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Common variants in *TMPRSS6* are associated with iron status and erythrocyte volume

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Abstract

We report a genome-wide association study to iron status. We identify an association of SNPs in *TPMRSS6* to serum iron (rs855791, combined $P = 1.5\times10^{-20}$), transferrin saturation (combined $P = 2.2\times10^{-23}$), and erythrocyte mean cell volume (MCV, combined $P = 1.1\times10^{-10}$). We also find suggestive evidence of association with blood haemoglobin levels (combined $P = 5.3\times10^{-7}$). These findings demonstrate the involvement of *TMPRSS6* in control of iron homeostasis and in normal erythropoiesis.

Mutations in genes which code for components of iron homeostasis mechanisms can cause overload in hereditary haemochromatosis (commonly associated with *HFE*, but also with *HJV*, *HAMP*, *TFR2*, *SLC40A1*) or deficiency in iron-refractory iron deficiency anaemia (associated with *TMPRSS6*). Although most attention has been paid to variants with major effects leading to inherited disease, variation in iron status within the general population 1,2

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The authors declare that they have no competing financial interests.

AUTHOR CONTRIBUTIONS

Study design and coordination: A.C.H., A.K.H., B.W.P., D.I.B., D.R.N., E.J.C. de G., G.W.M., I.H.F., J.B.W., N.G.M. and P.M.V. **Obtaining study funding:** A.C.H., B.W.P., D.I.B., D.R.N., E.J.C.de G., G.W.M., I.H.F., J.B.W., N.G.M. and P.M.V. **Sample collection and phenotype data collection**: A.C.H., A.K.H., D.R.N., G.W.M., I.H.F., J.B.W., L.W., M.J.C. and N.G.M. (**Australian**): B.W.P., D.I.B., E.J.C.de G., G.W. and J-J.H. (**Dutch**). **Data preparation**: A.K.H., B.B., B.P.M., D.R.N., J.B.W., J-J.H., L.W., M.J.C., R.P.S.M. and S.G. **Statistical analyses**: B.B., B.P.M., D.R.N., J.B.W., M.A.R.F., P.M.V. and S.G. **Results interpretation**: B.B., D.R.N., G.W.M., J.B.W., M.A.R.F., N.G.M. and P.M.V. **Manuscript writing**: B.B., M.A.R.F. and J.B.W. **Review and revision of the manuscript**: A.C.H., B.B., B.W.P., D.I.B., D.R.N., E.J.C.de G., G.W., G.W.M., J.B.W., M.A.R.F., N.G.M. and P.M.V. **All authors contributed to the final version of the paper.**

is important in relation to risk of iron deficiency anaemia, oxidative stress, liver disease and metabolic syndrome.

Investigation of iron status in humans can be based on quantitative tests including serum iron, serum transferrin, transferrin saturation with iron, and serum ferritin. We aimed to identify polymorphisms causing variation in iron status in the general population using genome-wide association methods in 2,516 adolescent and 2,302 adult individuals from 2,277 Australian twin families (Supplementary Table 1). The genotyping was performed using Human610-Quadv1 chips (~582K SNPs) and HumanCNV370-Quadv3 chips (~351K SNPs) (Supplementary Methods). We replicated previously reported SNPs in *TF* (rs3811647) with serum transferrin and in *HFE* (rs1800562) with iron, transferrin and transferrin saturation 2 in both the adolescent and adult samples (Table 1, Supplementary Fig. 1 & 2).

We identified significant associations between SNPs in *TMPRSS6* and serum iron (adolescents $P = 1.6\times10^{-11}$, adults $P = 9.9\times10^{-11}$) and transferrin saturation (adolescents $P = 9.7\times10^{-13}$, adults $P = 1.8\times10^{-11}$) (Table 1). The strongest *TMPRSS6* association was with rs855791, a non-synonymous coding SNP (A736V) in exon 17 (Fig. 1). The effects of rs855791 on serum iron and transferrin saturation were additive with each T allele decreasing serum iron and transferrin saturation by 0.18 and 0.20 SD of the mean phenotypes, respectively. The SNP explains 2.2% and 2.5% of the variation in the mean of serum iron and transferrin saturation in the adolescent cohort and 1.9 % and 2.0 % in the adult cohort.

Because of the involvement of *TMPRSS6* mutations in iron-refractory iron deficiency anaemia 3 and because low values for blood haemoglobin (Hb) and erythrocyte mean cell volume (MCV) occur in iron-deficiency states, we next checked for associations between rs855791 and Hb or MCV in the adolescents. Strong associations were found ($P = 2.3\times10^{-6}$ and 1.3×10^{-5}, respectively). The T allele of rs855791 decreased mean Hb and MCV by 0.15 and 0.14 SD, respectively. These correspond to 1.1 and 0.9% of the total variance in mean Hb and MCV. We replicated these associations in an independent Adult Dutch cohort 4 consisting of 3,470 unrelated individuals ($P = 7.7\times10^{-3}$ and 1.8×10^{-6} for Hb and MCV, respectively) (Table 1).

Genotypic means for each of the marker phenotypes for the most significant SNPs in *TF, HFE and TMPRSS6*, by sex and study cohort, are presented in Supplementary Fig. 3A & 3B. The direction of the allelic effects was consistent with expectation, in that the alleles or genotypes causing low iron or transferrin saturation were the same in the adults and adolescents and were also those associated with lower Hb and MCV. The estimated effect sizes stratified by gender after correcting for the effect of age are presented in Supplementary Table 2. Except for the effect of rs1800562 on Hb in the adult cohort, there appear to be no significant differences between the estimates for males and females.

To assess whether the effects of rs855791 on Hb and MCV are explained by effects on iron status, or whether there may be direct effects of *HFE* or *TMPRSS6* variants on erythropoesis, we repeated the SNP association analysis for Hb and MCV in the Australian

adolescents using the residuals after adjustment for the effect of transferrin saturation (chosen because this measure shows the strongest association with rs855791). This first required assessment of the relationships between transferrin saturation, Hb and MCV (Supplementary Fig. 4); there were essentially linear relationships across the range found in this population sample. Results showed that linear adjustment for transferrin saturation reduced but did not eliminate the effects of rs855791 in *TMPRSS6* on Hb (from $R^2 = 1.2\%$, $P = 1.4\times10^{-6}$ to $R^2 = 0.5\%$, $P = 0.003$), and MCV (from $R^2 = 0.8\%$, $P = 6.9\times10^{-5}$ to $R^2 = 0.4\%$, $P = 0.008$). Similar adjustment essentially eliminated the effects of rs1800562 in *HFE* (Hb from $R^2 = 0.8\%$, $P = 1.1\times10^{-4}$ to $R^2 = 0.2\%$, $P = 0.09$, MCV from $R^2 = 0.4\%$, $P = 0.007$ to $R^2 < 0.1\%$), $P = 0.40$). The residual allelic association with rs855791, together with the strong overall association between transferrin saturation and Hb or MCV (Supplementary Table 3 and Supplementary Fig. 4), suggest that the effects of this *TMPRSS6* polymorphism on Hb and MCV may not be solely due to the availability of transferrin-bound iron being rate-limiting for erythropoesis.

We have shown genome-wide significant association of a common SNP in *TMPRSS6* with serum iron, transferrin saturation and MCV, and suggestive association with Hb, extending previous data 2 implicating *TMPRSS6* in variation in serum iron and transferring saturation in the general population. Data from other sources 3,5–7 shows that knockout (in mice) or mutations with major effect (in humans) in *TMPRSS6* greatly affect iron status. Loss of function in this gene produces iron deficiency and anaemia, probably through a combination of protease action on hemojuvelin 6 and regulation of hepcidin expression 8. We have now found significant and consistent *TMPRSS6* effects on both iron status (serum iron and transferrin saturation) and erythropoesis (Hb and MCV) in adolescents and adults from the general population. The effects of this non-synonymous coding variant on both Hb and MCV raise important questions about the relationship between iron status and normal haemoglobin synthesis. One implication is that transferrin saturation may play a role in the control of erythropoesis, and alleles which increase it allow an increase in haemoglobin concentration and MCV even in people who show no evidence of iron deficiency.

There are now confirmed effects on iron markers in three genes, each previously known from human case studies or mouse experiments to affect iron homeostasis. *TF* mainly affects transferrin concentration, and this may in turn affect the concentration of diferric transferrin, which regulates hepcidin expression in the liver by interacting with *HFE* and *TFR2* gene products 9. *HFE* likewise regulates hepcidin, and clinical or experimentally induced mutations lead to iron overload; the effects in these population samples are significant for serum iron, serum transferrin and transferrin saturation but not (genome-wide) on serum ferritin. The effects of the three SNPs on serum ferritin were weak compared to other traits. With the current sample size we do not yet have power to detect any significant ferritin-associated SNPs at genome-wide significance levels. Note that rs855791 and rs1800562 were associated with serum ferritin with combined *P* values around 10^{-4} (Table 1).

Loss-of-function mutations in the protease domain of *TMPRSS6* gene product, matripase-2, lead to increased hepcidin expression and iron deficiency 5,6,7. The variant which has the largest effect in this study produces an amino acid change, from alanine to valine at amino acid 736. This too is in the protease domain and we speculate that it may lead to changes in

protease activity. Other variants of *TMPRSS6* may also affect iron status. A report on gene expression in human liver 10 found a cis-acting effect of rs2160906 ($P = 8.4\times10^{-8}$, HapMap CEU r^2 with rs855791 is 0.06) at 35.82 MB on *TMPRSS6* expression and we found strong supportive evidence (combined $P = 9.8\times10^{-8}$ with transferrin saturation) for an effect of this polymorphism on iron status (Fig. 1).

Estimates of heritability 1 indicate other loci or gene interactions must exist, but their identification will require larger studies or meta-analysis of multiple studies. Polygenic effects on iron status may have clinical importance for iron overload states; both primary (as risk modifiers in *HFE* C282Y homozygotes) and secondary to liver disease or metabolic syndrome, and case-control studies on the effects of *TMPRSS6* variation in these conditions are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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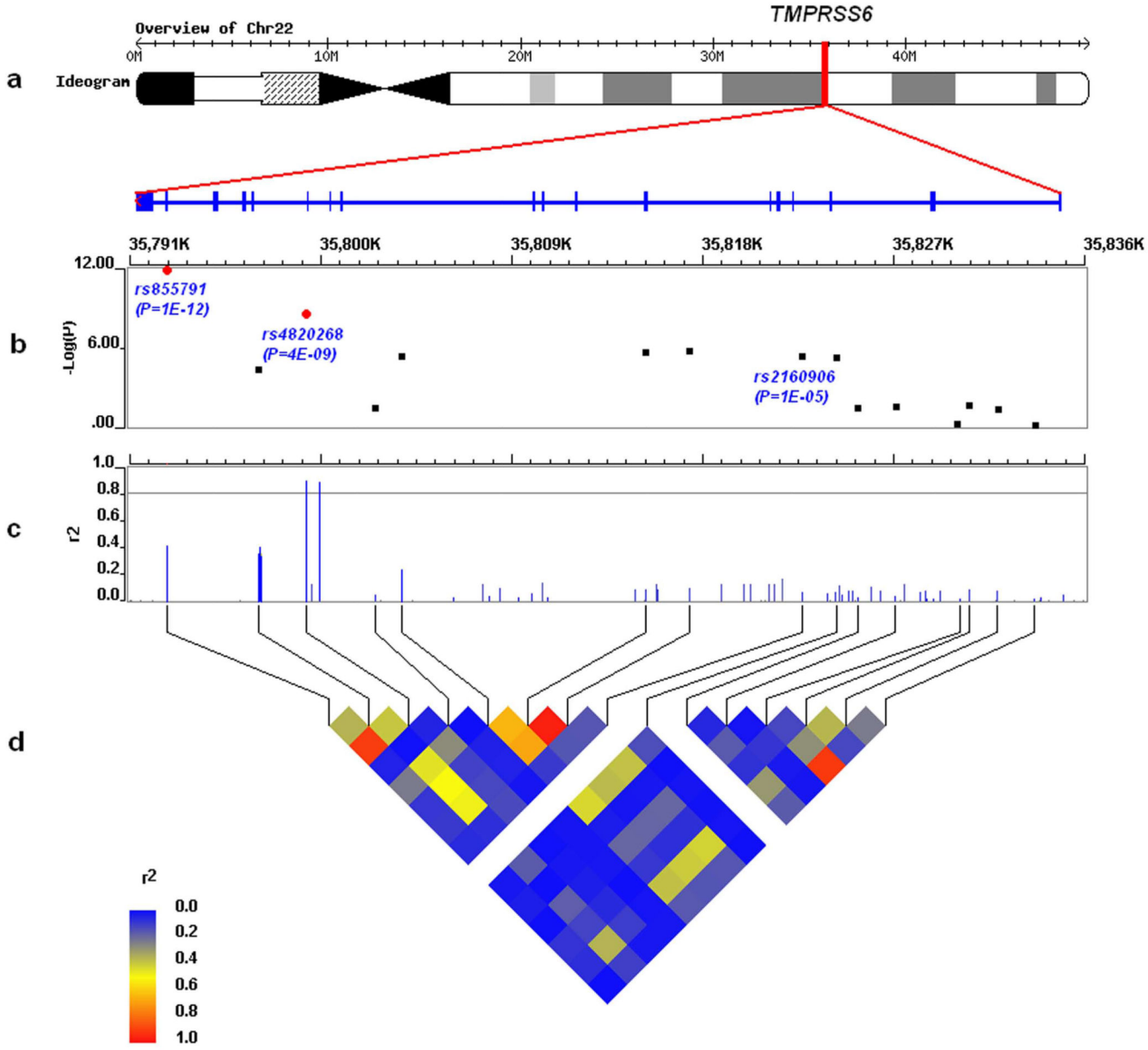

Figure 1.

Detailed results of the associations between SNPs within *TMPRSS6* and transferring saturation in the adolescent cohort: (**a**) The genomic location of *TMPRSS6* at 22q12-q13 on Chromosome 22; (**b**) $-\log_{10}(P)$ of the association tests between SNPs and transferring saturation, where red dots indicate genome-wide significant SNPs. rs2160906 has a significant cis-acting effect on *TMPRSS6* expression ($P = 8.4\times10^{-8}$) 10 ; (**c**) Linkage disequilibrium (LD) (r^2) between rs855791 and all other HapMap SNPs within *TMPRSS6*; (**d**) HapMap-LD plot (r^2) between genotyped SNPs within *TMPRSS6*.

Table 1

Additive effects (in SD) of the three SNPs in *TMPRSS6*, *HFE* and *TF* on serum iron, transferrin, transferrin saturation, ferritin, and blood haemoglobin (Hb) and erythrocyte mean cell volume (MCV)

	Adolescent					Adult					Combined		
Trait	**N**	**Beta**	**SE**	**R^2 (%)**	***P***	**N**	**Beta**	**SE**	**R^2 (%)**	***P***	**Beta**	**SE**	***P***
						rs855791(TMPRSS6)							
Iron	2505	−0.183	0.027	2.2	1.6×10^{-11}	2298	−0.191	0.030	1.9	9.9×10^{-11}	−0.187	0.020	1.5×10^{-20}
Transferrin	2513	0.078	0.029	0.4	7.1×10^{-3}	2299	0.069	0.031	0.2	0.023	0.074	0.021	4.9×10^{-4}
Saturation	2503	−0.196	0.027	2.5	9.7×10^{-13}	2294	−0.198	0.029	2.0	1.8×10^{-11}	−0.197	0.020	2.2×10^{-23}
Log_{10}(ferritin)	2512	−0.092	0.028	0.5	8.8×10^{-4}	2301	−0.040	0.030	0.1	0.17	−0.068	0.021	9.3×10^{-4}
Hb	2468	−0.151	0.032	1.1	2.3×10^{-6}	3188	−0.067	0.025	0.2	7.7×10^{-3}	−0.099	0.020	5.3×10^{-7}
MCV	2467	−0.139	0.032	0.9	1.3×10^{-5}	3181	−0.120	0.025	0.7	1.8×10^{-6}	−0.127	0.020	1.1×10^{-10}
						rs1800562 (HFE)							
Iron	2502	0.343	0.052	2.2	4.7×10^{-11}	2299	0.315	0.053	1.5	2.5×10^{-9}	0.329	0.037	8.2×10^{-19}
Transferrin	2510	−0.602	0.056	6.0	4.3×10^{-27}	2300	−0.605	0.055	5.4	2.9×10^{-28}	−0.604	0.039	2.2×10^{-53}
Saturation	2500	0.598	0.053	6.6	2.3×10^{-29}	2295	0.523	0.054	4.2	3.0×10^{-22}	0.561	0.038	8.5×10^{-50}
Log_{10}(ferritin)	2509	0.168	0.053	0.5	1.6×10^{-3}	2302	0.103	0.054	0.2	0.06	0.136	0.038	3.4×10^{-4}
Hb	2465	0.236	0.061	0.8	1.2×10^{-4}	3451	0.169	0.051	0.3	9.0×10^{-4}	0.197	0.039	5.1×10^{-7}
MCV	2464	0.148	0.061	0.3	0.015	3442	0.273	0.051	0.8	7.7×10^{-8}	0.222	0.039	1.5×10^{-8}
						rs3811647 (TF)							
Iron	2505	0.073	0.029	0.3	0.012	2299	0.042	0.032	0.1	0.18	0.059	0.022	0.006
Transferrin	2513	0.460	0.031	11.2	4.4×10^{-50}	2300	0.371	0.032	6.1	2.1×10^{-30}	0.417	0.022	2.1×10^{-78}
Saturation	2503	−0.089	0.029	0.5	2.4×10^{-3}	2295	−0.093	0.031	0.4	3.1×10^{-3}	−0.091	0.021	1.8×10^{-5}
Log_{10}(ferritin)	2512	−0.053	0.029	0.2	0.07	2302	−0.030	0.032	0.1	0.35	−0.044	0.022	0.04
Hb	2468	−0.019	0.034	0.02	0.58	3470	0.032	0.026	0.04	0.21	0.013	0.021	0.52
MCV	2467	−0.041	0.034	0.1	0.23	3461	−0.042	0.026	0.1	0.10	−0.042	0.021	0.04

Note: The results are from the Australian cohorts, except for Hb and MCV in adults (which are from the Dutch cohort). The results in the adolescent cohort are from the mean of measurements from up to 4 visits. The rs855791 variant was not genotyped in the Dutch panel and so was imputed using PLINK [11] as described previously [12]. Genotypes were imputed with high confidence (information score of 0.97) in 92% of individuals. Minor allele frequencies (MAFs) for rs855791 (T), rs1800562 (A) and rs381164 (A) in the adolescent cohort are 0.42, 0.08 and 0.33, respectively. R^2 is the proportion of the phenotypic variance explained by each SNP. After correction for multiple testing, there is no significant evidence for heterogeneity between the effect sizes in adolescent and adult data.