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Peculitaries of carbohydrate metabolism in patients with metabolic syndrome depending on C/T polymorphism in the DIO 1 gene

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

According to the WHO, the prevalence of metabolic syndrome (MS) is 20–40 %. Thyroid hormones are involved in the regulation of almost all physiological processes in the body, including carbohydrate metabolism.

The aim of the research was to study the dependence of carbohydrate metabolism on C/T polymorphism in the DIO 1 gene in patients with metabolic syndrome.

Material and methods. The C/T polymorphism in the DIO1 gene was studied in 102 patients and 97 healthy subjects. To assess the dependence of carbohydrate metabolism on the C/T polymorphism in the DIO 1 gene, the following groups has been formed: 19 patients with CC genotype, 69 individuals – with CT and 14 ones – with TT genotypes.

Results. Disorders of distribution of genotype frequencies contributed by the reduction of CC genotype frequency was revealed in the group of enrolled patients comparing to the control group ($\chi^2 = 6.8$, $P < 0.05$), while there was no significant difference between the frequencies of CT and TT genotypes in the main and control groups ($\chi^2 = 2.4$, $P > 0.05$ and $\chi^2 = 1.2$, $P > 0.05$). Taking into account that the difference in genotypes frequencies occurs mainly due to a decrease in the number of patients homozygous for C allele, it can be assumed that the C allele has protective properties against deiodinase 1 activity reduction, that indicates the assotiation of C/T polymorphism in the DIO1 gene with the development of thyroid hormone disturbances in the patients with metabolic syndrome as compared to the control group. Elevation of HOMA-IR index was established in patients with TT genotype. Insulin resistance develops in the carriers of T allele, that causes disturbances in carbohydrate metabolism.

Conclusions. Presence of the T allele in genotype is associated with HOMA-IR index elevation as a consequence of the triiodothyronine level reduction and further development of insulin resistance.

Key words: C/T polymorphism in the DIO 1 gene, carbohydrate metabolism, insulin resistance, metabolic syndrome.

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Особливості вуглеводного обміну в пацієнтів із метаболічним синдромом залежно від C/T поліморфізму гена DIO 1

Н. О. Абрамова, Н. В. Пашковська

За даними ВООЗ, поширеність метаболічного синдрому (МС) становить 20–40 %. Тиреоїдні гормони беруть участь у регуляції майже всіх фізіологічних процесів в організмі, зокрема та вуглеводного обміну.

Мета роботи – вивчити залежність показників вуглеводного обміну в пацієнтів із метаболічним синдромом від C/T поліморфізму гена DIO 1.

Матеріали та методи. У 102 осіб із метаболічним синдромом і 97 практично здорових осіб дослідили C/T поліморфізм гена DIO 1. Для оцінювання залежності вуглеводного обміну від C/T поліморфізму гена DIO 1 хворих поділили на групи: 19 – із CC, 69 – із CT, 14 – із TT генотипом.

Результати. Порівняння розподілу частот генотипів гена DIO1 показало, що C/T поліморфізм гена DIO1 асоційований із розвитком порушення обміну тиреоїдних гормонів у пацієнтів, яких обстежили, порівняно з групою контролю ($p < 0,05$). Виявили, що такі зміни зумовлені зменшенням частоти CC генотипу у групі осіб із метаболічним синдромом порівняно з групою контролю ($\chi^2 = 6,8$, $p < 0,05$), вірогідної різниці між частотами CT і TT генотипів в основній і контрольній групах не було ($\chi^2 = 2,4$, $p > 0,05$; $\chi^2 = 1,2$, $p > 0,05$). Враховуючи, що різниця частот генотипів виникає переважно внаслідок зменшення кількості осіб, гомозиготних за алелем C, можна припустити: C-алель характеризується протекторними властивостями, а це свідчить про роль C/T поліморфізму гена DIO1 у розвитку тиреоїдного дисбалансу. Аналізуючи дані показників вуглеводного обміну залежно від генотипу, у групі осіб із TT генотипом встановили вірогідне зростання HOMA-IR порівняно з групою осіб із CC генотипом ($p < 0,05$).

Висновки. Носійство «мутантного» T-алеля гена DIO 1 асоційоване з вірогідним зростанням HOMA-IR як наслідок зниження рівня стимулювального трийодтироніну з розвитком інсулінорезистентності.

Ключові слова: C/T поліморфізм гена DIO 1, вуглеводний обмін, інсуліно-резистентність, метаболічний синдром.

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Особенности углеводного обмена у пациентов с метаболическим синдромом в зависимости от C/T полиморфизма гена DIO 1

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По данным ВОЗ, распространенность метаболического синдрома (МС) составляет 20–40 %. Тиреоидные гормоны участвуют в регуляции практически всех физиологических процессов в организме, в том числе и углеводного обмена.

Ключевые слова: С/Т полиморфизм гена DIO 1, углеводный обмен, инсулинорезистентность, метаболический синдром.

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Цель работы – изучить зависимость показателей углеводного обмена у пациентов с метаболическим синдромом от С/Т полиморфизма гена DIO 1.

Материалы и методы. У 102 пациентов с метаболическим синдромом и 97 практически здоровых лиц исследовали С/Т полиморфизм гена DIO 1. Для оценки зависимости углеводного обмена от С/Т полиморфизма гена DIO 1 больных разделили на группы: 19 – с СС, 69 – с СТ, 14 – с ТТ генотипом.

Результаты. Сравнение распределения частот генотипов гена DIO1 показало, что С/Т полиморфизм гена DIO1 ассоциирован с развитием нарушения обмена тиреоидных гормонов у обследуемых пациентов по сравнению с группой контроля ($p < 0,05$). Обнаружено, что такие изменения обусловлены уменьшением частоты СС генотипа в группе пациентов с метаболическим синдромом по сравнению с группой контроля ($\chi^2 = 6,8$, $p < 0,05$), тогда как достоверной разницы между частотами СТ и ТТ генотипов в основной и контрольной группах не было ($\chi^2 = 2,4$, $p > 0,05$; $\chi^2 = 1,2$, $p > 0,05$). Учитывая, что разница частот генотипов возникает преимущественно за счет снижения количества лиц, гомозиготных по С-аллели, можно предположить, что С-аллель обладает протекторными свойствами, что свидетельствует о роли С/Т полиморфизма гена DIO1 в развитии тиреоидного дисбаланса. Анализируя данные показателей углеводного обмена в зависимости от генотипа, в группе лиц с ТТ генотипом установлено достоверное увеличение показателя HOMA-IR по сравнению с группой лиц с СС генотипом ($p < 0,05$).

Выводы. Носительство «мутантной» Т-аллели гена DIO 1 ассоциировано с достоверным увеличением HOMA-IR в результате снижения уровня стимулирующего трийодтиронина с развитием инсулинорезистентности.

According to the WHO, the prevalence of metabolic syndrome (MS) is 20–40 % [11]. MS most often occurs in people of middle and senior age (30–40 %). Besides, about 30 % (16.8 % of women and 14.9 % of men) of the planet's population are overweight [6].

The pathophysiological processes, that accompany obesity, cause the development of arterial hypertension, carbohydrate metabolism disorders, dyslipidemia, which are components of MS [8].

Thyroid hormones bind to their nuclear receptors α and β, which are present in all tissues of the human body and participate in the regulation of most physiological processes, including carbohydrate metabolism [2,4,10].

Particularly important in the activation of nuclear receptors is triiodothyronine (T_3), which partially regulates gene transcription by modulation of post-translational histone modifications [3,7,14].

The regulation of carbohydrate metabolism under the influence of thyroid hormones is directly influenced by the expression of genes that regulate the production of insulin in liver and skeletal muscles, and stimulate the expression of additional factors such as the complex of ChREBP (carbohydrate response element-binding protein), which subsequently affect the body's ability to secrete insulin in response to glucose intake [5,9].

Therefore, the enzymes deiodinase 1 (D1) and deiodinase 2 (D2) that catalyze the conversion of prohormone T_4 to T_3 are important for the functioning of this mechanism of carbohydrate metabolism [17].

D1 is one of the deiodinases family, which is active in liver, kidney, thyroid tissue. Transcripts of DIO1 are also found in the pituitary gland, intestine, placenta and gonads [12,13,20].

In our study we selected C/T polymorphism at position 785 in the DIO1 gene of complementary DNA [16].

R. Peeters et al. revealed that the presence of minor T-allele in the genotype is associated with an increase in the reverse T_3 (rT_3) content, an increase in rT_3/T_4 ratio and a decrease in the T_3/rT_3 ratio. Similar results were obtained by Frank Jan de Jong et al [1].

However, the effect of the polymorphism in the genes that regulates the metabolism of the thyroid hormones on the carbohydrate metabolism needs further investigation.

Aim

To investigate the dependence of carbohydrate metabolism in patients with metabolic syndrome on C/T polymorphism in the DIO1 gene.

Materials and methods

102 patients with MS were examined. The average age of patients was 52.5 ± 8.8 years: 35 patients (34.3 %) were women, 67 (65.7 %) patients – men. The control group included 97 practically healthy persons at the age of 48.90 ± 7.96 years: 58 persons (59.8 %) were men and 39 – women (40.2 %).

In the following study the principles of bioethics were respected: the main provisions of the European Convention on Human Rights and Biomedicine (from 04.04.1997), GCP (1996), Helsinki Declaration of the World Medical Association on the Ethical Principles of Human Medical Scientific Research (1964–2000) and the Ministry of Health of Ukraine Order No. 281 dated back to 01.11.2000. The study protocol and Informed Consent form for patient was approved by the Ethics Committee of the Bukovinian State Medical University, Ukraine.

Inclusion criteria: diagnosed arterial hypertension, combined with abdominal obesity, a violation of carbohydrate metabolism in the form of impaired tolerance to glucose or type 2 diabetes mellitus, dyslipidemia, informed consent of the patient to participate in the study.

Exclusion criteria: secondary arterial hypertension, hypothyroidism, thyrotoxicosis, decompensated kidney and liver damage, chronic heart failure above FC III, left ventricular ejection fraction up to 45 %, acute cerebrovascular accident and acute coronary syndrome less than 3 months before the study, mental disorders, pregnant women, lactating, any chronic diseases in the acute stage and acute inflammatory processes, other comorbid diseases in the stage of decompensation or acute conditions capable to influence research results.

MS was determined according to the recommendations of the International Diabetic Federation (IDF), 2005.

The research was conducted on the basis of clinical and diagnostic laboratories of the Department of Internal Medicine, the Department of Medical Biology, Genetics

and Pharmaceutical Botany of the Bukovinian State Medical University, the Regional Endocrinologycal Center, the Chernivtsi Regional Hospital of Veterans of War.

C/T polymorphism in the DIO1 gene was studied in 102 patients and 97 healthy individuals by isolation of genomic DNA from peripheral blood leukocytes, after that amplification of the polymorphic area in the state of polymerase chain reaction (PCR) was performed on the programmed PCR thermal cyclers "Amply-4L" ("Biocom", Moscow, RF) at individual temperature response. Reagents «DNA sorb-V» option 100 (Federal State Institution of Science «Central Research Institute of Epidemiology» of the Federal Service for Supervision in the Sphere of Protection of Human Rights and Human Welfare, RF) were used for DNA isolation from lymphocytes according to instructions. PCR samples were prepared by means of the set "AmpliSens-200-1" (Federal State Institution of Science "Central Research Institute of Epidemiology" of the Federal Service for Supervision in the Sphere of Protection of Human Rights and Human Welfare, RF).

The following primer set were used: to determine the C/T polymorphism in the DIO1 gene – forward – 5'-GAACTTGATGTGAAGGCTGGA-3' and reverse – 5'-TAACCTCAGCTGGGAGTTGTTT-3'. Discrimination of DIO1 gene alleles was performed using the specific restriction enzyme Bcl I ("Fermentas^{R}", USA).

Products of PCR were separated using electrophoresis in 3 % agarose gel in the presence of tetraborate buffer, concentrated with ethidium bromide. Fragments were visualized by transilluminator in the presence of a marker of molecular mass 100–1000 bq ("Fermentas^{R}", USA).

Pearson's χ^2-criterion was used to estimate the correspondence of the genotype frequencies in the study to theoretically expected distribution at Hardy-Weinberg's equilibrium. Odds ratio (OR) with determination of 95 % confidence interval (CI) was calculated with the aim to establish the association of polymorphic variant of the gene with a pathological phenotype.

To evaluate the dependence of carbohydrate metabolism depending on C/T polymorphism in the DIO 1 gene we divided the patients into groups in the following way: 19 patients with CC, 69 individuals with CT and 14 ones with TT genotypes. The control group included 20 healthy individuals.

Disorders of carbohydrate metabolism were diagnosed according to WHO criteria (1999). Fasting immunoreactive insulin (IRI), C-peptide were determined by immunoassay method on analyser of immune-enzymatic reactions "Uniplan" using DRG (Germany) reagents.

Glucose content was determined by glucose oxidase method using standard set of reagents of the "Fylisyt diagnosis" (Ukraine).

Content of glycated hemoglobin (HbA_{1C}) was studied by the method of ion-exchange high performance liquid chromatography (HPLC) on Automatic analyzer of glycated hemoglobin D10 "Bio-Rad Laboratories Inc.", using the "Biomedinvest" reagents (Ukraine).

To study the proinflammatory activity of adipocytes, the level of leptin was studied by immunoassay method on analyser of immune-enzymatic reactions "Uniplan" using DRG (Germany) reagents.

To assess the degree of insulin resistance a small model of homeostasis (Homeostasis model assessment – HOMA) was used, calculated by means of the HOMA Calculator Version 2.2 Diabetes Trials Unit at the University of Oxford (UK).

Anthropometric indices were calculated: such as waist to hip (W/H) ratio and body mass index (BMI) by Quetelet, 1832.

All statistical computations were performed by means of the licensed software package Statistica for Windows 6.0, serial number 31415926535897 and Microsoft Excel 2016 table processor (Microsoft Corp., USA). For all parameters, in the groups of patients with CC, CT and TT genotypes and control group, the arithmetic mean (M), its dispersion and mean error (m) were calculated. All data corresponded to the normal distribution law. To determine the significance of differences between the results of research in groups of patients with CC, CT and TT genotypes and control group, the Student's coefficient (t) was calculated, after that the significance of the difference between the samples (P) and the confidence interval of the mean according to the Student distribution tables were determined. Valid values for $P < 0.05$ were considered statistically significant.

Results

When assessing the distribution of genotype frequencies in the DIO 1 gene, it was revealed that C/T polymorphism in the DIO1 gene is associated with the violation of thyroid hormones metabolism in patients with MS (*Table 1*).

The frequency of CC genotype in patients with MS was significantly lower, compared with the control group ($\chi^2 = 6.8$, $P < 0.05$), while there was no significant difference between the frequencies of CT and TT genotypes in the group of patients and control group ($\chi^2 = 2.4$, $P > 0.05$ and $\chi^2 = 1.2$, $P > 0.05$).

In patients with CT polymorphism the risk of disturbance of D1 activity increases by 5.7 times ($P < 0.05$; OR = 1.89, 0.95 % CI =1.06–3.35) than in those with CC genotype.

So, the risk of reduction of D1 activity is associated with the presence of minor T allele, while homozygous for C allele had significantly lower risk of this disturbance development. Considering that the difference of genotypes frequency occurs mainly due to decrease in the number of individuals homozygous for C allele, it can be assumed that C allele has protective properties concerning the development of thyroid hormones metabolism violation.

When studying the dependence of carbohydrate metabolism on the C/T polymorphism in the DIO1 gene, it was found that carriers of minor T allele had significantly higher HOMA-IR index than homozygous for C allele ($P < 0.05$) (*Table 2*). No significant changes in glucose, HbA1C, leptin, C-peptide, IRI levels and anthropometric parameters were revealed.

Discussion

According to our data, the risk of reduction of D1 activity is associated with the presence of minor T allele, while homozygous for C allele had significantly lower risk of this

Table 1. The distribution of genotype frequencies depending on CT polymorphism in the DIO1 gene in patients with metabolic syndrome and the control group

Genotypes	Cases	Controls	χ^2	P	OR	0,95 % CI
	102	97				
Genotype frequency CC	0.186	0.412	6.8	P < 0.05	0.33	0.17–0.62
Genotype frequency CT	0.676	0.526	4.7	P < 0.05	1.89	1.06–3.35
Genotype frequency TT	0.137	0.062	1.2	P > 0.05	2.41	0.89–6.56

χ^2: Pearson criterion, **OR:** odds ratio, **CI:** confidence interval.

Table 2. Peculitaries of carbohydrate metabolism indices and anthropometric parameters in patients with metabolic syndrome according to C/T polymorphism in the DIO1 gene (M ± м)

Index, units of measurement	Genotypes of the GPX 1 gene, n = 102			Control group, n = 20
	CC, n = 19	CT, n = 69	TT, n = 14	
Glucose, mmol/l	5.27 ± 0.28*	5.64 ± 0.26*	6.18 ± 0.39*	4.73 ± 0.17
Immunoreactive insulin, IU/ml	17.45 ± 2.24*	19.79 ± 2.32*	20.89 ± 1.52*	6.11 ± 1.31
HOMA-IR	4.09 ± 0.24*/**/***	4.96 ± 0.32*	5.74 ± 0.23*	0.97 ± 0.04
C-peptide, ng/ml	4.85 ± 0.17*	5.12 ± 0.16*	5.62 ± 0.15*	1.29 ± 0.12
BMI, kg/m^2	30.88 ± 1.46*	32.64 ± 1.63*	34.62 ± 3.76*	24.88 ± 2.28
Leptin, ng/ml	24.12 ± 3.24*	27.93 ± 4.10*	30.38 ± 4.58*	4.72 ± 0.15
HbA_{1C}, %	5.96 ± 0.61*	6.14 ± 0.38*	6.58 ± 0.47*	4.42 ± 0.58
W/H ratio	0.97 ± 0.05*	1.01 ± 0.06*	1.13 ± 0.05*	0.68 ± 0.03

n: number of observations; ***:** the probability of changes in relation to control; ****:** the probability of changes in relation to the group with CT-genotype; *****:** the probability of changes in relation to group with TT genotype.

disturbance development. Considering that the difference of genotypes frequency occurs mainly due to a decrease in the number of individuals homozygous for C allele, it can be assumed that C allele has protective properties concerning the development of thyroid hormones metabolism violation.

Our data coincide with the statement that thyroid hormones participate in the regulation of carbohydrate metabolism by stimulation of expression of genes that regulate the production of insulin in liver and skeletal muscles, and stimulate the expression of additional factors such as the complex of ChREBP (carbohydrate response element-binding protein), which subsequently affect the body's ability to secrete insulin in response to glucose intake [5,15].

In our study HOMA-IR was higher in the group of patients with TT genotype (homozygous for "mutant" allele) as compared to the group with CC genotype (homozygous for "wild" allele) of the DIO1 gene (P < 0.05).

In the study of Sikandar Hayat Khan et al. carbohydrate metabolism in euthyroid and hypothyroid patients was examined. It was revealed that patients with hypothyroidism had significantly higher HOMA-IR index, compared to control group, suggesting that reduced thyroid hormones levels contribute to insulin resistance [18].

Vyakaranam S. at al. also revealed that HOMA-IR index values were higher in subjects with subclinical hypothyroidism compared with euthyroid subjects. Moreover, TSH positively correlated with insulin and HOMA-IR values, whereas fT_4 and fT_3 inversely correlated with insulin and HOMA-IR [19].

Authors of both these studies suggested that these changes develop as a reflection of alterations of tissue thyroid hormones metabolism – deiodination impairment, with further tissue hypothyroidism development in hepatocytes, which leads to insulin resistance thus leading to decreased glucose metabolism

Ana C. Panveloski-Costa et al. found that T_3 treatment of obese rats leads to improvement of insulin sensitivity (T_3-treated rats presented higher constant rate for the insulin tolerance test (kITT)) and negative modulation of inflammatory cytokine expression in adipose tissue. Furthermore, T_3 treatment reduced the serum levels of triglycerides and cholesterol, body weight gain was decreased in these animals [20]. The results of this study suggests positive effect of thyroid hormones on components of metabolic syndrome and necessity to keep the levels of thyroid hormones in normal ranges not only in serum but also in tissues, taking into account the fact of impaired thyroid hormones metabolism in them as result of deiodinases inhibition.

It can be assumed, that elevation of HOMA-IR index in our study is caused by decreased level of the most active hormone of thyroid gland – T_3 due to the inhibition of D1 activity in patients with MS, with subsequent reduction of insulin receptors sensitivity and development of IR.

Conclusions

1. Taking into account that the difference in genotypes frequencies occurs mainly due to a decrease in the number of patients homozygous for C allele, it can be assumed that the C allele has protective properties against deiodinase 1 activity reduction, whilst T allele presence is associated with the inhibition of peripheral conversion of thyroid hormones.

2. The presence of T alleles in the genotype of patients with metabolic syndrome is associated with a violation of carbohydrate metabolism as a result of insulin resistance development.

Prospects for further research. The survey results indicate the necessity of effective measures for carbohydrate dysfunction correction development in patients with metabolic syndrome.

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