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Angiotensin II Type 1 Receptor $A^{1166}C$ Polymorphism and Prophylactic Indomethacin Treatment Induced Ductus Arteriosus Closure in Very Low Birth Weight Neonates

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ABSTRACT

Altered pulmonary vascular resistance might be a factor for delayed closure of the ductus arteriosus (DA) in preterm infants. Angiotensin II plays a central role in the elevation of pulmonary vascular resistance. Angiotensin II exerts its vasoconstrictor effect on the angiotensin II type 1 receptor (AT1R). Homozygous carriers of the AT1R $A^{1166}C$ genetic variant present an exaggerated vasoconstrictor response to angiotensin II. We have investigated whether the presence of AT1R CC^{1166} influences the effect of prophylactic indomethacin treatment on the closure of DA until the fifth postnatal day in preterm infants. In this retrospective study detailed medical history of the first postnatal week was obtained in 159 infants born before the 33rd gestational week. All were treated by prophylactic indomethacin to induce permanent closure of the DA. On the sixth postnatal day the DA was still open in 56, whereas it was permanently closed in 103. The AT1R $A^{1166}C$ genotype of the infants was determined from Guthrie spots. Stepwise binary logistic regression analysis was used to assess the effect of medical conditions and genotype on the risk of patent DA (PDA). Birth weight, infantile respiratory distress, and severe hypotension were independent risk factors for PDA ($p < 0.01$, $p < 0.05$, $p < 0.05$, respectively). The carrier state of AT1R CC^{1166} was protective against PDA ($p < 0.05$; odds ratio, 0.067). AT1R AC^{1166} genotype was not associated with PDA. Our results indicate that the risk of PDA might be lower in infants of AT1R CC^{1166} than in those with AC or AA genotypes. **(*Pediatr Res* 54: 753–755, 2003)**

Abbreviations

AngII, angiotensin II
AT1R, AngII type 1 receptors
AT1R $A^{1166}C$, polymorphism substituting C for A at position +1166 of *AT1R* gene
DA, ductus arterious
PDA, patent ductus arteriosus
ACE, angiotensin-converting enzyme
PKU, phenylketonuria
IRDS, infantile respiratory distress syndrome

Endogenous vasoactive peptides play a central role in the regulation of pulmonary hemodynamics. So far the effect of AngII on the development of pulmonary hypertension has been studied most extensively (1). Evidence suggests that AngII produces proportionately greater vasoconstriction in the pulmonary than in the systemic vascular bed of healthy humans (2). Plasma concentrations of AngII, the main vasoactive component of the renin-angiotensin system, are elevated in pulmonary hypertension and may interact with hypoxemia to cause further pulmonary vasoconstriction.

Animal experiments indicate that the vasoconstrictor effect of AngII is mediated by AT1R in the pulmonary artery (3, 4). Clinical studies also support that specific inhibition of AT1R is associated with reduced pulmonary vascular resistance in adult patients with heart failure (5–7).

Genetic polymorphisms of the *AT1R* gene might influence the vasoconstrictor effect of AngII (8). The polymorphism substituting C for A at position +1166 was related to enhanced systemic vasoconstriction leading to hypertension (9, 10). Data are missing about the impact of the C^{1166} variant of the AngII gene on the effects of AngII at pulmonary vessels, but it seems reasonable to postulate that the presence of this gene polymor-

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phism might be associated with higher pulmonary vascular resistance.

In the neonate the difference between pulmonary and systemic blood pressure influences the complex process of the closure of the DA. Conditions known to be associated with pulmonary hypotension (*i.e.* pulmonary hemorrhage, low $Paco_2$ levels, intrauterine ACE inhibitor exposure) augment the long-term patency of the DA (11). Assuming that the presence of the C^{1166} allele of the *AT1R* gene is associated with elevated pulmonary vascular resistance in the neonate, we have investigated in our pilot study the independent effect of the AT1R genotype on the risk of PDA in preterm neonates.

METHODS

Patients. The study population was selected from a cohort of neonates born between January 1, 1998, and September 1, 2002, at the Second Department of Gynecology and Obstetrics and at the Schöpf Mérei Women's Hospital. The medical history of low-birth-weight neonates during the first 6 postnatal d was analyzed in detail. In this retrospective study the following selection criteria were applied: gestational age $\leq$ 32 wk; prophylactic dose of indomethacin (0.1 $mg \cdot kg^{-1} \cdot d^{-1}$, at least during the first 3 postnatal d); absence of blood transfusion before collecting blood samples for PKU analysis; death before the 28th postnatal d; and excessive volume loading.

The PDA group consisted of 56 neonates in whom echocardiography or clinical signs indicated the presence of PDA after the fifth postnatal day. In these patients, the PDA was closed permanently before the 10th postnatal d in 11 (35%), was closed after repeated indomethacin treatment in 39 (54%), or was resolved by surgical closure in 6 (11%) patients. The non-PDA group consisted of those 103 neonates in whom the DA had been permanently closed before the sixth postnatal day and had not reopened thereafter.

Surfactant need (at least two doses during the first postnatal day) indicated the presence of IRDS. Severe hypotension was defined as need for catecholamine treatment (with dopamine dose of $\geq$ 5 $\mu g \cdot kg^{-1} \cdot d^{-1}$ or dobutamine dose of $\geq$ 9 $\mu g \cdot kg^{-1} \cdot d^{-1}$). Perinatal infection was established by clinical and laboratory signs according to the criteria of Modi *et al.* (12). Neurologic and ultrasound signs indicated the presence of intraventricular bleeding.

The study was approved by an institutional ethical committee. Informed consent of parents was obtained to collect dried blood samples for diagnostic and scientific purposes at the beginning of the therapy at the neonatal intensive care unit. Blood spots were taken on the fifth postnatal day for screening of PKU and hypothyreosis, then were stored at the PKU laboratory of the Metabolic Screening Program. We used remnant dried blood samples for genotyping. The parents of each patient were Hungarian citizens.

Genotyping. The Hb content of samples was denatured by heat inactivation according to the method of Szalai *et al.* (13). For the determination of AT1R $A^{1166}C$ polymorphism, 5'-ATA ATG TAA GCT CAT CCA CCA AGA AG-3' and 5'-TCT CCT TCA ATT CTG AAA AGT ACT TAA-3' primers were used (14). PCR began with denaturation at 94°C (4 min), followed by 35 cycles of denaturation at 94°C (0.5 min), annealing at 50°C (1 min), and extension at 72°C (1 min) and a final extension at 72°C (10 min). PCR products were restricted with Afl-II (New England Biolabs, Beverly, MA, U.S.A.). PCR products were separated on 3% agarose gels and visualized under UV illumination.

Statistical analysis. Clinical characteristics between PDA and non-PDA patients were compared by Mann-Whitney *U* test. Categorical data were analyzed by χ^2 test. Stepwise binary logistic regression analysis was applied to determine the independent effect of birth weight, intraventricular bleeding, Apgar scores, perinatal infection, severe hypotension, IRDS,and AT1R C^{1166} genotype on PDA. The level of significance was set at $p < 0.05$.

RESULTS

Our results are summarized in Table 1. Factors significantly affecting the development of PDA are birth weight (odds ratio [confidence interval]; 0.996 [0.994–0.999], $p = 0.002$), IRDS (2.976 [1.052–8.415], $p = 0.040$), severe hypotension (3.858 [1.348–11.044], $p = 0.012$), and the presence of CC^{1166} (0.067 [0.005–0.821], $p = 0.035$). Apgar scores, intracerebral bleed-

Table 1. *Clinical characteristics and AT1R $A^{1166}C$ genotype of low-birth-weight infants with or without PDA after the fifth postnatal day*

Characteristic	Infants with PDA after the fifth postnatal day ($n = 56$)	Infants without PDA after the fifth postnatal day ($n = 103$)
Permanent closure of DA after fifth postnatal day	0	103
Gestational age (wk) [median, range]	28 [24–32]	30 [25–32]*
Tested factors for PDA at the sixth postnatal day		
Birth weight (g) [median, range]	1100 [650–1500]	1240 [870–1500]*
Apgar 1/Apgar 5	5.75/7.92	6.25/8.25
IRDS	38	55
Severe hypotension†	25	33
Perinatal infection	20	25
Intraventricular hemorrhage stage	21	25
Prevalence of AT1R C^{1166} genotype	0.18	0.27
Number of patients with $AT1R^{1166}$ (AA/AC/CC) genotype	36/20/00	61/29/13*

* Significantly different ($p < 0.05$) between PDA and non-PDA infants.

† Catecholamine treatment (with dopamine dose of $\geq$ 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ or dobutamine dose of $\geq$ 9 $\mu g \cdot kg^{-1} \cdot min^{-1}$).

ing, and perinatal infection were not associated with higher risk in this study.

The prevalence of AT1R C^{1166} alleles was 0.18 in PDA and 0.27 in non-PDA infants ($p < 0.05$). The prevalence of carriers of AT1R C mutant allele was 0.36 in PDA and 0.41 in non-PDA neonates. AT1R CC genotype occurred significantly more frequently in non-PDA than in PDA neonates (13 of 103 *versus* 0 of 56, $p < 0.05$). PDA did not develop in any of the 13 infants with the AT1R CC genotype. No difference was found between the risk factors in infants with or without CC^{1166} genotype (data not shown).

DISCUSSION

Persistent PDA is a frequent complication of immaturity, especially in babies born before the 33rd week of gestation. In this pilot study we have investigated the prevalence and distribution of the AT1R C^{1166} genotype in low-birth-weight infants with PDA and tested whether carrying this genotype is independently associated with PDA risk. Our results show that in addition to immaturity and IRDS, the AT1R CC^{1166} genotype might play an independent role in the development of PDA.

The underlying mechanism is still to be elucidated. Speculatively, there are two possibilities. On the one hand, AngII might have a direct effect on the DA itself. However, data are not available about whether AT1R is expressed on smooth muscle cells of the ductal wall at all. The indirect effect of AngII on the patency of the DA seems to be more reasonable. Literary data indicate that AngII is a potent vasoconstrictor of pulmonary arteries. Preventing the production of AngII, ACE inhibitor therapy leads to pulmonary hypotension in adults (15). Indirect data also support the significance of the renin-angiotensin system in closure of the DA. ACE inhibitor treatment of pregnant women was associated with PDA in the neonate (16).

CONCLUSIONS

AngII exerts its hemodynamic effects mainly on the *AT1R* gene. The AT1R CC genotype was related to a markedly increased response to exogenously given AngII (17), but data supporting the significance of AngII receptor polymorphism in the regulation of pulmonary vascular bed are not available. On the basis of our preliminary results it is tempting to speculate that the AT1R CC^{1166} genotype in neonates born before the 33rd gestational week might be associated with more favorable hemodynamic conditions for the cessation of the left-to-right shunt and for the permanent closure of the DA.

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