Genetic factors associated with iron storage in Australian blood donors

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Background. Blood donors are at risk of developing iron deficiency and/or iron deficiency anaemia. This may affect their health and affect their eligibility to give subsequent donations. Investigating genetic factors that may predispose donors to high or low iron stores is of interest; this may assist with providing optimal management strategies for maintaining donor health. This study aimed to investigate whether the presence of selected single nucleotide polymorphisms (SNPs) affecting parameters of iron status were associated with ferritin levels in Australian donors.

Materials and methods. Samples (n=800) were collected from non-first-time blood donors in Queensland. Plasma ferritin levels were quantified and the genotypes for ten SNPs, identified by a review of relevant literature, were determined for each sample. Associations between SNPs and ferritin levels were investigated.

Results. Three SNPs were associated with ferritin levels. In male donors, high ferritin levels were associated with the variant allele (G) of the SNP rs3923809 in the *BTBD9* gene. An association with ferritin levels was also identified with the SNP rs235756 in the *BMP2* gene in males. The SNP rs4820268 in the *TMPRSS6* gene was associated with ferritin levels in females, with donors with the AG genotype being three times more likely to have low ferritin levels.

Discussion. Variants in the genes *TMPRSS, BTBD9* and *BMP2* were associated with ferritin levels in Australian blood donors. These findings provide support that genetic testing may be useful for the generation of predictive algorithms that may allow for management strategies to be tailor-made for individual donors.

Keywords: ferritin, single nucleotide polymorphism, iron deficiency, health, safety.

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Introduction

Blood donation leads to iron loss in blood donors. This increases the risk of developing iron deficiency (ID) or iron-deficiency anaemia (IDA) in regular donors, especially young females, and those with low initial ferritin levels[1,2]. Clinically significant ID can be associated with a number of health issues, including fatigue, pica, restless leg syndrome, and impaired cognitive performance[3]. In addition, ID-induced donor deferral imposes a negative experience on enthusiastic donors, and may result in permanent donor loss and a long-term adverse impact on blood collections[4].

The current donor selection procedure in Australia, as well as in other countries including the USA and Canada, uses capillary haemoglobin (Hb) level as the only criterion to assess whether the iron status of a donor is acceptable for blood donation[5-7]. However, Hb does not reflect body iron stores and discrepancies exist between Hb measuring devices[8]. Various approaches have been explored to predict the suitability of a donor for blood donation, including adding pathology tests or developing suitability models in donor screening[9,10]. However, these predictors/predictive models increase cost and lack large-scale validation[11,12].

Despite the fact that the same management strategies, such as minimum Hb requirements and pre-determined donation intervals (12 weeks for whole blood and 2 weeks for plasmapheresis in Australia)[5], are applied, some blood donors become iron deficient more easily than others after donation[13]. In addition, the ability of individuals to recover normal iron levels varies, which indicates that there are contributing factors in addition to dietary iron intake or supplementation[14]. In recent decades, various studies have reported the associations between genetic variants and iron metabolism. Single nucleotide polymorphisms (SNPs) associated with parameters of iron status and red cell indices have been identified in genes involved in iron regulation in various population studies. For example, variants in the *TMPRSS6* gene are associated with decreased serum iron[15], serum ferritin[16] and Hb[15,17]. SNPs in the *TF* gene are associated with increased serum transferrin levels[17,18], total iron binding capacity[17] and serum ferritin levels[18]. The SNP rs7385804 in the

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TFR2 gene is associated with iron status and red cell indices[17,19]. In addition, SNPs have been discovered in other genes involved in iron metabolism or storage, including the genes *HFE*[20], *BMP2*[21], *CYBRD1*[22], *HIF1A*[23], and *EPO*[19]. Some SNPs that are located on genes not directly involved in iron regulation may also be associated with parameters of iron status. For example, the SNPs rs3923809, and rs9296249 in the *BTBD9* gene are associated with decreased serum ferritin, and restless leg syndrome, respectively[24,25]. The SNP rs1175550 in the *SMIM1* gene influences the expression of the red blood cell antigen Vel, and is also associated with lower mean corpuscular Hb concentration in Europeans[26,27]. These polymorphisms may directly affect iron metabolism through yet unknown mechanisms, or be associated with proteins that participate in iron regulation signalling pathways. Evidence as such raises the question of whether generic factors contribute to differences in responses to iron loss between individuals. This study aims to investigate whether the presence of genetic variants in parameters of iron status were associated with ferritin levels in Australian blood donors. With a focus on genetic factors associated with changes in iron metabolism and storage that would be of relevance in blood donors in Australia, we focused on ten SNPs in six different genes identified through a review of large population-wide published studies: rs9296249 and rs3923809 in the *BTBD9* gene[24,25]; rs855791 and rs4820268 in the *TMPRSS6* gene[15-17,28]; rs7385804 in the *TFR2* gene[17,28]; rs3811647, rs1799852 and rs2280673 in the *TF* gene[18]; rs235756 in the *BMP2* gene[21], and, rs1175550 in the *SMIM1* gene[27].

Materials and methods

Samples

Samples (n=800) were collected from non-first-time blood donors in Queensland, Australia. Samples were collected in tubes containing ethylenediamine tetra-acetic acid (EDTA) anticoagulant (BD Vacutainer® Whole Blood Collection tube with spray-coated di-potassium EDTA 6 mL; Becton Dickinson, Franklin Lakes, NJ, USA) before centrifugation at 2,500 rpm for 5 minutes, as per routine procedures. These samples were collected for routine infectious disease screening as part of the normal blood donation procedure. They were recovered for use in this study after such testing was complete, and prior to their usual discard. All were investigated within 1 week of collection and were stored at 4 °C before use in this study. The following data were collected for each donor/sample: age, sex, Hb level, number and type of prior donations (ever and in the previous 12 months) and time since previous donation. Donation-induced iron loss (in mg, in the preceding 12 months) for each donor was calculated using the following formula: number of whole blood donations × 220 mg (average iron loss in one whole blood donation) + number of plasma donations × 18 mg (average iron loss in one plasmapheresis donation), and was recorded as one of the parameters[5]. Samples were anonymised prior to any laboratory testing. Ethical approval was provided by the Blood Service Human Research Ethics Committee (2014#12) and the University of Queensland School of Medicine Ethics Committee (2014-SOMILRE-0107).

Ferritin testing

Plasma ferritin levels were quantified with the Human Ferritin ELISA kit (Sigma-Aldrich, Castle Hill, NSW, Australia), in accordance with the manufacturer's instructions. Colorimetric changes were read on a plate reader (Synergy H1 Multi-Mode Reader; BioTek, Winooski, VT, USA) at 450 nm immediately, and the ferritin result of each sample was determined automatically by the in-built Gen5™ software (BioTek).

DNA extraction

Genomic DNA was extracted from whole blood (separated plasma was resuspended with the cell pellet to recreate a whole blood sample) using the EZ1 DNA Blood kit (QIAGEN, Hilden, Germany) on an EZ1 Advance machine (QIAGEN), according to the manufacturer's directions. The concentration of DNA in each sample was quantified with a NanoDrop™ 2000 (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Single nucleotide polymorphism analysis

The genotypes of the candidate SNPs were determined using TaqMan SNP genotyping assays (Thermo Fisher Scientific, Mulgrave, Australia) according to the manufacturer's instructions (assay details are provided in the Online Supplementary Content, Table SI) using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Scoresby, VIC, Australia). Each reaction well had a total volume of 15 µL, which included DNA template (10 ng), TaqMan SNP reagent (0.375 µL) and genotyping Master Mix (7.5 µL). Standard cycling conditions were used: 40 cycles of 15 seconds at 95 °C and 1 minute at 60 °C. Due to weak expression of alleles for one of the SNPs (rs1175550), modified conditions were used and included 50 ng of DNA template followed by 50 cycles of 15 seconds at 95 °C and 1 minute at 60 °C for the polymerase chain reaction amplification. An automated genotype call from the StepOnePlus™ instrument (StepOne™ Software v2.2.2, Thermo Fisher Scientific Inc., Waltham, MA, USA) was used.

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Data analysis

All SNPs were tested for Hardy-Weinberg equilibrium (HWE) using the Court Lab Calculator[29]. Only SNPs that did not differ from HWE (p>0.05) were included in subsequent analyses. Samples were stratified into three groups based on plasma ferritin levels: high, medium and low. Males and females were analysed separately due to known differences in ferritin levels[5]: for male donors the three groups were ≤15 ng/mL (low), >15 and ≤100 ng/mL (medium), and >100 ng/mL (high), while for female donors they were ≤8 ng/mL (low), >8 and ≤60 ng/mL (medium), and >60 ng/mL (high). A multinomial regression analysis was performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics 19; IMB Australia Ltd., St. Leonards, NSW, Australia) to analyse the association between ferritin levels and donor parameters (age, sex, Hb level, number and type of prior donations (ever and in the previous 12 months) and time since previous donation) as well as each individual SNP. Both high and low ferritin groups were compared with the medium group to identify SNPs that may be associated with high or low ferritin levels. The homozygous wild-type allele for each SNP was used as the reference genotype to compare the effect of having one or two variant alleles on donor ferritin levels. Donors with missing demographic or genotypic information were excluded from analyses. SNPs with an overall p-value less than 0.05 were adjusted for significant donor parameters.

Results

A total of 800 non-first-time donors were tested in this study. Of these, 54% were male (Online Supplementary Data, Table SII), and more than half of the donors (484 individuals, 61%) were greater than 45 years old. Almost half of all donors studied (377) had given a pervious donation within the preceding 3 months, and the majority were men. A total of 31% of donors had an iron loss of less than 220 mg (equal to 1 whole blood donation) within the last 12 months, while 28% had an iron loss of between 440 and 880 mg (equal to 2 to 4 whole blood donations). Interestingly, ten donors, nine of whom were male, had iron loss equal to greater than four whole blood donations (880 mg) per year, but maintained normal Hb levels.

All 800 samples were successfully genotyped for the selected ten SNPs (Online Supplementary Content, Table SIII). Most SNPs were frequent in our donor cohort (mean allele frequency: 0.22-0.46), except for the SNP rs1799852 (mean allele frequency: 0.10). SNP frequencies obtained in Australian donors were similar to those that were reported in the National Centre for Biotechnology Information (NCBI) by previous studies. None of the SNPs differed from the HWE.

Not surprisingly, Hb levels were associated with ferritin levels in both male and female donors (overall p=0.024, and p<0.001, respectively) (Online Supplementary Content, Table SIV). For both sexes, increased amounts of iron loss had a negative effect on ferritin level (p<0.001), while a longer donation interval was positively associated with iron stores (overall p<0.001 for males and p=0.013 for females). Age was significantly associated with ferritin levels in female donors only (p=0.018).

Out of the ten SNPs analysed, rs3923809 (p=0.027) and rs235756 (p=0.029) were significantly associated with ferritin levels in male donors (Table I). Male donors with one G allele within the SNP rs3923809 were two times more likely to have high ferritin levels (p=0.011), while having two G alleles increased this likelihood to three times (p=0.004) (Table II). After adjusting for donation-induced iron loss and days since previous donation, the G allele of the SNP rs3923809 remained significantly associated with the high ferritin group, although this SNP was not significant overall (p=0.073) (Table II). For the SNP rs235756, the impact of heterozygosity or homozygosity of the variant genotype on ferritin levels could not be determined (individual p>0.05) (Table II). This SNP remained significantly associated with ferritin levels (p=0.038) after adjusting for donor parameters, although the significance of heterozygosity and homozygosity of the variant allele remained to be determined (p>0.05) (Table II). Low numbers of homozygous variant genotypes were present in high and low ferritin groups (Online Supplementary

Table I - Association between SNP and ferritin levels.

Gene	SNP name	Overall p-value*			
		Male		*Female*	
		Before†	*After‡*	*Before†*	*After‡*
BTBD9	rs9296249	0.641		0.996	
	rs3923809	0.027	0.073	0.748	
TMPRSS6	rs855791	0.874		0.869	
	rs4820268	0.980		0.043	0.025
TFR2	rs7385804	0.512		0.480	
TF	rs3811647	0.824		0.686	
	rs1799852	0.260		0.736	
	rs2280673	0.473		0.377	
BMP2	rs235756	0.029	0.038	0.711	
SMIM1	rs1175550	0.961		0.425	

*Overall p-value <0.05 indicates this particular SNP has a statistically significant impact on ferritin levels; †before adjusting for donor parameters; ‡after adjusting for significant donor parameters (donation-induced iron loss and time since previous donation for male donors; age, donation-induced iron loss and time since previous donation for female donors); Shaded cells show SNP significantly (p<0.05) associated with ferritin levels. SNP: single nucleotide polymorphism.

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Table II - Association between ferritin levels and *BTBD9* rs3923809 or *BMP2* rs235756 in male donors.

SNP	**Before adjusting for donor parameters**			
	Ferritin ≤15 ng/mL			
	GG vs *AA*		*AG* vs *AA*	
	OR (95% CI)	*p-value*	*OR (95% CI)*	*p-value*
rs3923809	1.491(0.395-5.632)	0.556	1.438 (0.651-3.174)	0.369
rs235756	0.297 (0.037-2.406)	0.255	1.967 (0.865-4.471)	0.106
	Ferritin >100 ng/mL			
rs3923809	3.253 (1.443-7.333)	0.004	2.091 (1.186-3.688)	0.011
rs235756	0.434 (0.172-1.091)	0.076	0.742 (0.436-1.262)	0.271
	After adjusting for significant donor factors§			
	Ferritin ≤15 ng/mL			
rs3923809	1.949 (0.493-7.709)	0.342	1.565 (0.682–3.592)	0.291
rs235756	0.327 (0.039-2.768)	0.305	2.077 (0.868-4.973)	0.101
	Ferritin >100 ng/mL			
rs3923809	2.715 (1.164-6.330)	0.021	1.967 (1.097-3.525)	0.023
rs235756	0.448 (0.176-1.141)	0.092	0.724 (0.417-1.254)	0.249

Shaded cells indicate the genotype was significantly (p<0.05) associated with this particular ferritin group compared to the wild-type (AA) genotype; §Significant donor factors include time since previous donation and donation-induced iron loss in the previous 12 months. SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

Data, Table SV), suggesting that this could be due to the small sample size.

Out of the ten SNPs analysed, only one SNP, rs4820268, was significantly associated with ferritin levels, specifically low ferritin levels, in female donors (Table I). The overall association remained significant (p=0.025) after adjusting for significant donor parameters. Females with the AG genotype within this SNP were three times more likely to have low ferritin levels (p=0.010) (Table III), with this observation remaining statistically significant (p=0.012) after adjusting for donor parameters.

Among the seven other SNPs investigated, the SNP rs1799852 (*TF* gene) could not be analysed because of the very low frequency (8/800 homozygous variant [TT]genotype) (Online Supplementary Content, Table SVI). However, males with the TT genotype of this SNP had the highest mean ferritin values (145 ng/mL) among those with all genotypes, in both sexes. Given that this SNP is proposed to be associated with increased serum ferritin in Australians of European descent[18], analysis of this SNP in a larger number of samples would allow determination of whether there is indeed an association between this SNP and donor ferritin levels. The SNP rs1175550 was not shown to be associated with ferritin levels (Table I); however, female donors with the variant allele had a smaller ferritin range of 4-68 ng/mL, compared to those in other genotype groups (Online Supplementary Data, Table SVI). This is likely to be a result of the small numbers of this genotype identified in our study population, or could be due to the impact of the homozygous variant allele. Additional studies are required to assess the impact of this SNP on ferritin levels of female donors.

Table III - Association between ferritin levels and *TMPRSS6* rs4820268 in female donors.

Before adjusting for donor parameters			
Ferritin ≤8 ng/mL			
GG vs *AA*		*AG* vs *AA*	
OR (95% CI)	*p-value*	*OR (95% CI)*	*p-value*
1.808 (0.580-5.641)	0.307	3.348 (1.328-8.438)	0.010
Ferritin >60 ng/mL			
0.775 (0.339-1.769)	0.545	0.890 (0.379-1.462)	0.391
After adjusting for significant donor factors*			
Ferritin ≤8 ng/mL			
2.073 (0.646-6.647)	0.220	3.443 (1.314-9.021)	0.012
Ferritin >60 ng/mL			
0.573 (0.238-1.378)	0.214	0.548 (0.265-1.133)	0.104

Shaded cells indicate the genotype was significantly (p<0.05) associated with this particular ferritin group compared to the wild-type (AA) genotype; *Significant donor factors include age, time since previous donation and donation-induced iron loss in the previous 12 months. SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

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Discussion

ID is a significant issue for frequent whole blood donors, with being female, particularly a younger female, time since previous donation and number of donations in the past 2 years identified as independent predictors for ID erythropoiesis[2]. ID is implicated in a range of adverse effects, ranging from fatigue[30] to impaired cognitive performance, especially in young individuals who are undergoing neurological and physical development[31,32]. Given that blood donation is associated with ID and IDA, donors may suffer from these adverse effects depending on the severity of iron depletion. In this study, we investigated whether SNP involved in parameters of iron status were associated with ferritin levels in a cohort of Australian blood donors. We show that the SNPs rs4820268, rs235756 and rs3923809 were potentially associated with changes in iron metabolism and lead to variations in ferritin levels.

The SNP rs4820268 has been shown to be associated with decreased serum iron, serum ferritin and red cell indices in Australians of European descent[18], Chinese Han[17], and Italian populations[28]. In our study, a similar finding was obtained, whereby this SNP was significantly associated with low ferritin levels in female donors. This demonstrates the presence of a common variant that may potentially lower iron stores in healthy individuals. The SNP rs4820268 is a synonymous substitution found in exon 13 of the *TMPRSS6* gene[33]. The *TMPRSS6* gene encodes a membrane serine protease, Matriptase-2, which can strongly suppress the expression of hepcidin - the principal negative iron regulator in human[34]. A synonymous substitution does not result in amino acid changes, although it may cause inefficient translation of the variant codon, and lead to altered structure of clinically important proteins[35]. The SNP rs4820268 may, therefore, lead to decreased production or function of Matriptase-2 and thus result in variations in iron stores. Further analyses including measuring the mRNA or total protein levels may assist in understanding the mechanism.

The SNP rs235756 was identified in a study of hemochromatosis patients, in which individuals with this SNP had increased serum ferritin levels[21]. However, this result was not replicated in a study that involved 2,139 Chinese women[17]. In our study, an impact of this SNP was observed to have an impact on ferritin levels in males, however, the significance of homozygosity and heterozygosity of the variant allele could not be determined. This was probably a result of low statistical power or not enough samples in the variant genotype group. The SNP rs235756 is located in the 3' flanking region of the *BMP2* gene, which is rapidly removed while pre-mature RNA is processed into mature mRNA[36]. However, this region often has sequences that affect the formation of the 3' end of the mRNA, and may also contain enhancer or protein-binding sites[37]. The presence of the SNP rs235756 at this region may, therefore, lead to altered bone morphogenetic protein (BMP) 2 mRNA regulation and decreased function/production of encoded protein. The BMP2 signalling pathway is proposed to induce hepcidin expression and decrease systemic iron levels[38]. SNPs in the *BMP2* gene may, therefore, compromise the stimulation of hepcidin expression, leading to increased iron absorption and accumulation. Additional studies with a larger sample size would be necessary to further understand the role of this SNP in Australian donors.

The SNP rs3923809 is found in the intronic region of the *BTBD9* gene, which is proposed to exert diverse biological functions, although it has not so far been shown to be involved in iron metabolism[39-41]. This SNP was associated with decreased serum ferritin levels, 13% per A allele in an Icelandic population, and increased susceptibility to restless leg syndrome[25]. We found that although this SNP was initially associated with ferritin levels in male donors, the association was not significant overall after adjusting for donor parameters. However, the G allele of this SNP remained significantly associated with high ferritin levels after adjusting for significant donor parameters, which is in accordance with the Icelandic study[25]. The insignificant results of the overall analysis could be a consequence of a small sample size not being able to achieve statistical significance. Thus, additional studies with a larger sample size are necessary to confirm the impact of this SNP. Although introns are removed before mRNA is translated into mature proteins, an intronic SNP present in the regulatory region of the gene may affect alternative splicing of the mRNA and lead to the formation of an alternative protein isoform[42], or change the levels of gene expression of one or more genes[43]. The SNP rs3923809 may, therefore, lead to altered iron metabolic pathways: (i) by contributing to altered expression of BTBD protein, which may indirectly affect iron storage through an unknown mechanism; (ii) by affecting the expression of genes involved in iron metabolism; or (iii) through association with an as yet unidentified SNP that is associated with ferritin levels. Future investigations involving DNA sequencing and cDNA analysis would be required to elucidate possible mechanisms, with the outcome having the potential to provide additional evidence in relation to novel pathways involved in iron regulation.

Our study has a number of limitations. As this study utilised convenience samples remaining after routine testing, plasma rather than serum was investigated. Our initial analysis suggested plasma and serum ferritin levels are not interchangeable, with plasma ferritin levels estimated to be half of those in serum[44]. In the

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absence of a plasma ferritin reference range, we therefore assigned low, medium and high ferritin levels based on this approximate conversion. This allowed us to stratify donors based on ferritin levels, but did not allow direct comparison with the serum ferritin ranges used for assessing ID and IDA or suitability to donate blood. Additional studies using serum samples are required to further validate our findings. In addition, information on the donors' diet, use of iron supplementation, menopausal status (female donors) and other factors that may affect ferritin levels (such as infection or inflammation) were not obtained. A large longitudinal study is required to understand the contribution of such factors. Furthermore, we analysed the association between various SNPs and donors' ferritin levels at their current donation; additional studies could involve testing donors' ferritin levels at two sequential donations or longitudinally over many donations, so as to identify SNPs associated with iron recovery after donation. Moreover, as serum ferritin levels are influenced by infection and inflammation[45], additional studies could look at other parameters involved in iron status, such as transferrin iron-binding capacity or percent transferrin saturation[46].

Conclusions

This study provides evidence of an association between genetic factors in blood donors and plasma ferritin levels. Although further validation is required, the three SNPs identified, rs3923809, rs235756 and rs4820268, have the potential to be useful in identifying the most suitable donation frequencies for eligible blood donors who pass the initial Hb screen. For example, male donors with a variant SNP rs3923809 allele were more likely to have high ferritin levels and may, therefore, be suitable for more frequent donation or double red blood cell donation, while female donors heterozygous for the SNP rs4820268 were more likely to have low ferritin levels and thus may be better managed with a longer donation interval. In addition, significant genotypes discovered in future analyses may assist in generating a SNP panel for accurately predicting ferritin variations between blood donations. Overall, this study provides support that genetic testing may be useful for the generation of predictive algorithms to assist in maintaining iron levels in regular blood donors with varied donation patterns, and thus, allow for improved, individualised donor management that better caters for donor health and wellbeing.

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Authorship contributions

YJ performed the experimental work, analysed the data and prepared the first draft of this manuscript. All Authors contributed to the study design, data interpretation, data analysis/consolidation and drafting/approval of the manuscript.

The Authors declare no conflicts of interest.

References

1) Garry PJ, Koehler KM, Simon TL. Iron stores and iron absorption: effects of repeated blood donations. Am J Clin Nutr 1995; **62**: 611-20.
2) Cable RG, Glynn SA, Kiss JE, et al. Iron deficiency in blood donors: the REDS-II Donor Iron Status Evaluation (RISE) study. Transfusion 2012; **52**: 702-11.
3) Cancado RD, Langhi D. Blood donation, blood supply, iron deficiency and anemia - it is time to shift attention back to donor health. Rev Bras Hematol Hemoter 2012; **34**: 330-1.
4) Custer B, Johnson ES, Sullivan SD, et al. Quantifying losses to the donated blood supply due to donor deferral and miscollection. Transfusion 2004; **44**: 1417-26.
5) Australian Red Cross Blood Service. Anaemia and iron deficiency in blood donors. 2014. Available at: http://www.transfusion.com.au/anaemia_management/iron_deficiency_anaemia/anaemia_and_iron_deficiency_blood_donors. Accessed on 24/02/2015.
6) American Red Cross. Iron Information for All Donors. 2016. Available at: http://www.redcrossblood.org/learn-about-blood/iron-and-blood-donation/iron-info-all-donors. Accessed on 01/02/2016.
7) Canadian Blood Services. Hemoglobin. 2016. Available at: https://www.blood.ca/en/blood/hemoglobin. Accessed on 01/02/2016.
8) da Silva MA, de Souza RA, Carlos AM, et al. Etiology of anemia of blood donor candidates deferred by hematologic screening. Rev Bras Hematol Hemoter 2012; **34**: 356-60.
9) Semmelrock MJ, Raggam RB, Amrein K, et al. Reticulocyte hemoglobin content allows early and reliable detection of functional iron deficiency in blood donors. Clin Chim Acta 2012; **413**: 678-82.
10) Harthoorn-Lasthuizen EJ, Lindemans J, Langenhuijsen MM. Zinc protoporphyrin as screening test in female blood donors. Clin Chem 1998; **44**: 800-4.
11) Baart AM, de Kort WL, Moons KG, et al. Zinc protoporphyrin levels have added value in the prediction of low hemoglobin deferral in whole blood donors. Transfusion 2013; **53**: 1661-9.
12) Baart AM, Atsma F, McSweeney EN, et al. External validation and updating of a Dutch prediction model for low hemoglobin deferral in Irish whole blood donors. Transfusion 2014; **54**: 762-9.
13) Mast AE, Schlumpf KS, Wright DJ, et al. Demographic correlates of low hemoglobin deferral among prospective whole blood donors. Transfusion 2010; **50**: 1794-802.
14) Rigas AS, Sorensen CJ, Pedersen OB, et al. Predictors of iron levels in 14,737 Danish blood donors: results from the Danish Blood Donor Study. Transfusion 2014; **54**: 789-96.
15) Benyamin B, Ferreira MA, Willemsen G, et al.. Common variants in TMPRSS6 are associated with iron status and erythrocyte volume. Nat Genet 2009; **41**: 1173-5.

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16) Kloss-Brandstatter A, Erhart G, Lamina C, et al. Candidate gene sequencing of SLC11A2 and TMPRSS6 in a family with severe anaemia: common SNPs, rare haplotypes, no causative mutation. PLoS One 2012; **7**: e35015.
17) An P, Wu Q, Wang H, et al. TMPRSS6, but not TF, TFR2 or BMP2 variants are associated with increased risk of iron-deficiency anemia. Hum Mol Genet 2012; **21**: 2124-31.
18) Benyamin B, McRae AF, Zhu G, et al. Variants in TF and HFE explain approximately 40% of genetic variation in serum-transferrin levels. Am J Hum Genet 2009; **84**: 60-5.
19) Ganesh SK, Zakai NA, van Rooij FJ, et al. Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. Nat Genet 2009; **41**: 1191-8.
20) Chambers JC, Zhang W, Li Y, et al. Genome-wide association study identifies variants in TMPRSS6 associated with hemoglobin levels. Nat Genet 2009; **41**: 1170-2.
21) Milet J, Dehais V, Bourgain C, et al. Common variants in the BMP2, BMP4, and HJV genes of the hepcidin regulation pathway modulate HFE hemochromatosis penetrance. Am J Hum Genet 2007; **81**: 799-807.
22) Constantine CC, Anderson GJ, Vulpe CD, et al. A novel association between a SNP in CYBRD1 and serum ferritin levels in a cohort study of HFE hereditary haemochromatosis. Br J Haematol 2009; **147**: 140-9.
23) Torti L, Teofili L, Capodimonti S, et al. Hypoxia-inducible factor-1α(Pro-582-Ser) polymorphism prevents iron deprivation in healthy blood donors. Blood Transfus 2013; **11**: 553.
24) Sorensen E, Grau K, Berg T, et al. A genetic risk factor for low serum ferritin levels in Danish blood donors. Transfusion 2012; **52**: 2585-9.
25) Stefansson H, Rye DB, Hicks A, et al. A genetic risk factor for periodic limb movements in sleep. N Engl J Med 2007; **357**: 639-47.
26) Haer-Wigman L, Stegmann TC, Solati S, et al. Impact of genetic variation in the SMIM1 gene on Vel expression levels. Transfusion 2015; **55**: 1457-66.
27) Cvejic A, Haer-Wigman L, Stephens JC, et al. SMIM1 underlies the Vel blood group and influences red blood cell traits. Nat Genet 2013; **45**: 542-5.
28) Pichler I, Minelli C, Sanna S, et al. Identification of a common variant in the TFR2 gene implicated in the physiological regulation of serum iron levels. Hum Mol Genet 2011; **20**: 1232-40.
29) Court MH. Court Lab Calculator. 2008. Available at: http://emerald.tufts.edu/~mcourt01/lab_protocols.htm. Accessed on 10/03/2015.
30) Beutler E, Larsh SE, Gurney CW. Iron therapy in chronically fatigued, nonanemic women: a double-blind study. Ann Intern Med 1960; **52**: 378-94.
31) Khedr E, Hamed SA, Elbeih E, et al. Iron states and cognitive abilities in young adults: neuropsychological and neurophysiological assessment. Eur Arch Psychiatry Clin Neurosci 2008; **258**: 489-96.
32) Georgieff MK. Long-term brain and behavioral consequences of early iron deficiency. Nutr Rev 2011; **69** (Suppl 1): S43-8.
33) Tanaka T, Roy CN, Yao W, et al. A genome-wide association analysis of serum iron concentrations. Blood 2010; **115**: 94-6.
34) Du X, She E, Gelbart T, et al. The serine protease TMPRSS6 is required to sense iron deficiency. Science 2008; **320**: 1088-92.
35) Goymer P. Synonymous mutations break their silence. Nat Rev Genet 2007; **8**: 92.
36) Lin M, Stewart DJ, Spitz MR, et al. Genetic variations in the transforming growth factor-beta pathway as predictors of survival in advanced non-small cell lung cancer. Carcinogenesis 2011; **32**: 1050-6.
37) Hayashi S, Watanabe J, Kawajiri K. Genetic polymorphisms in the 5'-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. J Biochem 1991; **110**: 559-65.
38) Babitt JL, Huang FW, Wrighting DM, et al. Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nat Genet 2006; **38**: 531-9.
39) Ahmad KF, Melnick A, Lax S, et al. Mechanism of SMRT corepressor recruitment by the BCL6 BTB domain. Mol Cell 2003; **12**: 1551-64.
40) Bomont P, Cavalier L, Blondeau F, et al. The gene encoding gigaxonin, a new member of the cytoskeletal BTB/kelch repeat family, is mutated in giant axonal neuropathy. Nat Genet 2000; **26**: 370-4.
41) Pintard L, Willis JH, Willems A, et al. The BTB protein MEL-26 is a substrate-specific adaptor of the CUL-3 ubiquitin-ligase. Nature 2003; **425**: 311-6.
42) Moyer RA, Wang D, Papp AC, et al. Intronic polymorphisms affecting alternative splicing of human dopamine D2 receptor are associated with cocaine abuse. Neuropsychopharmacology 2011; **36**: 753-62.
43) Kim JM, Lim KS, Hong JS, et al. A polymorphism in the porcine miR-208b is associated with microRNA biogenesis and expressions of SOX-6 and MYH7 with effects on muscle fibre characteristics and meat quality. Anim Genet 2015; **46**: 73-7.
44) Ji Y, Faddy H, Hyland C, Flower R. A plasma ferritin is not always a serum ferritin. Pathology 2015; **47**: S89-90.
45) Torti FM, Torti SV. Regulation of ferritin genes and protein. Blood 2002; **99**: 3505-16.
46) Liu K, Kaffes AJ. Iron deficiency anaemia: a review of diagnosis, investigation and management. Eur J Gastroenterol Hepatol 2012; **24**: 109-16.

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