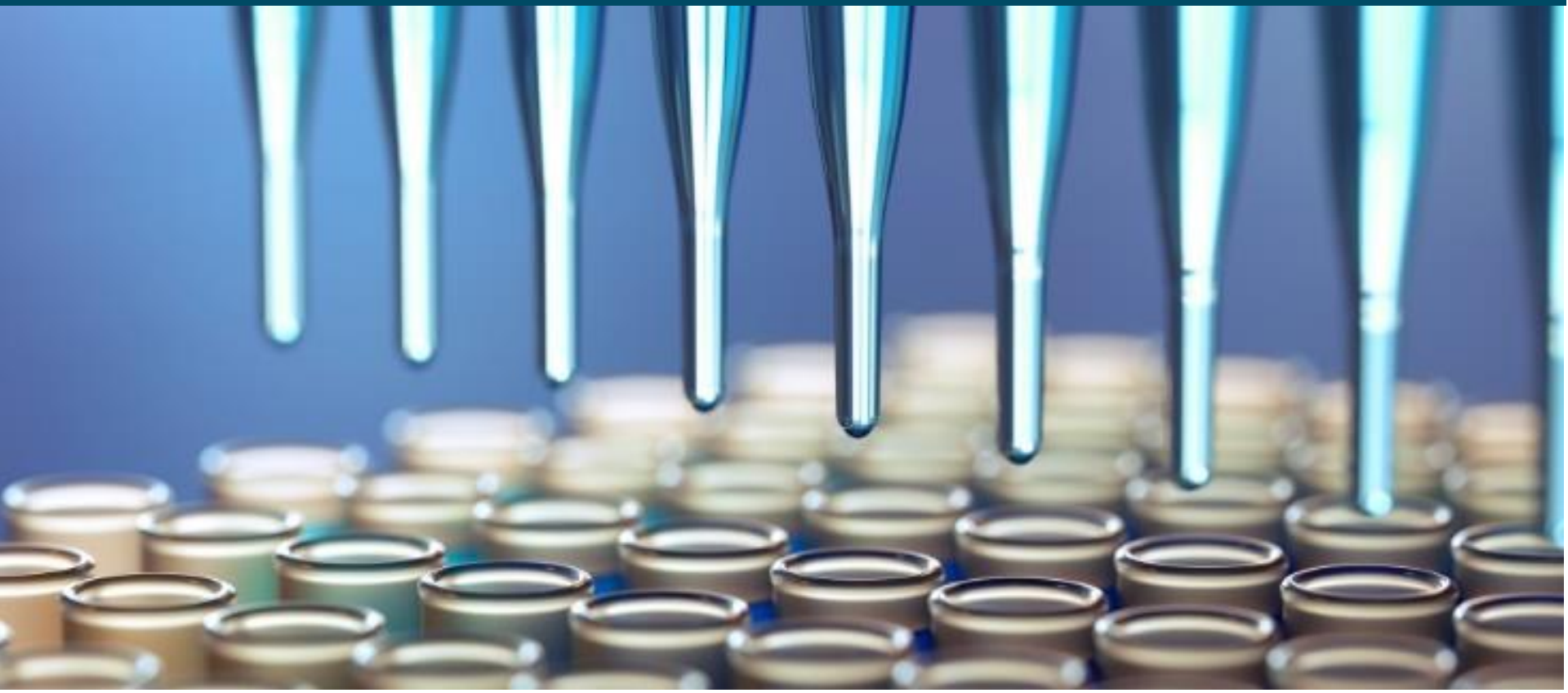
DISEASE EXOME, A POWERFUL DIAGNOSTIC TOOL: *POST-MORTEM* DIAGNOSIS OF DYSKERATOSIS CONGENITA

Pinto-Basto J, Melo F, Almeida LS, Garrido A, Barbosa C, Pereira SM, Cerqueira R, Tavares P



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DYSKERATOSIS CONGENITA, DKC

> BACKGROUND

1. **Rare ectodermal dysplasia** that often presents with the classic triad of:
    - Nail dysplasia,
    - Skin pigmentation changes
    - Oral leukoplakia associated with a high risk of bone marrow failure (BMF) and cancer.

2. **Onset and progression of manifestations of DKC varies:**

3. **Diagnosis:**
    - Telomere length – cytogenetics analysis (ex: FISH)
    - **Molecular Diagnosis -** mutations known to cause DKC in:

CTC1 ***DKC1*** ***TERC*** ***TERT*** ***TINF2*** ***NHP2*** ***NOP10*** ***WRAP53***

There are several variants of the disorder, each associated with a different pattern of inheritance

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CASE REPORT

> OVERVIEW

PATIENT:

> Deceased 60 year-old male

> Undiagnosed systemic disease (age at onset - 50 years):

- liver cirrhosis
- pulmonary fibrosis
- sick sinus syndrome
- thrombocytopenia

> Multiple tests had been previously performed with **no** diagnosis.

DNA from *post-mortem* tissue was obtained and *DISEASE EXOME* performed.

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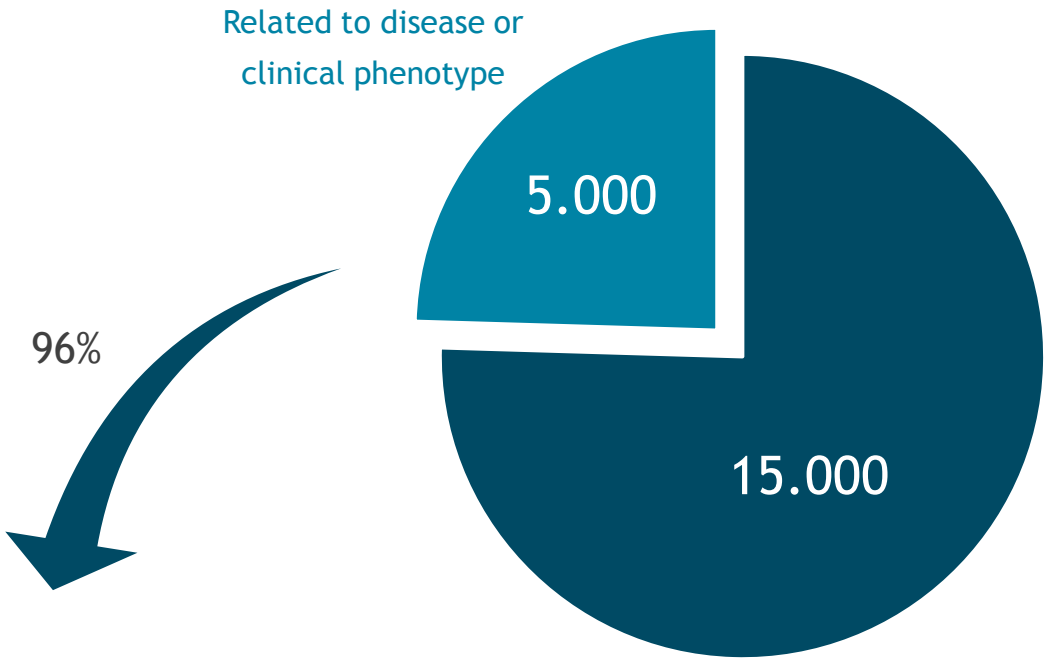
DISEASE EXOME

> DISEASE EXOME BY CGC GENETICS

- Uncertain or complex phenotype
- Unclear diagnosis



DISEASE EXOME panel is based on:

1. Human Gene Mutation Database (HGMD) (www.hgmd.cf.ac.uk/ac/index.php)
2. Online Mendelian Inheritance in Man (OMIM) (www.omim.org)
3. GeneTests (www.genetests.org)
4. Illumina TruSight Sequencing Panels (www.illumina.com/trusight)
5. Other commercially available sequencing panels

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METHODOLOGY PROCESS

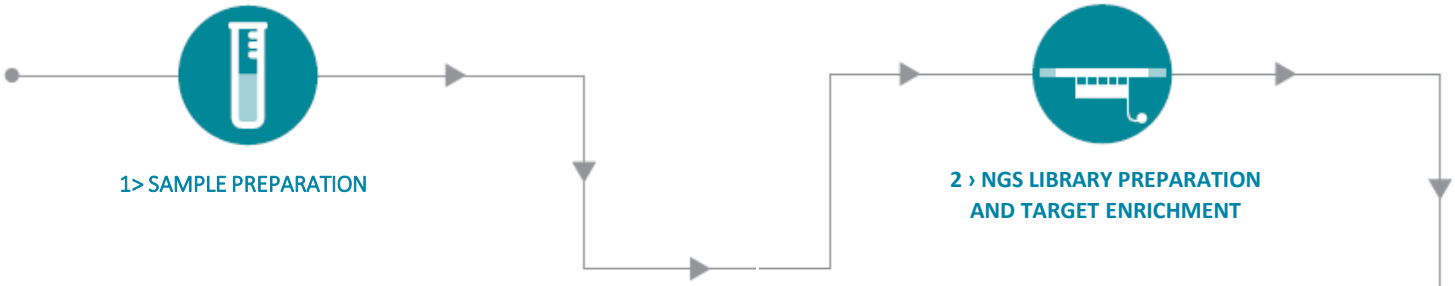
> NEXT GENERATION SEQUENCING (NGS)

1> SAMPLE PREPARATION

post-mortem collection
Skin
Skeletal muscle
Hepatic tissue
Pulmonary tissue
Pancreatic tissue

METHODOLOGY PROCESS

> NEXT GENERATION SEQUENCING (NGS)

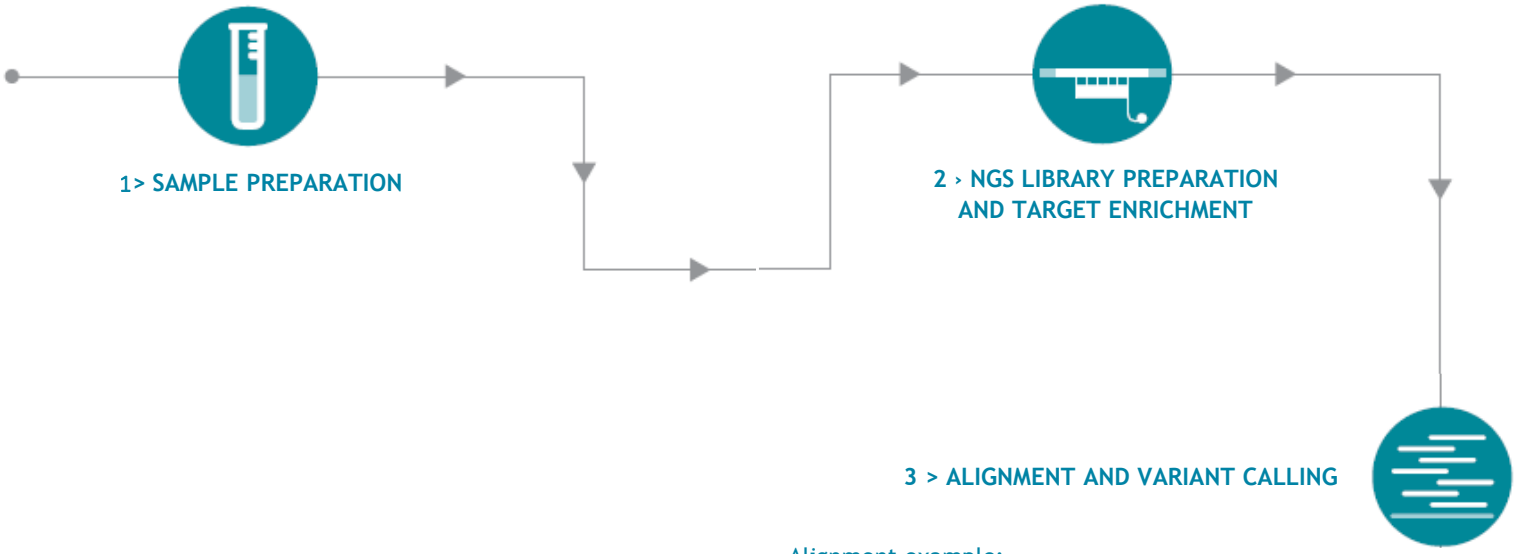


> Capture of target regions using oligonucleotide probes
TruSightOne, Illumina

> NGS of a panel composed by 4,813 clinically-relevant genes (Disease Exome)
MiSeq, Illumina

METHODOLOGY PROCESS

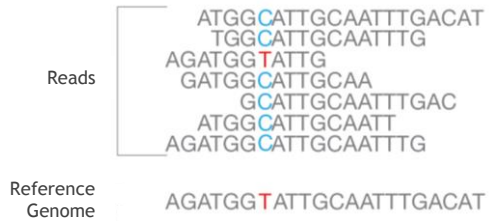
> NEXT GENERATION SEQUENCING (NGS)



> Alignment and variant calling
BWA and GATK

> Variants with MAF<1%
Bioinformatic analysis tools

Alignment example:



METHODOLOGY PROCESS

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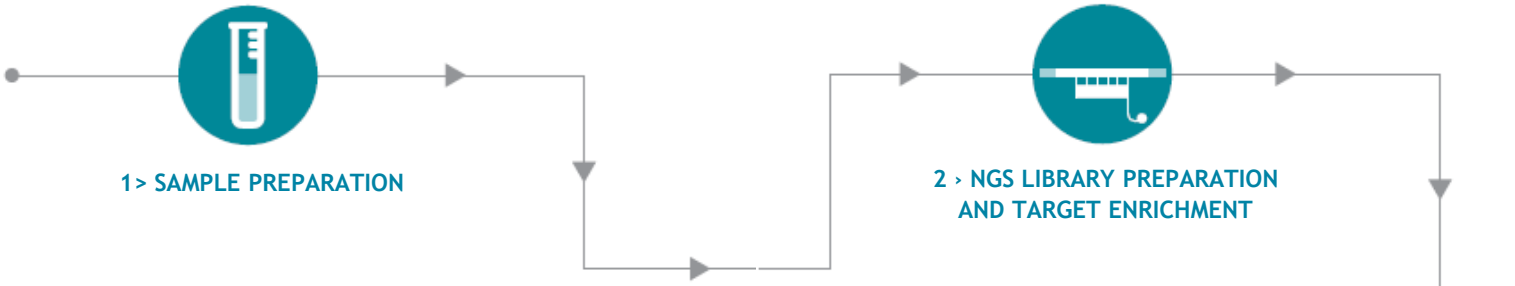

1> SAMPLE PREPARATION

2 › NGS LIBRARY PREPARATION AND TARGET ENRICHMENT

3> ALIGNMENT AND VARIANT CALLING

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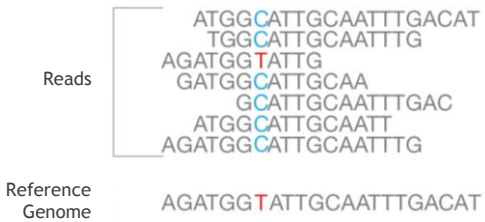

4 > DATA ANALYSIS

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METHODOLOGY PROCESS

> NEXT GENERATION SEQUENCING (NGS)

1> SAMPLE PREPARATION

2 › NGS LIBRARY PREPARATION AND TARGET ENRICHMENT

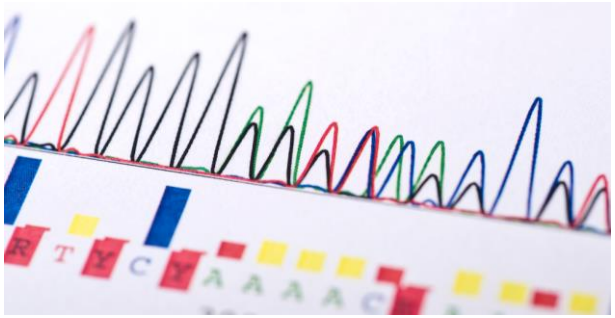
3 > ALIGNMENT AND VARIANT CALLING

4 > DATA ANALYSIS

5 › CONFIRMING VARIATIONS

Pathogenic and likely pathogenic variants

Confirmation with SANGER SEQUENCING



METHODOLOGY PROCESS

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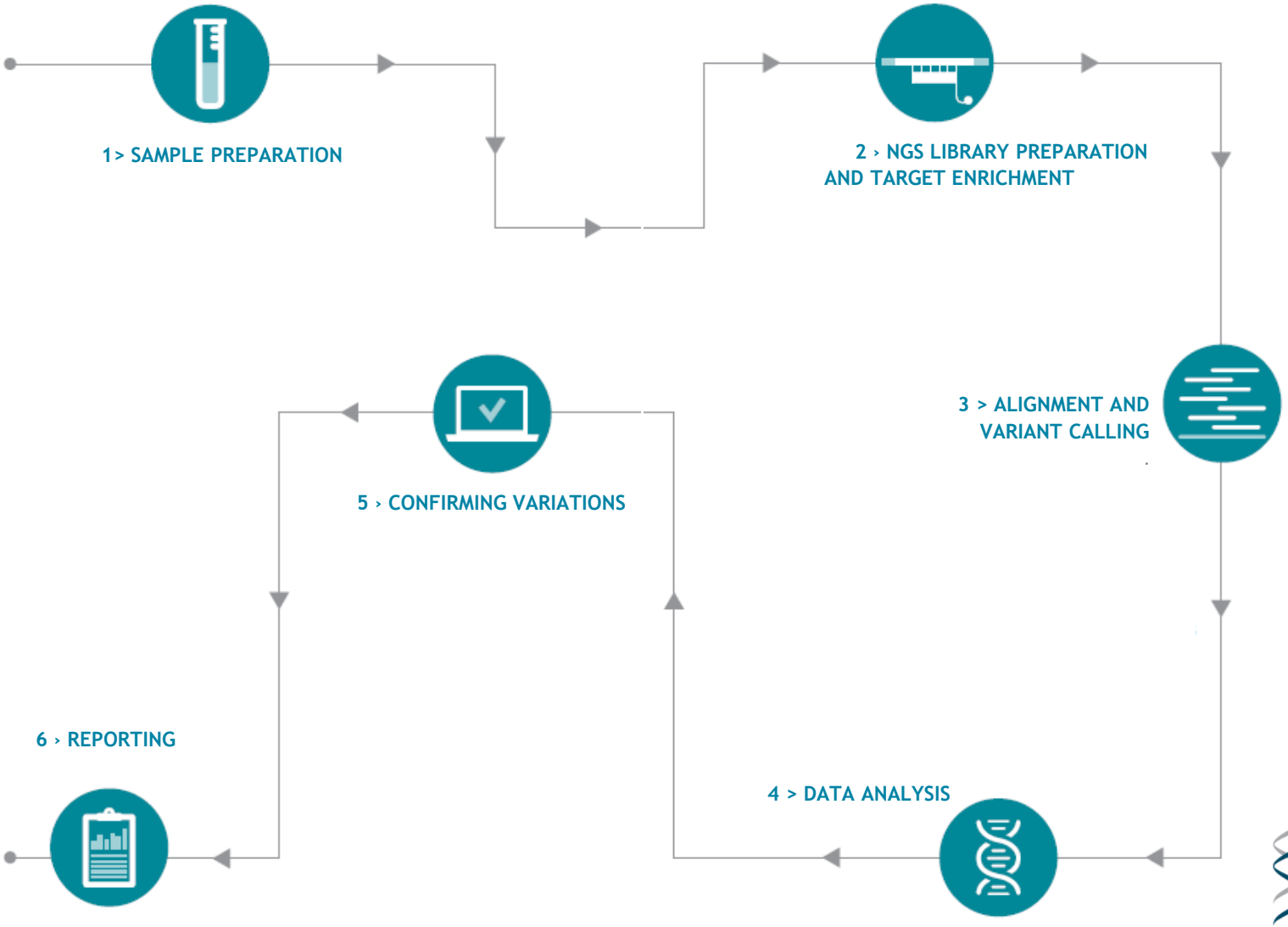

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GENETIC ANALYSIS

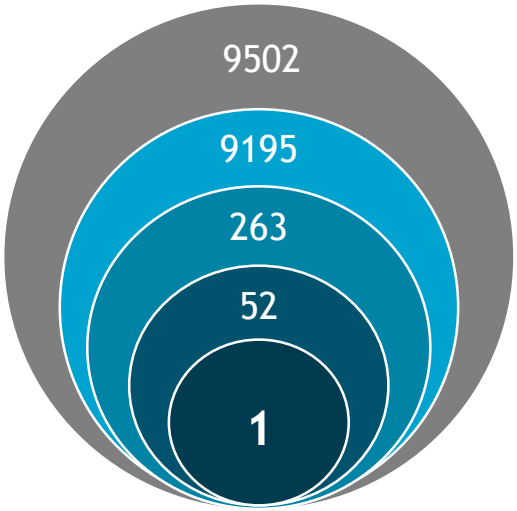
> PRIORITIZATION OF DISEASE EXOME DATA

Exclusion of:

- low quality reads
- non-genic, intronic and synonymous variants
- variants in in-house variant database

Inclusion of:

- Variants with less than 1%
- Variants in exonic, splice-site and canonical splice site regions



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GENETIC ANALYSIS

> DISEASE EXOME BY CGC GENETICS

Heterozygous variant in ***TERT*** gene

NM_198253.2: c.1492G>A (p.Gly498Arg)

> Variant not yet described in the literature

Not detected: dbSNP, ExAC, 1KGenomes, ESP

> Affects highly conserved residue

> Bioinformatic analysis - likely pathogenic variant

- **PoplyPhen-2:** probably damaging
- **SIFT:** deleterious

TERT

> **Autosomal Dominant *Dyskeratosis Congenita***
(type 2; MIM 613989)

> Autosomal Recessive Dyskeratosis Congenita
(type 4; MIM 613989)

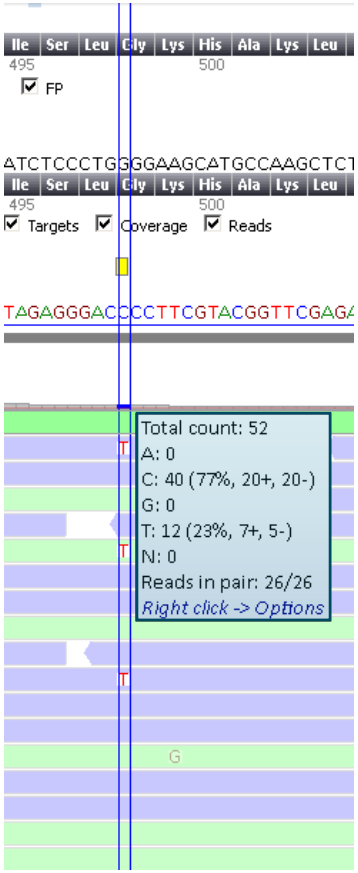
GENETIC ANALYSIS

> TERT - Telomerase reverse transcriptase (Chr 5)

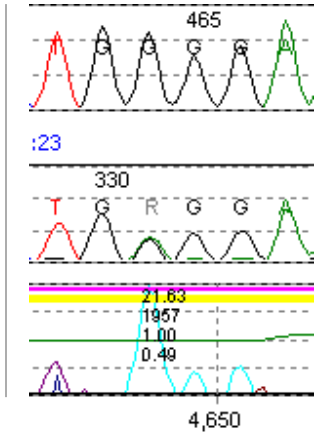
NM_198253.2:
c.1492G>A (p.Gly498Arg)

> NGS

BAM Alignment



> SANGER CONFIRMATION



Control

Subject

CONCLUSION

- ***post-mortem* diagnosis of autosomal dominant DKC type-2**
- **Novel variant in *TERT* gene**, expanding the mutational spectrum of *TERT*-related DKC
- Highlights the **importance of molecular diagnosis**, even *post-mortem*, as establishing the molecular etiology allows proper genetic counselling to at-risk relatives
- Clinical exome sequencing applied in **solving** complex diagnostic cases
  - **Directed to clinically relevant genes**
  - **High diagnostic yield**
  - **Clinical integration and interpretation**
  - **Lower cost and more useful comparatively to other exome testing**

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Thank you for your Attention!

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