



Investigating the relationship between iron and depression



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ABSTRACT

Lower levels of circulating iron have been associated with depression. Our objective was to investigate the phenotypic and genetic relationship between measures of circulating levels of iron (serum iron, transferrin, transferrin saturation, and ferritin) and depressive symptoms. Data were available from ongoing studies at QIMR Berghofer Medical Research Institute (QIMRB), including twin adolescents (mean age 15.1 years, standard deviation (SD) 3.2 years), and twin adults (mean age 23.2 years, SD 2.2 years). In the adolescent cohort, there were 3416 participants from 1688 families. In the adult cohort there were 9035 participants from 4533 families. We estimated heritabilities of, and phenotypic and genetic correlations between, traits. We conducted analyses that linked results from published large-scale genome-wide association studies (including iron and Major Depressive Disorder) with our study samples using single SNP and multi-SNP genetic risk score analyses, and LD score regression analyses. In both cohorts, measures of iron, transferrin, transferrin saturation, and log₁₀ of ferritin (L10Fer) were all highly heritable, while depressive measures were moderately heritable. In adolescents, depression measures were higher in those in the middle 10th versus top 10th percentile of transferrin saturation measures ($p = 0.002$). Genetic profile risk scores of the iron measures were not significantly associated with depression in study participants. LD score analyses showed no significant genetic relationship between iron and depression. Genetic factors strongly influence iron measures in adolescents and adults. Using several different strategies we find no evidence for a genetic contribution to the relationship between blood measures of iron and measures of depression.

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1. Introduction

Iron is an essential component of brain growth and development, and is needed for cell differentiation, protein synthesis, hormone production and important aspects of cellular energy

metabolism (Khedr et al., 2008). Iron deficiency is a major health problem worldwide, affecting more than a quarter of the world's population (Cook, 1995) and remains the leading cause of anaemia globally (Kassebaum et al., 2014).

Major Depressive Disorder (MDD) is also a major health problem worldwide, with considerable morbidity and mortality (Moussavi et al., 2007; Whiteford et al., 2013). This morbidity occurs from an early age, with MDD in adolescents often persisting or reappearing in adult life (Dunn and Goodyer, 2006). MDD has also been associated with inflammation (Maes et al., 2009), and changes in

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iron measures, such as decreased serum transferrin levels, are also seen in inflammatory states (Feelders et al., 1998). Therefore MDD may also be related to iron measures indirectly (Baune et al., 2010). In view of the health burdens associated with both iron deficiency anemia and MDD, and the association between these phenotypes, the relationship between iron and depression is an area worthy of further investigation.

Fatigue, lethargy, and depression can all be symptoms of iron deficiency (Verdon et al., 2003; Lomagno et al., 2014), which has led to studies investigating the relationship between circulating levels of iron and depression. However, these studies are few in number, particularly for adolescents. The results of published studies provide an unclear view of the relationship between circulating levels of iron and depression. In community samples, some studies have shown lower levels of serum ferritin (a measure of body iron stores) are associated with depression in adults (Yi et al., 2011; Shariatpanaahi et al., 2007), but other studies have not observed this relationship (Hunt and Penland, 1999). In the context of iron-deficiency anaemia, mothers with young infants were found to show an improvement in depressive symptoms with iron supplementation (Beard et al., 2005).

It is unknown if the reported phenotypic relationships between blood iron levels and depression has a genetic component, as there are no published studies investigating the genetic relationship between these measures. The variation between individuals in measures of iron in serum is partly under genetic control, with heritability estimated to be ~25%–50% (Njajou et al., 2006; Whitfield et al., 2000; Traglia et al., 2009). Furthermore, iron absorption, absorption-diet interactions, and variation in iron loss (particularly in women) are all potentially subject to genetic influences (Whitfield et al., 2000). Genome-wide association studies (GWAS) of iron phenotypes have identified 11 loci affecting the variation in iron status (Benyamin et al., 2014). Together these loci explained 3.4%, 7.2%, 6.7%, and 0.9% of the phenotypic variance in serum iron, transferrin, transferrin saturation and serum ferritin.

Likewise, genetic influences also contribute to individual differences in MDD. Heritabilities are estimated to be 31%–42% for depression (Sullivan et al., 2000). Genetic influences in MDD are also found in adolescents (Goodyer, 2008). A mega-analysis of GWAS for MDD found no genome-wide significant loci in Caucasians (Ripke et al., 2013), but it is expected (Levinson et al., 2014) that larger samples sizes will deliver MDD associated loci as has been found for schizophrenia (Ripke et al., 2014). Moreover, the era of genome-wide association studies provides a new experimental paradigm to explore the genetic relationship between traits using data sets independently collected for different measures (Wray et al., 2014; Bulik-Sullivan et al., 2015a). Genome-wide genetic association studies can generate a measure of genetic liability to a condition (e.g. MDD) even if no single locus achieves genome-wide significance.

The aim of this study is to: 1) investigate the phenotypic and genetic relationship between measures of circulating levels of iron and depressive symptoms in two large independent community cohorts of twins, and 2) investigate if SNPs that explain variation in iron phenotypes are associated with measures of depression. If there is a genetic association between iron measures and depression measures, this may provide useful information about the biology of MDD.

2. Methods

2.1. Cohorts

2.1.1. 1) Adolescent cohort

Participants are 16 year old twins from the Brisbane Adolescent

Twin Study (Wright and Martin, 2004). Participants completed the Somatic and Psychological Health Report (SPHERE) (Hickie et al., 1998), a self-report questionnaire that includes 14 anxiety and depression items. Items were recorded as binary responses, coded as 0 (less anxiety) and 1 (more anxiety) which sum to provide a quantitative measure of anxiety and depression (Hansell et al., 2012) giving greater power to detect genetic influence (Neale et al., 1994) than a binary diagnostic code. Some participants completed the SPHERE either before or after the collection of iron measures, with a mean age at completion of SPHERE of 15.1 years (standard deviation (SD) 3.2 years). The participants also provided a blood sample (mean age at time of blood collection 16.2 years (SD 0.2 years)). This allowed quantification of a number of iron phenotypes in the serum: iron (measured in $\mu\text{mol/L}$), transferrin (g/L), transferrin saturation (measured as a percentage of transferrin saturated with iron), and ferritin ($\mu\text{g/L}$). We used Roche methods on a Hitachi 917 analyzer to measure the levels of serum iron, transferrin and ferritin. Transferrin is an iron-binding blood plasma glycoprotein that controls the level of free iron (Crichton and Charleaux-Wauters, 1987). Ferritin is an intracellular protein that stores iron and releases it in a controlled manner (Crichton and Charleaux-Wauters, 1987). A log transformation was applied to the ferritin (L10Fer) measures to normalise the distribution. The sample size comprised between 1363 and 2890 adolescents from 1688 families, depending on the measure.

2.1.2. 2) Adult cohort

Participants are adult twins, taken from the Australian Twin Registry. In 1989, a Health and Lifestyle Questionnaire (HLQ) was mailed to twins born between the years of 1964–1971. The mean age of respondents was 23.2 years (standard deviation (SD) 2.2 years). The psychiatric symptom inventory section in the HLQ contained self-report questions, consisting of 14 anxiety and depression items from the Delusions Symptoms State Inventory (DSSI), which provides a quantitative score of depression (Bedford and Deary, 1999), as well as a 19 item subset of the 90-item Symptom Checklist (SCL-90) (Derogatis et al., 1976). When these 33 items are factor analysed, 4 factors are derived: depression, anxiety, somatic distress, and sleep difficulties (Gillespie et al., 1999). Study participants provided a blood sample approximately 10 years later, allowing quantification of serum iron ($\mu\text{mol/L}$), transferrin (g/L), transferrin saturation (percentage of transferrin saturated with iron), and ferritin ($\mu\text{g/L}$).

All procedures in both the adolescent and adult cohorts were approved by the Human Research Ethics Committee of QIMR Berghofer Medical Research Institute (QIMRB). The study was carried out in accordance with the latest version of the Declaration of Helsinki.

2.2. Statistical analyses

2.2.1. Estimation of genetic parameters

We estimated the genetic and environmental contributions to the traits using a classical twin design by contrasting the similarity of monozygotic (MZ) to dizygotic (DZ) twins. We first tested for one of the assumptions of the twin design, i.e. the equality of means and variances across zygosity and sex. We then calculated the MZ and DZ twin correlations. Broad sense heritabilities were calculated initially under a univariate additive genetic (A), common environment (C) and unique environment (E) model and then under bivariate models considering all pairwise combinations of traits. To examine the significance of the estimated univariate variance components, we also considered AE and CE reduced models. Goodness-of-fit of the reduced models was assessed using likelihood ratio tests. The sample size gave at least 99% power to detect

significant additive genetic variance (adolescents and adults) (Visscher, 2004; Visscher et al., 2008). Using this twin design, we also estimated the genetic and environmental correlations between the traits. These analyses were performed using the statistical program Mx (Neale et al., 2006).

2.2.2. Percentile analysis

We hypothesised that the relationship between iron and depression measures may be non-linear. Therefore, we also investigated whether there was a phenotypic association between the upper and lower range of circulating levels of iron measures with depressive symptoms (perhaps representing a non-linear relationship between iron and depression), by testing for differences between 1) the lowest 10th percentile and middle 10th percentile (i.e. 45th–55th percentile), 2) the highest 10th percentile and middle 10th percentile, 3) the lowest 10th percentile and highest 10th percentile, and 4) the lowest 5th percentile and highest 5th percentile (Welch Two Sample *t*-test). These choices reflect non-linear models that could be U-shaped (first two tests) as well as differences in the extremes (third and fourth tests).

2.2.3. Single nucleotide polymorphisms (SNPs) association analysis

Single nucleotide polymorphisms (SNPs) significantly associated with iron phenotypes in genome-wide association studies (GWAS) (Benyamin et al., 2009; McLaren et al., 2011; Tanaka et al., 2010; Benyamin et al., 2014) were identified: rs2698530, rs1799852, rs1830084, rs2280673, rs3811647, rs1799945, rs8177240, rs1800562, rs7787204, rs4820268, rs855791, rs9990333, rs987710, rs744653, rs7385804, rs235756, rs4921915, rs651007, rs6486121, rs174577, rs411988. The association statistics of these 21 SNPs (or their proxies, defined as in linkage disequilibrium $r^2 > 0.8$) with MDD were extracted from the Psychiatric GWAS Consortium (PGC) MDD GWAS summary statistics (Ripke et al., 2013). The sample size of the PGC MDD data gave at least 99% power to detect common (MAF > 0.1) variants that explain 0.5% of the variance at significance level 0.05 (Purcell et al., 2003).

2.2.4. Genomic risk profile score analysis

Genomic risk profile scores (GPRS) for iron, transferrin, transferrin saturation and L10Fer were generated for each individual in both the adolescent and adult ‘target’ samples using GWAS summary statistics data from the Genetics of Iron Status Consortium (GISC) (Benyamin et al., 2014) ‘discovery’ sample. The GISC data comprise association statistics between SNP genotypes and iron markers (serum iron, transferrin, transferrin saturation, and ferritin) from approximately 24,000 individuals from a total of 19 cohorts in 9 participating centres (Benyamin et al., 2014). QIMRB samples used here were part of the GISC. Since genetic prediction analysis requires independence between discovery and target samples, we recalculated effect sizes from the GISC cohorts after excluding QIMRB samples. GPRS were created (separately for adolescents and adults) as the sum of associated alleles of quasi-independent SNPs (pruned so that pairwise linkage disequilibrium between SNPs was less than $r^2 = 0.25$) weighted by their effect size estimated in the GISC meta-analysis.

GPRS for MDD were generated for individuals in the adolescent and adult target QIMRB samples using GWAS data from PGC MDD working group (Ripke et al., 2013). The PGC MDD ‘discovery’ sample has 9240 MDD cases and 9519 controls (Ripke et al., 2013). QIMRB samples were part of the PGC, so we recalculated effect sizes from the PGC MDD cohorts after excluding the QIMRB samples. In the calculation of GPRS, for SNP pruning we enforced pairwise linkage disequilibrium between SNPs to be $r^2 < 0.1$. GPRS for both iron measures and MDD were calculated using varying levels of discovery sample p-value thresholds in PLINK (Purcell et al., 2007).

The appropriate choice of p-value thresholds depend on the genetic architecture of the trait and the size and hence power of the sample. For each iron measure, we presented the p-value threshold from the GWAS results that maximised variance for the same iron measure in our data. Therefore, different thresholds were selected for different traits. To help in the interpretation of results, one individual per family was selected for inclusion in the profile scoring analysis ($n = 2394$ individuals for adult data, $n = 1028$ individuals for adolescent data).

Linear regression models were then used to predict how much of the variation in each of the phenotypes of our samples is explained by the GPRS and the direction of association. We used an age and sex adjusted regression model to test for an association between the profile scores (iron measures and MDD) and measures in the QIMRB samples. We also conducted the profile scoring analyses using all individuals, where the relatives were included using a mixed linear model by fitting family and twin IDs as random effects.

2.2.5. LD score analysis

Genetic correlations based on genome-wide SNPs between iron phenotypes and depression measures were estimated using LD score regression (v1.0.0) (Bulik-Sullivan et al., 2015b), based on GWAS summary statistics (effect size, direction of effect for each SNP, and sample size). The method exploits the expectation that SNPs in high LD regions (with large LD scores) will on average tag more causal variants than SNPs in low LD regions. Therefore the slope of the regression of the product of the association statistics for two traits on the LD score will provide an estimate of genetic covariance between the two traits, which can then be transformed to a genetic correlation estimate (Bulik-Sullivan et al., 2015b). LD score regression has previously been used to estimate genetic correlations for a wide range of traits, and the resulting estimates were consistent with estimates of genetic correlations obtained by bivariate GREML, which uses full genotype data to estimate genetic correlation (Bulik-Sullivan et al., 2015a). We used LD scores for each SNP calculated from 1000 Genomes, which are available on the LD Scores Regression github page (<https://github.com/bulik/ldsc>), both as the independent variable in LD Score regression, as well as for the regression weights (options “-ref-ld-chr” and “-w-ld-chr”). SNPs which were located in or around the iron metabolism related genes *TF*, *HFE*, and *TMPRSS6*, which had previously been shown to have large effect on variance of serum transferrin levels (Benyamin et al., 2009) were excluded from the analysis. However, including them in the analysis did not have a large impact on the estimates of genetic correlation. For MDD SNP heritability estimation, we used the file “pgc.mdd.full.2012-04.txt” (in “pgc.mdd.2012-04.zip”, from <https://www.med.unc.edu/pgc/downloads>). Fig. 1 represents the main strategies/types of analyses we used to investigate the genetic relationship between iron measures and depression measures.

3. Results

The numbers of study participants and mean iron and depression measures are shown in Table 1. With the exception of transferrin (in the adolescent and adult cohorts), all iron measures in males had significantly higher mean measures compared with females (Table 1). Mean depression measures were higher in females in both cohorts, however this difference was only significant in the adult cohort (Table 1). Sex effects were significant for all iron measures in the adolescent cohort. In the adult cohort, sex and age effects were significant for all iron and depression measures, so both sex and age were fitted as covariates in the estimation of heritability of all traits in both cohorts.

As expected for traits under genetic influences, MZ correlations

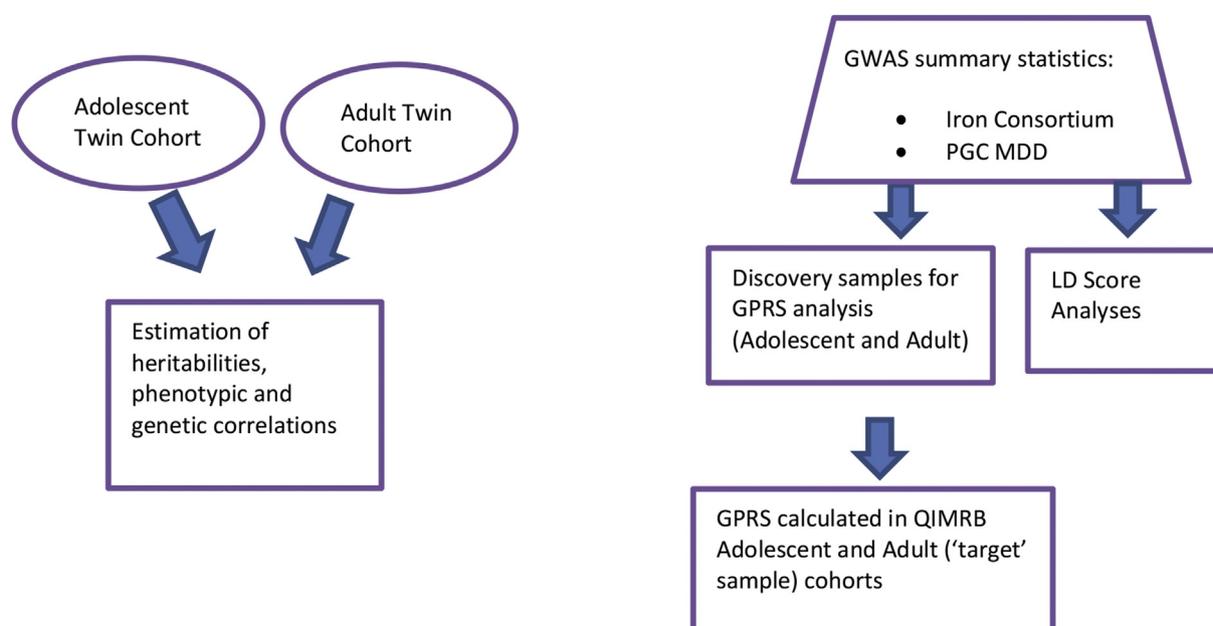


Fig. 1. Schematic representation of analyses. Key: GPRS = Genetic Profile Risk Score; MDD = Major Depressive Disorder; PGC = Psychiatric GWAS Consortium.

Table 1

Means and standard deviations for iron measures and depression measures.

Trait (Adolescents)	Total		Males		Females		p-value ^a
	Mean	SD	Mean	SD	Mean	SD	
Iron ($\mu\text{mol/L}$)	17.47	6.76	19.04	6.64	15.93	6.52	$<2.2 \times 10^{-16}$
Transferrin (g/L)	2.96	0.46	2.93	0.41	2.99	0.50	1.8×10^{-2}
Saturation (%)	24.05	9.69	26.35	9.60	21.79	9.24	$<2.2 \times 10^{-16}$
Log ferritin ($\mu\text{g/L}$)	1.62	0.34	1.74	0.26	1.51	0.38	$<2.2 \times 10^{-16}$
SPHERE	7.60	6.28	8.57	6.34	8.84	6.58	0.21
Trait (Adults)							
Iron ($\mu\text{mol/L}$)	19.44	6.63	20.77	6.42	18.69	6.64	$<2.2 \times 10^{-16}$
Transferrin (g/L)	2.78	0.49	2.66	0.37	2.85	0.47	$<2.2 \times 10^{-16}$
Saturation (%)	28.42	10.59	31.38	10.44	26.73	10.30	$<2.2 \times 10^{-16}$
Log ferritin ($\mu\text{g/L}$)	2.00	0.44	2.27	0.36	1.84	0.41	$<2.2 \times 10^{-16}$
Factor 1	0.00	1.06	-0.12	0.95	0.09	1.13	$<2.2 \times 10^{-16}$

Adolescents: number individuals iron measures = 1363 (males = 676, females = 687); depression measures = 2890 (males = 1327, females = 1563).

Adults: number individuals iron measures = 4366 (males = 1609, females = 2757); depression measures = 8072 (males = 2998, females = 5074).

^a p-value for difference between means for males and females; % = units for transferrin saturation = percentage of transferrin saturated with iron; Factor 1 = depression measure (adult cohort); SPHERE = Somatic and Psychological Health Report (depression measure adolescent cohort).

were higher than DZ correlations for all measures of iron (iron, transferrin, transferrin saturation, log of ferritin) in both the adolescent and adult cohort (Table S1 of supplementary material). MZ correlations were also significantly higher than DZ correlations for depression measures in the adolescent cohort. Twin correlations in the adolescent cohort were significantly different between sexes only for log of ferritin measures in MZ twins. In the adult cohort, twin correlations were significantly different between sexes for transferrin (MZ and DZ twins) and depression measures (MZ twins only).

We found heritability of the iron measures in both cohorts to be moderate to high (Table S2 of supplementary material). In contrast, estimates of variance attributable to the shared family environment (C) were not significantly different from zero. Heritability for depression measures in the adolescents (SPHERE) was 0.46 (95% CI 0.29–0.52), which was in keeping with that reported from an analysis in which data collected on multiple occasions were averaged (Hansell et al., 2012).

Bivariate analyses showed high phenotypic and genetic correlations among the iron phenotypes (except for the phenotypic

correlation between transferrin and iron), but that the phenotypic and genetic correlations between the iron phenotypes and depression measures were not significantly different from zero (Table 2). A bivariate AE model was used since the univariate analyses for these measures showed estimates of the common environmental components were small and not significantly different from zero.

3.1. Percentile analysis

In the adolescent cohort, depression measures were nominally significantly higher in those in the lowest 5th percentile of log ferritin measures compared to those in the highest 5th percentile of log ferritin measures ($p = 0.034$). We also found depression measures were higher in those in the middle 10th percentile of iron and transferrin saturation measures compared to those in the highest 10th percentile of iron and transferrin saturation measures ($p = 0.008$ and $p = 0.002$ respectively). In the adult cohort we did not find a phenotypic association between the upper and lower range of circulating levels of iron measures with depressive

Table 2
Estimates of proportion of variance attributable to additive genetic effects (A) or heritabilities (diagonals) from univariate ACE models; phenotypic correlations (above diagonals) and genetic correlations (below diagonals) from bivariate AE Models (95% confidence interval (CI) in parenthesis).

Adolescents:					
Trait	Iron	Transferrin	Saturation	Log10 ferritin	SPHERE
Iron	0.46 (0.15,0.66)	–0.03 (–0.10,0.05)	0.93 (0.92,0.94)	0.17 (0.10,0.25)	–0.06 (–0.14,0.02)
Transferrin	–0.10 (–0.34,0.07)	0.64 (0.42,0.81)	–0.34 (–0.41,–0.27)	–0.43 (–0.49,–0.36)	–0.02 (–0.10,0.06)
Saturation	0.94 (0.90,0.97)	–0.51 (–0.78,–0.36)	0.61 (0.39,0.70)	0.28 (0.21,0.35)	–0.05 (–0.13,0.03)
Log10 ferritin	0.32 (0.12,0.69)	–0.69 (–0.97,–0.51)	0.44 (0.27,0.68)	0.56 (0.28,0.72)	–0.04 (–0.12,0.04)
SPHERE	–0.19 (–0.51,0.04)	0.00 (–0.19,0.18)	–0.15 (–0.36,0.04)	–0.01 (–0.22,0.19)	0.46 (0.29,0.52)
Adults:					
Trait	Iron	Transferrin	Saturation	Log10 ferritin	Factor 1
Iron	0.35 (0.25,0.41)	–0.02 (–0.05,0.02)	0.90 (0.90,0.90)	0.24 (0.21,0.27)	–0.03 (–0.03,–0.02)
Transferrin	–0.15 (–0.25,–0.05)	0.52 (0.38,0.56)	–0.39 (–0.40,–0.38)	–0.39 (–0.39,–0.36)	0.00 (–0.04,0.03)
Saturation	0.95 (0.90,0.99)	–0.57 (–0.64,–0.57)	0.50 (0.44,0.55)	0.34 (0.31,0.37)	–0.03 (–0.03,–0.03)
Log10 ferritin	0.27 (0.15,0.41)	–0.33 (–0.42,–0.24)	0.38 (0.28,0.51)	0.42 (0.27,0.49)	0.03 (0.00,0.07)
Factor 1	0.02 (0.02,0.03)	0.10 (–0.01,0.24)	–0.03 (–0.17,0.10)	0.00 (–0.15,0.13)	0.30 (0.11,0.40)

Key: Factor 1 = depression measure (adult cohort); SPHERE = Somatic and Psychological Health Report (depression measure adolescent cohort).

symptoms. Investigation of the phenotypic relationship in males and females separately (in both adolescent and adult cohorts) showed no significant association between iron measures and depression measures after correcting for multiple testing (Table S3, S4, and S5 of supplementary material). To be conservative we used two-sided *t*-tests. However, given the multiple testing of 4 traits and 4 tests, the Bonferroni corrected significance level was 0.0031, so only the association between depression measures and transferrin saturation survived multiple testing ($p = 0.002$). Linear plots of depression measures for these percentiles of iron measures (adolescent and adult cohorts) are shown in the Supplementary material (Figure S1).

3.2. Association analysis

Of the 21 independent SNPs associated with iron phenotypes, 15 were included in the PGC MDD GWAS. The smallest *p*-value of association was rs744653 for MDD ($p = 0.027$, O.R. = 1.07, standard error (s.e.) = 0.030 prior to correction for multiple testing), and hence no association was significant after correcting for multiple testing. As expected, together the SNPs did not explain a significant proportion of the variance. Results of a sign test of direction of effect were consistent with there being no association between iron measures and MDD (*p*-values 0.94–1).

3.3. Genetic profile risk scores (GPRS)

Results of testing for an association between the profile scores (iron, transferrin, transferrin saturation, and log ferritin) and the measures of iron and depression in the QIMRB samples are shown in Table 3. Profile scores calculated for each individual in the QIMRB sample using GWAS association results from analyses of iron, transferrin, transferrin saturation, and log ferritin were each highly significantly associated with their respective trait measures (Table 3 column 3). Marginal associations were observed with genetic profile risk scores of iron and transferrin for depression

measures in adults at *p*-value thresholds (from the GWAS results) of $p < 0.05$ and $p < 0.1$ respectively (these *p*-value thresholds for iron and transferrin did not maximise variance, hence these thresholds were not chosen). However, these associations were not significant after correction for multiple testing (Bonferroni corrected significance level $p = 0.0013$). In both the adolescent and adult cohort, the direction of effect was not in the expected direction between transferrin genetic profile risk scores and depression measures (Table 3). Neither transferrin saturation profile scores nor log ferritin profile scores predicted depression status in adolescents or adults. Furthermore, profile scores (iron, transferrin, transferrin saturation, and log ferritin) did not predict depression measures in either cohort when relatives were included in the analyses (results not shown).

The results of testing for an association between MDD profile scores and the QIMRB adolescent and adult iron measures are shown in Table 4. The nominal associations ($p < 0.05$) listed in Table 4 do not survive correction for multiple testing. Furthermore, MDD profile scores did not predict iron measures in the adolescent or adult cohort when relatives were included in the analyses (results not shown).

3.4. LD score

Results of LD score analyses showed the same pattern for SNP genetic correlations between iron and transferrin, log ferritin and transferrin, and saturation and transferrin (Fig. 2) as expected from the whole genome genetic correlations estimated from the twin data (Table 2). The magnitude of the standard error (s.e.) of the correlation in Fig. 2, shows that despite the large sample sizes in the contributing GWAS, the data was only powered to detect very high correlations. The SNP-correlation between transferrin saturation and MDD ($0.29 \pm \text{se } 0.20$) was not significantly different from zero after accounting for multiple testing.

Table 3
Iron, transferrin, transferrin saturation, and log ferritin genetic profile risk scores – prediction of iron/depression measures.

Target	Adult/Adolescent	P-value Target	Variance explained Target (%)	β (S.E.)
Iron discovery sample: 212 SNPs with p-value threshold $P < 10^{-3}$ (Adolescent); 23 SNPs with p-value threshold $P < 10^{-5}$ (Adult)				
Iron	Adolescent	0.010	1.56	478.32 (185.66)
SPHERE	Adolescent	0.87	0	-22.15 (133.30)
Iron	Adult	1.81×10^{-8}	5.49	128.53 (22.49)
Depression	Adult	0.10	0.57	-2.67 (1.62)
Transferrin discovery sample: 27 SNPs with p-value threshold $P < 10^{-5}$ (Adolescent); 27 SNPs with p-value threshold $P < 10^{-5}$ (Adult)				
Transferrin	Adolescent	1.83×10^{-7}	7.09	7.95 (1.49)
SPHERE	Adolescent	0.50	0	10.25 (15.12)
Transferrin	Adult	8.20×10^{-10}	5.93	6.26 (1.003)
Depression	Adult	0.85	0.08	0.22 (1.15)
Transferrin saturation discovery sample: 227 SNPs with p-value threshold $P < 10^{-3}$ (Adolescent); 32 SNPs with p-value threshold $P < 10^{-5}$ (Adult)				
Transferrin sat	Adolescent	4.82×10^{-5}	4.30	742.63 (180.47)
SPHERE	Adolescent	1.00	0	-0.45 (92.43)
Transferrin sat	Adult	4.15×10^{-13}	9.07	200.27 (26.94)
Depression	Adult	0.20	0.38	-1.66 (1.29)
Log ferritin discovery sample: 212 SNPs with p-value threshold $P < 10^{-3}$ (Adolescent); 959 SNPs with p-value threshold $P < 5 \times 10^{-4}$ (Adult)				
Log ferritin	Adolescent	3.24×10^{-3}	2.14	40.85 (13.78)
SPHERE	Adolescent	0.52	0	-108.57 (170.04)
Log ferritin	Adult	2.26×10^{-3}	1.38	77.00 (25.10)
Depression	Adult	0.83	0.08	-7.60 (34.85)

Table 4
MDD genetic profile risk scores – prediction of iron measures.

P-value threshold of SNPs in MDD discovery GWAS	N SNPs	Target	P-value Target	Variance explained Target (%)	β (S.E.)
Adolescent sample					
$P < 10^{-6}$	11	Iron	0.17	0.24	-0.45 (0.32)
$P < 0.05$	17,699	Transferrin	0.066	0.61	0.043 (0.023)
$P < 10^{-6}$	11	Saturation	0.25	0.08	-0.53 (0.47)
$P < 0.10$	29,840	Log ferritin	0.46	0	0.014 (0.018)
Adult sample					
$P < 10^{-6}$	11	Iron	0.022	0.30	0.40 (0.18)
$P < 1.0$	119,734	Transferrin	0.43	0	-0.008 (0.011)
$P < 10^{-6}$	11	Saturation	0.093	0.13	0.46 (0.27)
$P < 10^{-4}$	733	Log ferritin	0.048	0.19	-0.017 (0.009)

Key: MDD = major depressive disorder.

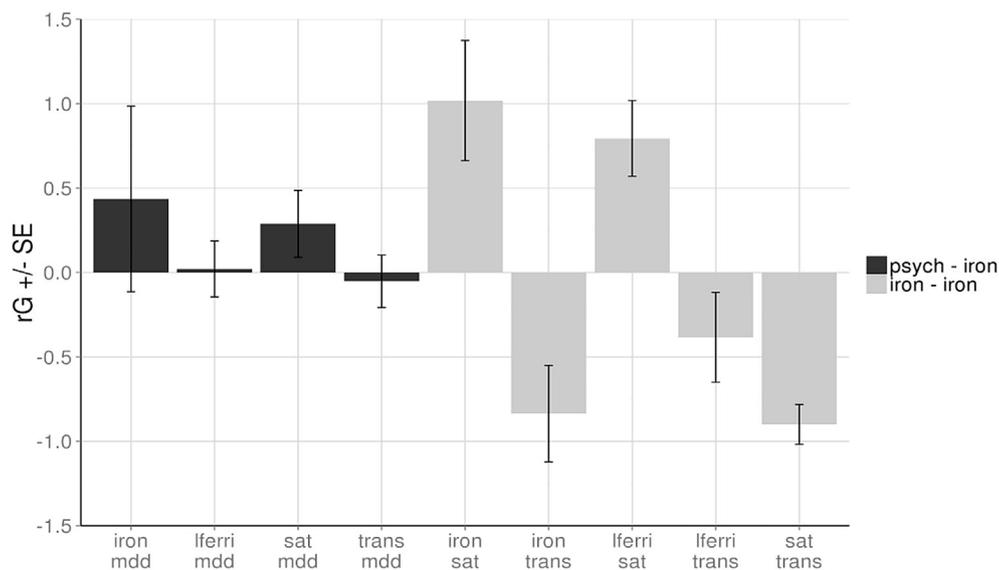


Fig. 2. Estimate of genome-wide SNP correlations (rG) between traits from LD score analyses using GWAS summary statistics (vertical lines represent standard error (SE)). Key: lferri = log ferritin; mdd = major depressive disorder; sat = transferrin saturation; trans = transferrin; iron – iron = correlations between iron traits; psych – iron = correlations between mdd and iron traits.

4. Discussion

This study examined the phenotypic and genetic relationship between measures of circulating levels of iron and depressive

symptoms. In both the adolescent and adult cohorts, phenotypic correlations of the iron measures with depression measures were not significantly different from zero.

We explored the possibility of a non-linear relationship

between iron and depression by testing the differences in adolescents and adults with measures for iron categorised into (i) the lowest 10th, middle 10th, and highest 10th percentiles, and (ii) the lowest 5th and highest 5th percentiles. In adolescents, depression measures were significantly higher (after correction for multiple testing) in those in the middle 10th percentile of transferrin saturation measures compared to those in the highest 10th percentiles of transferrin saturation measures ($p = 0.002$). We could not compare this finding to other studies as there were no published reports examining the relationship between iron measures and depression in community samples of adolescents. However, it is possible that a phenotypic relationship of serum iron measures with depressive symptoms is more likely to be observed in the presence of iron deficiency. In our study, the lowest iron level in adolescent females was $2.80 \mu\text{mol/L}$, and the lowest iron level in adolescent males was $4.0 \mu\text{mol/L}$ (normal reference range $14\text{--}32 \mu\text{mol/L}$ for males and females (Firkin and Rush, 1997)). These non-linear relationships have also been reported for other biological measures such as Vitamin D, with neonates who had either low or high levels of Vitamin D observed to have an increased risk of schizophrenia later in life (McGrath et al., 2010).

To examine the genetic relationship between measures of iron and measures of depression, we first used data from a community sample of twins to estimate heritabilities and genetic correlations of these measures. Heritabilities for circulating levels of iron measures were moderate to high, and somewhat higher than heritabilities estimated from previous studies (Fairweather-Tait et al., 2013; Traglia et al., 2009). These differences from previous studies may simply reflect sampling, but may also reflect differences in the ages of subjects between the studies. Both previous studies were in adults, but Traglia et al., 2009 reported the effect of age to be significant in the estimation of serum transferrin heritability (Traglia et al., 2009). Here, our estimates of heritability were higher in the adolescent than in the adult cohort.

As twin correlations were significantly different between sexes only in MZ adolescent twins for log of ferritin measures, MZ and DZ adult twins for transferrin, and MZ adult twins for depression measures, we did not perform a stratified analysis based on sex using a sex limitation model. In addition, our sample size was not large enough to be stratified into males and females. Dividing the samples into males and females would have reduced power, as well as precision of the variance component estimates, and increased the problem of the burden of multiple testing. Previous studies have shown that while there are some phenotypic differences between sexes, the genetic control of complex traits is largely the same between sexes (Yang et al., 2015; Rawlik et al., 2016). Even these studies required tens to hundreds of thousands of individuals to be able to detect any significant difference between males and females. For example, using genome-wide gene expression data in approximately 2000 individuals, genetic control was found to be shared between males and females (Kassam et al., 2016).

This study was well-powered ($\geq 99\%$) to detect significant additive genetic variance for iron and depression measures (Visscher, 2004; Visscher et al., 2008). However, we found that genetic correlations between iron measures and depressive measures were not different from zero. As expected, moderate to high genetic correlations were found between iron traits in both twin cohorts.

We used LD score analyses (Bulik-Sullivan et al., 2015b) to explore the genome-wide correlation between SNP effects for iron measures and MDD. This is likely the most powerful analysis based on currently available data, but still lacked power to detect small correlations as being different from zero. The SNP-correlations between the iron measures were consistent with the genetic correlations we had obtained using a different approach (see Table 2), with negative correlations between transferrin and iron, transferrin

and log ferritin, and transferrin and saturation. We found a positive correlation between MDD with iron and transferrin saturation, however these were not significant after accounting for multiple testing.

We used another independent strategy to explore the hypothesis of a genetic relationship between measures of circulating iron and depression. We undertook single SNP association analyses using published genome-wide association studies of iron and MDD. Despite these sample sizes giving at least 99% power to detect common ($\text{MAF} > 0.1$) variants (Purcell et al., 2003), none of the SNPs explaining variation in iron phenotypes showed significant association in the published MDD GWAS results (Ripke et al., 2013), and together the SNPs did not explain a significant proportion of the variance. We also conducted polygenic risk score analyses that linked results from published large-scale genome-wide association studies with our in-house adolescent and adult samples for which we also had genome-wide genotype data. As with single SNP association analysis, we found no significant association between iron measures and depression.

A limitation of this study was the time difference between iron measures and depression measures, particularly in the adult cohort. While mean age at completion of the SPHERE was 15.1 years for the adolescent cohort and blood collection was at mean age 16.2 years, in the adult cohort study participants provided a blood sample approximately 10 years after completing the DSSI. MDD is unremitting in 15% of individuals, and recurrent in 35% of individuals (Eaton et al., 2008). Furthermore, levels of circulating iron are tightly controlled (Donovan et al., 2006), with the same normal reference range for serum iron, serum transferrin, and transferrin saturation for males and females (Firkin and Rush, 1997). So while this time difference was important in the investigation of the phenotypic relationship, we would expect to see some association in the long term.

This time difference may also affect the estimate of the genetic correlation (using the classical twin design), between the iron and depression measures. However, this time difference will not affect the estimation of heritability of each trait. This time difference will also not affect the estimation of genetic correlation using LD score regression, as this method can estimate the genetic correlation between traits measured in completely different individuals.

A further limitation was that the depression measures did not use MDD DSM-IV diagnostic criteria. Therefore, the depression measures in the adolescent cohort are not comparable to those in the adult cohort.

5. Conclusion

We used multiple approaches to explore evidence for a genetic relationship between measures of circulating serum iron and depressive measures. Although each approach may have limitations, the results when taken together across the different approaches provide no compelling evidence for a genetic relationship between circulating iron and measures of depression, even though we were well-powered to detect a relationship through estimation of genetic correlation, association analyses, and LD score analyses. The reported phenotypic relationship between iron and depression may be more likely to be observed at times when the body requires higher amounts of iron, such as during times of rapid growth. In this way it may reflect a highly non-linear relationship, in which those with circulating levels of iron below an extreme threshold are more likely to experience symptoms of depression.

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Conflict of interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jpsychires.2017.07.006>.

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