

# Strong association between angiotensin I-converting enzyme insertion/deletion polymorphism and unexplained recurrent spontaneous abortion of Sudanese women: a case-control study

Asaad M.A. Babker,<sup>1</sup> Hanan Khalid Fadul Ahmed,<sup>2</sup> Rania Saad Suliman,<sup>3</sup> Ahmed Luay Osman,<sup>1</sup> Ayman Hussien Alfeel,<sup>1</sup> Praveen Kumar Kandakurti,<sup>4</sup> Salaheldeen G Elzaki<sup>2</sup>

<sup>1</sup>Department of Medical Laboratory Sciences, College of Health Sciences, Gulf Medical University, Ajman, United Arab Emirates; <sup>2</sup>Department of Epidemiology, Tropical Medicine Research Institute, National Center for Research, Khartoum, Sudan; <sup>3</sup>Department of Clinical Laboratory Sciences, Prince Sultan Military College for Health Sciences, Dhahran, Saudi Arabia; <sup>4</sup>College of Health Sciences, Gulf Medical University, Ajman, United Arab Emirates

## ABSTRACT

This study investigated the link between angiotensin I-converting enzyme insertion/deletion (ACE I/D) polymorphism and unexplained spontaneous abortion. This retrospective analytical case-control was conducted at the Omdurman Maternity Hospital in Sudan. The current study contained 230 individuals, including 119 cases (women who had at least three abortions) of unknown cause and 119 controls (healthy women who had at least two full-term deliveries without spontaneous abortion). Patients and controls were provided five ml of ethylenediaminetetraacetic acid blood and answered questionnaires about their demographics, personal lives, and family histories. ACE I/D polymorphisms were assessed using a conventional polymerase chain reaction approach after total genomic DNA was isolated from blood leukocytes using the GF-1 blood DNA extraction kit. Data was analyzed using the Statistical Package for the Social Sciences version 24. ACE I/D polymorphism is strongly linked to unexplained spontaneous abortion, and women with the I/D and D/D genotypes are more likely to have it than those with the I/I genotype. The current study reveals that ACEI/D polymorphism increases pregnancy problems. Sudanese women may have spontaneous abortions due to the ACE I/D polymorphism.

Correspondence: Asaad M.A. Babker, Department of Medical Laboratory Sciences, College of Health Sciences, Gulf Medical University, Ajman, United Arab Emirates.  
E-mail: azad.88@hotmail.com

Key words: spontaneous abortion; polymorphism; pregnancy loss; angiotensin converting enzyme.

Acknowledgments: the authors want to thank everyone who took part in the study, with special appreciation to the personnel at the Omdurman Maternity Hospital in Sudan.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: none.

Ethical approval and consent to participate: the ethical considerations and conformity to individuals in this study were considered by approving of ethical committees of Omdurman Maternity Hospital in Sudan, in addition to using documented agreement within the questionnaire signed by the participants.

Availability of data and material: data and materials are available by the authors.

Informed consent: each participant provided informed consent after receiving detailed information about the study's goals, the confidentiality of their data, and the intended use of the information solely for the study's purposes.

Received: 13 March 2024.

Accepted: 15 March 2024.

Publisher's note: all claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

©Copyright: the Author(s), 2024  
Licensee PAGEPress, Italy  
Italian Journal of Medicine 2024; 18:1717  
doi:10.4081/ijm.2024.1717

This work is licensed under a Creative Commons Attribution NonCommercial 4.0 License (CC BY-NC 4.0).

## Introduction

Recurrent spontaneous abortion (RSA) is defined as two or more consecutive pregnancy losses. A clinically confirmed pregnancy loss occurs when it spontaneously terminates before 20 weeks and affects 1-5% of fertile women.<sup>1</sup> RSA is a complex disorder with a poorly known pathogenesis and the treatment of RSA is a challenge both for clinicians and patients.<sup>2</sup> Several factors have been suggested to contribute to RSA pathogenesis, including chromosomal anomalies, anatomical, endocrine, immunological, and infectious disorders, are thought to affect pregnancy.<sup>3,4</sup>

The renin-angiotensin-aldosterone system (RAAS) is a crucial pathway for maintaining homeostasis, regulating blood pressure, and managing water balance in the body. It plays a significant role in controlling the fibrinolytic pathway, directly or indirectly impacting blood pressure regulation. This interaction between RAAS and fibrinolysis is associated with conditions such as pulmonary embolism (PE), thrombophilia, recurrent pregnancy loss (RPL), and infertility.<sup>5</sup> Although the exact mechanisms of this interaction during

pregnancy are not fully understood, this comprehensive review aims to shed light on the role and interaction of RAAS and fibrinolytic pathways in pregnancy complications. The review also delves into how this interaction can lead to pregnancy complications and ultimately infertility, as well as explores common markers linking RAAS and fibrinolysis in the development of thrombophilia, PE, and RPL.<sup>6</sup>

The most prevalent type of one of multiple angiotensin I-converting enzyme (ACE) polymorphisms is an insertion/deletion (I/D) of a 278 bp fragment in intron 16 of the gene, which controls the quantity of the enzyme. Levels of ACE enzyme circulation are highly associated with ACE I/D polymorphism. It was also discovered that those with the D/D genotype had almost doubled the average amount of ACE plasma as those with the I/I genotype. On the other hand, the average amount of enzyme circulation among people with the I/D genotype is average.<sup>7</sup>

A well-studied mutation in the ACE gene, the ACE I/D polymorphism involves the insertion or deletion of a particular DNA segment. This hereditary variable accounts for about half of the variance in ACE levels and has a substantial impact on blood ACE levels. Preeclampsia, intrauterine growth restriction, and recurrent spontaneous miscarriages are among the pregnancy issues that have been associated with the ACE I/D polymorphism, which is also strongly associated with female infertility. Impairment of placentation is a common component across these problems.<sup>6</sup> Critical to the onset of thrombophilia, the D/D genotype also affects inflammation and fibrinolytic system control. This genetic variant has also been linked to an increased risk of endometrial cancers, and other studies have linked the ACE gene D/D genotype to an increased risk of preeclampsia. Another indicator of insulin resistance, acanthosis, is strongly connected with ACE I/D polymorphism, which has also been associated with an increased risk of polycystic ovarian syndrome in young women.<sup>5,8</sup>

Several studies have been carried out to investigate the causes of recurrent pregnancy loss. Additionally, these studies aim to provide guidance on incorporating genetic testing to correlate with RSA in Sudanese women.<sup>9,10</sup> The aim of our study was to investigate the relationship between the ACE I/D polymorphisms and unexplained RSA in Sudanese women.

---

## Materials and Methods

The number of participants in this case-control research was limited to 232 due to financial constraints; the cases consisted of 119 women diagnosed with RSA, while the controls consisted of 113 healthy normal women. The research was conducted at Sudan's Omdurman Medical Hospital between 2019 and 2020. The women in the case group were all from Sudan and had a minimum of three unsatisfactory pregnancy outcomes. They were all between the ages of 25 and 45 and had visited Omdurman Maternity Hospital in Omdurman, Sudan. The women in the control group were randomly assigned to one of the hospitals, matched by age (25 to 45), had a history of at least two healthy pregnancies, and were free from recurrent miscarriages (RM) or other complications. The research only included women who had a string of three or more RPL outcomes without knowing what caused them. Women who were known to have a history of vascular thrombotic illness, fetal congenital deformities, fetal chromosomal

anomalies, uterine abnormalities, or a known cause for the abortion were not included in the research.

Before any samples were taken, each lady had to sign a permission form, which was authorized by the administration of the Omdurman Maternity Hospital as well as the professors of the College of Graduate Studies and Scientific Research at the Sudan University of Science and Technology.

Information on the patients' and controls' ages, medical histories, family histories, and obstetric histories was gathered using a standardized questionnaire. Each subject was interviewed and given written and verbal consent before five milliliters of venous blood was drawn and deposited into a designated container. For each sample, we noted the date of collection, the time of collection, the patient's name, and the medical record number.

## Molecular technique

### DNA extraction

Genomic DNA can be efficiently and quickly extracted from up to 200 l of whole blood using the GF-1 Blood DNA Extraction Kit. A total blood volume of 200 l was injected into the 1.5 ml microcentrifuge tube. After adding 200 ml of lysis buffer, and mixing it thoroughly with a pulsed vortex, it was incubated at 65°C for 10 min. After that, 20 ml of proteinase K were added. Due to the requirement for RNA-free DNA, 20 l of RNase A (DNase-Free, 20 mg/ml) was added. Ten minutes of 37°C mixing and incubation. 200 l of 100% ethanol were quickly added and stirred to form a homogeneous solution. The sample was added to a column that had been assembled in a spotless collection tube. After 1 minute of 5,000 x g centrifugation, the flow-through was removed. The flow-through was discarded after one minute of centrifuging the column at 5,000 x g while it was being cleaned with 500 l of Wash Buffer 1. The column was washed with 500 l of Wash Buffer 2 and the flow through was discarded while being centrifuged at 5,000 x g for one minute. After that, the column was put into a clean 1.5 ml microcentrifuge tube, washed once more with 500 l of Wash Buffer 2, centrifuged at maximum speed for three minutes, and then 100 l of hot Elution Buffer were injected right into the column membrane and allowed to stand for two minutes. Centrifugation was used to elute the DNA for 1 minute at 5,000 x g. Until polymerase chain reaction (PCR) analysis, DNA was stored in a -20°C freezer.

### DNA quantification

By measuring the absorbance at 260 nm spectrophotometer using Gene Quant, extracted genomic DNA was measured spectrophotometrically (Amersham BioSciences, UK).

### Molecular determination of angiotensin I-converting enzyme insertion/deletion genotyping

ACE I/D was genotyped using PCR-based DNA amplification primers flanking a specific region that was previously disclosed 20. The PCR reaction was run in a final volume of 25 l, which also contained 5 l of genomic DNA, 4 l of Green master mix (5X), and 0.4 pmol of each forward and reversed primer per sample. The DNA was amplified and first denatured at 94°C for 5 min, then heated for 35 cy-

cles at 94°C for 1 min, 58.5°C for 90 min, and 65°C for 4 min, with a final extension at 72°C for 7 min (Bio-RAD, Peltier thermal cycler). The PCR products were then electrophoresed for 45 minutes at 100 volts in 1.5% agarose solution in 1xTBE buffer, stained with 0.5 g Ethidium bromide, and visualized using a UV transilluminator. The PCR results were 490 bp for the II genotype, 190 bp for the D/D genotype, and 490,190 bp for the ID genotype.

## Statistical analysis

Data entry and analysis were performed using the Statistical Package for the Social Sciences application (version: 24.0). The prevalence of ACE I/D polymorphisms among patients and controls was compared using data analysis utilizing the Chi-square test (the test considered significant when P-value 0.05). The study population's demographic numbers of statistics were all given in the text as means. The strength of the link between the determinant and the result was determined using categorical frequencies (%) percentage.

## Results

Out of 232 women, 30 (representing instances) had a history of three or more recurrent fetal loss occurrences (abortion, miscarriage, or stillbirth), and their mean age SD was 31.3±5.9 (range 20-45). The 113 healthy women who made up the control group had a mean age of 30.3±5.4. Of patients,

30 (25.2) had four or more abortions whereas 89 (74.8) had fewer than four (Table 1).

The D/D genotype was more prevalent in patients 94 (79%) than in controls 69 (61.1%) and the difference was highly significant (P-value<0.001). Whereas the D/I polymorphisms were 18 (15.1%) in the patients and 33 (29.2%) in controls. and the normal homozygotes I/I polymorphism in patients 7 (5.9%) and 11 (9.7%) in controls. Concerning D allele frequency, it was significantly more prevalent among RM patients 206 (86.6%) than in controls 171 (75.7%), whereas the I was 32 (13.4%) and 55 (24.3%) in patients and controls respectively showing a significant difference (P-value<0.001) (Table 2). Table 3 shows the relationship between times of abortion with ACE I/D polymorphisms. ACE polymorphisms D/D 77 (78.6%) in patients <4 times of abortion vs. 17 (81%) in ≥4, D/I 15 (15.3%) in patients <4 times of abortion vs. 3 (14.3) in ≥4 while I/I 6 (6.1%) in <4 times of abortion patients and 1 (4.8%) in ≥4.

## Discussion

The most prevalent pregnancy problem is RSA, which occurs when a pregnancy is spontaneously terminated before the fetus reaches viability. For those who are passionate about bettering women's health and the outcomes of their pregnancies, enhancing the quality of the pregnancy itself is a top priority. Investigating the link between RSA and ACE I/D gene

**Table 1.** Baseline characteristics among patients with spontaneous abortion and control groups.

Characteristic	Patient (N=119)	Control (N=113)	P-value
Age, mean±standard deviation	31.3±5.9	30.3±5.4	0.285
Abortion time N (%)			
<4	90 (75)	-	-
≥4	29 (25)	-	-

**Table 2.** Angiotensin I-converting enzyme insertion/deletion polymorphism in spontaneous abortion patient and control groups.

ACE I/D	Patient N=119 (%)	Control N=113 (%)	Total N	P-value	Chix <sup>2</sup>
D/D	94 (79.0)	69 (61.1)	163	0.006*	19.93
D/I	18 (15.1)	33 (29.2)	51		
I/I	7 (5.9)	11 (9.7)	18	0.135	10.12
Allele D	206 (86.6)	171 (75.7)	377	0.003*	3.03 <sup>a</sup>
Allele I	32 (13.4)	55 (24.3)	87		

\*P-value<0.05, statistically significant association, highly statistically significant association; <sup>a</sup>Fisher exact test. ACE, angiotensin I-converting enzyme; I, insertion; D, deletion.

**Table 3.** Effects of angiotensin I-converting enzyme insertion/deletion genotypes on recurrent miscarriage patients according to abortion time.

Genotypes	Abortion time		P-value	Chix <sup>2</sup>
	<4 N=98 (%)	≥4 N=21 (%)		
ACE I/D			0.961	0.079
D/D	77 (78.6)	17 (81.0)		
D/I	15 (15.3)	3 (14.3)		
I/I	6 (6.1)	1 (4.8)		

ACE, angiotensin I-converting enzyme; I, insertion; D, deletion.

polymorphisms is difficult. This is because RSA has more than one cause, and genetic factors are one among those causes. The ACE I/D polymorphism has been linked to pregnancy problems in many studies.

When comparing the patient and control groups in this investigation, a very significant correlation was found between the D/D genotype and the D allele of the ACE gene. There is evidence that preeclampsia and pregnancy-induced hypertension, both of which may lead to abortion, are associated with a D/D genotype. The converse is also true: being homozygous for the I allele may reduce blood ACE levels, which in turn may slow the placental rate of bradykinin inactivation and provide protection against preeclampsia.<sup>8</sup> Consistent with a study by Zhang *et al.*,<sup>11</sup> we found that patients experiencing unexplained RM had a significantly different distribution of alleles and genotypes (ACE I/D) compared to healthy controls. The association between these factors and pregnancy loss was significant in both dominant (1.63-fold, D allele) and recessive modes (1.76-fold, D/D genotype). In addition, a Korean research by Kim *et al.*,<sup>12</sup> among Korean women suffering from RPL, was consistent with our results. Researchers found no significant difference between the RPL group and the control group in terms of the genotype distributions of the (ACE I/D) polymorphism, suggesting that this variant, either alone or in combination, is not a key factor in RPL formation. To investigate if there is a connection between the ACE gene variant and repeated abortions, Su *et al.* performed a meta-analysis and comprehensive review. We used the premise of dominant inheritance to examine 11 trials with a total of 1,275 cases and 2,049 controls. There was shown to be a strong correlation between the two. A greater risk was also shown for women with the I/D and D/D genotypes compared to those with the I/I genotype,<sup>13</sup> and they also showed a substantial link between the ACE I/D polymorphism and recurrent pregnancy loss. Studies from China have also shown that the significant proteinuria and renal impairment seen in preeclampsia patients are associated with the ACE gene I/D polymorphism. People who have preeclampsia and have the D variant of the ACE gene may also be at a higher risk of developing renal impairment.<sup>14</sup> Goodman *et al.* and Shakarami *et al.* found no significant connection between the ACE D allele or the D/D genotype with pregnancy loss, which contradicts our findings.<sup>15,16</sup> Additionally, Aisha *et al.*<sup>17</sup> discovered no statistically significant link between ACE genotypes and recurrent abortion in their research of Sudanese women who were evaluated for the relationship of ACE I/D polymorphism with RPL. The study by Fatimah Basil *et al.*<sup>18</sup> sought to identify any potential link between the I/D polymorphism of the ACE gene and RM in Saudi females. The researchers found that 56.7% of the females with RM had the D/D genotype, 29.5% had the ID genotype, and 4.9% had the II genotype. In comparison, the control group had 54.2%, 42.3%, and 3.3% of these genotypes, respectively. However, the difference between the two groups was not statistically significant.

There are noticeable variations in the frequency of I/D polymorphism in the ACE gene among different populations. It is crucial to acknowledge the limitations of the current study conducted on RSA within Sudan. While some studies call for the necessity of larger-scale investigations across diverse regions of Sudan and among various ethnic groups to enhance the generalizability of findings, it is essential to highlight a

notable deficiency. Many reported studies tend to overlook the evaluation of other potential causes of RSA within their predetermined inclusion and exclusion criteria. This limitation could potentially restrict the depth of understanding regarding the multifactorial nature of recurrent pregnancy loss in the Sudanese context. Future research endeavors should strive to incorporate a more comprehensive approach that considers a broader range of potential factors contributing to RSA, thereby enriching the overall knowledge base in this area.

## Conclusions

Our result indicates that the D and I allele of the polymorphism possibly be a risk factor for unexplained spontaneous abortion in Sudanese women. We suggest including these two genetic variations (ACE D/I) in the panel of thrombophilic mutations to be tested in individuals who have experienced multiple spontaneous miscarriages. According to the available data, further research is still required before providing therapy alternatives for patients who frequently miscarry.

## References

- Deng T, Liao X, Zhu S. Recent Advances in Treatment of Recurrent Spontaneous Abortion. *Obstet Gynecol Surv* 2022;77:355-66.
- Turesheva A, Aimagambetova G, Ukybassova T, et al. Recurrent pregnancy loss etiology, risk factors, diagnosis, and management. fresh look into a full box. *J Clin Med* 2023;12:4074.
- Cao C, Bai S, Zhang J, et al. Understanding recurrent pregnancy loss: recent advances on its etiology, clinical diagnosis, and management. *Med Rev* 2022;2:570-89.
- Hyde KJ, Schust DJ. Genetic considerations in recurrent pregnancy loss. *Cold Spring Harb Perspect Med* 2015;5:a023119.
- Thakur S, Sharma V, Kaur D, Purkait P. Angiotensin-Converting Enzyme (ACE) Insertion/Deletion (I/D) Polymorphism as a Conjoint Regulator of Coagulation, Fibrinolytic, and RAAS Pathway in Infertility and Associated Pregnancy Complications. *J Renin Angiotensin Aldosterone Syst* 2022;2022:1695769.
- Thakur S, Sharma V, Kaur D, Purkait P. Angiotensin-Converting Enzyme (ACE) Insertion/Deletion (I/D) Polymorphism as a Conjoint Regulator of Coagulation, Fibrinolytic, and RAAS Pathway in Infertility and Associated Pregnancy Complications. *J Renin Angiotensin Aldosterone Syst* 2022;2022:1695769.
- Vladeanu MC, Bararu Bojan I, Bojan A, et al. Angiotensin-converting enzyme gene D-allele and the severity of coronary artery disease. *Exp Ther Med* 2020;20:3407-11.
- Gintoni I, Adamopoulou M, Yapijakis C. The angiotensin-converting enzyme insertion/deletion polymorphism as a common risk factor for major pregnancy complications. *In vivo (Athens, Greece)* 2021;35:95-103.
- Babker AM, Gameel FE. Methylenetetrahydrofolate reductase c677t polymorphism in Sudanese women with recurrent spontaneous abortions. *Kuwait Med J* 2016;48:100-4.



10. Babker AM, Gameel FE. Molecular Characterization of Prothrombin G20210A gene Mutations In pregnant Sudanese women with spontaneous recurrent abortions. *Rawal Med J* 2015;40:207-9.
11. Zhang S, Wang J, Wang B, et al. Strong association between angiotensin I-converting enzyme I/D polymorphism and unexplained recurrent miscarriage of Chinese women--a case-control study. *Reprod Sci* 2011;18:743-6.
12. Kim JJ, Choi YM, Lee SK, et al. The PAI-1 4G/5G and ACE I/D polymorphisms and risk of recurrent pregnancy loss: a case-control study. *Am J Reprod Immunol* 2014;72:571-6.
13. Su MT, Lin SH, Chen YC, Kuo PL. Genetic association studies of ACE and PAI-1 genes in women with recurrent pregnancy loss: a systematic review and meta-analysis. *Thromb Haemost* 2013;109:8-15.
14. Li H, Ma Y, Fu Q, Wang L. Angiotensin-converting enzyme insertion/deletion (ACE I/D) and angiotensin II type I receptor (AT1R) gene polymorphism and its association with preeclampsia in Chinese women. *Hypertens Pregnancy* 2007;26:293-301.
15. Goodman C, Hur J, Goodman CS, et al. Are polymorphisms in the ACE and PAI-1 genes associated with recurrent spontaneous miscarriages? *Am J Reprod Immunol* 2009;62:365-70.
16. Shakarmi F, Akbari MT, ZareKarizi SH, Ajamian F. Association of angiotensin converting enzyme (ACE), D/I polymorphisms with recurrent pregnancy loss. *Armaghane Danesh*. 2014;19:442-9.
17. Fageer Hussian AM, Ali Mohammed NE, Mohamed Ahmed MA, Widaa Ali E. Angiotensin Converting Enzyme Insertion/Deletion (I/D) Polymorphism and Risk of Recurrent Pregnancy Loss among Sudanese Women. *Res Obstet Gynecol* 2016;4:7-10.
18. Al-Mukaynizi FB, AlKhuriji A, Bebay Z, et al. Lack of Association between Angiotensin Converting Enzyme I/D Polymorphism and Unexplained Recurrent Miscarriage in Saudi Arabia. *J Med Biochem* 2016;35:166-73.