A pedigree-based GWAS identifies UDP-glucuronosyltransferase variants associated with serum bilirubin concentration and risk of Type-2 Diabetes in the Norfolk Island genetic isolate

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The Norfolk Island Health Study

Why are we doing this study?

Increased risk of complex metabolic disorders within the NI population.

Aim:

Investigate the well established genetic isolate of Norfolk Island (NI) and leverage its unique genomic structure to increase the ability to detect related genetic markers.

This study revolves around the complex structure of the NI pedigree, but before we get there...
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A brief history lesson...
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The Reconstructed NI Core Pedigree...
Study Design

- Participants - 500 NIHS individuals

- Samples - Blood (circulating lymphocytes)

- Platforms:
  - SNPs: Illumina 610quad

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37 CVD and T2D-related traits
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Analysis: Pedigree-Based GWAS

- Heritability analysis (batched using GenABEL/R: Polygenic Model)
  - mmscore function - pedigree structure analysis
    - study-wide significance for NI pop = $1.84 \times 10^{-7}$
    - suggestive significance threshold = $1.0 \times 10^{-5}$
  
- R: logistic regression models
  - bilirubin concentration and T2D-risk
  - adjusting the model for genotype
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A range of metabolic-related traits show 'high' $h^2$ in NI
Lack of association?

HDL

LDL

CHOL
Bilirubin associations

A

T_BILI

-\log_{10}(P-value)

Chromosome

B

D_BILI

-\log_{10}(P-value)

Chromosome
A striking association peak located at chromosome 2q37.1 was observed for both total bilirubin and direct bilirubin, with 29 SNPs passing multiple correction ($1.84 \times 10^{-7}$). Strong linkage disequilibrium (LD) was observed across a 200 kb region spanning the UDP-glucuronosyltransferase (UDPGT) gene family, including UGT1A1, which codes an enzyme known to metabolise bilirubin.
Genotype effect on bilirubin concentration
Comparison of LD across populations

(a) NI

(b) CHD

(c) CEU

(d) JPT
Minor Allele Freq Comparisons
Bilirubin: A protective role?

Bilirubin:
- component of haemoglobin
- formed during metabolic breakdown in liver
- direct form is soluble and secreted via blood or urine

Protective role:
- potent antioxidant
- vital role in protecting the body from reactive oxygen species

Clinical Associations:
- well established that serum bilirubin shown to have protective effect on CVD
- more recently Metabolic Syndrome and T2D
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UDP-glucuronosyltransferase:
- family of enzymes
- large group of isoforms on Chr 2q37.1
- major importance in conjugation and elimination of potentially toxic xenobiotics and endogenous compounds
- UGT1A1 primarily metabolises bilirubin, UGT1A3 and UGT1A4 have also been shown to have some affinity

Associations:
- previous GWAS identified bilirubin associations with UDPGT
- don’t appear to be genotype associations with disease-risk or traits
- there are associations noted with Gilbert Syndrome - also decrease T2D incidence
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UDP-GGT region and associations

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What we found in the NIHS cohort...

Explored clinical associations in the NIHS cohort:

- logistic regression modelling
  - revealed a significant association between direct bilirubin concentration and T2D-risk\(^1\)

In NIHS increased direct bilirubin was associated with a 28% reduction in T2D risk

- (OR: 0.72, 95% CI: 0.57-0.91, p-value: 0.005).

When adjusted for genotype the overall model was validated:

- adjusted model predicting a 30% reduction in T2D-risk with increasing direct bilirubin concentrations

- (OR: 0.70, 95% CI: 0.53-0.89, p-value: 0.0001).

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- We were able to use the NI pedigree to associate CVD-related traits
  - Identified the UDPGT region (2q37.1) with bilirubin levels
  - Strong LD across the region
  - Genotypic effects on bilirubin levels
  - Identified 'protective' haplotypes (increased bilirubin levels)
  - To our knowledge this is the first association between disease-risk (T2D), clinical trait (bilirubin levels) and genotype.

In the NI population, polymorphic SNPs within the UDPGT enzyme family are directly related to serum bilirubin levels and also associated with reduced risk of developing T2D.
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Identification of potential blood biomarkers to predict bilirubin levels

Further explore population differences (additional populations)

Starting to collaborate with other groups that have populations/cohorts with bilirubin measures (USA and NZ)

Potentially modifiable outcome (can change bilirubin levels)

UDPGT region (2q371.1) contains many different isoforms, act on thousands of different compounds
- potential to screen other compounds against UDPGT variants
Future Directions

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The people of Norfolk Island