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INVESTIGATING THE RELATIONSHIP BETWEEN IRON AND DEPRESSION

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Running title: Iron measures in depression

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Abstract

Introduction: Measures of circulating levels of 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.

Methodology: Data were collected from ongoing studies at QIMR Berghofer Medical Research Institute (QIMRB). We report data from 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 3,416 participants from 1,688 families. In the adult cohort there were 9,035 participants from 4,533 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 in-house samples using single SNP and multi-SNP genetic risk score analyses, and LD score regression analyses.

Results: In both cohorts, measures of iron, transferrin, transferrin saturation, and log 10 of ferritin (L10Fer) were all found to be highly heritable, while depressive measures were found to be moderately heritable. In adolescents, depression measures were significantly higher in those in the middle 10^{th} versus top 10^{th} percentile of transferrin saturation measures ($p=0.002$). Genetic profile risk scores of the iron measures was not significantly associated with depression in study participants. LD score analyses showed no significant genetic relationship between iron and depression.

Conclusions: 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.

Keywords: iron, transferrin, transferrin saturation, ferritin, depression

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, Hamed 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, Jasrasaria et al. 2014).

Major Depressive Disorder (MDD) is also a major health problem worldwide, with considerable morbidity and mortality (Moussavi, Chatterji et al. 2007, Whiteford, Degenhardt et al. 2013). Major Depressive Disorder (MDD) has also been associated with inflammation (Maes, Yirmiya et al. 2009), and changes in iron measures, such as decreased serum transferrin levels, are also seen in inflammatory states (Feelders, Vreugdenhil et al. 1998). Therefore MDD may also be related to iron measures indirectly (Baune, Neuhauser et al. 2010). In view of the health burdens associated with both iron deficiency anemia and MDD, and the association between the phenotypes of these disorders, this suggests the relationship between iron and depression is an area worthy of further investigation.

Studies investigating the relationship between circulating levels of iron and depression are few, 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, lower levels of serum ferritin (a measure of body iron stores) have also been associated with depression in adults (Shariatpanaahi, Shariatpanaahi et al. 2007, Yi, Nanri et al. 2011), but other studies have not observed a relationship between iron and depression (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, Hendricks et al. 2005)

It is unknown if the reported phenotypic relationships between blood iron levels and depression has a genetic component, as to date 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% (Whitfield, Cullen et al. 2000, Njajou, Alizadeh et al. 2006, Traglia, Sala 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, Cullen et al. 2000). Genome-wide association studies (GWAS) of iron phenotypes have identified 11 loci affecting the variation in iron status (Benyamin, Esko 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, Neale 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, Wray et al. 2013), but it is expected (Levinson, Mostafavi et al. 2014) that larger samples sizes will deliver MDD associated loci as has been found for schizophrenia (Ripke, Neale 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, Lee et al. 2014, Bulik-Sullivan, Finucane et al. 2015).

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.

Methods:

Cohorts:

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, Hadzi-Pavlovic 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, Wright et al. 2012) giving greater power to detect genetic influence (Neale, Eaves et al. 1994) than a binary diagnostic code. Mean age at completion of SPHERE was 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 μmol/L), transferrin (g/L), transferrin saturation (measured as a percentage of transferrin saturated with iron), and ferritin (μg/L). Transferrin is an iron-binding blood plasma glycoprotein that controls the level of free iron (Crichton and Charloteaux-Wauters 1987). Ferritin is an intracellular protein that stores iron and releases it in a controlled manner (Crichton and Charloteaux-Wauters 1987). A log transformation was applied to the ferritin (L10Fer) measures to normalise the distribution. The sample size comprised between 1,363 and 2,890 adolescents from 1,688 families, depending on the measure.

2) Adult Cohort

Participants are adult twins, taken from the Australian Twin Register. 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, Rickels et al. 1976). When these 33 items are factor analysed, 4 factors are derived: depression, anxiety, somatic distress, and sleep difficulties. Study participants provided a blood sample approximately 10 years after, allowing quantification of serum iron (μmol/L), transferrin (g/L), transferrin saturation (percentage of transferrin saturated with iron), and ferritin (μg/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).

Statistical Analyses:

1) Estimation of Genetic Parameters

Data were analysed using the statistical program Mx. After standard testing of assumptions (equality of means and variances across zygosity and sex), twin correlations were calculated. 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 were assessed using likelihood ratio tests. The sample size gave at least 99% power to estimate additive genetic variance greater than zero for iron and depression measures at significance level 0.05 (adolescents and adults) (Visscher 2004, Visscher, Gordon et al. 2008).

2) *Percentile analysis*

We hypothesised that the relationship between iron measures 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 10^{th} percentile and middle 10^{th} percentile (i.e. 45^{th}-55^{th} percentile), 2) the highest 10^{th} percentile and middle 10^{th} percentile, 3) the lowest 10^{th} percentile and highest 10^{th} percentile, and 4) the lowest 5^{th} percentile and highest 5^{th} 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).

3) Association Analysis

Single nucleotide polymorphisms (SNPs) significantly associated with iron phenotypes in genome-wide association studies (GWAS) (Benyamin, McRae et al. 2009, Tanaka, Roy et al. 2010, McLaren, Garner et al. 2011, Benyamin, Esko 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, Wray 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, Cherny et al. 2003).

4) Genomic risk profile score analysis

Genomic profile risk 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, Esko 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, Esko 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, Wray et al. 2013). The PGC MDD 'discovery' sample has 9,240 MDD cases and 9,519 controls (Ripke, Wray 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. GPRS for both iron measures and MDD were calculated using varying levels of discovery sample p-value thresholds in PLINK (Purcell, Neale 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 selected 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=2,394 individuals for adult data, n=1,028 individuals for adolescent data).

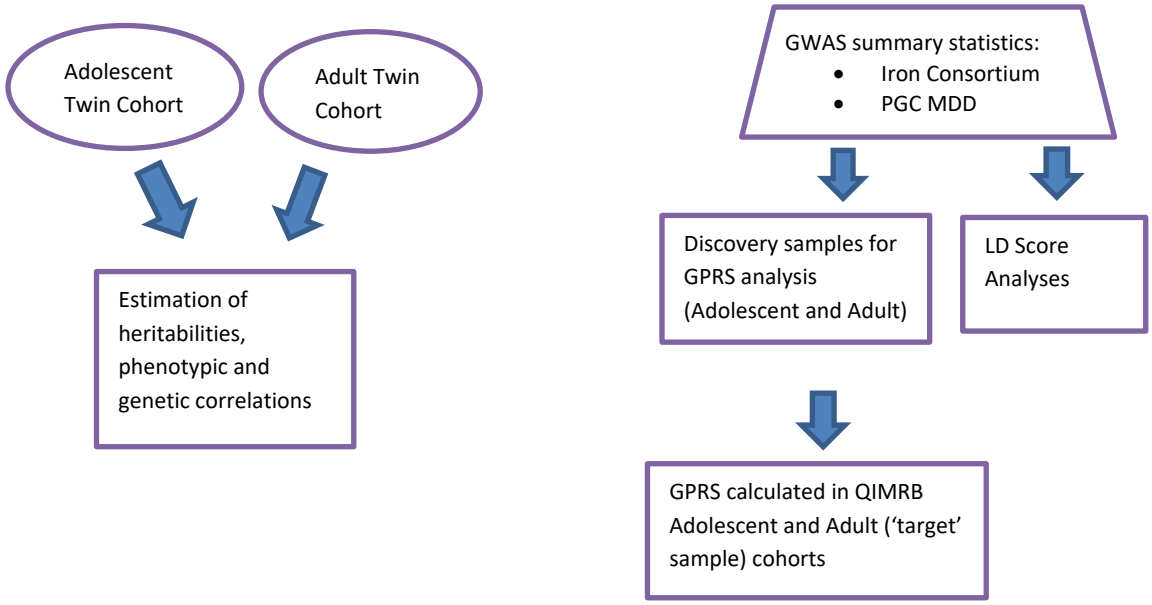
Linear regression models were then used to predict how much of the variation in each of the phenotypes of our samples is explained by the GPRS and the direction of association. Age was significantly associated with measures of iron and ferritin, whilst sex was significantly associated with measures of iron, transferrin saturation and ferritin. Therefore 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.

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, Loh et al. 2015), 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, Loh et al. 2015). 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, Finucane et al. 2015). 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, McRae 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). Figure 1 represents the main strategies / types of analyses we used to investigate the genetic relationship between iron measures and depression measures.

Figure 1: Schematic representation of analyses



Key: GPRS = Genetic Profile Risk Score; MDD = Major Depressive Disorder; PGC = Psychiatric GWAS Consortium

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), for all iron measures 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).

In assumption testing analyses that are preliminary to variance component estimation in twin samples, means and variances (both within and across zygosity groups) were not significantly different for all measures in the adolescent cohort. In the adult cohort, we observed a difference in birth order for iron and transferrin saturation means ($p=0.001$ for both traits), and a difference in variances between MZ and DZ twins for transferrin, even after adjusting for multiple testing. In view of the differences in variances for transferrin ($p=<9.8\times10^{-6}$), results from variance component analysis of this trait should be considered with more caution. 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.

Table 1: Means and standard deviations for iron measures and depression measures

Trait (Adolescents)	Total		Males		Females		p-value*
	Mean	SD	Mean	SD	Mean	SD	

Iron (µmol/L)	17.47	6.76	19.04	6.64	15.93	6.52	$<2.2x10^{-16}$
Transferrin (g/L)	2.96	0.46	2.93	0.41	2.99	0.50	$1.8\ x10^{-2}$
Saturation (%)	24.05	9.69	26.35	9.60	21.79	9.24	$<2.2x10^{-16}$
Log ferritin (µg/L)	1.62	0.34	1.74	0.26	1.51	0.38	$<2.2x10^{-16}$
SPHERE	7.60	6.28	8.57	6.34	8.84	6.58	0.21
Trait	**Total**		**Males**		**Females**		**p-value***
(Adults)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Iron (µmol/L)	19.44	6.63	20.77	6.42	18.69	6.64	< 2.2e-16
Transferrin (g/L)	2.78	0.49	2.66	0.37	2.85	0.47	< 2.2e-16
Saturation (%)	28.42	10.59	31.38	10.44	26.73	10.30	< 2.2e-16
Log ferritin (µg/L)	2.00	0.44	2.27	0.36	1.84	0.41	< 2.2e-16
Factor 1	0.00	1.06	-0.12	0.95	0.09	1.13	< 2.2e-16

*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)

Adolescents: number individuals iron measures = 1,363 (males=676, females=687); depression measures = 2,890 (males=1,327, females=1,563)
Adults: number individuals iron measures = 4,366 (males=1,609, females=2,757); depression measures = 8,072 (males=2,998, females=5,074)

As expected for traits under genetic influences, MZ correlations 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 1 of supplementary material). MZ correlations were also significantly higher than DZ correlations for depression measures in the adolescent cohort.

Using an ACE model, we found heritability of the iron measures in both cohorts to be moderate to high (Table 2 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, Wright et al. 2012).

Bivariate analyses showed high phenotypic and genetic correlations between the iron phenotypes 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.

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% CI in parenthesis)

Adolescents:

Trait	Iron	Transferrin	Saturation	Log10ferritin	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)
Log10ferritin	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	Log10ferritin	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)

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 symptoms. 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 is 0.0031, so only the association between depression measures and transferrin saturation survives 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 1).

Association Analysis: Of the 21 independent SNPs associated with iron phenotypes, 15 were in the PGC MDD GWAS. The smallest p-value of association was rs744653 for MDD (p = 0.027), 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 the hypothesis that there was not a significant association between iron measures and MDD (p-values 0.94 – 1).

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 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).

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 (%)	Direction of effect
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	+
SPHERE	Adolescent	0.87	0	-
Iron	Adult	$1.81x10^{-8}$	5.49	+
Depression	Adult	0.10	0.57	-
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	$1.83x10^{-7}$	7.09	+
SPHERE	Adolescent	0.50	0	+
Transferrin	Adult	$8.20x10^{-10}$	5.93	+
Depression	Adult	0.85	0.08	+
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	$4.82x10^{-5}$	4.30	+
SPHERE	Adolescent	1.00	0	-
Transferrin sat	Adult	$4.15x10^{-13}$	9.07	+
Depression	Adult	0.20	0.38	-
Log ferritin discovery sample: 212 SNPs with p-value threshold P<10^{-3} (Adolescent); 959 SNPs with p-value threshold P<$5x10^{-4}$ (Adult)				
Log ferritin	Adolescent	$3.24x10^{-3}$	2.14	+
SPHERE	Adolescent	0.52	0	+
Log ferritin	Adult	$2.26x10^{-3}$	1.38	+
Depression	Adult	0.83	0.08	-

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).

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

P-value of SNPs in MDD discovery GWAS	N SNPs	Target	P-value Target	Variance explained Target (%)	Direction of effect
Adolescent sample					
P <10^{-6}	11	Iron	0.17	0.24	-
P <0.05	17,699	Transferrin	0.066	0.61	+
P <10^{-6}	11	Saturation	0.25	0.08	-
P <0.10	29,840	Log ferritin	0.46	0	+
Adult sample					
P <10^{-6}	11	Iron	0.022	0.30	+
P <1.0	119,734	Transferrin	0.43	0	-

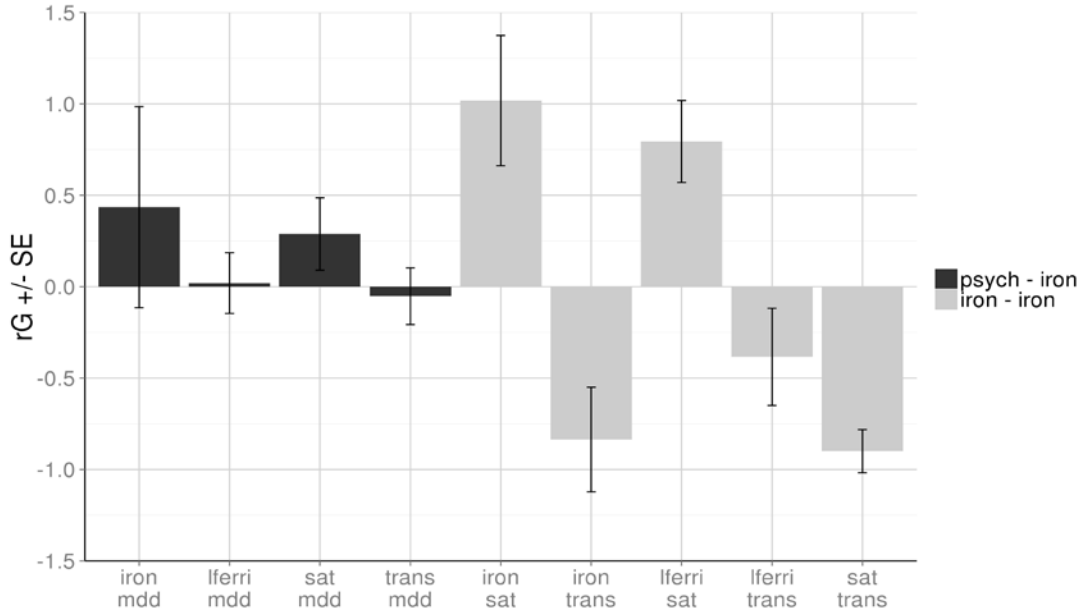
P <10^{-6}	11	Saturation	0.093	0.13	+
P <10^{-4}	733	Log ferritin	0.048	0.19	-

Key: MDD = major depressive disorder

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 (Figure 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 Figure 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 ± se 0.20) was not significantly different from zero after accounting for multiple testing.

Figure 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

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 µmol/L, and the lowest iron level in adolescent males was 4.0 µmol/L (normal reference range 14-32 µmol/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, Eyles 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 (Traglia, Sala et al. 2009, Fairweather-Tait, Guile et al. 2013). 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, Sala et al. 2009 reported the effect of age to be significant in the estimation of serum transferrin heritability (Traglia, Sala et al. 2009). Here, our estimates of heritability were higher in the adolescent than in the adult cohort.

This study was well-powered (≥99%) to estimate additive genetic variance greater than zero for iron and depression measures at significance level 0.05 (Visscher 2004, Visscher, Gordon 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, Loh et al. 2015) to explore the genome-wide correlation between SNP effects for different traits. 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. Using LD score analyses, SNP heritability for MDD (using "pgc.mdd.full.2012-04.txt" from https://www.med.unc.edu/pgc/downloads) and transferrin were higher than estimated heritability of these traits in the QIMRB twin cohorts.

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 (MAF > 0.1) variants (Purcell, Cherny et al. 2003), none of the SNPs explaining variation in iron phenotypes showed significant association in the published MDD GWAS results (Ripke, Wray 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. A further limitation was that the depression measures did not use MDD DSM-IV diagnostic criteria.

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 be impacted.

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