Polish Annals of Medicine

Research paper

The *ACE* rs4340 polymorphism as genetic modulator of gender-specific trends in diabetic ketoacidosis development at onset of type 1 diabetes in children

Małgorzata Pawłowicz[1,2] ID *, Rafał Filipów[3], Grzegorz Krzykowski[4,5], Julia Kulczycka[6], Anna Balcerska[1], Joanna Wojtkiewicz[2]*

[1] *Department of Paediatric, Haematology, Oncology and Endocrinology, Medical University of Gdańsk, Poland*
[2] *Department of Pathophysiology, Laboratory of Regenerative Medicine, Faculty of Medical Sciences, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Poland*
[3] *Institute of Mathematics, Faculty of Mathematics, Physics and Informatics, University of Gdańsk, Poland*
[4] *Institute of Informatics, Faculty of Mathematics, Physics and Informatics, University of Gdańsk, Poland*
[5] *Banking College of Gdańsk, Poland*
[6] *Laboratory of Immunology and Clinic Transplantology, University Clinical Centre of Gdańsk, Poland*

ARTICLE INFO

ABSTRACT

Introduction: Regulatory function of renin-angiotensin-aldosterone system in glucose metabolism may interfere with metabolic dysregulation associated with the development of diabetic ketoacidosis (DKA) at type 1 diabetes (T1DM) onset with further impact to clinical course of the disease.

Aim: Our aim was to evaluate the association between general and gender-specific distribution of the insertion/deletion polymorphism of *ACE* gene (rs4340) and DKA development at T1DM onset in paediatric patients.

Material and methods: 147 children with newly diagnosed autoimmune T1DM from Pomeranian Province (Poland) were qualified to prospective longitudinal study. The *ACE* rs4340 polymorphism was analysed using polymerase chain reaction – allele specific amplification (ASA-PCR). DKA diagnosis and its severity were evaluated based on the international guidelines. Patients' follow-up was conducted throughout 24 months with periodic re-evaluation of fasting C--peptide (FCP) and HbA1c level.

Results and discussion: In study group the *ACE* rs4340 polymorphism was distributed according to Hardy–Weinberg equilibrium, without any gender-specific allocation of genotypes. A significant lower frequency of DKA development at time of T1DM diagnosis was observed in girls ($P = 0.04$). In patients with DKA development at T1DM onset we identified significant decrease in percentage of male carriers of *ACE* rs4340 II genotype and female carriers of *ACE* rs4340 DD+ID genotypes in comparison to subjects without DKA in anamnesis. Simultaneously in above subgroups unfavourable dynamics of residual β-cell function were observed.

Conclusions: Modification of gender-specific trends in DKA development at T1DM onset associated with the *ACE* rs4340 polymorphism and its further impact to clinical course of disease requires further functional studies to development of new additive therapeutic strategies of disease.

Corresponding author: *Małgorzata Pawłowicz, Department of Pathophysiology, Laboratory of Regenerative Medicine, Faculty of Medical Sciences, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Warszawska 30, 10-082 Olsztyn, Poland. Tel: +48 609 866 488.*
E-mail address: malgorzatapawlowicz@gumed.edu.pl.

1. INTRODUCTION

The angiotensin converting enzyme (ACE) plays crucial role in the renin – angiotensin – aldosterone system (RAAS) by converting angiotensin I (ANG I) to metabolically active angiotensin II (ANG II).[1] ANG II interacts mainly with angiotensin receptors type 1 (AT1Rs) associated with the major effects of ANG II and stimulation of aldosterone production. The effects of stimulation of angiotensin receptors type 2 (AT2Rs) are less known, however AT2Rs expression is comparable to AT1R and increases during local inflammation and tissue remodeling.[2]

Stimulation of AT1R via ANG II in pancreatic -cells could lead to decreased local blood flow and reduced insulin secretion due to: (1) suppressing function of pancreatic glucose sensor – glucose transporter 2 (GLUT2), (2) promoting apoptosis and (3) production of reactive oxygen species (ROS) increasing oxidative stress in -cells.[2,3] Above effects of ANG II could be modified by the insertion/deletion (I/D) polymorphism of ACE gene (rs4340), which in the case of homozygous deletion carriers (genotype DD) could cause two-fold increase in plasma and tissue ACE activity in comparison to the insertion homozygotes (genotype II).[1] However, data concerning the relationship between I/D polymorphism of ACE gene and glucose metabolism are divergent, showing differences between populations and gender.[4]

In the case of glucose metabolism, further local RAAS could be additionally involved to insulin resistance/sensitivity pathomechanisms, which are modified by the same genetic factors.[2] The RAAS deleterious axis by overstimulation of AT1Rs could change the secretory profile of adipose tissue to more proinflammatory and insulin-resistant due to activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase – ROS pathway.[2,3,5] In skeletal myocytes overstimulation of AT1Rs decreases insulin-stimulated glucose uptake and impairs insulin signaling by inhibition of translocation of glucose transporter 4 (GLUT4) (via downregulation of phosphatidylinositol 3-kinase (PI3K) cascade and promoting ROS production).[2,3] AT1Rs over-signaling in hepatocytes leads to over-expression of phosphoenolpyruvate carboxykinase (PEPCK) catalysing conversion of oxaloacetate into phosphoenolpyruvate and carbon dioxide.[2]

Imbalance between insulin and counterregulatory hormones observed at type 1 diabetes (T1DM) onset, leading to diabetic ketoacidosis (DKA) development by enhanced lipolysis with reduced glucose utilization and altered gluconeogenesis, could interfere with the above RAAS pathomechanisms.[6] In the case of all above pathways associated with ATRs signaling there are observed some gender-specific differences in expression, levels and activity of particular proteins and enzymes.[5,7,8] Also gender-specific trends in frequency of DKA development at disease onset were noticed in many T1DM populations.[9,10]

Therefore, we conducted a longitudinal study to evaluate the association between general and gender-specific distribution of the I/D polymorphism of *ACE* gene and DKA development at T1DM onset in paediatric patients from Pomeranian Province in Poland with a following 24-monthly clinical observation.

2. AIM

Our aim was to evaluate the association between general and gender-specific distribution of the I/D polymorphism of *ACE* gene (rs4340) and DKA development at T1DM onset in paediatric patients.

3. MATERIAL AND METHODS

3.1. Study group and protocol
The consecutive 147 children (73 boys and 74 girls; mean age 8.91 years, range 1.08–17.9 years) with newly diagnosed autoimmune T1DM from Pomerania Province in Poland was classified to the prospective, longitudinal study. Inclusion and exclusion criteria with detailed synopsis of the patient group were given in our previous study.[11] After baseline hospitalisation at the time of T1DM diagnosis patients were followed-up by further 24 months.

3.1.1. Baseline hospitalisation at the time of T1DM diagnosis
The clinical status at the time of diagnosis (DKA or non-DKA presentation) in each patient was established using the American Diabetes Association (ADA)[6] and International Society for Pediatric and Adolescent Diabetes (IS-PAD) guidelines.[12]

The baseline biochemical and serological tests – fasting C-peptide (FCP) concentration as residual β-cell function evaluation, HbA1c level and specific autoantibodies titres – were performed in each patient after correction of disturbances in the acid-base balance as described previously.[11] Blood samples for genetic testing were collected at the same time and stored at $-20°C$ before use, no longer than 6 months.

3.1.2. Follow-up of patients
The biochemical parameters (FCP and HbA1c level) were controlled in each patient through 24-months of follow-up, with the previously described frequency:[11] at 3- and 6-months intervals in the 1st and 2nd year of T1DM, respectively.

3.2. *ACE* I/D polymorphism genotyping
The genomic DNA extracted from the whole blood samples of patients (Genomic Midi AX Kits, A&A Biotechnology, Gdynia, Poland) was used for *ACE* I/D polymorphism analysis (rs4340). The chosen *ACE* polymorphism was assessed via previously described polymerase chain reaction – allele specific amplification (ASA-PCR).[13]

3.3. Ethical considerations
The Local Ethics Committee on Clinical Investigation of the Medical University of Gdańsk, Poland approved the protocol of study (NKEBN/213/2011). According to the Declaration of Helsinki, written informed consent was obtained from all participants' parents/guardians, as well as the participants, when applicable.

Table 1. Clinical characteristics of patients depending on the gender in the study population.

	Boys n = 73	Girls n = 74	P value
Median of age at diagnosis, years	9.28 (IQR 4.37–12.78)	8.88 (IQR 4.9–12.71)	0.37
Tanner stage at diagnosis			
Prepubertal	44	43	0.9
Pubertal	29	31	
Patient's condition at diagnosis			
Without disorders in acid-base status	38	50	0.04
Diabetic ketoacidosis	31	21	
Median of T1DM symptoms duration, days	14 (IQR 7–14)	14 (IQR 7–30)	0.22
Median of BMI Z-score at diagnosis	0.21 (IQR 0.65–0.48)	0.28 (IQR 0.07–0.60)	0.21
Family history of type 1 diabetes	11	12	0.59
Place of residence			
Town	41	51	0.07
Countryside	32	23	
ACE rs4340 (I/D) polymorphism – allele distribution			
Allele D	70	57	0.06
Allele I	76	91	
ACE rs4340 (I/D) polymorphism – genotypes distribution			
DD	15	8	
ID	40	41	0.19
II	18	25	

Comments: BMI – body mass index; IQR – interquartile range; T1DM – type 1 diabetes.

3.4. Statistics[14]

The software R v. 3.1.1 (The R Foundation for Statistical Computing, Austria) was used for statistical analyses. The mode of data distribution was assessed using the Kolmogorov–Smirnov test. Non-parametric tests were performed for further analysis of data with non-normal distribution. The data was presented as a median with an interquartile range (IQR) from 25 to 75. The Fisher's exact test was performed for analysis of contingency tables. A *P* value of less than 0.05 reflected statistically significance. Missing values were handled by case-wise deletion.

4. RESULTS

4.1. Characteristics of the study population with clinical trends specific to the gender

A clinical comparison between boys and girls in the study population is presented in Table 1. A significant lower frequency of DKA development at the time of T1DM diagnosis was observed in girls (*P* = 0.04). Gender-specific significant differences in the dynamics of residual β-cell function were found only at the beginning of the disease (*P* = 0.03; girls 0.32 ng/mL vs. boys 0.21 ng/mL). Dynamics of HbA1c levels was comparable between both sexes throughout follow-up period.

4.2. Distribution of the *ACE* I/D polymorphism in the patient population

The allele frequency and genotypes distribution of the *ACE* I/D polymorphism in the patient group was consistent with Hardy–Weinberg equilibrium and is presented in Table 1.

4.3. *ACE* I/D polymorphism as a modulator of association between gender and DKA development at the time of T1DM diagnosis

In the entire patient population, no association between specified allele or genotype of the *ACE* I/D polymorphism and DKA development at the time of T1DM diagnosis was found. In the subgroup without DKA diagnosis at T1DM onset the boys and girls ratio (M : F) was similar, irrespective of the *ACE* I/D polymorphism (*P* = 0.45) (subgroup without DKA M : F = 1.0 : 1.3; genotypes DD+ID vs. genotype II: M:F=1.0 : 1.4 vs. M:F=1.0 : 1.2) (Figure 1a). The opposite gender ratio noticed in patients with DKA development (M : F = 1.5 : 1.0) was further modified by the genotype of the *ACE* I/D polymorphism, in the significantly completely adversative manner (*P* = 0.007): genotypes DD+ID vs. genotype II M : F=2.4 : 1.0 vs. M : F=1.0 : 2.4 (Figure 1b).
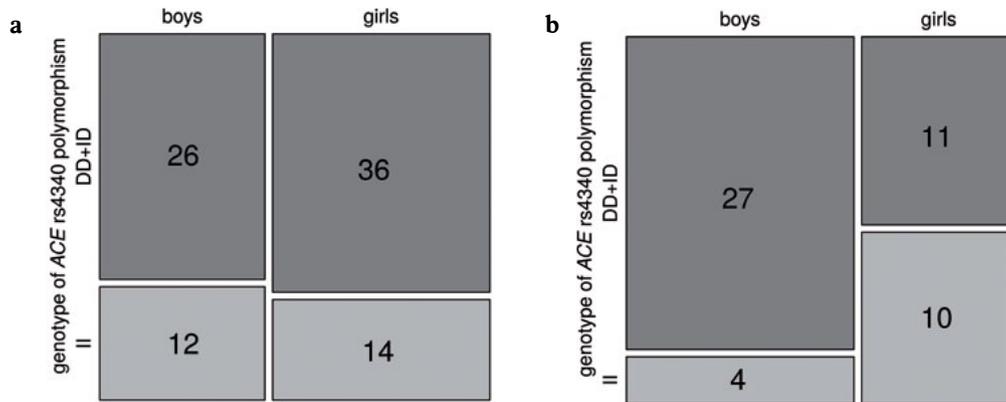
Figure 1. Distribution of the *ACE* rs4340 genotypes in the patient group, depending on the occurrence of diabetic ketoacidosis at type 1 diabetes onset: (a) subgroup of patients without DKA development at T1DM onset; (b) subgroup of patients with DKA development at T1DM onset.
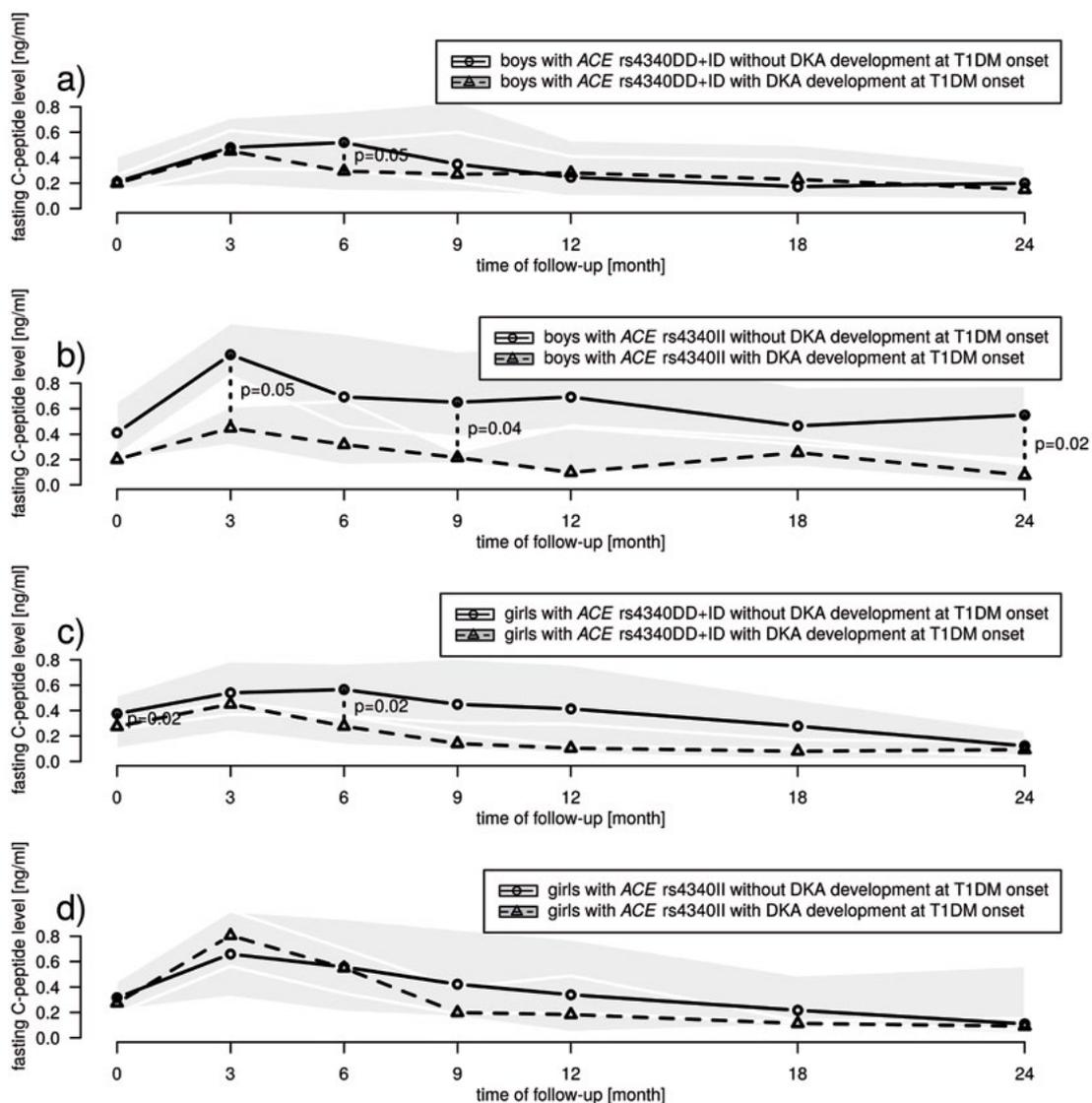

Figure 2. Dynamics of residual β-cell function in the patient group: (a) comparison of the male carriers of *ACE* rs4340 DD + ID genotypes depending on the occurrence of DKA at T1DM onset; (b) comparison of the male carriers of *ACE* rs4340 II genotype depending on the occurrence of DKA at T1DM onset; (c) comparison of the female carriers of *ACE* rs4340 DD+ID genotypes depending on the occurrence of DKA at T1DM onset; (d) comparison of the female carriers of *ACE* rs4340 II genotype depending on the occurrence of DKA at T1DM onset. Comments: black lines – median; grey pools – 25th–75th interquartile range.

4.4. General trends in residual β-cell function and HbA1c level dynamics in the patient group

The general dynamics of residual β-cell function and HbA1c level in the patient group throughout the 24-month follow-up period was previously described.[11] There were noticed no significant differences in dynamics trends of FCP and HbA1c level depending on the gender. The parallel time trends in FCP and HbA1c level were observed in the subgroups divided according to the DKA development at T1DM onset with significantly lower FCP levels throughout the follow-up and corresponding inversely proportional HbA1c levels in patients with DKA development.

4.5. The clinical effects of the *ACE* I/D polymorphism in the patient group

In analysis of residual β-cell function dynamics according to the allele of *ACE* I/D polymorphism there was noticed significantly higher FCP level in homozygotes II only in the 3rd month in comparison to DD+ID genotypes carriers ($P < 0.001$). Simultaneously in homozygotes II there were observed significantly higher HbA1c level at the T1DM onset ($P = 0.04$) and significantly lower in 12th month of disease when compared with the DD+ID genotypes carriers ($P = 0.03$).

4.5.1. The clinical effects of modulation of the association between gender and DKA development at the time of T1DM diagnosis by the *ACE* I/D polymorphism in the patient group

4.5.1.1. The dynamics of residual β-cell function

The specifically unfavourable dynamics of residual β-cell function was observed in patients with DKA development at T1DM onset, belonging to the gender represented in the lower percentage of carriers of a particular genotype of *ACE* I/D polymorphism (Figure 2): in male carriers of genotype II (Figure 2b) and in female carriers of genotypes DD+ID (Figure 2c).

In boys with genotype II of *ACE* I/D polymorphism and DKA development at T1DM diagnosis there were observed almost significantly lower FCP peak at 3rd month of disease ($P = 0.05$) with steady worsening of residual β-cell function up to undetectable FCP concentrations occurred after 12th month of disease. Above dynamics of residual β-cell function was especially detrimental and led to significant differences when compared to boys with genotype II and without DKA history, particularly in the second year of follow-up ($P \leq 0.04$) (Figure 2b).

The DKA occurrence at T1DM diagnosis in female carriers of genotypes DD+ID resulted in significantly lower FCP levels up to sixth month of disease ($P = 0.02$) in comparison to the girls with the same genetic constitution and without DKA in anamnesis (Figure 2c).

In patients with DKA diagnosis at T1DM onset belonging to the dominant gender represented in the carriers of a particular genotype of *ACE* I/D polymorphism, the dynamics of residual β-cell function indicated no specific alterations. In these patients were observed insignificantly lower or comparable FCP levels throughout follow-up in comparison to the subgroups without DKA, matched according to the gender and genotype of *ACE* I/D polymorphism (Figures 2a and 2d).

All described trends in FCP dynamics in the particular subgroups throughout follow-up were independent of patients' BMI z-scores. Considering daily insulin dose the significantly higher daily insulin doses were noticed only up to the 3rd month of disease in female carriers of genotypes DD+ID in the case of DKA diagnosis at T1DM onset.

4.5.1.2. The dynamics of HbA1c level

Significant differences in HbA1c level dynamics were observed only in male carriers of genotypes DD+ID according to the DKA occurrence at T1DM diagnosis and only for a limited period of time – up to 6th month of disease (3rd month of disease $P = 0.02$, 6th month of disease $P = 0.04$).

5. DISCUSSION

To the best of our knowledge, this is one of the first studies to show the modulating and gender-specific influence of the *ACE* I/D polymorphism (rs4340) on the clinical onset and further course of T1DM in paediatric patients. In patients with DKA development at T1DM onset we identified significant decrease in percentage of male carriers of *ACE* rs4340 II genotype and female carriers of *ACE* rs4340 DD+ID genotypes in comparison to the subjects without DKA in anamnesis. Simultaneously in above subgroups the unfavourable dynamics of residual β-cell function were observed.

The background for explanation of our study results could be the dose-dependent inhibitory effect of ANG II on insulin secretion modified functionally by the *ACE* I/D polymorphism with subsequent gender-specific differences in expression, levels and activity of particular proteins and enzymes associated with ATRs signaling pathways.[5,7,8]

In insertion homozygotes (genotype II), lower ACE activity promotes insulin secretion by inhibition of the Pi3K pathway due to reducing oxidative stress.[2,15] The above ACE effect is achieved by AT2Rs stimulation, the expression of which increases in inflammation and metabolic stress.[2] This promotion of insulin secretion could be limited in time by a direct auto-regulation of insulin receptors via insulin receptor substrate 1 (IRS-1).[16] Simultaneously lower ACE concentration in insertion homozygotes leads to increase stimulation of bradykinin B1 and B2 receptors (B1R and B2R) in peripheral tissue due to decreased bradykinin (BK) degradation. This effect is exacerbated in pro-inflammatory conditions by overstimulation of B1R by the active BK metabolite – Des-Arg9-BK, which is expressed exclusively during inflammation.[16,17] The rate of Des-Arg9-BK degradation is lower in men and could be associated with prolonged stimulation of B1R leading to over-activation of mitogen activated protein kinase (MAP kinase) – ROS pathway and therefore increased level of free fatty acid (FFA).[15,17] Above gender-differences in Des-Arg9-BK metabolism could modify intensity of lipolysis and interfere with the main DKA

pathomechanism, in which increased level of FFA generates further exacerbation of ketogenesis and gluconeogenesis.[6,15] By interference between Des-Arg9-BK – MAP – ROS pathway and DKA pathomechanism could be explained significantly unfavorable dynamics of FCP level in boys carrying genotype II of *ACE* I/D polymorphism, in whom DKA developed at T1DM onset, in comparison to the male carriers with genotype II and without DKA in anamnesis.

In female carriers of genotypes DD+ID with DKA diagnosis unfavorable dynamics of residual β-cells function in the first 6 months of disease could be associated with overlap of the negative effect of deletion ACE allele on insulin secretion and uncoupling between AT1R and NADPH oxidase, which is more expressed in female.[2,5] Above uncoupling, could lead to dysfunction of suppressive CD8+ T regulatory cells (CD8+ Treg) further exacerbated by DKA and thus to more aggressive autoimmune destruction of β-cells at the onset of T1DM associated with DKA presentation.[18,19]

Significant differences in HbA1c level dynamics noticed in male carriers of genotypes DD+ID depending on the DKA occurrence at T1DM diagnosis could be explained by the male-predominant expression of PEPCK, which is further upregulated in renal proximal tubule and hepatocytes under conditions of prolonged metabolic acidosis, leading to the exacerbation of hyperglycemia associated with insulin deficiency.[2,6,8]

6. CONCLUSIONS

Indicated modification of gender-specific trends in DKA development at T1DM onset associated with ACE I/D polymorphism and its further impact to the subsequent clinical course of disease requires further functional researches to the development of new additive therapeutic strategies (ACE modificators) to insulin or Treg therapy in patients with early T1DM phase.

Conflict of interest
None declared.

References

1. Sayed-Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Witteman JC. ACE polymorphisms. *Circ Res*. 2006;98(9): 1123––1133. https://doi.org/10.1161/01.RES.0000223145.74217.e7.
2. Favre GA, Esnault VL, Van Obberghen E. Modulation of glucose metabolism by the renin-angiotensin-aldosterone system. *Am J Physiol Endocrinol Metab*. 2015;308(6):E435–449. https://doi.org/10.1152/ajpendo.00391.2014.
3. Underwood PC, Adler GK. The renin angiotensin aldosterone system and insulin resistance in humans. *Curr Hypertens Rep*. 2013;15(1):59–70. https://doi.org/10.1007/s11906-012-0323-2.
4. Ryan AS, Nicklas BJ, Berman DM, Ferrell RE. The insertion/deletion polymorphism of the ACE gene is related to insulin sensitivity in overweight women. *Diabetes Care*. 2001;24(9): 1646–1652. https://doi.org/10.2337/diacare.24.9.1646.
5. Bhatia K, Elmarakby AA, El-Remessy AB, Sullivan JC. Oxidative stress contributes to sex differences in angiotensin II-mediated hypertension in spontaneously hypertensive rats. *Am J Physiol Regul Integr Comp Physiol*. 2012;302(2): R274–282. https://doi.org/10.1152/ajpregu.00546.2011.
6. Wolfsdorf J, Glaser N, Sperling MA. Diabetic ketoacidosis in infants, children, and adolescents: A consensus statement from the American Diabetes Association. *Diabetes Care*. 2006;29(5):1150–1159. https://doi.org/10.2337/diacare.2951150.
7. Komukai K, Mochizuki S, Yoshimura M. Gender and the renin-angiotensin-aldosterone system. *Fundam Clin Pharmacol*. 2010;24(6):687–698. https://doi.org/10.1111/j.1472-8206.2010.00854.x.
8. Gustavsson C, Yassin K, Wahlstrom E, et al. Sex-different hepaticglycogen content and glucose output in rats. *BMC Biochem*. 2010;11:38. https://doi.org/10.1186/1471-2091-11-38.
9. Usher-Smith JA, Walter FM, Thompson MJ, Sharp SJ. Factors associated with the presence of diabetic ketoacidosis at diagnosis of diabetes in children and young adults: A systematic review. *BMJ*. 2011;343:d4092. https://doi.org/10.1136/bmj.d4092.
10. Usher-Smith JA, Thompson M, Ercole A, Walter FM. Variation between countries in the frequency of diabetic ketoacidosis at first presentation of type 1 diabetes in children: a systematic review. *Diabetologia*. 2012;55(11):2878–2894. https://doi.org/10.1007/s00125-012-2690-2.
11. Pawlowicz M, Filipow R, Krzykowski G, et al. Coincidence of *PTPN22* c.1858CC and *FCRL3* β-169CC genotypes as a biomarker of preserved residual beta-cell function in children with type 1 diabetes. *Pediatr Diab*. 2016;18(8):696–705. https://doi.org/10.1111/pedi.12429.
12. Wolfsdorf J, Craig ME, Daneman D, et al. Diabetic ketoacidosis in children and adolescents with diabetes. *Pediatr Diab*. 2009;10(Suppl 12):118–133. https://doi.org/10.1111/j.1399-5448.2009.00569.x.
13. Borzyszkowska J, Stanislawska-Sachadyn A, Wirtwein M, et al. Angiotensin converting enzyme gene polymorphism is associated with severity of coronary artery disease in men with high total cholesterol levels. *J Appl Genet*. 2012;53(2):175–182. https://doi.org/10.1007/s13353-012-0083-3.
14. Altman DG, ed. Practical statistics for medical research. Boca Raton: Chapman & Hall; 2001.
15. Almeida SS, Corgosinho FC, Amorim CE, et al. Different metabolic responses induced by long-term interdisciplinary therapy in obese adolescents related to ACE I/D polymorphism. *J Renin Angiotensin Aldosterone Syst*. 2017;18(2):1470320317703451. https://doi.org/10.1177/1470320317703451.

16   Rhodes CJ, White MF, Leahy JL, Kahn SE. Direct auto-
     crine action of insulin on beta-cells: does it make physio-
     logical sense? *Diabetes*. 2013;62(7):2157–2163. https://doi.
     org/10.2337/db13-0246.

17   Cyr M, Lepage Y, Blais C Jr, et al. Bradykinin and des-Arg(9)-
     -bradykinin metabolic pathways and kinetics of activation of
     human plasma. *Am J Physiol Heart Circ Physiol*. 2001;281(1):
     H275–283. https://doi.org/10.1152/ajpheart.2001.281.1.H275.

18   Kitabchi AE, Stentz FB, Umpierrez GE. Diabetic ketoaci-
     dosis induces in vivo activation of human T-lymphocytes.
     Biochem Biophys Res Commun. 2004;315(2):404–407. https://
     doi.org/10.1016/j.bbrc.2004.01.065.

19   Giardino G, Cicalese MP, Delmonte O, et al. NADPH Oxidase
     Deficiency: A Multisystem Approach. *Oxid Med Cell Longev*.
     2017;2017:4590127. https://doi.org/10.1155/2017/4590127.