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Maternal *TMPRSS6* Gene Polymorphism rs855791SNP in Women with Preeclampsia

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Abstract

Introduction: Preeclampsia can lead to several maternal and perinatal adverse effects. There are few published data on the association between transmembrane serine protease 6 (*TMPRSS6*) gene polymorphism and preeclampsia. **Objective:** To assess the association between *TMPRSS6* gene polymorphism rs855791SNP in women with preeclampsia compared with healthy pregnant women. **Method:** A case-control study (60 women in each arm) was conducted at Saad Abuaela Maternity Hospital in Khartoum, Sudan. Sociodemographic and clinical data were gathered through a questionnaire. The participant was genotype for *TMPRSS6* gene rs855791SNP using Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP). The results were confirmed by DNA sequencing. **Result:** There was no significant difference in the median of age, parity, and body mass index. The distribution of the genotypes and alleles of *TMPRSS6* rs855791 was consistent with the HWE. The overall *TMPRSS6* rs855791 polymorphism was not significantly associated with preeclampsia. However, the proportion of heterozygotes (TC) was considerably higher in the women with preeclampsia (46.7%) than in the control group (23.3%) ($p = 0.001$; OR = 2.71; 95% CI = 1.21 - 6.07). The proportion of homozygotes (TT) and T alleles was not significantly different between women with preeclampsia and the control group. **Conclusion:** The overall *TMPRSS6* rs855791 polymorphism was not significantly associated with preeclampsia and healthy control.

Keywords

Preeclampsia, *TMPRSS6* Gene Polymorphism, rs855791SNP

1. Introduction

Preeclampsia is a multi-organ pregnancy syndrome characterized by the new onset of hypertension and proteinuria after 20 weeks of gestation [1]. It affects 2% - 8% of pregnancies globally [2]. It is one of the leading causes of maternal and neonatal mortality and morbidity in developed countries [2]. Although the exact pathophysiology of preeclampsia remains to be elucidated, preeclampsia is characterized by poor placentation (shallow cytotrophoblast invasion) which results in defective re-modeling of the maternal spiral arteries [3]. Poor placentation (under-perfused placenta) can lead to maternal angiogenic imbalance and results in the activation of an inflammatory response [3]. Hepcidin is a peptide hormone that plays an essential role in iron homeostasis to keep it tightly regulated for haemoglobin and erythropoiesis without allowing iron overload to occur in the body. It is an acute-phase reactant that can increase in response to inflammation [4]. It has been reported that hepcidin is lower during pregnancy to ensure optimum iron bioavailability to the mother and fetus. However, inflammatory states, such as preeclampsia and parasitic (malaria) infection, have been associated with higher hepcidin levels during pregnancy [5]. The elevated hepcidin level in women with preeclampsia could be a protective mechanism to counteract iron overload (mediated cytotoxicity), oxidative stress, and endothelial dysfunction that might occur in women with preeclampsia [6]. Preeclampsia's main treatment options are delivery of the infant or management of the condition until the ideal moment for delivery. The severity of preeclampsia, the gestational age of your child, and your overall health and that of your child will all play a role in the decision that your doctor and you make [7]. Magnesium sulfate remains the preventive and curative treatment and its early administration conditions the maternal and fetal prognosis [8]. Aspirin is effective in the secondary prevention of preeclampsia mainly in patients with a history of preeclampsia aspirin efficacy for the prevention of preeclampsia is dose-dependent, but the optimum dosage of 75 mg/day to 150 mg/day needs to be determined safety data at 150 mg/day are still limited [9]. Amaral stabilized the blood pressure and reduced the concentrations of sFlt-1 in four patients with preeclampsia less than 30 weeks gestation [10]. Maternal ages are one of the potential risk factors for preeclampsia. When compared to women between the ages of 25 and 29, women over 35 (Advanced Maternal Age, AMA) had a 4.5-fold increased risk of developing preeclampsia [11]. Similar research from China revealed that women over the age of 40 had a 3.80 and 7.46-fold increased chance of developing preeclampsia compared to women of normal reproductive age [12]. Preeclampsia and severe preeclampsia are 1.86 and 2.03 times more common in mothers over 45 in another Japanese study [13].

Several studies have shown the relation between serum iron and women with preeclampsia compared to healthy pregnant women [6] [14] [15] [16], and this goes with the previous study which showed similar findings. *TMPRSS6* gene and was recently described as the top-hit of Genome-Wide Association Studies (GWASs) to be associated with alterations of serum iron, transferrin saturation, erythro-

cyte mean cell volume, blood hemoglobin levels, and glycated hemoglobin [17] [18] [19]. To our knowledge, this is the first study to assess the relation between *TMPRSS6* gene rs855791SNP in women with preeclampsia. There is a need to understand the genetic factors associated with iron deficiency anemia in pregnant women [20]. Matriptase-2 is important and down-regulate hepcidin expression through cleaving membrane-bound hemojuvelin, which can enhance hepcidin transcription [21]. The reported causative mutations in *TMPRSS6* are spread throughout the gene sequence, disrupting the catalytic activity or protein-protein interactions [22]. Complete loss of function mutation of matriptase-2 leads to a rare disease, Iron-Refractory Iron Deficiency Anemia (IRIDA) [15]. It is of interest whether genetic variants with incomplete loss of function of matriptase-2 will be associated with IDA. Recently, genetic factors have been identified that may play an essential role in the pathogenesis of IDA [23]. Several polymorphisms have been previously reported in the Saudi population as causative Single Nucleotide Polymorphisms (SNPs) in diseases such as breast cancer [24], colon cancer [25], diffuse parenchymal lung disease [19] [26], and acute myeloid leukemia [24]. In addition, genetic risk factors for IDA and causative genes have also been identified including the *TMPRSS6* gene [26].

A family of proteolytic enzymes known as the human Type II Transmembrane Serine Proteases (TTSPs) is expressed on the surface of many different cell types. Matriptase-2, also known as *TMPRSS6*, is one member of this family that is primarily expressed in the liver [27]. The *TMPRSS6* gene is located on chromosome 22q12-q13 [21].

Recently, the Genome-Wide Association (GWA) studies identified several Single Nucleotide Polymorphisms (SNPs) of the *TMPRSS6* genes, and it was found that the rs855791 has strong robust relation with microcytic red blood cell phenotype [28]. The SNP occurs at nucleotide position 2207 of *TMPRSS6* exon 17, causing a missense mutation from Valine (V) to Alanine (A) as GTC > GCC; V736A [29]. The three SNP genotypes are T allele (T/T), homozygous C allele (C/C), and heterozygous C/T. There were several studies of allele frequency and SNP variation prevalence mentioned. Similar to the Rwandan population in Sub-Saharan Africa, the Caucasian population had a greatly scattered C allele frequency [30].

The data generated from this study will serve as a reference for future studies on IDA in the African population.

Preeclampsia is the high cause of maternal and perinatal mortality in Sudan a leading health problem [31]. In the African population, there was more complex genetic factor than in other populations because of the interaction between an environmental factor and genetic variation [32]. The effect of SNP rs855791 in Sudanese women with preeclampsia still needs to be fully understood, and to our knowledge no published data of *TMPRSS6* gene rs855791SNP in Sub-Saharan Africa including Sudan. Therefore, the current study was conducted to assess the association of *TMPRSS6* gene polymorphism specially rs855791SNP, in Sudanese women with preeclampsia.

To our knowledge, there is no study conducted in Sudan regarding the mutational analysis of the rs855791SNP *TMPRSS6* gene in patients with preeclampsia other than this study, and thus, this is the first study done to screen exon 17 of *TMPRSS6* gene rs855791SNP in Sudanese women with preeclampsia.

2. Material and Methods

2.1. Study Area

A case-control study was conducted at Saad Abualela Maternity Hospital in Khartoum, Sudan, Sudan, from June to December 2020. The cases were pregnant women who presented with preeclampsia. Preeclampsia was defined per American College of Obstetricians and Gynecologists criteria [1]: pregnant women with hypertension (an average blood pressure reading of ≥140/90 mmHg which was taken on two occasions, at least six hours apart), plus/and proteinuria (≥300 mg/24 hours). Preeclampsia was classified as severe in women with an average blood pressure reading of ≥160/110 mmHg on two occasions or proteinuria of ≥5 g/24 hours and (HELLP) syndrome which included hemolysis, elevated liver enzymes, and low platelet count, otherwise, preeclampsia was considered mild [1]. Moreover, preeclampsia was considered early and late-onset preeclampsia (before and after 34 weeks), respectively [33]. A healthy pregnant woman without any systemic disease, such as hypertension, diabetes mellitus, renal disease, or thyroid disease, served as the control for each preeclampsia case. Women with multiple pregnancies, diabetic smokers, and fetuses with significant abnormalities or died were excluded from the issues and the controls.

After signing an informed Consent, the women were asked about their sociodemographic, obstetrics, and clinical data, including age, parity, educational level, residence antenatal attendance, and history of miscarriage and preeclampsia/hypertension. Body Mass Index (BMI) was computed from the measured weight and height.

2.2. Sampling

Out of all Sudanese women diagnosed with preeclampsia and standard control pregnant (60 were collected from each arm), 15 and 7 samples, were randomly selected for genetic sequencing and analysis from women with preeclampsia and control respectively. Blood samples were collected in Ethylene Diamine Tetra Acetic (EDTA) acid and preserved at −20˚C.

2.3. Molecular Genetics Analysis

2.3.1. DNA Extraction

Genomic DNA is extracted by salting out methods Single Lysis-Salting Out (SLSO) according to published protocol, which gives quality and quantity of DNA in single steps [34]. Proteinase K was added at 56c to improve the breakdown of the white cell membrane then DNA was extracted an hour after, dissolved in EDTA buffer, kept at 4˚C four overnights, and preserved at −20 till use.

2.3.2. Polymerase Chain Reaction (PCR) Amplification

Sixty preeclampsia and sixty normal pregnant women were amplification using primer; the primers that will be used for the *TMPRSS6* gene in exon 17.

It was purchased from (EUROFINE Genomic Company, Germany). By using Maxime PCR PreMix Kit i-Taq 20 μl (INTRON Biotechnology, South Korea) the annealing temperature was adjusted on several runs of PCR.). The reaction condition for amplification was done after the addition of15 ul Distilled water, 3 ul sample DNA, and 1 ul of each forward and reverse to the ready-to-use master mix volume as follows: 95C for 5 minutes for initial denaturation, 30 cycles of 95C for 45 seconds for denaturation, annealing at 63C for 45 seconds and step of elongation 72C for 45 seconds, and final extension at 72C for 5 minutes. The results amplification will give a PCR product (249 bp). Only 15 patients and seven controls yielded sufficient quality bands, and were subsequently selected for sequencing by the Sanger sequencing technique.

The *TMPRSS6* rs855791SNP is determined by Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP). It is defined by an enzyme, a restriction endonuclease, that cuts the double-stranded DNA at a particular sequence of bases, a probe, a labeled, complementary segment of DNA that will anneal to a portion of the digested sample, and a set of variable fragment length bands that appear on a Southern blot [35]. Following amplification, the PCR products (249 bp) were digested with the restriction endonuclease *Stu* I (New England Biolabs, Inc. Hitchin, Herts, UK) (**Figure 3**).

2.3.3. Sequencing of *TMPRSS6* Gene Exon 17

Genotypes are determined by fragment size and 10% of the sample is directly sequenced to confirm the genotyping result. Sanger sequencing was performed for the PCR products. BGI Tech Company sequenced both DNA strands (Wuhan, China).

2.3.4. Statistical Analysis

The collected data were entered into SPSS (Statistical Package for Social Science) version 22.0 for Windows for data analysis. The observed and expected genotype distributions and their agreement with the Hardy-Weinberg equilibrium (HWE) were assessed using the chi-square (χ^2) goodness-of-fit test. Differences in allele frequencies between the preeclampsia and the controls were compared using Pearson's chi-square test. The risk associated with a specific genotype was estimated using the Chi-square or Fisher's exact test and using Odds Ratios (ORs), and it is expressed with a 95% confidence level. P-values of <0.05 were considered significant.

3. Results

There was no significant difference in the median (IQR) of age, parity, and BMI. However, the median (IQR) of the gestational age was significantly lower in women with preeclampsia (**Table 1**, **Figure 1**).

1) The overall *TMPRSS6* gene rs855791 polymorphism was not significantly

associated with preeclampsia (OR = 5.3; 95% CI = 2.6 - 10.7; p < 0.001) (Table 2). However, the proportion of heterozygotes (TC) was significantly higher in the women with preeclampsia (46.7%) than in the control group (23.3%) (p = 0.001; OR = 2.71; 95% CI = 1.21 - 6.07) (Table 2, Figure 2). The proportion of homozygotes (TT) was not significantly different between women with preeclampsia (46.7%) and the control group (23.3%) (p = 0.001; OR = 2.71; 95% CI = 1.21 - 6.07) (Table 2, Figure 2).

2) The proportion of T allele was not significantly different in the women with preeclampsia than in the healthy control group (30.0% vs. 25.0%; OR = 1.28, 95% CI = 0.72 - 2.27, p = 0.385) (Table 2, Figure 3).

Table 1. Shows compares the Median (interquartile range) of the clinical variables between women with preeclampsia and controls in Khartoum Sudan (2020).

Variables	Preeclampsia (60 women)	Controls (60 women)	P
Age, years	27 (24 - 31)	27 (25 - 32)	0.289
Parity	2 (1 - 4)	2 (1 - 3.75)	0.430
Body mass index, kg/m^2	25.3 (22.9 - 26.4)	24.9 (22.0 - 25.7)	0.678
Gestational age, week	38.0 (37.0 - 39.0)	39.0 (38.0 - 40.0)	<0.001

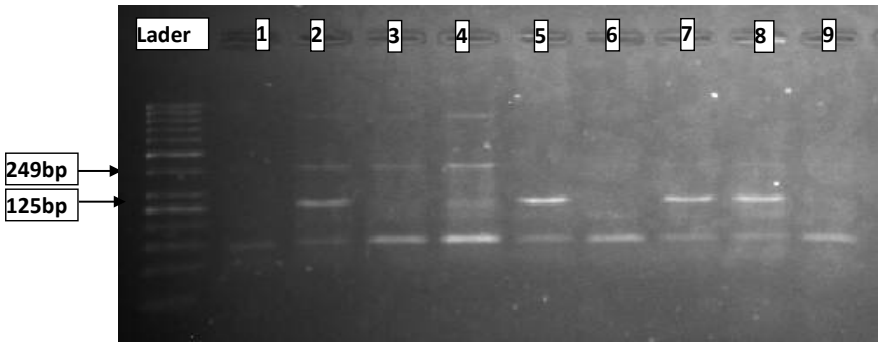

Figure 1. Genotyping of *TMPRSS6* rs855791 by restriction fragment length polymorphism (RFLP). The PCR products were digested with Stu I endonuclease. The single band at 249 bp represented C homozygous; the single band at 125 bp suggested T homozygous, and the two bands represented heterozygously.

Table 2. Genotype and allele frequencies for the *TMPRSS6* rs855791 gene polymorphism in women with preeclampsia and healthy controls, Sudan (2020).

Genotypes	Preeclampsia (n = 60) NUMBER (%)	Controls (n = 60) Number (%)	OR (95% CI)	P-value
CC	28 (46.7)	38 (63.3)	Reference	0.013
TC	28 (46.7)	14 (23.3)	TC vs. CC 2.71 (1.21 - 6.07)	
TT	4 (6.7)	8 (13.3)	TT vs. CC 0.67 (0.18 - 2.47)	0.555
TC + TT	32 (76.9)	22 (38.4)	TC + TT vs. CC 1.79 (0.95 - 4.09)	0.066
Allele C	84 (70.0)	90 (75)	Reference	0.385
Allele T	36 (30)	30 (25)	T vs. C 1.28 0.72 - 2.27)	

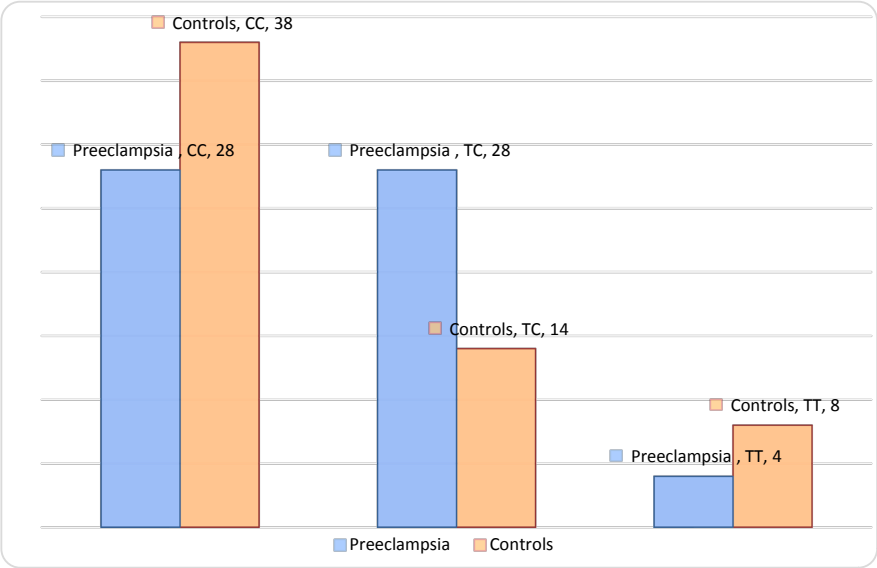

Figure 2. Genotype frequencies for the rs855791 gene polymorphism in women with preeclampsia and healthy controls, were CC, CT, and, TT were 28 to 38, 28 to 14 and 4 to 8 respectively.

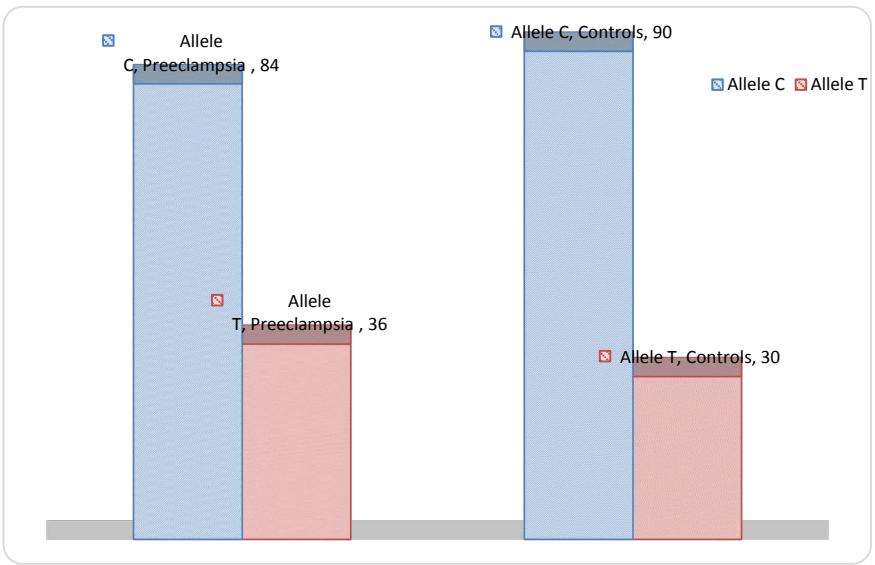

Figure 3. Allies frequencies for the *TMPRSS6* rs855791 gene polymorphism in women with preeclampsia and healthy controls, Sudan (2020), Allele C (84) and T (36) in preeclamsia, Allele C (90) and, Allele T (30) in controls.

4. Discussion

Our study found that the overall *TMPRSS6* rs855791 polymorphism was not significantly associated with preeclampsia. This agrees with the study which showed that there did not find statistically differences rs855791SNP between preeclampsia and normal control pregnant women [36] (**Table 2**). And this disagrees with the study which shows that there was a significantly associated between *TMPRSS6* rs855791 with IDA in pregnant women [37] [38]. However, the

proportion of heterozygotes (TC) was significantly higher in the women with preeclampsia (46.7%) than in the control group. The proportion of homozygotes (TT) and (T) alleles was not significantly different between women with preeclampsia and the control group.

The previous studies reported that there was association between *TMPRSS6* gene rs855791SNP and iron [21], also reported that there was significant association between *TMPRSS6* rs855791 and iron defiecncy anemia [37]. In *TMPRSS6* SNP, especially rs85579 has been linked to an increased risk of Iron Deficiency Anemia (IDA) in Genome-Wide Association Studies (GWASs) [38]. But we didn't find previous studies mansion the relationship between *TMPRSS6* gene rs855791 and preeclampsia, so to our best of knowledge this the first study to assess the association between preeclampsia and *TMPRSS6* gene rs855791.

It should be noted that our results should be compared to those of other studies with caution. First, highly prevalent anaemia (50%) among pregnant women could affect the whole picture [39]. Second, as previously mentioned, several communicable diseases (e.g. malaria and viral diseases) have been associated with preeclampsia in Sudan, and these diseases could have influenced the rs855791SNP [40].

It should be noted that our results should be compared to those of other studies with caution. First, highly prevalent anaemia (50%) among pregnant women could affect the whole picture [39]. Second, as previously mentioned, several communicable diseases (e.g. malaria and viral diseases) have been associated with preeclampsia in Sudan, and these diseases could have influenced the rs855791SNP [41]. To our knowledge, is the first study conducted in Sudan regarding the mutational analysis of the rs855791SNP *TMPRSS6* gene in patients with preeclampsia other than this study, and thus, this is the first study done to screen exon 17 of *TMPRSS6* gene rs855791SNP in Sudanese women with preeclampsia.

Sixty patients' DNA had been extracted, but only fifteen women with preeclampsia and seven healthy pregnant women extracts were sequenced owing to financial constraints. Also, due to these financial constraints, only the product of one primer with the highest stability was subjected to further analysis in this study.

Moreover, the sample size limits the generalizability of this study. Still, for this variant to be generalized to the Sudanese population, further studies using a larger sample size will be needed in the future. In a general context, *TMPRSS6* genes have not got broad assessment within our geographic region; thus, in such a scarce way of expression of preeclampsia genetic characteristics regarding some countries including Sudan, data presented in our study could be more raised. Next Generation Sequencing (NGS) or further testing for the frequently reported mutations in exons 17 is advised. The functional analysis of SNPs by using in silico is practical. The data generated from this study will serve as a reference for future studies on IDA in the African population.

5. Conclusion

Our main finding is that the rs855791SNP was a non-synonymous (V736A) change in the serine protease of *TMPRSS6*. In this study, the overall *TMPRSS6* rs855791 polymorphism was not significantly associated with preeclampsia. However, the proportion of heterozygotes (TC) was significantly higher in the women with preeclampsia than in the control group. The proportion of homozygotes (TT) was not significantly different between women with preeclampsia and the control group. The proportion of the T allele was not significantly different in the women with preeclampsia than in the healthy control group (**Table 2**, **Figure 3**).

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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