The association of rs7041 polymorphism with infertility and recurrent pregnancy loss in Iranian women with polycystic ovary syndrome

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Abstract

Background

Polycystic ovarian syndrome (PCOS) stands as the most prevalent endocrine disorder among women of reproductive age. Vitamin D binding protein (VDBP) is a polymorphic protein with a crucial role in vitamin D metabolism. This study aimed to examine the association of rs7041 polymorphism with PCOS, as well as infertility and recurrent pregnancy loss (RPL) in PCOS patients.

Methods

A total of 200 women diagnosed with PCOS, including 100 infertile and 100 with a history of recurrent pregnancy loss, and 100 fertile women were enrolled in this study. Blood samples were taken from these individuals, and their biochemical and hormonal profiles were assessed. Genotyping for the rs7041 polymorphism was carried out in the study population using the PCR-RFLP method.

Results

Genotype analysis showed that the GT genotype and the T allele of the rs7041 polymorphism were associated with an elevated risk of PCOS (OR: 2.8 95% CI [1.48–5.33], p = 0.002, and OR: 2.27 95% CI [1.2–4.28], p = 0.01, respectively). Furthermore, the GT genotype and T allele of the rs7041 polymorphism were associated with an increased risk of infertility in PCOS women (OR: 40.55 95% CI [5.34–307.7], p < 0.0001, and OR: 30.667 95% CI [4.05-232.19], p = 0.001, respectively). Conversely, no significant association was observed between genotypes and RPL in women with PCOS. LH levels were significantly higher in individuals with the T allele compared to those with the G allele.

Conclusion

The study results highlight a significant correlation between the rs7041 polymorphism in the VDBP gene and the risk of PCOS and infertility.

1. Introduction

Polycystic ovarian syndrome (PCOS) is the most prevalent endocrine disorder among women of reproductive age, affecting an estimated 5–7% of them (1). This syndrome is marked with various manifestations, including chronic anovulation or oligo-ovulation and irregular biochemical and metabolic alterations, chronic inflammation, obesity and hypertension (2, 3). PCOS patients commonly exhibit insulin resistance (IR) leading to offsetting hyperinsulinemia and higher glucose levels, significantly elevating the risk of developing type 2 diabetes (4). The diagnosis of PCOS requires the presence of two out of three key criteria called Rotterdam criteria: clinical and/or biochemical hyperandrogenism, oligomenorrhea or amenorrhea and a polycystic morphology in one or both ovaries (5). Although the exact etiology and pathogenesis of PCOS remain elusive, there is evidence suggesting that PCOS is linked to genetic, epigenetic, and environmental factors (3, 6).

Infertility and recurrent pregnancy loss (RPL) commonly appear as complications in women with PCOS (7, 8). The prevalence of infertility in PCOS women is 70–80% (9). Various lifestyle factors such as diet, smoking or obesity can lead to infertility disorders. Failure to ovulate can be one of the causes of infertility. Almost 70% of cases of ovulatory

dysfunction in women are due to PCOS (7). It is noteworthy that 36–82% of RPL cases occur in women with PCOS (8). It is suggested that insulin resistance serves as the link between PCOS and RPL (10).

Over the past decade, numerous studies have focused on vitamin D, revealing its insufficiency in women with PCOS; however, further investigation is still needed in this area (11). Various studies have demonstrated that vitamin D impacts glucose and insulin metabolism (12) and plays a consistent role in insulin sensitivity (13). A deficiency in vitamin D has been proposed as the potential link between excessing IR and metabolic dysfunction in PCOS (11, 14).

Vitamin D binding protein (VDBP), also known as group-specific component (GC) is encoded by a gene located in the long arm of chromosome 4 (4q12-q13) comprising 13 exons and 12 introns (15) which is one of the most polymorphic genes in the human genome (16). VDBP serves as the primary transporter of vitamin D and its metabolites in serum (15). In addition to its role in carrying vitamin D metabolites, this multifunctional protein is involved in various biological processes, including inflammatory response, macrophage activation, scavenging, fatty acid transport, osteoclast stimulation and differentiation (17). VDBP significantly influences vitamin D metabolism and status (18). A well-known single-nucleotide polymorphism (SNP) in exon 11 of VDBP gene named rs7041 is believed to have a substantial impact on low levels of 25-hydroxyvitamin D in diverse populations (19). Until now the association of rs7041 polymorphism with some metabolic disorders such as diabetes, obesity, pulmonary obstruction, asthma, Parkinson`s disease, and MS was investigated by some studies (20–26). However, a few studies have been carried out on the association between this polymorphism and PCOS (27, 28). Notably, no prior study has investigated the link between rs7041 genotypes and infertility and RPL in women with PCOS. The current study was conducted to investigate the association of rs7041 polymorphism with PCOS, as well as infertility and RPL among Iranian women with PCOS.

2. Materials and methods

2.1. Study population

This case-control study involved 300 women aged 15 to 35 years. Among them, 200 women were diagnosed with PCOS comprising 100 infertile patients and 100 individuals with a history of recurrent pregnancy loss. The remaining 100 participants were healthy female volunteers, matching the age criteria, exhibiting regular menstrual cycles (26–34 days), normal ovarian morphology, and normal total testosterone levels (to exclude hyperandrogenism). Infertile women were those who were unable to achieve pregnancy despite engaging in regular unprotected sexual intercourse for 12 months or longer (29). Recurrent pregnancy loss is identified by the presence of a minimum of two consecutive pregnancy losses before the 20th week (30). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1975 Helsinki declaration as revised in 2008. The present research was approved by the Ethics Committee of Ibn Sina Infertility Center. Informed consent was obtained by means of a written informed consent form prior to beginning the study. A comprehensive medical history was collected from all participants, and clinical, biochemical, and radiological examinations were conducted to identify PCOS symptoms and signs. PCOS patients were diagnosed according to the Rotterdam criteria (5). Individuals with Cushing’s syndrome, thyroid dysfunction, androgen-secreting tumors, or those who had undergone hormonal treatment or used drugs such as steroids that could induce insulin resistance for at least 6 months prior to the evaluation were excluded from the study. Additionally, participants with parathyroid disorders, chronic kidney disease or other chronic illnesses, as well as those taking calcium and vitamin D supplements were also excluded.

2.2. Laboratory analysis

Venous blood was collected under strict aseptic conditions after a 12-hour overnight fast. The samples were processed immediately and stored at − 80°C until further analysis was required. All biochemical and hormonal parameters were measured according as reported previously (31–33).

2.3 Genotyping

The genotyping of the *VDBP* SNP rs7041 (*HaeIII*; G > T) was performed using the restriction fragment length polymorphism (RFLP) technique. Common primers were utilized to amplify a 483-bp fragment for *HaeIII*: forward 5'-AAATAATGAGCAAATGAAAGAAGAC-3' and reverse 5'-CAATAACAGCAAAGAAATGAGTAGA-3. The PCR process included an initial denaturation step at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 90 s. The reaction concluded with a final extension step at 72°C for 5 min. The rs7041 PCR product underwent digestion with *HaeIII* restriction enzyme at 37°C for 2 hours and was subsequently separated on a 2% agarose gel. PCR products carrying the G allele were cleaved by the enzyme, resulting in two distinct bands measuring 186 bp and 297 bp. On the other hand, PCR products carrying the T allele, lacking the *HaeIII* restriction site, resulted in a single 483 bp product.

2.4 Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 25.0 (IBM 137 Corp., Armonk, NY, USA). The normality of the data was assessed using the Shapiro-Wilk test. Normal data were analyzed using the independent t-test and ANOVA tests, while the Mann-Whitney and Kruskal-Wallis tests were employed for non-normally distributed data. Post hoc comparisons were conducted using the Bonferroni method. For categorical data, group comparisons were made using the chi-squared test of Fisher`s exact test (for small sample size). The relationship between the frequency of genotypes/alleles of rs7041 with PCOS was investigated using logistic regression, and their association with infertility and RPL was assessed using multinominal regression. Statistical significance was defined as two-tailed P values < 0.05.

3. Results

The clinical characteristics of the study population are presented in Table 1. Significant differences were observed in age, insulin, HOMA-IR, HDL, FT, and FSH levels when comparing PCOS and related subgroups with the non-PCOS group. However, no significant differences were found in BMI, FBS, TC, LDL, and LH levels between cases and controls. In the PCOS-RPL subgroup, the TG level (136.71 ± 52.04) was significantly higher than in the non-PCOS (118.70 ± 38.35) and PCOS-infertile subgroup (118.51 ± 57.33) (p = 0.01). Additionally, the PCOS-infertile subgroup showed a significantly increased level of LH (7.81 ± 4.64) compared to the PCOS-RPL subgroup (5.6 ± 2.61) (p < 0.0001).

Table 1
Clinical characteristics of PCOS and non-PCOS groups.

	non-PCOS	PCOS	P-value†	PCOS-infertile	PCOS-RPL	P-value‡
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Age (year)	33.37 ± 4.61 [a,b]	30.24 ± 4.59	< 0.0001	30.3 ± 4.82[a]	30.17 ± 4.36[b]	< 0.0001
BMI (kg/m^2)	25.73 ± 4.30	26.74 ± 4.76	0.08	27.02 ± 4.51	26.46 ± 5	0.1
FBS (mg/dL)	89 (82,96.5)	87 (83,93.5)	0.1	87.75 (83,93)	87 (82,94)	0.2
Insulin (µU/mL)	2.88 (1.96,4.3) [a,b]	6.4 (2.85,8.1)	< 0.0001	5.1 (3.1,8.25)[a]	4.39 (2.51,7.92) [b]	< 0.0001
HOMA-IR	0.59 (0.44,1.08) [a,b]	1.02 (0.57,1.77)	< 0.0001	1.04(0.65,1.75)[a]	1(0.54,1.8)[b]	< 0.0001
TG (mg/dL)	118.70 ± 38.35[b]	127.65 ± 55.36	0.1	118.51 ± 57.33[c]	136.71 ± 52.04 [b,c]	0.01
TC (mg/dL)	168.62 ± 38.88	171.21 ± 35.95	0.5	169.91 ± 32.78	172.5 ± 38.96	0.7
LDL (mg/dL)	99.99 ± 29.42	97.5 ± 29.46	0.5	97.05 ± 27.73	97.95 ± 31.21	0.7
HDL (mg/dL)	46 (41,52) [a,b]	43 (37.75,49)	0.006	43 (37,50) [a]	44 (38, 48) [b]	0.02
Free_T (pg/mL)	1.48 (1.27,1.79)[&] [a,b]	3.25 (2.52, 3.96)	< 0.0001	3.1 (2.32, 3.7)[a]	3.29 (2.73,4.15)[b]	< 0.0001
LH (IU/L)	6.17 ± 2.31[&]	6.71 ± 3.92	0.9	7.81 ± 4.64[c]	5.6 ± 2.61[c]	< 0.0001
FSH (IU/L)	8.15 (6.39,9.6) [a,b]	5.9 (4.35,7.42)	< 0.0001	6.02 (4.75,7.2) [a]	5.9 (4.24,7.61) [b]	< 0.0001

†Independent t test for comparison between PCOS and non-PCOS groups

‡One-way ANOVA with Bonferroni post hoc test for comparison among PCOS-infertile, PCOS-RPL and non-PCOS

[&] LH and Free-T levels in 45 individuals have been measured in the non-PCOS group.

Similar uppercase letters indicate significant differences among pairwise groups with Bonferroni's approach.

PCOS, Poly Cystic Ovary Syndrome; BMI, Body Mass Index; FBS, Fasting Blood Sugar; TG, Triglyceride; TC, Total Cholesterol; LDL-C, Low Density Lipoprotein Cholesterol; HDL-C, High Density Lipoprotein Cholesterol; T, Testosterone; LH: luteinizing hormone; FSH: follicle-stimulating hormone.

Genotyping analysis of the VDBP rs7041 polymorphism was performed using the PCR-RFLP method (Fig. 1). The results detailing the genotype and allele frequencies of VDBP genotypes are outlined in Table 2. Genotypic and allelic frequencies of the rs7041 polymorphism were consistent with Hardy-Weinberg equilibrium ($P > 0.05$).

Table 2
Genotypic and allelic distribution of VDBP genotypes in PCOS and Non-PCOS groups

genotype/ allele	Non-PCOS N (%)	PCOS N (%)	PCOS-infertile N (%)	PCOS-RPL N (%)	PCOS vs Non- PCOS		PCOS-infertile Vs. Non- PCOS		PCOS-RPL Vs. Non-PCOS	
					OR (CI)	P-value	OR (CI)	P-value	OR (CI)	P-value
GG	24 24.2%	24 11.8%	1 1%	23 22.8%	ref	-	ref	-	ref	
GT	58 58.6%	163 80.3%	98 96.1%	65 64.4%	2.8 (1.48–5.33)	0.002 [a]	40.55 (5.34–307.7)	< 0.0001 [b]	1.16 (0.59–2.29)	0.6 [b]
TT	17 17.2%	16 7.9%	3 2.9%	13 12.9%	0.94 (0.38–2.28)	0.8 [a]	4.23 (0.4-44.27)	0.2 [b]	0.79 (0.31-2)	0.6 [b]
G	106 53.53%	211 51.97%	100 49.01%	111 54.95%	ref	-	ref		ref	
T	92 46.47%	195 48.03%	104 50.99%	91 45.05%	2.27 (1.2–4.28)	0.01 [a]	30.66 (4.05-232.19)	0.001 [b]	1.04 (0.53–2.01)	0.9 [b]

[a] Results of binary logistic regression.

[b] Results of multinomial logistic regression.

Genotype analysis underscored the prevalence of the GT genotype across all examined groups (Table 2). A significant difference in the infrequency of genotypes and alleles was observed between PCOS and non-PCOS groups. Interestingly, the presence of GT genotypes increased the risk of PCOS (OR: 2.933, 95% CI [1.538–5.593], P = 0.001) when compared to the GG genotype (Table 2). The presence of the T allele increased the likelihood of PCOS (OR: 2.274, 95%, CI [1.208–4.281], P = 0.011) compared to those with the G allele (Table 2). Additionally, GT genotypes and the T allele were associated with an increased risk of infertility among PCOS patients (OR: 40.552, 95% CI [5.344-307.704], P = 0.000, and OR: 30.667, 95% CI [4.050-232.190], P = 0.001, respectively). However, PCOS-RPL subgroup exhibited no significant differences in genotypic and allelic frequencies of rs7041 when compared to non-PCOS (Table 2).

The relationship between biochemical factors and genotypes is specified in Table 3. According to the obtained results, LH level was significantly higher in individuals with the TT + GT genotype (6.83 ± 3.82) than in those with the GG genotype (5.31 ± 2.30) (p = 0.002). Moreover, a significant correlation was observed between the GT + TT genotype of rs7041 and FBS levels (P = 0.018) However, no significant associations were found between other clinical characteristics and the rs7041 polymorphism.

Table 3
Association of Clinical characteristics with genotypic frequencies for VDBP genotypes.

Biochemical/hormonal parameters	GG	TT + TG	P-value
Age (years)	32 (28.25,35.75)	31 (27, 34)	0.3
BMI (kg/m^2)	26.75 (23.55,29.28)	25.94 (23.41,29.12)	0.7
FBS (mg/dL)	92 (85.75,97)	87 (82,93.75)	0.018
Insulin (µU/mL)	3.19 (2.27,6.27)	3.95 (2.39,6.93)	0.3
HOMA-IR	0.78 (0.5,1.43)	0.86 (0.51,1.52)	0.7
TG (mg/dL)	130.19 ± 49.93	123.83 ± 50.88	0.4 [a]
TC (mg/dL)	169.43 ± 41.08	170.57 ± 36.11	0.8 [a]
LDL (mg/dL)	95 (78.5,119.5)	96 (78,115)	0.9
HDL (mg/dL)	44 (39,50.25)	44 (39,50)	1.0
Free-T (pg/mL)	2.52 (1.39,3.88)	2.63 (1.79,3.69)	0.4
LH (IU/L)	5.31 ± 2.30	6.83 ± 3.82	0.002 [a]
FSH (IU/L)	6.81 (5.41,8.9)	6.1 (4.52,7.79)	0.1

[a]: Independent t test, the other tests are Mann-Whitney.

4. Discussion

PCOS is a multifactorial disorder that has been linked to genetic and environmental factors, including obesity and insulin resistance. A growing body of evidence suggests that PCOS has a genetic background and various biochemical pathways as well as their related genes in these pathways –including those involved in steroidogenesis, lipid metabolism, gonadotropin and sex hormones metabolism and many others- have crucial roles in the pathological development of PCOS (3, 6). Vitamin D is recognized for its vital roles in the metabolic and endocrine pathways linked to PCOS, including the insulin signaling pathway (34) and the production of sex hormones (35).

The GC gene contains instructions for a polymorphic plasma protein called GC, also referred to as group-specific component or vitamin D binding protein (VDBP). This protein, synthesized in the liver, serves as a carrier for vitamin D and its metabolites. The rs7041 polymorphism is one of the genetic variations associated with the VDBP gene, and various studies have investigated its correlation with several diseases, including diabetes (20, 36),obesity (21, 37), pulmonary obstruction (22, 38), asthma (23, 39), Parkinson's disease (25, 40), and MS (26). The results of these studies suggest that this polymorphism significantly impacts various aspects of diseases. To date, only a limited number of studies have addressed the association of the rs7041 polymorphism with PCOS (27, 28).

The findings of this study reveal a significant association between the GT genotype and PCOS. Analysis of the alleles related to this polymorphism indicates that the T allele significantly increases the risk of PCOS. Vitamin D deficiency and its metabolism disruption play a crucial role in the pathogenesis of PCOS (27, 28). In this context, studies have indicated the beneficial effects of vitamin D supplementation on insulin secretion and serum lipid profiles in PCOS patients (41, 42). The serum level of vitamin D is influenced by various factors, with one of the main factors being VDBP. Numerous studies have shown a significant association between the rs7041 polymorphism and serum

25(OH)D levels (18, 43). In this regard, some studies have reported that the T allele in this polymorphism is associated with vitamin D deficiency (44, 45). Therefore, the T allele of rs7041 polymorphism may be involved in the pathogenesis and progression of PCOS in women through decreased vitamin D levels. In this regard, a study conducted in India on 50 PCOS women showed that the GT genotype of the rs7041 polymorphism increases the risk of PCOS in women with vitamin D deficiency (27). However, two other studies did not report any association between genotypes of this polymorphism and the risk of PCOS (28, 46). Besides, in a study by Santos and colleagues (2017), it was shown that PCOS women with the TT genotype had a higher prevalence of metabolic syndrome, indicating a significant association between this genotype and metabolic syndrome (18). Therefore, the T allele of rs7041 polymorphism may play a role in the development of metabolic symptoms and potentially contribute to the pathogenesis of PCOS through this pathway.

This study, for the first time, investigated the association of the rs7041 polymorphism in the VDBP gene with infertility and RPL in women with PCOS. According to the results, the GT genotype and T allele show a significant association with infertility in women with PCOS. However, no association was observed between genotypes and alleles with RPL in women with PCOS. The differing results in the two subgroups suggest that different pathological pathways may be involved in infertility and RPL in PCOS patients, and this polymorphism may only affect specific metabolic pathways related to infertility.

PCOS is associated with a significantly higher risk of infertility, with a prevalence ranging from 70–80% among affected women (9). In this regard, studies have demonstrated that an elevated level of LH in women with PCOS is linked to infertility, as increased LH levels can disrupt ovulation (47). The present study revealed that women with the TT + GT genotype have higher LH levels compared to women with the GG genotype. Therefore, it can be suggested that the T allele may increase the risk of infertility in women with PCOS by elevating LH levels.

Moreover, individuals with the GG genotype were found to have higher FBS levels compared to those with the GT and TT genotypes. Consistent with these findings, Zhao et al. (2020) also indicated that individuals with the G allele have higher FBS levels than those with the T allele (20). Thus, it is plausible that individuals with the G allele may have a higher risk of insulin resistance and type 2 diabetes. However, some studies have reported conflicting results or found different outcomes. Kiani and colleagues (2019) reported that cardiovascular patients with the TT genotype have higher FBS levels (48). Given these contradictory results, further investigation in larger populations and different ethnicities is necessary.

All clinical features, except BMI, FBS, TC, and LDL showed significant differences between PCOS and its associated subgroups when compared to the non-PCOS group. These results have also been detailed in our earlier publications (31–33). No significant difference in BMI, FBS, TC, and LDL was observed in some studies (18, 28). However, certain studies reported significant variations in the level of FBS and BMI between PCOS and non-PCOS subjects (46, 49). These differences could stem from factors such as race, age, and sample size.

The present study has some limitations that should be taken into consideration. This research was conducted on a relatively small population of women. Therefore, to confirm the results of this study, further research with a larger sample size is needed. Additionally, this study was exclusively carried out on Iranian women and does not encompass racial and environmental diversity. Future studies should focus on larger and more diverse populations to validate the findings of this study. Another limitation of this study is not having measured serum vitamin D levels.

Conclusion

The frequency of rs7041 genotypes in women with PCOS, as well as its subgroups, significantly differed from the control group. Additionally, this study revealed an association between the GT genotype and T allele with the risk of developing PCOS and infertility in these women. Considering the elevated LH levels in individuals with the GT + TT genotype, it seems that this polymorphism increases the risk of both PCOS and infertility by influencing LH levels. However, further studies are necessary to comprehend the precise mechanism underlying the relationship between this polymorphism and PCOS, as well as infertility associated with PCOS.

Abbreviations

BMI (Body Mass Index); FBG (Fasting Blood Glucose); FSH (Follicle-Stimulating Hormone); FT (Free Testosterone); HDL-C (High-Density Lipoprotein Cholesterol); HOMA-IR (Homeostasis Model Assessment of IR); LDL-C (Low-Density Lipoprotein Cholesterol); LH (Luteinizing Hormone); PCOS (Polycystic Ovary Syndrome); TC (Total Cholesterol); VDBP (Vitamin D Binding Protein)

Declarations

Funding

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Competing Interests

The authors declare that they have no competing interests.

Authors' contributions

HH: Writing original draft, Investigation, Experiment; AK: Conceptualization and Supervision;
AV: Conceptualization and Statistical Analysis. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1975 Helsinki declaration as revised in 2008. The present research was approved by the Ethics Committee of Ibn Sina Infertility Center. Informed consent was obtained by means of a written informed consent form prior to beginning the study.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Figures

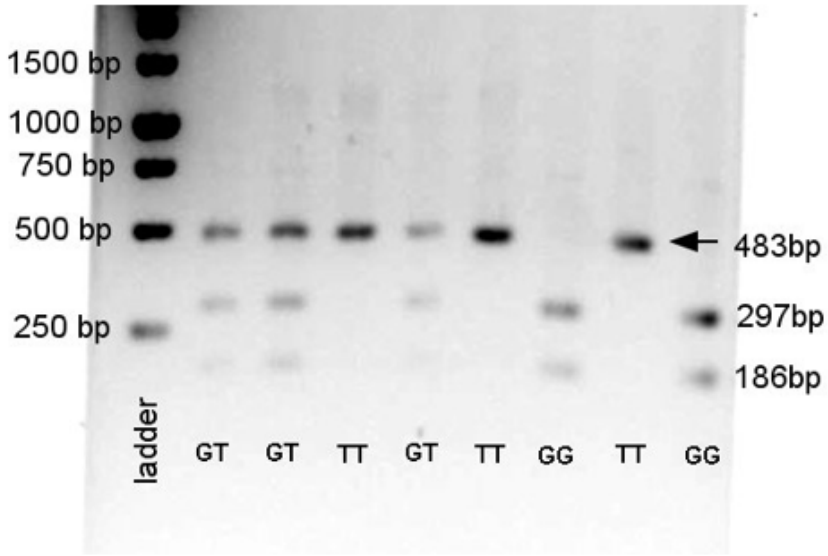

Figure 1

Genotype analysis of the VDBP rs7041 polymorphism using PCR-RFLP method. Homozygote TT (483 bp), homozygote GG (297 and 186 bp), and heterozygote GT (483, 297 and 186 bp) were visualized using the Syber safe stain on agarose 2%.