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Genetic contribution to iron status: SNPs related to iron deficiency anaemia and fine mapping of CACNA2D3 calcium channel subunit

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ABSTRACT

Numerous studies associate genetic markers with iron- and erythrocyte-related parameters, but few relate them to iron-clinical phenotypes. Novel SNP rs1375515, located in a subunit of the calcium channel gene *CACNA2D3*, is associated with a higher risk of anaemia. The aim of this study is to further investigate the association of this SNP with iron-related parameters and iron-clinical phenotypes, and to explore the potential role of calcium channel subunit region in iron regulation. Furthermore, we aim to replicate the association of other SNPs reported previously in our population. We tested 45 SNPs selected via systematic review and fine mapping of *CACNA2D3* region, with haematological and biochemical traits in 358 women of reproductive age. Multivariate analyses include back-step logistic regression and decision trees. The results replicate the association of SNPs with iron-related traits, and also confirm the protective effect of both A allele of rs1800562 (*HFE*) and G allele of rs4895441 (*HBS1L-MYB*). The risk of developing anaemia is increased in reproductive age women carriers of A allele of rs1868505 (*CACNA2D3*) and/or T allele of rs13194491 (*HIST1H2BJ*). Association of SNPs from fine mapping with ferritin and serum iron suggests that calcium channels could be a potential pathway for iron uptake in physiological conditions.

1. Introduction

Iron homeostasis is essential for numerous physiological processes. However, excess of iron could form toxic free radicals, and it thus requires precise regulation [1]. Because humans do not possess an active mechanism for iron excretion, this regulation is carried out mainly by modulating the uptake of iron from the diet by enterocytes and transfer of this iron to the systemic circulation [2]. The key proteins that control this process also control the release of stored iron to plasma, to supply iron for erythropoiesis and other metabolic activities [3]. Small deviations from correct iron levels are the ultimate cause of several disorders, of which iron deficiency anaemia (IDA) is the most prevalent. Indeed, it is considered a pandemic according to the WHO, which classifies women in fertile age as the most at-risk group [4]. Moreover, iron deficiency is associated with other disorders [5,6].

IDA is influenced by diet, iron intake, blood loss, physiological status and infections [7,8]. However, the evidence on several genetic disorders of iron metabolism in human and animals indicates a plausible genetic contribution to iron regulation [9–11]. Moreover, heritability of the biochemical parameters used to assess iron status, such as haemoglobin, haematocrit, ferritin, mean cell volume (MCV), transferrin and serum iron, supports this hypothesis [12–14]. Several studies on humans have associated many genetic markers, mainly SNPs, with iron-related parameters [15–19] and with anaemia itself [20–22]. Despite the vast amount of information that these genome-wide association studies (GWAS) have made available, it is still difficult to replicate or apply the obtained results in practical health contexts [23–25]. In this respect, the increasing complexities of health and illness issues render it necessary to broaden methodological strategies in research, and the use of data mining methods has been suggested as a means to integrate all of these associations [26].

Although some of the SNPs associated with iron metabolism parameters are found in protein codifying regions, most of them, as the Encode project is revealing [27,28], are not involved in structural changes but in functional aspects. The authors of the present study found a novel SNP (rs1375515), located in an intronic region of a gene that codifies a subunit of a calcium channel *CACNA2D3*, significantly associated with haemoglobin and ferritin, as well as with different probabilities of belonging to different iron-clinical phenotypes [21]. Calcium channels are associated with iron homeostasis through the modification of red cell volume that leads to disorders such as liver insufficiency, fibrosing

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disease and sickle cell anaemia [29]. Other studies have shown that these channels could be directly responsible for iron uptake, namely that the L-type voltage dependent calcium channel could be an alternate route for iron to enter different cell types [30,31].

The aims of this study are to replicate the association of the most relevant SNPs not only with iron-related parameters but also with iron-clinical phenotypes, using data mining and qualitative methods, and to explore the potential role of the calcium channel subunit region by means of fine mapping.

2. Material and methods

2.1. Subjects

A total of 358 women of reproductive age were recruited in a wider project aimed at understanding the interactions among iron, genes, nutrition and disease in menstruating women. This sample is an extension in 74 women of the sample analysed in Bertoncini et al. and Baeza-Richer et al. [21,32]. The subjects were Caucasian women born in Europe, aged between 18 and 45 years old, menstruating, non-pregnant, non-lactating and non-smoking. In this sample, 43.6% of the total individuals had normal haemoglobin (Hb,g/dl) and ferritin (Ft,μg/l) levels (Hb ≥ 12, Ft ≥ 20), 45.8% were iron deficient (Hb ≥ 12 and Ft < 20 or Hb ≤ 12 and Ft > 20) and 10.6% were iron deficient anaemic (Hb < 12 and Ft < 20). This classification follows WHO criteria [4]. All women were administered a health questionnaire and underwent a blood test. The study was approved by the ethics committee of the *Puerta de Hierro* Hospital and the Spanish National Research Council, Madrid, Spain.

2.2. SNP selection

Thirty five SNPs were analysed in this cohort for the first time, plus 10 SNPs already typed in Bertoncini et al. and Baeza-Richer et al. [21,32] where these 10 SNPs were analysed in 284 out the whole sample. The total 45 SNPs (Table 1S) were selected for this study according to the following criteria:

a) Fine Mapping: this technique allows us to look deeper into the possible implication of *CACNA2D3* in iron metabolism, by genotyping more extensively this region. In general terms, pairwise linkage disequilibrium (LD) gives you information of how two SNPs vary together, so that you need to select less SNPs to capture all the genetic variability (information) of a given region. Using Tagger [33] from the Haploview software [34] vs 4.1, we fine-mapped a 100 kb LD (linkage disequilibrium) block region in which rs1375515 is located (*CACNA2D3*). Haploview software will select the least SNPs needed to capture

Table 1
Descriptive and linear regression for SNPs and biochemical parameters. Only significant results were shown. Effect model analyses do not apply for those SNPs that do not present individuals homozygous for the rare allele

Reference	SNPs	Location		Parameter	Model	Effect	Coef (CI 95%)	p
		chr	Gene			Genotype		
[b]	rs16826756	2	–	logMCV	Rec	G/G	−0.02 (−0.04, 0.00)	0.033
[b]	rs2673289	2	–	Haemoglobin	Rec	T/T	−0.47 (−0.84, −0.09)	0.014
				logFerritin	Rec	T/T	−0.11 (−0.22, −0.01)	0.033
				Haematocrit	Rec	T/T	−1.15 (−2.14, −0.16)	0.024
[a]	rs13089763	3	CACNA2D3	logFerritin		C/G	0.19 (0.02, 0.36)	0.03
[b]	rs1375515	3	CACNA2D3	Haemoglobin	Rec	G/G	−0.49 (−0.98, −0.01)	0.046
				logFerritin	Rec	G/G	−0.16 (−0.29, −0.02)	0.022
[a]	rs1868498	3	CACNA2D3	logFerritin	Dom	A/G–G/G	0.12 (0.01, 0.22)	0.027
				Serum iron	Rec	G/G	60.67 (21.67, 99.67)	0.0025
[a]	rs1868505	3	CACNA2D3	logFerritin	Dom	A/G–A/A	−0.10 (−0.19, 0.00)	0.049
[a]	rs4974366	3	CACNA2D3	logFerritin	Dom	C/T–T/T	0.11 (0.01, 0.21)	0.038
				Serum iron	Rec	T/T	76.61 (31.95, 121.26)	0.0009
[a]	rs7653648	3	CACNA2D3	logFerritin	Dom	C/G–G/G	0.09 (0.01, 0.17)	0.03
[b]	rs1799852	3	TF	Haemoglobin		C/T	−0.39 (−0.71, −0.07)	0.019
				Haematocrit		C/T	−1.09 (−1.96, −0.23)	0.014
				Transferrin		C/T	−25.45 (−39.29, −11.61)	0.0004
[b]	rs2280673	3	TF	Transferrin	Rec	A/A	19.96 (1.49, 38.42)	0.035
[b]	rs3811647	3	TF	Transferrin	Cod	G/A	21.29 (8.74, 33.84)	<0.0001
						A/A	51.48 (30.88, 72.07)	
[17,38]	rs11970772	6	CCND3	Serum iron	Rec	A/A	−24.85 (−48.80, −0.89)	0.043
[38,41]	rs4895441	6	HBS1L	logFerritin	Rec	G/G	0.36 (0.11, 0.62)	0.0057
				Serum iron	Dom	A/G–G/G	11.04 (2.14, 19.94)	0.016
				Transferrin	Rec	G/G	−44.35 (−84.67, −4.03)	0.032
				logMCV	Rec	G/G	0.03 (0.01, 0.06)	0.0053
[b]	rs1799945	6	HFE	Transferrin	Dom	G/C–G/G	−16.79 (−29.64, −3.95)	0.011
[b]	rs1800562	6	HFE	logFerritin		G/A	0.17 (0.00, 0.34)	0.048
				Transferrin		G/A	−39.35 (−64.67, −14.02)	0.0025
[38,39]	rs198846	6	HIST1H1T	Transferrin	Dom	A/G–A/A	−16.06 (−28.65, −3.47)	0.013
[38,40]	rs13194491	6	HIST1H2BJ	Haemoglobin		C/T	−0.60 (−1.18, −0.03)	0.04
				Haematocrit		C/T	−1.64 (−3.16, −0.11)	0.036
				Serum iron		C/T	−19.49 (−36.35, −2.63)	0.024
[38,41,43,44]	rs17342717	6	SLC17A1	Transferrin	Dom	C/T	−21.84 (−38.63, −5.05)	0.011
[38,40]	rs12216125	6	TRIM38	Transferrin	Dom	C/T–T/T	−25.30 (−37.35, −13.24)	<0.0001
				logMCV	Rec	T/T	0.02 (0.00, 0.03)	0.0094
[38,42]	rs3184504	12	SH2B3	Haematocrit	Dom	C/T–T/T	0.92 (0.07, 1.76)	0.034
[38,39]	rs11089823	22	TMPRSS6	Haemoglobin	Dom	C/T–C/C	0.34 (0.04, 0.64)	0.025
				Haematocrit	Dom	C/T–C/C	0.95 (0.16, 1.74)	0.018
[b]	rs855791	22	TMPRSS6	Transferrin	Rec	T/T	15.43 (0.07, 30.78)	0.049

[] Literature.
Model analyses do not apply for those SNPs that do not present individuals homozygous for the rare allele.
[a] Fine mapping.
[b] Decaplex.