To Cascade or not to Cascade
Novel approaches to VUS classification in Familial Hypercholesterolemia

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The UKGTN FH testing laboratories
Aberdeen, Cardiff, GOSH, Sheffield, Liverpool, Belfast
Familial Hypercholesterolaemia

- Disorder of lipid metabolism.
- Monogenic (*LDLR, APOB, PCSK9, LDLRAP1*)
- Polygenic
- Estimated prevalence of 1/200-1/300 in the UK
- Elevated LDL-C; premature atherosclerosis ↑ risk of cardiac events
- Simon Broom Criteria: lipid levels, physical signs, family history

- Early detection and statin therapy prevents acute cardiac events

**Drivers for FH testing:**
Aug 2008: NICE Guidance CG71
Mar 2013: DoH CVD Outcomes Strategy – Key Target 5
2014/15 BHF Investment – FH Cascade programme

26.9 WTE nurses  13 centres
Population 21.3 million
Increased demand for both the **Diagnostic** and **Cascade** Service:

- >2300 diagnostic NGS panel tests
- 584 familial cases (522 cascade + 63 Segregations)

High throughput approach:
- Beckman Robot
- 96 patients
- Illumina NextSeq 500

Skill mix review

Template reports
**Exceptional healthcare, personally delivered**

**Personalised medicine in the genomic era**

**FH NGS v3 assay redesign:**

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<th>Transcript</th>
<th>Inheritance</th>
<th>Exons</th>
<th>Region of interest</th>
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**Monogenic FH** (intronic regions & LDLR copy number)
- LDLR, APOB, PCSK9, LDLRAP1

**SLCO1B1** – predicts adverse drug reactions

**Polygenic SNP score** 12 LDL-C raising SNPs*
- Genotype used in combination to generate a polygenic SNP score

- **Deciles 1-3** – Least Likely Polygenic FH
- **Deciles 4-5** – Intermediate / grey area
- **Deciles 6-10** – Likely Polygenic FH

- Patients in decile 10: 4x more likely to have polygenic FH (LDL-C >4.9mmol/L), than those in decile 1.
- Additional tool for **VUS pathogenicity**
- **Triage patients for 100,000 Genomes Project**

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>2300 FH diagnostic cases

Detection rate

- Negative 69%
- VUS 6%
- Pathogenic variant 25%

Positive Detection Rate = 25%

VUS per gene

- LDLR 37%
- APOB 47%
- PCSK9 16%

- 24 unique LDLR Class 3 variants
- 56 unique APOB Class 3 variants
- 15 unique PCSK9 Class 3 variants

VUS detected in 6% of patients.

VUS reclassification would allow entry into the cascade programme
Variant pathogenicity assessment in FH

Classification tools available for FH:

- **Bioinformatics tools, comprehensive literature searches**

- **External Databases**
  - British Heart Foundation Database
  - HGMD Database
  - LOVD
  - JOJO Genetics

- **Data sharing**
  - FH “e network” between UKGTN and FH research labs
  - VUS Data exchange (BGL, Aberdeen, Cardiff, GOSH, Belfast)

- **Segregation studies**
  - Cardiff Heart Research Wales funded project
  - Individual labs segregation studies

- **Functional studies and RNA splicing**
VUS and The Polygenic SNP assay

VUS  
\(n = 37\)

Deciles 6-10  
**Likely** polygenic FH  
\(n = 20\) (54%)

Deciles 1-3  
**Unlikely** polygenic FH  
\(n = 9\) (24%)

Deciles 4-5  
**Grey zone**  
\(n = 8\) (22%)

- No further work
- Further work: **Likely monogenic FH**
- Further work required

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North Bristol NHS Trust
Case Study One – Data sharing results in reclassification of VUS

Referral reason:
34yr old female
Pre-treatment: LDL-C 6.4mmol/L.

NGS assay results:
Heterozygous for novel *LDLR* missense variant, c.2311G>A, p.(Ala771Thr)

**Evidence**
Not reported in the literature or databases
Small physiochemical difference between amino acids
Amino acid moderately conserved
BI unconvincing - may affect splicing

**Segregation analysis:** insufficient to reclassify

Seen in 8 FH patients (Aberdeen, GOSH, BGL)

Reclassified: ‘Highly likely’ to be pathogenic

Facilitates cascade testing of index case’s children.
Case Study One – Data sharing results in reclassification of VUS

Harefield family
Since identified this variant in a second family

Index case: 60 year old male.
LDL-C 9.1
FH heart disease and high cholesterol

Allowed cascade testing

- 9 siblings

- Diabetes
- CV/IA 85

- MI 50
- RP 25

- 91

- Muscular

- Artery

- On cholesterol treatment

- Heart Disease

- High cholesterol

- On cholesterol treatment

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Case Study Two – *LDLR*-35C>G promoter Variant

**Case 1**
56yr old female; ?FH. Referred from Bournemouth

**Case 2**
54yr old female; raised cholesterol
Father and mother premature heart disease
Referred from Weston

**Results of the FH NGS assay**
Novel *LDLR* c.-35C>G promoter variant identified
Polygenic SNP score – 1\(^{st}\) and 2\(^{nd}\) decile

**Could Promoter variants be the cause of FH?**

**How can data sharing and segregation clarify?**
Case Study Two – *LDLR*-35C>G promoter Variant

Segregation Analysis at BGL (Case 2)

Segregated with 4 relatives.

Reclassified to ‘likely pathogenic’ – cascade testing available.

The 2nd BGL patient – no cascade testing yet, but this another large family which will benefit.
Case Study Three – Potential LDLR Intronic Putative Cryptic Splice Variant

Referral reason:
24 year old male
Polish
Raised total cholesterol 20.4mmol/l.

NGS assay results:
No causative FH mutation identified
2 x likely benign variants:
  LDLR Intron 13 c.1988-60G>T, p.(?)
  APOB c.2068-4T>A, p.(?)

Polygenic SNP score 0.826 (Decile 4) – grey zone

Follow up studies
Mother – 46 years
Polish
Cholesterol 12.9mmol/L
?FH

Unaffected brother: sample expected
Case Study Three – Potential **LDLR** Intronic Putative Cryptic Splice Variant

Analysis of LDLR mRNA in patients with familial hypercholesterolemia revealed a novel mutation in intron 14, which activates a cryptic splice site

Mari Ann Kulseth¹, Knut Erik Berge¹, Martin Prøven Bøgrud² and Trond P Leren³

Evidence potentially supporting this c.1988-60G>T variant

Not reported to population databases
- **BUT** outside ROI and area normally covered by exome data.
- Not seen in: - >2300 BGL cohort of referrals
  - 385 exomes (Exeter lab)
  - No other variation reported in this small intron
  - Polish laboratory cohort
- **In silico Splice Tools:** 1 tool predicts creation of a donor site

What Next?
**Further Segregation** Analysis - Test unaffected brother.

**RNA studies** – designed primers and awaiting samples

**Candidate for the 100,000 Genome Project** – Likely monogenic cause

Illustrates cryptic splice sites can be a mechanism for this gene.
Summary

- A comprehensive high throughput personalised medicine NGS assay which detects monogenic & polygenic FH and informing information regarding adverse drug reactions.

- Supports the genomic pathway for triaging patients into 100,000 genomes project

- Polygenic SNP tool can be used as an aid in classifying VUS

- Highlights the vital importance of data sharing (e-network), functional studies and segregation analysis in variant reclassification for FH.

- Need a central data resource for reporting of variants – what should this be?
  - Need an agreed mechanism for the reclassification of variants
  - How should people get updated?
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