Association study between BGLAP RS1800247-polymorphic variant and type 2 diabetes mellitus development among hypertensive and non-hypertensive Ukrainians

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The risk of type 2 diabetes mellitus (T2DM) development depends on a hereditary predisposition. According to the current data, bone tissue enhances insulin gene expression in pancreatic β-cells as well as increases insulin sensitivity of adipocytes, myocytes and hepatocytes through the secretion of undercarboxylated osteocalcin (unOCN).

The aim. To analyze the relation between rs1800247 SNP and T2DM occurrence depending on the arterial hypertension (AH) presence, as well as association between rs1800247 and systolic, diastolic, pulse, mean blood pressure among patients with T2DM.

Materials and methods. This study included 153 patients with diagnosed T2DM and 311 individuals without any carbohydrate metabolism disorders. Polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) was used for BGLAP rs1800247-genotyping. Logistic regression with interaction term “genotype × AH” was used to estimate the association between BGLAP rs1800247-genotypes and T2DM development in dominant, recessive, over-dominant and additive models of inheritance. Linear regression was performed to examine the influence of minor C-allele on the arterial blood pressure. Lipid profile characteristics of T2DM patients were stratified by rs1800247-genotype using ANOVA with Bonferroni post hoc test. All calculations were performed using Statistical Package for the Social Sciences software (SPSS, version 22.0, Chicago, IL, USA).

A value of P < 0.05 was considered as significant.

Results. No association was found between rs1800247 single nucleotide polymorphism and T2DM development neither in AH patients, nor in subjects without AH (P > 0.05). There was no impact of rs1800247 genotypes on systolic, diastolic, pulse and mean blood pressure among patients with T2DM (P > 0.05). It was showed that T2DM non-hypertensive CC-carriers had significantly lower levels of total cholesterol (P = 0.012) and LDL cholesterol (P = 0.04), but higher concentration of HDL cholesterol (P = 0.015) compared to the TT-genotype.

Conclusions. It was showed that CC-carriers had more favorable parameters of lipid metabolism among T2DM non-hypertensive Ukrainians. However, there was no association between rs1800247 SNP and T2DM development as well as blood pressure parameters.
grupi хворих на ЦД2 без АГ мають істотно нижчий рівень загального холестеролу (p = 0,012), ЛПНП (p = 0,04) і вищий рівень ЛПВП (p = 0,015) порівняно з ТТ-гетерозиготою.

Висновки. Виявлено, що носії СС-гетерозиготи мають сприятливіші показники ліпідного обміну серед українців із ЦД2 та без АГ. Але відсутній зв'язок між rs1800247-однонуклеотидним полиморфізмом, виникненням ЦД2 і показниками артеріального тиску.

Изучение связи между RS1800247-полиморфным вариантом гена BGLAP и развитием сахарного диабета 2 типа среди украинцев с артериальной гипертензией и нормальным артериальным давлением

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Риск возникновения сахарного диабета 2 типа (СД2) зависит от генетической предрасположенности. В соответствии с современными данными, костная ткань увеличивает экспрессию гена инсулина β-клетками поджелудочной железы, а также повышает чувствительность адипоцитов, миоцитов и гепатоцитов к инсулину путем продукции декарбоксилированного остеокальцина (unOCN).

Цель работы – проанализировать связь между rs1800247-однонуклеотидным полиморфизмом и возникновением СД2 в зависимости от наличия артериальной гипертензии (АГ), а также связь между rs1800247 и системическим, диастолическим, пульсовым, средним кровяным давлением у пациентов с СД2.

Материалы и методы. В исследование включили 153 пациента с диагностированным СД2 и 311 лиц без каких-либо нарушений углеводного обмена. Генотипирование участников исследования по rs1800247-полиморфизмом гена BGLAP проведено при помощи полимеразной цепной реакции с анализом длины рестрикционных фрагментов (PCR-RFLP). Для оценки связи между rs1800247-полиморфизмом гена BGLAP и развитием СД2 использована логистическая регрессия (с учётом независимой переменной «генотип x АГ») в пределах доминантной, рецессивной, сверхдоминантной и аддитивной моделей наследования.

Для исследования влияния минорного С-аллеля на уровень артериального давления использован метод линейной регрессии. Показатели липидного обмена у пациентов с СД2 в зависимости от rs1800247-гетерозиготы сравнивали при помощи однофакторного дисперсионного анализа и теста Бонферрони. Все расчёты проведены с использованием программы для статистической обработки данных SPSS 22.0. Значение p < 0,05 свидетельствовало о статистической значимости результатов.

Результаты. Установлено отсутствие связи между rs1800247-однонуклеотидным полиморфизмом и развитием СД2 среди лиц с АГ, а также в зависимости от наличия артериальной гипертензии (АГ), а также связь между rs1800247 и системическим, диастолическим, пульсовым, средним кровяным давлением у пациентов с СД2.

Выводы. Установлено, что носители СС-гетерозиготы имеют более благоприятные показатели липидного метаболизма среди украинцев с СД2 и без АГ. Однако отсутствует связь между rs1800247 и артериальным давлением.

Nowadays it is known about at least two proteins of bone tissue that could influence on systemic energy metabolism. The first one is undercarboxylated osteocalcin (unOCN), which directly stimulates insulin production in pancreatic β-cells and increases peripheral tissue sensitivity to this hormone. The second one, osteotesticular tyrosine phosphatase (OST-PTP), regulates OCN gene expression in accordance with metabolic requirements of the bone tissue [1]. Bone reparation was the priority process of energy sub [2]. Meta-analysis indicates the presence of reverse correlation between OCN plasma concentration and insulin, fasting glucose and glycated hemoglobin in patients with type 2 diabetes mellitus (T2DM) [7]. Moreover, rs1800247-single nucleotide polymorphism (SNP) of BGLAP gene was associated with decreased risk of arterial hypertension (AH) and lower diastolic blood pressure [8].

Aim

Therefore, the aim of this study was to analyze the relation between rs1800247 SNP and T2DM occurrence depending on the AH presence, as well as association between rs1800247 and systolic, diastolic, pulse, mean blood pressure among patients with T2DM.
Materials and methods

Study population and genotyping. This study included 153 patients (75 females and 78 males; mean age ± SD 64.67 ± 8.2 year) with diagnosed T2DM and 311 individuals (106 females and 205 males; mean age 65.65 ± 12.58 year) without any cardiovascular metabolic disorders. Final T2DM diagnosis was determined in the presence of specific clinical manifestations (polydipsia, polyuria, polyphagia and weight loss), fasting glucose level and glucose tolerance test results according to the World Health Organization criteria (WHO, 1999).

Materials and methods

Table 1. Characteristics of the study population

| Parameter                        | Units | T2DM (n = 107) | Control (n = 156) | P  
|----------------------------------|-------|----------------|------------------|------
| Age, years                       |       | 64.49 ± 8.11   | 69.53 ± 11.31    | <0.001 |
| Sex, female/male                 |       | 56:52          | 101:55           | 0.036 |
| Body mass index, kg/m²           |       | 29.7 ± 4.98    | 27.62 ± 4.69     | 0.001 |
| Current smokers, n (%)           |       | 36 (33.3)      | 48 (30.5)        | 0.66  |
| Fasting glucose, mmol/L          |       | 10.35 ± 3.77   | 5.22 ± 0.68      | <0.001 |
| Total cholesterol, mmol/L        |       | 5.24 ± 1.18    | 4.56 ±1.26       | <0.001 |
| HDL cholesterol, mmol/L          |       | 0.94 ± 0.28    | 1.09 ± 0.28      | <0.001 |
| LDL cholesterol, mmol/L          |       | 3.38 ± 1.16    | 2.8 ± 1.22       | 0.003 |
| Triglyceride, mmol/L             |       | 1.76 ± 0.68    | 1.44 ± 0.65      | 0.002 |
| Systolic BP, mmHg                |       | 151.71 ± 14.78 | 174.46 ± 22.69   | <0.001 |
| Diastolic BP, mmHg               |       | 92.31 ± 8.6    | 97.05 ± 13.89    | 0.002 |
| Pulse BP, mmHg                   |       | 59.4 ± 13.43   | 77.4 ± 19.32     | <0.001 |
| Mean BP, mmHg                    |       | 112.12 ± 9.06  | 122.65 ± 14.74   | <0.001 |

Table 1. Characteristics of the study population

| Parameter                        | Units | T2DM (n = 46) | Control (n = 155) | P  
|----------------------------------|-------|---------------|-------------------|------
| Age, years                       |       | 65.26 ± 8.49  | 61.75 ± 12.61     | 0.078 |
| Sex, female/male                 |       | 23/23         | 51/104            | 0.035 |
| Body mass index, kg/m²           |       | 28.35 ± 4.74  | 27.38 ± 4.72      | 0.222 |
| Current smokers, n (%)           |       | 14 (30.4)     | 43 (27.7)         | 0.722 |
| Fasting glucose, mmol/L          |       | 9.86 ± 2.57   | 5.23 ± 0.73       | <0.001 |
| Total cholesterol, mmol/L        |       | 5.65 ± 1.35   | 4.14 ± 1.15       | <0.001 |
| HDL cholesterol, mmol/L          |       | 0.96 ± 0.31   | 1.09 ± 0.19       | 0.038 |
| LDL cholesterol, mmol/L          |       | 3.54 ± 1.39   | 2.48 ± 1.12       | 0.001 |
| Triglyceride, mmol/L             |       | 2.22 ± 2.49   | 1.25 ± 0.51       | 0.051 |
| Systolic BP, mmHg                |       | 126.09 ± 8.02 | 126.84 ± 11.09    | 0.67  |
| Diastolic BP, mmHg               |       | 79.67 ± 4.76  | 79.58 ± 7.05      | 0.933 |
| Pulse BP, mmHg                   |       | 46.41 ± 6.02  | 47.26 ± 9.01      | 0.551 |
| Mean BP, mmHg                    |       | 95.14 ± 5.34  | 95.33 ± 7.49      | 0.874 |

Materials and methods

Study population and genotyping. This study included 153 patients (75 females and 78 males; mean age ± SD 64.67 ± 8.2 year) with diagnosed T2DM and 311 individuals (106 females and 205 males; mean age 65.65 ± 12.58 year) without any cardiovascular metabolic disorders. Final T2DM diagnosis was determined in the presence of specific clinical manifestations (polydipsia, polyuria, polyphagia and weight loss), fasting glucose level and glucose tolerance test results according to the World Health Organization criteria (WHO, 1999).

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Relatively healthy subjects without any carbohydrate metabolic disorders (which was confirmed by a fasting plasma glucose level less than 5.6 mmol/L and a 75g oral glucose tolerance test result less than 7.8 mmol/L) and negative family history of diabetes were enrolled in the control group. All the examined individuals were selected from hospital records in the 5th Sumy Clinical Hospital and Sumy Regional Diagnostic Center between 2011–2019. AH was diagnosed in 107 T2DM patients and 156 control subjects with systolic blood pressure higher than 140 mmHg and/or diastolic blood pressure higher than 90 mmHg and no antihypertensive therapy (according to the WHO, 1999). Polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) was used for BGLAP rs1800247 genotyping. Full information about genotyping protocol was presented in our previous research [9].

The study design complies with the Declaration of Helsinki and was approved by the Ethic Committee of Medical Institute of Sumy State University and the Ethic Committee of Medical Institute of Sumy State University (number 4/02.18.09). A written informed consent was obtained from all participants.

Statistical analysis. Continuous variables were presented as the mean ± SD, categorical – as absolute and percentage values. Chi square ($\chi^2$) test was used for comparing the categorical variables. Two-tailed Student's t-test was used to compare the mean values of two groups (with preliminary verification of the data distribution for normality through the Shapiro-Wilk test). The mean values of three groups were compared using ANOVA with further Bonferroni post hoc test. Logistic regression with interaction term "genotype × AH" was used for the association analysis between four models of inheritance: dominant, recessive, over-dominant and additive.

The adjustment for age, sex, smoking and body mass index (BMI) was used to exclude influence of other T2DM risk factors. Bonferroni correction was applied for accurate results. The impact of rs1800247-C minor allele on systolic, diastolic, pulse and mean arterial blood pressure among diabetic patients was estimated via linear regression. All calculations were performed using Statistical Package for the Social Sciences software (SPSS, version 22.0, Chicago, IL, USA). A value of $P < 0.05$ was considered as significant.

Results

The clinical characteristics of compared groups are shown in Table 1. Statistically significant differences in age, sex, BMI, fasting glucose, lipid profile and blood pressure parameters ($P < 0.05$), but not among smokers ($P = 0.66$), was found in groups with AH. In contrast, T2DM patients and controls without AH were comparable in age, BMI, smoking status, fasting glucose, lipid profile and blood pressure parameters ($P > 0.05$), but not in sex, fasting glucose levels, total cholesterol, high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol concentrations ($P < 0.05$).

Logistic regression with interaction term was used to study the influence of rs1800247 genotypes on the T2DM development. There was no statistically significant associations neither in AH patients, nor in non-AH individuals in all models of inheritance (Table 2).

Then we performed the linear regression models to compare the rs1800247 genotype impact on the arterial blood pressure values. No significant differences were found for systolic, diastolic, pulse and mean arterial blood pressure among T2DM patients (Table 3).

Table 4 indicates the parameters of lipid profile in T2DM patients with and without AH stratified by rs1800247 genotypes. Statistically significant differences were found between TT and CC carriers in total cholesterol ($P = 0.012$), HDL cholesterol ($P = 0.015$) and LDL cholesterol ($P = 0.04$) concentrations among T2DM individuals without AH.
**Table 2. Association analysis between BGLAP rs1800247 and T2DM development among AH and non-AH individuals**

| Regression model | Covariate | P \(_{\text{int}}\) | OR (95 % CI) | P | OR (95 % CI) | P | OR (95 % CI) | P | OR (95 % CI) | P |
|------------------|-----------|-----------------|--------------|---|--------------|---|--------------|---|--------------|---|
| Dominant         | TC+CC vs TT | 0.288 | 0.748 (0.448–1.25) | 0.976 | 0.199 | 0.708 (0.417–1.200) | 0.754 | 0.796 | 1 |
| Recessive        | CC vs TT+TC | 0.628 | 1.264 (0.413–3.873) | 0.412 | 0.674 | 1.279 (0.406–4.338) | 0.367 | 1 | 1 |
| Over-dominant    | CT vs TT+CC | 0.13 | 0.696 (0.408–1.187) | 0.504 | 0.131 | 0.655 (0.378–1.134) | 0.653 | 0.524 | 1 |
| Additive         | CT vs TT | 0.199 | 0.702 (0.409–1.205) | 0.622 | 0.149 | 0.664 (0.381–1.517) | 0.779 | 0.596 | 1 |
| CC vs TT         | 0.844 | 1.123 (0.361–3.873) | 0.468 | 0.833 | 1.136 (0.351–3.873) | 0.363 | 1 | 1 |

1: Upper row shows the results for individuals with AH and lower row -- for those without AH; Pc: crude value; Pcket: crude value for interaction term; Ps: value adjusted for age, sex, smoking status, and body mass index; Paint: value adjusted for age, sex, smoking status, and body mass index for interaction term; Pabal: value adjusted for Bonferroni correction; Paintb: value adjusted for Bonferroni correction for interaction term. T2DM: type 2 diabetes mellitus; AH: arterial hypertension, TT: homozygous dominant, TC: heterozygous, CC: homozygous recessive.

**Discussion**

It is known that T2DM patients have lower carboxylated osteocalcin (cOCN) and unOCN concentrations than relatively healthy subjects [10,11]. The meta-analysis showed significantly decreased baseline serum total OCN in T2DM compared with non-T2DM subjects. Moreover, a unit elevation in serum total OCN was correlated with a mean increase in HOMA-B, as well as mean reduction in Hba1c, fasting plasma glucose, HOMA-IR and BMI [12].

In this study, we continued to study the association between BGLAP rs1800247 SNP and T2DM development among Ukrainians. The lack of studied correlation matches both our previous research [9] and Das et al. study, which excluded BGLAP rs1800247 SNP as a T2DM potential risk factor [13].

Cardiovascular diseases are widespread chronic complications in patients with T2DM. Animal and in vitro studies showed the protective effect of unOCN on vessels. This was explained by the enhanced expression of endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) production [14]. Lower serum OCN concentration was found among hypertensive men, but not women. Moreover, serum OCN level was inversely associated with systolic blood pressure in Chinese men, but not women [15].

Another study showed that BGLAP rs1800247 was associated with lower risk of AH and diastolic blood pressure in Chinese population [8]. In contrast, our study indicates no relation between BGLAP rs1800247 and blood pressure level among T2DM Ukrainians that can be explained by ethnic differences. Despite this, in present study, we showed that T2DM non-hypertensive CC-carriers had significantly lower levels of total cholesterol and LDL cholesterol, but higher concentration of HDL cholesterol compared to those in the TT-genotype. The results obtained may indicate more favorable conditions for the lipid metabolism in CC-homozygous of the examined groups among Ukrainians.

**Conclusions**

1. Non-hypertensive T2DM CC-carriers had significantly lower levels of total cholesterol (P = 0.012) and LDL cholesterol (P = 0.04), but higher concentration of HDL cholesterol (P = 0.015) compared to the TT-genotype in Ukrainian population.

**Table 3. Association analysis between BGLAP rs1800247 and blood pressure value among T2DM patients**

| Regression model | B | P | r² |
|------------------|---|---|---|
| **Systolic blood pressure** | | | |
| TC vs TT | 2.363 | 0.476 | 0.01 |
| CC vs TT | -4.596 | 0.396 | <0.001 |
| Constant | 143.762 | | |
| **Diastolic blood pressure** | | | |
| TC vs TT | -0.239 | 0.895 | <0.001 |
| CC vs TT | -0.281 | 0.925 | |
| Constant | 88.614 | <0.001 | |
| **Pulse blood pressure** | | | |
| TC vs TT | 2.601 | 0.291 | 0.018 |
| CC vs TT | -4.315 | 0.284 | |
| Constant | 55.149 | <0.001 | |
| **Mean blood pressure** | | | |
| TC vs TT | 0.625 | 0.768 | 0.003 |
| CC vs TT | -1.722 | 0.62 | |
| Constant | 107 | <0.001 | |

B: regression coefficient; r²: r-squared value; T2DM: type 2 diabetes mellitus; TT: homozygous dominant; TC: heterozygous; CC: homozygous recessive.

**Table 4. Lipid profile in T2DM patients with and without AH stratified by BGLAP rs1800247 genotypes**

| With AH | Parameters, units | Genotype | TT (n = 71) | TC (n = 30) | CC (n = 6) |
|---------|------------------|----------|-------------|-------------|------------|
| Total cholesterol, mmol/L | 5.52 ± 1.22 | 5.19 ± 1.11 | 5.34 ± 1.28 | 0.057 | 0.945 |
| HDL cholesterol, mmol/L | 0.66 ± 0.28 | 0.66 ± 0.27 | 0.81 ± 0.32 | 2.004 | 0.14 |
| LDL cholesterol, mmol/L | 3.33 ± 1.17 | 3.42 ± 1.14 | 3.36 ± 1.22 | 0.065 | 0.937 |
| Triglyceride, mmol/L | 1.73 ± 0.66 | 1.88 ± 0.65 | 1.58 ± 0.75 | 0.763 | 0.469 |

Without AH

| Parameters, units | Genotype | TT (n = 30) | TC (n = 10) | CC (n = 6) |
|------------------|----------|-------------|-------------|------------|
| Total cholesterol, mmol/L | 5.95 ± 1.32 | 5.04 ± 1.23 | 4.27 ± 0.54 | 5.686 | 0.007* |
| HDL cholesterol, mmol/L | 0.94 ± 0.26 | 1.01 ± 0.39 | 1.32 ± 0.12 | 4.368 | 0.019* |
| LDL cholesterol, mmol/L | 3.8 ± 1.41 | 2.85 ± 1.07 | 2.33 ± 0.83 | 4.53 | 0.016* |
| Triglyceride, mmol/L | 2.43 ± 3.04 | 1.88 ± 0.74 | 1.68 ± 0.62 | 0.332 | 0.719 |

AH: arterial hypertension; T2DM: type 2 diabetes mellitus; HDL: high density lipoproteins; LDL: low density lipoproteins; 1: significant difference between the TT and CC genotypes (P = 0.012) by Bonferroni post hoc test; 2: significant difference between the TT and CC genotypes (P = 0.015) by Bonferroni post hoc test; 3: significant difference between the TT and CC genotypes (P = 0.04) by Bonferroni post hoc test; TT: homozygous dominant, TC: heterozygous, CC: homozygous recessive.
2. No association was found between rs1800247 SNP and T2DM development among hypertensive Ukrainians (P \text{adj} > 0.05).

3. No association was found between rs1800247 SNP and T2DM development among non-hypertensive Ukrainians (P \text{adj} > 0.05).

4. There was no relation between rs1800247 SNP and blood pressure parameters (systolic, diastolic, pulse and mean blood pressure) among T2DM Ukrainians (P > 0.05).

**Perspectives for further research.** Further studies with extended groups of comparison are needed for the confirmation of results. Moreover, it will be useful to study the association between other BGLAP SNPs and T2DM and AH development.

**Funding**

This research was a part of the scientific project “Molecular-genetic and morphological features of lower limb tissues regeneration under conditions of chronic hyperglycemia”, state registration No. 0117U003926.

**Conflicts of interest:** authors have no conflict of interest to declare.

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