ACE gene deletion/deletion polymorphism may be a protective factor for respiratory distress in preterm infants

Ercan Sivaslı[1], Murat Yurdakök[1], Elif Babaoğlu[2], Halil Karabulut[2], Şule Yiğit[1]
Melih Babaoğlu[3], Gülsevin Tekinalp[1], Ajlan Tükün[4]

Departments of [1]Pediatrics, and [3]Pharmacology, Hacettepe University Faculty of Medicine, and Departments of [2]Pharmacology, and [4]Medical Genetics, Ankara University Faculty of Medicine, Ankara, Turkey

SUMMARY: Sivaslı E, Yurdakök M, Babaoğlu E, Karabulut H, Yiğit Ş, Babaoğlu M, Tekinalp G, Tükün A. ACE gene deletion/deletion polymorphism may be a protective factor for respiratory distress in preterm infants. Turk J Pediatr 2007; 49: 69-74.

The objective in this study was to evaluate the angiotensin converting enzyme (ACE) insertion/deletion (I/D) gene polymorphism in premature infants with and without respiratory distress within the first 24 hours of life.

Totally, 87 premature babies who were followed up in the neonatal unit were included in the study. Of these babies, 41 had respiratory distress, and constituted the patient group. The remaining 46 babies who did not have respiratory distress constituted the control group. Blood samples were obtained from the babies within the first few days of life prior to administration of any blood product. The ACE gene insertion (I) and deletion (D) polymorphism was investigated using polymerase chain reaction method.

The I/I polymorphism was frequent in the patient group and the D/D polymorphism was frequent in the control group (p<0.05). There was no relationship between the ACE gene polymorphism and hospital stay, ventilation or oxygen consumption duration of the patients. In addition, taking into consideration the gestational age, no association was found between ACE gene polymorphism and birth weights of the babies. The I/I genotype was considered a risk factor for pulmonary disorders in neonates as the I/I variant was more frequent in the neonates with respiratory distress than in healthy newborns.

The ACE I/I genotype is associated with an increased risk of respiratory disorders among premature infants and the D/D genotype is a protective factor for respiratory disorders, but these infants with ACE D/D genotype might be at risk for the development of cardiovascular disorders later in life.

Key words: ACE gene polymorphisms, preterm infant, respiratory distress.

The human renin-angiotensin system (RAS) is one of the major regulators of blood pressure and fluid and electrolyte homeostasis. Angiotensin converting enzyme (ACE), a key component of RAS, cleaves angiotensin I (AT-I) to yield the potent vasoconstrictor angiotensin II (AT-II). In addition, ACE degrades vasodilator kinins. Therefore, RAS plays an important role in circulatory homeostasis[1].

Local RAS also exists in diverse human tissues including myocardium, vasculature, brain tissue and lymphocytes[2]. Additionally, RAS is present in the placental capillaries, infant lung endothelial and type II epithelial cells. The RAS, especially AT-II, acts as modulator of growth in a variety of cells and tissues[3-7]. High levels and activities of RAS were found in mice lung in the first week postpartum[8],

and therefore it has been suggested that RAS plays an important role in the development of the fetal and postnatal lung.

Angiotensin converting enzyme gene deletion (D) and insertion (I) polymorphism affects the level of serum and tissue ACE activity[9,10]. DD genotype is associated with the highest activity of ACE. Several studies in adults reported an association between increased RAS activation and pulmonary disorders such as adult respiratory distress syndrome (ARDS) and pulmonary hypertension (PH)[11-13]. This prospective study about the significance and existence of ACE I/D gene polymorphism in premature infants with RD was designed around the hypothesis that the change in ACE activity in serum and tissue related to the ACE gene polymorphism may impact pulmonary disease by affecting activation of the RAS. The aim of this study was to evaluate the ACE I/D gene polymorphism in premature infants with and without RD within the first 24 hours of life.

Material and Methods
Subjects

Eighty-seven premature infants who were admitted to the Neonatal Intensive Care Unit at Hacettepe University Ihsan Dogramaci Children's Hospital, with gestational age under 37 weeks, were included in the study. The premature neonates with RD during the first 24 hours of life constituted the patient group and those without any respiratory problem constituted the control group. All of the premature infants were treated with corticosteroids antenatally, and none had prophylactic surfactant treatment. Exclusion criteria were minor and major congenital anomalies.

The criteria for the diagnosis of RD were the presence of a respiratory rate of more than 60 per minute and dyspnea characterized by intercostal, subcostal or suprasternal retraction, grunting, nasal flare, and cyanosis[1,14]. The patient group consisted of neonates with respiratory distress syndrome (RDS) (n=25), wet lung (n=3), primary pulmonary hypertension (PPH) (n=8), and pneumonia (n=5). The presence of typical clinical and radiological signs of RDS in preterm infants revealed the diagnosis of RDS[15]. The diagnosis of wet lung was confirmed by the presence of RD occurring

after 1-2 hours of life and resolving within 2-3 days, and with the typical radiological findings[16]. PPH was diagnosed by the presence of mean pulmonary artery pressure >30 mmHg echocardiographically, radiologically normal lungs, no evidence of parenchymal lung disease and no cardiac anomaly[17]. The presence of typical radiological findings of pneumonia and evidence of infection in complete blood cell count and peripheric smear confirmed the diagnosis of pneumonia[18]. Duration of hospitalization, mechanical ventilation and oxygen use, number of surfactant use, and mortality in the RD group were recorded.

The study was approved by the Hacettepe University Human Ethics Committee and informed consent was obtained from all parents.

Genotyping Method

Venous blood samples obtained from the babies were anticoagulated in ethylenediamine tetraacetic acid (EDTA) tubes. Total genomic DNA was isolated using a standard method using QIAamp DNA blood kit (Qiagen, Hilden, Germany). ACE gene I/D polymorphism genotypes were determined by polymerase chain reaction (PCR) using the primers and conditions described earlier[10]. Reactions were performed with 10 pmol of each primer: sense oligo, 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', and antisense oligo, 5'-GAT GTG GCC ATC ACA TTC GTC AAG T-3'. Amplification was performed in a thermal cycler (Eppendorf Mastercycle, Hamburg, Germany) for 30 cycles with denaturation at 94°C for 40 s, annealing at 56°C for 40 s and extension at 72°C for 40 s, followed by a final extension at 72°C for 10 min.

Polymerase chain reaction products separated by means of electrophoresis on 1% agarose gel were identified with ethidium bromide (Sigma) staining. The polymorphisms detected by means of PCR were evident as an approximately 490-bp fragment in the presence of the insertion (I) allele and as an approximately 190-bp fragment in the absence of insertion (D) allele (Fig. 1).

Statistical Analysis

The proportions of alleles/genotypes represented in patient and control groups were compared with chi-square (χ2) test. Kruskal-Wallis and one-way analysis of variance (ANOVA) tests were used to compare the characteristics
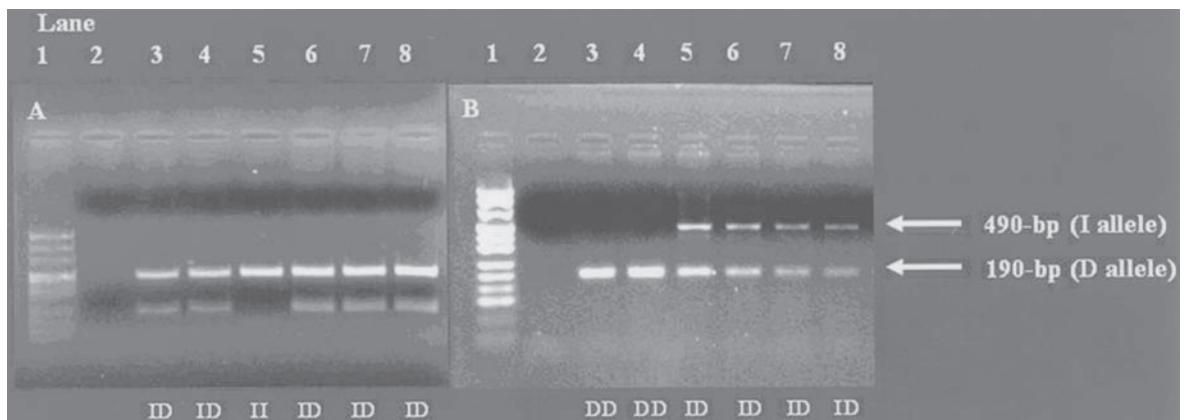
Fig. 1. Determination of angiotensin-converting enzyme (ACE) genotypes (DD, ID and II) by means of PCR using ACE primers. (A) Lane 1 is a marker, lane 2 is a negative control; lanes 3 and 4 are ID genotype; lane 5 is II genotype, lanes 6, 7 and 8 are ID genotype. (B) Lane 1 is a marker, lane 2 is a negative control; lanes 3 and 4 are DD genotype, lanes 5, 6, 7 and 8 are ID genotype.

of the patient and control groups. Data are expressed as means and standard deviations. All statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS for Windows©, version 11.0). P<0.05 was considered significant.

Results

Eighty-seven premature infants were included. The patient group consisted of 41 preterm infants who had RD in the first 24 hours of life (25 RDS, 8 PPH, 5 pneumonia, 3 wet lung). Four of 41 patients (9.7%) died in the follow-up period due to RDS. The control group consisted of 46 preterm infants with no RD. The demographic characteristics of the patient and control groups are shown in Table I. There was no statistically significant difference

between the demographic characteristics of the two groups except for Apgar scores at the 5th minute and maternal ages.

The ACE gene I/D genotypes in the patient and control groups are presented in Table II. The incidence of the I/I genotype was higher in the patient group. The incidence of the heterozygous (I/D) genotype was similar in both groups. The incidence of the D/D genotype was lower in RD cases when compared with the controls (Table II). The frequencies of D and I alleles were calculated from the genotype frequencies. There was a statistically significant difference between the patient and control groups. The incidence of the D allele was lower and of the I allele higher in RD cases compared with the controls (Table III). Also, there was no relation between

Table I. Demographic Characteristics of Infants With and Without Respiratory Distress

	Patient group (n=41)	Control group (n=46)	P value
Gestational age (weeks)[1]	32.0±3.7	33.6±2.1	p=0.07
Birth weight (g)[1]	1790.0±704.2	1893.9±538.5	p=0.61
Apgar score (5th min)	7.5±2.5	8.6±1.2	p=0.01
Male/female	25/16	23/23	p=0.31
Mode of birth (V/CS)	9/32	10/36	
Maternal age (years)	28.7±6.5	31.7±4.7	p=0.02

[1]Mean±standard deviation.
V: Vaginal delivery. CS: Cesarean section.

Table II. Frequency (%) of Angiotensin-Converting Enzyme (ACE) Gene I/D Polymorphism Genotypes in Premature Newborns with RD and in Non-RD Controls

Genotype	Patient group n (%)	Control group n (%)
D/D	11 (26.8)	20 (43.5)
D/I	17 (41.5)	23 (50.0)
I/I	13 (31.7)	3 (6.5)
Total	41 (100)	46 (100)

$\chi^2 = 9.507$, p=0.009

I: Insertion. D: Deletion. RD: Respiratory distress.

Table III. Frequency (%) of Angiotensin-Converting Enzyme (ACE) Gene D and I Alleles in Premature Newborns with RD and in Non-RD Controls

Allele	Patient group (n=41)	Control group (n=46)
D	39/82 (47.6%)	63/92 (68.5%)
I	43/82 (52.4%)	29/92 (31.5%)

$\chi^2 = 7.820$, p=0.005.

D: Deletion. I: Insertion. RD: Respiratory distress.

the ACE gene I/D genotypes and severity of clinical findings (Table IV). The genotypes in the population were in Hardy-Weinberg equilibrium (chi-square test, P>0.05).

via the AT1 receptor; and autocrine action of transforming growth factor β (TGF β)[19-21]. Wang and colleagues[22] identified AT-II as a pro-apoptotic factor for alveolar epithelial cells.

Table IV. Frequency (%) of Angiotensin-Converting Enzyme (ACE) Gene I/D Polymorphism Genotypes According to Disease Severity in Premature Newborns with RD

	Genotype			One-Way ANOVA	
	DD (n=11)	ID (n=17)	II (n=13)	F value	P value
Hospitalization (days)[1]	25.0±30.5	17.0±19.4	25.5±25.5	0.559	0.577
Mechanic ventilation (days)[1]	10.5±22.2	4.5±4.4	8.6±12.4	0.709	0.499
Oxygen use (days)[1]	20.4±35.5	11.1±14.2	22.1±30.6	0.737	0.485
Mean arterial pressure in first day (mmHg)	42.6±2.4	40.1±1.5	43.0±1.6	0.921	0.407
No. patients requiring vasopressor drug in first day	3	10	7	0.761	0.475

[1]Mean±standard deviation.
I: Insertion. D: Deletion. RD: Respiratory distress.

Discussion

We determined the prevalence of insertion/deletion (I/D) polymorphism of the ACE gene in premature infants with RD. Our data indicate that the I/I genotype was considerably overrepresented in RD cases compared to non-RD cases (p<0.05).

Activation of a pulmonary RAS might influence the pathogenesis of lung injury via a number of cellular impacts, like changes in vascular permeability via modulation of endothelial intracellular calcium ion levels; changes in vascular tone via calcium influx and contraction of vascular smooth muscles and fibroblast activity

Idell and colleagues[23] demonstrated that ACE is elevated in bronchoalveolar lavage fluid in ARDS. These data suggest that RAS activation is important for both initiation and progression of lung injury. However, these studies were usually performed in adults. In neonates, the findings of the limited number of studies are controversial[24]. Harding and colleagues[24] demonstrated that the ACE polymorphism has a role in the development of preterm cardiorespiratory disease and that the D/D genotype may adversely influence the early health status of preterm infants. Another study demonstrated that the ACE I/D polymorphism did not significantly influence the development of bronchopulmonary dysplasia (BPD) in ventilated

premature infants[25]. But in another study, Kazzi and colleagues[26] demonstrated that the D allele of ACE is associated with an increased risk and severity of BPD among preterm infants. In our study, the incidence of I allele and I/I genotype was higher in neonates with RD than in controls. These findings may suggest that the I allele and I/I genotype of the ACE gene increased the risk for RD, and the D allele and D/D genotype could be protective against RD in neonates. However, in our study, there was no relation between the ACE gene polymorphism and primary disorders, duration of hospital stay, ventilation, oxygen consumption, mean arterial pressure and number of patients who required vasopressor in the first day of life.

Pitt and colleagues[6] demonstrated that lung endothelial cells were rich sources of circulating ACE, and therefore the increased activities in infants might be related to rapid lung development that occurred during the last trimester and the immediate postpartum period. These data were supported by the adverse findings of growth retardation and pulmonary hypoplasia with ACE inhibitor medication when prescribed antenatally[27,28]. Lumbers and colleagues[29] demonstrated that maternal use of ACE inhibitor caused decreased fetal lung liquid flow and oligohydramnios. AT-II has a specific growth factor-like effect on target tissue, and serum ACE activities are higher in preterm infants, suggesting that ACE may be more active in the immature lung. This strong association between pulmonary development and serum ACE suggests that ACE or the RAS may be linked to mechanisms controlling pulmonary growth. Taking into consideration that serum ACE activity is increased in the presence of D/D genotype, and the DD genotype is higher in healthy newborns than in neonates with RD, it may be suggested that increased ACE activity is a protective factor from respiratory disorders in the neonatal period.

In our study, we concluded that ACE I/I phenotype increased the risk of RD; however, the D/D genotype may be a protective factor for RD in premature infants. Several genetic polymorphisms have been described in genes of the RAS. These include polymorphisms in the angiotensinogen, ACE, and type 1 angiotensin II receptor (AT1) genes. The ACE I/D polymorphism is the most frequently studied

in this respect[30,31]. The ACE I/D polymorphism influences tissue and plasma ACE activity. The ACE D/D genotype is associated with increased circulating ACE levels, which are generally twice as high as those found for I/I genotypes; I/D or heterozygote genotype is associated with intermediate ACE levels[9,32]. However, there is no evidence of an association of ACE genotypes with circulating AT-II levels[33]. In our study, we could not measure the ACE activity in serum and tissue. Although serum and tissue ACE activity is stipulated to change RAS variably, in the conclusions of our study we speculated that ACE activity in serum and/or bronchoalveolar lavage liquid should be measured in the same patient group.

In conclusion, we found that significant association exists between the development of RD in neonates and the ACE gene I/I genotype. We speculate that ACE D/D genotype is a protective factor for respiratory disorders, but that these neonates with ACE D/D genotype might be at risk for the development of a cardiovascular system disorder later in life. However, the lack of a relationship between the ACE gene polymorphism and the severity of the disease suggests that some factors other than ACE gene polymorphism might be involved in determining the disease severity. Further studies are required for confirmation of our results.

Acknowledgement

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