

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

**The significance of I/D polymorphism  
of the angiotensin-converting enzyme  
in the posttransplant patient and graft survival**

by Roland Fedor MD

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UNIVERSITY OF DEBRECEN  
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The Examination takes place at Library of Department of Pediatrics, Faculty of  
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Internal Medicine, Faculty of Medicine, University of Debrecen  
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## **Introduction**

The incidence of end stage renal disease (ESRD) is growing worldwide. However, the number of annual kidney transplantations (transplantation activity) does not increase proportionally. Renal transplantation provides longer life expectancy in patients with renal failure, than the other alternative therapeutic modalities, although this improved life expectancy is still shorter than that for the general population. The main cause of premature death in renal transplant patients compared to the general population is cardiovascular disease, which has about fifty times higher risk to occur after transplantation. There are several possible etiologic factors like obesity, the primary kidney disease, the quality of the transplanted organ, delayed graft function (DGF), acute rejection (AR), immunosuppressive therapy using calcineurin inhibitors (CNI), side effects of the glucocorticoids, transplant renal artery stenosis (TRAS) and chronic allograft nephropathy (CAN) leading to cardiovascular disease. Accordingly, hypertension occurs in 75-90% of the patients.

Renal transplantation is an alternative therapy for end stage renal disease patients, with the promise of a better quality of life. The effectivity and safety of immunosuppressive therapy improved persistently during the last decades. Acute rejection was the prevalent cause of kidney damage in the past decades while CAN is the most significant cause of graft loss.

Decreasing kidney function, rising creatinine level, proteinuria and hypertension could be the clinical symptoms of chronic allograft dysfunction. Histologically it is characterized by interstitial fibrosis, hyalinisation of the vessels and tubular atrophy. Chronic allograft dysfunction is a multifactorial syndrome, similarly to the cardiovascular diseases. The literature classifies the known etiologic factors into immunologic and non-immunologic groups. The immunologic factors include HLA mismatch, acute rejection episodes, high panel reactive antibody level (PRA). The non-immunologic group contains the ischemic-

reperfusion damage, recurrence of the primary kidney disease, hypertension, hyperlipidemia, toxicity of the immunosuppressive drugs and infections.

The polymorphisms of the genes in the renin-angiotensin system were correlated with both cardiovascular complications and CAN in clinical and experimental studies, although with contradictory results. The trials were performed involving distinctly related patient populations, vast differences in the number of participants and various other criteria. Therefore there is no consensus about the pathologic role of these genes and polymorphisms.

### *The Renin-Angiotensin System (RAS)*

The renin-angiotensin-aldosterone system is a fundamental part of the fluid and ion homeostasis. It plays an essential role in the regulation of blood pressure. It has an influence on the vascular tone via the regulation of the volume of body fluids, through fluid and electrolyte excretion. In particular, the RAS plays a central role in sodium excretion by direct regulation of sodium channels and by regulation of blood distribution within the kidneys.

The central role of kidney was supposed in RAS, as the name „renin” shows initially. Skeggs and his colleagues published a multi-step process in 1957. The first step is the formation of angiotensin I (Ang-I) from angiotensinogen. The second step was the formation of angiotensin II (Ang-II) from Ang-I. The angiotensinogen is a protein containing 453 amino acid residues, from what the renin creates the Ang-I decapeptid (representing the N-terminal amino acids of angiotensinogen). Ang-II is proteolysed to angiotensin II (Ang-II, octapeptide) by the angiotensin converting enzyme (ACE). The angiotensin II (Ang-II) is the responsible for the regulation of vascular contraction.

The discovery of the specific inhibitors of RAS started in the 70's and they reached the everyday clinical practice shortly.

### *Angiotensin-converting enzyme (ACE)*

The ACE could occur in membrane-bound form on the cell surface and in intracellular compartments. It is expressed in endothelial cells of the arteries and veins, in epithelial cells, in the cardiovascular system, in the brain, in the reproductive organs, and also in the fibroblasts and macrophages. It is present in the plasma and also in other body fluids. It requires chloride and zinc for substrate binding and enzymatic activity. It has a low substrate specificity, resulting in various di- and tripeptides released from larger proteins. The enzyme has two active centers. These catalytic centers are equally effective in bradykinin cleavage, but Ang-I is converted more effectively to Ang-II by the C-terminal catalytic site. ACE also cleaves (and therefore inactivates) the vasodilator bradykinin and kallidine.

### *Angiotensin II (Ang-II)*

The Ang-I has only a very limited effect on blood pressure, while Ang-II is one of the most effective vasoconstrictor molecule of the human body. It binds to vascular smooth muscle receptors (primarily to Type 1 Angiotensin-II Receptor) and results in constriction within seconds. It increases the total peripheral resistance and the blood pressure. Ang-II evoked constriction is more prominent in resistance arterioles than in veins. Ang-II evoked vasoconstriction is prominent in the kidney and the splanchnic vessels, weaker in the brain, negligible in the lung and in the skeletal muscles. Its role in the long term regulation of blood pressure is more important, than the short term responses, which are related to the regulation of blood distribution. This is mediated by complex mechanisms involving the regulation of kidney function.

The long term effects of Ang-II is rather proliferative, than regulative. Ang-II evokes morphologic changes, transformations in the structure of various elements of the cardiovascular system. It increases the fibrosis in the wall of the

vessels and in heart muscle, facilitates the progression of these processes. Ang-II evokes hypertrophy, proliferation and migration of vascular smooth muscle cells, fibroblasts, heart muscle cells by stimulating proto-oncogenes and growth factors. This leads to target organ damage which is not limited to the cardiovascular system, but also affects the kidneys, leading to fibrotic lesions.

#### *Subtypes of angiotensin II receptors*

Ang-II binds to and activates G-protein linked receptors, from which type 1 and type 2 (AT1R and AT2R) were characterized in detail. Angiotensin receptors expressed in the smooth muscle cells of the vessels, in the kidney, heart, brain, in the cortical and medullar part of suprarenal gland, liver, uterus and in hypopituitary gland as well as in the gonads.

Stimulation of AT1R leads to the characteristic effects through several signaling pathways including protooncogenes. Activation of this pathway results in cellular hypertrophy, especially in cardiomyocytes and in vascular smooth muscle cells.

The role and function of AT2R is ambiguous. AT2R appears to be involved in vascular remodeling, but mediates opposite effects than that for AT1R. It inhibits the processes leading to cellular hypertrophy and differentiation. AT2R play a role in modulation of extracellular matrix (ECM), regeneration of neurons, apoptosis, vasodilation.

The reports on type 3 and type 4 Ang-II receptors are inconclusive. It was suggested that AT4R is activated by angiotensin-IV, which is the metabolite of Ang-II, taking part in the regulation of ECM in the central nervous system by increasing the release of oxytocin.

### *Polymorphisms*

The polymorphism is a variation of the DNA, which has an incidence of at least 1 % in the population. This variation could be an insertion of a nucleotide sequence into the DNA, or deletion (insertion/deletion), besides others. This variation of the DNA molecule could have an effect on the amount, and activity of the protein encoded by the gene. Polymorphisms may therefore have a role in pathologic processes.

The insertion/deletion (I/D) polymorphism of the ACE was first described by Rigat and colleagues in 1990. They found variable circulating ACE-concentration in 80 healthy persons. They found a negative correlation of ACE expression with a 287 base pair long insertion sequence in the gene. The concentration of the enzyme was the lowest at the II genotype and highest at the DD homozygotes.

### *Chronic allograft nephropathy (CAN)*

The chronic allograft nephropathy is the most important hindrance of the long term success of renal transplantation. This is a process which starts asymptotically, and its severity shows positive correlation with the graft survival. Although a lot of risk factors have been already identified, the list is far to be complete. The risk factors are generally divided into immunological and non-immunological groups. The identified immunological factors are the HLA-mismatch, the number and severity of acute rejection episodes, the PRA level of the recipient, the occurrence and titer of donor specific antibodies and the administered immunosuppression therapy. Non-immunological factors are the age of donor, gender, type of donation (cadaver or living), cause of brain death, hypertension and kidney function of the donor. The occurrence of CAN is also affected by the delayed graft function (DGF), the length of cold ischemic time (CIT), the size of the kidney and the

recipient, diabetes mellitus, atherosclerosis of the patient and every atherosclerotic risk factor.

The histological measurement shows degenerative and fibrotic changes throughout of the kidney and nephrons. Fibrocytes are proliferating in the interstitia and a significant fibrosis develops. The tubular structure shows atrophy, the glomeruli is sclerotic and stenosis and hyalinisation is present in the vascular wall. These changes cause deterioration of graft function and irreversible damage in the kidney.

### *Left ventricular hypertrophy*

The cardiovascular diseases affect strongly the survival of transplant patients beside the infective complications of immunosuppressive therapy. The most frequent cause of death is cardiovascular complication in the kidney transplant population. The above mentioned risk factors also increase the risk of fatal outcome. In particular, a persistent increase of blood pressure by 1 mmHg increases the risk of cardiovascular event with 1-2%, while the occurrence of hypertension is 75-90% within the transplanted population.

Left ventricular hypertrophy (LVH) is a cardiac complication of hypertension, which develops at blood pressure values above 130/85 mmHg. The diagnosis of LVH is based on electro- and echocardiography. Risk factors for LVH are similar than those for hypertension, but additional risk factors are also identified, such as deteriorated kidney function, anemia and genetic factors. The decrease of kidney function leads to the morphological changes and dysfunction of heart by increased blood volume and the activation of RAS. This results in deterioration of pump function. Tissue located RAS is being activated in the myocardium and vessel wall, resulting in Ang-II mediated apoptosis, cardiomyocyte hypertrophy and interstitial fibrosis, leading to progression of heart failure.



### *RAS inhibition therapy*

ACE inhibitors are primary drugs in hypertension irrespectively of the cause. They inhibit ACE in the sera as well as in the tissues, resulting in decreased Ang-II and increased bradykinin concentrations. The peripheral resistance and systemic blood pressure decreases as a result of these changes. They improve endothelial function and inhibit the hypertension evoked vascular and myocardial complications. ACE inhibitors reduce proteinuria and delay kidney failure (some even found improvement of kidney function upon ACE inhibitory medication) in advanced nephropathy.

Several studies proved the nephroprotective and anti-atherosclerotic effects of angiotensin-II receptor antagonists (ARB). The major advantage of these drugs is that they have negligible side effects and also effective in almost all types of hypertension (except gravidity and renal artery stenosis). They have an exceptionally beneficial effect in reno-vascular hypertension.

The direct renin inhibitors (DRI) block the binding of angiotensinogen to the catalytic center of renin and therefore inhibit the production of Ang-I in the plasma and in the tissues. There is single drug in the clinical practice from this group, the aliskiren. It is a potent antihypertensive drug, which also inhibits the progression of left ventricular hypertrophy and has beneficial effects in heart failure in the initial studies. However, one of the later studies (ALTITUDE) has been terminated prematurely as a result of safety problems and the lack of therapeutic effects casting doubts of the clinical applicability of aliskiren.

## **Main objectives**

The main goals of our study was:

1. To identify a biomarker from the blood which has a correlation with the survival of the transplanted kidney.
2. We investigated the correlation of I/D polymorphism of the ACE gene with the survival of the transplanted kidney. To investigate the correlation of ACE I/D polymorphism with left ventricular hypertrophy (which is a severe cardiovascular risk factor).
3. In general, the aim was to find a biomarker, which can be measured non-invasively, reproductively, and shows a good correlation with the graft and with the recipient survival or with the quality of patient's life.

## **Patients and methods**

The kidney transplant program has started in 1991 in our Department. The transplant surgeons use a standard technique, for safety reasons (best clinical practice). The patients spend the first few postoperative days in the intensive care unit, and after they transferred into the specially built transplant ward, from which they are released to their home within a month. The transplantation is followed by the postoperative care.

Blood samples and the medical data were obtained from patients at the outpatient center upon their regular follow-up visits. 72 patients were enrolled into the study depending on their documented informed consent. The enrollment started in January 2009 and finished in April 2011. Patients were older than 18 at the time of the surgery and were at least 7 years after the kidney transplantation.

There were two patients' groups defined, based on the kidney function of the patients. One group contained the patients with preserved kidney function (normal renal function, NRF), while the other contained patients with definite chronic allograft nephropathy (CAN, diagnosed by renal biopsy).

Serum creatinine level, the glomerular filtration rate (GFR), urine protein excretion and the diuretic therapy were regularly recorded at the visits. In addition, the age, gender, body mass index (BMI) and abdominal circumference were also measured. A standardized method was used for blood pressure and pulse rate measurement. Mean arterial pressure (MAP) was calculated. Medical documentations were used as a source document for the treatments and for the existence and stage of additional diseases, such as diabetes mellitus. Smoking status was also recorded. Laboratory measurement of blood glucose and lipid levels (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerol) were performed at the regular visits.

Factors implicated in the etiology of CAN were documented and analyzed retrospectively. In particular, HLA-mismatch, the titer of cytotoxic antibodies, the number of acute rejection episodes, the age of donors, the occurrence of delayed graft function (DGF) and the cold ischemic time were taken into account. The immunosuppressive medication and the presence of cytomegalovirus (CMV) antigens were also recorded.

#### *Determination of ACE I/D polymorphism*

DNA was isolated from peripheral white blood cells using a FlexiGene<sup>®</sup> DNA Kit. The ACE I/D polymorphism was determined by polymerase chain reaction (PCR) according to the method by Rigat et al. Amplified PCR products were separated by electrophoresis on 5% polyacrylamid gel. The bands were stained with ethidium-bromid.

#### *Concentration of circulating ACE*

Blood samples were incubated in room temperature for an hour and the sera was separated with centrifugation (1,000 g, 15 minutes). Samples were stored until measurements at -20°C.

The concentration of ACE was determined with an ELISA method (Human ACE ELISA development system) according to the manufacturer's instructions. A recombinant ACE calibration curve was established in each individual experiment for the determination of ACE concentration. Data are the average of at least 3 independent measurements, but sometimes experiments were further repeated until the standard deviation reached 15% or lower.

#### *Circulating ACE activity*

ACE activity was determined by the method published by Beneteau et al. The activity of was calculated with the following formula:

$$Activity = (S / k) * D,$$

where  $S$  is the slope (1/min),  $k$  is the decrease in optical density following the cleavage of 1  $\mu\text{mol}$  FAPGG and  $D$  is the dilution of the sera. One unit of ACE activity results in 1  $\mu\text{mol}$  FAPGG hydrolysis in each minute.

### *Echocardiography*

Echocardiography was performed as a part of the general clinical follow up of the patients. Patients enrolled in this sub-study had a well documented echocardiography 4 months postoperatively, 1 year after and at least one more occasion during the follow up.

Two dimensional, M-mode and Doppler ultrasonography was performed using the standard section planes at the Echocardiographic Laboratory of the Cardiological Department of the University of Debrecen. The first echocardiography was performed 4 months postoperatively ( $0,29 \pm 0,08$  years), the second almost 1 year later ( $1,20 \pm 0,24$  years). In every cases we analyzed one more measurement which was the last during the follow up period. These were made in average 7 years after the transplantation ( $7,17 \pm 2,53$  years). Left ventricular mass index (LVMI) was calculated to diagnose left ventricular hypertrophy (LVH) and to quantitate its severity.

Calculations:

$$LVM = 0,8 \times \{1,04 [(LVIDd + PWTd + SWTd)^3 - (LVIDd)^3]\} + 0,6 \text{ (g)}.$$

Where LVM is the left ventricular mass, LVIDd is the left ventricular diastolic diameter, PWTd is the posterior wall thickness and SWTd is the septal wall thickness.

Body surface area (BSA) was calculated according to the DuBois and DuBois formula:

$$BSA = (W^{0,425} \times H^{0,725}) \times 0,007184 \text{ (m}^2\text{)},$$

where  $W$  is the weight and  $H$  is the height of the patients.

After that the left ventricular mass index (LVMI) was calculated:

$$\mathbf{LVMI} = \mathbf{LVM} / \mathbf{BSA} \text{ (g/m}^2\text{)}$$

### *Statistical analysis*

Parametric values were tested by t-test, while the categorical results were evaluated by the khi-squared test ( $\chi^2$ ). Linear regression analysis was made to determine the correlation between kidney function and LVMI.

Every calculation was performed with GraphPad Prism<sup>®</sup> 5.0 (GraphPad Software Inc., San Diego, CA). The result was statistically significant if the *p*-value were lower than 0,005.

## Results

### *Chronic allograft nephropathy (CAN)*

The CAN was diagnosed in 38 patients with biopsy (CAN group). We enrolled to the normal renal function group (NRF) 34 recipients with healthy graft function at least 7 years after transplantation. The serum creatinine level in this latter group was in average  $95,68 \pm 17,17 \mu\text{mol/l}$ .

The female to male ratio in the CAN group was 24 to 14 and 23 to 11 in the NRF group. The average age of the transplanted patients was 37 years in the CAN and 42 years in the NRF groups at the transplantation. There was a 5,2 years long period between the transplantation and the biopsy in the CAN group. There was a longer period between the operation and the enrollment in the NRF group (11,2 years), than in the CAN group (8,3 years).

The creatinine level and the urine protein excretion was significantly lower and the GFR higher in the NRF group. Almost all patients had diuretic therapy in the CAN group (37 out of 38). BMI, the abdominal circumference and the HDL-cholesterol level were higher in the CAN group compared to the NRF. There were more smokers in the CAN group. There was no difference between the groups regarding the HLA-mismatch, the cytotoxic antibody titer, the occurrence of delayed graft function (DGF), the average length of cold ischemic time and nor the cytomegalovirus state. The acute rejection (AR) episode was more frequent in CAN group and the average age of donors was higher. All patients enrolled to the study received calcineurin-inhibitor (CNI) based immunosuppression.

The frequency of ACE II genotype 13% in the CAN group and 12% in the NRF group ( $p=0,83$ ). The frequency of ID genotype was 55% in CAN group, compared to 71% in the NRF group ( $p=0,02$ ). The incidence of the DD genotype was higher in CAN group (32%), than in NRF (18%,  $p=0,02$ ).

ACE concentration showed the lowest levels in II homozygotes which was significantly elevated in patients with DD genotype ( $p=0,02$ ). The ACE concentration in ID heterozygotes were between these values.

The activity of ACE was also determined. The results showed good correlation with the ACE expression in patients without ACE-inhibition therapy ( $n=38$ ). The activity of the enzyme was the lowest in II genotype recipients, higher in ID heterozygotes and highest in DD homozygotes. The activity was significantly higher in patients with II genotype, compared to other genotypes (ID vs DD  $p<0,01$ , II vs DD  $p=0,01$ ).

The efficacy of ACE-inhibitor therapy was also determined. The average activity of the enzyme was 30 U/l in patients without ACE-inhibitor therapy, whereas in those with this treatment was significantly lower (11 U/l,  $p<0,01$ ).

#### *Left ventricular hypertrophy*

The left ventricular mass index (LVMI) correlated with creatinine levels or with the GFR. The correlations were statistically significant.

After calculated the LVMI, the patients were divided into two groups with different severity of LVMI values. The threshold limit for the diagnosis of LVH was 95 g/m<sup>2</sup> in females and 115 g/m<sup>2</sup> in male patients. LVH occurred in 67% in II genotype patients, all of the heterozygotes (100%) and 86% of the DD homozygotes at the first echocardiography. LVH was diagnosed in 67% of II homozygotes, in 82% of heterozygotes and in 86% of DD genotype recipients at the second measurement. The results of the third echocardiography were the following: 67% of II homozygotes, 76% of ID genotype patients and 86% of DD homozygotes had LVH.

LVH was ranked serious (following the guideline of the American Echocardiography Association), when LVMI was higher than 122 g/m<sup>2</sup> in females and higher than 149 g/m<sup>2</sup> in males. Nobody showed serious LVH in II



homozygotes (0%), but 82% in ID heterozygotes and 57% in DD genotype patients at the first echocardiography. 33% of II homozygotes had severe LVH, compared to 59% of heterozygotes and 57% of DD homozygote recipients at the second measurement. The last echocardiography made during the follow up showed the following results: 33% of II genotype patients had severe LVH and the same diagnosis in the ID and DD genotype group was 53 and 71%, respectively.

## Discussion

### *Chronic allograft nephropathy*

Renal transplantation provides longer life expectancy in patients with renal failure compared to other methods used for the management of renal failure. Nonetheless, this improved life expectancy is still shorter than that for the age-matched healthy population. The most frequent cause of death in renal transplant patients is cardiovascular disease, which can be explained by the higher incidence of hypertension and left ventricular hypertrophy. In respect of the transplanted organ, chronic allograft nephropathy (CAN) is the most significant cause of graft loss.

The renin-angiotensin aldosterone system (RAAS) has been implicated in the ethiology of hypertension and target organ damage in general and also in nephropathy. We looked for a valuable biomarker which can be easily obtained and may predict individual susceptibility for these diseases.

The I/D polymorphism of the angiotensin-converting enzyme was first described by Rigat and colleagues. They described its profound influence on the concentration of the circulating ACE. This initial report has been followed by numerous follow up studies in the last decades. Since ACE-inhibitory medication slows the progression of kidney disease it is conceivable that individuals with lower ACE concentrations can be less susceptible for kidney or cardiovascular diseases. However, this remained elusive in the clinical studies, probably related to the fact that the studies were designed aiming different end-points and used different methodology.

For example, Broekroelofs et al did not find any correlation between ACE I/D genotype and graft function while Lovati et al found that DD genotype recipients had significantly faster deterioration of kidney function, than heterozygotes or II homozygotes.

Akcay et al showed positive correlation between ACE DD genotype and chronic allograft dysfunction (CAD). Rodríguez-Moreno et al found a positive correlation not just in cases of DD homozygotes, but also in patients carrying the D-allele. The same was verified by Azarpira et al. They pointed out that the frequency of DD genotype was significantly higher in the failing group. However, Slowinski et al and Ayed et al did not confirm these results.

Taking in account the previous failures we have paid a special attention on the diagnosis of chronic allograft nephropathy (CAN) by evaluating biopsies from the clinically suspected patients. We believe that this unambiguous diagnosis is makes it possible to clearly differentiate between patient's populations and significantly improves the chance of the identification of genetical markers of post-transplant nephropathy. We set up a long enough interval between the transplantation and the occurrence of CAN to have a reliable control group of patients with maintained graft function. Reliable documentation and careful analysis had to be made to explore all the risk factors and the additive relationships of the multifactorial disease.

We based the diagnosis of CAN on histological evaluation in each cases. In the normal renal function group (NRF) kidney function inclusion criteria was maintained renal function at least 7 years after the transplantation. All of the known immunologic and non-immunologic factors of CAN were investigated. Our results showed positive relationship between CAN and donor age, as well as with acute rejection episodes. Besides to these known risk factors, we found a statistically significant correlation between the I/D polymorphism of ACE and CAN. The DD genotype was more frequent in the CAN group. Furthermore, our measurements confirmed a strong correlation among ACE genotype and circulating ACE concentration/activity.

### *Left ventricular hypertrophy*

The results of the Framingham Heart Study were published in 1990. In this study the left ventricular hypertrophy was determined by echocardiography and showed a positive correlation between left ventricular mass and the incidence of cardiovascular diseases having also a significant effect on mortality.

Again, the cornerstone of treatment is the inhibition of RAS. Morath et al measured the efficacy and safety of RAS-inhibition after kidney transplantation. They found that ACE inhibitors decrease left ventricular mass and the grade of left ventricular hypertrophy (LVH) in kidney transplant patients.

The successful renal transplantation could also reduce left ventricular mass index (LVMI). Iqbal et al found, that LVMI decreased significantly 3 months after transplantation. The results were explained by the decrease of the diameter of the left ventricle, which can be explained by reduced fluid load.

A majority of publications suggest a significantly higher frequency of deteriorated morphological and functional cardiac parameters after renal transplantation, compared to the age-matched not-transplanted population. One of these parameters is LVH. Although it is an independent risk factor with multifactorial etiology, there is no agreement in the role of genetic variations on the occurrence of LVH.

Our study was designed to reveal correlations between ACE I/D genotype and LVH in a kidney transplanted population. Important to note, that we included patients after at least 7 years of transplantation, therefore our results were not influenced by the early morphologic improvement, which is usually observed in the first years after transplantation. We found a clear correlation between kidney function and LVMI. Furthermore, not only LVH was more frequent in patients with ACE DD genotype, but its incidence also increased during the follow up period.

## **Conclusion**

Our results suggest that ACE I/D genotype predicts the expected survival of the transplanted kidney, as well as the patient. Patients with ACE DD genotype are more susceptible for transplant failure. These patients should be identified and a special attention should be made on their pharmacological treatment (RAS inhibition), and their compliance should be maintained.



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### List of publications related to the dissertation

1. **Fedor, R.**, Asztalos, L., Lőcsey, L., Szabó, L., Mányiné, I.S., Fagyas, M., Lizanecz, E., Tóth, A.:  
Insertion/deletion polymorphism of the angiotensin-converting enzyme predicts left ventricular hypertrophy after renal transplantation.  
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List of other publications

3. Úri, K., Fagyas, M., Mányiné Siket, I., Kertész, A., Csanádi, Z., Sándorfi, G., Clemens, M., **Fedor, R.**, Papp, Z., Édes, I., Tóth, A., Lizanecz, E.: New perspectives in the renin-angiotensin-aldosterone system (RAAS) IV: Circulating ACE2 as a biomarker of systolic dysfunction in human hypertension and heart failure.  
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