Angiotensin-converting enzyme and its insertion/deletion (I/D) polymorphism in women with spontaneous preterm birth

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Mentor: Doc. dr. sc. Sanja Dević Pavlić

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Angiotenzin-konvertirajući enzim i njegov insercijsko/delecijski (I/D) polimorfizam u žena sa spontanim prijevremenim porodom

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SUMMARY

The angiotensin-converting enzyme (ACE) plays crucial role in regulating blood flow to the placenta and promoting angiogenesis. Variations in the ACE gene have been linked to adverse pregnancy outcomes, including preterm birth (PTB), defined as delivery before 37 weeks of gestation. PTB affects approximately 10.6% of live births worldwide, posing significant health risks to newborns and long-term challenges for families. Despite its prevalence, the specific mechanisms leading to PTB remain unclear. Results of existing studies into the insertion/deletion (I/D) polymorphism of the ACE gene have been inconsistent across different populations. To further investigate this relationship, we conducted a case-control study on 120 Croatian women with PTB and 85 control subjects who delivered at term, using polymerase chain reaction for genotyping. Additionally, we performed comprehensive literature review conducted to gather studies that investigated association of ACE I/D polymorphism and PTB, yielding a total of six studies that we included in the meta-analysis. The case-control study found no significant genetic differences between the PTB and control groups. However, the meta-analysis showed a modest but statistically significant association between the II and DD genotypes, suggesting that individuals with the II genotype may have a lower risk of PTB. Although our study suggests that the ACE I/D polymorphism does not significantly contribute to PTB risk, the observed association in the meta-analysis emphasizes the need for further research. Given the serious consequences of PTB on neonatal health and family well-being, understanding genetic factors like I/D ACE polymorphisms is crucial for developing targeted interventions and improving maternal and neonatal outcomes.

KEYWORDS: angiotensin-converting enzyme, genetics, insertion/deletion polymorphism, meta-analysis, preterm birth

SAŽETAK

Angiotenzin-konvertirajući enzim (ACE) igra ključnu ulogu u regulaciji protoka krvi prema posteljici i poticanju angiogeneze. Varijacije u ACE genu povezane su s nepovoljnim ishodima trudnoće, uključujući prijevremeni porod (PP), koji se definira kao porod prije 37. tjedna gestacije. PP zahvaća približno 10,6% sve živorođene djece diljem svijeta, predstavljajući značajan rizik za zdravlje novorođenčadi i dugoročne posljedice za obitelji. Unatoč visokoj učestalosti PTB-a, točni mehanizmi koji do njega dovode još uvijek nisu razjašnjeni. Dosadašnja istraživanja o povezanosti insercijsko/delecijskog (I/D) polimorfizme ACE gena i PP-a dala su nedosljedne rezultate u različitim populacijama. Kako bismo dodatno istražili navedenu povezanost, proveli smo istraživanje na ukupno 120 žena s PP-om i 85 kontrolnih ispitanica koje su rodile u terminu iz Hrvatske, koristeći lančanu reakciju polimeraze za genotipizaciju. Dodatno, proveli smo i sveobuhvatan pregled literature s ciljem pronalaženja svih do sad provedenih istraživanja povezanosti ACE I/D polimorfizma i PP-a, koji je rezultirao uključivanjem ukupno šest studija u meta-analizu. Istraživanjem provedenim na Hrvatskoj populaciji nisu utvrđene značajne razlike u frekvencijama genotipova i alela između skupine s PP-om i kontrolne skupine. Međutim, meta-analiza je pokazala graničnu, ali statistički značajnu povezanost pri usporedbi II i DD genotipova, sugerirajući da bi osobe s II genotipom mogle imati manji rizik od PP-a. Iako naše istraživanje pokazuje da ACE I/D polimorfizam ne doprinosi značajno riziku od PP-a, uočena blaga povezanost u meta-analizi ukazuje na potrebu za daljnjim istraživanjima. S obzirom na ozbiljne posljedice PP-a na zdravlje novorođenčadi i dobrobit obitelji, razumijevanje genetičkih čimbenika, poput I/D ACE polimorfizma ključno je za razvoj ciljanih intervencija i poboljšanje ishoda za majku i dijete.

KLJUČNE RIJEČI: angiotenzin konvertirajući enzim, genetika, insercijsko/ delecijski polimorfizam, meta analiza, prijevremeni porod

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1. INTRODUCTION

1.1. Preterm birth: etiology, classification, and genetic influences

Preterm birth (PTB), defined as delivery before 37 weeks of gestation or before 259 days from the start of a woman's last menstrual cycle, poses a considerable global public health issue (1). Data from 107 countries estimated that in 2014, approximately 10.6% of live births worldwide (14.84 million) were preterm (2). The high occurrence of PTB and its severe effects on newborns and their families make it an important focus of attention. PTB is recognized as a major risk factor for adverse health outcomes in both the short and long term. In the short term, it is a leading cause of neonatal illness and death, often resulting in hospitalization within the first year of life (3,4). Over time, PTB is linked to an increased risk of conditions such as hypertension, cardiovascular and cerebrovascular diseases, type 2 diabetes, chronic kidney disease, respiratory issues like asthma and lung dysfunction, and neurocognitive problems. Furthermore, PTB is tied to higher health care expenses and socioeconomic challenges later in life (5).

The causes of PTB are multifactorial, involving both environmental and genetic factors. Genetic influences on PTB are underscored by its recurrence in families and high recurrence rates in women (6). Research highlights the genetic influence on spontaneous PTB, showing that while paternal genes have minimal impact, maternal and fetal genetics play significant roles. Estimates indicate fetal genetics account for 5% to 13.1% of spontaneous PTB variation, while maternal genetics contribute 20.6% to 25% (7). Additionally, women with a personal or family history of premature birth face a higher risk (7,8). Furthermore, twin studies suggest that the heritability of PTB ranges from 17% to 36%, emphasizing the genetic contribution to this complex condition (6). Therefore, our study focuses exclusively on maternal genetic factors in relation to spontaneous PTB.

The World Health Organization (WHO) categorizes PTB into the following groups based on gestational age: extremely preterm (<28 weeks), very preterm (28 to <32 weeks), and moderate or late preterm (32 to <37 weeks)(9). Between 2010 and 2020, around 15% of all preterm births worldwide occurred before 32 weeks of pregnancy, requiring more advanced neonatal care. Specifically, about 4.2% of these births occurred before 28 weeks, while 10.4% were between 28 and 32 weeks (3). This classification helps in understanding the diverse clinical and genetic factors that contribute to PTB.

Clinically, PTB can be either spontaneous or medically indicated. Spontaneous PTB occurs as a result of preterm labor (PTL) accompanied by cervical dilation or preterm premature rupture of membranes (PPROM). In contrast, medically indicated PTB is initiated by obstetricians due to complications, which can occur even in the absence of labor or PPROM. Recent studies indicate that the underlying causes of spontaneous and medically indicated late PTB frequently overlap, with vascular abnormalities in placental blood flow being a common contributing factor (9,10).

Various mechanisms involved in PTB are linked to different clinical conditions that trigger labor. These mechanisms include placental abruption causing decidual hemorrhage, maternal conditions such as cervical insufficiency and uterine overdistension, maternal stress activating the hypothalamic-pituitary-adrenal axis, infections (both intra- and extrauterine), inflammation without infection, and PPROM, which serves as a final common pathway to PTB (3) (Figure 1.).

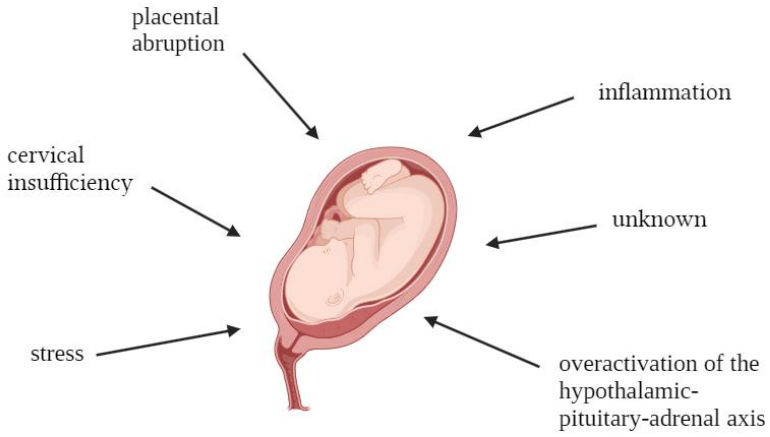

Figure 1. Various potential pathways leading to preterm birth (Adapted from Samuel TM et al. by BioRender software) (3).

Although the precise molecular mechanisms of labor initiation are not fully understood, it is widely recognized that the labor cascade involves spontaneous activation of pro-inflammatory pathways, resulting in various clinical conditions. At the molecular level, this process is mediated by progesterone and includes the activation of progesterone receptor-B (PR-B) and its less active nuclear isoform, progesterone receptor-A (PR-A). It is essential to sustain the balance between inflammation and progesterone throughout labor (3,6).

Genetic predispositions for PTB represent a complex area of research that encompasses various mechanisms and influences. In candidate gene studies, researchers have identified more than 750 SNPs within more than 240 genes affiliated with risk of PTB and gestational length (11). These genes are often involved in processes such as inflammatory reactions, immune responses, tissue remodeling and metabolic function (12).

One of the important mechanisms that have been investigated is the inflammatory response during pregnancy. Labor is believed to be triggered

in part by a shift from a balanced inflammatory state to a pro-inflammatory state (12). Examples of genes that have been shown to be associated with PTB include toll-like receptors (TLR1, TLR2, TLR7), which have a crucial role in pathogen recognition. Polymorphisms in these genes are associated with an increased risk of premature birth both in the mother and in the newborns (11,12). In addition, research has focused on the gene encoding pro-inflammatory and anti-inflammatory cytokines. For instance, interleukin 6 (IL-6) and interleukin 10 (IL-10) are frequently investigated for their role in regulating inflammatory responses (12). Polymorphisms in these genes are associated with PTB, suggesting that individual differences in these genes may influence the likelihood of PTB. Studies has also shown the importance of genes involved in tissue remodeling, such as MMP-9 (matrix metalloproteinase 9) COL1A1 (collagen type I), which play a key role in structural changes during pregnancy. These genes are associated with mechanisms that allow the cervix to soften and prepare the body for childbirth. Metabolic pathways are also of importance; such examples are insulin-like growth factor 1 (IGF1) and IGF2, which are important for fetal growth and development. Polymorphisms in these genes are connected to elevated risk of PTB, which increases the role of metabolic homeostasis in maintaining pregnancy (11,12).

Given all these findings, although candidate gene studies have provided significant insights, results are often inconsistent due to small samples and lack of repeated analyses (12). Further research, including larger studies with carefully selected phenotypes, is needed to enhance the identification of women at elevated genetic risk for PTB. Understanding genetic predispositions could help develop new strategies for the prevention and management of PTB.

Understanding the genetic risk factors associated with PTB is critical for unraveling its etiology and developing preventive strategies. Despite significant research into genetic polymorphisms related to PTB, clear associations with high-risk cases remain elusive (5). This underscores the

necessity for continued research and the development of personalized approaches to prevent and manage this complex medical condition.

1.2. Angiotensin-converting enzyme (ACE)

Angiotensin-converting enzyme (ACE) is essential in human physiology due to its multifaceted role in several systems. It is directly involved in the renin-angiotensin-aldosterone system (RAAS), the kinin-kallikrein system, the breakdown of amyloid-beta peptides in vitro, the activity of glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD), and signal transduction (13).

ACE is zinc dipeptidyl carboxypeptidase and can be found in different isoforms, somatic and testicular, which differ in structure and function (Figure 2.)(14). The somatic isoform (sACE, 150–180 kDa) can be found in endothelial, epithelial and neuroepithelial cells and smaller testicular isoform (tACE, 90–110 kDa) can only be found in male germinal cells. sACE isoform contains two catalytic domains, N-terminal domain (ACE_N) and C-terminal domain (ACE_C), that are responsible for the degradation of different peptides, while the tACE isoform has only one active center (15).

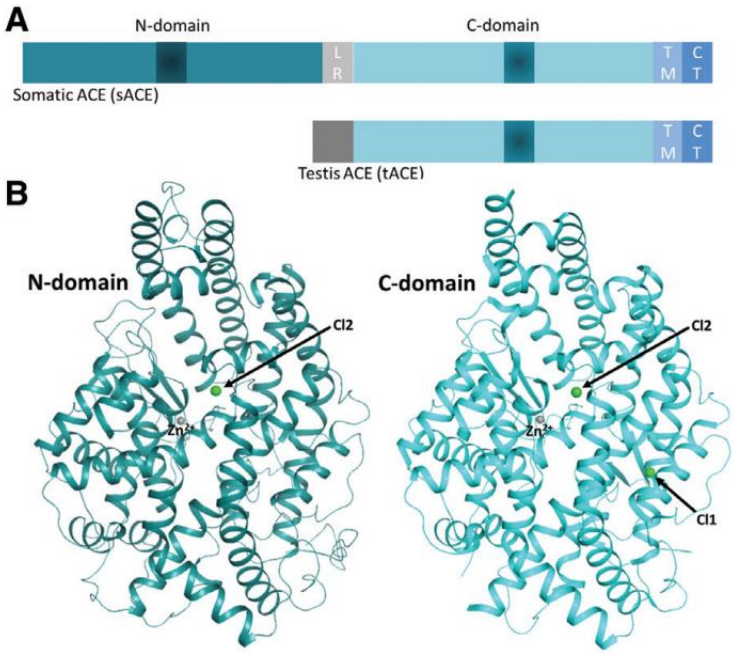

Figure 2. Structure of human ACE (A) Diagram illustrating the structure of ACE isoforms, highlighting the N-domain (left) and C-domain (right) (B) Crystal structures of N-domain ACE (PDB code: 2C6F) and tACE (PDB code: 1O8A) (Taken from Masuyer G. et. al.) (14).

ACE plays crucial role in RAAS (Figure 3.). When ACE comes into contact with angiotensin I, an inactive decapeptide, it acts on C-terminal end of the peptide and removes two amino acid residues, creating the active octapeptide angiotensin II. This process is crucial in the control of blood pressure, because angiotensin II acts as a powerful vasoconstrictor, narrowing blood vessels and elevating blood pressure. RAAS is also crucial for body fluid balance (16).

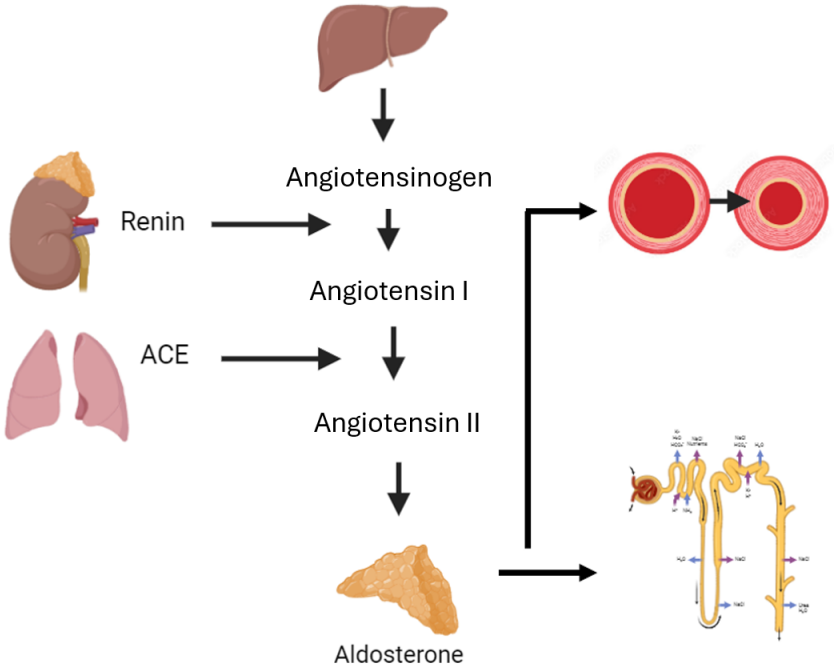

Figure 3. Schematic Representation of the Renin-Angiotensin-Aldosterone System (RAAS) (Adapted from Patel S. et al. by BioRender software) (16).

In the kinin-kallikrein system, ACE breaks down bradykinin, turning it into inactive metabolites. Bradykinin increases the permeability of blood vessels, causes pain and participates in inflammatory processes. ACE inactivates bradykinin, which reduces its effects such as dilation of blood vessels, inflammation and pain. Thus, ACE controls the duration and intensity of bradykinin effects (13,15).

Amyloid-beta peptides are proteins that accumulate in the brain in Alzheimer's disease and form harmful plaques. ACE can degrade these peptides under laboratory conditions. ACE catalyzes the breakdown of amyloid-beta peptides by removing certain parts from them, thus reducing their amount and potential harm; potential treatment (15,17).

Studies suggests that ACE can also modify the activity of GPI-PLD. ACE may be associated with modification or regulation of GPI-PLD activity through

various mechanisms, including changes in cell signaling or interactions with other proteins (17).

1.3. ACE insertion/deletion (I/D) polymorphism

The ACE gene is located on chromosome 17q23, spans 20.5 kb, and consists of 26 exons and 25 introns of varying length (Figure 4.)(18). Within this gene, which encodes for a functional enzyme, there are multiple genetic variations that can influence its function and regulation referred to as polymorphisms and mutations.

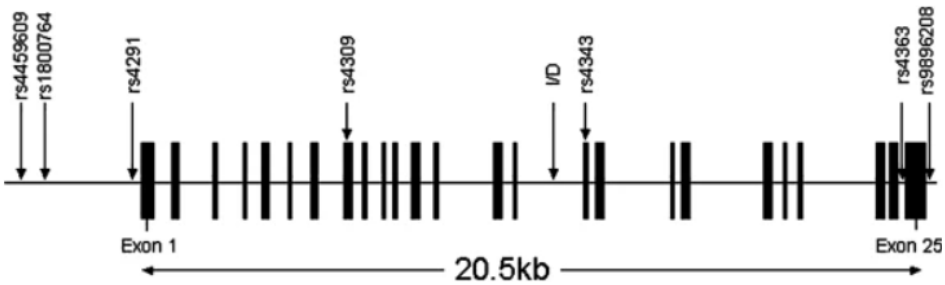

Figure 4. Diagram of the ACE gene showing the distribution of exons, introns and several polymorphisms: rs4459609: A>C, rs1800764: C>G/C>T, rs4309: C>T, rs4343: A>G, rs4363: G>A/G>C, rs9896208: T>A/T>C (Taken from Bell et al.) (19).

Gene polymorphisms represent variations in the DNA sequence that occur in a relatively high frequency, usually above 1% population and can have various functional effects (20). They can influence different traits such as susceptibility to diseases, drug metabolism, and responses to environmental factors, ultimately contributing to genetic diversity and individual variability in physiology and health outcomes. These variations can include changes in a single nucleotide (known as SNP - single nucleotide

polymorphisms), as well as longer sequences that can be duplicated, deleted or rearranged (21).

On the other hand, a mutation is a change in the DNA sequence that occurs as a result of an error in DNA replication or exposure to mutagenic agents. Mutations are relatively rare and usually occur at a lower frequency than polymorphisms and are often considered abnormal or pathological. These can have serious functional effects, such as disorders or diseases, by causing changes in protein structure or function. Examples of mutations include genetic disorders such as cystic fibrosis and Huntington's disease (22).

Polymorphisms and mutations play a crucial role in genetic diversity and can significantly affect an individual's phenotype, including enzyme function. Polymorphism can cause changes in the amino acid sequence of an enzyme, which can affect its three-dimensional structure and, consequently, its function. Polymorphisms can also affect the level of enzyme expression. Variations in the promoter or other regulatory regions of a gene can lead to increased or decreased enzyme production. A third way polymorphisms affect is by changing the way the enzyme reacts with its substrate. If the polymorphism causes a change in the active site of the enzyme, it can result in reduced or increased affinity for the substrate, which directly affects the speed and efficiency of the chemical reaction that the enzyme catalyzes (23). Moreover, polymorphisms can also affect drug metabolism, which can result with various clinical consequences. For example, variations in genes encoding drug-metabolizing enzymes can result in different responses to therapy, including side effects or reduced drug efficacy (23).

According to the National Center for Biotechnology Information (NCBI), over 160 ACE gene polymorphisms have been identified, with the majority being single nucleotide polymorphisms. Of these polymorphisms, only 34 are located in coding regions, and 18 represent missense mutations (24). ACE gene SNPs (examples in Figure 4.) can affect the expression of the enzyme

and its function and thus lead to numerous changes in the physiology of the enzyme. Such modifications have different consequences and risks for the development of the diseases (Table 1).

Table 1. ACE polymorphisms and their association with pathological conditions in humans.

ACE SNP	Disease Association	Reference
rs4291	Alzheimer's disease	(25,26)
rs4309	Alzheimer's disease	(25)
rs4340	Metabolic syndrome Type 2 diabetes	(27,28)
rs4343	Preeclampsia Alzheimer's disease SARS-CoV-2 infection	(25,29) (30)
rs4363	Myocardial infarction	(31)
rs1800764	Severe neuropathy in type 1 diabetes	(32)
rs9896208	Severe neuropathy in type 1 diabetes	(32)
rs1799752	Hypertension Migraine Coronary Artery Disease (CAD) Cancer Diabetes Diabetic Nephropathy Alzheimer's Disease Pregnancy Complications SARS-CoV-2 infection	(8,33–37)
rs12449782	Myocardial infarction	(31,38)

Previous research shows that some ACE SNPs, especially rs12449782 and rs4363, are potentially associated with the risk of myocardial infarction, but these effects are complex and dependent on interactions with other genetic variants and environmental factors (31). The research also confirmed the association of SNPs rs1800764 and rs9896208 with the progression of severe neuropathy in type 1 diabetes (32). Moreover, it has been found that the rs4343 polymorphism is linked with an increased risk of preeclampsia in pregnant women. SNPs rs4343, rs4291 and rs4309 have been connected with the development of Alzheimer's disease, but a recent meta-analysis did not find a significant link between these SNPs and the development of Alzheimer's disease (25). In addition to all of the above, ACE SNPs have also been associated with SARS-CoV-2 infection (30), hypertension (8), metabolic syndrome, excess body weight (27), and the risk of type 2 diabetes (39). Some ACE SNPs increase susceptibility to migraine, suggesting a possible genetic component in the development of this condition (40).

The most notable and extensively researched polymorphism of the ACE gene, the I/D polymorphism (rs1799752), has been identified as a critical genetic variation that influences ACE plasma levels and activity (41,42). The ACE I/D polymorphism consists of the presence or absence of a 287-base fragment within intron 16 of the ACE gene, resulting in three possible genotypic combinations: II (homozygous for the I allele), ID (heterozygous) and DD (homozygous for the D allele) (43). The insertion allele of ACE gene that features an insertion of 287 pb Alu repetitive sequence in intron 16, is associated with lower ACE plasma activity. Deletion allele does not have this insertion and is associated with higher ACE plasma activity. The DD genotype produces the highest levels of ACE in plasma, the ID genotype results in moderate levels, and the II genotype yields the lowest levels (44).

ACE I/D polymorphism are connected with various conditions, including cardiovascular diseases, asthma and cancer. Studies have shown that the DD genotype of the ACE enzyme is associated with an increased risk of

coronary heart disease (CAD)(45). ACE polymorphisms are also associated with arterial hypertension and proteinuria (46). These findings suggest that the polymorphism may be useful for predicting response to therapy (34).

Recent research has also focused on the impact of ACE polymorphisms on the severity and outcome of COVID-19 disease. The research showed that certain polymorphisms of ACE2, which is related to ACE, could influence the clinical picture of the disease, especially in younger patients (37). These results indicate a complex interaction between genetic variation and infections (47,48).

ACE polymorphisms have also been investigated in the context of other diseases, including diabetes (36), Alzheimer's disease (49), and cancer (35,50). The association between the I/D polymorphism and diabetic nephropathy suggests that genetic variation may influence disease progression and response to treatment (51).

Considering its connection to the RAAS and different pregnancy complications, dysregulated ACE activity has also been associated with negative pregnancy outcomes, such as PTB, which is a major risk factor for infant mortality (52).

Taking the above into account, genotyping of the ACE I/D polymorphism has an important role in researching the association between genetic variants and predispositions to different diseases, such as hypertension and other cardiovascular disorders. Various research has demonstrated that the D allele may be connected with an increased risk of these conditions, while the I allele may have a protective effect (45). These findings contribute to a clearer insight of the genetic factors that influence health and can help in the development of personalized approaches to the treatment and prevention of diseases.

1.4. ACE I/D polymorphism in preterm birth

The ACE I/D polymorphism has been a central point of numerous scientific investigations, particularly concerning its association with pregnancy and the potential adverse effects on both maternal health and newborn outcomes. This genetic variant, which involves an insertion or deletion in the ACE gene, has been linked to various health complications during pregnancy and after delivery.

Earlier studies have established a connection between the ACE I/D polymorphism and health issues in children born prematurely. Notably, Harding et al. (2003) found that the DD genotype, which is associated with increased ACE activity, plays a significant role in the development of preterm cardiorespiratory diseases (53). Following this, in 2005, the same researchers explored the relationship between the ACE I/D polymorphism and the motor and cognitive development of children born prematurely. Their investigation aimed to determine whether any of the ACE polymorphisms were linked to developmental challenges in these children and considered the potential of ACE inhibitors as a neuroprotective treatment. However, this study did not find any statistically significant correlations (54).

In addition to these findings, a wealth of research has examined the implications of the ACE I/D polymorphism for women during pregnancy. Studies have established associations between this polymorphism and conditions such as preeclampsia, infertility, endometrial cancer and more. For instance, González-Garrido et a. (2017) demonstrated that the D allele significantly raises the risk of developing preeclampsia among Mexican women, aligning with the observation that the DD genotype correlates with heightened ACE enzyme activity (55). The hypothesis has also been confirmed by studies on the Chinese population (56).

The ACE I/D polymorphism is also suspected to contribute to infertility, primarily due to its involvement in RAAS regulation. Dysregulation of RAAS

can lead to complications such as thrombophilia, preeclampsia, and recurrent pregnancy loss. The RAAS may influence these complications through the fibrinolytic pathway, which affects blood clotting and vascular function. Furthermore, the ACE I/D polymorphism, in conjunction with other molecules like PAI-1 and AT1R, may significantly impact these processes, potentially leading to fertility issues (57). Moreover, evidence suggests that the ACE I/D polymorphism increases the likelihood of developing polycystic ovary syndrome (PCOS) at an early age and is significantly associated with acanthosis, a marker of insulin resistance (58).

Additionally, previous studies have also explored the potential link between the I/D polymorphism of the ACE gene and PTB, but the results have been inconsistent across different populations.

Valdez et al (2007) found a notable association between D allele and increased risk of PROM in Mexican population (59). Similarly, Uma et al. (2008) reported a correlation between the DD genotype and PTB in a Caucasian group (60). Conversely, Uvuz et al. (2009) did not observe any significant links between the ACE gene I/D polymorphism and PTB in a Turkish population (61).

A meta-analysis focusing on Asian populations suggested that the ID genotype of the ACE gene I/D polymorphism may have a protective effect against PTB (62). However, another meta-analysis focusing on Slovenian cohort found no significant association between ACE I/D polymorphisms and PTB (63). These discrepancies highlight the complexity of genetic influences on PTB and suggest that further research is necessary to clarify these associations and their implications.

Since the relationship between ACE I/D polymorphisms and PTB remains contested, this thesis centers on further elucidation of its role in this specific pathological state. Some studies, particularly those conducted in Asian population (62), have found significant associations between the ACE polymorphism and PTB risk, while others have shown no correlation (63).

These discrepancies may be due to genetic diversity between populations, environmental factors, or variations in study design. By concentrating on the Croatian population, this study aims to fill the gaps in existing research, providing a clearer understanding of how ACE gene polymorphisms may contribute to PTB risk in this specific population. This research will further explore the underlying mechanisms by which ACE activity may influence PTB. As ACE is involved in the regulation of blood pressure and vascular tone, its role in placental function and uterine environment during pregnancy is of particular interest. Understanding mechanisms of dysregulation of ACE levels at a molecular level may reveal how specific ACE genotypes interact with other risk factors, such as inflammation, infection, or maternal stress, to trigger early labor.

Moreover, if particular ACE genotypes are identified as increasing the likelihood of PTB, this information could be applied to improve prenatal care by allowing for earlier identification of women at higher risk. These women could then benefit from targeted interventions, such as closer monitoring or specialized treatments aimed at preventing premature labor. This approach aligns with the growing emphasis on personalized medicine, where genetic insights help tailor medical care to individual risk profiles. The findings from this research may contribute to our ability to predict and prevent PTB and also support the development of more targeted and effective prenatal care practices, ultimately improving maternal and neonatal health outcomes.

2. AIMS

The primary aim of this thesis is to investigate the association ACE I/D polymorphisms and the risk of preterm birth, through a combined approach of a case-control study and a meta-analysis. The ACE I/D polymorphism, which affects the regulation of angiotensin II and its impact on blood pressure and vascular function, is hypothesized to contribute to an increased risk of preterm birth due to its influence on placental development and maternal hemodynamics.

Specific aims of the thesis were:

- To determine and compare the frequencies of genotypes and alleles of the ACE I/D polymorphism between individuals who experienced preterm birth and a healthy control group in the Croatian population.
- To assess the relationship between different genotypes and alleles of the ACE I/D polymorphism with susceptibility to preterm birth across various genetic models in the Croatian population.
- To perform a meta-analysis of existing studies to assess the overall association between ACE I/D polymorphisms and preterm birth across different populations.
- To statistically analyze and compare the findings from both the case-control study and meta-analysis, evaluating the role of the ACE I/D polymorphism in contributing to preterm birth.

3. MATERIALS AND METHODS

3.1. Case-control study

3.1.1. Subjects

A total of 205 subjects were included in the analysis: 120 patients diagnosed with PTB and 85 controls (women at ≥37 weeks gestation). Blood samples were collected in cooperation with doctors from Clinical hospital center (KBC) Rijeka after childbirth. All participants in this case-control study were Croatian, having given written informed consent prior to their involvement. Inclusion criteria for test group comprised women who underwent spontaneous labor before completing 37 weeks of gestation. Controls were healthy mothers of similar age who had an uncomplicated pregnancy, delivered after 37 weeks, and had neonates with appropriate-for-gestational-age birth weight.

3.1.2. DNA extraction

For DNA analysis, leukocyte-isolated DNA from maternal blood postpartum was utilized by Flexigene method. We started by preparation of reagents, including proteinase dissolution in FG3 buffer and preparation of the FG2/proteinase mixture, which must be used within one hour. After the blood is dissolved and heated in a water bath to 65°C, it is mixed with FG1 buffer, centrifuged, and the supernatant is removed. The pellet is then treated with FG2/proteinase mixture and homogenized by vortexing.

After incubation at 65°C, the DNA is precipitated with isopropanol, centrifuged, washed with ethanol, and air-dried. Last, the precipitate is dissolved in FG3 buffer, incubated at 65°C and transferred to a test tube for further analysis. Genotyping of the ACE I/D polymorphism was performed using polymerase chain reaction (PCR).

3.1.3. Genotyping

Genotyping was conducted using PCR, a key technique in molecular biology used to amplify specific DNA sequences. In the context of ACE I/D

polymorphism genotyping, PCR enables the detection of different alleles that can have a significant impact the risk of various diseases.

After genomic DNA isolation, PCR is performed using specific oligonucleotide primers that are designed to amplify the region containing the polymorphism. A standard PCR protocol includes three main stages: denaturation, annealing and extension. In the denaturation phase, the DNA sample is heated to a high temperature (94-96 °C), which causes the DNA double strands to separate. In the annealing phase, the temperature is reduced (50-65 °C) to allow binding of primers to complementary DNA sequences. Finally, in the extension phase, the temperature is increased to 72 °C, which allows Taq polymerase to synthesize a new strand of DNA, using the existing strands as a template (64). Protocol of temperature cycles used in the PCR for the ACE I/D polymorphism is shown in Table 2.

Table 2. Protocol of temperature cycles used in the PCR for the ACE I/D polymorphism.

Step	Temperature	Time	Cycles number
Denaturation	94°C	5 min	1
Hybridization	94°C	1 min	30
	60°C	1 min	
	72°C	2 min	
Elongation	72°C	5 min	1

The PCR reaction was conducted in a total volume of 10 µL. Content of the mixture for PCR analysis of ACE I/D polymorphism for one sample is shown in Table 3.

Table 3. Content of the mixture for PCR analysis of ACE I/D polymorphism for one sample.

	quantity (1 sample)
10 × PCR buffer	1 µl
25 mM MgCl	0,6 µl
10 mM dNTP	0,2 µl
10 x primer F	0,5 µl
10 x primer R	0,5 µl
Bidistilled water	6,07 µl
Taq polymerase	0,13 µl
DNA	0,2 µl

PCR amplification was carried out using a Mastercycle Personal thermal cycler, Eppendorf (Hamburg, Germany) with elongation cycle repeating 30 times.

After PCR amplification, the products are analyzed by agarose gel electrophoresis. This method enables the separation of amplified fragments according to their size. Fragments of different lengths correspond to different genotypes. Visualization of the results is usually done under UV light after staining the gel with ethidium bromide or other dyes that bind to DNA (64).

PCR products were separated on agarose gel using electrophoresis and then were visualized (Figure 5). Visualization was done under UV light after staining the gel with ethidium bromide. We recognized the II genotype on an agarose gel as a single large fragment. In this genotype, the DNA sequence has the insertion polymorphism that would alter the size of the amplified DNA fragment. The II genotype is typically observed at approximately 490 bp. We saw DD genotype on an agarose gel as a single

smaller fragment, which represents a PCR product that is shorter due to the presence of a deletion polymorphism. The DD genotype is typically observed at approximately 190 bp. In the case of the ID polymorphism, we were able to see two distinct fragments, which represent a combination of the first two genotypes.

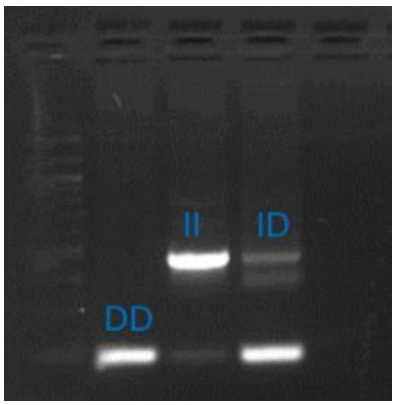

Figure 5. The gel after visualization with UV light: three different genotypes are distinguished: DD, II, ID (left to right).

3.1.4. Statistical analysis

Statistical data analysis was performed using Statistica software for Windows, version 12 (StatSoft, Inc., Tulsa, OK, USA) and MedCalc for Windows, version 14.12.0. (MedCalc Software, Mariakerke, Belgium. We calculated the frequency of alleles, as well as the frequency of individual genotypes. A Chi-square test was conducted to examine the association between preterm delivery and specific allele and/or genotype.

3.2. Meta-analysis

3.2.1. Search strategy, study selection and data extraction

We initiated the literature selection process for the meta-analysis by searching the PubMed, Google Scholar, and Scopus databases using keywords and their combinations, such as "preterm birth," "ACE enzyme," and "ACE polymorphism."

After the initial database search, we reviewed the search results and selected relevant literature based on predefined inclusion criteria. The exclusive criteria for exclusion were studies that did not provide data on the frequency of genotypes and/or alleles or did not adhere to the Hardy-Weinberg equilibrium (HWE) in control groups ($p<0.05$).

Data were thoroughly extracted from the selected studies, including sample sizes, genotype distributions, and demographic information.

3.2.2. Conduction of meta-analysis

The meta-analysis of data was performed using Comprehensive Meta-Analysis 3.0 software, a powerful tool designed for conducting systematic reviews and meta-analyses. This software facilitates the integration of data from multiple studies, allowing for a comprehensive evaluation of the overall effect size and the assessment of heterogeneity among studies.

To begin, we entered the relevant data from each selected study, including sample size and genotype frequencies. The software supports various statistical models, enabling us to choose between fixed-effects and random-effects models based on the level of heterogeneity observed in the data. We assessed heterogeneity using the I^2 statistic, which quantifies the percentage of variation across studies that is attributable to heterogeneity rather than chance.

In addition to calculating pooled odds ratios and confidence intervals, CMA 3.0 provided tools for conducting sensitivity analyses to evaluate the robustness of our findings.

Furthermore, we performed subgroup analyses to explore potential differences in effect sizes based on demographic factors, such as ethnicity and geographical location. This comprehensive approach allowed us to identify patterns and draw more nuanced conclusions regarding the association between the ACE gene I/D polymorphism and preterm birth.

Overall, the use of Comprehensive Meta-Analysis 3.0 software significantly enhanced the rigor and clarity of our analysis, ensuring that our findings are both reliable and informative for future research in this area.

4. RESULTS

4.1. Case-control study

4.1.1. Subjects

120 PTB and 85 control subjects participated in the study. Clinical characteristics of those two tested groups are shown in the Table 4.

Table 4. Maternal characteristics of participants in PTB and control groups

	Cases (N=120)	**Controls** (N=85)	**P**
Mean age at delivery / median (range)	33 (20-45)	34 (20-45)	0.092
Prepregnancy BMI / median (range)	24 (17-44)	24 (16-39)	1.000
Gestational age at delivery / median (range)	34 (23-36)	39 (37-41)	<0.001
Extremely preterm <28 weeks / N (%)	6 (8.1)		
Very preterm 32-28 weeks / N (%)	5 (6.8)		
Moderate to late preterm 32-36 weeks / N (%)	63 (85.1)		
Smoking during pregnancy			
Yes / N (%)	16 (13.3)	14 (16.4)	0.531
No / N (%)	104 (86.7)	71 (83.6)	

There was no statistically significant difference of the mean age between tested groups (mean age at delivery for the PTB group was 33 years, while for the controls, it was 34 years, with a p-value of 0.092. Pregnancy BMI was identical for both groups (24), with the p-value of 1.000. Of course, the gestational age at delivery significantly differs between the groups, with an average gestational age of 34 weeks for cases compared to 39 weeks for controls, with a p-value less than 0.001. Regarding preterm births, 6 cases (8%) were extremely preterm (<28 weeks), 5 cases (7%) were very preterm (28-32 weeks), while 63 cases (85%) were moderate to late

preterm (32-36 weeks). In relation to smoking during pregnancy, the percentage of smokers was 16% in the controls and 13% in the cases, with a p-value of 0.531 indicating that there is no significant difference between the groups.

4.1.2. Genotyping

After PCR was conducted under conditions display in table 1. and table 2., our samples were visualized on 11 gels using UV light after PCR analysis. Figure 6. shows example of one of the gels obtained after visualization under UV light. Shorter fragment represents the II genotype, the larger fragment represents the DD genotype, while the ID genotype is distinguished as a combination of these two genotypes.

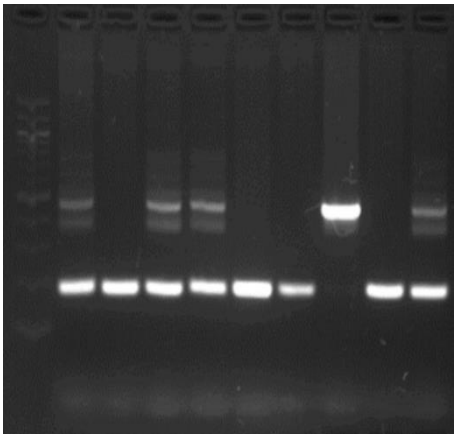

Figure 6. The example of a gel after visualization with UV light: three different genotypes (DD, II, ID) are clearly visible.

Genotyping results were obtained for a total of 205 subjects from the Croatian population, of which 85 were controls and 120 subjects in the PTB group (Table 5). In the control group, 42 (49%) subjects had the ID genotype, 30 (35%) of them had the DD genotype, while 13 subjects had the II (14,5%) genotype. In the PTB group, 59 (50%) subjects had the ID

genotype, 39 (31%) subjects had the DD genotype, and 22 (19%) subjects had the II genotype. The analysis of allele frequencies revealed that in the PTB group the frequencies of alleles I and D were 43% and 57%, while in the control group they were 40% and 60%. To investigate the association between ACE polymorphisms and PTB, χ^2 tests were performed. The chi-square value was 0.39, and the p- value was 0.83. This p-value is significantly higher than 0.05, which indicates that there is no statistically significant difference between the expected and actual frequencies in the analyzed groups.

Table 5. Frequencies of genotypes and alleles of the ACE I/D polymorphism in PTB and control groups. Results of ACE I/D polymorphism genotyping.

	PTB mothers N (%)	**Controls N (%)**	**χ2**	**OR (95% CI)**	**p**
Alleles					
I (%)	103 (40%)	68 (43%)	0,34	0.83 (0,52-1,34)	0,56
D (%)	137 (60%)	102 (57%)			
Genotype					
ID (%)	59 (50%)	42 (49%)	0,39	0,80 (0,51-1,26)	0,83
II (%)	22 (19%)	13 (14,5%)			
DD (%)	39 (31%)	30 (35%)			

Furthermore, analysis was performed using different genetic models, including allelic, dominant, recessive, and overdominant models, to investigate the potential association between the ACE I/D SNP and the risk of PTB (Table 6.).

Table 6. Association of ACE I/D polymorphism with the risk of PTB under different genetic models.

GENETIC MODEL		OR (95% CI)	p	χ^2
Allelic model	I vs. D	0.92 [0.78;1.16]	0.8255	0.0486
Dominant model	DD + ID vs. II	1.27 [0. 58;2.56]	0.6635	0.1892
Recessive model	DD vs. ID+II	0.84 [0.69;1.02]	0.6618	0.1913
Overdominant model	II+DD vs. ID	1.02 [0.59;1.79]	1.0	0.0

In the allelic model, the odds ratio (OR) for allele I versus allele D was 0.92, indicating that there is no significant difference between these two alleles in the risk of PTB. Similar results were reported in the dominant model, where the OR was 1.27, which also suggests a lack of statistically significant association between genotypes and risk of PTB. In the recessive model, the OR was 0.84, while the overdominant model showed an OR of 1.02. These results confirm that none of the examined genetic models indicate a significant influence of ACE polymorphisms on the risk of premature birth.

Based on the results of the χ^2 test (Table 4.), we cannot reject the null hypothesis (H_0) which states that there is no statistically significant association between ACE polymorphisms and preterm birth. P-values indicate that there is no evidence to support the alternative hypothesis (H_1) of the existence of a significant association. Overall, the results suggest that ACE genetic polymorphisms do not play a significant role in the risk of preterm birth in the analyzed populations.

4.2. Meta-analysis

A thorough and systematic literature search was performed using the PubMed, Google Scholar, and Scopus databases, yielding 5902 articles. After removing duplicates, non-English articles, and non-relevant publications such as reviews, case reports, book chapters, and incomplete publications like conference abstracts, 77 articles remained for evaluation based on their titles and abstracts. Of these, 43 articles were excluded due to the lack of data on genotype and allele frequencies or failure to meet the Hardy-Weinberg equilibrium (HWE) criteria in control groups ($p < 0.05$). The detailed process of the literature search is illustrated in the PRISMA flow diagram presented in Figure 7. Ultimately, 5 articles were deemed eligible for data extraction on sample sizes, genotype distributions, and demographic information, and were included in the meta-analysis.

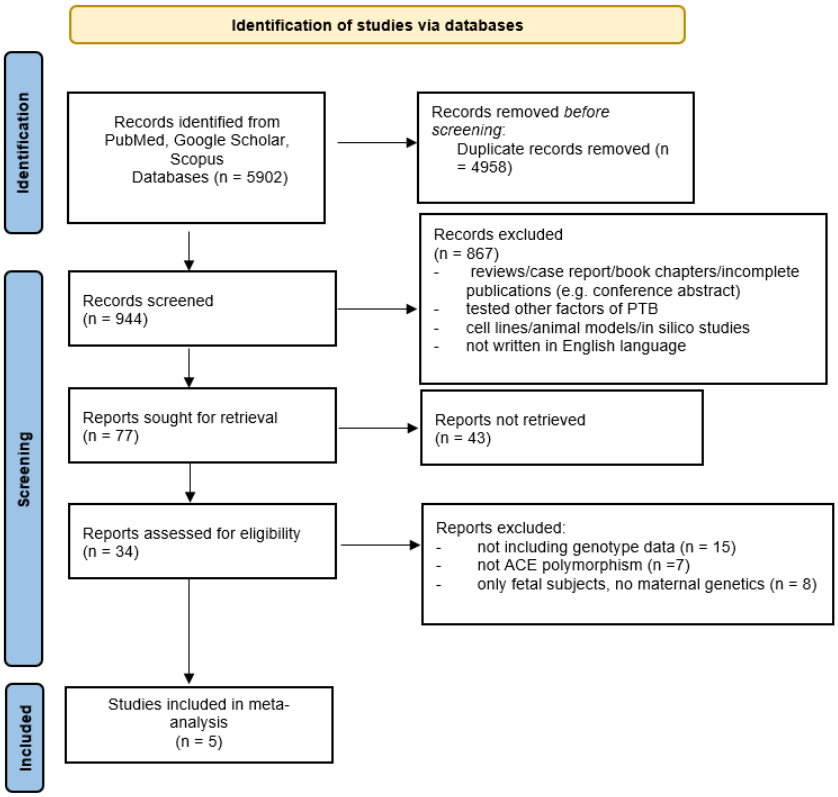

Figure 7. PRISMA flow diagram showing illustrating the study selection process in this meta-analysis. (Figure done by PRISMA flow diagram generator software)

After screening and selection, our meta-analysis included data from a total of 600 PTB mothers and 602 controls (incorporating our case-control study) (Table 7.). This expanded sample size provided greater statistical power to investigate the association between ACE genetic polymorphisms and preterm birth risk.

Table 7. An overview of studies linking ACE I/D polymorphism to PTB included in the meta- analysis.

Authors	Year	Country	PTB	Control	Conclusion	P
Valdez et al.	2004	Mexico	288	89	D allele increased risk of PROM	0,02
Uma et al.	2008	UK	18	83	DD allele increased risk of PTB	0,04
Uvuz et al.	2009	Turkey	50	50		0,40
Hočevar et al.	2019	Slovenia	217	158		0,15
Noo Ri Lee et al.	2019	Asia	111	143	ID genotype protective	0,03
Our study	2024	Croatia	120	85		0,83

We examined the association between a genetic variant and preterm birth (PTB) using various genetic models: allelic, genotypic (all three genotypes), dominant, recessive, and overdominant. The findings of the meta-analysis are presented in Table 8. and Figure 8.

Table 8. Meta-analysis of ACE I/D polymorphism in association with PTB under different genetic models. (Statistic was done by Comprehensive Meta-Analysis 3.0 software)

	I vs D		**II vs DD**		**II vs ID**		**DD vs ID**		**II vs DD + ID**		**DD+ II vs ID**		**ID+ II vs DD**	
Study	OR [CI]	P	OR [CI]	P	OR [CI]	P	OR [CI]	P	OR [CI]	P	OR [CI]	P	OR [CI]	P
Hočevar et al	0,772 [0,577-1,033]	0,082	0,564 [0,310-1,029]	0,062	0,634 [0,367-1,097]	0,103	0,124 [0,697-1,810]	0,632	0,608 [0,362-1,021]	0,060	0,897 [0,595-1,352]	0,605	0,785 [0,595-1,352]	0,294
Noo Ri Lee et al	1,148 [0,7981-1,651]	0,456	1,000 [0,467-2,142]	1.000	1,774 [1,014-3,000]	0,044	1,744 [0,821-3,704]	0,148	1,525 [0,917-2,534]	0,104	1,744 [1,054-2,886]	0,030	0,744 [0,367-1,508	0,412
Uma et al	1,509 [0,739-3,082]	0,259	1,130 [0,253-5,042]	0,872	1,237 [0,347-4,406]	0,743	1,094 [0,309-3,868]	0,889	1,204 [0,359-4,040]	0,764	1,161 [0,418-3,228]	0,775	0,971 [0,292-3,235]	0,962
Uvuz et al	0,585 [0,332-1,031]	0,064	0,542 [0,186-1,575]	0,260	1,306 [0,799-2,136]	0,288	0,546 [0,320-0,931]	0,026	0,777 [0,410-1,471]	0,438	0,997 [0,539-1,845]	0,922	0,598 [0,221-1,616]	0,311
Valdez et al	0,776 [0,586-1,029]	0,079	0,475 [0,229-0,984]	0,045	0,678 [0,355-1,292]	0,238	1,426 [0,789-2,578]	0,240	0,604 [0,327-1,166]	0,108	0,997 [0,607-1,636]	0,989	0,643 [0,366-1,129]	0,124
Our study	1,362 [0,902-2,057]	0,142	1,372 [0,594-3,172]	0,459	1,205 [0,546-2,659]	0,645	0,878 [0,471-1,637]	0,682	1,269 [0,599-2,680]	0,534	0,977 [0,599-1,705]	0,934	1,194 [0,661-2,156]	0,556
Pooled	0,906 [0,778-1,056]	0,206	0,718 [0,516-1,000]	**0,050**	1,205 [0,734-1,515]	0,773	1,029 [0,726-1,458]	0,874	0,903 [0,629-1,296]	0,580	1,080 [0,876-1,345]	0,493	0,803 [0,618-1,043]	0,100

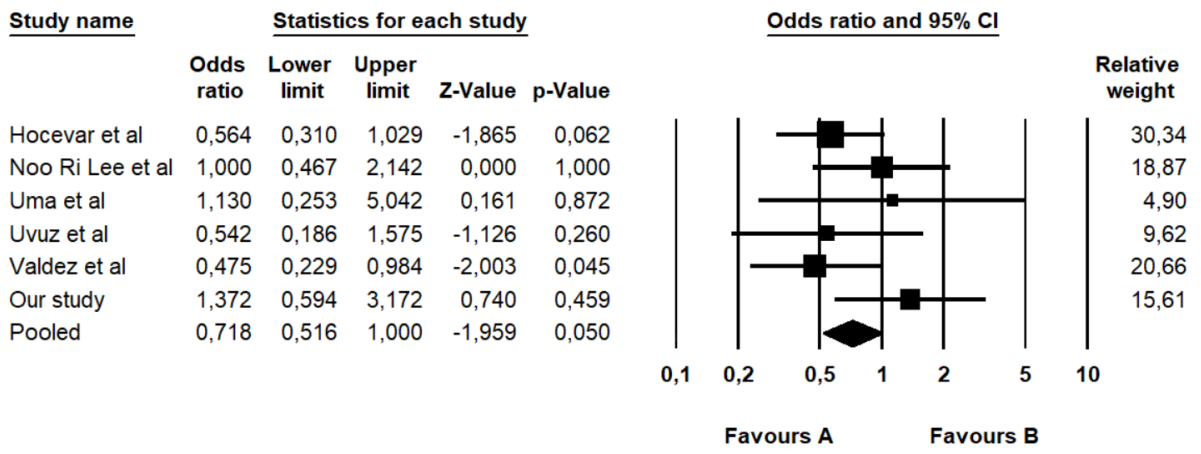

Figure 8. Forest plot of ACE II vs. DD genotype in association with PTB (Statistic was done by Comprehensive Meta-Analysis 3.0 software)

The results indicate a marginal statistical significance in the genotypic model comparing the II genotype to the DD genotype. Our analysis yielded a p-value of 0.050, which is borderline significant, with a 95% confidence interval of 0.718 [0.516-1.000]. This suggests a potential association between the II genotype and a reduced risk of preterm birth compared to the DD genotype.

In the allelic, dominant, recessive, and overdominant models, no statistically significant differences were observed between the PTB group and the control group. The p-values in these models did not reach the threshold for statistical significance, indicating that the genetic variant may not have a strong association with preterm birth when analyzed using these alternative models.

In addition to our previous analyses, we conducted a further investigation by focusing to Caucasian populations to explore potential differences specific for its demographic. The results of this analysis are detailed in Table 9.

Table 9. Meta-analysis of ACE I/D polymorphism in association with PTB in Caucasian population. (Statistic was done by Comprehensive Meta-Analysis 3.0 software)

	I vs D		**II vs DD**		**II vs ID**		**DD vs ID**		**II vs DD + ID**		**DD+ II vs ID**		**ID+ II vs DD**	
Study	OR [CI]	P	OR [CI]	P	OR [CI]	P	OR [CI]	P	OR [CI]	P	OR [CI]	P	OR [CI]	P
Hočevar et al	0,772 [0,577-1,033]	0,082	0,634 [0,367-1,097]	0,103	0,634 [0,367-1,097]	0,062	0,124 [0,697-1,810]	0,632	0,608 [0,362-1,021]	0,060	0,897 [0,595-1,352]	0,605	0,785 [0,595-1,352]	0,294
Uma et al	1,509 [0,739 -3,082]	0,259	1,237 [0,347-4,406]	0,734	1,237 [0,347-4,406]	0,872	1,094 [0,309-3,868]	0,889	1,204 [0,359-4,040]	0,764	1,161 [0,418-3,228]	0,775	0,971 [0,292-3,235]	0,962
Uvuz et al	0,585 [0,332-1,031]	0,064	0,848 [0,437-1,644]	0,625	1,306 [0,799-2,136]	0,260	0,546 [0,320-0,931]	0,026	0,777 [0,410-1,471]	0,438	0,997 [0,539-1,845]	0,922	0,598 [0,221-1,616]	0,311
Valdez et al	0,766 [0,586-1,029]	0,076	0,678 [0,355-1,292]	0,238	0,678 [0,355-1,292]	0,045	1,426 [0,789-2,578]	0,240	0,604 [0,327-1,166]	0,108	0,997 [0,607-1,636]	0,989	0,643 [0,366-1,129]	0,124
Our study	1,362 [0,674-1,201]	0,142	1,205 [0,546-2,659]	0,645	1,205 [0,546-2,659]	0,459	0,878 [0,471-1,637]	0,682	1,269 [0,599-2,680]	0,534	0,977 [0,599-1,705]	0,934	1,194 [0,661-2,156]	0,556
Pooled	0,899 [0,674-1,201]	0,472	0,791 [0,579-1,081]	0,142	0,834 [0,531-1,308]	0,429	1,031 [0,717-1,482]	0,868	0,866 [0,006-1,672]	0,068	0,945 [0,690-1,294]	0,722	0,921 [0,653-1,298]	0,638

Our findings did not reveal any statistically significant differences between the PTB group and the control group in any of the models when examining the Caucasian population. Furthermore, the p-values obtained did not meet the criteria necessary for rejecting the null hypothesis, indicating that there is insufficient evidence to suggest a difference between these groups.

5. DISCUSSION

Research on the association between the I/D polymorphism of the ACE gene and PTB brings interesting insights, especially considering the inconsistent findings in the literature so far. Despite extensive research, the precise genetic causes contributing to PTB remain unclear, highlighting the complexity of this condition.

Our results from the case-control study suggest that the ACE I/D polymorphism does not have a significant impact on the risk of PTB within the Croatian population. Statistical analysis showed that the p-value obtained was notably higher than the limit for significance ($p < 0.05$) for both genotypes and alleles. This indicates a lack of evidence to support a meaningful association between the ACE I/D polymorphism and PTB in this population. Furthermore, the odds ratio calculated in our analysis was not statistically significant, reinforcing the conclusion that the presence of this polymorphism does not correlate with the risk of PTB in the studied cohort.

Previous studies conducted on the ACE I/D polymorphism in the Croatian population have various results. One study reported an association between the deletion homozygote genotype (DD genotype) of the ACE gene and hypertension within the Croatian population (65). Conversely, another study examining the Croatian population found a significantly higher prevalence of D allele carriers among the elderly compared to the general population, suggesting that the ACE D allele may contribute to better health and longevity (66). These findings indicate a complex relationship between the ACE I/D polymorphism and various health conditions within the Croatian population.

Furthermore, our results indicate no statistically significant difference in the distribution of genotypes and alleles between the control and PTB groups. These findings differ from those of Uma et al (2008), who reported an association between the DD genotype and an increased risk of PTB.

However, it is important to note that their conclusions may have been affected by limitations in their study design, particularly the small sample size, with only 18 participants in the case group (60). Similarly, our findings are not aligned with those of Valdez et al (2004), who identified a connection between the D allele and a heightened risk of PROM (59). Uvuz et al (2009) also suggested that women carrying the D allele may be predisposed to PTB, although they did not find a statistically significant association (61). On the other hand, Noo Ri Lee et al. found that the ID genotype may have a protective effect, further highlighting the variability in study outcomes (62).

The observed frequencies of both genotypes and alleles in our study groups closely align with the expected frequencies for a population of this size, as predicted by Hardy-Weinberg equilibrium. This consistency confirms that the genetic composition of our control group accurately reflects the general population, ensuring the reliability of our comparisons. The fact that our control group follows Hardy-Weinberg equilibrium strengthens the validity of our findings, as it suggests that there is no selection bias within our sample, allowing for more generalizable conclusions.

This, together with previously stated lack of statistically significant difference in the distribution of genotypes and alleles between the control and PTB groups, supports the conclusion that the PTB group does not show abnormal or unusual patterns in the distribution of genotypes and alleles compared to the control group or the general population.

Using different genetic models: the allelic model (I vs. D), the dominant model (DD + ID vs. II), the recessive model (DD vs. ID + II), and the overdominant model (II + DD vs. ID), we found no statistically significant associations. Specifically, the odds ratios (OR) for the allelic model, the dominant model, the recessive model, and the overdominant model suggest lack of significant differences between the PTB group and the control group. The p-values obtained from these analyzes were above the conventional

significance criteria, further supporting our conclusion that ACE polymorphisms do not play a key role in PTB risk. In a similar analysis, case-control study by Hočevar et al (2018) reported borderline statistical significance in the dominant model in Slovenian population, indicating a potential weak association between the II genotype and a reduced risk of PTB. They found that all other genetic models analyzed did not demonstrate statistical significance (63). Furthermore, a case-control study by Noo Ri Lee et al (2019) identified statistical significance in the overdominant model in Korean women, suggesting that individuals carrying the ID genotype may experience a protective effect against PTB. Notably, Noo Ri Lee et al (2019) did not find significant associations in other genetic models, highlighting the complexity and variability of the relationships between ACE I/D polymorphisms and PTB risk across different populations (62).

Given the inconsistency of previous results regarding the association between the ACE I/D polymorphism and PTB, we decided to conduct a meta-analysis to clarify this relationship. There are two existing meta-analysis with conflicting findings. Hočevar et al (2018), identified a positive association between the D allele and increased PTB risk is Slovenian population while Noo Ri Lee et al (2019) reported a protective effect associated with the ID genotype in Korean population (62,63). Given the observed differences in genetic associations between populations, we also wanted to perform a statistical analysis that excluded the Asian population. The goal of this approach was to explore whether a more consistent pattern would emerge specifically within the Caucasian population.

In our own meta-analysis, which combined findings from six studies, including our case-control study, we observed borderline significance in one genotypic model, suggesting that individuals with the II genotype might have a reduced risk of PTB compared to those with the DD genotype. Although this observation hints at a possible protective effect of the II genotype, the overall evidence remains inconclusive. This finding aligns with meta-analysis performed by Hočevar et al (2018) who also found that

individuals carrying the D allele or the DD genotype are at an elevated risk for PTB (63). Additionally, studies by Valdez et al (2004) and Uma et al (2008) also support the theory that D allele and DD genotype are associated with an increased risk of PTB (59,60). In study by Rahimi et al (2013), homozygous deletion genotype (DD) has been identified as a risk factor for abnormal placentation (67). Fazelnia et al (2016) proposed that the DD genotype of the ACE gene is significantly associated with spontaneous recurrent pregnancy loss (68). Similarly, Yang et al (2012) demonstrated that the presence of the D allele in the ACE gene I/D polymorphism increases the risk of spontaneous recurrent pregnancy loss (69).

Furthermore, our analysis, which excluded data from the Asian population, revealed consistent results within the Caucasian population, highlighting the reliability of our findings across different demographic groups.

Collectively, these findings suggest a pattern where the I allele may have a protective effect, while the D allele is more frequently associated with adverse pregnancy outcomes, such as PTB, abnormal placentation, and recurrent pregnancy loss. The consistent association between the D allele and these complications indicates that genetic factors, particularly those related to the ACE gene, may play a significant role in determining pregnancy outcomes. However, while the association is compelling, it is important to note that these findings also highlight the complexity of the genetic influence on pregnancy, as evidenced by the inconclusiveness of certain models and the variability in risk across different populations and studies.

In addition, functional studies investigating the biological mechanisms through which ACE polymorphisms may influence pregnancy outcomes are needed. The RAS plays a crucial role in regulating uteroplacental circulation and placental vascularization, and its disruption has been linked to impaired placentation. One potential mechanism through which the ACE I/D polymorphism may influence the risk of preterm birth (PTB) involves insufficient blood flow in the placenta (70,71). The ACE DD genotype, in

particular, has been suggested to affect uteroplacental and umbilical circulation, potentially increasing the risk of adverse pregnancy outcomes, especially in women with a history of preeclampsia. Uteroplacental ischemia is a known risk factor for spontaneous PTB, accounting for roughly 15% of cases, and studies have shown that failure in the transformation of spiral arteries is common in patients with preterm labor. Understanding the role of ACE in placental circulation and angiogenesis could provide valuable insights into its potential impact on PTB (70–72).

Limitations of our case-control study and our meta-analysis should also be taken into account. In the case-control study, we must consider the size of the sample. Our case- study included a relatively small number of participants, which may affect the power of the analysis and the possibility of detecting statistically significant associations. Smaller samples can lead to false negative results, where real associations remain undetected. Likewise, there is a possibility of bias in the selection of participants, considering that only patients from certain health institutions are included. This may limit the generalizability of the results to the wider population.

One limitation of our meta-analysis is the variability between the included studies. The use of different methodological approaches across studies can contribute to differences in the results and introduce heterogeneity. Different populations, definitions of PTB and methods of analysis may make it difficult to draw definitive conclusions. The quality of individual studies included in a meta-analysis may vary. Low quality studies can distort the overall results and reduce the reliability of the meta-analysis. Also, it may be difficult to compare the results if different statistical approaches and models were used in the studies. Furthermore, there is a possibility that studies with positive results are more likely to be published, which may lead to bias in the available data and affect the conclusions of the meta-analysis.

Another significant limitation is that external factors such as age, comorbidities, socioeconomic status, smoking, alcohol consumption, and

others, were not considered in our analysis, similar to the two existing meta-analyses on this topic (62,63). While we analyzed the statistical significance of some external factors in Croatian study population (BMI, age, smoking during pregnancy), we didn't conduct an overall analysis that considers these factors in pooled populations together with the presence of specific alleles or genotypes. We didn't investigate how the interactions between these external factors and genetic variables (specific allele or genotype) might influence the results. This could imply that we might be missing the combined effect of these factors on the outcomes, which could be important in the context of gene-environment interactions. Integrating genetic, environmental, and social factors may provide a comprehensive framework for understanding PTB and enable the development of strategies aimed at reducing risk and improving the health of pregnant women and newborns.

Further research is essential for several key reasons. First, the inconsistency in existing findings on the relationship between the ACE I/D polymorphism and PTB highlights the complexity of genetic factors in pregnancy outcomes. While some studies suggest that the D allele or DD genotype may increase the risk of PTB, others point to a protective role of the ID genotype (59,60,62). These conflicting results, seen even across meta-analyses, suggest that the genetic influence on PTB is not straightforward and likely involves interactions with other genetic or environmental factors. Without more comprehensive research, it is difficult to draw definitive conclusions or create effective strategies for predicting or preventing PTB based on genetic data alone.

Second, many previous studies did not fully account for external factors that can influence pregnancy outcomes, such as age, comorbidities, socioeconomic status, and lifestyle choices like smoking and alcohol consumption. These factors could interact with genetic predispositions in ways that either increase or decrease the risk of PTB. For instance, the effect of the ACE I/D polymorphism might vary depending on the overall

health or lifestyle of the mother, or other underlying genetic conditions that were not assessed (73). To better understand how these interactions contribute to PTB risk, future research should adopt a more holistic approach, integrating both genetic and environmental variables.

Furthermore, investigating the biological mechanisms underlying the relationship between the ACE I/D polymorphism and pregnancy outcomes is critical. Disruptions in RAS system could lead to conditions such as preeclampsia or placental insufficiency, both of which are risk factors for PTB. Understanding how specific genotypes affect RAS function could help explain why some individuals are more susceptible to PTB (70). This knowledge would not only clarify genetic associations but also gave the way for potential therapeutic interventions targeting the RAS to reduce PTB risk.

Lastly, the impact of PTB on neonatal and long-term health outcomes underscores the importance of identifying risk factors and preventative strategies. PTB is associated with higher rates of infant morbidity and mortality, as well as developmental delays and chronic health conditions later in life (4,5). Understanding the genetic and environmental contributors to PTB could lead to more personalized approaches to prenatal care, enabling healthcare providers to identify women at higher risk and tailor interventions to improve maternal and infant health outcomes. In the long term, this could help reduce the overall incidence of PTB and its associated complications, ultimately improving public health outcomes.

In summary, further research is vital not only to resolve the current inconsistencies in the literature but also to develop a more comprehensive understanding of how genetic and environmental factors combine to influence PTB risk. This integrated approach has the potential to lead to more effective preventive strategies and better health outcomes for mothers and their babies.

6. CONCLUSION

Our study did not find a statistically significant association between the ACE I/D polymorphism and the risk of PTB, neither in the case-control study conducted on the Croatian population nor in the meta-analysis that combined data from all previously conducted studies.

Based on our specific research objectives, the following conclusions can be drawn:

- There were no statistically significant differences in the frequencies of the ACE I/D polymorphism genotypes and alleles between the PTB group and the healthy control group ($p > 0.05$) in the Croatian population.
- No statistically significant associations were found between different genotypes and alleles of the ACE I/D polymorphism and PTB when assessed across various genetic models ($p > 0.05$) in the Croatian population.
- Findings from our meta-analysis indicate a borderline significance in the genotypic model, suggesting that individuals with the II genotype might have a reduced risk of PTB.
- However, the overall evidence remains inconclusive, as previous studies showed varying results regarding the association between ACE I/D polymorphisms and PTB risk.

This research contributes to the understanding of the genetic factors influencing preterm birth, particularly within the Caucasian population, by providing evidence that the ACE I/D polymorphism may not significantly affect PTB risk. The findings highlight the importance of further genetic and environmental research, which could ultimately inform clinical practices and public health strategies aimed at reducing the incidence of preterm births.

7. REFERENCES

1. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. The Lancet. 2016 Dec;388(10063):3027–35.

2. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health. 2019 Jan;7(1):e37–46.

3. Samuel TM, Sakwinska O, Makinen K, Burdge GC, Godfrey KM, Silva-Zolezzi I. Preterm Birth: A Narrative Review of the Current Evidence on Nutritional and Bioactive Solutions for Risk Reduction. Nutrients. 2019 Aug 6;11(8):1811.

4. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller AB, et al. Born Too Soon: The global epidemiology of 15 million preterm births. Reprod Health. 2013 Nov 15;10(S1):S2.

5. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. The Lancet. 2008 Jan;371(9608):261–9.

6. Mead EC, Wang CA, Phung J, Fu JY, Williams SM, Merialdi M, et al. The Role of Genetics in Preterm Birth. Reproductive Sciences. 2023 Dec 14;30(12):3410–27.

7. York TP, Eaves LJ, Lichtenstein P, Neale MC, Svensson A, Latendresse S, et al. Fetal and Maternal Genes' Influence on Gestational Age in a Quantitative Genetic Analysis of 244,000 Swedish Births. Am J Epidemiol. 2013 Aug 15;178(4):543–50.

8. Do AN, Lynch AI, Claas SA, Boerwinkle E, Davis BR, Ford CE, et al. The effects of genes implicated in cardiovascular disease on blood pressure response to treatment among treatment-naive hypertensive African Americans in the GenHAT study. J Hum Hypertens. 2016 Sep 21;30(9):549–54.

9. Wadon M, Modi N, Wong HS, Thapar A, O'Donovan MC. Recent advances in the genetics of preterm birth. Ann Hum Genet. 2020 May 19;84(3):205–13.

10. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. The Lancet. 2016 Dec;388(10063):3027–35.

11. Myking S, Myhre R, Gjessing HK, Morken NH, Sengpiel V, Williams SM, et al. Candidate gene analysis of spontaneous preterm delivery: New insights from re-analysis of a case-control study using case-parent triads and control-mother dyads. BMC Med Genet. 2011 Dec 30;12(1):174.

12. Mead EC, Wang CA, Phung J, Fu JY, Williams SM, Merialdi M, et al. The Role of Genetics in Preterm Birth. Reproductive Sciences. 2023 Dec 14;30(12):3410–27.

13. Skeggs LT, Kahn JR, Shumway NP. THE PREPARATION AND FUNCTION OF THE HYPERTENSIN-CONVERTING ENZYME. J Exp Med. 1956 Mar 1;103(3):295–9.

14. Masuyer G, Yates CJ, Sturrock ED, Acharya KR. Angiotensin-I converting enzyme (ACE): structure, biological roles, and molecular basis for chloride ion dependence. Biol Chem. 2014 Oct 1;395(10):1135–49.

15. Tzakos AG, Galanis AS, Spyroulias GA, Cordopatis P, Manessi-Zoupa E, Gerothanassis IP. Structure-function discrimination of the N- and C- catalytic domains of human angiotensin-converting enzyme: implications for Cl- activation and peptide hydrolysis mechanisms. Protein Engineering Design and Selection. 2003 Dec 1;16(12):993–1003.

16. Patel S, Rauf A, Khan H, Abu-Izneid T. Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies. Biomedicine & Pharmacotherapy. 2017 Oct;94:317–25.

17. Masuyer G, Yates CJ, Sturrock ED, Acharya KR. Angiotensin-I converting enzyme (ACE): structure, biological roles, and molecular basis for chloride ion dependence. Biol Chem. 2014 Oct 1;395(10):1135–49.

18. Hubert C, Houot AM, Corvol P, Soubrier F. Structure of the angiotensin I-converting enzyme gene. Two alternate promoters correspond to evolutionary steps of a duplicated gene. Journal of Biological Chemistry. 1991 Aug;266(23):15377–83.

19. Bell CG, Meyre D, Petretto E, Levy-Marchal C, Hercberg S, Charles MA, et al. No contribution of angiotensin-converting enzyme (ACE) gene variants to severe obesity: a model for comprehensive case/control and quantitative cladistic analysis of ACE in human diseases. European Journal of Human Genetics. 2007 Mar 13;15(3):320–7.

20. Chiarella P, Capone P, Sisto R. Contribution of Genetic Polymorphisms in Human Health. Int J Environ Res Public Health. 2023 Jan 4;20(2):912.

21. Shen LX, Basilion JP, Stanton VP. Single-nucleotide polymorphisms can cause different structural folds of mRNA. Proceedings of the National Academy of Sciences. 1999 Jul 6;96(14):7871–6.

22. Laura Bull. Genetics, Mutations, and Polymorphisms. Madame Curie Bioscience Database [Internet].

23. Sripichai O, Fucharoen S. Genetic polymorphisms and implications for human diseases. J Med Assoc Thai. 2007 Feb;90(2):394–8.

24. Sayed-Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Witteman JCM. *ACE* Polymorphisms. Circ Res. 2006 May 12;98(9):1123–33.

25. Xin XY, Lai ZH, Ding KQ, Zeng LL, Ma JF. Angiotensin-converting enzyme polymorphisms AND Alzheimer's disease susceptibility: An updated meta-analysis. PLoS One. 2021 Nov 24;16(11):e0260498.

26. Sleegers K, den Heijer T, van Dijk EJ, Hofman A, Bertoli-Avella AM, Koudstaal PJ, et al. ACE gene is associated with Alzheimer's disease and atrophy of hippocampus and amygdala. Neurobiol Aging. 2005 Aug;26(8):1153–9.

27. Younes S, Ibrahim A, Al-Jurf R, Zayed H. Genetic polymorphisms associated with obesity in the Arab world: a systematic review. Int J Obes. 2021 Sep 15;45(9):1899–913.

28. Feng Y, Niu T, Xu X, Chen C, Li Q, Qian R, et al. Insertion/Deletion Polymorphism of the ACE Gene Is Associated With Type 2 Diabetes. Diabetes. 2002 Jun 1;51(6):1986–8.

29. Dmitrenko OP, Karpova NS, Nurbekov MK, Papysheva O V. I/D Polymorphism Gene ACE and Risk of Preeclampsia in Women with Gestational Diabetes Mellitus. Dis Markers. 2020 Dec 28;2020:1–7.

30. Alimoradi N, Sharqi M, Firouzabadi D, Sadeghi MM, Moezzi MI, Firouzabadi N. SNPs of ACE1 (rs4343) and ACE2 (rs2285666) genes are linked to SARS-CoV-2 infection but not with the severity of disease. Virol J. 2022 Mar 19;19(1):48.

31. Kulminski AM, Culminskaya I V., Ukraintseva S V., Arbeev KG, Akushevich I, Land KC, et al. Polymorphisms in the *ACE* and *ADRB2* Genes and Risks of Aging-Associated Phenotypes: The Case of Myocardial Infarction. Rejuvenation Res. 2010 Feb;13(1):13–21.

32. Boright AP, Paterson AD, Mirea L, Bull SB, Mowjoodi A, Scherer SW, et al. Genetic Variation at the ACE Gene Is Associated With Persistent Microalbuminuria and Severe Nephropathy in Type 1 Diabetes. Diabetes. 2005 Apr 1;54(4):1238–44.

33. AL-Eitan L, Al-Khaldi S, Ibdah R k. ACE gene polymorphism and susceptibility to hypertension in a Jordanian adult population. PLoS One. 2024 Jun 25;19(6):e0304271.

34. Terzić R, Letonja M, Terzić I, Sehić A, Merić M, Teran N. Insertion/deletion polymorphism of angiotensin-converting enzyme gene--risk factor for coronary artery disease in the Tuzla region population (Bosnia and Herzegovina). Coll Antropol. 2003 Dec;27(2):537–40.

35. Dević Pavlić S, Ristić S, Flego V, Kapović M, Radojčić Badovinac A. Angiotensin-Converting Enzyme Insertion/Deletion Gene Polymorphism in Lung Cancer Patients. Genet Test Mol Biomarkers. 2012 Jul;16(7):722–5.

36. Ha SK. ACE Insertion/Deletion Polymorphism and Diabetic Nephropathy: Clinical Implications of Genetic Information. J Diabetes Res. 2014;2014:1–8.

37. Dević Pavlić S, Nadalin S, Starčević Čizmarević N, Buretić-Tomljanović A, Radojčić Badovinac A, Ristić S. Could angiotensin-converting enzyme 1 *I/D* polymorphism be a modificator of COVID-19 response in different populations, diseases, and/or conditions? Journal of the Renin-Angiotensin-Aldosterone System. 2020 Jul 14;21(3):147032032095715.

38. Hmimech W, Idrissi HH, Diakite B, Korchi F, Baghdadi D, Tahri Joutey Hassani Idrissi H, et al. Impact of I/D polymorphism of angiotensin-converting enzyme (ACE) gene on myocardial infarction susceptibility among young Moroccan patients. BMC Res Notes. 2017 Dec 21;10(1):763.

39. Schüler R, Osterhoff MA, Frahnow T, Möhlig M, Spranger J, Stefanovski D, et al. Dietary Fat Intake Modulates Effects of a Frequent ACE Gene Variant on Glucose Tolerance with association to Type 2 Diabetes. Sci Rep. 2017 Aug 23;7(1):9234.

40. Abedin-Do A, Pouriamanesh S, Kamaliyan Z, Mirfakhraie R. Angiotensin-converting enzyme gene rs4343 polymorphism increases susceptibility to migraine. CNS Neurosci Ther. 2017 Aug 18;23(8):698–9.

41. 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. Journal of the Renin-Angiotensin-Aldosterone System. 2022 Jan 11;2022.

42. Bartakova J, Deissova T, Slezakova S, Bartova J, Petanova J, Kuklinek P, et al. Association of the angiotensin I converting enzyme (ACE) gene polymorphisms with recurrent aphthous stomatitis in the Czech population: case–control study. BMC Oral Health. 2022 Dec 19;22(1):80.

43. Kwon I. Angiotensin-converting enzyme gene insertion/deletion polymorphism is not associated with BMI in Korean adults. Phys Act Nutr. 2020 Mar 31;24(1):24–8.

44. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. Journal of Clinical Investigation. 1990 Oct 1;86(4):1343–6.

45. Chen YH, Liu JM, Hsu RJ, Hu SC, Harn HJ, Chen SP, et al. Angiotensin converting enzyme DD genotype is associated with acute coronary syndrome severity and sudden cardiac death in Taiwan: a case-control emergency room study. BMC Cardiovasc Disord. 2012 Dec 15;12(1):6.

46. Ha S, Yong Lee S, Su Park H, Ho Shin J, Jung Kim S, Hun Kim D, et al. ACE DD genotype is more susceptible than ACE II and ID genotypes to the antiproteinuric effect of ACE inhibitors in patients with proteinuric non-insulin-dependent diabetes mellitus. Nephrology Dialysis Transplantation. 2000 Oct 1;15(10):1617–23.

47. Beyerstedt S, Casaro EB, Rangel ÉB. COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. European Journal of Clinical Microbiology & Infectious Diseases. 2021 May 3;40(5):905–19.

48. Ristić S, Pavlić SD, Nadalin S, Čizmarević NS. ACE I/D polymorphism and epidemiological findings for COVID-19: One year after the pandemic outbreak in Europe. Journal of Infection. 2021 Sep;83(3):381–412.

49. Jochemsen HM, Teunissen CE, Ashby EL, van der Flier WM, Jones RE, Geerlings MI, et al. The association of angiotensin-converting enzyme with biomarkers for Alzheimer's disease. Alzheimers Res Ther. 2014;6(3):27.

50. Holman DM, Ports KA, Buchanan ND, Hawkins NA, Merrick MT, Metzler M, et al. The Association Between Adverse Childhood Experiences and Risk of Cancer in Adulthood: A Systematic Review of the Literature. Pediatrics. 2016 Nov 1;138(Supplement_1):S81–91.

51. Cordonnier DJ, Zaoui P, Halimi S. Role of ACE Inhibitors in Patients with Diabetes Mellitus. Drugs. 2001;61(13):1883–92.

52. Agerholm-Larsen B, Nordestgaard BG, Tybjærg-Hansen A. *ACE* Gene Polymorphism in Cardiovascular Disease. Arterioscler Thromb Vasc Biol. 2000 Feb;20(2):484–92.

53. Harding D, Dhamrait S, Marlow N, Whitelaw A, Gupta S, Humphries S, et al. Angiotensin-converting enzyme DD genotype is associated with worse perinatal cardiorespiratory adaptation in preterm infants. J Pediatr. 2003 Dec;143(6):746–9.

54. Harding DR, Dhamrait S, Davedason D, Humphries SE, Whitelaw A, Marlow N, et al. Does angiotensin-1 converting enzyme genotype influence motor or cognitive development after pre-term birth? J Neuroinflammation. 2005;2(1):6.

55. González-Garrido JA, García-Sánchez JR, Tovar-Rodríguez JM, Olivares-Corichi IM. Preeclampsia is associated with ACE I/D polymorphism, obesity and oxidative damage in Mexican women. Pregnancy Hypertens. 2017 Oct;10:22–7.

56. Zhong WG, Wang Y, Zhu H, Zhao X. Meta analysis of angiotensin-converting enzyme I/D polymorphism as a risk factor for preeclampsia in Chinese women. Genetics and Molecular Research. 2012;11(3):2268–76.

57. 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.

58. Correa-Noronha SAA, Ribeiro de Noronha SM, Alecrim C, Mesquita A de C, Brito GS da S, Junqueira MG, et al. Association of angiotensin-converting enzyme I gene I/D polymorphism with endometrial but not with ovarian cancer. Gynecological Endocrinology. 2012 Nov 10;28(11):889–91.

59. Valdez-Velazquez LL, Quintero-Ramos A, Perez SA, Mendoza-Carrera F, Montoya-Fuentes H, Rivas F, et al. Genetic polymorphisms of the renin-angiotensin system in preterm delivery and premature rupture of membranes. Journal of the Renin-Angiotensin-Aldosterone System. 2007 Dec 23;8(4):160–8.

60. Uma R, Forsyth JS, Struthers AD, Fraser CG, Godfrey V, Murphy DJ. Correlation of angiotensin converting enzyme activity and the genotypes of the I/D polymorphism in the ACE gene with preterm birth and birth weight. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2008 Nov;141(1):27–30.

61. Uvuz F, Kilic S, Yilmaz N, Tuncay G, Cakar E, Yuksel B, et al. Relationship between Preterm Labor and Thrombophilic Gene Polymorphism: A Prospective Sequential Cohort Study. Gynecol Obstet Invest. 2009;68(4):234–8.

62. Lee NR, Hwang IW, Kim HJ, Kang YD, Park JW, Jin HJ. Genetic Association of Angiotensin-Converting Enzyme (ACE) Gene I/D Polymorphism with Preterm Birth in Korean Women: Case-Control Study and Meta-Analysis. Medicina (B Aires). 2019 Jun 10;55(6):264.

63. Hočevar K, Peterlin A, Jovanović AM, Božović A, Ristanović M, Tul N, et al. Association between angiotensin-converting enzyme gene insertion/deletion polymorphism and susceptibility to preterm birth: A case-control study and meta-analysis. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2018 Dec;231:122–8.

64. Khehra N, Padda IS, Swift CJ. Polymerase Chain Reaction (PCR). 2024.

65. Barbalić M; ŠJT; SNN; RI; TS; RP. Association of ACE I/D polymorphism with hypertension in Croatia and its isolate. European Journal of Human Genetics. 2005;11.

66. Zajc Petranović MTŽKPMJŠJTTRSSNN. ACE I/D polymorphism and human longevity - Croatian senescence study. European Journal of Human Genetics. 2010;18.

67. Rahimi Z, Rahimi Z, Mozafari H, Parsian A. Preeclampsia and angiotensin converting enzyme (ACE) I/D and angiotensin II type-1 receptor (AT1R) A1166C polymorphisms: association with ACE I/D polymorphism. Journal of the Renin-Angiotensin-Aldosterone System. 2013 Jun 19;14(2):174–80.

68. Fazelnia S FTHSS. ignificant correlation of angiotensin converting enzyme and glycoprotein IIIa genes polymorphisms with unexplained recurrent pregnancy loss in north of Iran. Int J Reprod Biomed. 2016;

69. Yang C, Fangfang W, Jie L, Yanlong Y, Jie W, Xuefei L, et al. Angiotensin-converting enzyme insertion/deletion (I/D) polymorphisms and recurrent pregnancy loss: a meta-analysis. J Assist Reprod Genet. 2012 Nov 7;29(11):1167–73.

70. Mello G, Parretti E, Gensini F, Sticchi E, Mecacci F, Scarselli G, et al. Maternal-Fetal Flow, Negative Events, and Preeclampsia. Hypertension. 2003 Apr;41(4):932–7.

71. Sheikh IA, Ahmad E, Jamal MS, Rehan M, Assidi M, Tayubi IA, et al. Spontaneous preterm birth and single nucleotide gene polymorphisms: a recent update. BMC Genomics. 2016 Oct 17;17(S9):759.

72. Romero R, Espinoza J, Kusanovic J, Gotsch F, Hassan S, Erez O, et al. The preterm parturition syndrome. BJOG. 2006 Dec 19;113(s3):17–42.

73. GINTONI I, ADAMOPOULOU M, YAPIJAKIS C. The Angiotensin-converting Enzyme Insertion/Deletion Polymorphism as a Common Risk Factor for Major Pregnancy Complications. In Vivo (Brooklyn). 2021;35(1):95–103.

8. CURRICULUM VITAE

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EDUCATION

10/2024 to Current
Master of Science: Drug Research and Development
University of Rijeka
Faculty: Faculty of Biotechnology

09/2018 to 07/2022
Bachelor of Science: Biotechnology and Drug Research, Department of Biotechnology
University of Rijeka
- Grade: cum laude
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9/2015 to 07/2019
Pharmacy technician
Viktorovac High School - Sisak

EXPERIENCE

06/2024 to Current
Pharmaceutical Sales Representative
Salvus
- Providing expert advice on pharmaceutical products to healthcare professionals
- Developing and maintaing strong relationships with clients to drive sales growth

05/2024
Intern
Jadran Galenski Laboratorij
- Research and development department
- Participated in the formulation and testing of new drugs.
- Collected and analyzed data from laboratory tests.

03/2022 to 05/2024
Sales Promotor
Avemed d.o.o., Rijeka
- Providing expert advice and information to patients on dietary supplements best suited to their individual needs and goals
- Providing unique consumer experience through personalized routines
- Meeting qualitative and quantitative sales targets.

12/2023 to 01/2024
Student demonstrator
Introduction to Bioessays, Faculty of Biotechnology

- Assistance in lesson preparation and conducting laboratory exercises

10/2023 to 12/2023 Volunteer
High-Throughput Analysis Laboratory
- Experience in chromatography and mass spectrometry

06/2022 to 06/2022 Intern
Institute for Medical Research and Occupational Health, Zagreb
- Cell culturing: maintenance, propagation; and manipulation of cell lines
- Measuring enzyme activity using a spectrophotometer, data analysis, and graphical representation of results.

10/2020 to 12/2020 Co Project Manager
Co Project Manager Biotechnology Student Association at University of Rijeka (USBRI)
- Co-organization of the Conference "Future and Perspective"

11/2019 to 03/2020 Sales Promotor
Almagea, Rijeka
- Providing expert advice and information to patients on dietary supplements best suited to their individual needs and goals
- Providing unique consumer experience through personalized routines
- Meeting qualitative and quantitative sales targets.

09/2015 to 07/2019 Pharmacy technician
Viktorovac High School, Sisak

06/2018 to 06/2018 Intern
Pharmacy Kovačićek, Petrinja
- Maintaining pharmacy inventory by checking pharmaceutical stock to determine inventory level, anticipating needed medications and supplies, and removing outdated drugs
- Organizing medications for pharmacist to dispense by reading medication orders and prescriptions, preparing labels, calculating quantities, and assembling medications with recipes.

VOLUNTEER EXPERIENCE

- Co Project Manager Biotechnology Student Association at University of Rijeka (USBRI), Co-organization of the Conference "Future and Perspective", 10/2020, 12/2020
- High-Throughput Analysis Laboratory, Experience in chromatography and mass spectrometry, 10/2023, Present
- Introduction to Bioessays, Faculty of Biotechnology, Assistance in lesson preparation and conducting laboratory exercises, 12/2023, 01/2024