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Common *TMPRSS6* mutations and iron, erythrocyte, and pica phenotypes in 48 women with iron deficiency or depletion

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Abstract

Background—*TMPRSS6* A736V is associated with lower transferrin saturation (TS), hemoglobin (Hb), and mean corpuscular volume (MCV) levels in general adult populations. We sought to identify relationships of *TMPRSS6* K253E, A736V, and Y739Y to iron, erythrocyte, and pica phenotypes in women with iron deficiency or depletion.

Methods—We tabulated observations on 48 outpatient non-pregnant women who had iron deficiency (serum ferritin (SF) <14 pmol/L and TS <10%) or iron depletion (SF<112 pmol/L). We performed direct sequencing of *TMPRSS6* exons 7 and 17 in each patient. We used age, TS, SF, Hb, MCV, pica, and *TMPRSS6* allele positivity (dichotomous) or mutation genotypes (trichotomous) as variables for analyses.

Results—Forty-six women were white; two were black. 58.3% had iron deficiency. 45.8% had pica (pagophagia, each case). Allele frequencies were 41.7% (K253E), 36.5% (A736V), and 39.6% (Y739Y). K253E frequency was greater in women with TS ≥10% ($p = 0.0001$). Y739Y was more frequent in women with TS <10% ($p = 0.0135$). Mean TS was also lower in women positive for Y739Y (6 ± 4% vs. 13 ± 16%, respectively; $p = 0.0021$). In multiple regressions, neither K253E, A736V, nor Y739Y genotypes were significantly associated with other variables.

Conclusions—*TMPRSS6* K253E frequency was greater in women with TS ≥10%. Frequency of Y739 was greater in women with TS <10%. Mean TS was lower in women with Y739Y. We observed no other significant relationship of *TMPRSS6* K253E, A736V, or Y739Y with iron, erythrocyte, or pica phenotypes.

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Author contributions

PLL performed mutation analyses, interpreted results of DNA studies, performed statistical analyses, and contributed to the manuscript.

JClB tabulated patient data, collected blood specimens, performed statistical analyses, and contributed to the manuscript.

PLK interpreted mutation analyses and tabulated results of DNA studies.

SYB interpreted mutation analyses and tabulated results of DNA studies.

JCB conceived the study, performed statistical analyses, and wrote the manuscript.

Key Words for Indexing

hemoglobin; iron absorption; matriptase-2; mean corpuscular volume; pagophagia; pica

Introduction[1]

TMPRSS6 (OMIM *609862; chromosome 22q12-q13) encodes matriptase-2 (transmembrane serine protease 6). Matriptase-2 is an essential component of a pathway that detects iron deficiency, represses hepcidin transcription in the liver by cleaving membrane-bound hemojuvelin, and permits enhanced dietary iron absorption [8,9]. The *TMPRSS6* allele A736V (rs855791) is related to significantly lower levels of serum iron (SI), transferrin saturation (TS), hemoglobin (Hb), and mean corpuscular volumes (MCV) in genome-wide association studies of twins and general population subjects, respectively [4,6]. This demonstrates that *TMPRSS6* A736V influences iron homeostasis and erythropoiesis in normal subjects. In another study, allele frequencies of *TMPRSS6* A736V and K253E in persons with iron deficiency, grouped according to the presence or absence of anemia, did not differ significantly from those of control subjects, but independent variables that could affect specific measures of iron and erythrocyte phenotypes were not evaluated [5].

Pica, the daily compulsive eating of food or non-food items not part of one's habitual diet or preferences, is a distinctive but poorly understood accompaniment of iron deficiency or depletion in some adults, although most pica items contain little or no iron [2,7,13,16]. In the US, compulsive ice eating (pagophagia) is the most prevalent pica manifestation in adults with iron depletion or deficiency [2,16]. In 262 non-pregnant adult patients with iron deficiency or depletion, the prevalence of pica was greater in women and the most common manifestation of pica was pagophagia. Mean SF, mean Hb, and mean MCV were lower in patients with pica [2].

We postulated that the *TMPRSS6* alleles K253E, A736V, or Y739Y could influence the iron, erythrocyte, and pica phenotypes of women with iron deficiency or depletion. Thus, we studied 48 consecutive women referred to a hematology clinic for management of iron deficiency or depletion. We sequenced *TMPRSS6* exons 7 and 17 in each subject. We tabulated the variables age at diagnosis, TS, serum ferritin (SF), Hb, MCV, pica reports, and K253E, A736V, or Y739Y positivity and genotypes in each woman. We performed univariable and multivariable analyses to identify positive and negative predictors of iron, erythrocyte, and pica phenotypes. Our results are discussed in the context of the action of *TMPRSS6* alleles and the interpretation our observations in understanding iron deficiency and its manifestations.

Materials and Methods

Patient selection

The performance of this study was approved by the Institutional Review Board of Brookwood Medical Center and Scripps Research Center. We performed retrospective reviews of the charts of a convenience sample of 48 consecutive adult female outpatients (≥18 years of age) who were treated with intravenous iron dextran in a referral hematology and medical oncology practice. The present patients were treated with intravenous iron dextran (INFed®; Watson Pharma, Inc., Morristown, NJ) because they could not tolerate

[1]SI = serum iron concentration; TIBC = total iron-binding capacity; TS = transferrin saturation; SF = serum ferritin concentration; Hb = hemoglobin concentration; and MCV = mean corpuscular volume.

oral iron supplements; their iron deficiency or depletion did not resolve with trials of oral iron supplementation; or they had anemia or another manifestation(s) that was too severe to manage with oral iron supplements [1].

We excluded patients who were pregnant; were hospitalized; had serum creatinine >133 μmol/L; had been treated with erythrocyte transfusion to alleviate anemia; had types of acquired anemia other than that due to iron deficiency or depletion; had erythrocytosis, polycythemia, or other bone marrow disorder not in remission; were receiving anti-cancer chemotherapy or radiation therapy; or had hyperferritinemia due to acute phase reaction, chronic inflammation, liver injury, malignancy, or other cause [1].

Laboratory techniques

Complete blood counts were performed using Cell-Dyn® 1800 or 1500 automated blood counters (Abbott Laboratories, Chicago, IL). Anemia was defined as Hb below lower reference limit 117 g/L [3]. Reference range for MCV was 80.0–97.0 fL. Serum iron measures were determined using automated clinical laboratory methods. Total iron-binding capacity (TIBC) was defined as the sum of serum iron concentration and unbound iron-binding capacity, and TS as the quotient of serum iron concentration by TIBC. Iron deficiency was defined as both SF <45 pmol/L (<20 μg/L), and TS <10%. Iron depletion was defined as SF <112 pmol/L (<50 μg/L) [2].

TMPRSS6 mutation analysis was performed using DNA isolated from whole blood using QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). DNA amplification was performed as previously described [5]. The primers and annealing temperatures used to amplify *TMPRSS6* exons associated with the variants K253E (exon 7) and A736V and Y739Y (exon 17) are displayed in Table 1. After primer removal with ExoSAP-IT (GE Healthcare, Piscataway, NJ) according to manufacturers' recommendations, sequencing was performed on amplified DNA products with an ABI 3730 Genetic Analyzer (Carlsbad, CA) at Retrogen, Inc. (San Diego, CA).

Definition of pica

This condition was defined as the daily compulsive eating of food or non-food items not ordinarily part of the patient's habitual diet or preferences for more than one month, and not reasonably attributable to other cause by the patient or treating physician [2]. We tabulated pica food and non-food items in each case. A report of pica or no pica was available in the chart of each of the 48 women.

Statistical considerations

We tabulated these observations at diagnosis in all 48 patients: age at initial intravenous iron treatment, presence (or absence) of pica reports, and *TMPRSS6* genotypes for the respective alleles we detected. We also tabulated pre-treatment levels of TS, SF, Hb, and MCV. Levels of SF were normalized for analyses by natural logarithm (ln) transformation. Descriptive statistics are displayed as enumerations, percentages, or mean ± 1 standard deviation (SD) or 95% confidence interval (CI), as appropriate. Comparisons were made using either Student's two-sided t-test or chi-square or Fisher's exact test, as appropriate. In comparisons that involved *TMPRSS6* positivity and student's t tests, we allele positivity as dichotomous variables (positivity or negativity for the respective mutant alleles). We performed multiple forward regression analyses to identify predictors of TS, SF, Hb, and MCV. We performed logistic regression analysis on pica using all other variables. For regression analyses, *TMPRSS6* genotypes were expressed as trichotomous variables (wild-type genotype, heterozygosity, or homozygosity for a mutant allele). Analyses were performed using GB-

Stat® v 8.0 (Dynamic Microsystems, Inc., Silver Spring, MD) and Microsoft Excel 2000® (Microsoft Corp., Redmond, WA). Values of $p < 0.05$ are defined as significant.

Results

General characteristics of study subjects

There were 48 women (46 white, 2 black) whose mean age was 55 ± 15 years. Their mean TS was 12 ± 15% and their mean SF level was 32 pmol/L (23, 43). Their mean Hb level was 104 ± 18 g/L and their mean MCV was 80.0 ± 10.0 fL. Twenty women (41.7%) had iron depletion and 28 other women (58.3%) had iron deficiency. Two women diagnosed to have hemochromatosis with *HFE* C282Y homozygosity were referred for additional management because they had developed iron depletion and deficiency, respectively, due excessive phlebotomy therapy; both had pica.

Subjects with pica

Twenty-two women (45.8%) reported having pica. Each of the 22 women had pica for ice (pagophagia). One woman each also reported pica for coconut icicles and hard candy, respectively. The mean age of women with pica was lower than that of women without pica (52 ± 12 y vs. 61 ± 15 y, respectively; $p = 0.0300$). The mean TS levels of women with and without pica did not differ significantly. The mean Hb level of patients with pica was slightly lower than that of women without pica (98 ± 18 g/L vs. 110 ± 15 g/L, respectively; $p = 0.0157$). The mean SF levels of women with and without pica did not differ significantly. The mean MCV values of women with pica were lower than those of women without pica (75.5 ± 11.1 fL vs. 83.7 ± 7.3 fL, respectively; $p = 0.0067$).

TMPRSS6 allele frequencies

We compared frequencies of each mutant *TMPRSS6* allele in subjects grouped by TS, SF, Hb, MCV and occurrence of pica (Table 2). The allele frequency of *TMPRSS6* K253E was greater in women with TS ≥10%. The allele frequency of *TMPRSS6* Y739Y was greater in women with TS <10%. We observed no other significant differences in allele frequencies in these respective phenotype groups (Table 1).

Univariable and regression analyses

We compared respective values of mean TS, mean SF, mean Hb, and mean MCV in patients with and without positivity for *TMPRSS6* K253E, A736V, and Y739Y (dichotomous variable). Mean TS was lower in women with Y739Y positivity than in women without Y739Y (6 ± 4% vs. 13 ± 16%, respectively; $p = 0.0021$).

We performed forward stepwise regressions of TS, SF, Hb, and MCV on the remaining variables, including age at diagnosis, pica, and positivity for *TMPRSS6* alleles (as trichotomous variables). TS was positively associated with MCV ($p = 0.0171$). lnSF was positively associated with age ($p = 0.0101$) and MCV ($p = 0.0035$). Hb was positively associated with MCV ($p = 0.0003$). MCV was positively associated with Hb and lnSF ($p = 0.0009$ and 0.0008, respectively). In multiple or logistic regression analyses, neither *TMPRSS6* K253E, A736V, nor Y739Y genotypes expressed as trichotomous variables were predictors of other variables we studied.

Discussion

Frequencies of the respective *TMPRSS6* alleles we studied are similar to those reported in other women with iron deficiency with or without anemia [5]. The frequency of *TMPRSS6* K253E was greater in women with TS ≥10%. The frequency of *TMPRSS6* Y739Y was

greater in women with TS <10%. Mean TS was lower in women with Y739Y. We found no other association of these alleles with iron, erythrocyte, or pica phenotypes. Frequencies of the *TMPRSS6* allele A736V did not differ significantly in women subgrouped according to TS, SF, Hb, or MCV values at diagnosis, or with the presence of pica. Multiple regression analyses did not detect significant associations of *TMPRSS6* K253E, A736V, or Y739Y genotypes with iron, erythrocyte, or pica phenotypes.

In genome-wide association studies, the *TMPRSS6* A736V has a significant association with lower TS, Hb, and MCV levels in adults in general populations [4,6]. This effect is due in part to decreased ability of *TMPRSS6* A736V to down-regulate hepcidin transcription and thus decrease circulating hepcidin levels [14,17]. On the other hand, serum hepcidin levels are low in adults with iron deficiency [11,15]. Taken together, the lack of apparent iron or erythrocyte phenotype effect attributable to *TMPRSS6* A736V in the present study suggests that subnormal quantities of body iron exert a greater effect on TS, Hb, and MCV levels than does *TMPRSS6* A736V.

In the present study, we observed lower mean age, mean Hb level, and mean MCV in women with pica (pagophagia) by univariable analyses. This agrees with previous reports of adults with pica, and demonstrates that the iron, erythrocyte, and pica phenotypes of the present cohort of 48 women were typical of other subjects with iron depletion or deficiency [2,12,13]. Their lower mean Hb and MCV levels are similar to erythrocyte phenotypes attributed to *TMPRSS6* A736V reported in population studies [4,6]. Thus, we postulated that pica susceptibility in persons with iron deficiency could be associated with *TMPRSS6* A736V. Further, patients with iron deficiency or depletion who experience pica develop recurrent pica with recurrent iron deficiency or depletion [2]. Our informal experience also indicates that some mother-daughter pairs with iron deficiency also develop pica. Matriptase-2 is expressed in the nasopharynx or olfactory epithelium [8,10]. Thus it is possible that *TMPRSS6* mutations could alter taste or smell and induce pica. Nonetheless, our multiple regression analyses revealed no significant relationship of pica to the occurrence of A736V or other *TMPRSS6* alleles that we studied. It is also possible that iron deficiency or depletion may cause dysregulation of central nervous system iron or iron proteins that leads to pica manifestations in some persons, although this is unproven.

We conclude that the frequency of *TMPRSS6* K253E was greater in women with TS ≥10%. The frequency of *TMPRSS6* Y739Y higher in women with TS <10%, and that mean TS was also lower in women with Y739Y. *TMPRSS6* Y739Y may be linked to a gene or mutation that negatively influences TS values. We observed no other significant relationship of *TMPRSS6* alleles K253E, A736V, or Y739Y with iron, erythrocyte, or pica phenotypes of women with iron depletion or deficiency.

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Table 1

Primers and annealing temperatures for *TMPRSS6* mutation analyses

Name	Sequence	Size (bp)	Temp °C	DMSO
TMPRSS6 Ex 7F	CTGCCTGGTGGAGGACCTTG	385	62	5%
TMPRSS6 Ex 7R	CTAAGAATGCTGTGTGTGAC			
TMPRSS6 Ex 17F	AGAAGTAGGCTCCTGAGATG	335	64	5%
TMPRSS6 Ex 17R	AGGCTTCAGCAGGCTGATGT			

Table 2

Frequencies of common *TMPRSS6* alleles in 48 women with iron depletion or deficiency[a]

Subjects (n)	K253E (c.757A→G; rs2235324) frequency (allele counts)	A736V (c.2207C→T; rs855791) frequency (allele counts)	Y739Y (c. 2217 C→T; rs2235321) frequency (allele counts)
All subjects (48)	41.7 (40/96)	36.5 (35/96)	39.6 (38/96)
Transferrin saturation <10% (28)	25.0 (14/56)[b]	30.4 (17/56)	50.0 (28/56)[c]
Transferrin saturation ≥10% (20)	65.0 (26/40)[b]	45.0 (18/40)	25.0 (10/40)[c]
Serum ferritin <45 pmol/L (26)	46.2 (24/52)	38.5 (20/52)	42.3 (22/52)
Serum ferritin ≥45 pmol/L (22)	36.4 (16/44)	34.1 (15/44)	36.4 (16/44)
Hemoglobin <117 g/L (34)	42.6 (29/68)	33.8 (23/68)	44.1 (30/68)
Hemoglobin ≥117 g/L (14)	39.3 (11/28)	42.9 (12/28)	28.6 (8/28)
Mean corpuscular volume <80.0 fL (26)	46.2 (24/52)	28.8 (15/52)	44.2 (23/52)
Mean corpuscular volume ≥80.0 fL (22)	36.4 (16/44)	45.5 (20/44)	34.1 (15/44)
Pica (22)	43.2 (19/44)	34.1 (15/44)	38.6 (17/44)
No pica (26)	40.4 (21/52)	38.5 (20/52)	40.4 (21/52)

[a] One woman was heterozygous for the *TMPRSS6* V795I polymorphism (rs139105452).

[b] The difference between these frequencies was significant (p = 0.0001).

[c] The difference between these frequencies was significant (p = 0.0135).