Effect of SNPs on iron metabolism

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Introduction

Single nucleotide polymorphisms (SNPs) account for 90% of all polymorphisms in humans. There are about 3 million SNPs in the human genome (1 per 1,000 nucleotides), two thirds of which are in non-coding DNA. Mutations that affect the composition of proteins involved in iron metabolism may have an impact on human health. There are no active pathways for iron excretion, therefore regulation of iron absorption is the primary determinant of iron balance; tightly controlled homeostatic mechanisms exist to ensure that sufficient iron is acquired and retained by the body and excess iron is not accumulated. Rare genetic mutations and knock-out animal models have been invaluable tools for elucidating the molecular events controlling iron metabolism (see reviews [6], [18]) but more common SNPs have a wider impact in both public health and personalised nutrition. The C282Y mutation of the *HFE* gene (chromosome 6), carried in approximately 13% of individuals from predominantly Caucasian populations, is closely associated with the iron-loading disease,

hereditary haemochromatosis, but its mechanism of action is not clear, and the low penetrance [21] suggests that there may be other modifier genes.

Iron absorption

The availability of iron from digestive chyme in the intestinal lumen is modulated by the presence of dietary substances with varying iron-binding strengths (e.g. phytate, tannin) and reducing properties (e.g. ascorbic acid), and there appears to be competition with other divalent cations for uptake into the cell (e.g. calcium). Haem and non-haem iron are absorbed by independent pathways and enter a common pool in the intestinal mucosal cell (Fig. 1); the non-haem transporter is DMT1. Rodent models of the G185R *DMT1* mutation (mk mice and the Belgrade rat) exhibit severe anaemia because of impaired transport activity. The first human mutation of *DMT1* was reported earlier this year [19] but unlike the animal models, the patient was iron overloaded. The phenotype difference is thought to be related to the fact that the rodent mutation results in an amino acid substitution whereas the effect of the human mutation is exon 12 skipping. Urinary hepcidin was low in the patient, which might explain the raised iron absorption. Alternatively, iron may be transported by a non-DMT1 route, or there may be increased basolateral transport (involving ferroportin and haephaestin). Numerous mutations in the *ferroportin* gene have been reported in haemochromatosis patients, some of which reduce iron export from cells (e.g. A77D, V162del, G490D) and others (Y64N, C326Y) that are associated with resistance to hepcidin [8]. The role of hephaestin in removing electrons from Fe(2+) to generate Fe(3+) ions that can bind to transferrin has been determined using a mutant mouse

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model: sex-linked anaemia (sla) mice have a deletion in the gene for hephaestin and develop microcytic hypochromic anaemia.

Iron homeostasis

Iron is conserved and recycled within the body (20–25 mg/day), mainly for red blood cell formation (Fig. 2), but in addition there is an obligatory loss of approximately 1–2 mg iron originating from blood (menstrual and other sources of bleeding), sweat, intestine, skin and hair which needs to be replenished with dietary iron. Hepcidin, a 25 amino acid protein produced in the liver, is involved in the systemic regulation of iron absorption. It directly binds to ferroportin causing it to be internalised and degraded, thereby blocking cellular iron export [9]. When iron stores are adequate or high, the liver produces hepcidin which reduces iron efflux from duodenal cells and macrophages. Hepcidin expression is inappropriately low in haemochromatosis, suggesting that the *HFE* gene is required for normal regulation of hepcidin synthesis, but clinical penetrance is low in C282Y homozygotes so phenotype is not related to *HFE* alone. Hepcidin expression is also low in juvenile haemochromatosis, a rare but severe ironloading disorder associated with mutations of the *HFE2* (hemojuvelin) gene [3].

Iron status

The balance between iron absorption and iron loss determines the quantity of iron that is available for incorporation into metabolic pathways and hence affects iron status. Serum/plasma ferritin is widely used to predict the level of iron stores (mainly in the liver), whereas haemoglobin concentration (the ironcontaining protein in red blood cells which transports oxygen) is a functional measure of iron status. When the iron supply is insufficient for red blood cell formation, serum/plasma ferritin values fall and iron is

Fig. 2 Overview of iron balance in adults

released from the liver. Once the stores are exhausted, the haemoglobin content of newly formed red cells cannot be maintained at optimal levels and a microcytic hypochromic anaemia presents. There is, as yet, no explanation for the well-documented large inter-individual variation in “normal” values for haemoglobin and serum ferritin concentration in healthy individuals. In order to investigate the relative importance of diet and menstrual blood loss in determining iron status of women of child-bearing age, a detailed study of the habitual diet and menstrual blood loss of 90 apparently healthy women was undertaken [10]. In the study population, 11.5% of the variation in iron status was attributable to menstrual blood loss and 6.7% to the overall type of diet (red meat, poultry and fish, or vegetarian). More detailed dietary analysis is required to investigate the effect of dietary composition, not just the type of diet, on iron phenotype. However, the results of the study have raised an important question, namely what other factors determine iron status? It is very likely that genotype and specific SNPs, most of which have not yet been identified, play an important role (Fig. 3) as well as the availability of iron in the diet.

Fig. 1 Schematic diagram of iron absorption in duodenal mucosal cells

Fig. 3 Iron and health

Hereditary haemochromatosis

Progressive accumulation of iron as a result of inappropriately high absorption from the diet may have serious clinical consequences including diabetes, heart disease, arthritis and cirrhosis, the classic sequelae of hereditary haemochromatosis. Most patients (~90%) are homozygous for the C282Y mutation of the *HFE* gene and another 4% are compound heterozygotes (C282Y, H63D), but the clinical penetrance is low [2, 4]. A number of other mutations are also known to be responsible for hereditary haemochromatosis so that the condition is now separated into four categories:

- Type 1 (*HFE* mutations)
- Type 2 [*HFE2* (hemojuvelin) mutation]
- Type 3 (*TfR2* mutation)
- Type 4 (*Ferroportin* mutation)

A SNP database is required to explain the incomplete penetrance of the C282Y mutation. Bioinformatics can be used to extract candidate SNPs from different data sources, but because the *in silico* approach has a low SNP prediction efficiency, the recommendation is to sequence selected genes systematically in an ethnically homogenous sample [7]. Frequent SNPs probably occurred a long time ago and are shared across ethnic groups whereas most rare SNPs have appeared more recently and are restricted to geographically defined populations, as is the case with the C282Y mutation. A recent study compared iron status and morbidity in first-degree relatives of C282Y homozygotes who were asymptomatic with those who had haemochromatosis [17]. Only 57% of the variability in iron phenotype was explained by the genetic, physiological and lifestyle factors included in the model, indicating that there are other unidentified influential factors such as diet. However these are unlikely to be of great clinical importance as morbidity was low in both groups of relatives.

Diet, genotype and phenotype

Diet plays a modulating role in determining the iron phenotype in people with *HFE* mutations [11]. Iron stores in

C282Y homozygotes are positively associated with high alcohol and low fresh fruit consumption [17] and regular tea drinking with meals reduces the frequency of phlebotomies [14]. The role of diet in the management of haemochromatosis is controversial as many haematologists consider regular phlebotomy to be less invasive than dietary modification and therefore more acceptable for their patients. There is, however, an important public health consideration with respect to possible adverse effects of diet, including food iron fortification, in carriers of *HFE* mutations as this affects approximately 13% of people of northern European descent. About 15 years ago, it was reported that apparently healthy, close relatives of haemochromatosis patients absorbed more iron from a high iron bioavailability meal than control subjects [16]. This raised concerns about the possible harmful effects of a diet high in iron for carriers of *HFE* mutations, the putative genotype of the subject group recruited for the 1989 study. More recently, iron absorption from fortified cereals and a high iron bioavailability meal was measured in C282Y heterozygotes and found to be similar to that of wildtype controls [20]. Carriers also exhibit no difference in the efficiency of haem iron absorption [13]. Iron absorption studies are informative with respect to iron bioavailability and homeostasis, but subtle differences are not detectable, and over time these may have an impact on iron loading. Therefore a detailed study was undertaken to examine the relative importance of C282Y heterozygosity, diet [12] and iron losses as independent determinants of iron status in 44 C282Y heterozygote and 85 wildtype men aged 40 years or over (Heath et al. personal communication). C282Y heterozygosity was associated with 17% higher transferrin saturation (95% CI: 6%, 29%) but no evidence of a difference in serum ferritin or serum transferrin receptor concentration. Interestingly, blood loss was a more powerful predictor of iron status than either C282Y heterozygosity or diet. Blood donation was associated with 58% lower serum ferritin concentration, self-reported faecal blood loss was associated with 35% lower serum ferritin concentration, and body mass index was positively associated with serum ferritin concentration. The only dietary variable associated with iron status was alcohol

intake (3% higher transferrin saturation, 7% higher serum ferritin concentration and 2% lower soluble transferrin receptor concentration). Neither total iron intake nor haem iron intake were associated with increased iron status in these C282Y heterozygote and wildtype men who had experienced at least 40 years of potential iron accumulation. The lack of association of haem iron intake with serum ferritin concentration contrasts with results obtained in a cohort of UK women [5] in which haem iron intake (estimated using the UK EPIC FFQ) was found to be a significant positive predictor of serum ferritin concentration in post-menopausal women. However, the effects of alcohol (+), blood donation (–) and BMI (+) on serum ferritin concentration were consistent between the two studies. Further investigation is required to clarify the possible impact of meat (haem iron) on iron status in individuals with *HFE* mutations.

Iron deficiency

Much less is known about the genetic basis of non-diet related iron deficiency. A polymorphism in the transferrin protein (G277S) has been associated with iron deficiency [15] but no effect of the SNP on the iron-binding capacity of transferrin was observed from in vitro studies [1]. A human iron absorption study also failed to show a significant difference between heterozygote and wildtype individuals, although there was a difference in the slope of the curve describing the relationship between body iron stores (serum ferritin) and % iron absorption.

Conclusions

Mutations of proteins involved in iron metabolism will continue to help advance our understanding of iron metabolism and homeostasis. However, addressing individual variability requires additional data on genotype–phenotype–disease progression. This includes the synergistic effects of known and, as yet, undiscovered, SNPs, and how their impact is modulated by dietary, physiological and life-style factors.

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