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The G277S transferrin mutation does not affect iron absorption in iron deficient women

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ABSTRACT

Background. Iron deficiency anaemia is one of the most important nutritional diseases, with high prevalence worldwide. The G277S transferrin mutation has been implicated as a risk factor for iron deficiency in menstruating women. However, the subject is controversial and there are no data concerning the possible influence of this polymorphism on iron absorption.

Aim of the study. To undertake a pilot study to investigate the effect of carrying the G277S transferrin mutation on non-haem iron absorption from a meal in young menstruating women compared to wild-type controls.

Methods. Menstruating women with low iron stores (serum ferritin < 30 µg/L) or who had suffered from iron deficiency anaemia or had a family history of anaemia were recruited (n=162). Haematological parameters were analysed, including haemoglobin, ferritin, total-iron binding capacity and transferrin saturation. Non-haem iron absorption from a meal was measured in 25 non-anaemic women either with the G277S/G277G (n=10) or the wild type G277G/G277G (n=15) genotype. The incorporation of stable isotopes of iron into erythrocytes was used to measure absorption.

Results and Conclusions. There were no significant differences in iron status indices or non-haem iron absorption between genotypes. However, G277S carriers did not show the usual inverse association between iron stores and non-haem iron absorption. Further studies should focus on the effects of a combination of polymorphisms in iron metabolism genes on iron absorption.

KEY WORDS Iron absorption, G277S transferrin mutation, iron deficiency, stable isotopes, humans.

INTRODUCTION

The prevalence of iron deficiency has been estimated to be between 8 and 33% of young women in Europe [1], 10 to 16% in the United States [2], and 42% in developing countries [3].

In addition to dietary factors which strongly affect the amount of non-haem iron absorbed, host-related factors are key determinants of the efficiency of iron absorption in humans [4]. The regulatory mechanism for iron homeostasis is still uncertain, but several proteins, and thus several genes, have been identified as contributors to the process [5]. Genetic variants in the iron regulatory systems influence the likelihood of iron deficiency, as is seen in mouse models [5]. The transferrin G277S variant of the transferrin gene has been found primarily in people of European ancestry [7], with a frequency of 0.0634 (111 of 1752 alleles) and according to Lee et al. [6], the G277S mutation is the only mutation found to influence iron metabolism and predispose menstruating white women to iron deficiency anaemia. However the significance of the mutation is unclear and in pregnant women there was no relationship between iron status or transferrin concentration in G277S carriers compared to wild-type controls [8] and moreover when the mutation was included in a human transferrin expression vector the biological activity of the transferrin was not altered [9].

We investigated the effect of carrying the G277S transferrin mutation on non-haem iron absorption from a meal in non-anaemic iron deficient women compared to wild-type controls.

SUBJECTS, MATERIALS AND METHODS

Subjects

This study was approved by the Ethics Committee of the Hospital Clinica Puerta de Hierro and Spanish Council for Scientific Research, in Madrid (Spain). Volunteer recruitment was carried out through advertisements in the Complutense University campus and by giving short talks about the study between lectures. One hundred and sixty-two, healthy, 18-45 year-old, non-smoking, non-pregnant, non-anaemic (haemoglobin >110g/L), menstruating women with low iron stores (serum ferritin <30 µg/l) or who had suffered from iron deficiency anaemia and had a family history of anaemia underwent a pre-study screening, which included a blood test and health questionnaire. None had taken iron supplements in the 12 months before the start of the study, were blood donors or were taking any medication that could influence iron metabolism.

Haematological analysis

Blood samples were collected by venepuncture into EDTA tubes. Haemoglobin concentration, serum ferritin, haematocrit, mean corpuscular volume, serum transferrin (TRF) and serum iron were determined following standard laboratory techniques and using the Symex NE 9100 automated haematology (Symex, Kobe, Japan) and the Modular Analytics Serum Work Area (Roche, Basel, Switzerland) analyzers. Total iron binding capacity (TIBC (µmol/L) = 25.1 x TRF (g/L)) and transferrin saturation (serum Fe/TIBC x 100) were calculated.

Genetic analysis

DNA sequencing was carried out in order to identify the G277S mutation in the transferrin gene. DNA was extracted from whole blood using standard phenol-chloroform methodology with proteinase K [10]. Amplification of exon 7 was performed using polymerase chain reaction carried out in an eppendorf mastercycler in a 25µl reaction volume containing 1X Standard Buffer (Tris-HCl (pH 9,0) 75mM, KCl 50 mM, (NH₄)₂SO₄ 20 mM), 2.5mM MgCl₂, 200µM dNTPs mix, 1U Taq polymerase (Biotools) and 0.200 µM of each primer. Exon 7 of the transferrin gene was amplified using primers described by Beutler et al [11]. The PCR product was purified with a QIAquick gel extraction kit (QIAGEN) and sequenced on an Applied Biosystems automatic sequencer model 3730.

Iron absorption study

Ten out of the sixteen subjects with the G277S mutation completed the study. Two dropped out and four showed incompatibility with study conditions and could not attend the Instituto del Frío (Madrid) for test meals. Fifteen wild-type subjects were selected using a simple randomized procedure and completed the study.

A meal containing 50 g of red kidney beans (raw weight) and 100 g (cooked weight) of cooked salmon fish was prepared using standardized techniques. The meal choice was made to be realistic and practical, beans are a typical Spanish meal, that is widely consumed mainly in combination with fish or meat, and the amounts of beans and fish used are average portion sizes.

Total iron content of the meal was 4.6±0.2 mg. The non-haem iron content of the meal was estimated to be 96% of the total iron [12].

Non-haem iron in the meal was extrinsically labelled by simultaneously consuming isotopically enriched iron in 100 ml of an isotonic drink (Powerade, Coca-Cola Company, Madrid) with the meal. The drink contained citric acid as a preservative and antioxidant and its pH was 3.56. Labelled ferric chloride solutions were prepared by weighing 0.4980 g and 0.1609 g of elemental Fe-57 and Fe-58 respectively (Chemgas, Boulogne, France), adding 8.9 mL and 9.5mL of 6M HCl respectively and heating the mixture slowly to dryness. The resulting powder was dissolved in 5mL of 0.01M HCl and left overnight. The solutions were made up to 100mL with Milli-Q water and were filtered through a 0.22 μ m filter (Osmonics, Lancashire, United Kingdom). The iron chloride solutions were sub-sampled into 1mL individual doses with final concentrations of 5 mg/mL of Fe-57 and 1.67 mg/mL of Fe-58. The final total iron content of the extrinsically labeled meals was adjusted to the same amount using unlabelled ferric chloride solutions prepared from ultrapure iron powder (Sigma-Aldrich, Steinheim, Germany).

On day 1, a baseline blood sample was taken to measure basal iron isotope contents and iron-status indices. On three consecutive days volunteers attended Instituto del Frio, after an overnight fast, and consumed the bean and fish meal extrinsically labeled with 5mg or 1.67 mg of Fe-57 and Fe-58, respectively. G277S/wild and wild type volunteers were randomly assigned to consume the meal with either Fe-57 or Fe-58.

Fourteen days after the labeled meal was consumed, a blood sample was taken and iron absorption from the test meal was determined by measuring the enrichment of Fe-57 and Fe-58 in red blood cells following the method by Roe et al [13]. Absorption was calculated from the enrichment of isotopes in the blood, the total iron concentration in whole blood and an estimate of blood

volume [14]. Isotope ratio analysis was carried out on a focusing Multi Collector Mass Spectrometer (MC-ICP-MS) (Isoprobe; Micromass, Manchester, United Kingdom). The iron content of whole blood ($\mu\text{g/mL}$) was calculated as: haemoglobin (g/dL) \times 34.7

Statistical analysis

In order to detect a difference in iron absorption of 5% with a standard deviation of 4%, $\alpha=0.05$ and power calculation of 80%, a sample size of 15 subjects per group was estimated.

Absorption data were analyzed by using a Student's T-test and the SPSS statistical package (version 13.0). Values of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Very few studies have focused on genetic disorders as an explanatory variable for risk of iron deficiency. To our knowledge, the possible influence of the G277S transferrin mutation on iron absorption has not yet been investigated. There were no significant differences in iron status indices between the genotype groups nor in iron absorption, $p=0.279$ (table 1). There was no significant effect of isotope (Fe-57 or Fe-58) used to label the meal on iron absorption (Fisher's test: $p=0.678$). G277S carriers had a wider range in iron absorption values than the wild type and only 10 G277S subjects completed the trial. The data obtained yielded a difference in absorption of 1.9, eg. $0.48 \times \text{SD}$, which was not significant and would not have been significant with 15 subjects in each group. A post-hoc power calculation using data generated from the study gives an estimated sample size of 70 G277S carriers for a statistical

power of 80% to confirm the apparent influence of the mutation. Interestingly, there was the expected significant negative correlation between tissue iron stores (log serum ferritin) and iron absorption in the wild-type group (Fig. 1, $R^2 = 0.636$, $p < 0.001$), but not in the G277S heterozygotes (R^2 , 0.476, $p > 0.05$). This could, however, be due to the small number in the group.

Our results are in agreement with studies performed in pregnant women who did not show any particular relationship between the G277S polymorphism and iron status compared to wild-type controls [8], and with the in vitro results by Aisen [9]. However, we cannot exclude the possibility that there may be a subtle effect of the G277S mutation on iron absorption. Further research is required on the effects of a combination of polymorphisms on iron absorption in relation to increased risk of iron deficiency.

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Table 1. Anthropometric, haematological and iron absorption data of the subjects¹.

	Genotype	
	G277G/G277G (n=15)	G277S/G277G (n=10)
Age (y)	23.7±3.0	24.9±5.8
Height (cm)	166.9±6.0	163.8±3.1
Weight (kg)	61.9±6.0	59.8±7.5
Body Mass Index (kg/m ²)	22.2±2.0	22.3±2.8
Haemoglobin (g/dL)	13.1±1.0	13.2±0.7
Ferritin(µg/L)*	13.7 (5.7-21.7)	19.1 (6.4-31.8)
Haematocrit (%)	39.5±2.6	39.8±1.9
Mean corpuscular volume (fl)	87.4±4.6	87.1±5.5
Total-iron binding capacity (µmol/L)	79.6±13.8	71.3±11.1
Serum iron (µmol/L)	15.6±6.1	13.0±4.0
Transferrin saturation (%)	22.2±8.5	20.4±6.3
Iron absorption (%)	6.8±4.0	4.9±3.6

¹All values are arithmetic mean ± standard deviation except * which is geometric mean (0.5-0.95% CI).

There were no significant differences between groups.

Figure 1. Relation between log serum ferritin and iron absorption in wild-type and G277S/wild groups.

▲, _____ wild genotype group ($R^2 = 0.643$, $p < 0.001$)

□, ----- G277S/wild genotype group ($R^2 = 0.234$, NS)

