DISORDERS OF IRON REGULATION

INTRODUCTION

Haemochromatosis:
Defined as systemic iron overload of genetic origin, caused by a reduction in the concentration of the iron regulatory hormone hepcidin, or a reduction in hepcidin-ferroportin binding. Hepcidin regulates the activity of ferroportin, which is the only identified cellular iron exporter. The most common form of haemochromatosis is Type 1 due to HFE gene mutations, most commonly homozygous p.Cys282Tyr (C282Y). Non-HFE forms of haemochromatosis due to mutations in HAMP, HJV or TFR2 are much rarer. Mutations in SLC40A1 (Ferroportin) that prevent hepcidin-ferroportin binding also cause haemochromatosis. Cellular iron excess in HFE and non-HFE forms of haemochromatosis is caused by increased concentrations of plasma iron, which can lead to the accumulation of iron on parenchymal cells, particularly hepatocytes, pancreatic cells and cardiomyocytes.

Other genetic disorders leading to systemic iron load:
Hereditary aceruloplasminaemia is caused by mutations in CP (ceruloplasmin) and these patients have systemic iron excess; the liver and brain have iron deposits and these patients have neurodegeneration. Mutations in TF (transferrin) lead to the development of anaemia owing to the absence of transferrin bound iron available for erythroid cells; hepcidin expression is decreased as a result of the anaemia; subsequent improved iron absorption together with NTBI contributes to the development of iron overload in the liver, pancreas and heart. Mutations in SLC11A2 (DMT1) lead to DMT1-related iron overload which affects both the absorption and intracellular iron trafficking; anaemia is one of the major consequences of DMT1 mutations and presents early in life.

Hyperferritinaemia:
Loss of function mutations in SLC40A1 (Type 4A haemochromatosis) lead to raised ferritin with normal serum iron and transferrin saturation. Mutations in the 5' UTR IRE of FTL are associated with Hereditary Hyperferritinaemia Cataract Syndrome (HHCS). Mutations in exon 1 of FTL are associated with Hereditary Hyperferritinaemia.

Iron-refractory iron deficiency anaemia (IRIDA):
IRIDA is a rare autosomal recessive disorder is due to mutations in TMPRSS6 and is characterized by hypochromic microcytic anaemia, low transferrin saturation and inappropriate high levels of the iron hormone hepcidin.

Iron and neurodegeneration:
Hereditary ferritinopathy has been associated with mutations in FTL and FTH1; defects in these ferritin proteins can lead to this group of neurodegenerative diseases.

X-linked sideroblastic anaemia:
Mutations in ALAS2 lead to X-linked sideroblastic anaemia which is characterized by anaemia with the emergence of ring sideroblasts (erythroblasts with iron accumulation in perinuclear mitochondria due to impaired iron utilisation) in the bone marrow.

Iron deficiency:
Mutations in HEPH, encoding Hephaestin, are associated with sex-linked anaemia. Hephaestin is involved in the metabolism and homeostasis of iron and possibly copper. It is a transmembrane copper dependent ferroxidase responsible for transporting dietary iron from intestinal enterocytes into the circulatory system.

BMP/SMAD pathway:
BMP6 mutations have been associated with mild-moderate iron overload, and may also act as a modifier of iron overload in combination with HFE gene mutations.

Classical Type 1 haemochromatosis, which is common in populations of Northern European descent, is the predominant cause of iron overload. Approximately 85% of patients in the UK with a genetic iron overload are homozygous for the HFE C282Y mutation and a smaller proportion are compound heterozygous for HFE C282Y & H63D mutations.

STRATEGY
1. Initial screen is a targeted PCR for the 2 common HFE mutations, C282Y and H63D.
2. NGS 16 gene panel (further details are provided on page 2)
3. Dosage analysis for 5 of the most common genes (HFE, HFE2, HAMP, TFR2, SLC40A1)
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The NGS panel covers 16 genes commonly involved within iron metabolism including the HH genes.

<table>
<thead>
<tr>
<th>Panel</th>
<th>No of Genes</th>
<th>Conditions</th>
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| DIR   | 16          | Haemochromatosis Type 1  
|       |             | Haemochromatosis Type 2A |
|       |             | Haemochromatosis Type 2B |
|       |             | Haemochromatosis Type 3  
|       |             | Haemochromatosis Type 4A |
|       |             | Haemochromatosis Type 4B |
|       |             | X-linked sideroblastic anaemia |
|       |             | Aceruloplasminaemia |
|       |             | Atransferrinaemia/Hypotransferrinaemia |
|       |             | Hereditary Hyperferritinaemia cataract syndrome |
|       |             | Hyperferritinaemia without iron overload |
|       |             | Hyperferritinaemia |
|       |             | Hereditary ferritinopathy |
|       |             | Sex-linked anaemia |
|       |             | Iron-refractory iron deficiency anaemia |
|       |             | BMP6 iron overload |

NGS panel includes the following genes:

- HFE, HFE2, HAMP, TFR2, SLC40A1, FTL, FTH1, CP, TF, ALAS2, TMPRSS6, SLC11A2, HEPH, BMP6, SMAD4, BMP4

MLPA analysis will also be undertaken for HFE, HFE2, HAMP, TFR2 & SLC40A1, if clinically indicated.

TESTING

Diagnostic: Clinically affected patients
Carrier: Relatives of clinically affected patients

REFERRALS

All samples must be accompanied by a completed proforma:
- Disorders of iron regulation request form
Child and adult referrals are accepted from Hospital Consultants

TECHNICAL INFORMATION

- PCR and restriction digest for specific point mutations within the HFE gene (C282Y and H63D)
- Next generation sequencing (NGS) using Illumina MiSeq sequencing
- MLPA for dosage analysis

TARGET REPORTING TIMES

- HFE (2 common mutations): 14 calendar days
- NGS Iron Panel: 56 calendar days

N.B. Details are correct for the date of printing only – last updated 27/04/2018