

The association of angiotensin-converting enzyme gene polymorphism with respiratory distress syndrome in premature neonates

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ABSTRACT

Background. There was a contradiction in the previous literature on whether the D/D genotype of angiotensin-converting enzyme (ACE) is a protective or risk factor for respiratory distress syndrome (RDS) in premature neonates. To solve this debate, we intended to examine the association between ACE gene polymorphism and RDS in premature neonates.

Methods. We enrolled a total of 100 premature neonates with gestational age below 37 weeks. They were divided into 2 groups, the case group included 50 premature neonates diagnosed with RDS. While the control group included 50 premature neonates with no signs of RDS. We assessed ACE gene polymorphism using polymerase chain reaction. All neonates underwent chest x-ray, echocardiography, and routine laboratory investigations.

Results. D/D and D/I genotypes were higher in the control group (48% and 50%) than in the case group (26% and 40%). Whereas, I/I genotype was lower in the control group (2%) than in the case group (34%) ($p < 0.001$). By counting D alleles among members of both groups, D-alleles were significantly higher in the control group (73%) than in the case group (46%) ($p < 0.001$).

Conclusions. In premature neonates, D/D and D/I genotypes and D-alleles are protective factors for RDS. Whereas, I/I genotype and I-alleles are associated with the incidence of RDS with complications.

Key words: angiotensin-converting enzyme, gene polymorphism, neonates, premature, respiratory distress syndrome.

Respiratory distress syndrome (RDS) is the most prevalent cause of comorbidity and mortality in preterm neonates. It is mainly triggered by the deficiency of alveolar surfactants in the lung. It commonly presents in premature neonates below 37 weeks of gestation. The severity of RDS increases over the first two days of life, which ends with death due to progressive hypoxia and respiratory failure if was not appropriately managed.¹⁻³

The Renin-angiotensin system (RAS) is a crucial modulator of blood pressure, liquid, and electrolyte homeostasis.⁴ Angiotensin-converting enzyme (ACE) is a membrane-bound exopeptidase enzyme. It has a crucial role in converting angiotensin-1 to angiotensin-2, which is a growth factor and vasoconstrictor.⁵ ACE degrades bradykinin and kallidin, vasodilator peptides. Consequently, ACE increases growth and vasoconstriction and decreases vasodilation.⁶

ACE is highly presented in the vascular endothelial cells of the neonatal lung.^{7,8} The human ACE gene is located on chromosome 17. It consists of 26 exons and 25 introns with a

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total length of 21 kbp.¹ ACE has many types of gene polymorphism, where the most common form is the existence or absence of 287bp to the intron 16, known as the insertion/deletion (I/D).^{1,9,10} The ACE insertion/deletion gene polymorphism results in three genotypes: deletion/deletion (D/D) and insertion/insertion (I/I) homozygotes, and deletion/insertion (D/I) heterozygote.¹⁰

It has been suggested that the D/D genotype and D-alleles genotype is associated with RDS patients with sepsis, increased risk of bronchopulmonary dysplasia, myocardial infarction and ischemic heart disease, and diabetic kidney disease.^{4,11,12} Furthermore, the D/D genotype is associated with the high activity of ACE in serum and tissues of the preterm neonates with RDS.¹³

Sivasli et al.¹⁴ revealed that the D/D genotype is a protective factor from RDS in preterm neonates. Whereas, Yimenicioglu et al.¹ and Hussein et al.⁴ demonstrated that it is a risk factor for RDS in preterm neonates. Satar et al.¹⁵ reported no link between D/D genotype and RDS. Consequently, to solve this debate, we intended to assess the association between ACE gene polymorphism and RDS in premature neonates.

Material and Methods

We performed this case-control study between January 2019 and June 2021 at the Menoufia University hospital, Egypt. The study protocol was approved by the Menoufia Faculty of Medicine Committee for Medical Research Ethics (IRB: 4-2018PEDI32). We followed the Helsinki Declaration of 1964, as revised in 2013. Written informed consent was obtained from the neonates' parents included in the study.

Eligibility criteria

We included preterm neonates with gestational age below 37 weeks, examined for RDS within the first 48 hours after delivery, and both sex. We excluded neonates with genetic

and/or metabolic disorders, minor or major anomalies, pulmonary hypertension, congenital pneumonia, wet lung, and meconium aspiration.

Study Process and Evaluations

The study was performed on 100 preterm neonates at gestation age below 37 weeks admitted to the neonatal intensive care units at Menoufia University Hospital. Of them, the case group consisted of 50 neonates with RDS and 50 premature neonates served as the control group with no signs of RDS. All neonates were subjected to a detailed history, clinical examination, chest x-ray, echocardiography, as well as laboratory investigations.

RDS was diagnosed according to the following standards: (1) respiratory rate of more than 60 per minute, (2) dyspnea, (3) grunting, nasal flare, cyanosis, (4) respiratory acidosis with pH < 7.25, pCO₂ > 60 mmHg, and PaO₂ < 50mmHg in blood gases test, (5) radiological and clinical signs of RDS.^{1,4}

Genotyping analysis

Peripheral venous blood was aseptically sampled in EDTA tubes. Total genomic DNA was extracted using Gene JET Whole Blood Genomic DNA Purification Mini Kit (Thermofisher) according to the manufacturers' instructions. We performed polymerase chain reaction using the primers, sense 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and antisense 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'.⁹ Depending on the D/I flanking sequence, two DNA fragments were amplified, D-alleles (190 bp) and I-alleles (490bp). The reaction (total volume 25µl) was conducted in 2720 thermal cycler Singapore Applied Biosystem. Following the 5 min initial denaturation step at 95°C, 35 cycles were conducted as the following: 30s for denaturation at 95°C, 45s for annealing at 58°C, and 90s for extension at 72°C followed by 5 min for the final extension. DNA products were separated by gel electrophoresis, then stained by ethidium bromide (Sigma), Fig. 1.

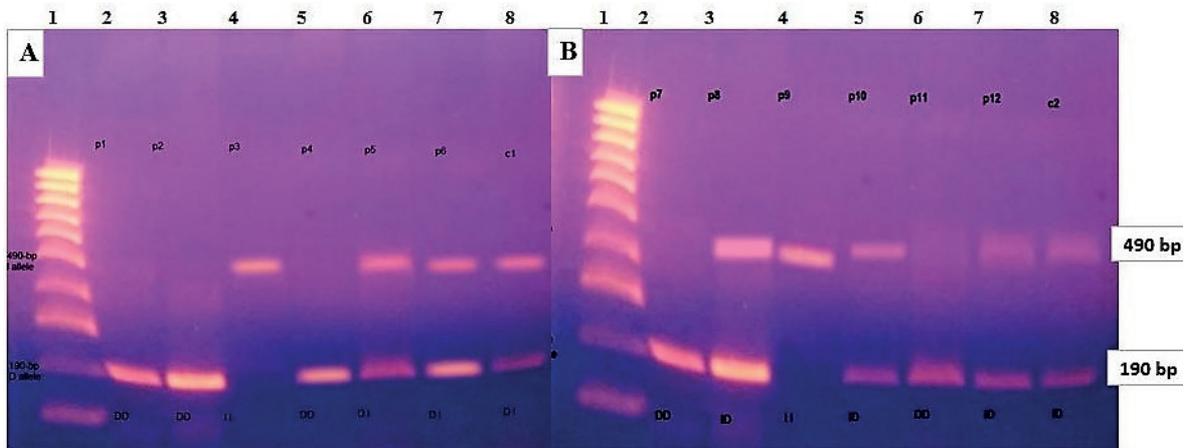


Fig. 1. Agarose gel visualization of the angiotensin-converting enzyme genotypes (DD, ID and II) fragments results from the polymerase chain reaction..(A) Lane 1 is the ladder; lane 8 is a control; lanes 2, 3, and 5 are the D/D genotype; lanes 6, 7 are D/I genotype; and lane 4 is the I/I genotype. (B) Lane 1 is the ladder; lanes 2 and 6 are the D/D; lanes 5, 7, and 8 are the D/I genotype; and lane 4 is the I/I genotype.

Statistical Methods

We analyzed the data using IBM SPSS advanced statistics version 25 (IBM Corp, NY, US). We presented the qualitative data in frequencies and percentages. Chi-squared test was used to measure the association between two or more qualitative variables. We performed the Shapiro-Wilk test to determine the type of data distribution.^{16,17} Parametric data were stated as mean \pm SD. The Student's t-test was performed for parametric data to compare quantitative variables between two groups. P-value <0.05 was considered significant.

Results

General characteristics of the included population

The study included 100 preterm neonates distributed into two groups. The case group included 50 premature neonates with RDS, with a mean gestational age of 32.16 weeks (± 1.95), while the control group included 50 premature neonates with no signs of RDS, with a mean gestational age of 34.74 weeks (± 0.94). Of the case and control group 62% and 56% were males, respectively. The control group participants had a significantly higher mean gestational age, birth weight, birth length, and

head circumference than neonates with RDS ($p < 0.05$). Premature neonates with RDS had significantly lower mean Apgar scores at 1 minute (2.74 vs. 5.86, $p < 0.001$) and 5 minutes (5.36 vs. 7.26, $p < 0.001$). Among the case group, three neonates (6%) developed intra-ventricular hemorrhage, twelve neonates (24%) had sepsis, and during follow-up, ten neonates (20%) died, Table I.

Regarding premature neonates with RDS, respiratory distress grade two was the most frequent in the case group (70%). Further, weak muscle tone was encountered in 4 neonates (8%) and absent moro-reflex was detected in 19 neonates (38%) of the case group. In contrast, none of them were detected in the premature neonates without RDS ($p = 0.041$ and $p < 0.001$, respectively). Weak suckling was more prevalent in the case group than in the control group (58% vs. 30%, $p = 0.005$). Convulsions were detected only in one neonate, which is in the case group, Table I.

ACE gene genotyping

ACE gene D/D and D/I genotypes were significantly lower in the premature neonates with RDS than in the premature neonates without RDS (26% and 40% vs. 48% and 50%). On the other hand, the I/I genotype was

Table I. General and clinical characteristics of the study groups.

Neonates Data	Case group (n=50)	Control group (n=50)	P-value
Sex (male/female)	31/19 (62%/38%)	28/22 (56%/44%)	0.542
Gestational age (weeks)	32.16 ± 1.95	34.74 ± 0.94	<0.001 ^a
Birth weight (g)	1872.8 ± 316.9	2035.2 ± 386	0.024 ^a
Birth length (cm)	42.42 ± 1.85	43.28 ± 1.62	0.015 ^a
Head Circumference (cm)	31.36 ± 1.27	31.94 ± 1.20	0.021 ^a
APGAR Score (at 1 min)	2.74 ± 0.78	5.86 ± 0.35	<0.001 ^a
APGAR Score (at 5 min)	5.36 ± 0.48	7.26 ± 0.44	<0.001 ^a
IntraVentricular Hemorrhage	3 (6%)	-	-
Sepsis	12 (24%)	-	-
Mortality	10 (20%)	-	-
<i>Respiratory distress (RD) grades</i>			
RD I	5 (10%)	-	-
RD II	35 (70%)	-	-
RD III	7 (14%)	-	-
RD IV	3 (6%)	-	-
<i>Muscle tone</i>			
Normal	46 (92%)	50 (100%)	0.041 ^a
Weak	4 (8%)	0 (0%)	
<i>Moro-reflex</i>			
Normal (Positive)	31 (62%)	50 (100%)	<0.001 ^a
Abnormal (Absent)	19 (38%)	0 (0%)	
<i>Suckling</i>			
Normal	21 (42%)	35 (70%)	0.005 ^a
Weak	29 (58%)	15 (30%)	
<i>Convulsions</i>			
No	49 (98%)	50 (100%)	0.315
Yes	1 (2%)	0 (0%)	

Parametric data are expressed as mean ± SD. Qualitative data are expressed as frequency (percentage). ^a p<0.05 is significant.

significantly higher in the case group than in the control group (34% vs. 2%), p<0.001, Table II.

By counting D alleles and I-alleles in all neonates in both groups, D-alleles were significantly lower in the case group than the control group (46% vs. 73%), Whereas the I-alleles were higher in the case group than the control group (54% vs. 27%), p<0.001, Table II.

Association of ACE with morbidity and mortality

There was no significant association between the D/D, D/I, and I/I genotypes of the ACE gene

with intraventricular hemorrhage, sepsis, and mortality (0.423, 0.328, and 0.342, respectively), Table III.

Discussion

ACE gene polymorphism is correlated with the prevalence of several diseases and morbidities. However, only few studies have been conducted on premature neonates. These studies revealed a contradictory association between the ACE gene and RDS.^{4,11-13} Consequently, we measured the ACE gene polymorphism in premature neonates with and without RDS.

Table II. Distribution of ACE gene polymorphism in the study groups.

Gene testing Data	Case group (n=50)	Control group (n=50)	p-value
Genotype			
• D/D	13 (26%)	24 (48%)	
• D/I	20 (40%)	25 (50%)	<0.001 ^a
• I/I	17 (34%)	1 (2%)	
Allele (n=100)			
• D	46 (46%)	73 (73%)	
• I	54 (54%)	27 (27 %)	<0.001 ^a

Qualitative data are expressed as frequency (percentage). ^a p<0.05 is significant.

Table III. Complications of RDS according to the genotypes.

Complications of RDS	D/D (n=13)	D/I (n=20)	I/I (n=17)	p-value
Intra Ventricular Hemorrhage				
Yes	1 (7.69%)	2 (10%)	0 (0%)	
No	12 (92.31%)	18 (90%)	17 (100%)	0.423
Sepsis				
Yes	2 (15.39%)	7 (35%)	3 (17.65%)	
No	11 (84.61%)	13 (65%)	14 (82.35%)	0.328
Mortality				
Yes	2 (15.39%)	6 (30%)	2 (11.75%)	
No	11 (84.61%)	14 (70%)	15 (88.25%)	0.342

Qualitative data are expressed as frequency (percentage). RDS: Respiratory distress syndrome.

Our study showed that D/D and D/I genotypes and D-alleles were significantly higher in premature neonates without RDS than those with RDS. In comparison, I/I genotype and I-alleles were significantly higher in the RDS premature neonates than in those without RDS. ACE gene polymorphism was not associated with intraventricular hemorrhage, sepsis, and mortality in premature neonates.

RAS activation may influence lung injury by modifying vascular permeability by changing the intracellular calcium levels in the endothelial cells and vascular tone by changing the calcium inflow and vascular smooth muscles activity. Moreover, RAS has been detected with high levels in the bronchoalveolar lavage of acute respiratory distress syndrome patients.^{14,18}

D/ D genotype and D-alleles may increase ACE activity, which increases the risk of RDS through its increased levels of ACE and its product angiotensin-2, increasing lung

inflammation and modifying the pulmonary vascular status.¹⁹ Angiotensin-2 increases pulmonary inflammation by stimulating interleukin-6, tumor necrosis-alpha, and chemoattractant protein-1 production in the pulmonary endothelial cells and vascular smooth muscles, which may cause edema in the lungs through increasing pulmonary endothelial permeability.¹⁹ Furthermore, the D/D genotype has a significant role in developing a cardiorespiratory disease in premature neonates.²⁰ D/D genotype may upsurge the risk of neonatal bronchopulmonary dysplasia.¹⁹

Our findings are in line with those of Sivasli et al.¹⁴, who reported that D/D and D-alleles were significantly higher in premature neonates without RDS than in neonates with RDS (43.5% and 68.5% vs. 26.8% and 47.6%, p<0.05), which suggested that D/D and D-alleles are protective factors from RDS in premature neonates. In contrast, Yimenicioglu et al.¹ and Hussein et al.⁴

demonstrated that D/D genotype and D-alleles were significantly higher in the RDS neonates than in the control ($p < 0.05$), revealing that they are risk factors for RDS. In addition, Satar et al.¹⁵ demonstrated no link between D/D genotype and RDS.

Rigat et al.²¹ studied the frequency of the genotypes and alleles of the ACE gene polymorphism in healthy subjects. They observed that D/D genotype and D-alleles were higher than I/I genotype and I-alleles (36% and 59.4% vs. 18% and 40.6%). These results are matching with the findings of the present study. Taking all this together, this disparity may be attributed to the different genetic factors between the different populations and the limited number of studies.^{4,19,22}

Cardinal-Fernandez et al.¹¹ demonstrated that D-alleles were associated with sepsis. However, Hou et al.²³ observed that D/D was a protective factor for sepsis in both the adult and pediatric populations in their meta-analysis study, and ACE inhibitor drugs may be harmful to patients with sepsis. These findings matched with our results that there is no relationship between ACE polymorphism and sepsis. Also, Hou et al.²³ elucidated no association between the I/D genotype and infant mortality, which enhances our results. However, during sepsis, ACE activity is decreased with the blood pressure.^{24,25} This decrease may have resulted from the damage of endothelial cells or ACE inhibition by lipopolysaccharide of the bacterial infection.²⁵

In the current study, multivariate analysis showed birth weight, birth length, and head circumference did not interfere with the difference in the ACE gene polymorphism between the case and control group except for the gestational age, which was proposed by other studies to be associated with ACE gene polymorphism.^{26,27}

We concluded that in premature neonates, D/D genotype and D-alleles are protective factors from RDS. I/I genotype and I-alleles are

associated with more severity and complications of RDS. In addition, there is no link between the ACE gene polymorphism and intraventricular hemorrhage, sepsis, and mortality. Further studies are required to examine the correlation of ACE gene polymorphism and activity with racial and genetic distribution.

Ethical approval

The study protocol was approved by the Ethics Committee of Menoufia Faculty of Medicine (IRB 4/2021PEDI2).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DM, SAE; data collection: MT; analysis and interpretation of results: DM, AK, MT; draft manuscript preparation: DM, SAE. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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