QIMR Berghofer
Medical Research Institute

Genetic Modifiers of Hereditary Haemochromatosis

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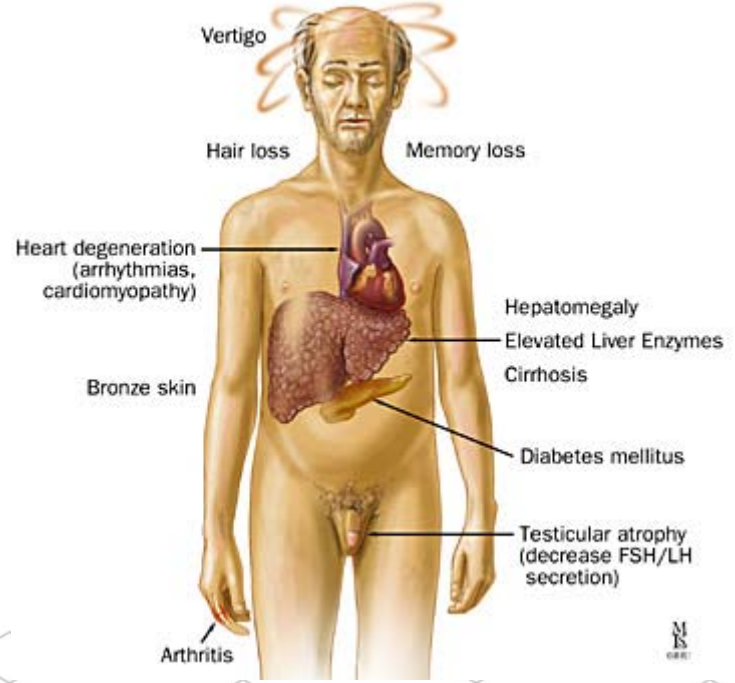
Haemochromatosis

- **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

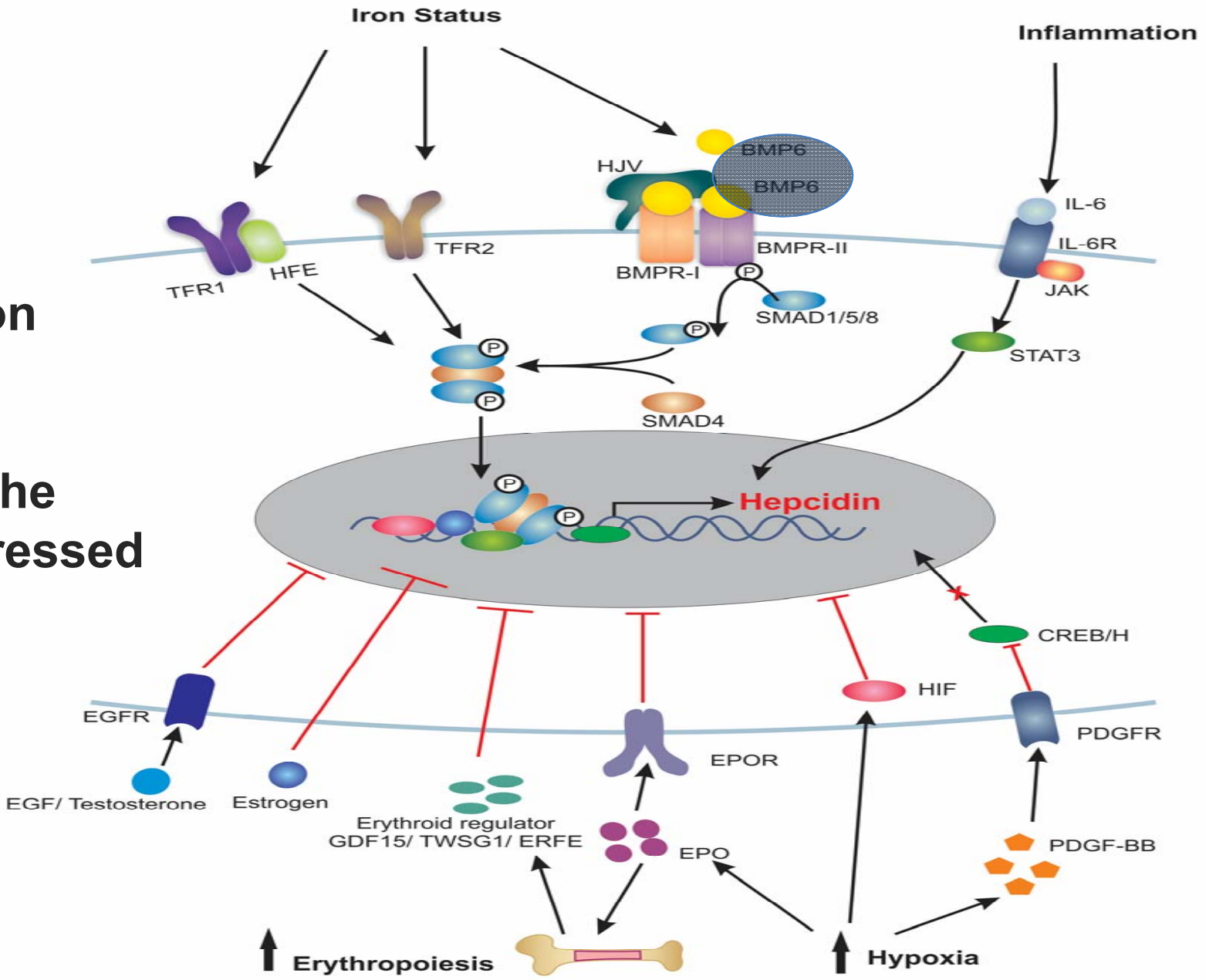
HFE HH (Typical)

- 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)

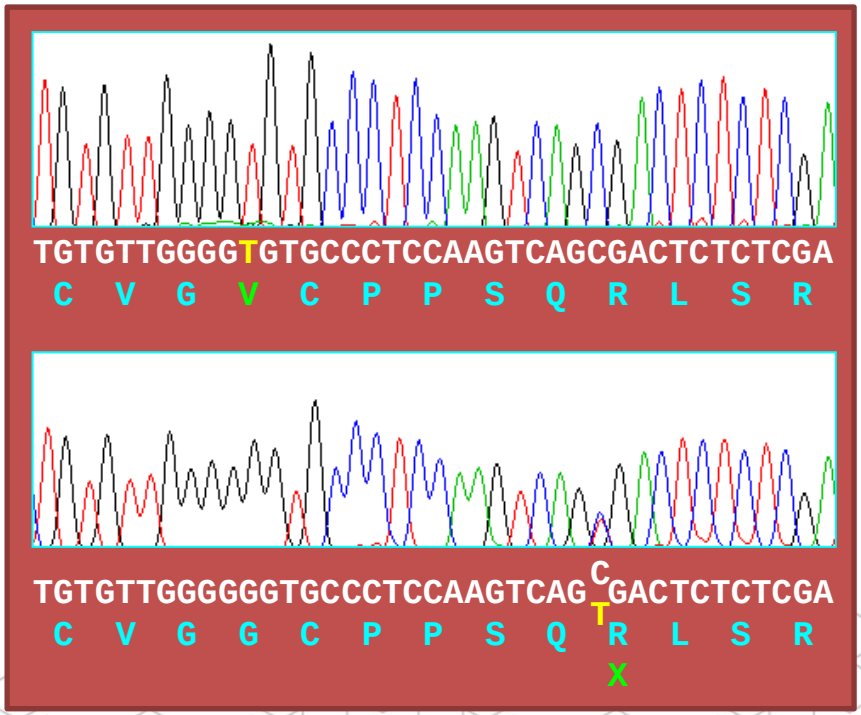
- Account for 5-30% of iron overload.
- **Increasing identified in non-Europeans.**
- **Type 2 – 4 HH genes**
  - *Hemojuvelin*
  - *Hepcidin*
  - *TFR2*
  - *Ferroportin*

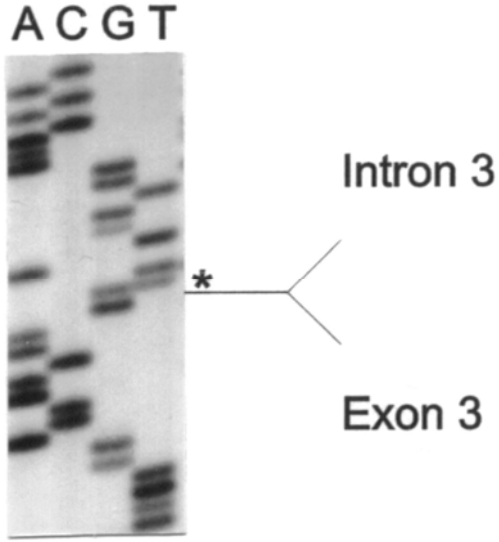
Regulation of iron through the liver-expressed peptide Hepcidin



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 pentrance

Our Sequencing Approach

Clinical data form

Patient consent form

2 x 5ml blood in EDTA

Report to clinician

Clinic

Bioinformatic Analysis

Laboratory

Finding the cause

Genes and SNPs with a standardised test:

HFE - H63D
- C282Y

Gene clinically associated with disease:

HFE
HAMP
HJV
TRF2
SLC40A1

SNPs clinically associated with disease:

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.(Gly93Arg)
p.(Trp94Glyfs*117)
p.(Ile105Thr)
p.(Glu114Lys)
p.(Tyr138*)
p.(Ala158Glnfs*53)
p.(Arg161Glyfs*50)
p.(Glu168Gln)
p.(Glu168*)
p.(Trp169*)
p.(Leu183Pro)
intronic - splicing (skipping of exon 3)
p.(Arg224Gln)
p.(Tyr230Phe)
p.(Tyr231del)
p.(Gln233*)
p.(Val256Ile)
p.(Trp267Leufs*80)
p.(Cys282Tyr)
p.(Cys282Ser)
p.(Gln283Pro)
p.(Val295Glu)
p.(Arg330Met)
intronic - splicing (skipping of exon 5)
p.(His341Leufs*119)

HJV

intronic (may affect splicing)
p.(Gln6His)
p.(Thr19Ala)
p.(Leu28Serfs*24)
p.(Arg54*)
p.(Gly66*)
p.(Gly69dup)
p.(Arg70Trp)
p.(Val74Trpfs*40)
p.(Cys80Arg)
p.(Cys80Tyr)
p.(Ser85Pro)
p.(Gly99Val)
p.(Gly99Arg)
p.(Leu101Pro)
p.(Ser105Leu)
p.(Gln116*)
p.(Cys119Phe)
p.(Arg131Phefs*111)

p.(Leu135Arg)
p.(Asp149Thrfs*97)
p.(Leu165*)
p.(Ala168Asp)
p.(Phe170Ser)
p.(Asp172Glu)
p.(Arg176Cys)
p.(Trp191Cys)
p.(Pro192Leu)
p.(Leu194Pro)
p.(Asn196Lys)
p.(Ser205Arg)
p.(Ile222Asn)
p.(Lys234del)
p.(Asp249His)
p.(Gly250Val)
p.(Ser264Leu)
p.(Asn269Lysfs*43)
p.(Ile281Thr)
p.(Arg288Trp)
p.(Glu302Lys)
p.(Ala310Gly)
p.(Gln312*)
p.(Cys321Valfs*21)
p.(Gly320Val)
p.(Cys321Trp)
p.(Cys321*)
p.(Arg326*)
p.(Ser328Aspfs*10)
p.(Arg335Gln)
p.(Ala343Profs*24)
p.(Cys361Valfs*6)
p.(Asn372Asp)
p.(Arg385*)

TFR2

p.(Val22Ile)
p.(Arg30Profs*31)
p.(His33Asn)
p.(Leu85_Ala96delinsPro) (also deletes splicing site and part of intron)
p.(Leu99Val)
p.(Arg105*)
p.(Met172Lys)
intronic - splicing (skipping of exon 4)
intronic (may affect splicing)
p.(Tyr250*)
p.(Val277Leu)
p.(Phe280Leu)
p.(Gln317*)
p.(Gly373Asp)
p.(Ala376Asp)
p.(Arg396*)
p.(Asn412del)
p.(Asn412Ile)
p.(Arg420His)
p.(Gly430Arg)
p.(Ala444Thr)
p.(Ile449Val)
p.(Arg455Gln)
p.(Arg468His)
p.(Leu490Arg)
p.(Glu491Glu) (may affect splicing)
p.(Tyr504Cys)
intronic - splicing
p.(Ser531Glnfs*6)
p.(Ser556Alafs*6)
p.(Val583Ile)
p.(Leu615Profs*177)
p.(Ala621_Gln624del)
p.(Arg678Pro)
p.(Gln690Pro)
p.(Met705Hisfs*87)
intronic - splicing
p.(Arg730Cys)
p.(Gly735Ser)
p.(Thr740Met)
p.(Ala743Val)
p.(Arg752His)
p.(Trp781*)
p.(Gly792Arg)

HAMP

promoter
promoter
5'UTR (creates new ATG)
p.(Gly32Aspfs*88)
p.(Arg42Serfs*78)
p.(Met50fs) (also deletes first base of intron)
p.(Trp49Cysfs*)
p.(Arg56*)
p.(Arg59Gly)
p.(Cys70Arg)
p.(Gly71Asp)
p.(Arg75*)
p.(Cys78Tyr)
p.(Lys83Arg)

SLC40A1

5'UTR
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.(Asn144Asp)
p.(Asn144Thr)
p.(Ile152Phe)
p.(Asp157Asn)
p.(Asp157Ala)
p.(Asp157Gly)
p.(Asp157Tyr)
p.(Trp158Leu)
p.(Trp158Cys)
p.(Val162del)
p.(Asn174Ile)
p.(Arg178Gln)
p.(Ile180Thr)
p.(Asp181Val)
p.(Gln182His)
p.(Asn185Asp)
p.(Asn185Thr)
p.(Gly204Ser)
p.(Thr230Asn)
p.(Ala232Asp)
p.(Leu233Pro)
p.(Lys240Glu)
p.(Gln248His)
p.(Met266Thr)
p.(Gly267Asp)
p.(Asp270Val)
p.(Gly323Val)
p.(Cys326Ser)
p.(Cys326Tyr)
p.(Ser338Arg)
p.(Leu345Phe)
p.(Ile351Val)
p.(Arg371Trp)
p.(Arg371Gln)
p.(Pro443Leu)
p.(Gly468Ser)
p.(Arg489Lys)
p.(Arg489Ser)
p.(Gly490Ser)
p.(Gly490Asp)
p.(Tyr501Cys)
p.(Asp504Asn)
p.(His507Arg)
p.(Arg561Gly)

Genes experimentally associated with disease

HFE
HAMP
HFE2
SLC40A1
FTL
FTH1
BMP2
BMP6
TMPRSS6
TFRC
TFR2
TF
CP
SLC11A2
CYBRD1
HEPH
SLC39A14
SMAD4
GDF15
TWSG1
APP
HEPHL1
SLC11A1
NDFIP1
NDFIP2
EPAS1
BMPR1A
ACO1
IREB2
B2M
IL6
ACVR2A
ACVR1
SMAD1
SMAD5
SMAD6
SMAD7
SMAD9
NFE2L2
HMOX1
HIF1A

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

McDonald *et al*, (2015) *Journal of Hepatology*

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

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Moving

- **Mid-August 2016**
- **Prof Nathan Subramaniam**
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