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### Authors

Mast, Alan E  
Lee, Tzong-Hae  
Schlumpf, Karen S  
[et al.](#)

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## The impact of *HFE* mutations on haemoglobin and iron status in individuals experiencing repeated iron loss through blood donation

Alan E. Mast<sup>1,2</sup>, Tzong-Hae Lee<sup>3</sup>, Karen S. Schlumpf<sup>4</sup>, David J. Wright<sup>4</sup>, Bryce Johnson<sup>4</sup>, Danielle M. Carrick<sup>4</sup>, Ritchard G. Cable<sup>5</sup>, Joseph E. Kiss<sup>6</sup>, Simone A. Glynn<sup>7</sup>, Whitney R. Steele<sup>4</sup>, Edward L. Murphy<sup>8</sup>, Ronald Sacher<sup>9</sup>, and Michael P. Busch<sup>3</sup> for the NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II)

<sup>1</sup>Blood Research Institute, Blood Center of Wisconsin, Milwaukee, WI

<sup>2</sup>Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI

<sup>3</sup>Blood Systems Research Institute, San Francisco, CA

<sup>4</sup>Westat, Rockville, MD

<sup>5</sup>New England Region, American Red Cross Blood Services, Farmington, CT

<sup>6</sup>Institute for Transfusion Medicine, Pittsburgh, PA

<sup>7</sup>Transfusion Medicine and Cellular Therapies, National Heart, Lung and Blood Institute, Bethesda, MD

<sup>8</sup>University of California, San Francisco and Blood Systems Research Institute, San Francisco, CA

<sup>9</sup>Hoxworth Blood Center, University of Cincinnati Academic Health Center, Cincinnati, OH

### Summary

Frequent blood donors become iron deficient. *HFE* mutations are present in over 30% of donors. A 24-month study of 888 first time/reactivated donors and 1537 frequent donors measured haemoglobin and iron status to assess how *HFE* mutations impact the development of iron deficiency erythropoiesis. Donors with two *HFE* mutations had increased baseline haemoglobin and iron stores as did those with one mutation, albeit to a lesser extent. Over multiple donations haemoglobin and iron status of donors with *HFE* mutations paralleled those lacking mutations. The prevalence of *HFE* mutations was not increased in higher intensity donors. Thus, in general, *HFE* mutations do not temper donation-induced changes in haemoglobin and iron status. However, in Black donors there was an increase of H63D carriers at baseline, from 3.7% in first time/reactivated donors to 15.8% in frequent donors, suggesting that the relative effects of *HFE* mutations on iron absorption may vary between racial/ethnic groups. In secondary analyses, venous haemoglobin decreased more slowly in donors with ferritin  $\geq 12 \mu\text{g/l}$ ; and haemoglobin recovery time was shorter in donors with reticulocyte haemoglobin (CHR)  $\geq 32.6 \text{ pg}$ , indicating that these biochemical measures are better indicators of a donor's response to phlebotomy than their *HFE* mutation status.

## Keywords

*HFE*; blood donor; iron deficiency; haemoglobin; ferritin

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## Introduction

In the United States the minimal allowable interval between whole blood donations is 56 days and applies to all allogeneic donors without regard to sex, age, race/ethnicity or other demographic factors, as long as their haemoglobin is  $\geq 125$  g/l or their haematocrit is  $\geq 38\%$ . Each blood donation results in the loss of 200 and 250 mg of iron and high frequency donors are prone to depletion of iron stores and iron deficiency anaemia (Cable *et al*, 2011). One means for preventing iron deficiency in donors is to develop individualized donation intervals for different demographic groups. For example, the minimum inter-donation interval is longer for women than men in some countries (Karp & King, 2010).

A study of high intensity blood donors who donate blood six times per year without deferral for low haemoglobin revealed that this is a self-selected population that is resistant to development of blood donation-induced anaemia (Mast *et al*, 2008a). This finding was confirmed and quantified in a large multicentre study of haemoglobin deferral of blood donors that demonstrated the odds for deferral of female donors with six successful whole blood donations in the previous 12 months were less than one-half that of females with only one donation (Mast *et al*, 2010). While there are several potential explanations for the self-selection of these individuals, including the use of oral iron supplements, it is possible that many of them have genetic polymorphisms that allow for increased absorption of dietary iron, particularly when iron stores are depleted. One candidate is *HFE*, a gene that when mutated can produce hereditary haemochromatosis (Feder *et al*, 1996). Patients homozygous for mutations in *HFE* do not produce adequate amounts of hepcidin, the hormone that dampens dietary iron absorption in response to elevated iron stores (Nicolas *et al*, 2001; Nemeth & Ganz, 2006). Therefore, they absorb dietary iron regardless of available iron stores and may develop life-threatening iron deposits in the heart and liver.

The two most common mutations in *HFE* are C282Y and H63D. Carriers for these mutations are very prevalent (10% and 24%, respectively) in Whites (Adams *et al*, 2005). These individuals have slightly altered biochemical measures of iron metabolism in peripheral blood tests, such as ferritin, an indicator of total body iron stores, but the values remain within normal reference intervals, and they do not develop iron overload (Beutler *et al*, 2003; Jackson *et al*, 2001). Although the C282Y mutation has a greater impact on iron balance than H63D, high intensity donors that carry the H63D mutation have decreased hepcidin to ferritin ratios when compared to those without the mutation (Mast *et al*, 2008a). A separate study found that the prevalence of H63D was higher among female than male donors (Konig *et al*, 2003). These data suggest that carriers of H63D may absorb more dietary iron when iron stores are depleted than those without the mutation, potentially protecting them from blood donation-induced iron deficiency anaemia. Despite these findings, several studies have determined that the prevalence of carriers for *HFE* mutations in high intensity blood donors is similar to the general population, rather than increased, as would be expected, if these mutations allowed individuals to repeatedly donate blood without development of anaemia (Mast *et al*, 2008a; Konig *et al*, 2003; Boulton *et al*, 2000).

The REDS-II donor Iron Status Evaluation (RISE) study was a 24 month, multicentre, longitudinal study of iron metabolism and anaemia in 2425 blood donors performed to assess demographic, behavioural, genetic and donation intensity determinants that influence the development of iron deficiency and iron deficiency anaemia in blood donors. The results

from this study suggest beneficial changes for how donors can be managed to prevent iron deficiency, such as altering the inter-donation interval and/or recommending use of iron supplements. As carriers of *HFE* mutations are prevalent within the blood donor population and there is potential for these mutations to alter dietary iron absorption, we undertook a detailed study to determine how *HFE* mutations impact measures of iron metabolism and haemoglobin in RISE subjects as they underwent the stress of repeated iron loss from blood donation.

## Methods

### Participating Blood Centres

The data presented here were collected as part of the National Heart, Lung and Blood Institute (NHLBI) Retrovirus Epidemiology Donor Study-II (REDS-II). There are six centres that participate in REDS-II: Blood Center of Wisconsin (Milwaukee, WI); the American Red Cross New England Region (Dedham, MA); Hoxworth Blood Center/ University of Cincinnati Academic Health Center (Cincinnati, OH); American Red Cross Southern Region (Douglasville, GA); Blood Centers of the Pacific (San Francisco, CA); and the Institute for Transfusion Medicine (Pittsburgh, PA). The REDS-II centres represent geographically and demographically diverse populations and collectively account for over 8% of annual blood collections in the United States. To keep the identity of each blood centre anonymous within the analysis, centres are referred to with alphabetical notation (A-F) in the results. The REDS-II coordinating centre is Westat (Rockville, MD) and Blood Systems Research Institute (San Francisco, CA) serves as the REDS-II Central Laboratory.

### Study Participants

Participants were recruited at all six REDS-II centres as part of the longitudinal, multicentre study called RISE conducted between December 2007 and December 2009. A detailed description of the RISE study has previously been published (Cable *et al*, 2011). There were four cohorts recruited in RISE: female and male first time/reactivated (first time donor or donor with no donations in the previous two years) cohorts and female and male frequent blood donor (men with at least three, and women with at least two donations in the previous year) cohorts. Participants were followed for 15 to 24 months in the longitudinal phase of the study. At enrollment, all subjects completed a survey that assessed smoking history, menstrual/reproductive history (females only), diet and use of vitamins/iron supplements. Race or ethnic group was determined by self-reported answer in the survey. All subjects were encouraged to donate frequently (males at least three and women at least two times per year) during the study period and blood samples were collected at each visit for laboratory testing. In addition, subjects were requested to return to complete a final questionnaire and provide a blood sample at the end of the study period.

### Laboratory Testing

Blood samples obtained at enrollment were tested for *HFE* mutation status. Determination of C282Y and the H63D mutations in the *HFE* gene, as well as the G277S polymorphism in transferrin (*TF*) (Lee *et al*, 2001), was performed using DNA isolated from frozen whole blood samples and SyBr green based real-time polymerase chain reaction (PCR) using techniques similar to those previously published (Gill *et al*, 2008). For each single nucleotide polymorphism (SNP) two pairs of primers differing by only a single nucleotide were designed; one specific to the wild type sequence and another specific to the mutation sequence. The two primer pairs were then used simultaneously in separate PCR amplifications of donor DNA. After amplification, the polymorphisms were determined by assessment of the difference in amplification efficiency between the two primer pairs. If both primer pairs were equally efficient in amplification of wild type and mutation

sequences (Cycle threshold difference ( $\Delta Ct$ ) < 2 cycles), the donor was classified as heterozygous; if one primer pair had high efficiency and the other low efficiency ( $\Delta Ct$  > 5 cycles), the sample was determined to be homozygous for the primer sequence with high amplification efficiency. After the PCR amplification conditions were optimized, the assays were validated by analysing a coded panel of 44 blood donors and 6 haemochromatosis patient samples. Four of the haemochromatosis patients tested as homozygous for C282Y and one as homozygous for H63D. To further validate the performance of these assays, we sequenced samples obtained from Dr. Ernest Beutler (San Diego, CA). These included 13 without *HFE* mutations, 2 heterozygous for C282Y, 4 homozygous for C282Y, 7 heterozygous for H63D and 1 homozygous for H63D. The sequence results were 100% identical with data obtained from the real time PCR assay. PCR testing of the 2425 RISE subjects was successful for all except for 3 samples tested for the C282Y genotype and 6 samples tested for the H63D genotype, for which sequencing was performed to obtain the genotype.

Plasma ferritin, plasma soluble transferrin receptor (sTfR) and complete blood count (CBC) with reticulocyte indices were measured on enrollment samples and on additional samples obtained during blood donations throughout the study. Ferritin, a measure of total body iron stores, was determined by immunoassay (ADVIA Centaur, Siemens Healthcare Diagnostics, Deerfield, IL). sTfR, a measure of cellular iron depletion, was determined turbidimetrically (Tina-quant sTfR assay, Roche Diagnostics, Indianapolis, IN). CBC with reticulocyte indices were measured using Advia 2120 or Advia 120 Hematology Autoanalyzers (Siemens) at four of the six centres (Milwaukee, Cincinnati, New England and Pittsburgh). Reticulocyte analysis included determination of the reticulocyte haemoglobin content (CHr), a measure of iron available for haemoglobin synthesis over the previous four days that detects iron-restricted erythropoiesis (Mast *et al*, 2008b). Fingerstick haemoglobin or haematocrit measurements were obtained to qualify subjects for blood donation using methods that varied at different centres as previously described (Mast *et al*, 2010).

## Statistical Analyses

Descriptive analyses include box and whisker plots; displaying the mean (solid bar), the 25<sup>th</sup> and 75<sup>th</sup> percentiles (box), and the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles (whiskers).

The *HFE* genotype distribution of first time/reactivated blood donors enrolled in the RISE study were compared to that of subjects enrolled in the Haemochromatosis and Iron Overload Screening (HEIRS) study (Adams *et al*, 2005) using chi-square statistics. The HEIRS study screened 99,711 primary care patients for *HFE* genotype from five field centres in the United States and Canada and was chosen for comparison to RISE subjects because of the large number of multiethnic subjects enrolled at geographically diverse sites across North America. The comparison was adjusted for race/ethnicity because the HEIRS study oversampled minorities. The first time/reactivated RISE donors were compared to frequent RISE donors using Fisher's exact tests.

Those with two *HFE* mutations (double heterozygous H63D and C282Y, and homozygous mutations) were combined into one group to increase power for some statistical analyses. The haemoglobin and iron measures of interest were CHr, venous haemoglobin, sTfR, ferritin and log (sTfR/ferritin). Baseline analysis (using enrollment visit data only) of each measure (on the log<sub>10</sub> scale for sTfR, ferritin, and sTfR/ferritin ratio and untransformed for CHr and haemoglobin) was done using analysis of variance (ANOVA). However, ANOVA models only indirectly measured longitudinal donation effects by comparing first time/reactivated donors with frequent donors.

To directly measure longitudinal donation effects (due to both donation intensity and recovery time), multivariate repeated measures regression models were developed for each haemoglobin/iron measure. The covariates in these repeated measures models were selected from the statistically significant predictors in the baseline analysis of RISE (Cable *et al*, 2011). The models included longitudinal effects for donation variables that changed at each donation (i.e. dynamic variables); recovery time measured as “time since last donation (in weeks)”, and donation intensity measured as “number of donations in the past 24 months.” Additional covariates were applied as used previously and include race/ethnicity, gender, age, weight, smoking, iron supplementation, menstrual status, pregnancy history, *HFE* genotype, *TFG277S* transferrin genotype (Lee *et al*, 2001), and centre.

All analyses were done using SAS (SAS 9.2 (2008) SAS Institute Inc, Cary NC).

## Results

### Study Population

There were 887 first time/reactivated donors (481 females and 407 males) and 1538 frequent blood donors (769 females and 768 males) for a total of 2425 subjects enrolled. The demographic characteristics of enrolled subjects, including the overall prevalence of the *HFE* mutations, are presented in Table I. According to self-reported race and ethnicity, 76 Asians (3.1%), 116 Blacks (4.8%), 76 Hispanics (3.1%), 2,111 Whites (87.1%) and 29 (1.2%) who reported their race as ‘Other’ were enrolled in the study. Race/ethnicity data was missing for 17 (0.7%) participants. The ethnic/racial distribution of study participants reflects that of blood donors at the six REDS-II blood centres (Mast *et al*, 2010). In the longitudinal phase of the study, 2,155 (88.9%) subjects returned to donate blood at least one additional time and 1,340 (55.3%) returned for a final visit at the end of the study.

### HFE genotype of participants

The prevalence of the different combinations of *HFE* mutations for first time/reactivated and frequent donors separated by race/ethnicity is presented in Table II. There were 39 donors homozygous for H63D, 7 donors homozygous for C282Y and 41 double heterozygous donors enrolled in the study. The previously reported prevalence of these mutations in the HEIRS study (Adams *et al*, 2005) is included in Table II for comparison with the RISE dataset. Comparison of the frequency of the *HFE* mutations in the individual race/ethnicity groups of the first time/reactivated RISE and HEIRS datasets revealed an overall difference in *HFE* frequencies ( $p=0.0006$ ); 76.0% of HEIRS study subjects showed no *HFE* mutations, whereas only 66.7% of first time/reactivated RISE donors showed no *HFE* mutations. Contrasts to identify specific racial/ethnic group differences found differences between Hispanic first time/reactivated RISE donors and HEIRS subjects ( $p=0.02$ ); with 67.5% and 79.9%, respectively, showing no *HFE* mutations. Comparison of the frequency of the *HFE* mutations in the individual race/ethnicity groups of the first time/reactivated RISE donors and frequent RISE donors revealed no overall difference in *HFE* frequencies ( $p=0.09$ ). However, contrasts to identify specific racial/ethnic group differences found differences between Black donors ( $p=0.01$ ); the prevalence of H63D carriers increased from 2 of 53 (3.8%) in first time/reactivated Black donors to 10 of 63 (15.8%) in frequent Black donors.

### Impact of HFE mutation status on baseline iron status in first-time/reactivated and frequent donors

An initial assessment of the effect of *HFE* mutations on the haemoglobin and iron status of blood donors was obtained through ANOVA of baseline CHr, sTfR, ferritin, sTfR/ferritin and haemoglobin values in first time/reactivated and frequent donors according to their *HFE* genotype. These data are presented in box and whisker plots in Fig 1A-E. Comparison of

these values between first time/reactivated and frequent donors that do not carry an *HFE* mutation showed that all are altered, demonstrating that repeated blood donation produces decreased iron stores, as evidenced by decreased ferritin (Fig 1D), increased sTfR (Fig 1C) and most notably increased  $\log(\text{sTfR}/\text{ferritin})$  (Fig 1E), and decreased haemoglobin, as evidenced by decreased CHr (Fig 1A) and decreased venous haemoglobin (Fig 1B). In general, similar changes were present for all measures of haemoglobin and iron status when comparing first time/reactivated and frequent donors with *HFE* mutations, suggesting that the *HFE* mutations do not provide a protective effect from blood donation-induced anaemia and iron deficiency. A second ANOVA adjusting for race/ethnicity, gender, menstrual status, age, weight, and centre showed similar *HFE* genotype by donor status differences (data not shown). As these baseline analyses were performed comparing first time/reactivated donors with frequent donors (as a surrogate for a longitudinal effect of repeated donations), additional analyses of longitudinal data were performed to examine changes of haemoglobin and iron status within individual donors as they experienced the stress of repeated blood donation.

### Impact of HFE mutation status on iron status in longitudinal analysis

Repeated measures regression models were developed to determine the impact of *HFE* mutations on five primary outcome measures: 1) CHr; 2) venous haemoglobin; 3)  $\log_{10}$  sTfR; 4)  $\log_{10}$  ferritin; and 5)  $\log_{10}$  (sTfR/ferritin) (Table III). In each of the regression models the *HFE* mutations have a significant impact on the five measures of haemoglobin and iron balance pre-donation (p-values <0.0001 for all except ferritin with p-value of 0.003). For example, after adjusting for other factors, CHr was higher by 0.40, 0.50 and 1.76 pg in carriers of H63D, carriers of C282Y and those with two mutations, respectively, when compared to wild type donors. Similarly,  $\log_{10}$  ferritin was higher than that in wild type donors by 0.02, 0.04 and 0.13 (equivalent to increases of 5%, 10% and 35%) in carriers of H63D, carriers of C282Y and those with two mutations, respectively. The magnitude of these changes can be compared to differences between males and females in the same regression models to better understand the effects of the *HFE* mutations (Table III). Males have higher CHr (+0.41g) and 100% higher ferritin (0.30  $\log_{10}$  ferritin) compared to female donors. As a result, the impact of carrier status for the *HFE* mutations on these two measures are of approximately equal magnitude as the impact of gender, where having two mutations has the largest impact among the *HFE* mutations.

Additionally, the models included a donation intensity (“number of donations in the past 24 months”) by *HFE* genotype interaction to test if the longitudinal donation effect varied by *HFE* genotype (e.g. the iron stores of a favourable *HFE* genotype donor may not decline with repeated donations while the iron stores of a donor without the C282Y or H63D polymorphisms may indeed decline with repeated donations). This interaction effect is exhibited in Fig 2. In carriers of the *HFE* mutations each of the five measures trended roughly in parallel with those of donors without a mutation indicating that *HFE* carrier status does not protect donors from blood donation-associated alterations in haemoglobin and iron status (while patterns were similar,  $\log_{10}(\text{ferritin})$  (p=0.01, Fig 2D) and  $\log_{10}(\text{sTfR}/\text{ferritin})$  (p=0.02, Fig 2E) had statistically significant interactions, meaning trends are not parallel). Similarly, those with two mutations are not protected from blood donation-associated alterations in haemoglobin and iron status. Although these donors had greater initial measures of haemoglobin and iron stores, the impact of donation intensity was similar for all genotypes. Consistent with these findings the prevalence of *HFE* mutations was not increased in high intensity donors (>10 donations in the previous 24 months) participating in RISE (data not shown).

The impact of the *HFE* mutations on changes in haemoglobin and iron parameters with time since last donation was also analysed. These interactions were not statistically significant in any of the haemoglobin or iron parameter models (data not shown).

### Impact of iron stores on haemoglobin status

Given that donors with *HFE* mutations have higher iron stores and haemoglobin values than other donors, a secondary haemoglobin repeated measures regression model was postulated, where the longitudinal donation effect depends on iron stores (Fig 3). Specifically, the donation intensity by *HFE* interaction of Table II was replaced with a donation intensity by baseline ferritin interaction and a time since last donation by baseline CHr interaction. When the donor's initial ferritin was  $\geq 12 \mu\text{g/l}$ , venous haemoglobin decreased significantly more slowly with repeated donation than in those with ferritin  $<12 \mu\text{g/l}$  (Fig 3A). When the donor's CHr was  $>32.6 \text{ pg}$ , their post-donation venous haemoglobin returned to baseline more rapidly (14–19 weeks) than in those with CHr was  $\leq 32.6 \text{ pg}$  (26+ weeks) (Fig 3B), demonstrating that these measures of iron stores and haemoglobin, which are often elevated in those with *HFE* mutations, have a significant impact on an individual donor's ability to repeatedly donate blood without iron deficient erythropoiesis or low haemoglobin deferral.

### Discussion

The relationship between the presence of *HFE* mutations and haemoglobin values and iron status was examined in a longitudinal study of individuals experiencing repeated iron loss through blood donation over a period of 15 to 24 months. It has previously been shown that individuals with iron deficiency anaemia have increased dietary iron absorption in response to their anaemia, and that the rate of dietary iron absorption in anaemic individuals is equal in those with or without *HFE* mutations (Beutler *et al*, 2003). In contrast, a study of 235 donors found that those with two *HFE* mutations maintained higher iron stores with frequent blood donation than donors without two *HFE* mutations (Boulton *et al*, 2000). Based on findings from these studies, we originally hypothesized that *HFE* mutations would slow the onset of iron deficient erythropoiesis in blood donors by allowing increased dietary iron absorption when they had depleted iron stores but had not yet developed anaemia. Thus, we performed continuous longitudinal analyses of data from the RISE study to assess the impact of *HFE* mutations on the gradual change in haemoglobin and iron stores. However, we were not able to confirm our hypothesis as measures of both haemoglobin and iron status changed essentially in parallel among donors with or without *HFE* mutations as they underwent repeated blood donation. Because of these parallel rates of change, with repeated donation those with *HFE* mutations will eventually succumb to iron deficient erythropoiesis in a manner similar to those without the mutations.

As expected, those with two *HFE* mutations had increased haemoglobin and iron stores at baseline. Perhaps more interestingly, carriers of either the C282Y or the H63D *HFE* mutations, who make up over 30% of the blood donor pool, also have increased haemoglobin and iron stores, albeit to a lesser extent, than those with two *HFE* mutations (Fig 2). These findings confirm previous reports demonstrating mildly increased ferritin and transferrin saturation in carriers of *HFE* mutations that is associated with a decreased prevalence of non-anaemic iron deficiency in carriers of the C282Y mutation (Beutler *et al*, 2003). The higher baseline levels of haemoglobin and iron stores suggest that donors with *HFE* mutations will initially be able to donate more frequently than other donors before development of iron deficiency erythropoiesis. This was indirectly assessed by examining how venous haemoglobin changes with increasing donation intensity, based on baseline ferritin and how long the recovery time for venous haemoglobin is following donation based on CHr at donation. These analyses indicate that donors lacking bone marrow iron stores, defined as ferritin  $<12 \mu\text{g/l}$ , have greater decreases in venous haemoglobin than donors with



higher ferritin. Conversely, donors with high iron availability for new red blood cell synthesis, defined as CHr  $\geq 32.6$  pg, have shorter post-donation venous haemoglobin recovery time than donors with lower CHr. These findings are consistent with the presence of *HFE* mutations allowing first time donors to initially donate more frequently than first time donors without *HFE* mutations. However, there are many factors that can influence the baseline haemoglobin and iron status of blood donors besides *HFE* mutation status. Thus, ferritin, CHr, or perhaps other measures of iron status, are better choices for the initial assessment and monitoring iron status of frequent blood donors and for making recommendations for individualized donation frequency intervals or iron supplementation that will limit development of iron deficiency in blood donors. In this regard, diagnostic testing for plasma hepcidin has been recently developed and is undergoing study to determine situations where it will be clinically useful (Ganz *et al*, 2008). One such situation may be assessment of iron status in blood donors.

We have previously reported that high intensity blood donors who donate blood six times per year are a self-selected population that is resistant to the development of iron deficiency anaemia (Mast *et al*, 2008a; Mast *et al*, 2010). However, even in the large population of donors studied here, the prevalence of donors with *HFE* mutations was not different among the high intensity and lower intensity donors. This finding is consistent with previously published studies of smaller numbers of donors (Konig *et al*, 2003; Boulton *et al*, 2000) and confirms that it is unlikely that these mutations are responsible for potential genetic contributions to the ability of high intensity donors to repeatedly donate blood without developing iron deficiency anaemia. Another mutation studied in RISE is *TFG227S*. This mutation is associated with a reduction in total iron binding capacity and is a risk factor for iron deficiency anaemia in menstruating White women (Lee *et al*, 2001). However, it did not have an impact on haemoglobin or iron balance in the baseline or longitudinal analyses performed in RISE (Table III). Another interesting, recently identified candidate gene that may alter iron and haemoglobin status in blood donors is *TMPRSS6*, a membrane-associated serine protease that regulates hepcidin production and, therefore, indirectly regulates dietary iron absorption (Du *et al*, 2008). A polymorphism in *TMPRSS6* associated with decreased iron stores and haemoglobin has been identified in four genome-wide association studies (Benyamin *et al*, 2009; Chambers *et al*, 2009; Tanaka *et al*, 2010; Ganesh *et al*, 2009). Further studies are needed to assess how this polymorphism alters risk for development of iron deficiency anaemia in frequent blood donors and whether or not it is selected for in high intensity donors.

Although the analyses performed here did not detect a significant impact of *HFE* mutations on iron status in the general donor population, examination of the frequency of different *HFE* genotypes in first time and repeat donors by race showed that H63D carriers were four-fold more prevalent in the frequent Black donors than would be expected based on the prevalence of H63D in first time/reactivated donors or in the HEIRS study (Adams *et al*, 2005). These data suggest that Black carriers of the H63D mutation may be more resistant to development of anaemia when under the stress of repeated blood donation than are Blacks that do not carry this mutation. There are numerous adaptive changes in iron metabolism and haemoglobin production that have occurred in different human gene pools and Blacks have lower average haemoglobin than other racial/ethnicity groups (Beutler & West, 2005). Although this is a preliminary finding that deserves further study, the selection for H63D carriers in frequent Black donors was not present in other races and suggests that H63D may interact with other genetic polymorphisms prevalent in Blacks to significantly increase dietary iron absorption and make this racial group resistant to anaemia. Unfortunately, there were not sufficient Black subjects enrolled in RISE for additional meaningful analyses of the potential significance of this finding to be conducted in the longitudinal phase of the study. Hispanic donors in RISE also had increased prevalence of H63D when compared to HEIRS,

but this increase was present in both the first time/reactivated and frequent donors, and is not surprising because Hispanics have a great deal of variation in *HFE* genotype across geographic regions in the United States (Acton *et al*, 2006).

These studies of the impact of *HFE* mutation status on the haemoglobin and iron balance in blood donors have found that although the mutations produce initially elevated haemoglobin and iron stores, there is no effect on their rate of decline when individuals are subjected to haemoglobin and iron loss with repeated blood donation. These findings indicate that *HFE* mutation status of blood donors should not be used as a determinant of individualized donation intervals to prevent iron deficiency in frequent blood donors. Utilization of CHr, ferritin or other measures of iron status to assess the current haemoglobin or iron stores of donors represent are better choices for making individualized recommendations for donation intervals or iron supplementation to frequent blood donors.

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Tzong-Hae Lee performed genotyping studies.

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Ritchard G. Cable designed the research study.

Joseph E. Kiss designed the research study.

Simone A. Glynn designed the research study.

Whitney R. Steele managed the research study and performed statistical analyses.

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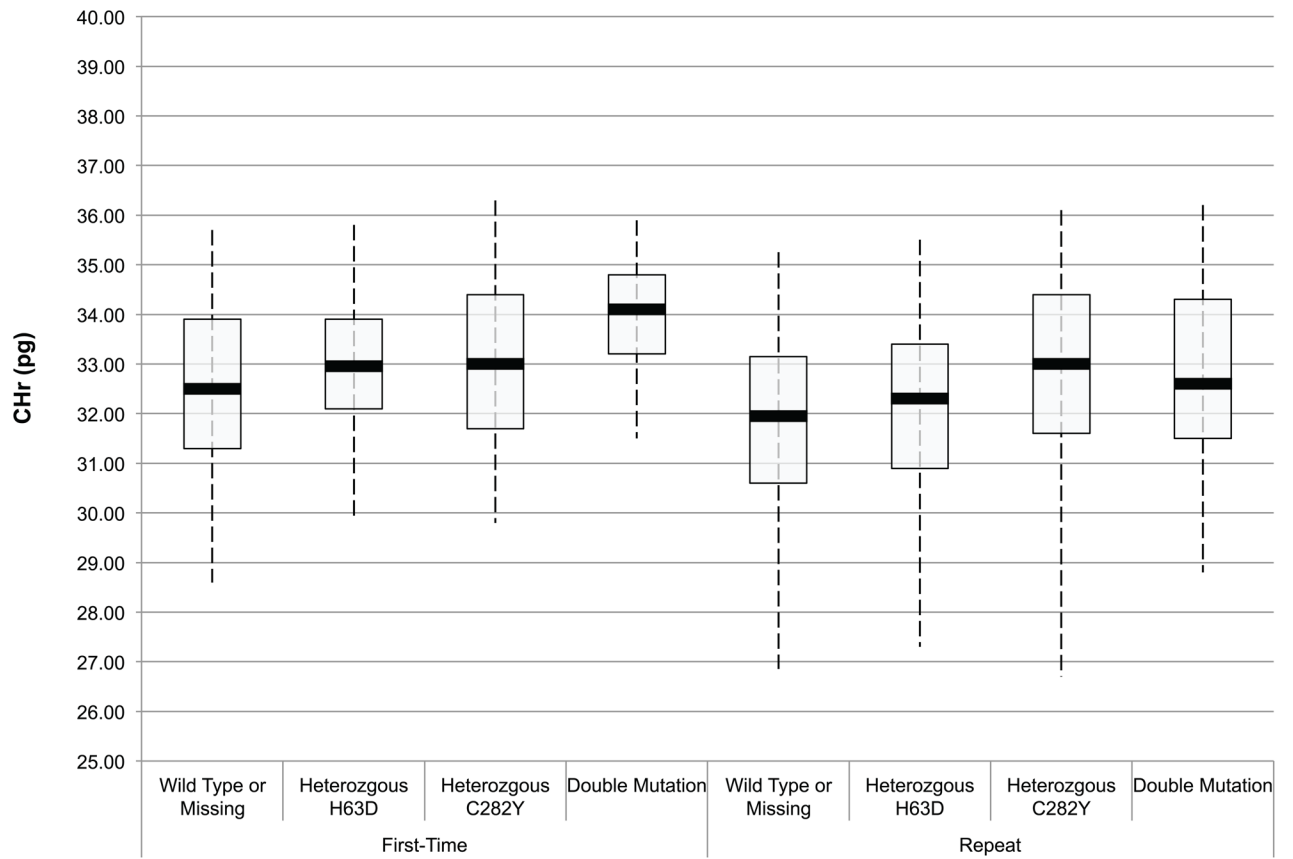
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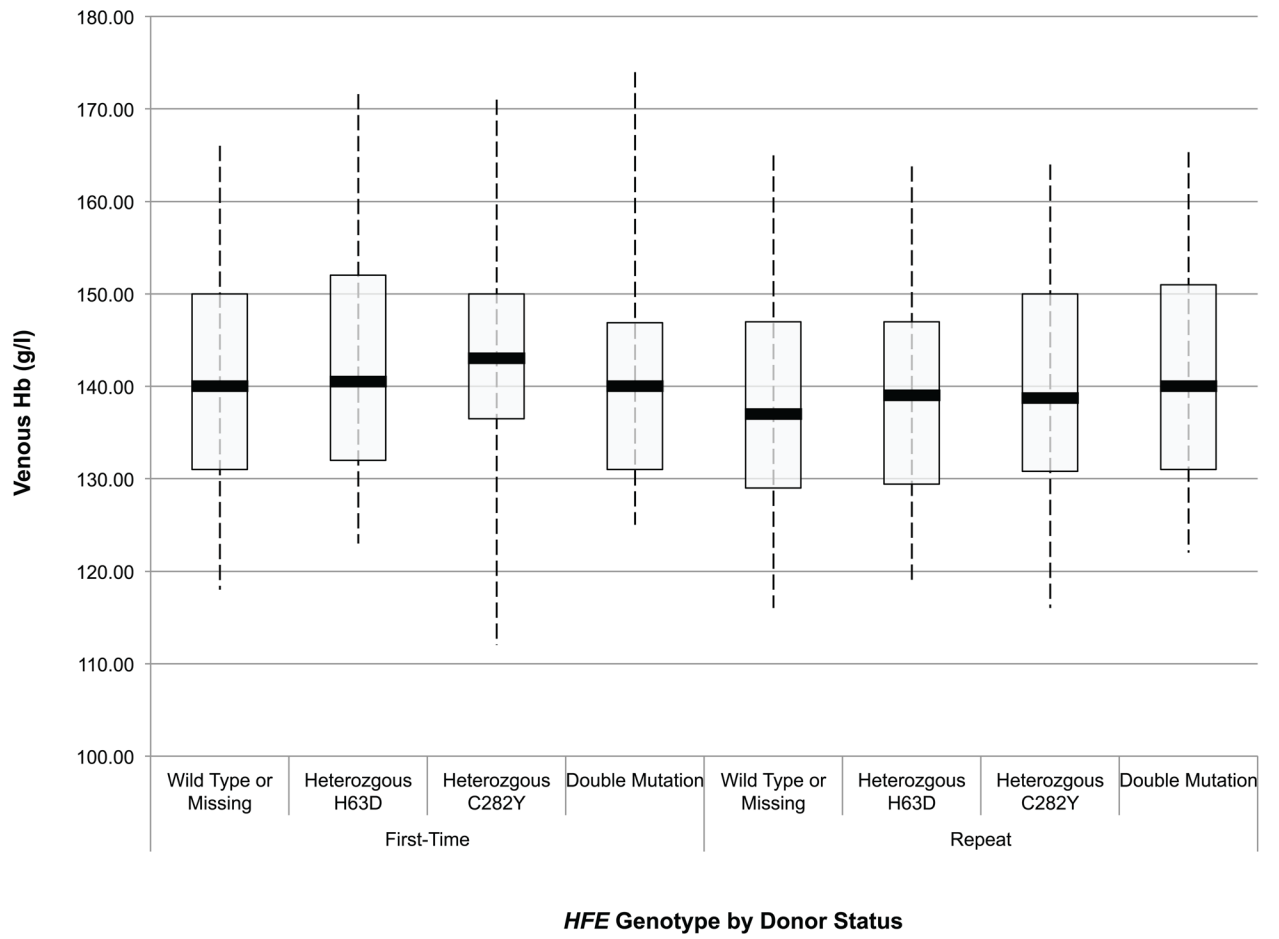
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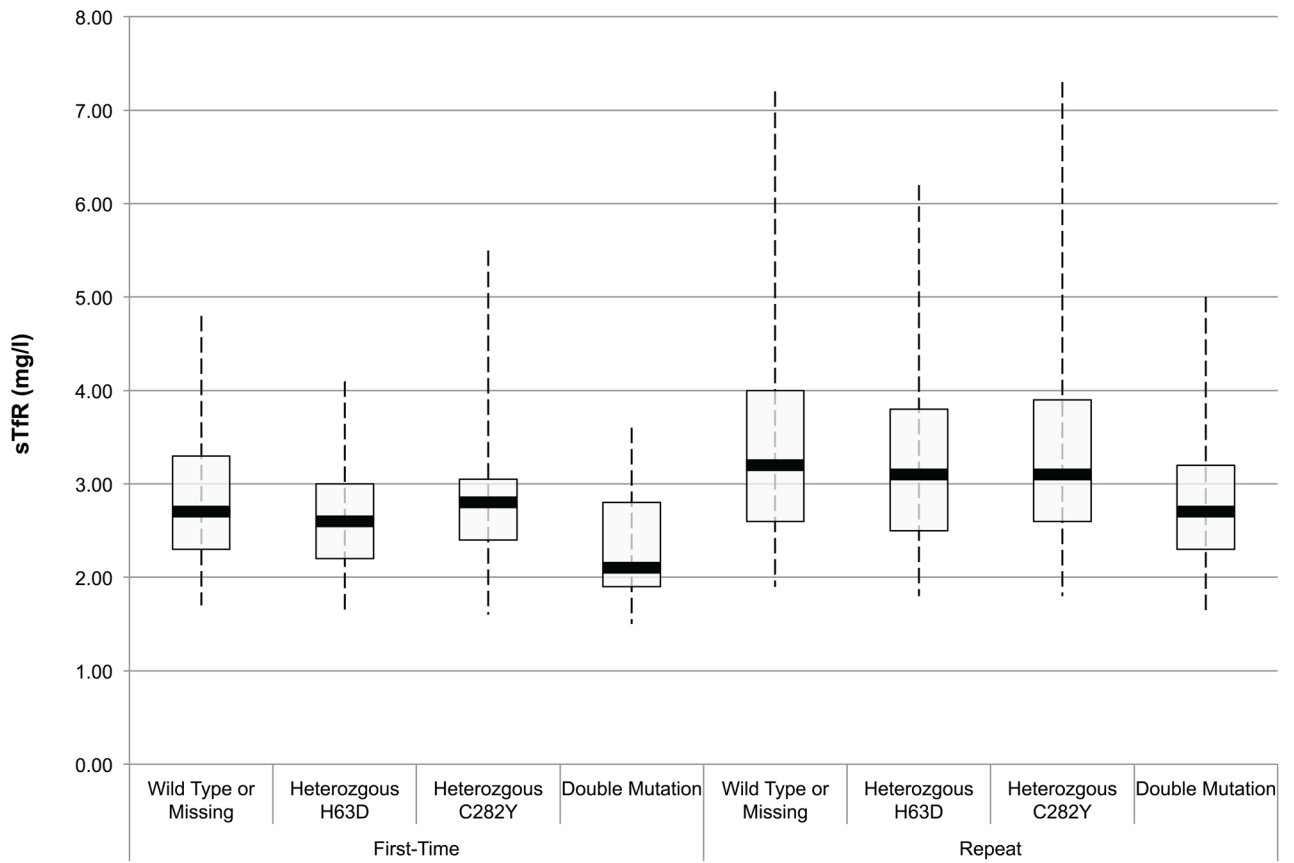
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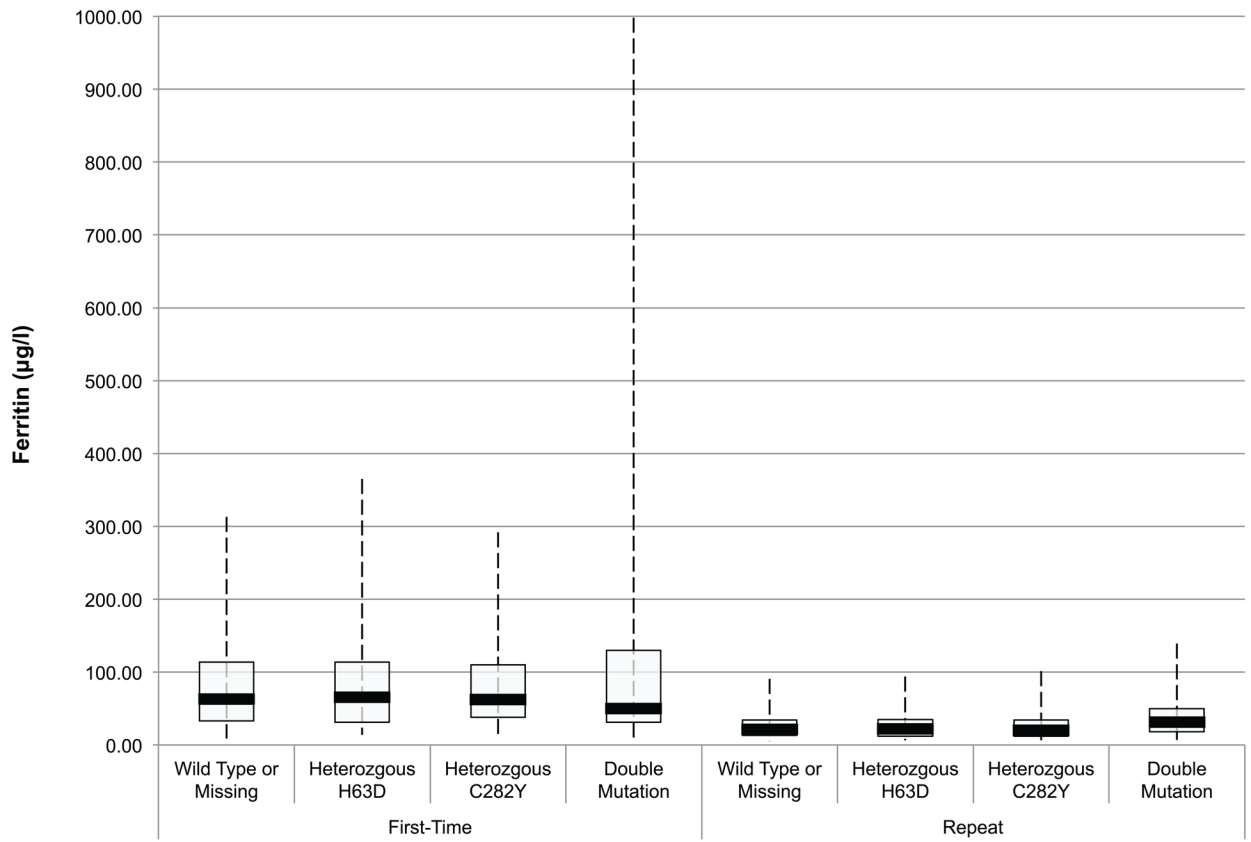


**HFE Genotype by Donor Status**



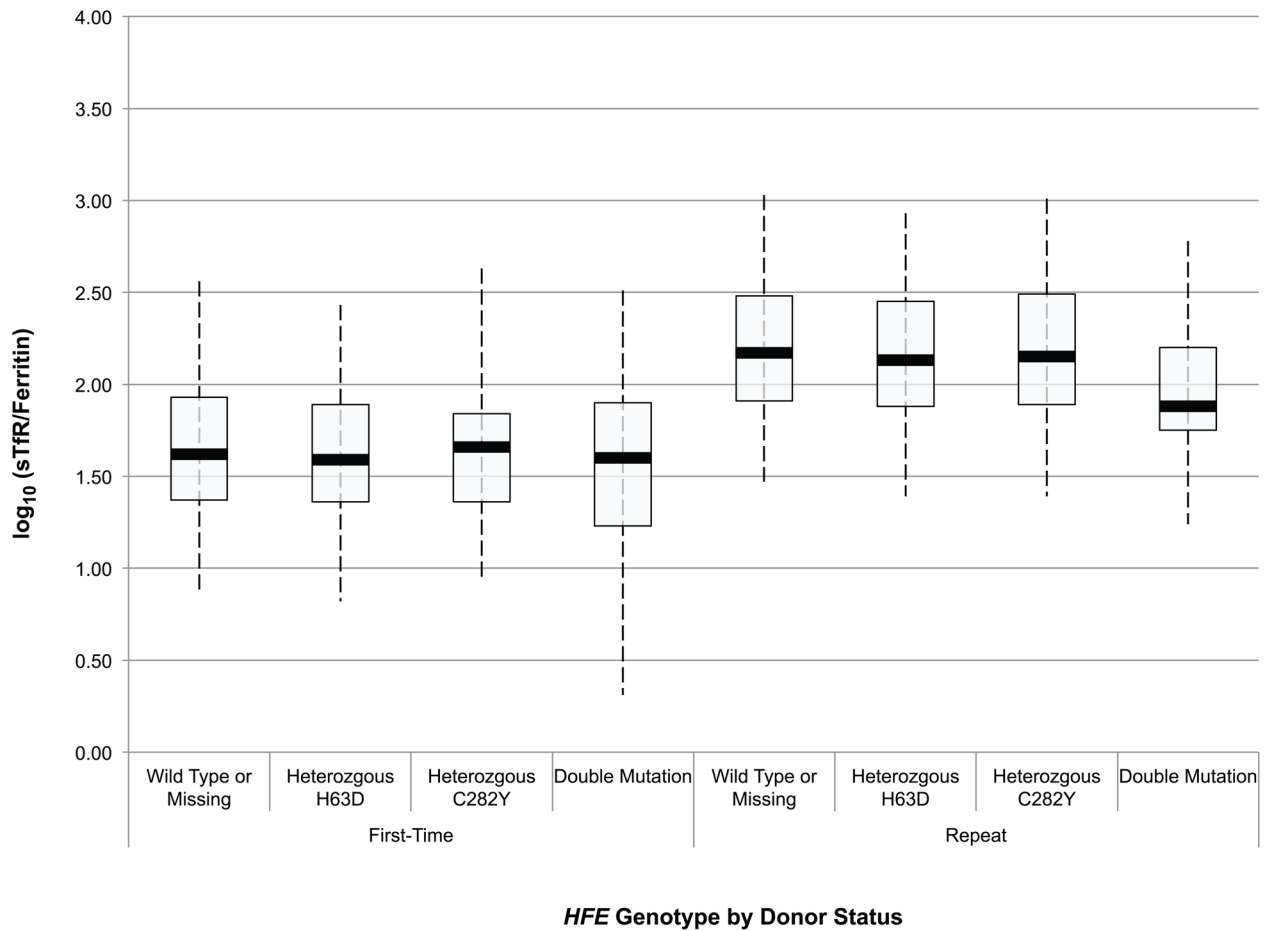


**HFE Genotype by Donor Status**

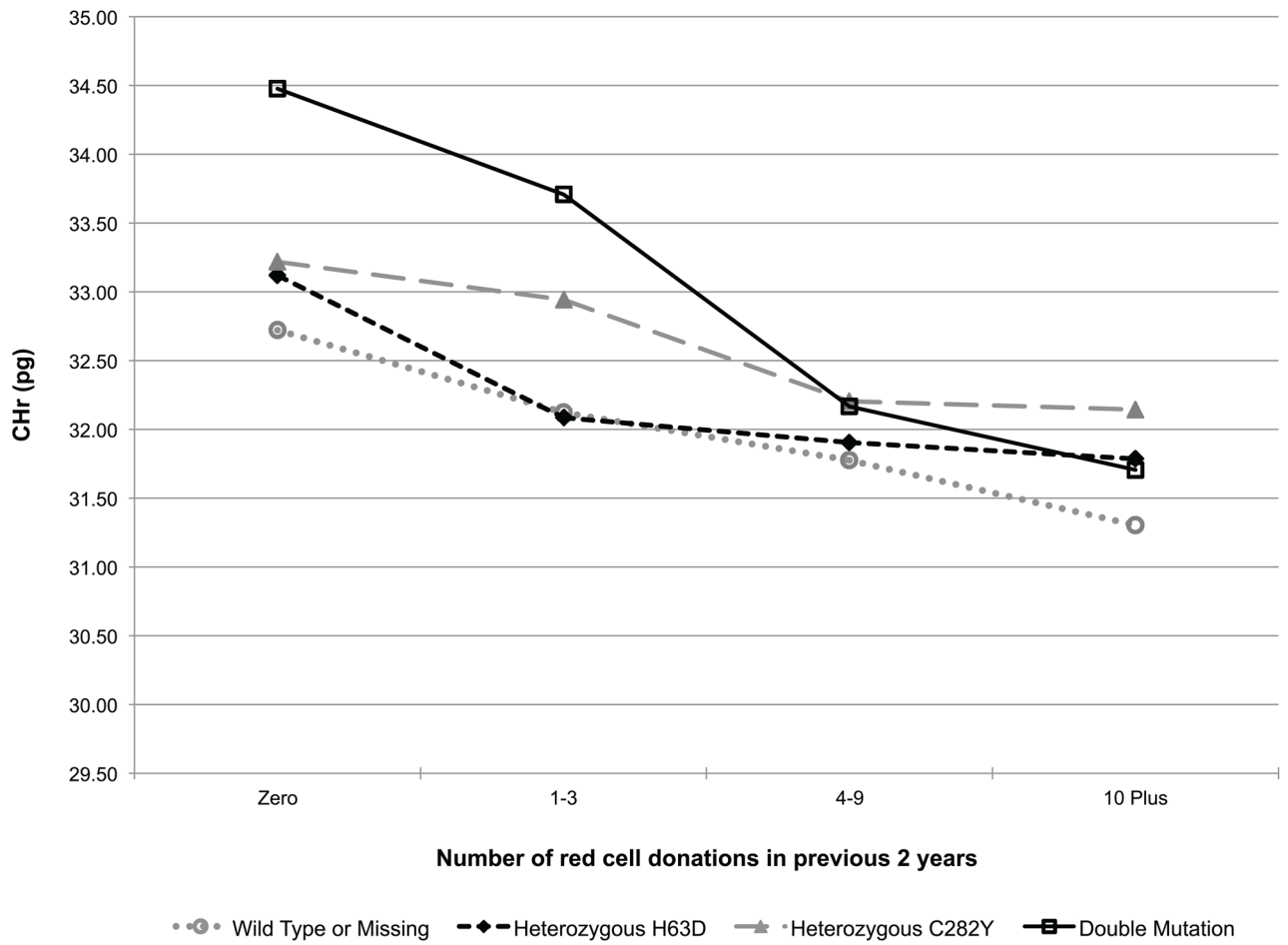


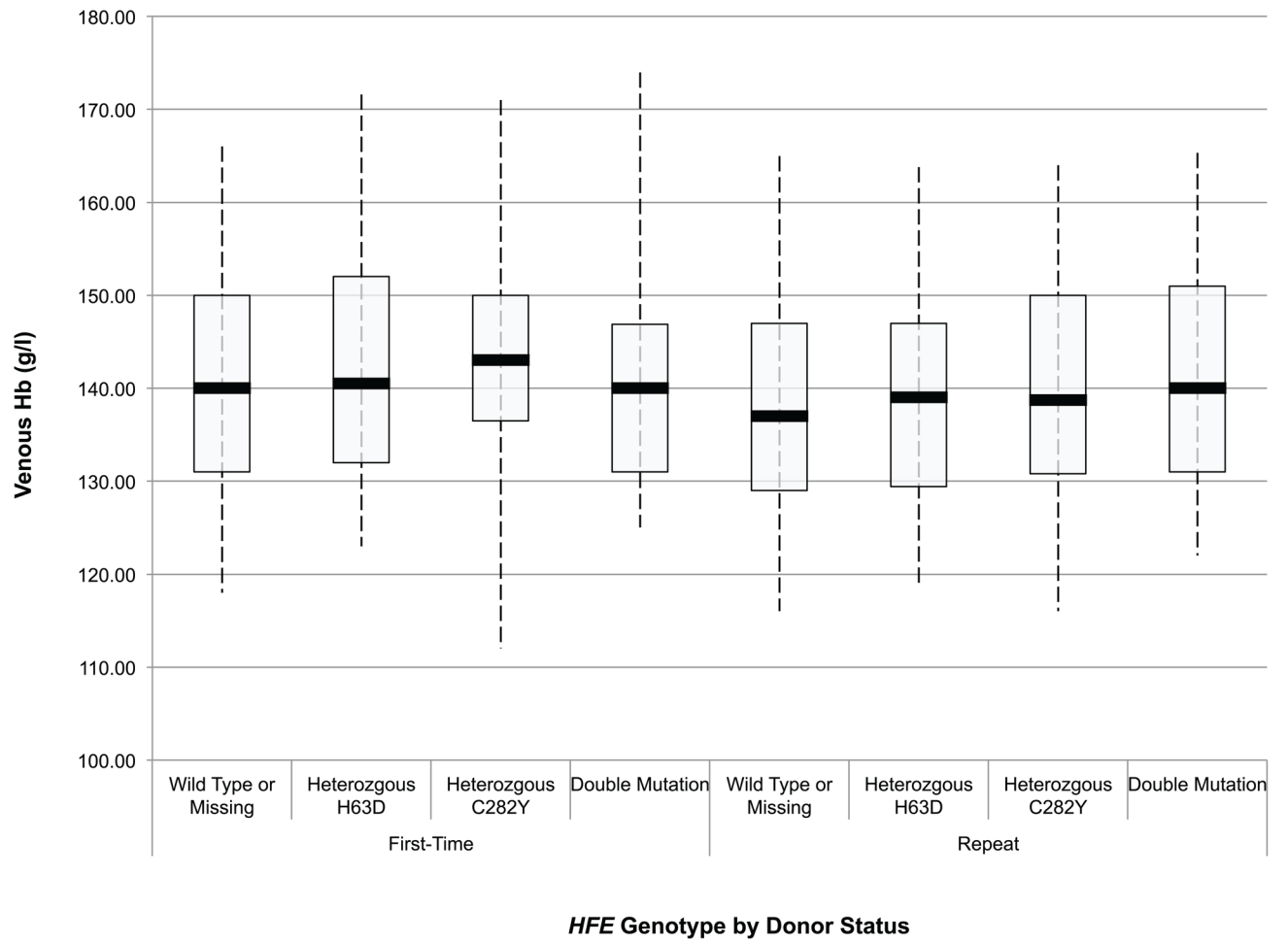
**HFE Genotype by Donor Status**

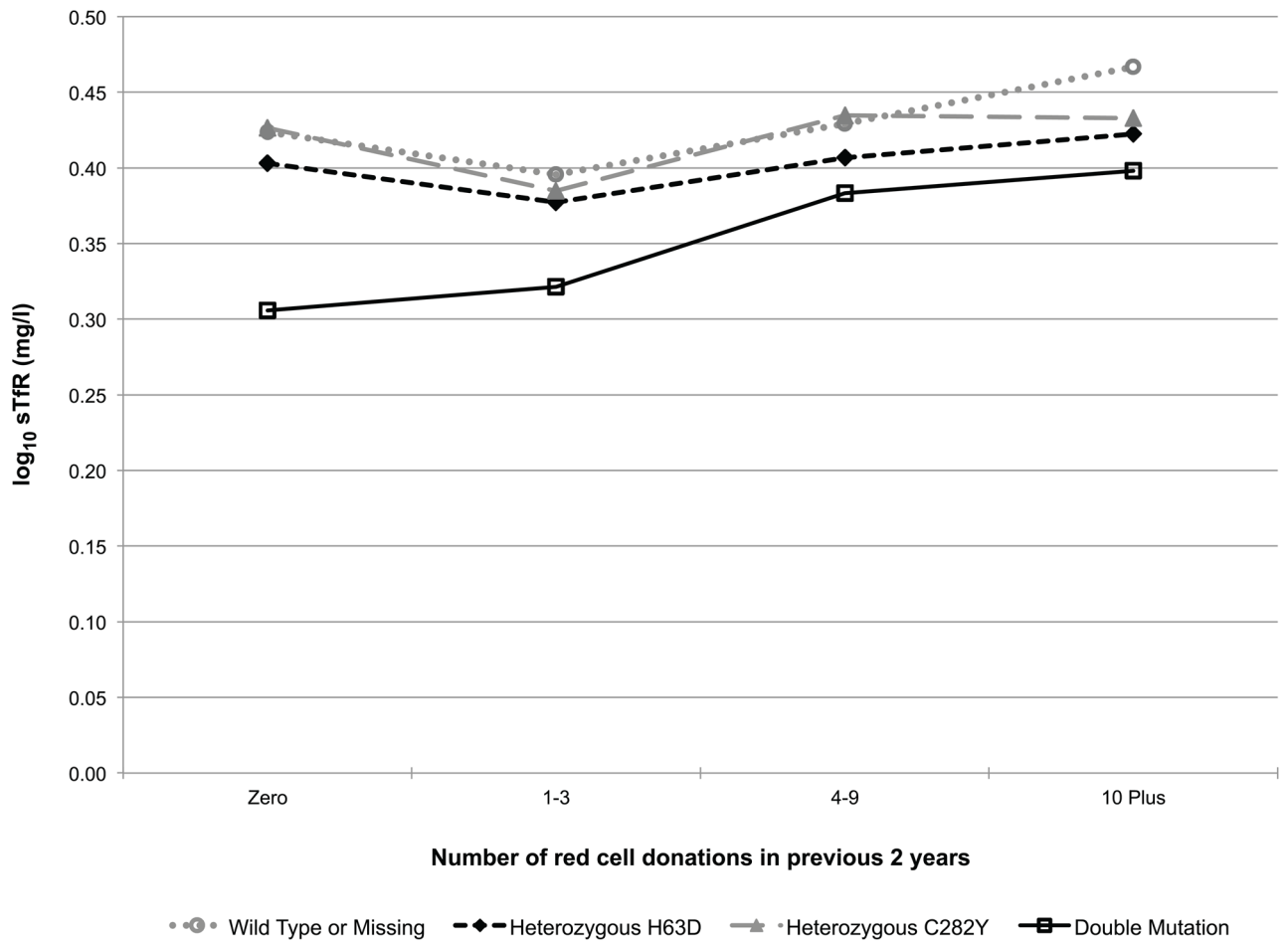


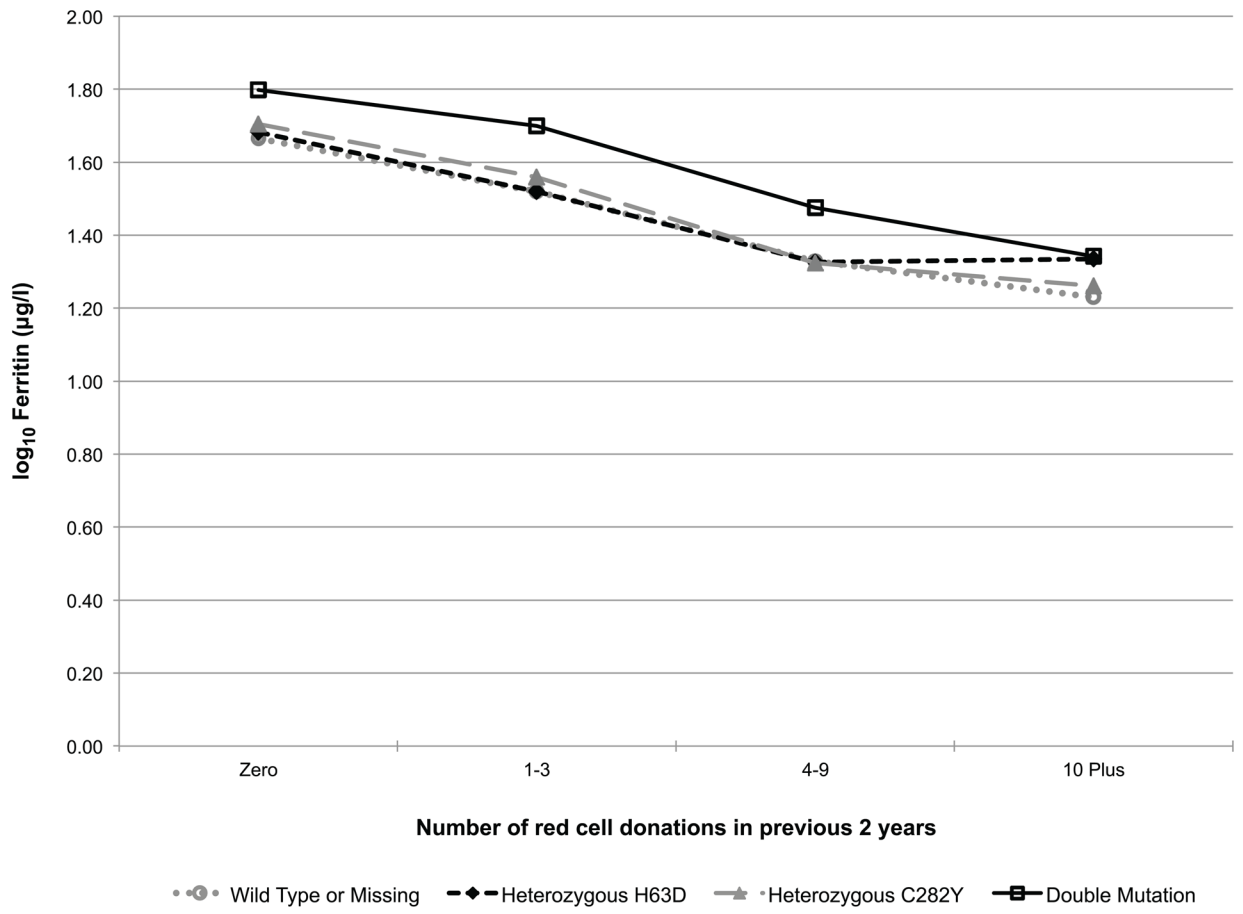
**Fig 1.**

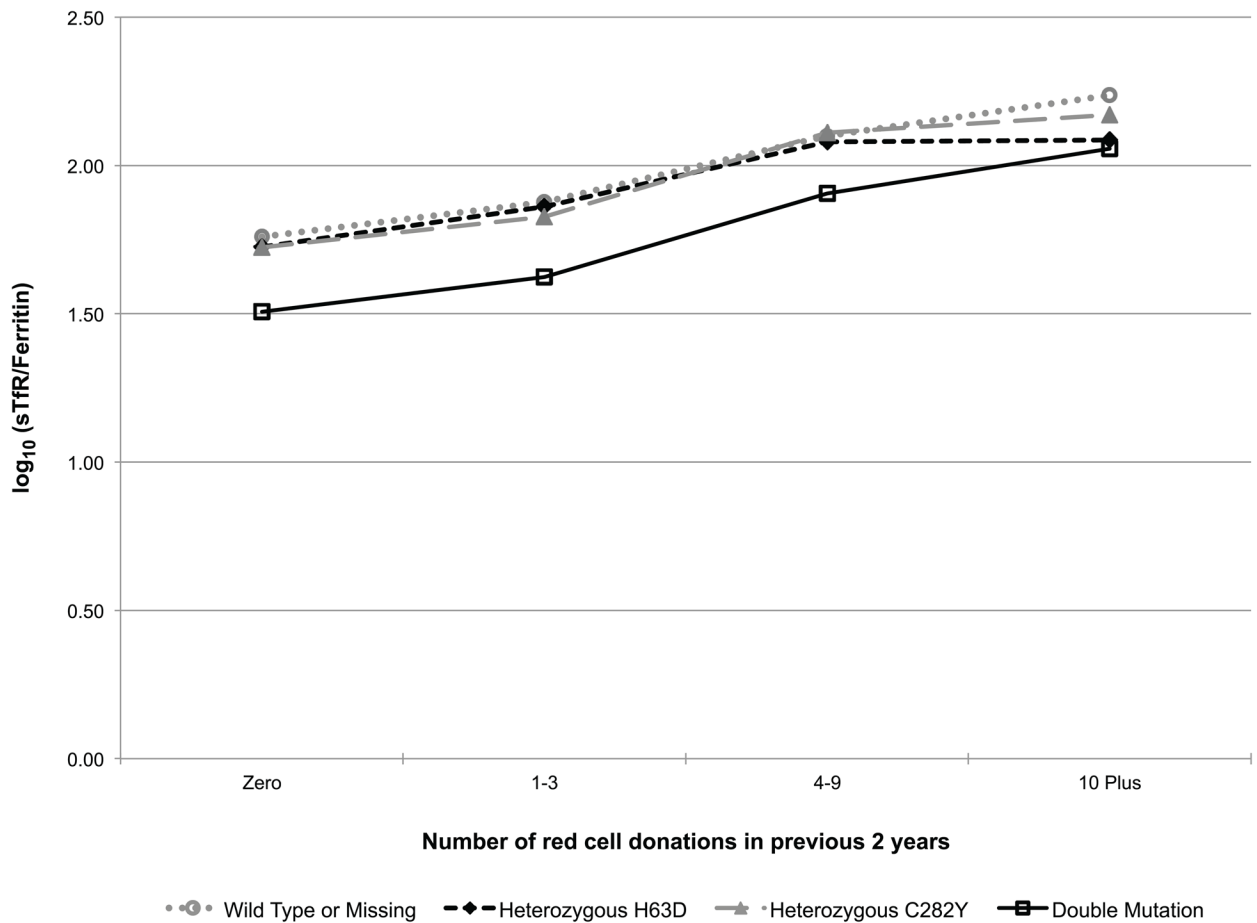
Box and whisker graphs comparing haemoglobin and iron parameters in first time/ reactivated and frequent donors grouped by *HFE* genotype at baseline. The boxes stretch from the 25<sup>th</sup> to the 75<sup>th</sup> percentile. Median values are indicated by the horizontal line across the box. The whiskers stretch to the 5<sup>th</sup> and 95<sup>th</sup> percentile. A) CHR; B) venous haemoglobin; C) sTfR; D) ferritin; E)  $\log(sTfR/ferritin)$ .





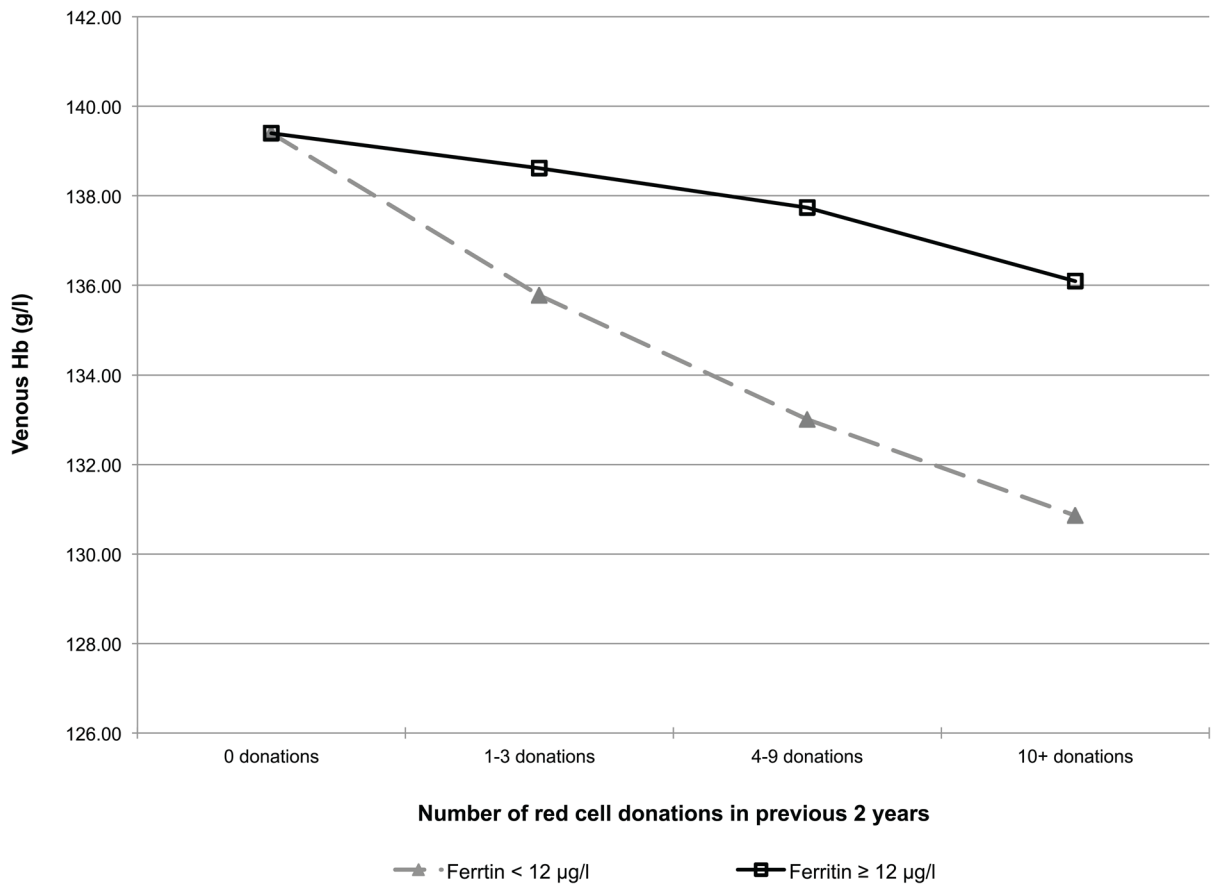


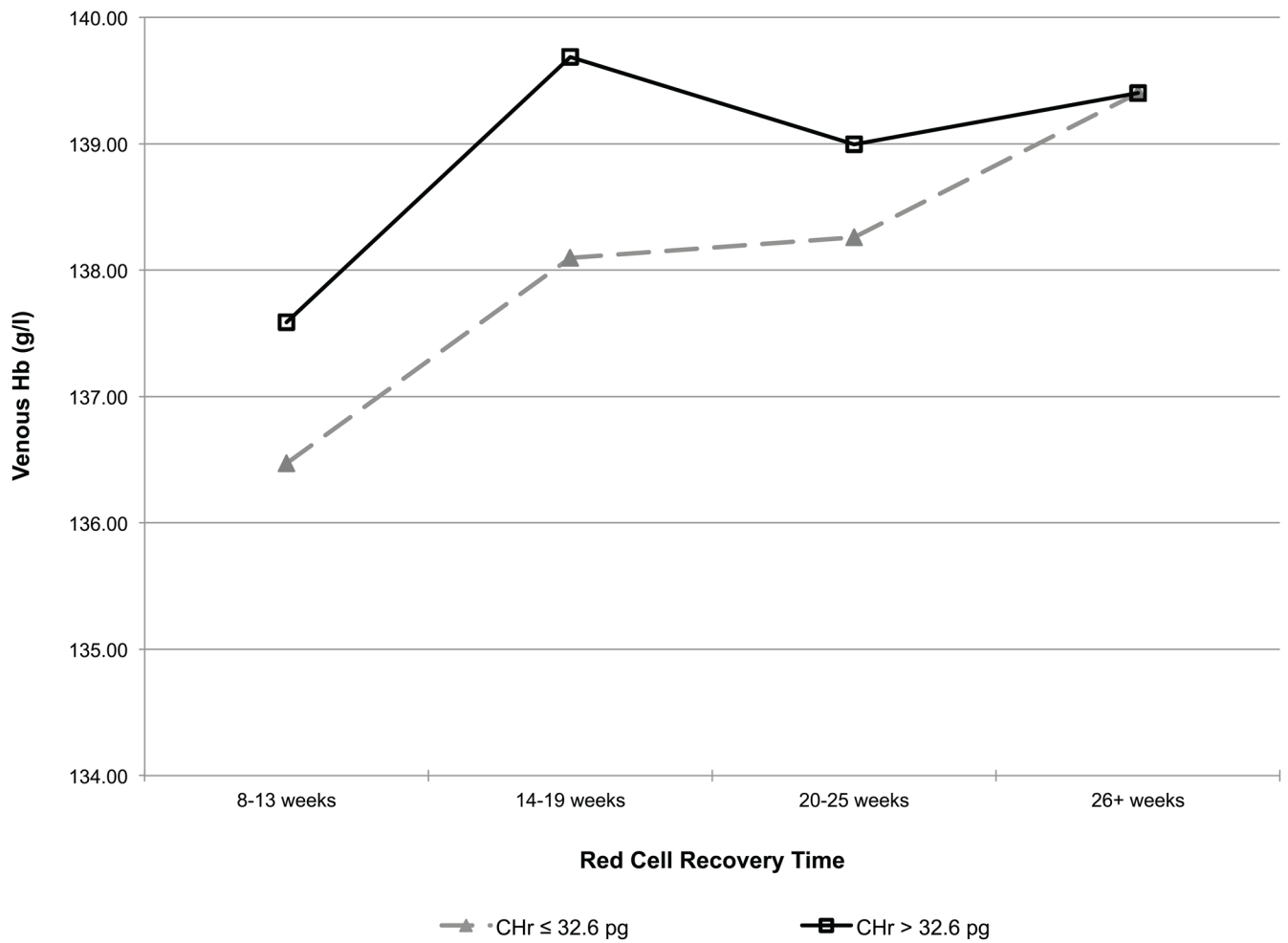




**Fig 2.**

Predicted changes in measures of haemoglobin and iron status with increasing donation intensity by *HFE* genotype. The analyses to generate the lines used information from the REDS-II donation database to determine the 24-month donation intensity for the frequent donors at each donation during the study. Individual donors contributed to different data points depending on changes in their 24-month donation intensity at the time of their study donations. A) CHr; B) venous haemoglobin; C)  $\log(\text{sTfR})$ ; D)  $\log(\text{ferritin})$ ; E)  $\log(\text{sTfR}/\text{ferritin})$ .





**Fig 3.**

A) Predicted changes in venous haemoglobin with increasing donation intensity based on ferritin. (■) ferritin <12 ng/ml; (◆) ferritin ≥ 12 ng/ml. B) Predicted venous haemoglobin recovery time following blood donation based on CHr. (■) CHr <32.6 pg; (◆) CHr ≥ 32.6.



**Table I**

Demographic and other characteristics of Baseline Retrovirus Epidemiology Donor Study-II (REDS-II) donor Iron Status Evaluation (RISE) donors (n=2,425)

	N (%)
<b>Race/Ethnicity</b>	
Asian	76 (3.1)
Black	116 (4.8)
Hispanic	76 (3.1)
White	2,111 (87.1)
Other	29 (1.2)
Missing	17 (0.7)
<b>Gender</b>	
Female	1,250 (51.6)
Male	1,175 (48.4)
<b>Age (years)</b>	
16 – 29	476 (19.6)
30 – 39	340 (14.0)
40 – 49	497 (20.5)
50 – 59	626 (25.8)
60+	486 (20.0)
<b>HFE genotype</b>	
Wild Type	1,568 (64.7%)
Heterozygous H63D	573 (23.6%)
Heterozygous C282Y	194 (8.0%)
Homozygous H63D	39 (1.6%)
Homozygous C282Y	7 (0.3%)
Double Mutation	41 (1.7%)
Missing	3 (0.1%)
<b>Donor Status</b>	
First-Time/Reactivated	888 (36.6)
Frequent/Repeat	1,537 (63.4)
<b>Weight (kg)</b>	
< 68	595 (24.5)
68 – 78	623 (25.7)
79 – 90	531 (21.9)
91	659 (27.2)
Missing	17 (0.7)
<b>Smoking</b>	
Current smoker	313 (12.9)
Past/Non/Unknown	2,112 (87.1)

	N (%)
<b>Iron Supplementation</b>	
Iron Supplement	954 (39.3)
No iron Supplement	1,471 (60.7)
<b>Menstrual Cycle (females)</b>	
Periods have stopped	585 (46.8)
Still having periods	665 (53.2)
<b>Pregnancy Status (females)</b>	
Ever Pregnant	831 (66.5)
Unknown pregnancy status	13 (1.0)
Never pregnant	406 (32.5)
<b>TF G277S Genotype</b>	
Wild Type	2,107 (86.9)
Hetero- or Homozygous	254 (10.5)
Missing	64 (2.6)
<b>Red Cell donations in previous 2 years</b>	
First-Time/Reactivated Donors	888 (36.6)
3 donations	317 (13.1)
4–9 donations	1,011 (41.7)
10+ donations	209 (8.6)
<b>Blood Centre</b>	
A	436 (18.0)
B	390 (16.1)
C	376 (15.5)
D	392 (16.2)
E	415 (17.1)
F	416 (17.2)

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**Table II**  
Comparison of RISE and HEIRS *HFE* genotypes in first time and repeat donors, stratified by race

	Wild Type or Missing n (%)	Heterozygous H63D n (%)	Heterozygous C282Y n (%)	Homozygous H63D n (%)	Homozygous C282Y n (%)	Double Mutation n (%)	Total
<b>All Donors</b>							
RISE Study*	1,571 (64.8)	573 (23.6)	194 (8.0)	39 (1.6)	7 (0.3)	41 (1.7)	2,425
FT/Reactivated	592 (66.7)	210 (23.7)	60 (6.8)	10 (1.1)	3 (0.3)	13 (1.5)	888
Frequent/Repeat	979 (63.7)	363 (23.6)	134 (8.7)	29 (1.9)	4 (0.3)	28 (1.8)	1,537
HEIRS Study	73,066 (76.0)	15,326 (15.9)	5,207 (5.4)	1,242 (1.3)	292 (0.3)	991 (1.0)	96,124
<b>Asian Donors</b>							
RISE: FT/Reactivated	42 (93.3)	2 (4.5)	1 (2.2)	0	0	0	45
RISE: Frequent/Repeat	31 (100.0)	0	0	0	0	0	31
HEIRS Study	11,657 (91.3)	1,070 (8.4)	16 (0.1)	29 (0.2)	0	0	12,772
<b>Black Donors</b>							
RISE: FT/Reactivated	49 (92.4)	2 (3.8)	2	0	0	0	53
RISE: Frequent/Repeat	53 (84.1)	10 (15.9)	0	0	0	0	63
HEIRS Study	24,930 (91.9)	1,520 (5.6)	608 (2.2)	30 (0.1)	4 (0.0)	35 (0.1)	27,127
<b>Hispanic Donors</b>							
RISE: FT/Reactivated	27 (67.5)	12 (30.0)	0	0	0	1 (2.5)	40
RISE: Frequent/Repeat	22 (61.1)	11 (30.5)	2 (5.6)	0	0	1 (2.8)	36
HEIRS Study	9,700 (79.9)	2,199 (18.1)	35 (0.3)	154 (1.3)	7 (0.1)	48 (0.4)	12,143
<b>White Donors</b>							
RISE: FT/Reactivated	451 (62.6)	188 (26.1)	57 (7.9)	10 (1.4)	3 (0.4)	11 (1.5)	720
RISE: Frequent/Repeat	859 (61.8)	340 (24.4)	132 (9.5)	29 (2.1)	4 (0.3)	27 (1.9)	1,391
HEIRS Study	26,779 (60.8)	10,537 (23.9)	4,548 (10.3)	1,029 (2.3)	281 (0.6)	908 (2.1)	44,082

\* 29 RISE donors report their Racial/Ethnic group as "Other" and 17 are missing Race/Ethnicity (and not presented in this table); 3 are missing *HFE* genotype (and are pooled with WT).

RISE, Retrovirus Epidemiology Donor Study-II (REDS-II) donor Iron Status Evaluation; HEIRS, Haemochromatosis and Iron Overload Screening; FT, first time donor

Table III

Parameter estimates\* for CHr (pg), venous haemoglobin (g/l), log<sub>10</sub> sTfR (mg/l), log<sub>10</sub> Ferritin (µg/l) and log<sub>10</sub> ratio of sTfR/Ferritin

	CHr Model estimate (SE)		Venous Haemoglobin Model estimate (SE)		log <sub>10</sub> sTfR Model estimate (SE)		log <sub>10</sub> Ferritin Model estimate (SE)		log <sub>10</sub> (sTfR/Ferritin) Model estimate (SE)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Mean**	31.69 (0.60)		139.4 (1.5)		0.45 (0.02)		1.62 (0.05)		1.84 (0.06)	
Intercept***	32.72 (0.62)		146.6 (2.7)		0.42 (0.03)		1.67 (0.07)		1.76 (0.09)	
Race/Ethnicity										
<i>p</i> -value	0.001		<0.0001		<0.0001		0.007		0.06	
Asian	-0.39 (0.64)		0.14 (0.11)		0.01 (0.02)		0.12 (0.03)		-0.11 (0.04)	
Black	-1.39 (0.32)		-0.49 (0.09)		0.07 (0.01)		0.03 (0.03)		0.04 (0.04)	
Hispanic	-0.76 (0.38)		-0.20 (0.11)		-0.01 (0.02)		-0.04 (0.03)		0.03 (0.04)	
White	0.00		0.00		0.00		0.00		0.00	
Other	-0.64 (0.47)		-0.20 (0.15)		0.00 (0.02)		0.03 (0.06)		-0.03 (0.07)	
Gender										
<i>p</i> -value	0.07		<0.0001		0.61		<0.0001		0.001	
Female	-0.41 (0.35)		-1.66 (0.20)		0.05 (0.02)		-0.30 (0.05)		0.34 (0.05)	
Male	0.00		0.00		0.00		0.00		0.00	
Age (years)										
<i>p</i> -value	0.0001	0.002	<0.0001	0.03	0.001	0.14	0.003	<0.0001	0.005	<0.0001
16 – 29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30 – 39	0.38 (0.28)	0.42 (0.26)	-0.18 (0.09)	0.09 (0.08)	0.00 (0.01)	-0.01 (0.01)	0.04 (0.03)	0.11 (0.03)	-0.03 (0.04)	-0.13 (0.03)
40 – 49	0.58 (0.22)	0.88 (0.22)	-0.21 (0.09)	0.18 (0.08)	-0.01 (0.01)	-0.03 (0.01)	0.07 (0.03)	0.19 (0.03)	-0.08 (0.03)	-0.22 (0.03)
50 – 59	0.47 (0.21)	0.69 (0.24)	-0.33 (0.09)	0.21 (0.09)	0.00 (0.01)	-0.03 (0.01)	-0.02 (0.03)	0.23 (0.03)	0.02 (0.03)	-0.26 (0.04)
60+	1.01 (0.22)	0.63 (0.27)	-0.46 (0.10)	0.35 (0.11)	-0.04 (0.01)	-0.04 (0.02)	0.02 (0.03)	0.27 (0.03)	-0.05 (0.03)	-0.30 (0.04)
HFE genotype										
<i>p</i> -value	<0.0001		<0.0001		<0.0001		0.003		<0.0001	
Wild Type or Missing	0.00		0.00		0.00		0.00		0.00	
Heterozygous H63D	0.40 (0.12)		0.14 (0.07)		-0.02 (0.01)		0.02 (0.02)		-0.03 (0.02)	
Heterozygous C282Y	0.50 (0.25)		0.09 (0.12)		0.00 (0.01)		0.04 (0.03)		-0.04 (0.04)	
Double Mutation	1.76 (0.42)		0.57 (0.17)		-0.12 (0.02)		0.13 (0.06)		-0.25 (0.06)	

Centre	CHr Model estimate (SE)		Venous Haemoglobin Model estimate (SE)		log <sub>10</sub> sITR Model estimate (SE)		log <sub>10</sub> Ferritin Model estimate (SE)		log <sub>10</sub> (sITR/Ferritin) Model estimate (SE)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
<i>p</i> -value	<0.0001		<0.0001		<0.0001		<0.0001		0.002	
A	0.77 (0.13)		0.15 (0.06)		0.01 (0.01)		0.01 (0.02)		0.00 (0.03)	
B	—		-0.72 (0.07)		-0.02 (0.01)		0.03 (0.02)		-0.05 (0.03)	
C	—		0.02 (0.07)		-0.02 (0.01)		0.00 (0.02)		-0.01 (0.03)	
D	-0.66 (0.12)		-0.16 (0.06)		-0.04 (0.01)		-0.02 (0.02)		-0.02 (0.03)	
E	0.20 (0.12)		-0.75 (0.06)		-0.01 (0.01)		-0.07 (0.02)		0.06 (0.03)	
F	0.00		0.00		0.00		0.00		0.00	
<b>Smoking</b>										
<i>p</i> -value	0.10		<0.0001		<0.0001		0.005		<0.0001	
Current smoker	0.22 (0.13)		0.39 (0.06)		-0.04 (0.01)		0.05 (0.02)		-0.09 (0.02)	
Past/Non/Unknown smoker	0.00		0.00		0.00		0.00		0.00	
<b>Weight (kg)</b>										
<i>p</i> -value	0.008	<0.0001	0.08	0.57	0.08	0.0002	<0.0001	0.001	<0.0001	0.23
< 68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
68 – 78	-0.29 (0.31)	-0.20 (0.14)	0.01 (0.19)	0.01 (0.05)	0.04 (0.02)	0.01 (0.01)	0.04 (0.04)	0.04 (0.02)	0.00 (0.05)	-0.03 (0.02)
79 – 90	-0.50 (0.30)	-0.63 (0.16)	0.04 (0.19)	0.08 (0.07)	0.05 (0.02)	0.02 (0.01)	0.06 (0.04)	0.05 (0.02)	-0.01 (0.05)	-0.03 (0.03)
91	-0.77 (0.29)	-1.21 (0.18)	0.18 (0.19)	0.07 (0.08)	0.05 (0.02)	0.05 (0.01)	0.17 (0.04)	0.11 (0.03)	-0.12 (0.05)	-0.06 (0.03)
<b>Iron Supplementation</b>										
<i>p</i> -value	0.23		0.86		0.19		0.001		0.001	
Iron Supplement	0.11 (0.09)		-0.01 (0.04)		-0.01 (0.01)		0.04 (0.01)		-0.05 (0.02)	
No iron Supplement	0.00		0.00		0.00		0.00		0.00	
<b>Pregnancy Status</b>										
<i>p</i> -value	0.04		0.12		0.04		0.009		0.01	
Ever Pregnant	-0.40 (0.15)		-0.11 (0.06)		0.02 (0.01)		-0.05 (0.02)		0.07 (0.03)	
Female unknown preg status	-1.87 (1.33)		-0.23 (0.22)		0.03 (0.03)		-0.25 (0.08)		0.28 (0.09)	
Male or never pregnant	0.00		0.00		0.00		0.00		0.00	
<b>Menstrual Cycle (female)</b>										
<i>p</i> -value	0.21		0.001		0.09		<0.0001		0.01	

	CHr Model estimate (SE)	Venous Haemoglobin Model estimate (SE)	log <sub>10</sub> sTfR Model estimate (SE)	log <sub>10</sub> Ferritin Model estimate (SE)	log <sub>10</sub> (sTfR/Ferritin) Model estimate (SE)
Periods have stopped	0.21 (0.17)	0.22 (0.07)	0.02 (0.01)	0.09 (0.02)	-0.08 (0.03)
Still having periods	0.00	0.00	0.00	0.00	0.00
<b>TF G277S Genotype</b>					
<i>p</i> -value	0.34	0.97	0.93	0.59	0.97
Wild Type	0.00	0.00	0.00	0.00	0.00
Heterozygous or Homozygous	0.149 (0.124)	0.007 (0.061)	-0.006 (0.008)	0.001 (0.018)	-0.005 (0.023)
Missing	-0.565 (0.166)	0.212 (0.106)	-0.005 (0.010)	-0.003 (0.023)	-0.004 (0.028)
<b>Red Cell Donations in the previous 2 years</b>					
<i>p</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
0 donations	0.00	0.00	0.00	0.00	0.00
1–3 donations	-0.60 (0.16)	-0.16 (0.05)	-0.03 (0.01)	-0.15 (0.02)	0.12 (0.02)
4–9 donations	-0.94 (0.17)	-0.26 (0.06)	0.01 (0.01)	-0.34 (0.02)	0.34 (0.02)
10+ donations	-1.42 (0.20)	-0.54 (0.08)	0.04 (0.01)	-0.44 (0.02)	0.48 (0.03)
<b>Time Since Last Donation</b>					
<i>p</i> -value	0.01	<0.0001	<0.0001	<0.0001	<0.0001
8–13 weeks	-0.26 (0.10)	-0.25 (0.04)	0.06 (0.005)	-0.23 (0.01)	0.29 (0.01)
14–19 weeks	-0.14 (0.10)	-0.08 (0.04)	0.02 (0.005)	-0.16 (0.01)	0.19 (0.01)
20–25 weeks	0.05 (0.12)	-0.13 (0.04)	0.02 (0.004)	-0.08 (0.01)	0.10 (0.01)
26+ weeks	0.00	0.00	0.00	0.00	0.00
<b>Red Cell Donations in the previous 2 years by HFE genotype</b>					
<i>p</i> -value	0.08	0.24	0.05	0.01	0.02

\* Parameter estimates are from repeated measures regression model with the fixed effects shown in the table and with random effects for within and between donor variation.

\*\* Mean is model least square mean at 0 donations in past 2 years.

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Intercept is the model prediction for the reference donor (white, male, aged 16–29 years, no HFE mutation, at centre F, non-smoker, weight less than 68 kg, not taking iron supplements, without the TF G277S genotype, and having made no donations in the previous 2 years).