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**BRIEF REPORT**

**HFE genotype modifies the influence of heme iron intake on iron status**

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**Abbreviated running title:** Iron intake, HFE genotype, and iron status

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## **Summary**

**Background:** Public health policy to prevent iron deficiency through food fortification or other measures may be disadvantageous to people with hereditary hemochromatosis.

**Methods:** 2531 women from a cohort of United Kingdom women were typed for C282Y and H63D mutations in the hemochromatosis gene. These women completed food frequency questionnaires and provided blood for iron status.

**Results:** C282Y homozygotes (n=31) had serum ferritin concentrations 2.4 times higher (95% confidence interval = 1.9 - 3.1) than wild types (n=1774) but heterozygotes (n=726) were not different from wild types. H63D genotype had no effect on its own. The effect of heme iron intake (from meat, fish and poultry) was 2.0 times greater (1.2 - 3.2) on C282Y homozygotes than other groups. Non-heme iron had little effect.

**Conclusions:** There may be scope for dietary intervention in women homozygous for the C282Y mutation. C282Y heterozygotes and H63D homozygotes and heterozygotes have similar serum ferritin concentrations to wild type and need not reduce their meat intake, other than as part of a normal healthy diet.

### **Keywords**

Hemochromatosis, HFE gene, iron overload, dietary iron, hereditary diseases, genetics

Public health policy to prevent iron deficiency may be disadvantageous for people with hereditary hemochromatosis. This is an autosomal recessive disorder in which there is iron accumulation as a result of increased dietary absorption. Most patients with hereditary hemochromatosis are homozygous for a single mutation (C282Y) of the HFE gene.<sup>1</sup> In the UK over 90% of patients with hemochromatosis have this genotype<sup>2</sup> as do about 1 in 150 people in the general population.<sup>3</sup> About 15% of the population are carriers of C282Y. A second variant, H63D, is also common in the general population (carrier frequency about 25%) and may cause iron accumulation if present with C282Y. Although the clinical penetrance of homozygosity for C282Y is low,<sup>4,5</sup> those patients where unidentified iron accumulation leads to iron overload may develop organ damage leading to arthritis, diabetes mellitus, heart disease, liver cirrhosis and hepatocellular carcinoma.<sup>6</sup> We therefore set out to address the question of whether an individual's response to long-term dietary practice differs according to their genotype. Previous research suggests that heme iron (found in meat, fish and poultry) should be the focus of this investigation in hemochromatosis.<sup>7</sup>

## **Methods**

### **Subjects**

Participants were sampled from the UK Women's Cohort Study, a cohort of 35,372 UK women aged 35-69 in 1995. The Cohort was designed to cover a broad range of dietary intakes with equal proportions of self-reported vegetarians, fish-eaters and red meat-eaters.<sup>8</sup> 15,000 women were randomly selected to receive two cytology brushes and asked to return cheek cell samples by post for DNA assays. Potential C282Y homozygotes and heterozygotes were then asked to provide blood samples taken at

their local general practice or hospital phlebotomy clinic. Another 3000 Cohort women were randomly selected to provide blood samples.

## **Measurements**

Details of DNA extraction and analysis of blood samples for serum ferritin concentration are available with the online version of this article. Long-term diet was measured using a 217-item food frequency questionnaire<sup>9,10</sup> based on the one used by the UK EPIC study.<sup>11</sup> Nutrient values for food items, including total iron, were derived from standard UK Food Composition tables.<sup>12</sup> Estimated heme iron intake from meat, fish and poultry was based on meat-specific concentrations of heme iron.<sup>13</sup> Supplemental intake of vitamins and minerals, including iron, was assessed by separate questionnaire.

## **Statistical methods**

We initially compared C282Y and H63D homozygotes with wild types for log-transformed serum ferritin, serum iron, unbound iron binding capacity, total iron binding capacity, transferrin saturation, and hemoglobin using two-sample t-tests. In this exploratory analysis, 99% confidence intervals (CIs) were used as an acknowledgement of the multiple testing involved. We used multiple linear regression to investigate the relationship between log-transformed serum ferritin concentrations and both total iron intake and heme iron intake. Adjustment was made for age, genotype, smoking status, blood donor status, menopausal status, body mass index, and intakes of total energy, alcohol, Englyst fiber, calcium, non-heme iron, and vitamin C, including supplemental intakes of iron, calcium and vitamin C as continuous variables. Any influence of genotype on the relationship between heme

iron intake and serum ferritin (i.e. the gene-diet interaction) was formally tested by adding the appropriate interaction term to the model and using a likelihood ratio test. The power calculation is available with the online version of this article.

Ethical approval for the study was obtained from 173 local research ethics committees and written consent for DNA analysis was obtained from all individuals taking part.

## **Results**

Women had a mean age of  $53 \pm 9$  years at recruitment with mean body mass index of  $25 \pm 4$ . Almost all the women were white. Geometric mean total iron intake was 18.0 mg/day and 0.3 mg/day for heme iron.

Of the 15,000 women contacted for cheek cell screening, 5349 (36%) provided cytology brush samples. Of the 3000 women contacted solely for blood, 1877 (63%) returned blood samples. Combining the results from cheek cell and blood samples, C282Y and H63D genotype information was available for 6747 (93%) and 6766 (94%), respectively, of the subjects returning samples. Of those successfully genotyped, 31 subjects (0.5%) were homozygous for the C282Y mutation, 901 (13.4%) were heterozygous for the C282Y mutation, and 5815 (86.2%) were wild type for the C282Y mutation. 167 (2.5%) were homozygous for the H63D mutation, 1745 (25.8%) were heterozygous for the H63D mutation, and 4854 (71.7%) were wild type for H63D. Of those who were heterozygous for either C282Y or H63D, 173 subjects were heterozygotes for both (compound heterozygotes). HFE genotypes were consistent with Hardy-Weinberg equilibrium, and phase analysis confirmed the two variants to be segregating on distinct haplotypes.

There were 2573 analyzable blood samples, with 2531 successfully typed for C282Y (31 homozygous, 726 heterozygous, 1774 wild types) and 2535 successfully typed for H63D (41 homozygous, 662 heterozygous, 1832 wild types). Blood iron status on these subjects is shown by genotype in Table 1. Twelve of the 31 (39%) women homozygous for the C282Y mutation had serum ferritin concentrations above the upper limit of the reference range. This was a substantially higher proportion than the 17 (2%) of heterozygotes and 18 (1%) of wild types with blood measurements. Of the C282Y homozygotes, 28 (90%) had transferrin saturation above 50%, compared with 191 (27%) of heterozygotes and 181 (10%) of wild types with this measure.

Adjusting for the potential confounders listed, an increment of 1 mg/day in heme iron intake (equivalent to approximately doubling intake) was associated with a 41% increase in serum ferritin concentrations (95% CI = 32% - 51%). Being homozygote for C282Y was associated with serum ferritin concentrations 2.4 times higher (1.9 - 3.1) than the wild type. C282Y heterozygotes had similar serum ferritin concentrations to wild type (ratio = 1.06; 0.99 - 1.13). The effect of the heme iron intake was 2.0 times greater (1.2 - 3.2) for C282Y homozygotes than other groups, indicating a statistically significant gene-diet interaction ( $p=0.006$ ). C282Y homozygotes also still had higher ferritin concentrations overall. Serum ferritin concentrations predicted by the model for each woman are shown in Figure 1. The stronger relationship between heme iron intake and serum ferritin for the C282Y homozygotes illustrates the interaction effect. Estimates of the interaction remained essentially unchanged after adjustment for socio-economic status and highest



educational level. Exclusion of self-reported vegetarians did not substantially change the results. There were insufficient data to investigate any threshold effect.

After adjustment for confounding factors, there was no evidence of subjects homozygous for H63D having higher serum ferritin concentrations than wild type (ratio = 0.97; 0.74 - 1.27). No interaction was evident with H63D genotype (P=0.23). Compound heterozygotes (heterozygote for both C282Y and H63D) had serum ferritin concentrations 1.2 times higher (1.0 - 1.5) than subjects who were wild type for both mutations.

When we investigated total iron intake we found no substantial association with serum ferritin concentrations. Only the heme iron component appeared to be related to serum ferritin concentration.

## **Discussion**

We characterized blood iron status in relation to both genotype and dietary iron intakes in a large cohort. The estimated prevalences of the mutations were similar to previous published studies of hemochromatosis in the United Kingdom,<sup>3,14</sup> northern Europe,<sup>15,16</sup> and the United States.<sup>4</sup> If we define biochemical expression of hereditary hemochromatosis as serum ferritin concentrations above the upper limit of the reference range, then penetrance of this disease in our cohort was 39% (though we have no measure of morbidity in this study).

The relative roles of heme and non-heme iron are discussed further in the online version of this article, along with a comparison of intakes recorded in other studies.

Heme iron intake was strongly related to iron status. Women consuming more heme iron had higher serum ferritin concentrations. This relationship was exacerbated by homozygosity for the C282Y mutation; the influence of heme iron intake was more than twice as strong among C282Y homozygotes than wild types. This resulted in substantially raised serum ferritin concentrations in this group. In contrast, even the highest intakes of heme iron were not associated with excessive serum ferritin concentrations among women who were wild type or heterozygous for C282Y. The presence of the H63D mutation was not associated with higher serum ferritin concentrations except in those who were compound heterozygotes with the C282Y mutation.

These findings confirm results from a series of small studies investigating heme and non-heme iron absorption in controlled conditions.<sup>7</sup> Lynch et al. found in healthy volunteers that absorption of both heme and non-heme iron was lower among those with higher serum ferritin concentrations. In patients with hemochromatosis, absorption of both types of iron was higher, but only heme iron absorption was free of any inverse association with serum ferritin. In their study, absorption of iron from normal diets in heterozygotes appeared little different from healthy volunteers. We have also formally demonstrated the observation of Rossi et al.<sup>17</sup> that there is no difference in serum ferritin concentrations between wild type and heterozygous C282Y meat-eaters.

Women who are homozygous for the C282Y mutation should be advised to limit their meat (heme iron) intake to reduce the rate of iron accumulation, providing they are not anaemic. The larger group of women who are heterozygous for the C282Y

mutation do not have substantially higher serum ferritin concentrations than wild type and need not reduce their intake on the basis of this study, other than as part of a normal healthy diet.

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## Figure legend

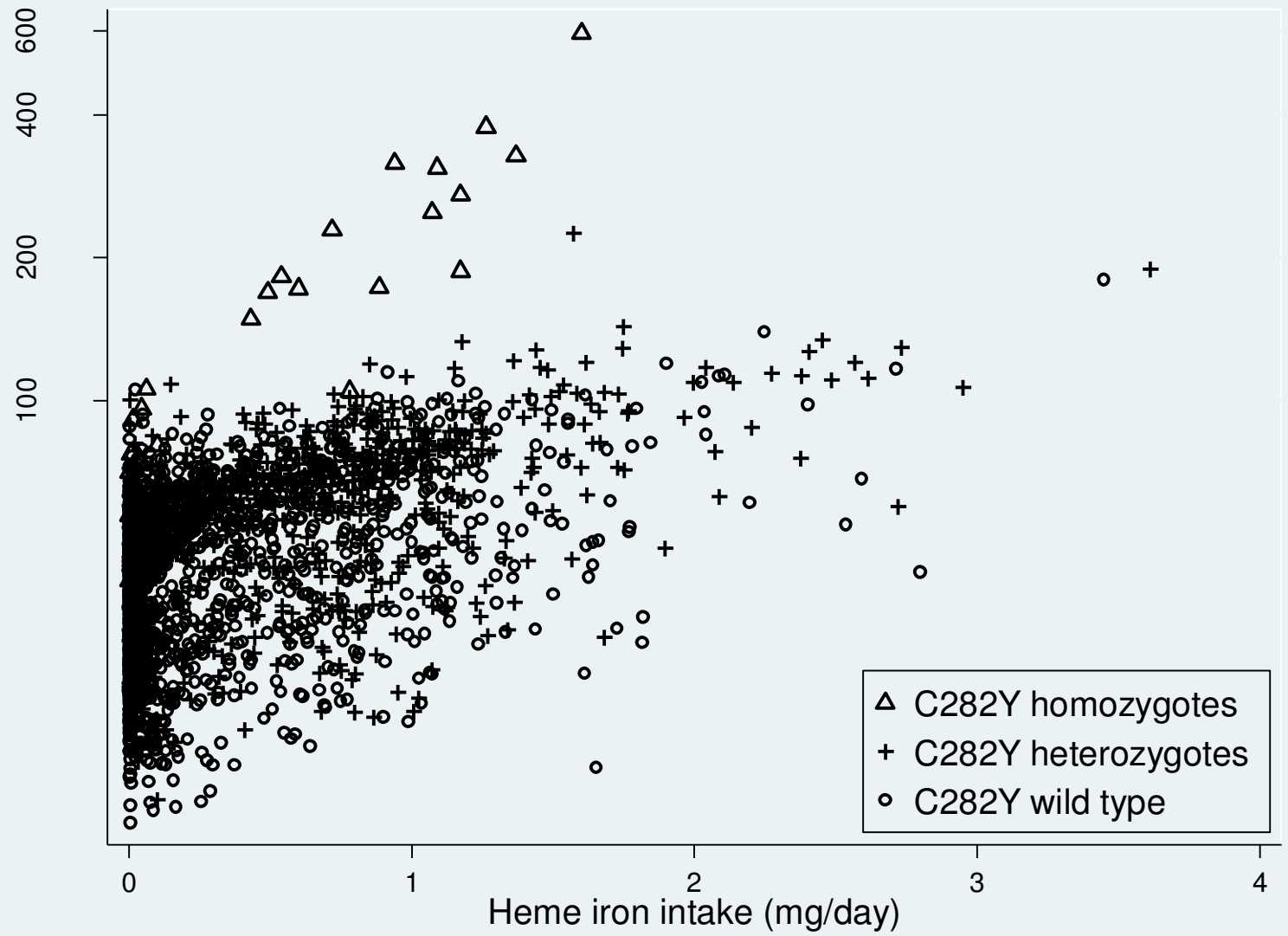
Figure 1. Plot of predicted serum ferritin concentrations for regression model including genotype by heme iron interaction.

Table 1. Iron status by genotype for 2528 women returning blood samples.

Genotype	Reference range	C282Y mutation			H63D mutation			Compound heterozygote
		C282Y / C282Y (n=31)*	C282Y / WT (n=726)*	WT / WT (n=1774)*	H63D / H63D (n=41)*	H63D / WT (n=662)*	WT / WT (n=1832)*	C282Y / H63D (n=137)*
Serum ferritin (µg/l); mean† (95% CI)	12-250	131 (83 - 204)	55 (52 - 58)	44 (43 - 46)	44 (35 - 57)	48 (45 - 51)	48 (46 - 50)	64 (56 - 75)
Serum iron (µmol/l); mean (95% CI)	11-29	34 (31 - 37)	22 (22 - 23)	20 (20 - 21)	23 (21 - 25)	22 (21 - 23)	21 (20 - 21)	25 (23 - 26)
Unbound Iron Binding Capacity (µmol/l); mean (95% CI)	23-65	13 (10 - 16)	30 (30 - 31)	36 (35 - 36)	32 (29 - 36)	33 (32 - 33)	35 (34 - 35)	26 (25 - 28)
Total Iron Binding Capacity (µmol/l); mean (95% CI)	54-80	47 (45 - 49)	53 (52 - 53)	56 (56 - 57)	55 (53 - 58)	55 (54 - 55)	55 (54 - 56)	51 (50 - 52)
Transferrin saturation (%); mean (95% CI)	≤ 50	73 (67 - 79)	43 (42 - 44)	37 (36 - 37)	42 (38 - 46)	41 (40 - 42)	38 (38 - 39)	49 (46 - 51)
Hemoglobin (g/dl); mean (95% CI)	11.5-16.0	14.5 (14.0 - 15.0)	13.9 (13.8 - 14.0)	13.6 (13.5 - 13.6)	13.9 (13.5 - 14.2)	13.7 (13.6 - 13.7)	13.7 (13.6 - 13.7)	14.0 (13.8 - 14.2)
Total dietary iron (mg/day); Mean† (95% CI)	-	16.7 (14.2 - 19.6)	18.0 (17.6 - 18.5)	17.9 (17.7 - 18.1)	18.3 (17.2 - 19.3)	18.0 (17.7 - 18.4)	17.9 (17.7 - 18.1)	18.1 (17.2 - 19.1)
Dietary heme iron (mg/day); Mean† (95% CI)	-	0.19 (0.09 - 0.44)	0.25 (0.22 - 0.29)	0.29 (0.27 - 0.30)	0.37 (0.28 - 0.49)	0.29 (0.27 - 0.32)	0.28 (0.26 - 0.29)	0.30 (0.22 - 0.41)

\* Numbers of people with each genotype refer to sampled individuals providing blood and do not reflect proportions in the general population.

† Geometric mean



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