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Angiotensin-converting enzyme insertion/deletion polymorphism is associated with severe hypoxemia in pediatric ARDS

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Abstract Purpose: The D allele of the insertion/deletion (I/D) polymorphism of a 287-bp sequence in the angiotensin-converting enzyme (ACE) gene has been associated with an increased activity of this enzyme. Its role in susceptibility to acute respiratory distress syndrome (ARDS) has not been well defined. We hypothesized that ACE I/D genotype in pediatrics is associated with ARDS and plasma levels of angiotensin II. **Methods:** Prospective case-control study in patients under 15 years of age from a mixed

Chilean population. Sixty patients with ARDS and 60 controls were included. Association between ACE genotype and ARDS was evaluated as the primary outcome; mortality and severe hypoxemia were examined as secondary outcomes. Plasma angiotensin-II concentration was measured by immunoassay at admission. **Results:** Frequency of ACE I/D genotype was similar in ARDS and control groups ($p = 0.18$). In the ARDS group, severe hypoxemia was less frequent in D allele carriers ($p < 0.05$). Plasma angiotensin-II levels were associated with genotype in the ARDS group, but not controls, being higher in D allele carriers ($p = 0.016$). **Conclusion:** These data do not support the association between ACE I/D genotype and ARDS, although severe hypoxemia was less frequent in D allele carriers. ACE I/D polymorphism modified angiotensin-II levels in pediatric ARDS, but its pathogenic role is not well understood and needs to be addressed in future studies.

Keywords Angiotensin-converting enzyme · Angiotensin II · Acute respiratory distress syndrome · Genetic polymorphism · Pediatrics

Introduction

Acute respiratory distress syndrome (ARDS) is the most severe form of respiratory failure. It is considered the final path for many lung insults, but the exact mechanisms implicated in its development are not completely understood. Eventually, any acute lung insult may result in respiratory failure, but only a small percentage of subjects, even when exposed to the same insult, develop ARDS [1, 2]. On this basis, genetic factors have been associated with increased risk of developing ARDS. Angiotensin-converting enzyme (ACE) is involved in intravascular volume and systemic blood pressure regulation [3]. Experimental data suggest that an increase in ACE activity could be deleterious to patients with ARDS. High levels of ACE and angiotensin (AT) II, its main biological effector, have been associated with increased inflammatory response and impaired lung parenchyma remodeling [4–8]. There has been a growing interest in ACE polymorphisms and risk of ARDS. The D allele of the insertion (I)/deletion (D) polymorphism, a 287-bp fragment in intron 16 of the ACE gene, has been associated with higher plasma and tissue activity of this enzyme in Caucasian populations [9, 10], although there are differences in others ethnic groups [11]. The D allele has been associated with an increased risk of developing ARDS in critically ill adults [12, 13], but this association has not been found in other studies [14]. In children, there is only one study that found no significant difference in D allele frequency between patients with and without acute hypoxic respiratory failure [15].

We hypothesized that the presence of the D allele would increase susceptibility to ARDS in pediatric patients and be associated with higher plasma angiotensin-II concentrations.

Methods

Patients, definitions, and data collection

Approval was obtained from the Ethics Research Committee at Servicio de Salud Metropolitano Surorientado. Written informed consent was obtained from parents or legal guardians according to the Declaration of Helsinki's guidelines. This was a case–control study of pediatric patients with ARDS. Patients were prospectively recruited from a mixed Chilean population consulting at a single center (Hospital Padre Hurtado's Pediatric Service) during the period from 1 March 2008 to 31 July 2009. Inclusion criteria were age up to 15 years and admission due to pulmonary disease. The case group included all patients admitted to the Critical Care Area who met the inclusion criteria of the American-European Consensus Conference (AECC) on ARDS [16]. The control group included patients admitted to the general ward with pneumonia, not

fulfilling ARDS criteria, and for whom the attending physician had requested a blood sample analysis during their hospital stay. Exclusion criteria were presence of congenital cardiac disease, chronic respiratory failure at the time of developing ARDS, immunodeficiency, malignancies, systemic immunosuppressive drug therapy other than corticosteroids, ACE inhibitors use, persistent pulmonary hypertension of the newborn, and corrected gestational age less than 34 weeks at birth. To assess severity in the case group, pediatric index of mortality 2 (PIM2) [17], pediatric logistic organ dysfunction (PELOD) [18], and lung injury score (LIS) [19] were recorded during the first 24 h after ARDS was diagnosed. Severe hypoxemia was defined as persistent $\text{PaO}_2/\text{FiO}_2$ less than 100 mmHg for at least 12 h. Sixty-day mortality and days on mechanical ventilation were recorded as well.

Blood sampling

After informed consent was obtained, blood samples were collected for DNA extraction and plasma storage.

ACE genotyping

Patients were genotyped for the I/D polymorphism using a three-primer method [20] yielding amplification products of 65 bp (I allele) and 84 bp (D allele). The DD genotype was confirmed by a second polymerase chain reaction (PCR), because the D allele is preferentially amplified in heterozygous samples. All DD genotype samples were negative for I allele-specific PCR. Staff involved in patient care and management was blinded to patient genotype throughout the study and personnel involved in genotyping were blinded to case–control status.

Angiotensin-II assay

For AT-II detection in plasma, a commercial AT-II enzyme immunoassay kit from SPI-Bio (Montigny le Bretonneux, France) was used [21]. Total AT-II values were directly calculated on the internal calibration curve and corrected according the original volume of plasma extracted. Personnel involved in AT-II measurement were also blinded to clinical data, genotype, and case–control status.

Data and statistical analysis

Estimated sample size was 60 patients per group to observe a difference of greater than 30% in the allelic frequencies with an α error of 0.05 and a β error no greater than 0.2, based on a known 0.43 D allele

frequency among healthy adult Chileans [22]. The chi-square (χ^2) test was used to compare the observed frequency of each allele and genotype with the expected frequency for a population in Hardy–Weinberg equilibrium and to compare the frequency of alleles and genotypes between the ARDS group and the control group. To evaluate the recessive or dominant effect of the D allele, we independently compared the frequency of D allele carriers (DD and ID) and I allele carriers (II and ID) with homozygous subjects. Secondary outcomes were assessed with χ^2 test, odds ratio (OR), and 95% confidence intervals (CI). Continuous data were evaluated with Anderson–Darling test for normality and Kruskal–Wallis or Student's *t* test were applied as required. Statistical analysis was performed with the Stata[®] program (Statacorp, Texas, USA). For all tests statistical significance was set at a *p* value of less than 0.05.

Results

A total of 60 cases and 60 controls were enrolled and genotyped. Both groups were comparable in their demographic characteristics (Table 1).

Primary outcome: association between genotype and ARDS

D allele frequency was 0.37 in the overall group, similar to that previously reported in a Chilean population [22]. There was no difference in the frequency of D allele which was 0.31 in controls and 0.43 in the ARDS group (*p* = 0.08, OR 1.87, 95% CI 0.84–4.18). The genotype

distribution in control and ARDS groups was not different (*p* = 0.18) (Table 2, top). We found no evidence of statistically significant difference among controls and ARDS subjects with D allele carriers (DD/ID vs. II, *p* = 0.19) and I allele carriers (DD vs. ID/II, *p* = 0.09). Subgroup analysis comparing pneumonia-ARDS and controls did not show difference in genotype distribution and allele frequencies (Table 2, bottom).

Secondary outcomes

Among subjects with ARDS, there was no significant association between ACE genotype and PIM2, PELOD, or LIS scores and days on mechanical ventilation (Table 3). Severe hypoxemia was present in 17% of cases. The I allele was associated with severe hypoxemia (OR 5.33, 95% CI 1.65–20.04) (Table 4).

Angiotensin II

Plasma AT-II levels were 229 (157–319) pg/ml in controls and 134 (18–353) pg/ml in ARDS patients (*p* = 0.15). Subjects homozygous for I allele had lower AT-II plasma levels in the ARDS group than in controls (29 [6–171] and 200 [30–291] pg/ml, respectively; *p* = 0.048). No differences in AT-II levels were found between cases and controls in other genotypes. In analysis within groups, D allele presence was associated with higher AT-II levels in both control and ARDS groups (Fig. 1). There was no significant association between plasma AT-II levels and secondary outcomes (data not shown).

Table 1 Patients' demographic and clinical data

	Controls	Cases	<i>p</i> value
<i>n</i>	60	60	
Age (months)	10 (5–24)	7.5 (2–16)	0.07
Male (%)	60	61	0.9
Pneumonia (%)	100	93	
Viral (%)	47	40	0.22
Bacterial (%)	8	13	0.19
Coinfected (%)	10	22	0.04
No etiology (%)	35	25	0.12
PIM2 (%)	N/A	7.1 (1.7–11.7)	
PELOD (%)	N/A	16 (0.1–23.0)	
LIS	N/A	2.5 (2.5–2.9)	
Days on MV	N/A	4.5 (3–6)	
Severe hypoxemia (%)	N/A	17	
Mortality (%)	0	8.3	0.011

Data are presented as median (interquartile range, IQR) ARDS acute respiratory distress syndrome, PIM2 pediatric index of mortality 2, PELOD pediatric logistic organ dysfunction, LIS lung injury score, days on MV mechanical ventilation (days)

Discussion

Our data do not support an association between the ACE I/D polymorphism and the presence of pediatric ARDS. The D allele frequency was similar in ARDS and control groups. The D allele was associated with increased plasma AT-II levels in cases and controls, but paradoxically with less severe hypoxemia in patients with ARDS.

The D allele has been previously associated with alveolar-capillary interface dysfunction phenotypes [13]. Recent studies in critically ill adults have found a significant association between DD genotype and ARDS prevalence compared to the general intensive care unit (ICU) population and also higher mortality rates [12, 23]. Nevertheless, as in many genetic association studies, there are conflicting data from other negative results [14]. ACE I/D polymorphism has been studied in many diseases in children but only one study, that by Plunkett et al. [15], looked at its association with respiratory failure. It was

Table 2 ACE I/D polymorphism genotype and D allele frequency for control vs ARDS, and control vs pneumonia-ARDS

	DD n (%)	ID n (%)	II n (%)	D allele frequency (95% CI)
Control vs ARDS				
Controls	6 (10)	25 (42)	29 (48)	31 (23–40)
ARDS	11 (18)	29 (48)	20 (33)	43 (34–51)
	$\chi^2 = 3.42$ DF = 2 p value = 0.181			$\chi^2 = 3.52$ DF = 1 p value = 0.061 Fisher's exact = 0.081
Control vs pneumonia-ARDS				
Controls	6 (10)	25 (42)	29 (48)	31 (23–40)
Pneumonia-ARDS	10 (18)	28 (50)	18 (32)	43 (34–52)
	$\chi^2 = 3.6$ DF = 2 p value = 0.164			$\chi^2 = 3.61$ DF = 1 p value = 0.058 Fisher's exact = 0.076

ARDS acute respiratory distress syndrome, 95% CI confidence interval, DF degrees of freedom

Table 3 ACE I/D polymorphism genotype and analysis of severity scores and days on mechanical ventilation of ARDS group

	DD	ID	II	p value
PIM2	8.0 (2.8–10.9)	6.7 (1.7–10.4)	4.4 (1.2–12.1)	0.49
PELOD	1.3 (0.1–9.7)	1.3 (0.1–2.3)	1.2 (0.1–23.0)	0.55
LIS	2.5 (2.3–3.3)	2.5 (2.3–2.7)	2.6 (2.5–3.5)	0.7
Days on MV	4.0 (3.0–6.0)	3.0 (3.0–5.0)	3.5 (3.0–6.5)	0.60

Data are presented as median (IQR)

ARDS acute respiratory distress syndrome, PIM2 pediatric index of mortality 2, PELOD pediatric logistic organ dysfunction score, LIS lung injury score, MV mechanical ventilation

Table 4 ACE I/D polymorphism genotype and analysis of categorical secondary outcomes, severe hypoxemia and 60-day mortality, of patients with ARDS

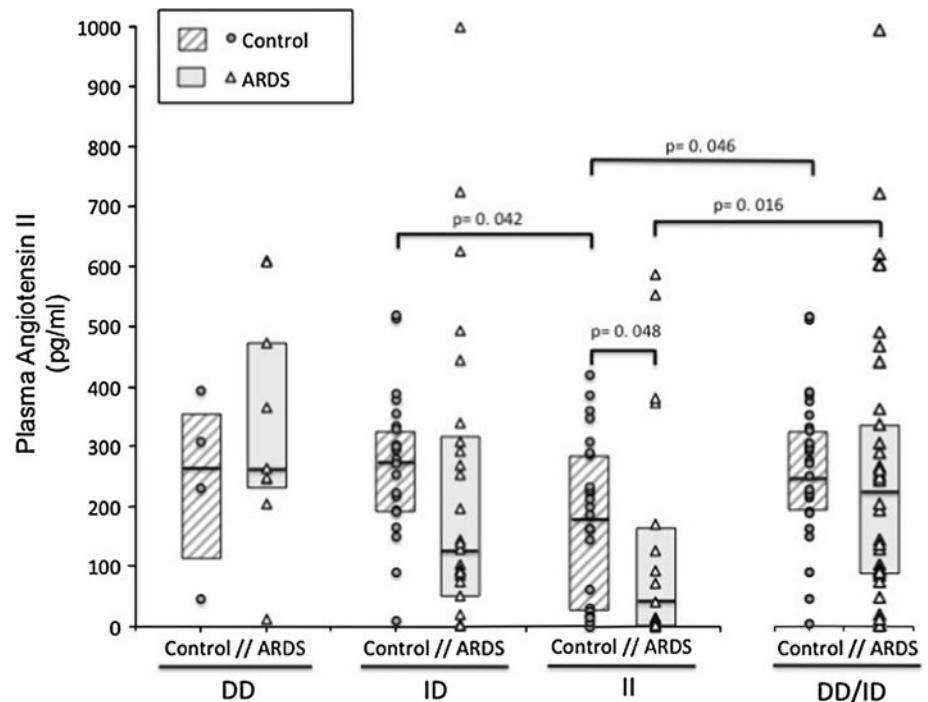
	DD n (%)	ID n (%)	II n (%)		D allele frequency	
Severe hypoxemia						
Yes	1 (10)	3 (30)	6 (60)	$\chi^2 = 14.2$	5 (0.25)	$\chi^2 = 10.4$
No	19 (38)	26 (52)	5 (10)	$p = 0.001$	64 (0.64)	$p = 0.001$
60-day mortality						
Yes	0 (0)	4 (80)	1(20)	$\chi^2 = 2.42$	4 (0.80)	$\chi^2 = 0.028$
No	11 (20)	25 (45)	19 (35)	$p = 0.3$	47 (0.85)	$p = 0.87$

limited to Caucasian populations and concluded that the D allele does not predispose children to acute hypoxemic respiratory failure (AHRF). Patients included in that study were heterogeneous, 54% had infection, and most were admitted for conditions other than pneumonia (encephalitis/meningitis, bronchiolitis, and bacterial septic shock). Also, primary outcome was infrequent, only 14% ($n = 30$) had AHRF and 6% ($n = 13$) had ARDS. Our case definition was children fulfilling AECC criteria for ARDS, focusing on patients with severe lung dysfunction.

AECC criteria have a poor correlation with specific histological findings of ARDS (i.e., diffuse alveolar damage), but at present no better diagnostic test is available, and these criteria are routinely used in clinical practice and in patient selection for clinical trials [24]. Given the socioeconomic characteristics of our population, all but one of the cases and all the controls had an infectious etiology. Despite our different design, case definition, etiologies, and ethnic background, our results are in accordance with Plunkett et al. in reaffirming the lack of association between ACE I/D genotype and pediatric ARDS.

On the basis of secondary outcomes, surprisingly we found that D allele carriers had less severe hypoxemia. As mentioned before, D allele has been associated with increased ACE activity in plasma [10]. But ACE is also present in endothelial cells of lung capillaries as well as other vascular beds, such as the kidneys, brain, heart, and skeletal muscle. Experimental data support that tissue ACE activity may be more important than plasma levels in ALI/ARDS pathogenesis [25, 26]. To date ACE I/D polymorphism association with ACE activity in lung tissue and endothelium has not been studied in humans. In addition, severe hypoxemia and lung dysfunction were not addressed in previous studies of ACE I/D genotype and ARDS, reporting almost exclusively mortality [12, 14, 23]. Two of them found association between D allele presence and higher mortality [12, 23]. We found no significant association between genotype and mortality of patients with ARDS, which is not surprising because this study was not powered to examine this. It is important to note that clinical studies have reported this discrepancy between severe hypoxemia and mortality. Hypoxemia itself is the cause of death in only 9–27% in different series of patients with ARDS, and multiorgan failure is the major cause of the remaining deaths [24, 27, 28]. Mortality was 9% in the ARDS group, similar to other pediatric studies [29, 30] and with

Fig. 1 Levels of plasmatic angiotensin II in control and ARDS subjects homozygous for the deletion (DD), heterozygous (ID), and homozygous for the insertion (II). D allele presence (DD and ID genotypes) is added as a fourth category for comparisons. *Box plots* represent median and IQR (interquartile range)



mortality predicted by PIM2 and PELOD scores. Currently, we do not have enough clinical information to relate specific ACE polymorphisms to a given prognosis for pediatric ARDS. A cohort study will be required to determine this.

Another significant finding is the association between ACE genotype and AT-II plasma levels at admission. As in previous studies, we found a wide distribution of plasma AT-II in all genotypes and both groups, control and ARDS. We measured AT-II as a surrogate of ACE plasmatic activity, being the most important biological effector of the renin-angiotensin system (RAS) in pulmonary circulation. AT-II has been shown to be involved in the development of pulmonary edema, increasing vascular tone and vascular permeability [31, 32]. Several authors described a reduction in ACE plasma level in patients with ARDS [33–36], reflecting lung damage severity (decreased synthesis) and impairment of systemic release of the enzyme [25]. We found lower AT-II plasma levels at admission in subjects with ACE II genotype, being more strikingly reduced in ARDS patients. The former result may be due to a lower ACE activity determined by the ACE I/D polymorphism genotype (genetic factor) and the latter due to an additive effect of the disease.

As pointed out by many authors, conclusions from studies in critically ill adults cannot be extrapolated to the pediatric population, not only because of the underlying pathophysiologic differences between adults and children, but also because ARDS triggering insults in the former are usually extrapulmonary, such as sepsis and trauma,

whereas in children direct pulmonary insult, such as pneumonia, are the main causes of ALI/ARDS cases [37]. In extrapulmonary ARDS, systemic RAS activation leading to increased vascular permeability may play a major role, contrary to primary ARDS in which ACE local activity and AT-II nonvascular effects (induction of coagulation, apoptosis, and fibrosis pathway, generation of reactive oxygen species, chemotaxis of neutrophils, among others) may be more important. Also, the role of AT-II in ARDS may not be limited to the acute phase. Locally activation of RAS and AT-II production may be involved in the fibroproliferative phase of the disease due to profibrotic properties [32]. Reaffirming this hypothesis, a study in preterm infants associated the D allele of the ACE gene with increased risk and severity of pulmonary sequela after a neonatal ARDS [38].

This study has some limitations. The ACE I/D polymorphism has only been demonstrated to be functional in Caucasians [10]. Data linking this polymorphism and ACE activity in other ethnic groups are either lacking or suggest the polymorphism is nonfunctional [32]. As this polymorphism lies within an intron, it is unlikely to be a functional variant itself, but rather in linkage disequilibrium with a functional variant either within the ACE gene or in a neighboring region of the chromosome [39], or alternatively, it may have a regulatory function. Also, the current view of RAS is a complex network of enzymes and substrates, including ACE 2 (homologue enzyme of ACE that acts on different peptide substrates) and other intermediate substrates with synergism or antagonism with AT-II [31, 32]. The final effect of AT-II is related to

a balance between all of these components and is not possible to correlate values with a single effect in vivo. Additionally, AT-II was measured only at admission, so it was not possible to analyze its tendency over time. Power and sample size were not calculated to find differences in mortality.

In conclusion, these data do not support association between ACE I/D genotype and pediatric ARDS, although severe hypoxemia was less frequent in D allele carriers. ACE I/D polymorphism modified AT-II levels in pediatric ARDS, but its pathogenic role is not well understood and needs to be addressed in future trials.

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