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Original Research Article

Relationship between Trans-Membrane Protease Serine 6 Gene Polymorphism (C1795T) and Danger of Iron Deficiency Anemia in Khartoum State

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Abstract

Iron deficiencies anemia has been broadly discovered and understood as a nutritional disorder but there is a limited understanding of iron deficiency anemia, which is due to genetic alterations in iron homeostasis, which is a recently recognized recessive disorder that causes microcytic hypochromic anemia. It's due to mutations in the trans-membrane protease serine 6 genes (C1795T), which encodes matriptase-2, and mainly expressed by hepatocytes. In this analytical study case- control, we registered hundred patients with iron deficiency anemia and 100 healthy volunteers as a control group and analyzed their complete blood counts and TMPRSS6 genotypes. TMPRSS6 C/T polymorphism was analyzed using polymerase chain reaction. The TT genotype (mutant gene) of TMPRSS6 C1795T polymorphism was higher frequent in IDA patients (71.4%). The results show low hemoglobin concentration and MCV while RDW was increase significant difference (P≤0.00) in IDA patients compare with normal individual when interacted with the TMPRSS6 genotypes. In peroration there were statistically significant association between TMPRSS6 C/T polymorphism and risk of IDA among Sudanese patients in Khartoum state.

Keywords: Iron deficiencies anemia, TMPRSS6-sudan.

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1. Introduction

TMPRSS6 is a member of type 2 trans-membrane serine proteases family diagramed on 22q12-13 with unique structural features including a serine protease domain, a trans-membrane domain, a short cytoplasmic domain, and a stem region containing an LDL [1].

TMPRSS6 gene synthesis protein (Matriptase 2), which plays an essential role in iron hemostasis that negatively regulates hepcidin expression by cleaving membrane-bound hemojuvelin.

Nonsense mutations were identified by sequencing the TMPRSS6 gene cDNA1795C T substitution in exon 15 introducing another protein nonsense codon R599X This mutation to delete the serine protease domain from the encoded protein unless the mRNA protecting premature translation termination codon is rapidly degraded through the nonsense-mediated RNA decay surveillance pathway[2,3].

To identify the nonsense mutations, sequencing of TMPRSS6 gene cDNA1795C T substitution in exon 15 has been done, it introduced different protein nonsense codon R599X. This is due to deletion of serine protease domain in the encoded protein, unless protection of premature translation termination codon is degraded, and this happened via nonsense mediated RNA decay surveillance pathway.

Homozygous inactivation of the TMPRSS6 gene leads to excessive HAMP production, impaired dietary iron absorption and microcytic anemia in mice and iron-refractory iron deficiency anemia (IRIDA) in humans.

Common TMPRSS6 genetic variants, as rs855791, associate with serum iron and transferrin saturation, hemoglobin (Hb) and erythrocyte (MCV and MCH) traits in different populations. TMPRSS6 gene dosage may modify erythropoiesis and influence HAMP expression.[4]

Missense mutations in TMPRSS6 gene are spread along the entire gene sequence, affecting not only the protease catalytic domain, but also other domains that could affect protein-protein interaction.[5- 8]

The deficient of TMPRSS6 activity up-regulates hepcidin transcription and reduces the expression of ferroportin thereby resulting in retention of iron within the cells. [9,10]

Most mutations are isolated. In vitro studies have shown that causal mutations have decreased activity and are unable to inhibit hepcidin promoter at the same rate of the wild type protein in a luciferase-based assay in cells transfected with haemojuvelin. Hepcidin is a small peptide hormone produced by the liver that is detectable in serum and urine, is a central regulator of iron homeostasis [11].

Only heterozygous TMPRSS6 mutations have been found in a few patients, although regulatory regions are not usually explored by sequencing 7. It is possible that single nucleotide polymorphisms (SNPs) or specific haplotypes play some role in the disease, and that cases showing microcytosis without anaemia are due to mild TMPRSS6 mutations. TMPRSS6 haplo-insufficiency renders mice more susceptible to iron deficiency in conditions of iron restriction or in the presence of increased requests, such as pregnancy [12].

Common genetic variants in the TMPRSS6 gene in several populations are associated with changes in the normal erythrocyte (microcytic hypochromic cells) and iron parameters. [13,14]

The SNP rs855791 with a missense change in the serine protease domain may influence serum hepcidin levels and iron parameters in normal subjects. The ethnic background and the environment are also important: in Chinese the TMPRSS6 genetic variant rs855791 is associated with iron deficiency in the aging. [15,16]

2. Method

2.1 Study populations

The analytical case-control study was conducted to investigate the association between TMPRSS6 gene polymorphism and risk of IDA. Two groups of 100 anemic patients and 100 normal control samples were enrolled in this study in Khartoum state.

Patients with microcytic anemia were screened at hematologic clinics (Mohamed Elamin Paediatric Hospital and Al-zahra care center, through the period of May to July 2018) for IDA patients. IDA defined by hemoglobin <10 g\dl, mean corpuscular volume <70 fl and red cell distribution width >17 %. And excluded other known inherited microcytic anemia (thalassemia). Questionnaire was run to every subject in this study. This study was accepted by collage of medical laboratory science of Sudan University of Science and Technology. A verbal informed consent was obtained from each participant before samples collection.

2.2 Hematology analyzer

Hemoglobin, mean corpuscular volume and red distribution width were determined by Sysmexkx 21.

2.3 Genotyping

Genomic DNA was extracted from whole blood using Soult out method, The TMPRSS6 (C1795T) polymorphism was determined by PCR, The primers used were as flow:

Name of the gene	Primer sequences
TMPRSS6	Forward-5'-TAG AGA ACA GGG GCT CCA GG - 3'
	Reverse-5'-ATG TGG GCA GCA TCC TTT C - 3'

The reaction conditions for amplification were as follows: 95°C for 5 min; 30 cycles of 95°C for 45 S, 63°C for 45 S, and 72°C for 45 S; and a final extension at 72°C for 5 min. PCR products were electrophoresed on 2% agarose gel containing ethidiumbromide and analyzed under UV light, 100bp deoxyribonucleic acid (DNA) ladder applied with each batch of patients samples. Amiphliction fragment produce single band at 249 bp represented the well gene (CC).

2.4 Statistical analysis

Data was analyzed by statistical package for social sciences (SPSS) version 16. Association between qualitative variables was tested using Pearson's Chi square (χ^2) and association between quantitative variables was tested using t-test. Binary logistic regression analysis was used to investigate the association between genotypes and risk of IDA.

3. Results

TMPRSS6 gene investigate by PCR, produce single band at 249 bp represented the well gene (figure 1). The mutant (TT) genotype of TMPRSS6 C/T polymorphism was higher frequent in IDA patients (71.4%). The result showed significant difference in genotype distribution when compared to anemic patients with normal control groups (P≤ 0.00) (Table 1).

Comparison of value of CBC sub-parameter (Hb, MCV and RDW) in anemic patients and normal control with TMPRSS6 (TT and TC/CC genotypes) polymorphism showed statistically significant difference (P≤ 0.00) but

when compare the mutant gene and well gene in anemic group there were no statistically significant difference (Table 2, 3 and 4) respectively.

The study investigate the risk of TMPRSS6 gene and IDA by logistic regression test, which show no interaction between TMPRSS6 genotypes with age and gender in IDA patients, but there were interaction between TMPRSS6 genotypes and hematological parameters (Hb, MCV and RDW) (Table 5).

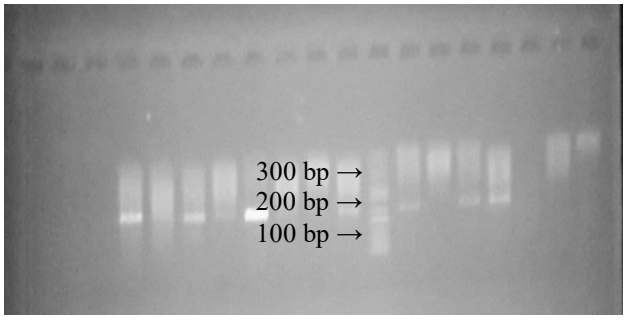

Figure 1: Amplified fragments of TMPRSS6

Table 1: Genotype distribution in study groups

Variable	TMPRSS6		Total	P value
	Mutant gene	Well gene		
Patient	68(77.4%)	32(28.6%)	100	≤ 0.00
Control	20(22.7%)	80(71.4%)	100	

Table 2: Comparison of mean Hb with case and control according to genotype

Variables	Mean of Hb g/dl		P value
	Mutant gene	Well gene	
Case	8.93±1.4	9.22±1.8	≤ 0.40
Control	12.9±0.8	12.68±1.3	

Table 3: Comparison of MCV value with anemic patients and control according to genotype

Variables	Mean of MCV (fl)		P value
	Mutant gene	Well gene	
anemic	66±5.5	68.4±5.7	≤ 0.08
Control	91.7±6.7	89.8±6.8	

Table 4: Comparison of RDW value with anemic patients and control according to genotype

Variables	Mean of RDW (%)		P value
	Mutant gene	Well gene	
anemic	19.83±2.8	19.75±4	≤ 0.91
Control	12.58 ±1.8	13.07±2.1	

Table 5: Interaction between TMPRSS6 polymorphism with age, gender, HB, MCV and RDW in IDA patients

Variables	95% C. I.		Odd ratio	P value
	Lower	Upper		
Gender	0.437	1.713	0.865	0.055
Age	0.989	1.029	1.009	0.053
Hb	0.822	1.354	1.055	0.022
MCV	0.963	1.069	1.015	0.011
RDW	0.892	1.165	1.020	0.009

4. Discussion

There were statistically significant relationship between TMPRSS6 C1795T polymorphism genotypes and risk of IDA (P≤0.03), also reported means of Hb, MCV and RDW in patents and control (9g/dl,12.7g/dl), (66 fl ,90 fl) and (19.8%, 12.9 %) respectively were significantly different (P ≤ 0.00) The explanation of lowing Hb concentration and MCV value and the increase of RDW is that TMPRSS6 polymorphisms imbalance iron hemostasis (low hem decrease Hb synthesis) which lead to IRIDA. The present results are in agreement with Sung *et al* who reported (8.5 g/dl, 69 fl and 18%) values for Hb concentration, MCV and RDW respectively in IDA patients [17]. An *et al* conducted study in Chinese population, reported that TMPRSS6 polymorphisms are significantly associated with decreased iron status which associated with lower hemoglobin levels and there were a common variants in TMPRSS6 as being a genetic risk factors for IDA (P≤ 0.00). Consistent with their associations to increased iron deficiency and anemia risk which agree with current result [15].

A study conducted on Italy population, observed that TMPRSS6 mutation leads to overproduction of hepcidin and defective iron absorption and utilization, which is a high risk factor for iron deficiency anemia. [14] TMPRSS6 homozygous mutation increases the risk of iron deficiency anemia by inappropriately elevated hepcidin expression in Tmprss6-/- results in chronically impaired uptake of dietary iron, reflected in decreased hepatic iron stores. Significantly fewer C homozygotes in the IDA group compared to the healthy group has been reported by Sung *et al*[17], suggesting that homozygote for TMPRSS6 T genotype has a high risk of IDA. In conclusions the present of TT genotype have the risk of iron deficiency anemia. There were interactions observed between TMPRSS6 C/T genotypes with means of Hb, MCV and RDW with IDA patients.

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References

[1]. Nai, A., Pagani, A., Silvestri, L. and Camaschella, C. Increased susceptibility to iron deficiency of Tmprss6-haploinsufficient mice. *Blood*, 2010; 116:851–852.

[2]. National Center for Biotechnology Information (NCBI). Entrez-Gene. *http://www.ncbi.nlm.nih.gov/sites/entrez/dbgene.* Accessed May 1, 2008.

[3]. Chang, Y. F., Imam J.S. and Wilkinson, M.F. The nonsense-mediated decay RNA surveillance pathway. *Annu Rev Biochem*. 2007; 76:51-74.

[4]. Nai, A., Pagani, A., Mandelli, G., Lidonnici, M. R., Silvestri, L., Ferrari, G. and Camaschella C. Deletion Of TMPRSS6 Attenuates the Phenotype in a mouse model of β thalassmia, Role of Tmprss6 in ß-thalassemia, American Society of Hematology, *Blood*, 2012; DOI 10.1182/blood-2012-01-401885

[5]. Finberg, K.E., Heeney, M.M., Campagna, D.R., Aydinok, Y., Pearson, H.A., Hartman, K.R., Mayo, M.M., Samuel, S.M., Strouse, J.J., Markianos, K., Andrews, N.C. and Fleming, M.D. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). *Nature Genetics* 2008; 40: 569–571.

[6]. Guillem, F., Lawson S., Kannengiesser C., Westerman M., Beaumont, C. and Grandchamp, B. Two nonsense mutations in the TMPRSS6 gene in a patient with microcytic anemia and iron deficiency. *Blood*, 2008; 112: 2089–2091.

[7]. Silvestri, L., Guillem, F., Pagani, A., Nai, A., Oudin, C., Silva, M., Toutain, F., Kannengiesser, C., Beaumont, C., Camaschella, C. and Grandchamp, B. Molecular mechanisms of the defective hepcidin inhibition in TMPRSS6 mutations associated with iron-refractory iron deficiency anemia. *Blood*, 2009; 113: 5605–5608.

[8]. Guillem, F, Kannengiesser C. and Oudin, C. Inactive matriptase-2 mutants found in IRIDA patients still repress hepcidin in a transfection assay despite having lost their serine protease activity. *Human Mutation*, 2012; 33: 1388-1396

[9]. Du, X., She, E., Gelbart, T., Truksa, J., Lee, P., Xia, Y., Khovananth, K., Mudd, S., Mann, N., Moresco, E.M., Beutler, E. and Beutler, B. The serine protease TMPRSS6 is required to sense iron deficiency. *Science*, 2008; 320: 1088–1092.

[10]. Nemeth E and Ganz T. Regulation of iron metabolism by hepcidin. *Annual Review Nutrition*. 2006; 26323-342.

[11]. Finberg, K.E., Whittlesey, R.L., Fleming, M.D. and Andrews, N.C. Down-regulation of Bmp/ Smad signaling by Tmprss6 is required for maintenance of systemic iron homeostasis. *Blood*, 2010; 115: 3817–3826.

[12]. Benyamin, B., Ferreira, M.A., Willemsen, G., Gordon, S., Middelberg, R.P., McEvoy, B.P., Hottenga, J.J., Henders, A.K., Campbell, M.J., Wallace, L., Frazer, I.H., Heath, A.C., de Geus, E.J., Nyholt, D.R., Visscher, P.M., Penninx, B. W., Boomsma, D.I., Martin, N.G., Montgomery, G.W. and Whitfield, J.B. Common variants in TMPRSS6 are associated with iron status and erythrocyte volume. *Nature Genetics*, 2009; 41: 1173–1175.

[13]. Tanaka, T., Roy, C.N., Yao, W., Matteini, A., Semba, R.D., and *et al*. A genome-wide association analysis of serum iron concentrations. *Blood*, 2010; 115:94 – 96.

[14]. Nai, A., Pagani, A., Silvestri, L., Campostrini, N., Corbella, M., Girelli, D., Traglia, M., Toniolo, D. and Camaschella, C. TMPRSS6 rs855791 modulates hepcidin transcription in vitro and serum hepcidin levels in normal individuals. *Blood,* 2011; 118: 4459–4462.

[15]. An, P., Wu, Q., Wang, H., Guan, Y., Mu, M., Liao, Y., Zhou, D., Song, P., Wang, C., Meng, L., Man, Q., Li, L., Zhang, J. and Wang, F. TMPRSS6, but not TF, TFR2 or BMP2 variants are associated with increased risk of iron-deficiency anemia. *Human Molecular Genetics*, 2012; 21: 2124–2131.

[16]. Soranzo ,N., Sanna, S. and Wheeler, E. Common variants at 10 genomic loci influence hemoglobin A(C) levels via glycemic and nonglycemic pathways. *Diabetes* 2010; 59: (12): 3229-3239.