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

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**DYSLERATOSIS 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:

   ![DCK spectrum diagram]

   - **Mild**: minimal physical findings with normal bone marrow function
   - **Severe**: diagnostic triad and early-onset BMF

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.
CASE REPORT

> OVERVIEW

PATIENT:

> Deceased 60 year-old male

> Undiagnosed systemic disease (age at onset - 50 years):
  
  o liver cirrhosis
  o pulmonary fibrosis
  o sick sinus syndrome
  o thrombocytopenia

> Multiple tests had been previously performed with no diagnosis.

DNA from *post-mortem* tissue was obtained and *DISEASE EXOME* performed.
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)
4. Illumina TruSight Sequencing Panels (www.illumina.com/trusight)
5. Other commercially available sequencing panels
METHODOLOGY PROCESS

> NEXT GENERATION SEQUENCING (NGS)

1> SAMPLE PREPARATION

<table>
<thead>
<tr>
<th>post-mortem collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
</tr>
<tr>
<td>Skeletal muscle</td>
</tr>
<tr>
<td>Hepatic tissue</td>
</tr>
<tr>
<td>Pulmonary tissue</td>
</tr>
<tr>
<td>Pancreatic tissue</td>
</tr>
</tbody>
</table>
**METHODOLOGY PROCESS**

> NEXT GENERATION SEQUENCING (NGS)

1. **SAMPLE PREPARATION**

2. **NGS LIBRARY PREPARATION AND TARGET ENRICHMENT**

   - 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)

1. SAMPLE PREPARATION

2. NGS LIBRARY PREPARATION AND TARGET ENRICHMENT

3. ALIGNMENT AND VARIANT CALLING

Alignment example:

- Alignment and variant calling
  BWA and GATK
- Variants with MAF<1%

Bioinformatic analysis tools

Reference Genome:
AGATGGTATTGCAATTTGACAT

Reads:
- ATGGCATTTGCAATTTGACAT
- TGCCATTGCAATTTG
- AGATGCTATTG
- GATGGCATTTGCA
- GATGGCATTTGAC
- ATGGCATTTGCAATT
- AGATGCTATTGCAATTTG
- AGATGGTATTGCAATTTGACAT
METHODOLOGY PROCESS

> NEXT GENERATION SEQUENCING (NGS)

1> SAMPLE PREPARATION

2> NGS LIBRARY PREPARATION AND TARGET ENRICHMENT

3> ALIGNMENT AND VARIANT CALLING

- Alignment and variant calling
  - BWA and GATK
- Variants with MAF<1%
  - Bioinformatic analysis tools

4> DATA ANALYSIS

Alignment example:

```
Reads
ATGCCATTGCAATTTGACAT
TGGCATTGCAATTTTG
AGATGGAATTG
GATGCCATTGCAA
GACATTGCAATTTGAC
ATGCCATTGCAATT
AGATGGAATTGCAATTTTG

Reference Genome
AGATGCCATTGCAATTTGACAT
```
SAMPLE PREPARATION

NGS LIBRARY PREPARATION AND TARGET ENRICHMENT

ALIGNMENT AND VARIANT CALLING

DATA ANALYSIS

CONFIRMING VARIATIONS

Pathogenic and likely pathogenic variants

Confirmation with SANGER SEQUENCING
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
6 > REPORTING
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
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

*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

> SANGER CONFIRMATION

BAM Alignment

Total count: 52
A: 0
C: 40 (77%, 20+, 20-)
G: 0
T: 12 (23%, 74, 5: )
NI: 0
Reads in pair: 26/26
Right click -> Options
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!