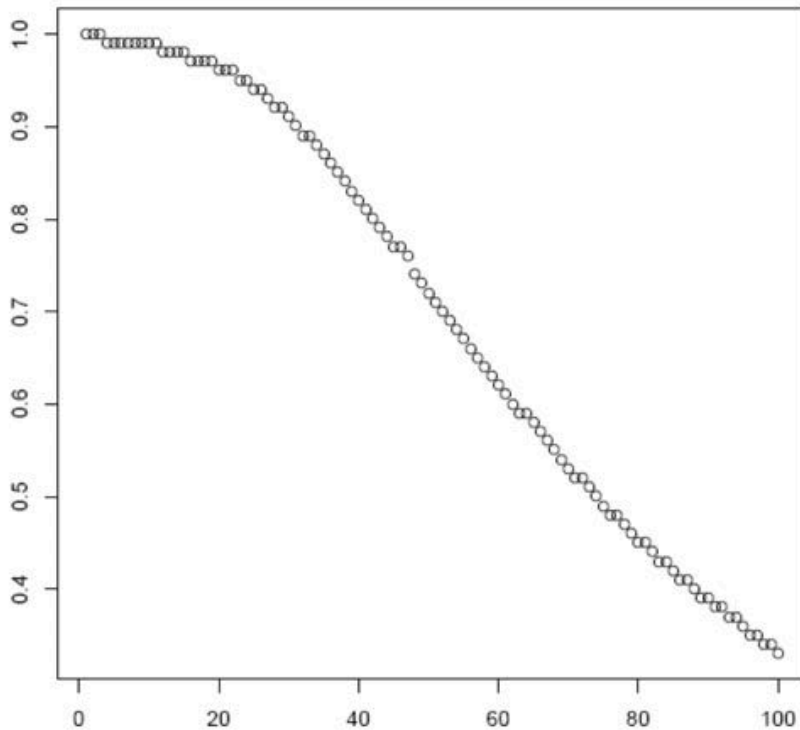
Mean coverage: **69x**

Median coverage: **73x**

Uniformity: **97%**

C20: **98.6%** bases covered at 20x

Coverage characteristic looks good

Q2 – Patient with blepharophimosis: What to do next?

1. The coverage is still to low, perform additional sequencing

2. Include the parental samples (trio exome)

3. Perform whole genome sequencing

4. Perform a literature search to understand the genetic and mutational spectrum of blepharophimosis

5. Conclude the case as negative

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What causes blepharophimosis?

Summary

Clinical characteristics. *B*lephariphimosis, *p*tosis, and *e*picanthus inversus syndrome (BPES) is a complex eyelid malformation invariably characterized by four major features: blepharophimosis, ptosis, epicanthus inversus, and telecanthus. BPES type I includes the four major features and premature ovarian insufficiency (POI); BPES type II includes only the four major features. Other ophthalmic manifestations that can be associated with BPES include lacrimal duct anomalies, amblyopia, strabismus, and refractive errors. Minor features include a broad nasal bridge, low-set ears, and a short philtrum. Individuals with BPES and an intragenic *FOXL2* pathogenic variant are expected to have normal intelligence, in contrast to affected individuals with cytogenetic rearrangements that involve *FOXL2* and additional genes.

Diagnosis/testing. The diagnosis of BPES is primarily based on clinical findings. Occasionally individuals with BPES have cytogenetic rearrangements, such as interstitial deletions and translocations involving 3q23. *FOXL2* is the only gene currently known to be associated with BPES.

Management. *Treatment of manifestations:* Timing of eyelid surgery involves balancing the benefits of early surgery to prevent deprivation amblyopia versus late surgery to allow for more reliable ptosis measurements. Surgery

FOXL2 gene coverage in WES data

Median coverage 64
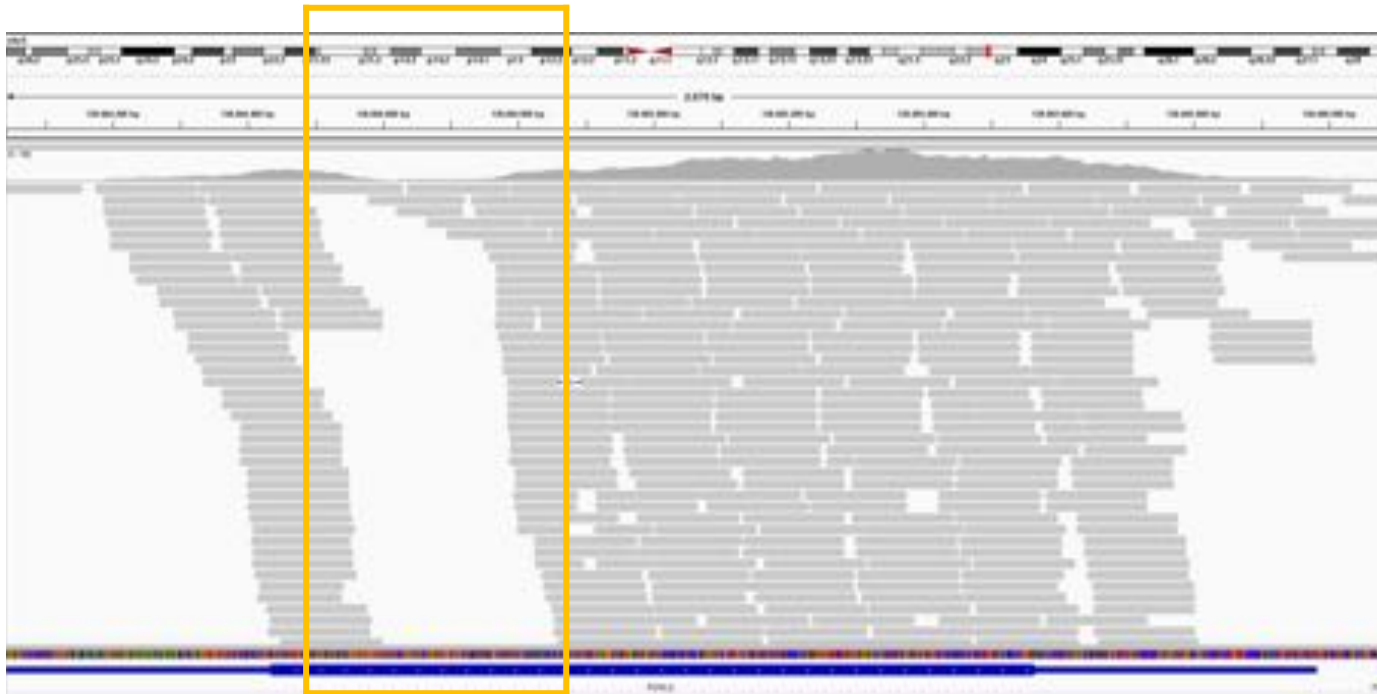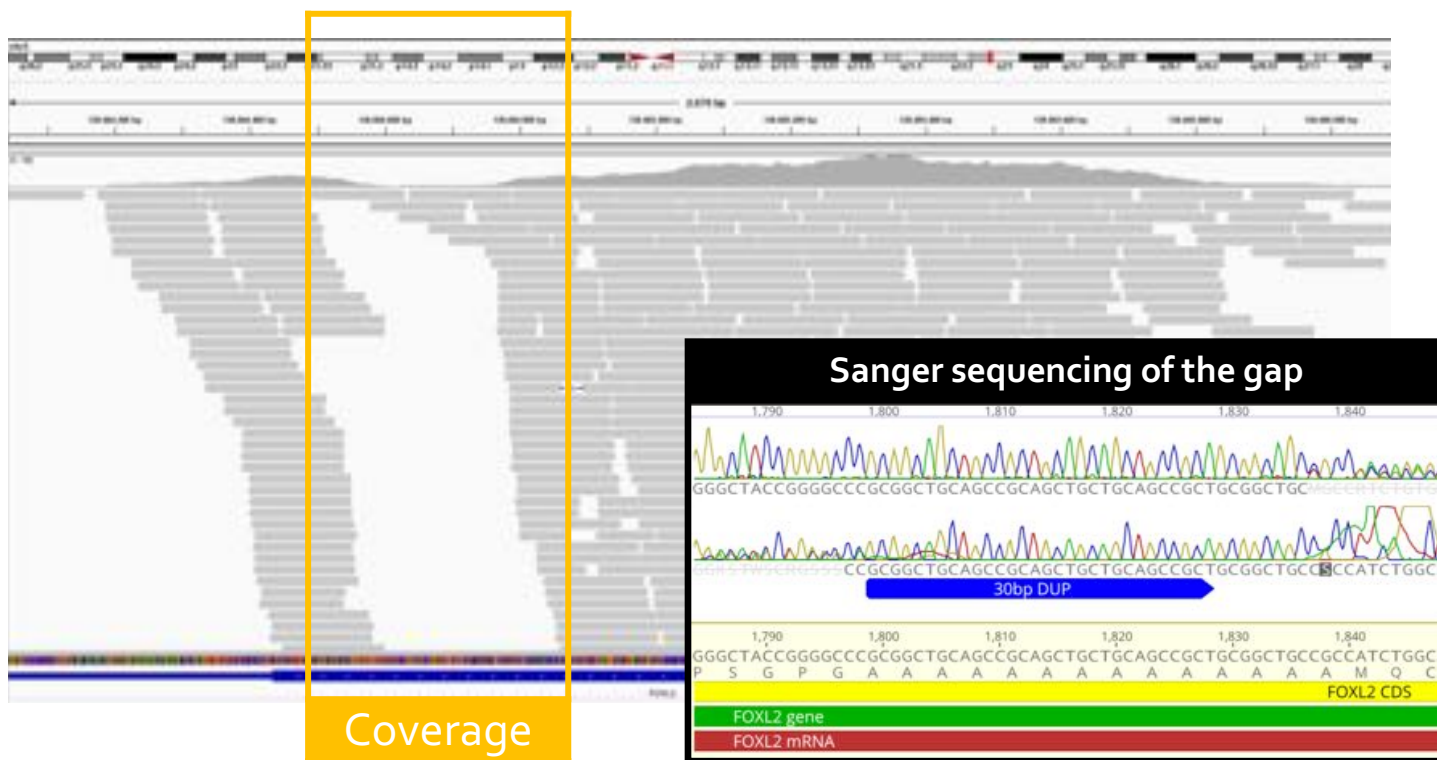10x coverage: 0.83
5x coverage: 0.9
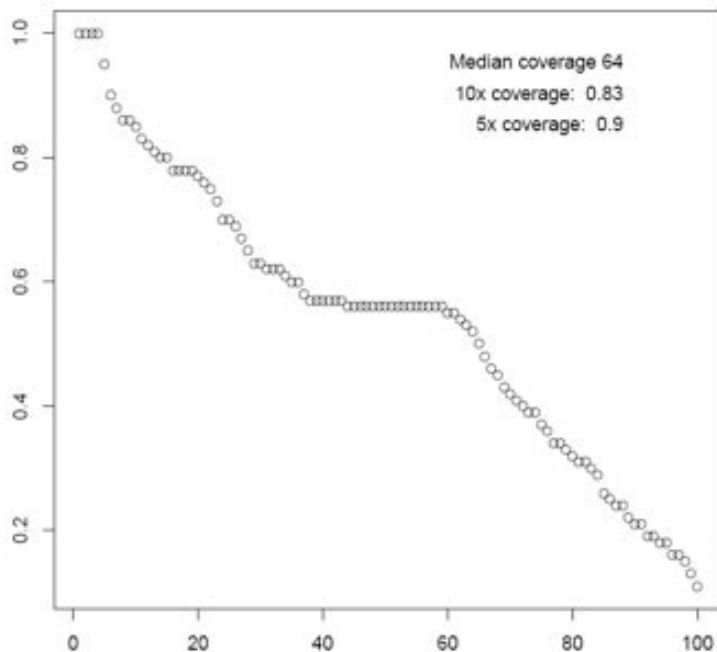
Mean coverage: **54x**

Median coverage: **65x**

Uniformity: **78%**

C20: **82.8%** bases covered at 10x

Coverage characteristic reveals incomplete coverage of the FOXL2 gene

FOXL2 gene coverage

Coverage

Several pathogenic variants have been reported in this region

Q3 – Patient with blepharophimosis: What to do next?

1. Use Sanger sequencing to bridge the gap

2. Perform additional sequencing of the exome library

3. Check for deletions in the FOXL2 gene

4. Perform whole genome sequencing

5. Conclude the case as negative

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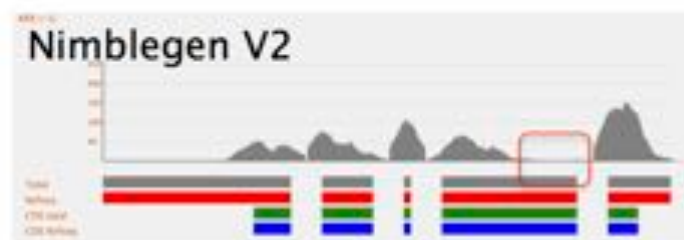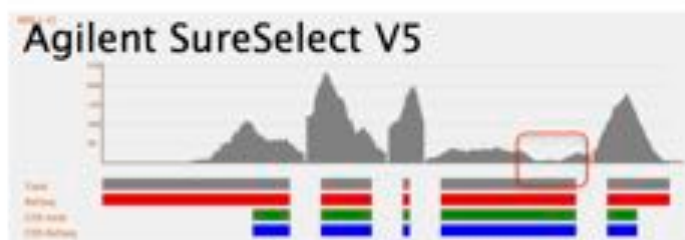
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FOXL2 gene coverage

Coverage

Several pathogenic variants have been reported in this region

Sanger sequencing of the gap

30bp DUP

FOXL2 CDS
FOXL2 gene
FOXL2 mRNA

A pathogenic 30 bp duplication in FOXL2 gene

Epileptic encephalopathy due to ARX mutations

This region contains the most common mutation of ARX gene!

Coverage gap

Coverage of ARX in exome enrichment kits

Taken from: Beyond the Ion Channel
http://epilepsygenetics.net/2014/05/19/the-arx-problem-how-an-epilepsy-gene-escapes-exome-sequencing/

39
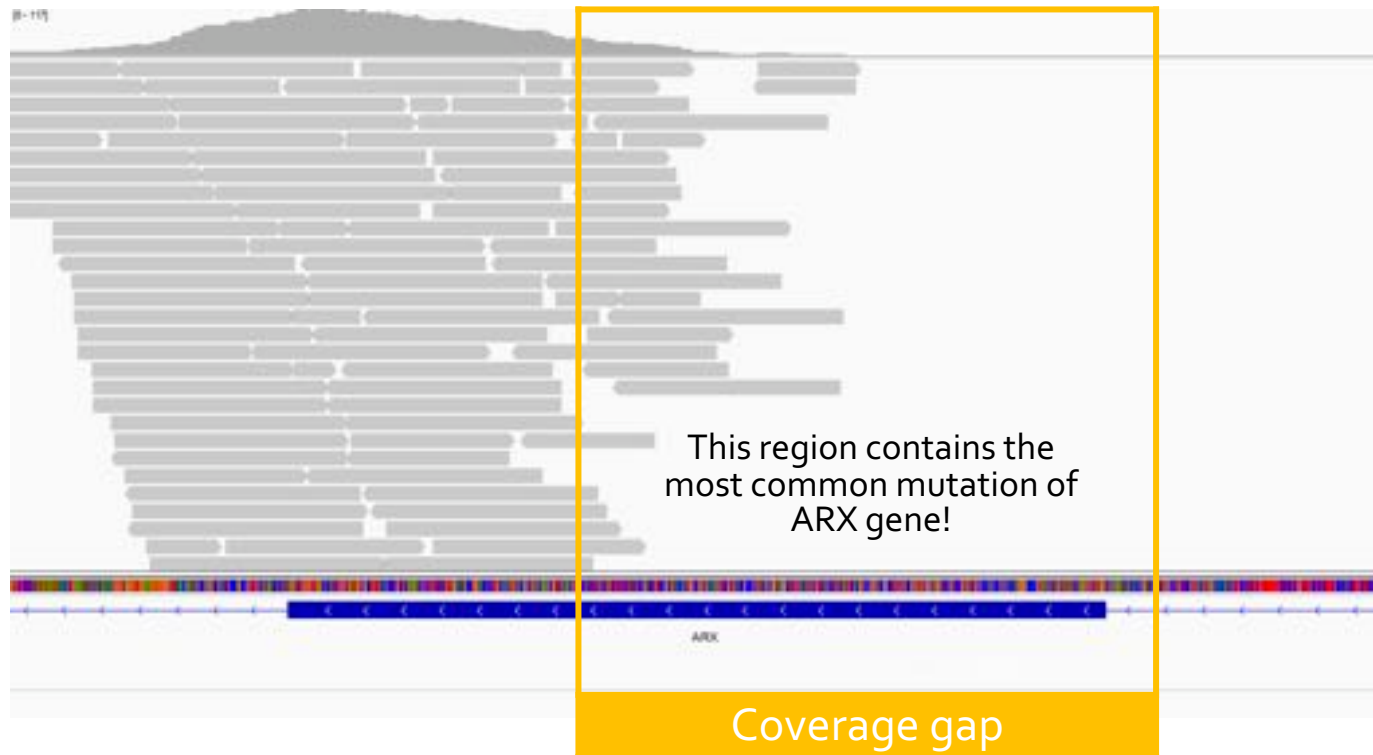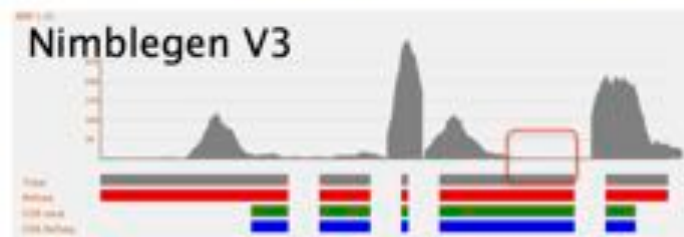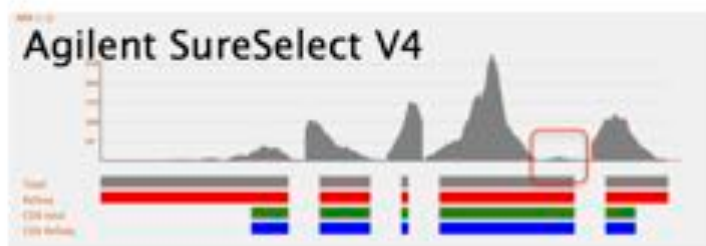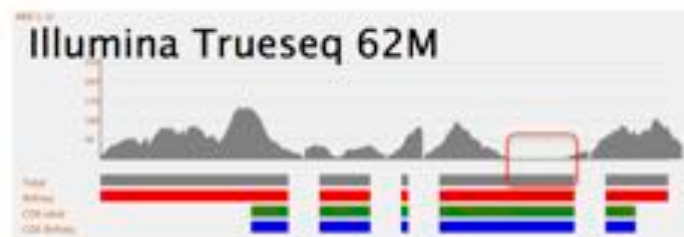
Patient with retinal dystrophy and a single mutation in CDHR1 gene (AR inheritance)

Initiating codon variant

Coverage gap
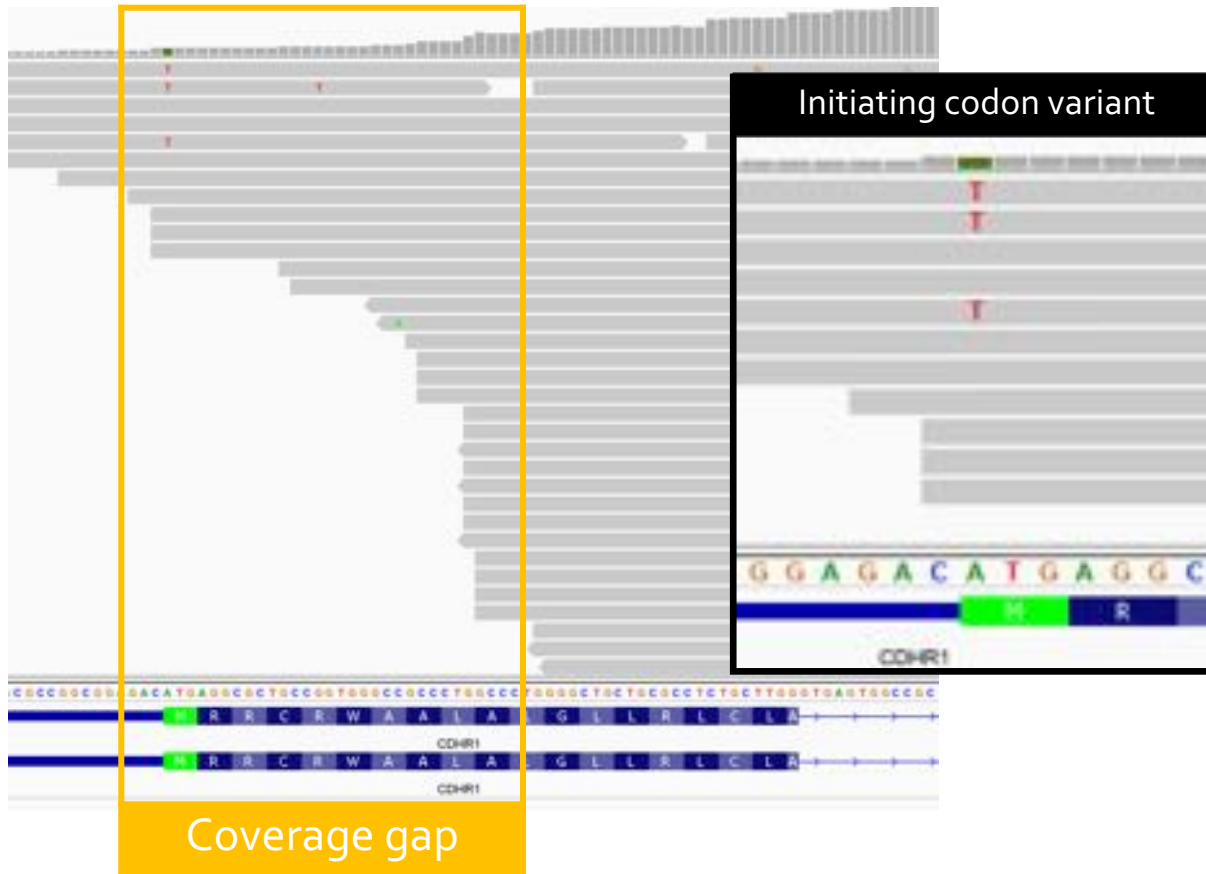
Targeted inspection reveals a likely pathogenic initiating codon variant
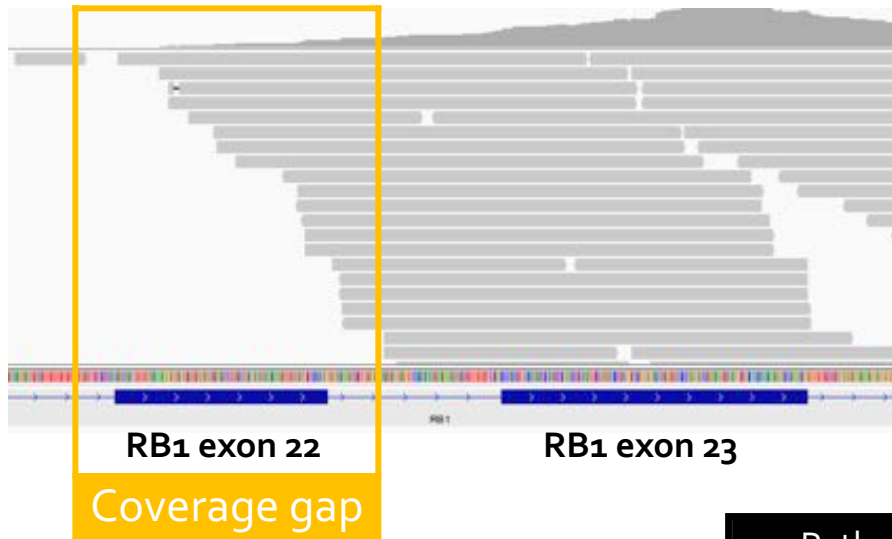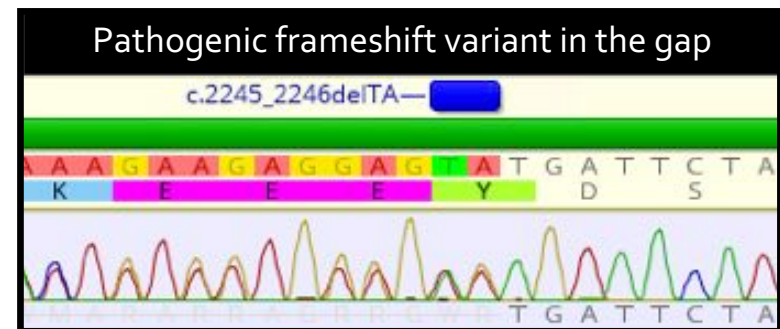
c.1A>T
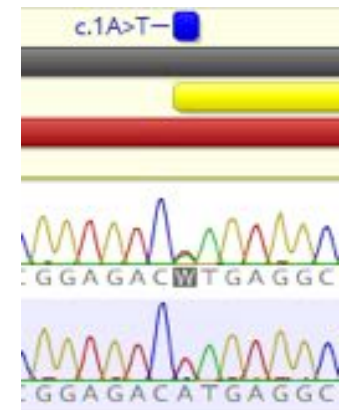
Sanger validation confirms the variant

Beware of poorly covered 1st exons in exome sequencing data

Genes with consistent gaps in WES

Patient with suspected hereditary retinoblastoma

RB1 exon 22   **RB1 exon 23**

Coverage gap

Exon 22 in RB1 gene is consistently poorly covered in exome sequencing data

Pathogenic frameshift variant in the gap

c.2245_2246delTA—

Considerations in selection of the NGS approach

Am I sequencing the region of interest?

Can I trust the results in my region of interest?

Can I detect the mutations associated with the referral condition?

How much can I trust the referral diagnosis?

Case 2: Family with a pigmentation disorder, developmental delay

Initial suspicion was of **Incontinentia pigmenti**

Incontinentia pigmenti is an X-linked condition that occurs due to heteroyzgous mutations in IKBKG gene in females (hemiyzgous state is lethal)

Clinical presentation includes blistering in the early life, wart like rash, then macular pigmentation and ultimately linear hypopigmentation

Clinical presentation in is consistent with IP, but some affected members display developmental delay
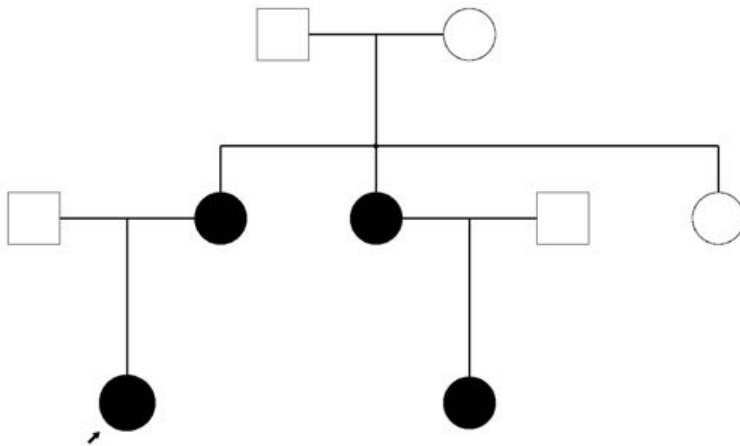
FIGURE 2: Verrucous, hyperkeratotic lesion on the right palm

Marcela A. C. An. Bras. Dermatol. vol.85 no.3 Rio de Janeiro June 2010

Whorly linear skin lesions, https://www.aao.org/disease-review/incontinentia-pigmenti

Case 2: Family with a pigmentation disorder, developmental delay

- Targeted testing for the common deletion of exons 4-10 in the IKBGKG gene

Negative result!

- Due to presence of developmental delay in some affected members and because a targeted test for sequencing of the IKBKG coding region was not available, the clinician ordered exome sequencing

Negative result!

Q4 - What do you advise next?

1. Check if the coverage of the IKBKG gene is sufficient

2. Advise WES in affected members to better detect a novel genes not yet associated with this condition

3. Perform WGS to detect intronic/regulatory variants

4. Advise PCR and Sanger sequencing of the IKBKG gene

5. Conclude the report as negative and advise reinterpretation after 2 years

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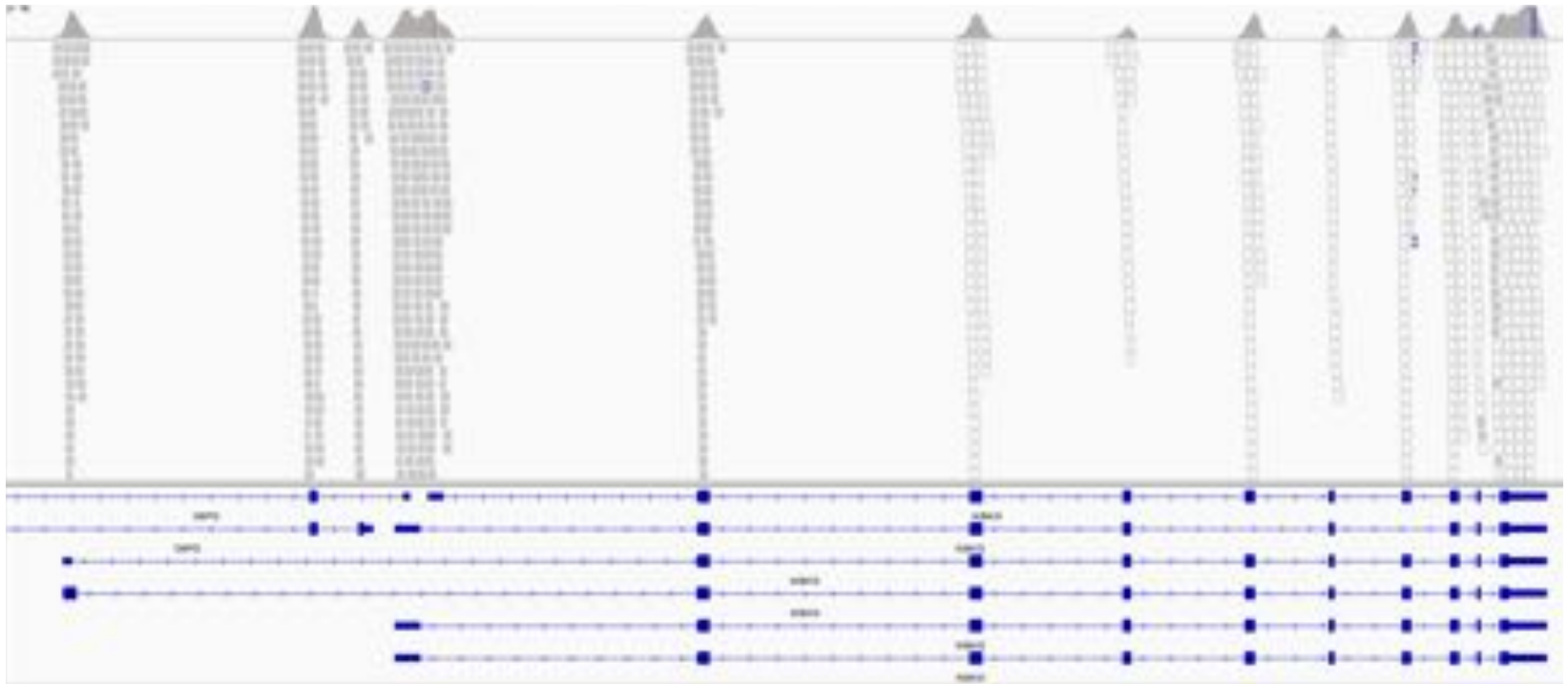| https:// | directpoil.com/Y7XDbzPBd3ixYqg8C5fs7H0DQfOB4WMbze4dJjuY1a8 |

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Coverage profile of the IKBKG gene

Q5 - What do you advise next?

1. Advise WES in other affected members to better detect a novel genes not yet associated with this condition

2. Perform WGS to detect intronic/regulatory variants

3. Advise long range PCR and Sanger sequencing of the IKBKG gene

4. Conclude the report as negative and advise reinterpretation after 2 years

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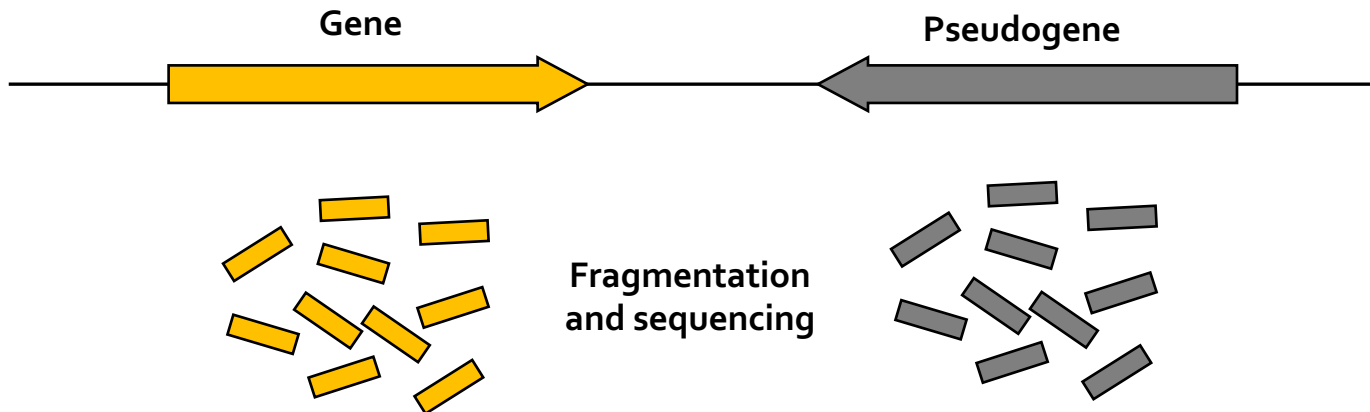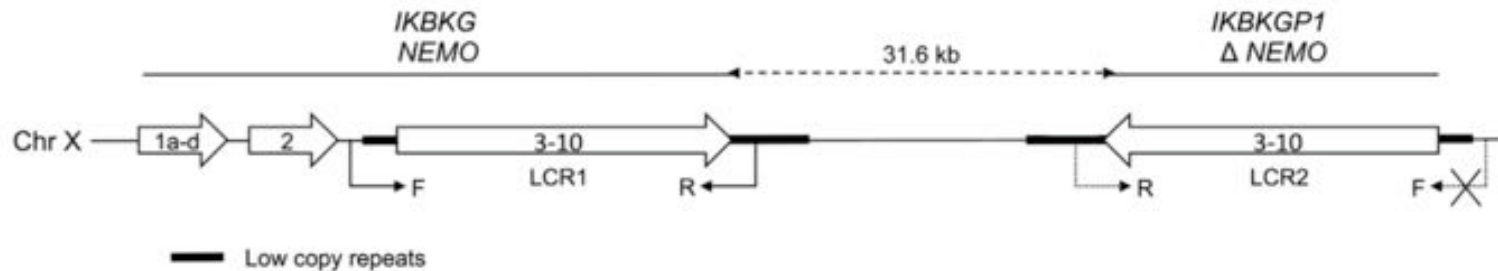
Please enter the URL below.

| https:// | directpoll.com/r7XDbzPBd3ixYqg8C5fs7H0DQfOB4WMbze4dJjuY1a8 |

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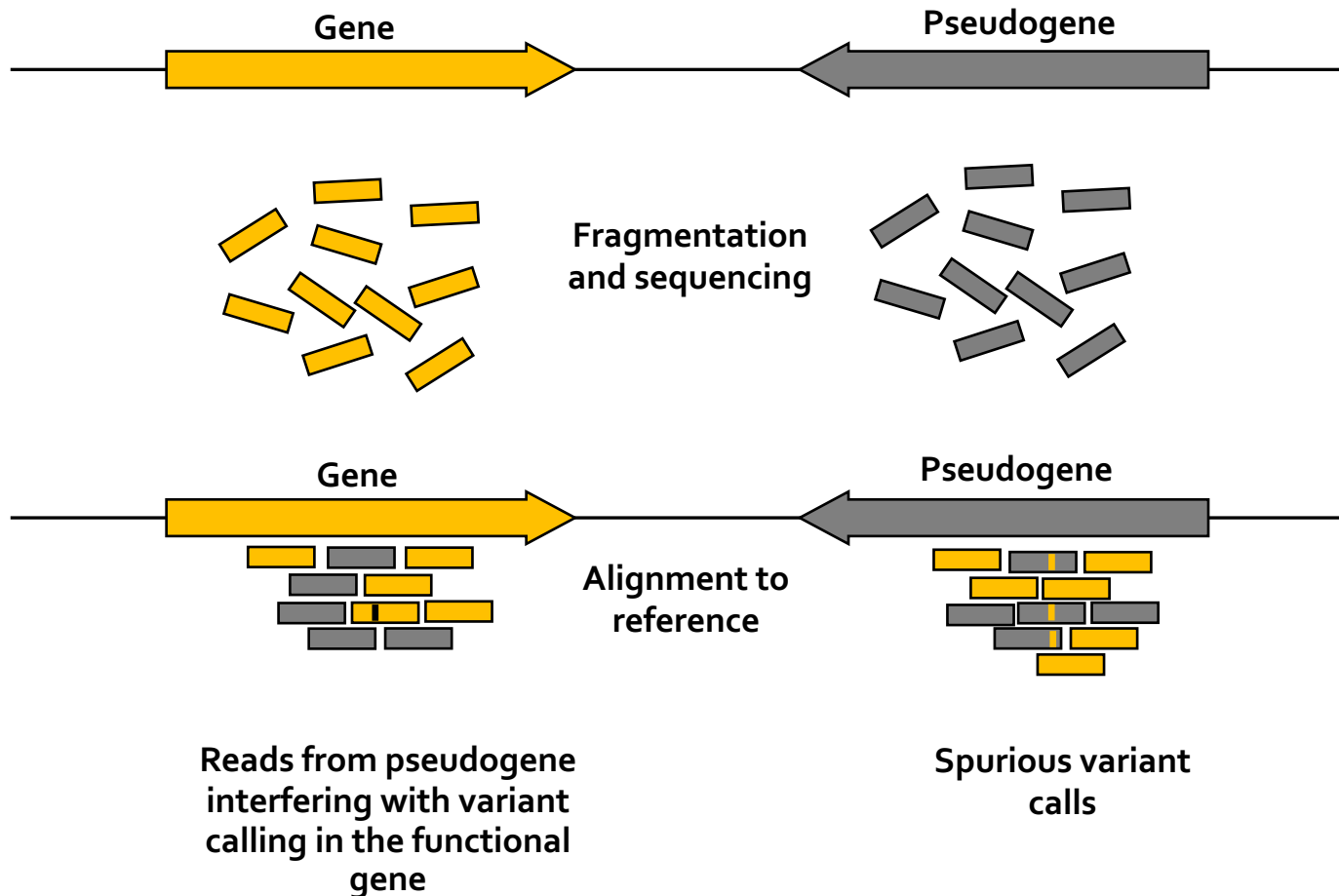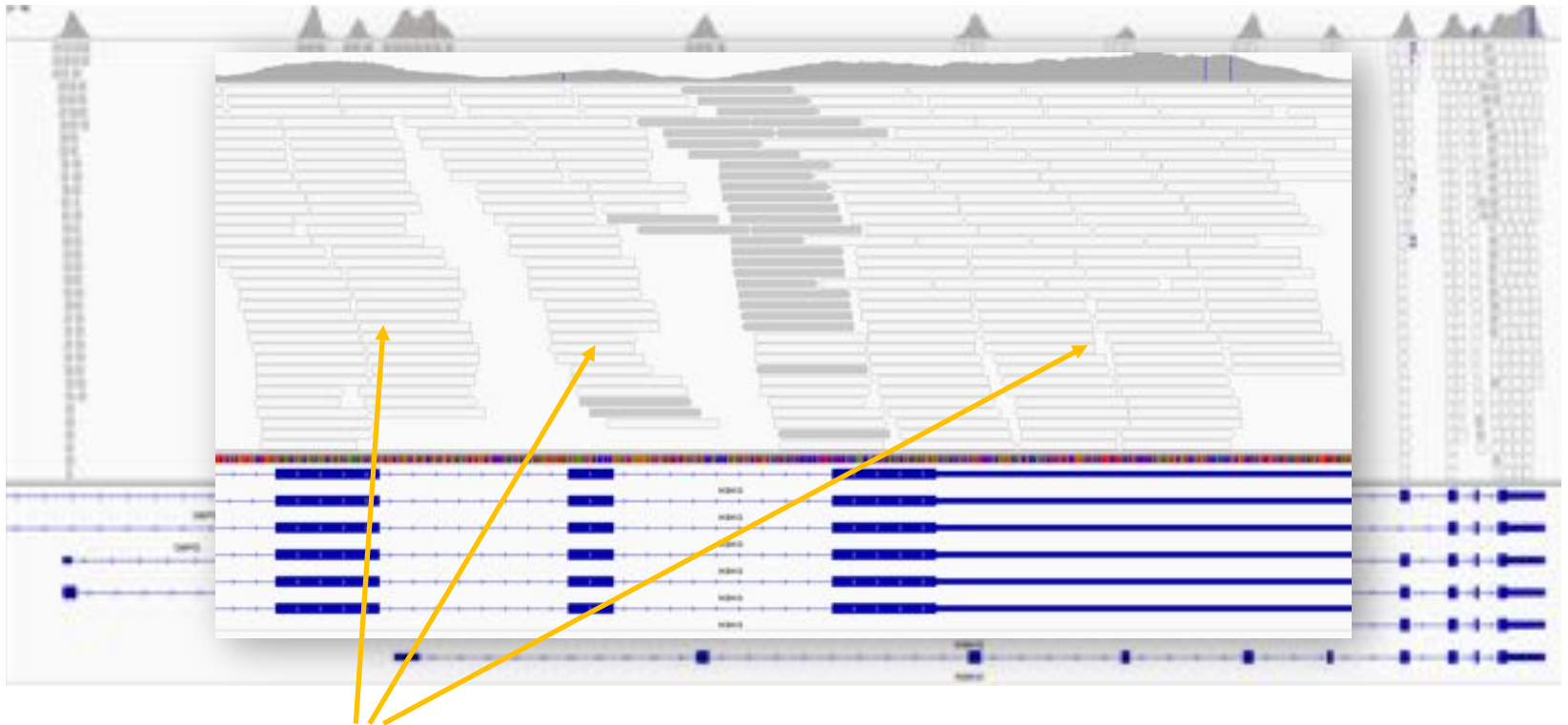
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Duplicated/repetitive sequences and next-generation sequencing

Duplicated/repetitive sequences and next-generation sequencing

Gene | Pseudogene

Fragmentation and sequencing

Gene | Pseudogene

Alignment to reference

Reads from pseudogene interfering with variant calling in the functional gene
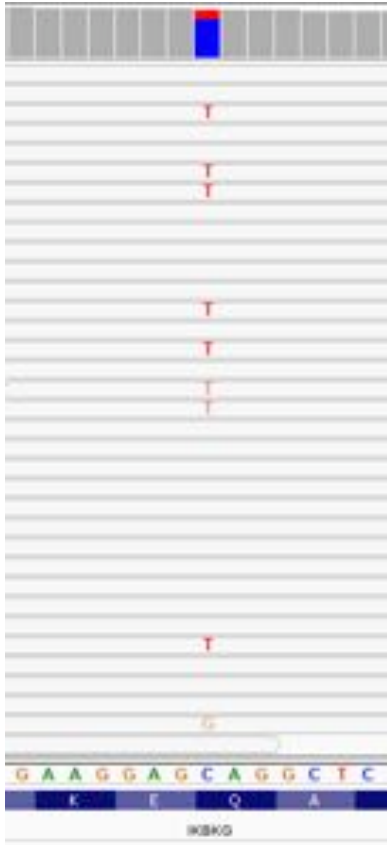
Spurious variant calls

Coverage profile of the IKBKG gene

Blank reads represent reads with mapping quality equal to 0 (MAQ=0)
BWA assigns this to reads that do not map uniquely to the genome reference

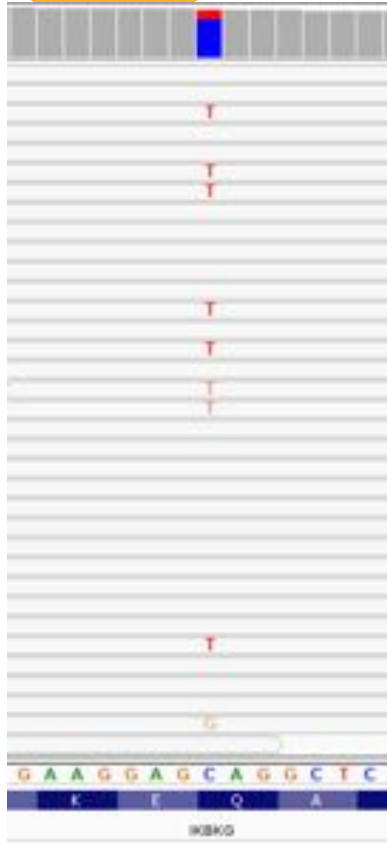Variant callers will also ignore these reads

Variants in repeated sequences

- There was a variant not called by GATK variant caller

- NM_003639.4:c.358C>T, p.Gln120Ter. Classifies as ACMG class 5
- Taken to Sanger after long-range PCR

Did we validate it?

Q6 - Does this variant validate using Sanger?

1. **No**, the variant is a sequencing artifact

2. **No**, the variant likely arose from the pseudogene of the IKBKG

3. **Yes**, this variant was confirmed as heterozygous

4. **Yes**, this variant was confirmed as a mosaic variant

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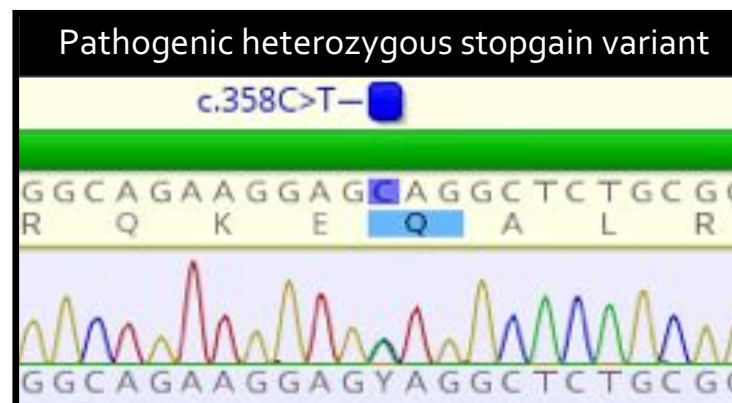| https:// | directpoll.com/r7XDbzPBd3ixYqg8C5fs7H0DQfOB4WMbze4dJjuY1a8 |

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Sanger after the long range PCR confirms the presence of this variant

Pathogenic heterozygous stopgain variant

c.358C>T—

GGCAGAAGGAGCAGGCTCTGCGG
R   Q   K   E   Q   A   L   R

GGCAGAAGGAGYAGGCTCTGCGG

Be careful^b ... **next—generati** ...

- Incontine...
- Spinal m...
- Congenit... P21A1P)
- Thalasse...
- Gaucher...
- Polycysti...
- X-linked...
- Several c...

Ebbert et al. Genome Biology 2019, **20**:97
**Systematic analysis of dark and camouflaged genes reveals
disease-relevant genes hiding in plain sight**

Considerations in selection of the NGS approach

Am I sequencing the region of interest?

Can I trust the results in my region of interest?

Can I detect the mutations associated with the referral condition?

How much can I trust the referral diagnosis?

Case 3: 1-year old boy with apnea

- Suspected congenital central hypoventilation syndrome

- Inability to control breathing, patients need life-long ventilatory support during sleep in some patients or all the time in others

- Associated with pathogenic variants in 15 genes

https://img.wikinut.com/img/3w1lcg9jmezlt 1u4/jpeg/0/Congenital-Central-Hypoventilation-Syndrome.jpeg
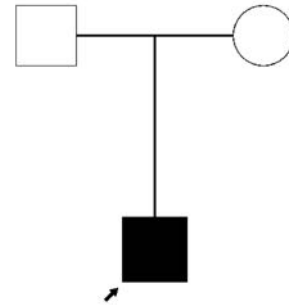
Q7 - What is your diagnostic approach of choice

1. Perform NGS based-panel sequencing
2. Perform exome sequencing
3. Perform whole genome sequencing
4. Perform a classical PCR-based test for the most common for of this syndrome

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Case 3: 1-year old boy with apnea

- Whole exome sequencing

Negative result!

Q8 – Why is the exome result negative?

1. The patient does not have pathogenic variants in the analyzed genes

2. The relevant genes are likely not covered well

3. Intronic/regulatory variants are likely the cause

4. We cannot detect pathogenic mutations is the causative gene(s)

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Case 3: 1-year old boy with apnea

- Whole exome sequencing

Negative result!

The coverage is sufficient, the targeted genes are not located in duplicated regions.

What to do next?

Congenital central hypoventilation

- The most common cause of this disorder are polyalanine expansions in the PHOX2B gene
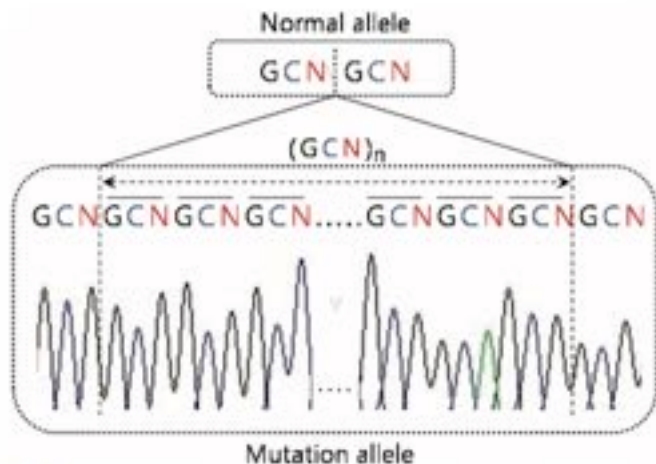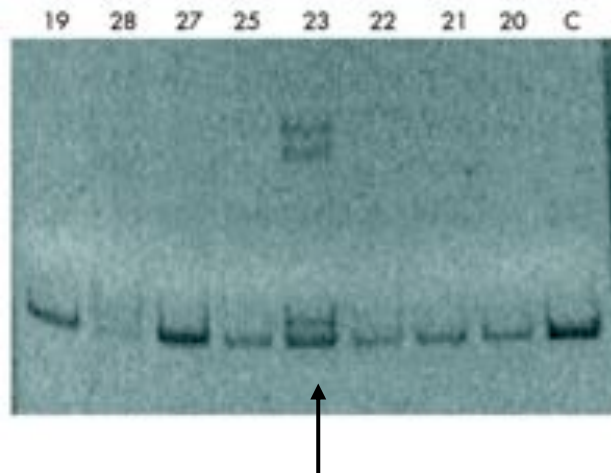- The patient had a typical expansion in PHOX2B

Fig. 1. Heterozygous mutation of the *PHOX2B* gene in the congenital central hypoventilation syndrome. Mutated alleleshave a greater number of alanine repeats (GCN) than the normal allele.

Jae-Ho Lee and Dae-Kwang Kim
J Genet Med 2014;11:11-15

Classical PCR test reveals polyalanine expansions. Matera et al. J Med Genet 2004;41:373–380.

Due to GC-richness of the expanded allele, there is a drop-out of the pathogenic variant from the NGS data.

Mutational mechanisms poorly or not detectable by panel/exome approaches

Repeat expansion disorders

ALS (C9orf72)
Frontotemporal dementia (C9orf72)
Multicystic kidney disease (MUC1)
Myoclonic epilepsy (SAMD12, TNRC6A and RAPGEF2)
CANVAS syndrome (RFC1)
Mental retardation (DIP2B)
Congenital central hypoventilation (PHOX2B)
Corneal dystrophy (TCF4)

And several other, including the classical SCA, FMR1, FXN, DM2, HTT…

Non-coding regions

Retinitis pigmentosa in boys (RPGR)
Hearing loss (GJB2 non-coding 1st exon)
Corneal dystrophy (GRHL2 and OVOL2)
Brain calcification (UFM1)
…several others

Small CNVs

Neurodegeneration (LMNB1 duplication)
Parkinson disease (PARK2 exonic deletions)
Skeletal dysplasia (SHOX upstream deletion)

Imprinting disorders

Silver Russel syndrome (H19/IGF2)
Transient Neonatal Diabetes Mellitus (PLAGL1)
Bechwith-Widemann syndrome(IH19/IGF2)
Kagami-Ogata syndrome (MEG3)
Angelman syndrome (UBE3A)

Although these genes are captured in panel/exome, you are missing the (sometimes the most common) pathogenic variants in those genes

Considerations in selection of the NGS approach

Am I sequencing the region of interest?

Can I trust the results in my region of interest?

Can I detect the mutations associated with the referral condition?

How much can I trust the referral diagnosis?

Case 4: Suspected hereditary motor neuropathy

A 69-year old male was referred for genetic testing with suspicion of hereditary motor neuropathy due to progressive lower limb weakness. EMG showed neuropathic changes, consistent with axonal neuropathy
He also has cataract and dilated cardiomyopathy

Q9 - What diagnostic approach will you choose

1. PMP22 duplication testing
2. Panel sequencing for neuropathies
3. Whole exome sequencing
4. No genetic testing, considering the patients age and no familial anamnesis of neuropathy

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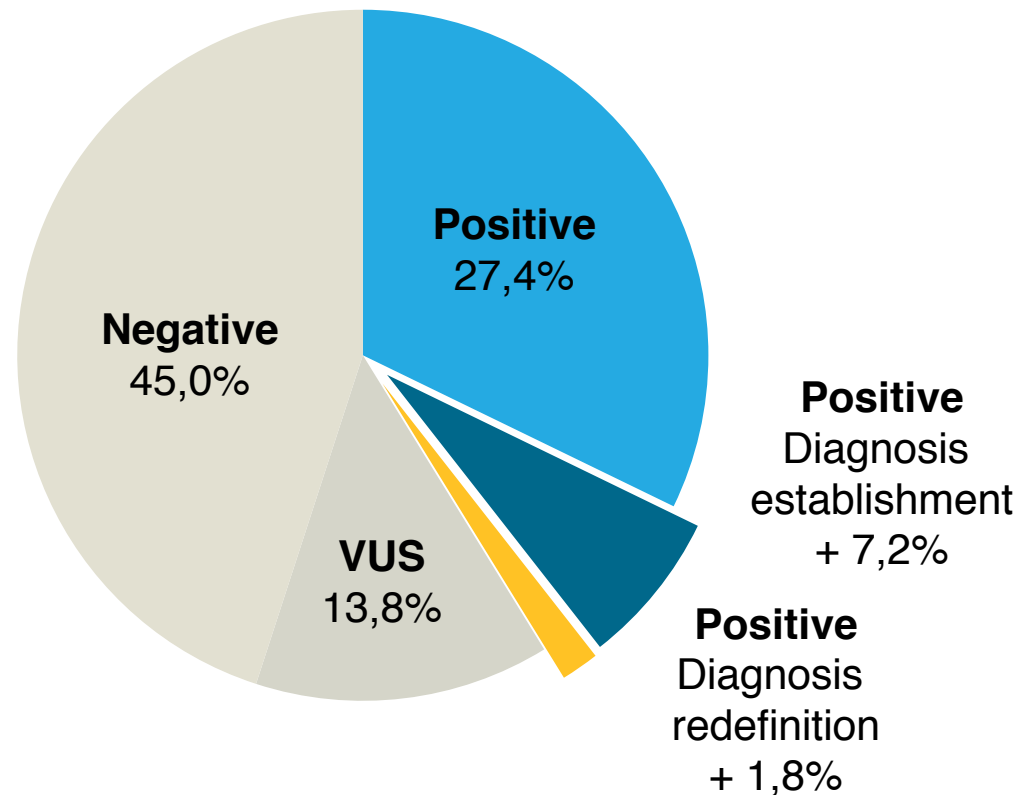| https://	directpoll.com/r7XDbzPBd3ixYqg8C5fs7H0DQfOB4WMbze4dJjuY1a8

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Case 4: Suspected hereditary motor neuropathy

Exome sequencing result
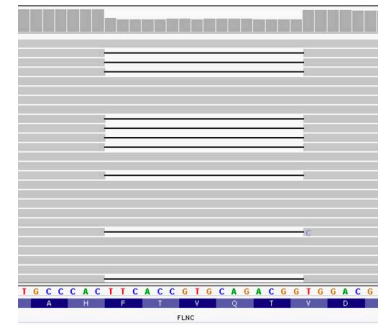
A likely pathogenic heteroyzgous
FLNC variant in the proband
NM_001458.4:c.892del, p.Phe298fs

Pathogenic variants in this gene
associated with:
**Distal and myofibrillar myopathy
Hypertrophic and restrictive
cardiomyopathy**

Q10 - What is your interpretation of this variant?

1. It is not related to neuropathy, thus it is likely **unrelated to original referral**

2. It is not related to neuropathy, but **could be related to dilated cardiomyopathy**

3. This variant is related to both – **the muscle weakness and cardiomyopathy**

4. This variant is related to both – **the muscle weakness and cardiomyopathy**, and is related to the cardiac disease in the patient's son

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Note: Many popular websites allow secure access. Please click on the preview button to ensure the web page is accessible.
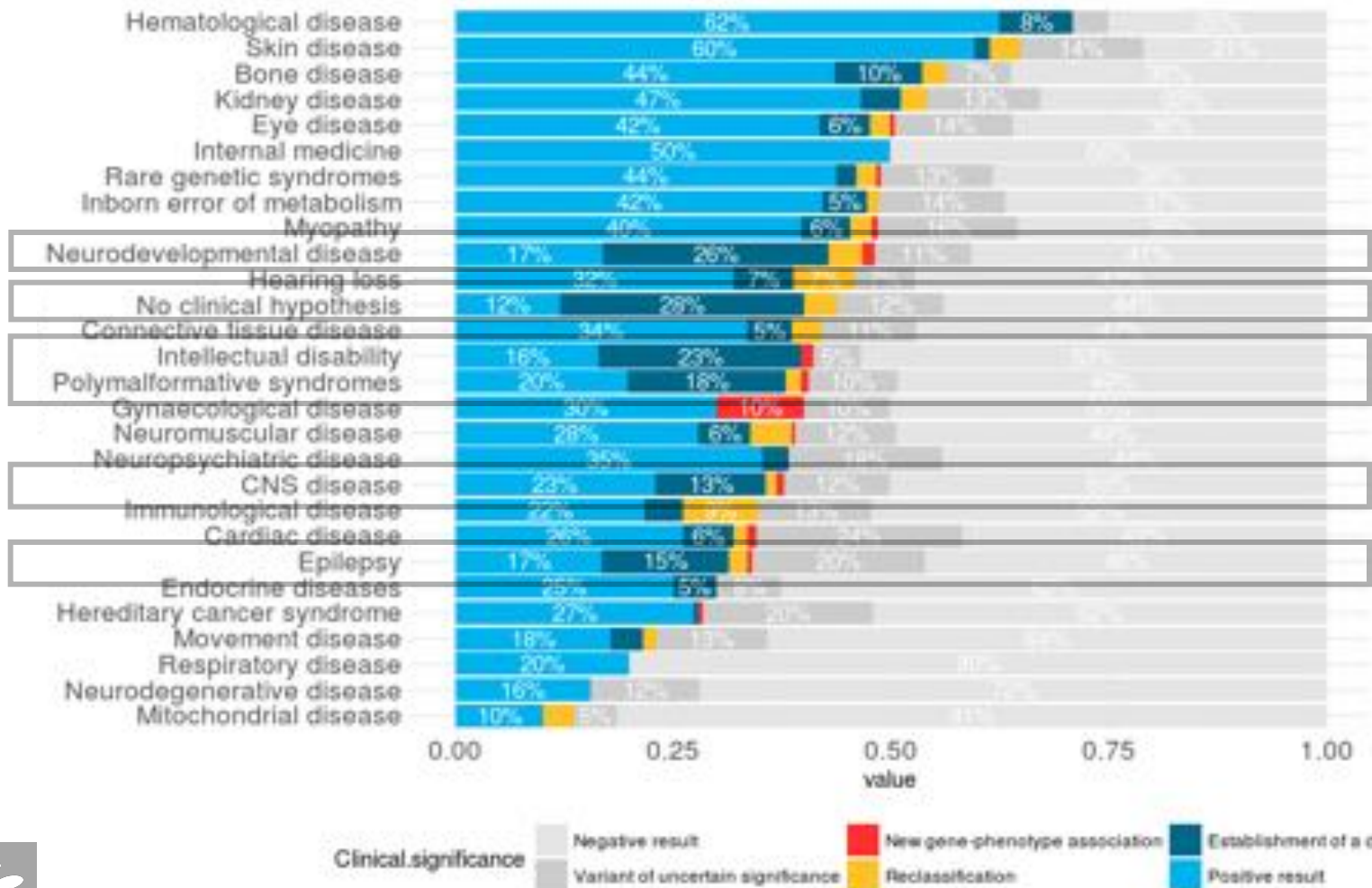
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In comparison to panels, exome and/or genome sequencing are robust in poorly defined and incorrect diagnostic hypotheses

Years 2012-2019, 3300 exomes, 2820 probands

Positive
27,4%

Negative
45,0%

VUS
13,8%

Positive
Diagnosis
establishment
+ 7,2%

Positive
Diagnosis
redefinition
+ 1,8%

Diagnostic yield varies across disease categories

N=2820 families

Patient and child with hyperammonemia

Hyperammonemia
Suspected ornithine
transcarbamoilase deficiency (OTC)
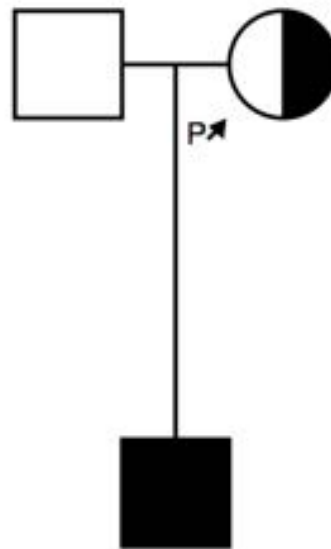
Negative targeted gene sequencing, MLPA
Negative exome sequencing

Q11 – Which approach will you choose next

1. Sanger sequencing of intronic and regulatory regions in the OTC gene

2. RNA analysis in peripheral blood

3. Whole genome methylation analysis

4. Whole genome sequencing

5. Perform the exome sequencing in the affected child

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https:// directpoll.com/r7XDbzPBd3ixYqg8C5fs7H0DQfOB4WMbze4dJjuY1a8

Note: Many popular websites allow secure access. Please click on the preview button to ensure the web page is accessible.
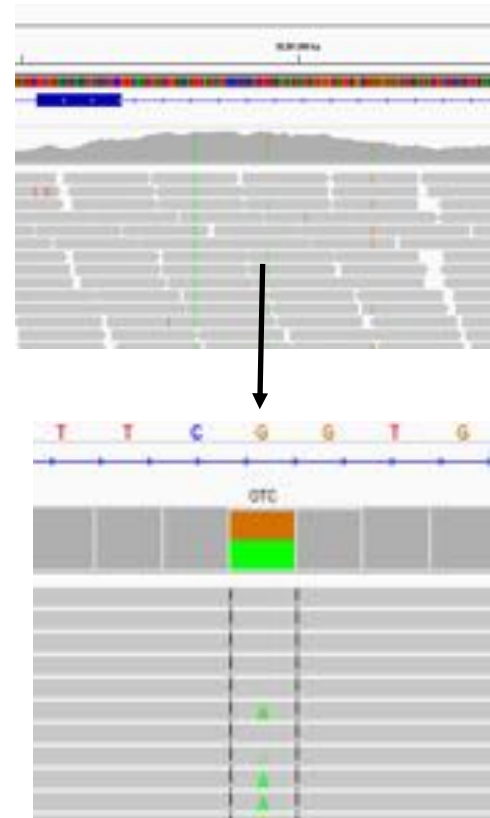
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Patient and child with hyperammonemia

**Hyperammonemia
Suspected ornithine
transcarbamoilase deficiency (OTC)**

**Pathogenic deep intronic
variant in OTC gene**

**Negative targeted gene testing,
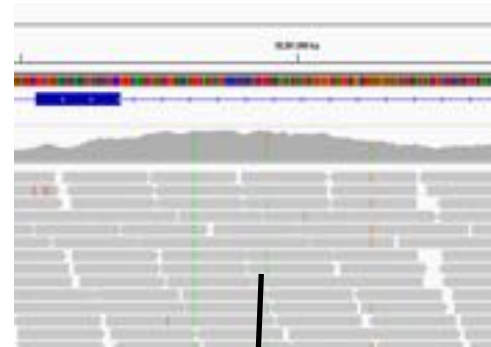Negative exome sequencing**

NM_000531.5:c.540+265G>A

Patient and child with hyperammonemia

**Hyperammonemia
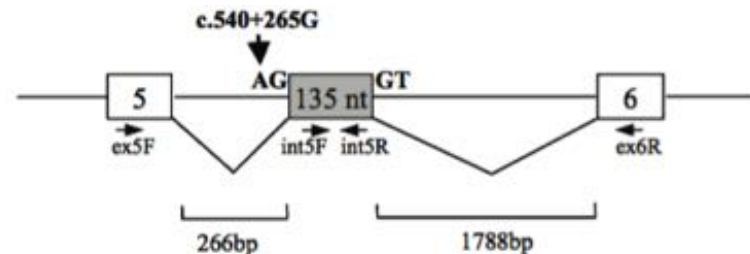Suspected ornithine
transcarbamoilase deficiency (OTC)**
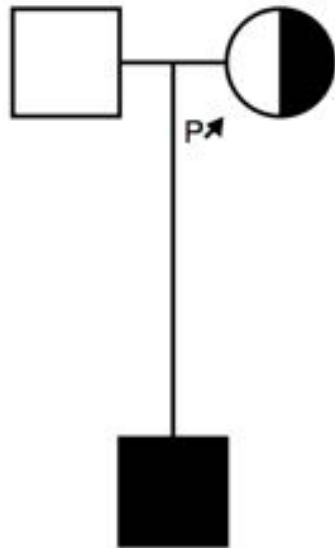
**Pathogenic deep intronic
variant in OTC gene**

**Activation of a cryptic
splice site in intron 5**

**Negative targeted gene testing,
Negative exome sequencing**

c.540+265G

Ogino et al. 2007

Regulatory regions | Intronic regions | Regulatory regions

Small CNVs

Complex rearrangements
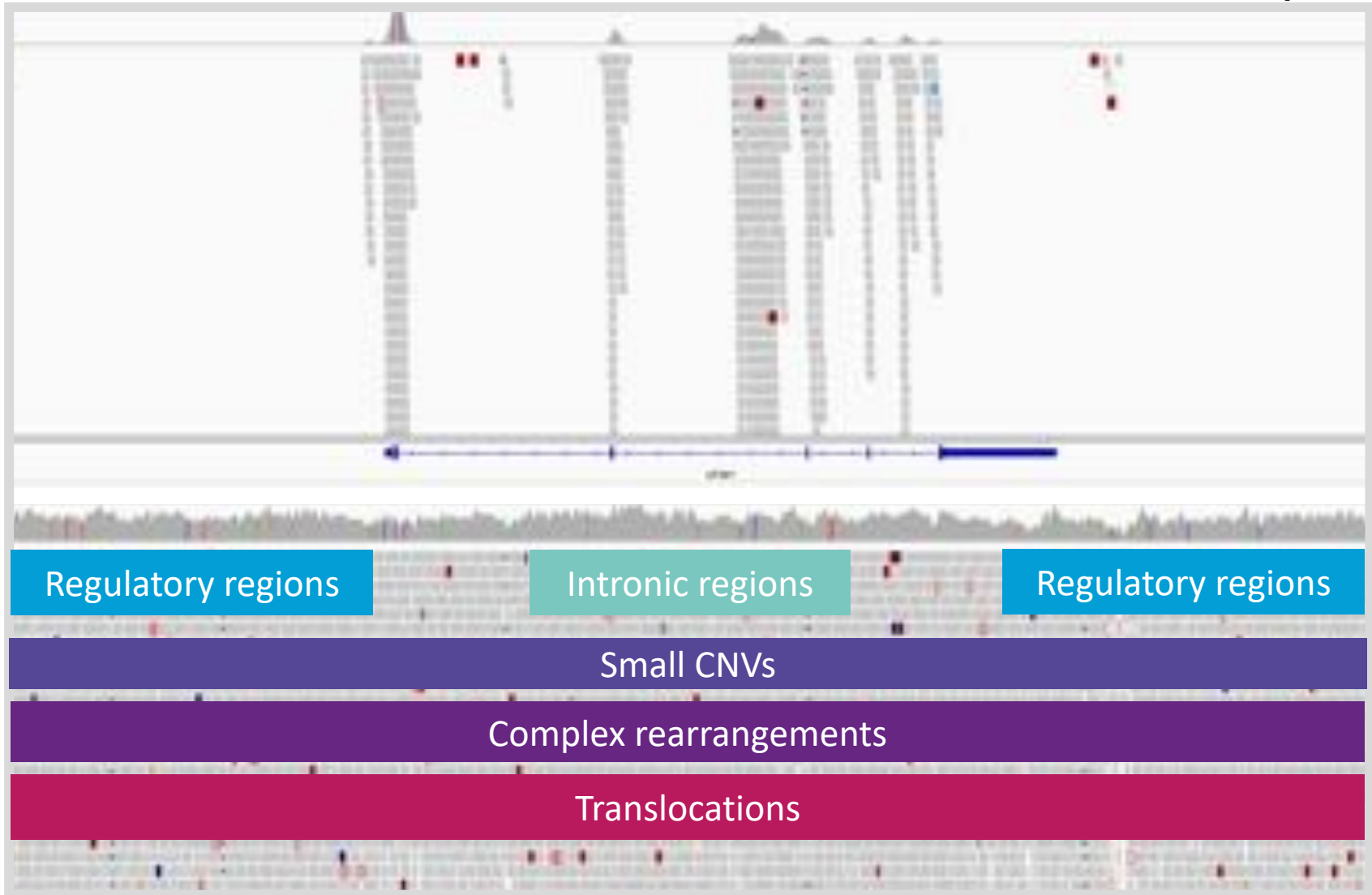
Translocations

Whole genome sequencing

Intronic regions | Regulatory regions | Translocations | Small CNVs | Complex rearrangements

Regulatory regions | Intronic regions | Regulatory regions

Small CNVs

Complex rearrangements

Translocations
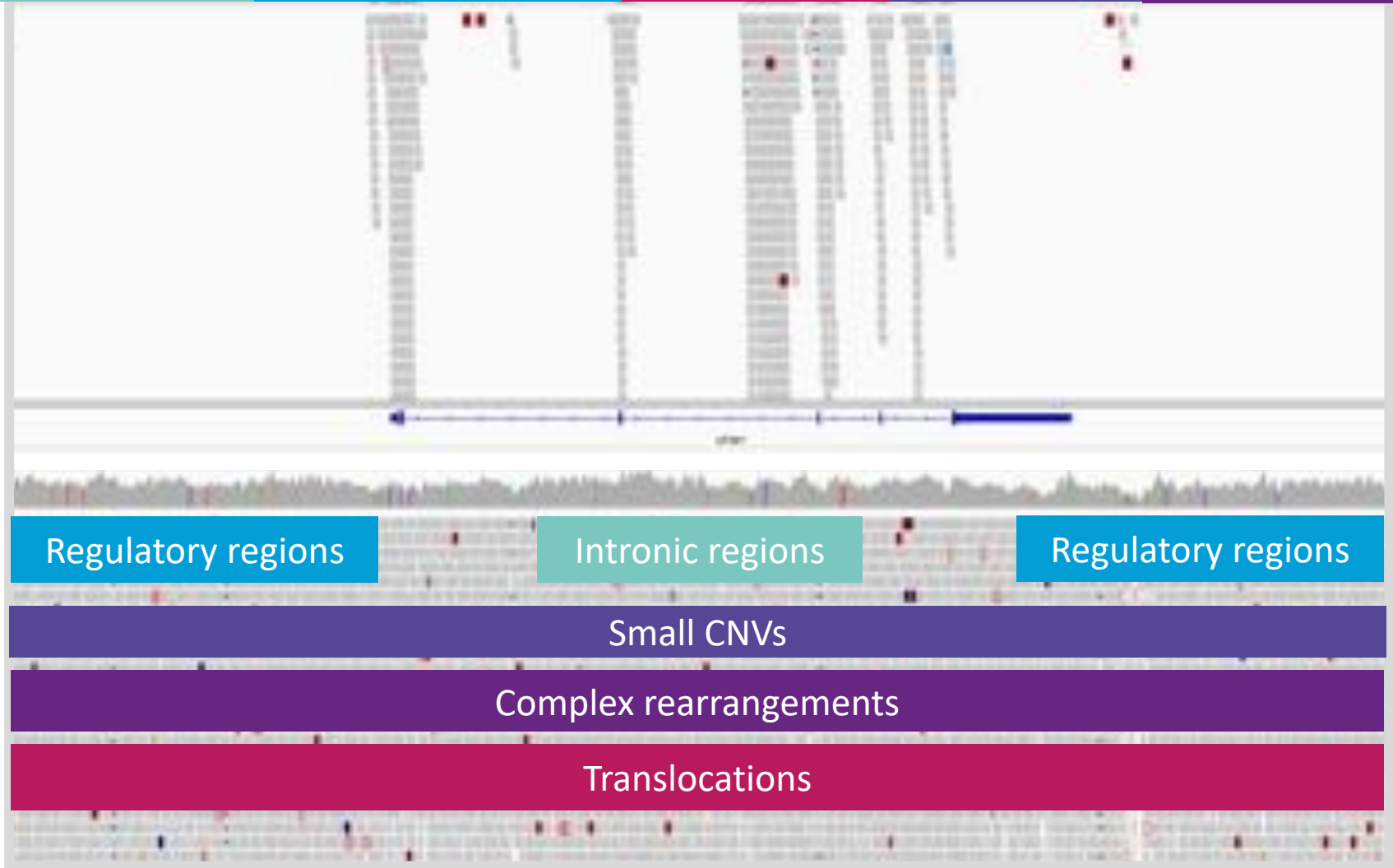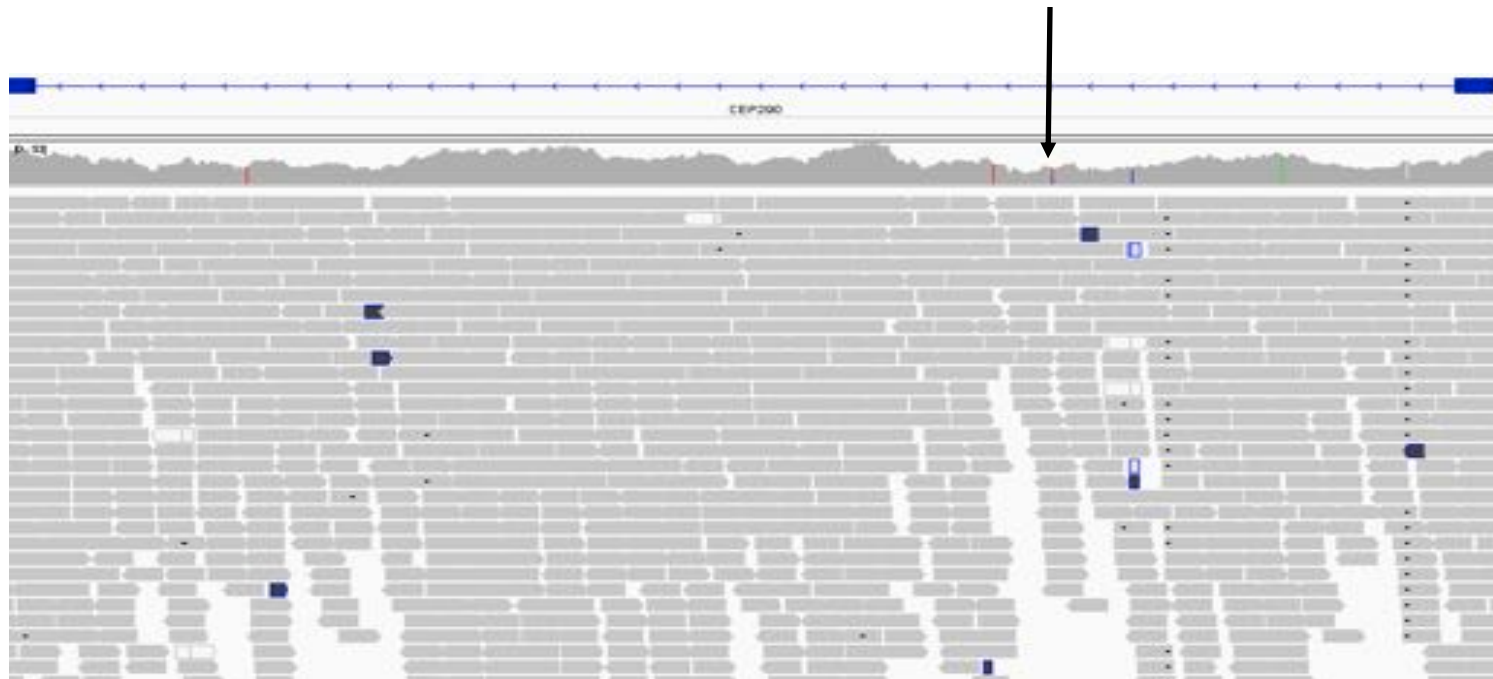
Whole genome sequencing

Patient with suspected Lebers optic amaurosis

Clinical exome sequencing reveals presence of a single pathogenic CEP290 variant

Patient with suspected Lebers optic amaurosis

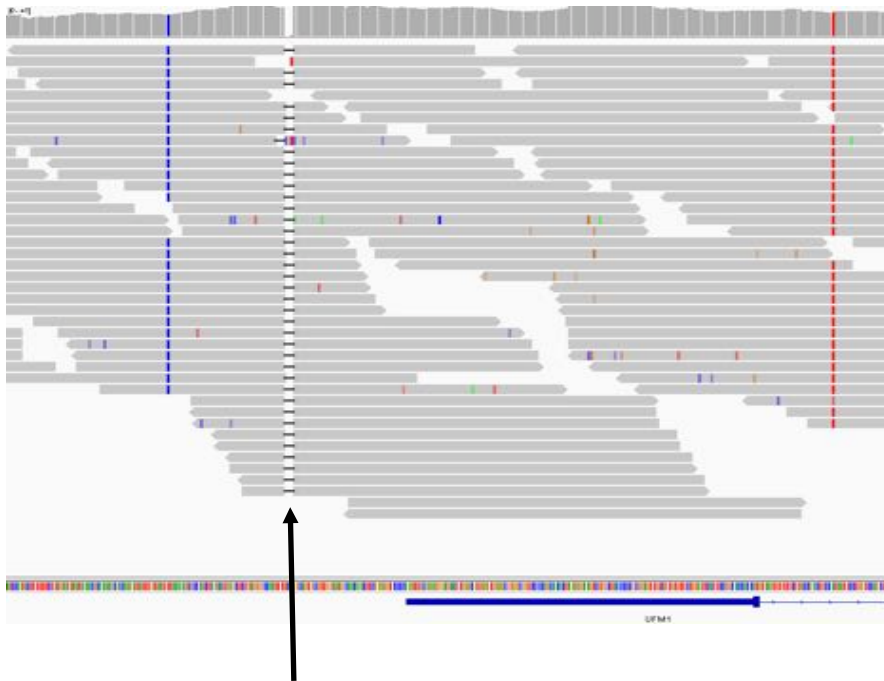**The second - deep intronic CEP290 variant**

CEP290:c.2991+1655A>G

The most common pathogenic CEP290 variant

Undiagnosed neurodegenerative disease in multiple Roma patients in Slovenia
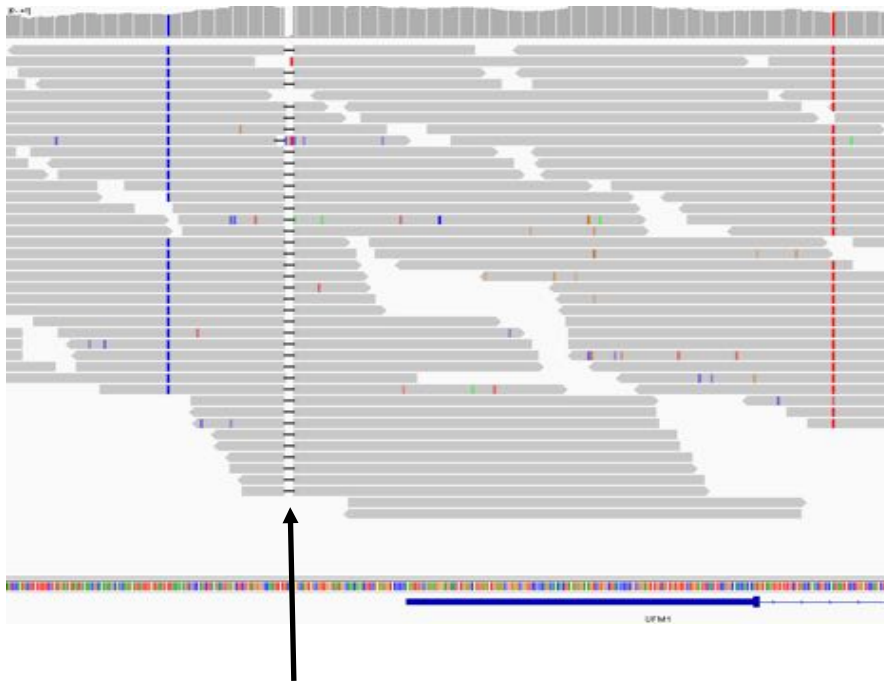
Več Multiple patients with severe developmental delay, neurodegeneration, basal ganglia calcinations in block of homozygositz on chromosome 13

3-base pait deletion in UFM1 gene promotor
UFM1:c.-273_-271delTCA

Undiagnosed neurodegenerative disease in multiple Roma patients in Slovenia

Več Multiple patients with severe developmental delay, neurodegeneration, basal ganglia calcinations in block of homozygosity on chromosome 13
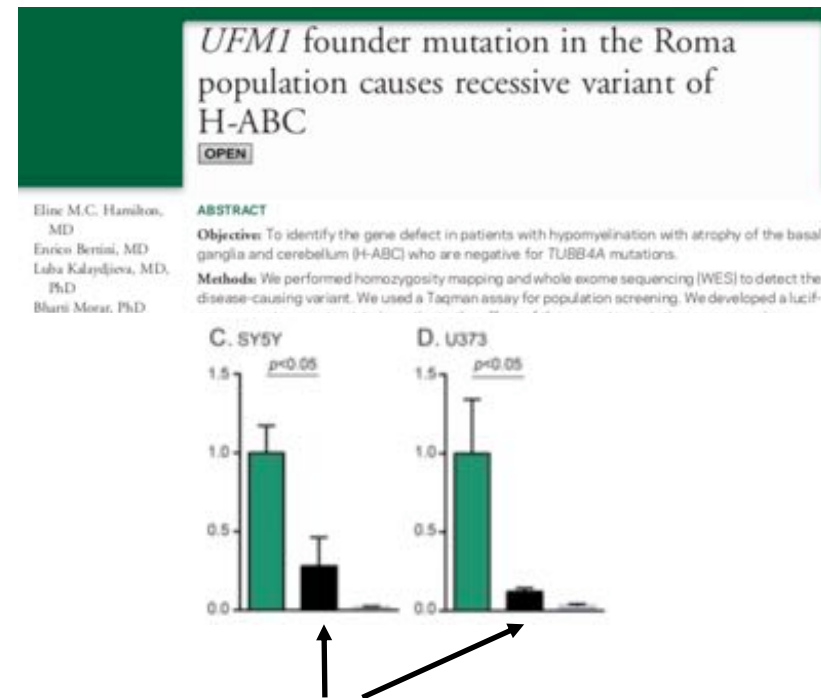

UFM1 founder mutation in the Roma population causes recessive variant of H-ABC

OPEN

Eline M.C. Hamilton, MD
Enrico Berini, MD
Luba Kalaydjieva, MD, PhD
Bharti Morar, PhD

ABSTRACT

Objective: To identify the gene defect in patients with hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) who are negative for TUBB4A mutations.

Methods: We performed homozygosity mapping and whole exome sequencing (WES) to detect the disease-causing variant. We used a Taqman assay for population screening. We developed a lucif-

3-base pait deletion in UFM1 gene promotor
UFM1:c.-273_-271delTCA

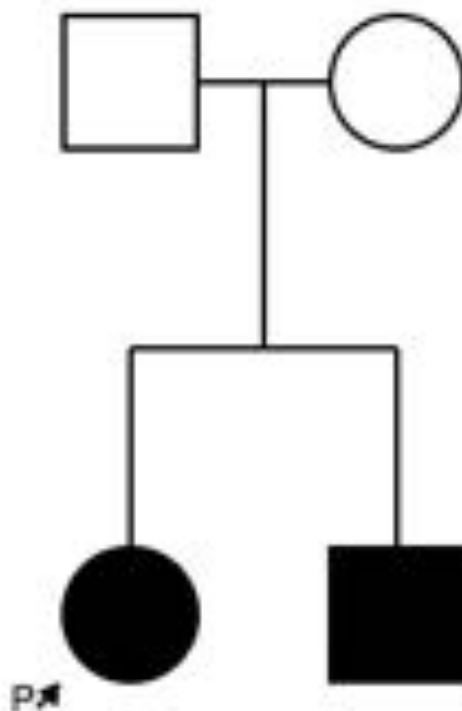The mutation reduces the UFM1 gene expression in CNS

Hamilton 2017, Neurology.

87

Translocation in a child with developmental delay, stereotypies and epilepsy
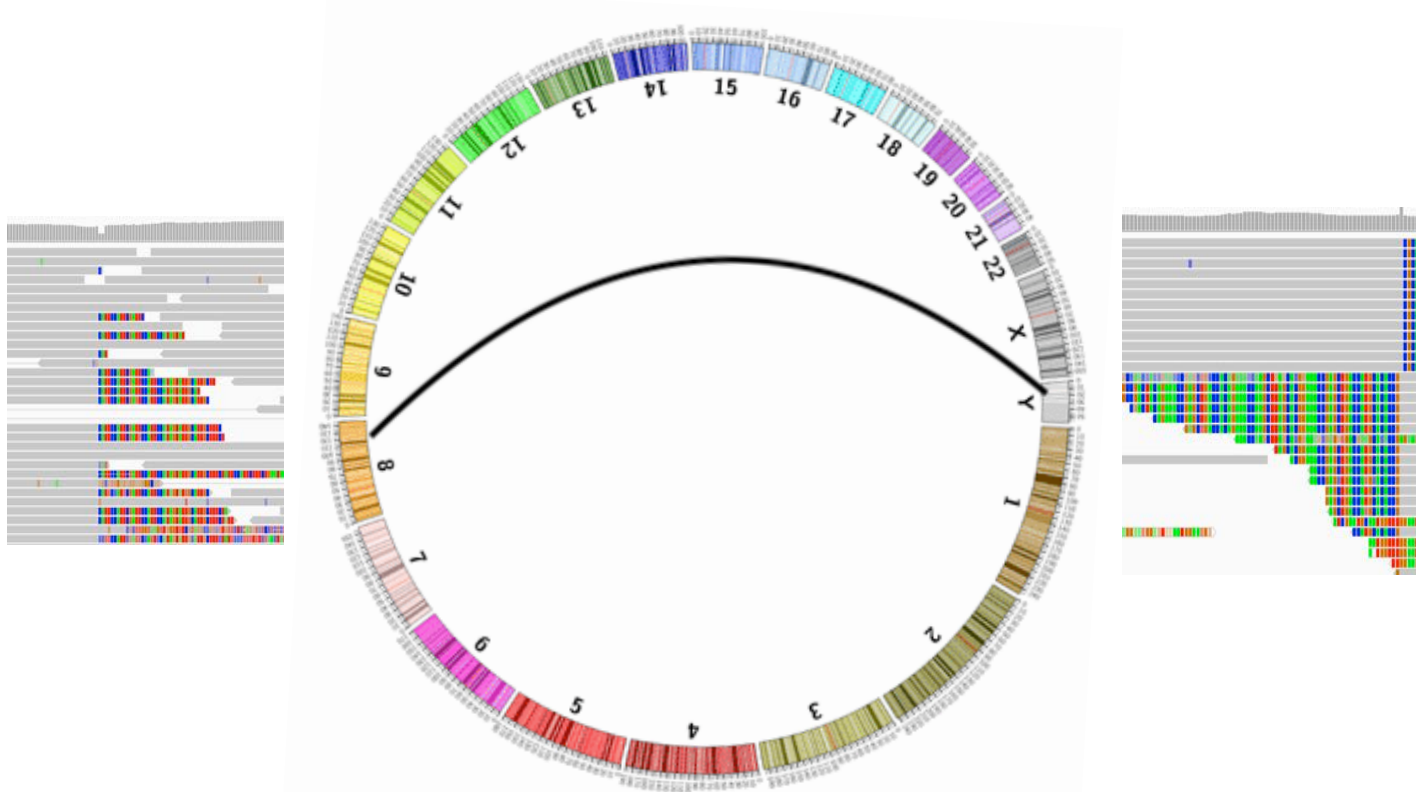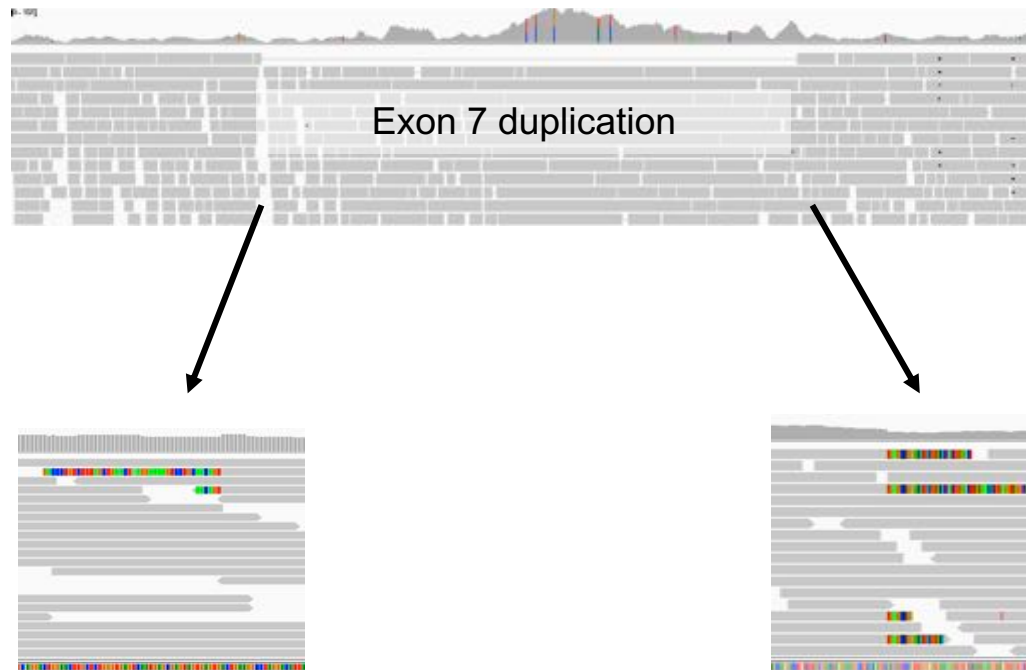

Disruption of a long non-coding RNA on chromosome 8, where structural rearrangements were reported previously in patients with autism (Pinto et al. 2010, Nature)

Cone-rod dystrophy in two sibs

Both affected sibs carry a single CNGB3 pathogenic variant
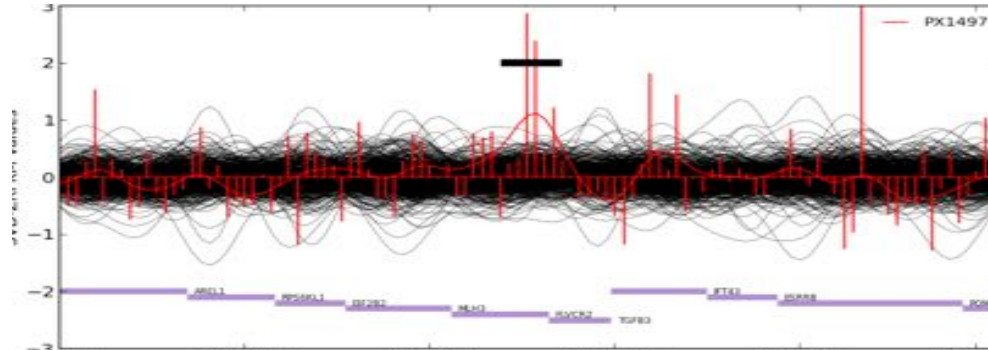NM_019098.4(CNGB3):c.819_826delCAGACTCC

CNGB3

Exon 7 duplication

Family with recurrent fetal hydrops, ventriculomegaly and contractures

Family with recurrent fetal hydrops, ventriculomegaly and contractures

CNV analysis of exome data shows a small duplication at the edge of FLVCR2


FLVCR2 gene is associated with recessive Fowler syndrome,
which includes fetal hydrops, ventriculomegaly and contractures

Family with recurrent fetal hydrops, ventriculomegaly and contractures

The read-level data shows an intragenic deletion in FLVCR2 gene that appears to be heterozygous

Homozygosity analysis of exome shows a loss of heterozygosity in the region of FLVCR2 gene
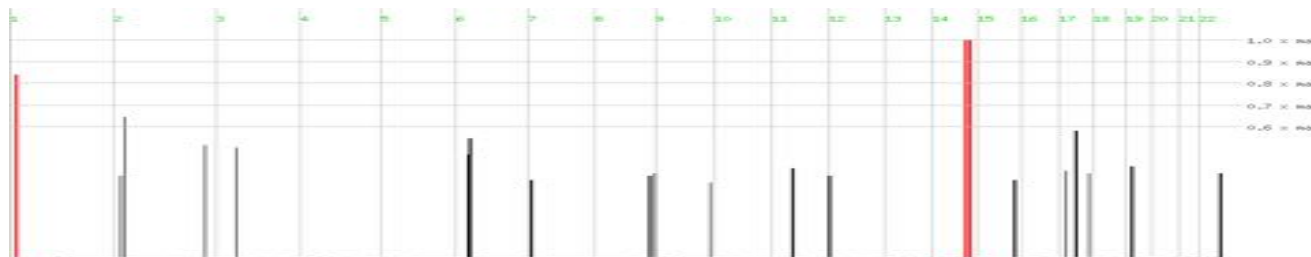
Q12 – The variant in proband is a:

1. Heterozygous duplication

2. Heterozygous deletion

3. Homoyzgous duplication

4. Compound heterozygous duplication on one allele and a deletion on the other

5. Homoyzgous inverted duplication with a partial loss of duplicated sequence

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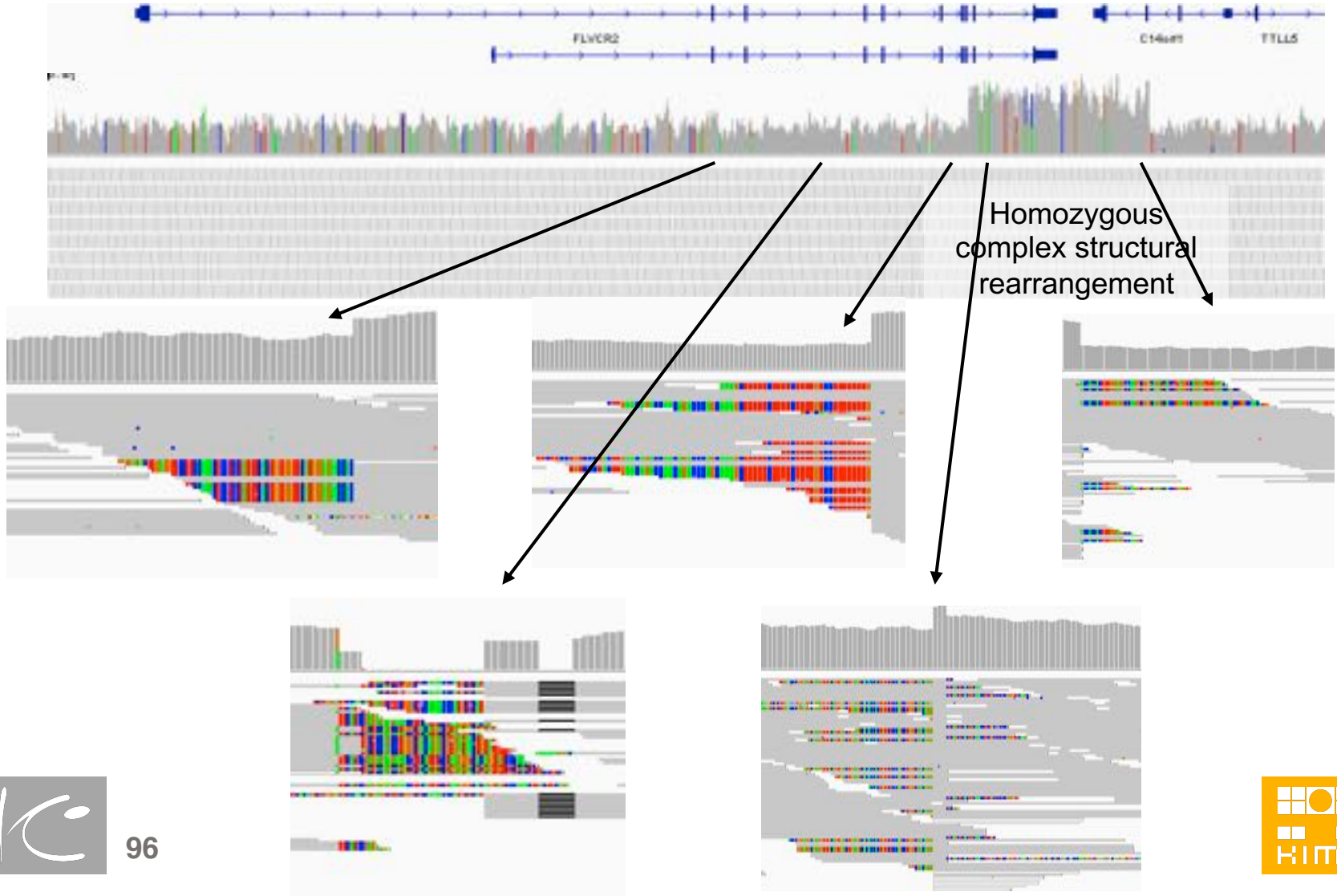
Please enter the URL below.

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Whole genome sequencing

Homozygous complex structural rearrangement

Take home messages

- Selection of a NGS diagnostic approach should be an informed decision based on the knowledge of the genetic spectrum of the condition

- Always check the coverage/mapping and mutational spectrum in the region of interest in advance

- Focused approaches (panels) my give sufficient coverage, but have limited utility in poorly defined (or wrong) diagnosis

- Broad approaches (WES/WGS) are powerful, but may miss critical variants in unnoticed dark/camouflaged regions

Seeing the trees but not seeing the forest

Seeing the forest but not seeing the trees

Thank you!

Prof. Borut Peterlin

CMG team

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Gaber Bergant
Barbara Golob
Tanja Višnjar
Ivana Babič Božović

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Nuša Trošt

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