Genetic Modifiers of Hereditary Haemochromatosis

Prof. Nathan Subramaniam
Membrane Transport Laboratory
Haemochromatosis

- Haemochromatosis – genetic iron overload disorder
- Iron accumulation in tissues
  - Liver, pancreas, heart, pituitary, joints
- Clinical features
  - Liver disease – cirrhosis
  - Hepatocellular carcinoma
  - Diabetes
  - Arthropathy
  - Hypogonadism
  - Cardiomyopathy
  - Skin pigmentation
What are genetic modifiers?

- Two individuals carry the identical mutation in a gene which is linked to a disorder – e.g C282Y HFE – haemochromatosis
- Despite this (and assuming everything else is equal) they have different presentations e.g. significantly different serum and liver iron levels
- Possibility that changes in another gene affects (modifies) expression of the HFE gene
- Identified by comparing the DNA profile of the 2 different individuals
- Why –
  - Diagnosis – Identify who might get the disorder
  - Prognosis – “predict” the course of the disorder
  - Treatment
Iron Overload

HFE HH (Typical)

- 1:200 people of European heritage.
- 90-95% of cases in Australia.
- Only standard test performed in Path labs is the HFE gene test.
- HFE C282Y change is most common

non-HFE HH (Atypical)

- Account for 5-30% of iron overload.
- Increasing identified in non-Europeans.
- Type 2 – 4 HH genes
  - Hemojuvelin
  - Hepcidin
  - TFR2
  - Ferroportin
Regulation of iron through the liver-expressed peptide Hepcidin
DNA sequencing to detect genetic variants

- Sanger chain terminator sequencing - 1977
- Gold standard for detecting novel genetic variants
New sequencing technologies – Next-Generation Sequencing

• Limitations of Sanger method
  – Small number of patients
  – Small number of genes
  – Manual inspection of sequence
  – Time and costs

• Next-Generation Sequencing (NGS)
  – New technologies – pyrosequencing (454), Illumina, SOLiD, Ion Torrent
  – High throughput
  – Multiplexing – more patients, more genes
  – More sequence coverage
  – More economical ↓$/base
First approach

- International collaborative study funded by the National Institutes of Health, USA. Investigators from:
  - Australia
    - Brisbane (Powell, Ramm, Anderson, Subramaniam)
    - Melbourne (Gurrin, Allen)
  - USA
    - Irvine (McLarens – Chris and Gordon)
    - New York (Phatak)
    - Birmingham (Barton)
  - Canada
    - London (Adams)
- Comparing the DNA profile – changes/variations in DNA between two groups of patients with haemochromatosis and C282Y mutations
  - First group – high iron levels
  - Second group – low/normal iron levels
  - Sequence the DNA which codes for proteins – exome sequencing
  - Compare the 2 groups and identify the differences
Exome sequencing in HFE C282Y homozygous men with extreme phenotypes identifies a GNPAT variant associated with severe iron overload

- Cases – High Iron = 22; Controls – Low Iron = 13
- Exome sequencing of DNA from patients
- 16/22 – had a variant in the GNPAT gene p.D519G
- 0/13 – controls
- The variant affects activity of an peroxisomal enzyme involved in synthesis of lipids in cell membranes
- Functional studies suggest that loss of GNPAT affects expression of hepcidin (levels are decreased)
- Lower hepcidin = increased iron absorption
- Suggests that individuals with p.C282Y HFE and the p.D519G GNPAT change have higher iron levels.
- Published in top-ranked international liver journal Hepatology.
Second approach –
Targeted Next-Generation Sequencing

- Panel covers 39 genes and promoters of 11
  - Clinically associated
  - Mouse models with primary alteration
  - Mechanistically demonstrated roles
  - Regulatory influence in response to challenge
  - Promoters - variability in disease penetrance
Our Sequencing Approach

Clinical data form + Patient consent form + 2 x 5ml blood in EDTA → Clinic Laboratory → Report to clinician → Bioinformatic Analysis
<table>
<thead>
<tr>
<th>Genes and SNPs with a standardised test:</th>
<th>Genes experimentally associated with disease:</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFE - H63D - C282Y</td>
<td>HFE - H63D - C282Y</td>
</tr>
</tbody>
</table>

### Gene clinically associated with disease: HFE

**HFE**

**5'UTR** (creates new ATG)
- p.(Gly43Asp)
- p.(Leu46Trp)
- p.(Leu50Cysfs*31)
- p.(His63Asp)
- p.(Ser65Cys)
- p.(Arg66Cys)
- p.(Arg67Cys)
- p.(Val68Glyfs*20)
- p.(Arg71*)
- p.(Gly ... - splicing (skipping of exon 5)
- p.(His341Leufs*119)

**HJV**

**intronic** (may affect splicing)
- p.(Val91Phe)
- p.(Glu100Glu)
- p.(Asp149Thrfs*97)
- p.(Leu165*)
- p.(Ala168Asp)
- p.(Phe170Ser)
- p.(Asp172Glu)
- p.(Arg176Cys)
- p.(Trp191Cys)

**SLC40A1**

**5'UTR**
- p.(Ala45Glu)
- p.(Tyr64His)
- p.(Ala69Thr)
- p.(Ala69Val)
- p.(Ser71Phe)
- p.(Val72Phe)
- p.(Ala77Asp)
- p.(Gly80Ser)
- p.(Gly80Val)
- p.(Arg88Gly)
- p.(Arg88Thr)
- p.(Leu129Pro)
- p.(Asn144His)
- p.(Asn144Thr)
- p.(Leu149Ile)
- p.(Asn151Ser)
- p.(Glu154Glu)
- p.(Glu154Glu)
- p.(Glu154Glu)
- p.(Glu154Glu)

### SNPs clinically associated with disease: HFE

**HFE**

**5'UTR** (creates new ATG)
- p.(Gly43Asp)
- p.(Leu46Trp)
- p.(Leu50Cysfs*31)
- p.(His63Asp)
- p.(Ser65Cys)
- p.(Arg66Cys)
- p.(Arg67Cys)
- p.(Val68Glyfs*20)
- p.(Arg71*)
- p.(Gly ... - splicing (skipping of exon 5)
- p.(His341Leufs*119)

**HJV**

**intronic** (may affect splicing)
- p.(Val91Phe)
- p.(Glu100Glu)
- p.(Asp149Thrfs*97)
- p.(Leu165*)
- p.(Ala168Asp)
- p.(Phe170Ser)
- p.(Asp172Glu)
- p.(Arg176Cys)
- p.(Trp191Cys)

**SLC40A1**

**5'UTR**
- p.(Ala45Glu)
- p.(Tyr64His)
- p.(Ala69Thr)
- p.(Ala69Val)
- p.(Ser71Phe)
- p.(Val72Phe)
- p.(Ala77Asp)
- p.(Gly80Ser)
- p.(Gly80Val)
- p.(Arg88Gly)
- p.(Arg88Thr)
- p.(Leu129Pro)
- p.(Asn144His)
- p.(Asn144Thr)
- p.(Leu149Ile)
- p.(Asn151Ser)
- p.(Glu154Glu)
- p.(Glu154Glu)
- p.(Glu154Glu)
- p.(Glu154Glu)
Case study

- 56-year-old female of Mediterranean origin
- Serum ferritin of 780 μg/L
- Transferrin saturation of 95%
- HFE gene test was negative
- Sister also has iron loading
- Identified a new DNA change in the Transferrin Receptor 2 gene
- Causes iron overload

Summary

• New technologies, well-characterised patient resources and collaborative studies (and of course good funding)
• Enable the identification of genetic modifiers of haemochromatosis
• Demonstrated utility of targeted Next Generation Sequencing for identifying causative mutations in individuals and families
• Significantly improves the chance of a genetic diagnosis in atypical cases
• Identification that genetic iron disorders are more diverse than previously recognised
• Likely that more genes affecting this disorder are “out there”.
Thank you
Patients and their families
Haemochromatosis Australia

Membrane Transport Laboratory
Dr. Cameron McDonald
Dr. Daniel Wallace
Dr. Gautam Rishi
Eriza Secondes
Lesa Ostini
Justin Goh

Collaborators
Ms Jeannette Dixon
Prof Lawrie Powell
Prof Grant Ramm
Prof Greg Anderson
NIH Collaborators
Prof. Darrel Crawford
Prof. Graham Cooksley
Prof. Barbara Leggett
Prof. Hugh Harley
Prof. Graeme Macdonald
Dr. Paul Clark
Dr. Sally Bell
Dr. Alison Lyons
Dr. Joy Ho
Dr. Hanlon Sia
Moving

- Mid-August 2016
- Prof Nathan Subramaniam
  - Group Leader, Liver Disease and Iron Disorders Research
  - Professor in Biomedical Sciences (Molecular Medicine)
    - Institute of Health and Biomedical Innovation
    - Queensland University of Technology,
      - Kelvin Grove, Brisbane
  - nathan.subramaniam@qut.edu.au