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SKA2 gene – A novel biomarker for latent anxiety and preterm birth prediction

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ABSTRACT

Background: There is a relationship between preterm birth (PTB) and anxiety. Spindle and Kinetochore Associated Complex Subunit 2 (*SKA2*) gene polymorphism (NC_000017.11: g.59110368 G > A) has also been associated with the development of anxiety. The current study was designed to evaluate the relationship between *SKA2* gene SNP (NC_000017.11: g.59110368 G > A) with the occurrence of anxiety and PTB which might be considered a predictive biomarker for the prediction of preterm delivery.

Methods: SKA2 gene (SNP rs7208505) genotyping was performed in 300 women with term birth (TB) and 293 women with PTB using PCR-RFLP method and then followed by DNA sequencing. Cortisol level was analyzed with ELISA method and the presence of anxiety was detected using Spielberg Inventory.

Results: The AA genotype of *SKA2* gene significantly increased the risk of PTB compared to the GG genotype by 9.6 fold ([CI] 4.5–20.2, $P < 0.001$) according to codominant model. Also, the frequency of A allele was significantly higher in PTB group ($\chi 2 = 20.4$, df = 1, $P < 0.001$) in comparison with the control group that increased the risk of PTB by 1.703 fold ([CI] 1.39–2.23, $P < 0.001$). Women with higher cortisol level with average 343.7 ± 3 nmol/L had AA genotype, while, the concentrations of cortisol in women with AG, and GG genotypes were 244.2 ± 3.1 nmol/L and 192.6 ± 2.5 nmol/L, respectively ($P < 0.001$). The score of apparent and latent anxiety in women with the AA genotype was higher compared to the AG and GG genotypes and also this score in women with the AG genotype was higher than the GG genotypes ($P < 0.001$). The history of preterm delivery was higher in women with the AA genotype (42.1%) in comparison with the GG (14.9%) and AG (22%) genotypes ($P < 0.05$).

Conclusion: The results of the current study suggest that prognosis of women with the AA genotype are more susceptible to be spontaneous preterm birth. Therefore, the A allele of SKA2 gene (NC_000017.11: g.59110368 G > A) could be as a predictive biomarker for the risk of PTB.

Introduction

Preterm birth (PTB) has a variable frequency in the world, with a prevalence ranging from 5% to 25% of all births in developed and developing countries. Furthermore, the prevalence of PTB has been reported about 5.9–13.4% in Iran [1]. PTB has a key role in neonatal morbidity and mortality and could lead to cerebral palsy, necrotizing enterocolitis, bronchopulmonary dysplasia, and longer hospitalization [2–4]. Despite the great amount of information obtained about PTB such as hormonal and tissue changes occur long before preterm delivery and in addition to the many efforts that have been made to prevent and cure this complication, they have not yet managed to prevent this complication [5]. One of desirable methods to manage PTB is to use the predictive biomarkers [6]. Genetic variants could influence the PTB occurrence; for example by changing the inflammatory factors in mother, therefore genetic changes may be a potential marker for infection-induced PTB [7]. Women with a history of previous PTB are at greater risk for spontaneous PTB recurrence [8]. Another factor influencing the PTB is hormonal responses, though the major cause of PTB is still unknown. The most important factor leading to delivery initiation is cortisol hormone which is increased in the third trimester of pregnancy. Cortisol and cortisol releasing hormone (CRH) are proposed as a fetal-placental clock for the timing of parturition and delivery [9].

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A number of considerable evidence demonstrated that stress and anxiety during the pregnancy might increase CRH levels associated with PTB [10,11]. Moreover, CRH impacts on adrenocorticotropic hormone (ACTH) to increase steroid hormone production, especially fetal cortisol biosynthesis through affecting the placental–adrenal endocrine axis [12]. Researchers assumed that premature increased levels of cortisol and estrogens lead to loss of the uterine quiescence and uterus contraction [13]. Furthermore, CRH induces the prostaglandin synthesis, increases the intracellular calcium and uterus contraction [14,15]. As a result, the peak of the cortisol rhythm occurs before parturition and consequently could cause uterus contraction by releasing prostaglandin. Some studies have shown that prostaglandin inhibitors could delay the onset of labor; however, it cannot prevent delivery [16,17].

PTB can be predicted, prevented and treated if the genetic predisposing factors could be identified. Recent findings have shown that Spindle and Kinetochore Associated Complex Subunit 2 (*SKA2*) gene regulates the effect of cortisol by transferring glucocorticoid receptor from cytoplasm to nucleus [18]. Another study has shown that SNP rs7208505 (NC_000017.11:g.59110368 G > A;NG_009298.1:g.1538C > T) in *SKA2* gene increases cortisol and anxiety [19]. We evaluated the relationship between *SKA2* SNP (NC_000017.11: g.59110368 G > A) and the risk of PTB to find out whether this SNP can be used as a predictive marker of preterm birth in women who are pregnant.

Methods

Study design and patient selection criteria

This is a prospective multicenter case control study. Among the pregnant women who were referred to five referral Hospitals of Tehran University of Medical Sciences (Moheb Yas, Arash, Imam Khomeini, Shariati, Mahdieh), which are located in Tehran, Iran. The present study was carried out between 2015 and 2016. In this study, 593 cases were randomly selected and categorized into two groups: 293 mothers with PTB and 300 mothers with term delivery. PTB is the birth of an infant at fewer than 37 weeks gestational age that is subdivided to early (24–33 weeks) and late preterm birth (34–36 weeks) [20]. The sampling was performed based on the clustering probable sampling. The pregnant women with an average age of 20–35 years had a history of pregnancy 1–3 times, middle family income, and high school or bachelor degree.

The exclusion criteria from the study were the presence of depression and mental illness accompanied by hospitalization, hypertension, and preeclampsia. In addition, acute fatty liver, all kinds of genital infections, antepartum hemorrhage, emergency bleeding, placental abruption, placenta previa, oligohydramnios, hydramnios, placental abnormalities, intrauterine growth restriction (IUGR), inappropriate maternal weight gain during pregnancy or low weight in pre-pregnancy period, smoking, alcohol and cocaine consumption during pregnancy, diabetes, cardiovascular and kidney diseases and previous cervical conization.

Evaluation of serum cortisol

After 24 h of delivery, 5 ml of whole blood sample was collected in which half of the whole blood was added to ethylenediaminetetraacetic acid (EDTA) containing tubes and stored at −20 °C for DNA extraction, and the rest of blood sample was added in the tubes without any anticoagulant agent. The second tube was immediately centrifuged for 10 min at 3500 rpm to separate the serum, and then the serum was stored at −20 °C until use.

Cortisol hormone was measured using IBL kit (Germany) and read by ELX800 ELISA Reader.

DNA extraction

Genomic DNA was extracted from the whole blood samples using the standard salting-out method. The purity of DNA was assessed by 1% agarose gel stained with ethidium bromide, and the quantity of DNA samples was measured at 280 nm wavelength using a Thermo 2000 Nanodrop spectrophotometer [21].

PCR-RFLP

PCR-RFLP was performed to determine the genotype of polymorphism in *SKA2* gene to show the presence of G/A alleles at rs7208505. The following primers used for this aim were: sense 5′-TCCAGACAACAGAATAGTGGC-3′ and antisense 5′-CCCAGGTTCAAGCAGTTCTC-3′

The PCR reactions were carried out in the final volume of 25 μl containing: Taq DNA polymerase 2x master mix red (Amplicon, 2 mM MgCl2 final concentration), 0.8 pmol of each primer, 50 ng template DNA and sterile distilled water up to 25 μl. PCR program was set as the following procedure: initial denaturation step at 95 °C for 5 min, followed by 30 cycles of 30 s denaturation (95 °C), 45 s annealing (58 °C) and 45 s extension (72 °C), finished by an ultimate extension for 10 min (72 °C) and finally cooling to 4 °C. All PCR products were subjected to electrophoresis on 1% agarose gel prepared in 1 × TBE, stained with ethidium bromide, and visualized by exposure to ultraviolet light. The PCR products of *SKA2* rs7208505 G/A were digested with TaaI (HpyCH4III) restriction enzymes (Fermentas) at 65 °C overnight. The DNA fragments were subjected to electrophoresis on 3% agarose gel stained with ethidium bromide. The G allele produced three fragments with sizes of 350, 200, and 150 bp, whereas the A allele produced two fragments with sizes of 500 and 200 bp and remained undigested (500 bp).

DNA sequencing

To confirm genotyping, three samples (with GG, GA and AA genotype) were amplified by PCR technique and then the sequencing of the corresponding genotypes was carried out. These samples were utilized as homozygote and heterozygote controls for verification of PCR-RFLP.

All DNA sequences were assembled using CodonCode Aligner v 5.1.1 (CodonCode Corporation; www.codoncode).

Anxiety detection

Silberberg Inventory was used to study the relationship among the anxiety, polymorphism of *SKA2* gene, and cortisol level in women with preterm and term birth. There were 40-items in two sections of the State-Trait Anxiety Inventory (STAI). Items were rated on a four-point Likert scale. Each item ranged from 1 to 4 and the number of overall scores varied form from 20 to 80. Inventory was used with high reliability (Cronbach alpha 0.94 and 0.87, respectively) [22].

Statistical analyses

Shapiro-Wilk analysis was used to survey the normal distribution of data. The quantitative information with normal distribution was expressed as the mean ± standard error of the mean (SEM). Baseline data and demographic characteristics of participants were compared using Student's *t*-test or Mann Whitney U test where appropriate. The multiple comparisons were performed between groups and genotypes via one-way analysis of variance (ANOVA) supplemented by chi-square post hoc test, or Kruskal-Wallis test supplemented by Bonferroni post hoc test. Allele and genotype

frequencies between PTB and TB were compared by chi-square test ($\chi 2$) or Fisher's exact test if suitable. The odds ratio is an estimate relative risk for disease development that was calculated and a 95% confidence interval was obtained using binary logistic regression analysis. The Hardy–Weinberg equilibrium for SNP was tested by Pearson's chi-square test. Statistical analyses were carried out using SPSS for Windows (version 20, IBM SPSS Inc., USA).

Results

Characteristics of the study population

A total of 293 women diagnosed with preterm delivery and 300 women with term delivery participated in this study. All the subjects were Iranians. The basic characteristics of the patients have been shown in Table 1. There was no significant difference in BMI and anxiety status between the PTB and TB groups. However, significant differences were observed between the case and control groups in terms of maternal age ($P < 0.045$), gestational age at delivery (weeks) ($P < 0.001$), history of preterm birth ($P < 0.009$), birth weight (g) ($P < 0.001$), 5-min Apgar score (5 min after birth) ($P < 0.001$), and cortisol level ($P < 0.001$).

Genotyping

Electrophoresis on PCR samples show by restriction enzyme that the 3-bands of 350, 200 and 150 bp, 4-bands of 500, 150, 350 and 200 bp and 2-bands of 500 and 200 bp are seen for normal homozygous (GG) samples, heterozygous (AG) samples and homozygous mutant (AA) samples, respectively in Fig. 1. Among these samples, three samples with genotypes of AA, AG, and GG were amplified for the sequencing and genotype confirmation. These samples were used as the positive control in enzyme digestion reaction. The results of Electropherograms for each sample have been shown in Fig. 2.

Association between SKA2 genotypes and PTB

The association between *SKA2* gene polymorphisms and PTB was evaluated by comparison between the presence of allele A and the risk of preterm birth in pregnant women. The genotype and allele frequencies of *SKA2* polymorphism in the study population were demonstrated in Tables 2 and 3. The distribution of rs7208505 genotypes was in agreement with Hardy-Weinberg equilibrium ($P > 0.1$).

There was no significant difference in the AG and GG genotypes frequencies between case and control groups, but according to the codominant model, the AA genotype significantly increased the risk of PTB when compared to the GG genotype by 9.6 fold ($P < 0.001$). With respect to dominant model, the A/A + A/G significantly increased the risk of PTB as compared to the G/G genotype by 1.85 fold ($P < 0.002$). Regarding the recessive model, the A/A genotype significantly increased the risk of PTB in comparison to the G/G + A/G genotype by 5.3 fold ($P < 0.001$). Also, allele frequency analysis demonstrated that the frequency of the A allele was significantly higher in preterm group (χ [2] = 20.4, df = 1, $P < 0.001$) while the frequency of allele G was higher in women with term birth. The results indicated that the allele A increased the risk of PTB by 1.703 fold (95%CI 1.39–2.23, $P < 0.001$) compared to the allele G.

Table 1
The demographic and biochemical characteristics of women with term and preterm delivery.

Characteristic	Preterm Delivery N = 293 (mean ± SD)	Term Delivery N = 300 (mean ± SD)	P value
Maternal age (years)	25.52 ± 0.28	26.27 ± 0.24	0.045
Body mass index (kg/m2)	23.91 ± 0.22	24 ± 0.22	0.772
Gestational age at delivery (weeks)	32.24 ± 0.14	38.85 ± 0.04	<0.001
History of previous preterm (% in each group)	27 (27%)	19 (19%)	0.009
Birth weight (gram)	1810.2 ± 28.2	3220.6 ± 17.3	<0.001
Apparent anxiety	50.6 ± 0.76	49.47 ± 0.76	0.292
Latent anxiety	50.83 ± 0.77	49.5 ± 0.78	0.225
Apgar score	6.99 ± 0.07	8.76 ± 0.03	<0.001
Cortisol (nmol/L)	261.58 ± 4	225.8 ± 3.4	<0.001

Association between SKA2 genotypes and other variables

The presence of the AA and AG genotypes statistically ($P < 0.001$) increased the risk of preterm delivery in comparison to the GG genotype. The mean cortisol levels in the AA and AG genotypes were more frequent compared with the GG genotype.

These results showed that women with the AA genotype in the preterm group had a higher concentration of cortisol over that of women with the GG genotype (approximately two folds increase). The levels of cortisol have been shown in Tables 4.

Also, the obtained data showed that the average gestational age at delivery or week of pregnancy in women carrying the AA genotype was found to be 32.4 ± 0.4 week ± days, while in women carrying the AG or GG genotypes were 35.8 ± 0.2 and 36.6 ± .27 week ± days, respectively, ($P < 0.001$) (Table 4).

The average of neonatal birth-weight in women carrying the AA genotype was 1823.6 g which was significantly ($P < 0.001$) lower than women bearing the AG and GG genotypes with the mean neonatal birth-weight of 2584.3 g and 2726.6 g, respectively. The data were summarized in Table 4.

Moreover, the anxiety status including anxiety apparent and anxiety latent was also examined. The average of the state anxiety score in individuals bearing the AA genotype was 57.26, and 50.4 and 45.5 in whom carrying the AG and GG genotypes, respectively. Also, the average of the trait anxiety score in individuals with the AA genotype was 58.2, and in the presence of AG and GG genotypes, it was 50.85 and 44.5, respectively (Table 4). Our findings indicated that the score of apparent anxiety and latent anxiety in individuals with the AA genotype was higher compared with women possessing the AG and GG genotypes ($P < 0.001$). In addition, apparent anxiety and latent anxiety scores were higher in subjects with the AG genotype compared with women with the GG genotype ($P < 0.001$) (Table 4).

Strikingly, the findings demonstrate that a history of preterm was higher in women carrying the AA genotype (42.1%) compared with the GG (14.9%) and AG (22%) genotypes ($P < 0.05$, Table 4). In accordance of Table 4, the Apgar scores revealed a lower grade ($P < 0.001$) in infants of postpartum women possessed the AA genotype (7.25 ± 0.1) in comparison to the neonates of women bearing the AG (7.8) and GG genotypes (8.24 ± 0.1).

Furthermore, of all PTB samples, 63% and 37% samples were early preterm and late preterm, respectively. The genotype frequencies of AA, AG and GG were 29%, 55%, and 16% in the early PTB group, respectively. While, in the late PTB group, the frequencies were 6%, 65%, and 29%, respectively. Table 5 shows the differences between early and late preterm labor in terms of cortisol levels and anxiety scores and Table 6 present the *SKA2* genotypes ratios (AA, AG, GG) between early and late preterm labor. The cortisol levels, the anxiety scores as well as the genotypes of AA and AG were significantly higher in the early PTB group than the late PTB group ($P < 0.001$).

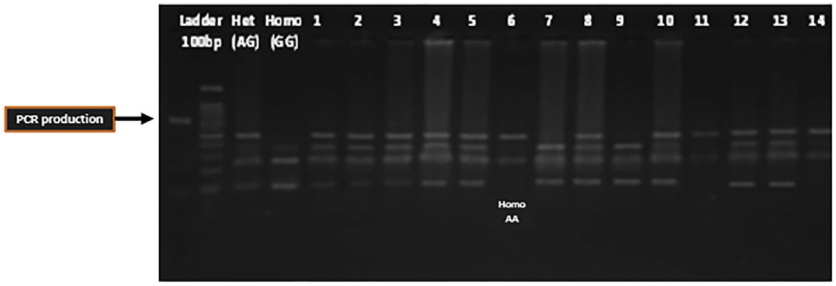

Fig. 1. Shows the PCR products of SNP rs7208505 in SKA2 gene, 3% agarose gel. The lanes are: the lane 1 DNA production (693 bp); the lane 2 DNA ladder with sizes of 100 bp; the lane 3 AG sample with sizes of 150, 200, 350 and 500 bp; the lane 4 GG sample with sizes of 150, 200 and 350 bp; the lane 10 AA sample with sizes of 200 and 500 bp, the lanes 5 to 18 (the numbers 1 to 14) show the results of Electrophoresis on some of the RFLP samples.

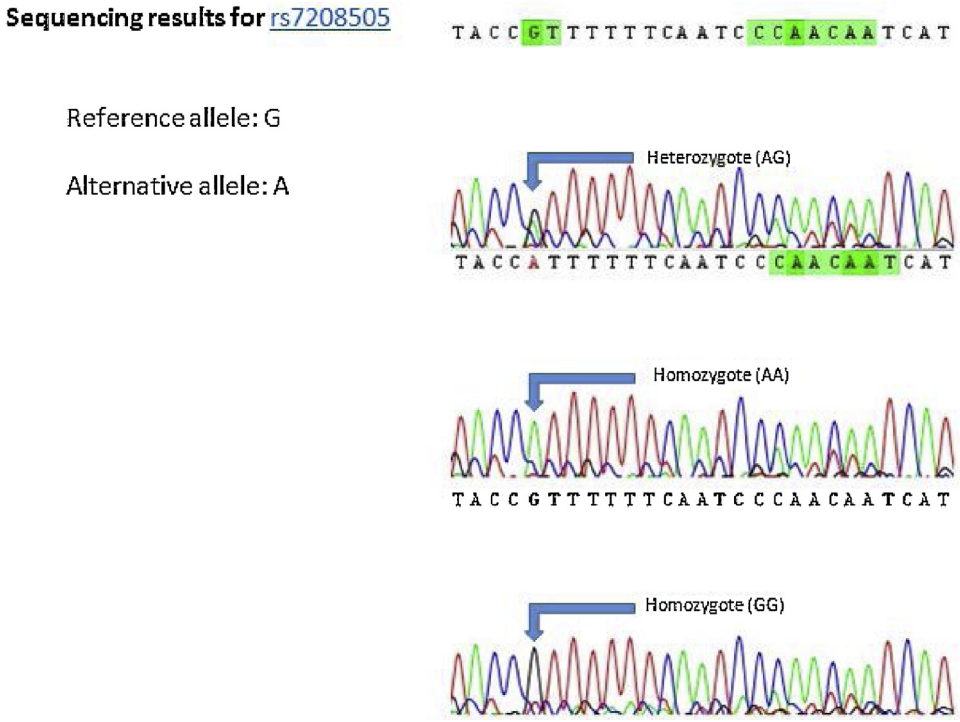

Fig. 2. Sequencing results show three person with Homozygous genotypes GG, heterozygous AG, and homozygous AA. The sequencing graph of three samples confirms the genotypes which obtained from the restriction enzyme digestion by (*TaaI*).

Table 2
Frequency of SKA2 genotypes and alleles in preterm and term subjects.

	Genotypes			Allele frequency	
	GG	GA	AA	G	A
Preterm N = 293 (%)	61 (20.8%)	171 (58.4%)	61(20.8%)	0.58	0.42
Term N = 300 (%)	93 (31%)	192 (64%)	15 (5%)	0.63	0.37
	$\chi2 = 35.06$, df = 2, p= <0.001			$\chi2 = 20.39$, df = 1, p= <0.001	

Table 3
Odds ratio and distribution of SKA2 genotypes with respect to codominant, dominant and recessive model in preterm patients after adjusting for BMI and age.

Genotype of SKA2		Preterm reference group OR (95%confidential interval)	Term reference group
Codominant	**A/A vs. G/G**	9.6 (4.5–20.2, p= <0.001) (n = 61 vs. n = 61) ($\chi2 = 35.3$, df = 1, p= <0.001)	(n = 15 vs. n = 93)
	A/G vs. G/G	1.45 (0.98–2.15, p = 0.06) (n = 171 vs. n = 61) ($\chi2 = 3.54$, df = 1, p = 0.06)	(n = 192 vs. n = 93)
Dominant	**A/A + A/G vs. G/G**	1.85 (1.26–2.7, p=<0.002) (n = 232 vs. n = 61) ($\chi2 = 10$, df = 1, p=<0.002)	(n = 207 vs. n = 93)
Recessive	**A/A vs. G/G + A/G**	5.3 (2.9–9.6, p= <0.001) (n = 61 vs. n = 232) ($\chi2 = 29.9$, df = 1, p= <0.001)	(n = 15 vs. n = 285)

Table 4
Association of SKA2 genotypes with various variables in both groups.

Variables	GG (154)	AG(363)	AA(76)	P value
Mother's age	24.66 ± 0.3	26.37 ± 0.25[a,**]	26.16 ± 0.53[b,*]	<0.001
BMI	23.39 ± 0.27	24.33 ± 0.21[a,*]	23.33 ± 0.4	0.013
Baby's weight	2726.6 ± 60.9	2584.3 ± 40.7	1823.6 ± 83.9[b,**,c,**]	<0.001
Gestational age at delivery	36.6 ± 0.27	35.8 ± 0.2[a,*]	32.4 ± 0.4[b,**,c,**]	<0.001
Apgar	8.24 ± 0.1	7.8 ± 0.07[a,*]	7.25 ± 0.15[b,**,c,**]	<0.001
Apparent anxiety	45.5 ± 1	50.4 ± 0.7[a,**]	57.26 ± 1.1[b,**,c,**]	<0.001
Latent anxiety	44.5 ± 1	50.87 ± 0.7[a,**]	58.2 ± 1.2[b,**,c,**]	<0.001
History of previous Preterm	14.9%	22%	42.1%[b,*,c,*]	<0.001
Cortisol	192.65 ± 2.5	244 ± 3.1[a,**]	343.7 ± 3[b,**,c,**]	<0.001

[a] Comparison between GG and AG.
[b] Comparison between GG and AA.
[c] Comparison between AG and AA.
[*] $p < 0.05$.
[**] $p < 0.001$.

Table 5
Comparison of cortisol levels and anxiety scores between the early and late preterm labor.

Variables	Preterm	Mean	SD	P value
Cortisol	Early	290.95	68.35	<0.001
	Late	205.05	65.39	
Latent anxiety	Early	55.80	11.15	<0.001
	Late	42.33	11.03	
Apparent anxiety	Early	55.09	10.53	<0.001
	Late	42.97	11.45	

Table 6
Comparison of SKA2 genotypes ratios (AA, AG, GG) between the early and late preterm labor.

SKA2 genotypes ratios				
	Early	AA (%)	29	<0.001
		AG (%)	55	
		GG (%)	16	
	Late	**AA (%)**	6	<0.001
		AG (%)	65	
		GG (%)	29	

Discussion

The causations of PTB remained elusive [23]. The parturition process is an uncontrollable and incurable condition because the parturition is a one-way process. Hence, the best way to control PTB is finding the predictive biomarkers in asymptomatic women [24]. We did an association of *SKA2* gene polymorphism (NC_000017.11: g.59110368 G > A) with anxiety, cortisol level and *SKA2* gene, in order to find possible clinical significance of this SNP as a predictive biomarker. So, any change in this allele (G) is considered a polymorphism. The *SKA2* gene was first discovered in liver cancer. This gene encodes a protein called SKA2 which interacts with the glucocorticoid receptor by chaperoning and transmitting it from the cytoplasm into the nucleus. Rice L et al. reported that *SKA2* reduced dexamethasone transactivation and increased the proliferation of cancer cells [18]. In the previous study, an epigenetic variation occurred at rs7208505 was introduced as a predictive biomarker for suicide. They reported that individuals with the TT genotype at rs7208505 secreted more salivary cortisol and had more thought of suicide. Also, it was shown that the methylation of the *SKA2* gene reduced the neuronal expression of *SKA2* [19]. The methylation of *SKA2* gene and its effects were also evaluated on post-traumatic stress disorder (PTSD). Reduction in *SKA2* methylation is linked to the development of PTSD; therefore, *SKA2* methylation level is suggested as a potential factor for the prediction of PTSD [25]. In another study implemented on the epigenetic variations at *SKA2* and suicide, there were significant differences between *SKA2* methylation status and clinical symptom interviews. Their findings showed no information about the clinical symptom interviews on suicide, but *SKA2* methylation status demonstrated some variances which were correlated with the risk of suicide [26].

Cortisol and CRH hormone are considered a fetal-placental clock for the timing of parturition and delivery [27,28]. There is a reverse relationship between the level of glucocorticoid hormone and the duration of pregnancy.

Hobble et al., have shown that the stress affects the PTB [12]. In their findings, some women with premature delivery had increased CRH level, but they did not have stress, obtained from the questionnaire; hence it is assumed that there may be the other causes for cortisol secretion. Based on the present findings, women with AG and AA genotypes of rs7208505 A/G polymorphism in *SKA2* gene were among those who had PTB. The current results indicated that cortisol levels in both preterm and term groups bearing the AA and AG genotypes were higher than individuals carrying the GG genotype.

Hobble et al. suggested that maternal stress affects the fetal adrenal axis and results in more cortisol production, therefore, the fetal genotype for *SKA2* gene polymorphism can play a crucial role in the production of more cortisol levels. Considering the role of the placenta and fetal genotypes in increasing cortisol level, it should be noted in the future studies.

In contrast, there is a contradictory study indicated no significant difference in serum corticotrophin-releasing levels between women with preterm birth and normal women implying that the polymorphism in this gene cannot be considered a predictive factor in PTB [29], however, the research showed that corticotrophin-releasing levels were increased close to delivery.

In a meta-analysis study comprised of 12 studies, a total of 17,304 pregnant women were investigated to find a correlation among the premature delivery, maternal anxiety, and PTB [30]. Several studies have shown that maternal stress and anxiety result in the elevation of placental CRH in plasma. There are a few theories describing the interactions between the maternal HPA axis and the fetal-placenta and define how maternal stress can pertain to PTB. On the other hand, placental CRH stimulates the pituitary-adrenal axis of both mother and fetus. CRH is related to prostaglandins and oxytocin, which are both mediators for delivery [31]. CRH helps prostaglandins be released from the placenta and fetal membranes. Thus, CRH initiates a cascade of endocrine events.

Stress and anxiety are two distinct conditions. Stress has a particular causation while the anxiety may emerge without any problems and be triggered by genetic or psychological trauma [32]. Stress has temporary mental and physical signs; however, the anxiety is a kind of stress accompanied by chronic and progressive symptoms. Moreover, the anxiety during the pregnancy has

adverse effects, such as premature labor, emotional or cognitive problems, hyperactivity, language developmental delay, and anxiety in childhood [33,34]. The Spielberg questionnaire is designed in two parts: state anxiety and trait anxiety. State anxiety is defined as an unpleasant emotional arousal in the face of threatening demands or dangers; therefore, it is an acute and variable emotion of anxiety. Trait anxiety refers to the permanent pattern of anxiety and pertains a relatively stable individual difference in anxiety-proneness.

Since there is a correlation between state and trait anxiety, according to these findings, the degree of state and trait anxiety in women bearing the AA and AG genotypes was higher than those with the GG genotype in both preterm and term groups.

There is evidence about the anxiety and depression during the pregnancy showing no association with the obstetric complications [35]. Also, a systematic review study implicated that the anxiety and depression during the pregnancy are associated with spontaneous PTB but cannot be medically specific for PTB [34].

The Apgar score is established for the diagnosis of asphyxia and the best test for the prediction of neonatal death. Apgar score is related to gestational age and the degree of infant maturity. So infants with PTB have lower Apgar scores. Apgar score is used to evaluate the physical condition of infants shortly after delivery [36,37]. We found that the mean of all findings such as increased anxiety, increased blood cortisol, decreased last menstrual period, decreased Apgar score, and decreased newborn birth-weight were intense in individuals carrying the AA genotype compared with individuals with the AG genotype, however, the findings in women with the AG genotype was more intense in comparison to those with the GG genotype. In the current assessment, the Apgar score of infants with mothers possessed the AA genotype was lower than neonates with mothers carrying the genotypes of AG and GG. It appears that mothers with the AA genotype are more prone to develop PTB and deliver neonates with lower weight compared with the other genotypes.

The current study has the following strengths: the good sample size, the evaluation of the *SKA2* gene as a novel biomarker for latent anxiety and preterm birth prediction, sampling is carried out in the capital city in Iran and in 4 different regions. Therefore, all segments of the society with an average income participate in it. Furthermore, the limitations of the research were failure to follow the participants in subsequent pregnancies, as well as the lack of evaluation of *SKA2* gene expression.

Based on the obtained results, we propose further investigations are warranted to illuminate the role of *SKA2* gene in the association of this gene with PTB. In conclusion, the prognosis of women with the AA genotype is poorer than the others. Therefore, the allele A may be taken into account as a biomarker for the risk of PTB. We hope that with further investigation on the implication of these findings could be assessed the gene of *SKA2* for preterm birth prediction in pregnant women and even before pregnancy.

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Ethics approval

Ethics approval was obtained from the research ethics committee of Gorgan

University of Medical Sciences (code: IR.goums.rec.1394.79) in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all participants included in the study.

Conflict of interest

The authors have no conflict of interest.

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