Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Jul 22; 10(A):1315-1320.
https://doi.org/10.3889/oamjms.2022.8145
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Genetics*

Effect of SHBG Polymorphism on the Levels of Bioavailable Testosterone and Lipid Metabolism in Older Men of the Kazakh Population

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Edited by: Slavica Hristomanova-Mitkovska
Citation: Akkaliyev M, Aukenov N, Massabayeva M, Apsalikov B, Rakhyzhanova S, Kuderbaev M, Sadykov N. Effect of SHBG Polymorphism on the Levels of Bioavailable Testosterone and Lipid Metabolism in Older Men of the Kazakh Population. Open Access Maced J Med Sci. 2022 Jul 22; 10(A):1315-1320. https://doi.org/10.3889/oamjms.2022.8145
Keywords: SHBG; Bioavailable testosterone; Hypogonadism; Lipid metabolism disorders in men
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Received: 02-Dec-2021
Revised: 09-Jul-2022
Accepted: 12-Jul-2022

Funding: The research has been carried out within the framework of Ministry of Education and Science of the Republic of Kazakhstan PhD Program under the grant agreement No. 246-C dated 28.08.2017.
Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: This study is aimed at investigating the effect of SHBG (rs727428; rs10822184) and LPL (rs754493647) single nucleotide polymorphisms on the concentration of the bioavailable fraction of testosterone in older men.

MATERIALS AND METHODS: To study gene mutations, 417 residents of the East Kazakhstan region of Kazakh nationality were examined. The main group included 135 men with signs of hypogonadism (AMS 37-49), and the control group consisted of 282 healthy men (AMS 17–26) of the corresponding age (p = 0.5). Single nucleotide polymorphisms rs 727428 [C/T]; rs10822184 [T/C]; rs754493647 [T/C], was determined by the TaqMan method.

RESULTS: Analysis of the rs727428 polymorphism has revealed that the TT allele (rs727428) has a lower level of albumin (p = 0.03), bioavailable testosterone (p = 0.04), and free testosterone (p = 0.6) than in carriers of the CC and CT genotypes. Furthermore, it has shown a decrease in total testosterone (p = 0.001) and an increase in SHBG levels (p = 0.07) in men with the TT genotype of the rs727428 gene polymorphism. The rs10822184 polymorphism demonstrated an increase in triglyceride and LDL levels in TT genotype (p ≤ 0.04), in comparison with CC and CT genotypes.

CONCLUSION: It has been proven that rs727428 (p = 0.001) is associated with testosterone levels and therefore can determine the concentration of bioavailable testosterone. Decreased levels of bioavailable testosterone are a sign of male hypogonadism. This study confirms the effect of rs10822184 on LDL (p = 0.01) and triglyceride (p = 0.04) levels, but its association with androgen levels has not been proven. Our results may be of interest for understanding the etiology of early development of hypogonadism and lipid metabolism disorders in men. To confirm the conclusions, a more detailed study with a large sample of men from the Kazakh population may be required.

Introduction

Early diagnosis, prevention, and treatment of many diseases in male body are an important public health task, often associated with the maintenance of reproductive and sexual functioning. Many studies are aimed at solving these issues, including studies of genetic predictors. Testosterone is the major hormone that is responsible for the formation and maintenance of male sex characteristics and sexual performance of men [1]. With age testosterone levels begin to decrease that is a natural physiological process. In this case the testicles reduce, libido decreases and adipose tissue increases [2]. Many physiological processes occurring in the body of an aging man are determined by family predisposition, population and individual characteristics, and also some gene polymorphisms play an important role. The genome–wide association search (GWAS) method is widely used to search for marker genes [3], [4], [5], [6].

The SHBG level is one of the most important parameters that govern the action testosterone ensuring the availability of hormones to target tissues [7], [8].

Testosterone in the male body is presented in the form of three fractions: strongly associated with SHBG (44%), weakly associated with albumin (54%), and free (1–3%). In majority the researches focus on total and free testosterone. The least studied fragment remains the albumin-bound testosterone fraction. Recent data confirm that testosterone bound to albumin is readily degraded in the capillary bed and absorbed by target cells [4]. The sum of albumin-bound and free testosterone is called the bioavailable fraction (BiT).

The level of globulin binding hormone (SHBG) is one of the important parameters to detect testosterone delivery to target tissues [5].

Sex hormone binding globulin (SHBG) is synthesized and secreted into the bloodstream by hepatocytes. SHBG specifically binds biologically

active androgens and regulates their bioavailability for target tissues [6].

The SHBG level is one of the most important indicators that regulate the action of testosterone ensuring the availability of hormones to the target tissues [7], [8].

Sex hormones can be one of the factors that determine the distribution of body fat. The deposition of adipose tissue occurring mainly in the abdominal region in men is the most critical risk factor for the development of hormonal and metabolic disorders, which leads to the development of hypogonadism [9]. A direct link between hypogonadism and excess weight has been proven [10], [11], [12]. Obesity is the main reason for the aggravating physiological course of age-related decline in the level of total testosterone and its bioavailable fraction [13]. One of the key factors in lipid metabolism is LPL [11], [12]. Three single nucleotide polymorphisms (SNPs), rs12150660, rs727428, and rs10822184 are thought to be associated with testosterone levels in populations of European origin [13]. However, genetic polymorphisms can differ in different ethnic groups.

Our study is aimed at studying the effect of SHBG (rs727428; rs10822184) and LPL (rs754493647) single nucleotide polymorphisms on the concentration of the bioavailable fraction of testosterone. The change in the concentration of the bioavailable fraction of testosterone determines the predisposition to the early development of hypogonadism and overweight in men of the Kazakh population.

Materials and Methods

Subjects

In total, 417 people of Kazakh nationality, living in Semey city, East Kazakhstan region, took part in the case–control study, including 135 men with signs of hypogonadism and 282 men of the control group with normal weight without erectile dysfunction. The presence of hypogonadism was diagnosed using the Aging Male Screening (AMS) questionnaire. Evaluation criteria for this questionnaire: 17-26 points - there are no signs of testosterone deficiency; 27–36 points - mild signs of testosterone deficiency; 37–49 points - signs of testosterone deficiency of moderate severity; and 50 or more points - there are pronounced signs of testosterone deficiency.

The body mass index (BMI) was calculated as the body weight in kilograms divided by the square of the height in meters. Exclusion criteria for participants from the study: Age less than 18 and more than 79 years; diabetes; decompensated heart and/or renal failure; malignant neoplasm; cardiovascular failure, current and past cigarette smoking; and taking drugs that affect erectile function (antihypertensive drugs, antidepressants, and hormonal drugs).

Ethics approval and consent to participate

Informed consent to participate in the study was obtained from all participants in the study in accordance with the Protocol of the Ethical Committee of the Medical University of Semey (No. of registration 11) and the requirements of the World Medical Association of Helsinki Declaration.

Laboratory experiments

The level of biochemical analyses, high-density lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides, and albumins was determined using commercial kits obtained from COBAS (Roche Diagnostics GmbH, Germany) on *Cobas* 8000 analyzers (Roche Diagnostics, Switzerland). Reference values HDL (0.78–2.2 mM/L), LDL (2.33–5.31 mM/L), triglycerides (1.7–2.25 mM/L), and albumin (35–55 g/L).

Immunological studies of sex hormone-binding globulin (SHBG), luteinizing hormone (LH), and total testosterone were performed on Architect i2000SR equipment (Abbott Laboratories, IL, USA) using commercial diagnostic kits (Abbott Laboratories, USA) in accordance with the manufacturer's instructions.

The calculation of free (FT) and bioavailable (BiT) testosterone was carried out on the basis of the equation of the Vermeulen *et al*. Equation, developed at the Endocrinology Department of the University Hospital of Ghent (Belgium) and recommended by the International Society for the Study of Aging Men (ISSAM) http://www. issam.ch/freetesto.htm, the calculation is based on an algorithm that takes into account the constants of total testosterone, albumin, and SHBG.

Reference values for SHBG (10–57 nM/L), LH (1.14–8.75 mIU/ml), testosterone total (5.41–19.54 nM/L), free testosterone (0.16–0.47 nmol/l), bioavailable testosterone (4.0–15.5 nmol/l).

Reference values for SHBG (10–57 nM/L), LH (1.14–8.75 mIU/ml), total testosterone (5.41–19.54 nM/L), free testosterone (0.16–0.47 nmol/L), and bioavailable testosterone (4.0-15.5 nmol/l).

To conduct the genetic study, we used peripheral blood plasma of the subjects, collected in vacuum tubes with K2/K3 EDTA. Genomic DNA was isolated from the plasma using commercial GeneJET Mini kit kits (Thermo Scientific, Vilnius, Lietuva) following the manufacturer's instructions. DNA concentration was measured using a Qubit 4 fluometer (Thermo Scientific, Walthem, MA, USA). The isolated DNA was frozen and stored at –20°C.

Genotyping of 147 DNA samples, after a preliminary quality and quantity check, was carried

out by real-time PCR on a CFX 96 amplifier (BioRad, CA, USA) using ready-mixed mixtures of primers and TaqMan probes master mix of rs754493647, rs727428, rs5934505, and rs10822184 gene polymorphisms. The total volume for 96 well cell-plates was 25 μl: 2x TaqMan master mix - 12.50 μl, 20x master mix - 1.25 μl, DNA - 11.25 μl (20 ng) (all reagents manufactured by Life Technologiest, Foster City, CA, USA). The amplification program was 10 min at 95°C, 50 denaturation cycles of 15 s at 92°C, and annealing for 90 s at 60°C for all polymorphisms.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics Version 21 software (International Business Machines Corp., Armonk, NY, USA).

To evaluate descriptive statistics of quantitative variables compared with independent samples, the non-parametric Mann–Whitney criteria and the Kruskal–Wallis criterion was used, the differences between the samples were considered to be statistically significant at a value for $p < 0.05$. The distribution of alleles and genotypes was compared using the χ^2 criterion, where the differences between the samples were considered statistically significant at a value for $p \leq 0.004$ (taking into account the correction for the multiplicity of comparisons). Deviations in the frequencies of alleles and genotypes of each polymorphism from the Hardy-Weinberg equilibrium were evaluated using the χ^2 test ($p > 0.05$).

Results

A group of 135 patients with signs of AMS hypogonadism (37–50) and 282 men from the control group of AMS men (17–36) were involved in the study.

The age difference between the groups of patients in the main group and those in the control group was 5 years, this indicator was not statistically significant ($p = 0.5$). The average body mass index in the main group (35.4) is significantly higher ($p = 0.004$) than in the comparison group (24.7). When comparing biochemical analyses, we paid attention to the increase in triglycerides in patients of the main group (2.76) above the reference value (1.7–2.25 mM/L). The study of the hormonal status among men from the main group showed a decrease in testosterone total (10.62) and SHBG (25.96) in comparison with the control group (11.98) (33.43), respectively, without achieving statistical significance. The result of bioavailable testosterone, calculated according to the formula of Vermeulen *et al*., was also worth considering. Bioavailable testosterone is higher in men with hypogonadism (5.7) than in the control group (5.56), but this result did not reach statistical significance (Table 1).

Table 1: Demographic and results

	Case (n = 135)	Control (n = 282)	p value
Age	46 ± 7.9	51.43 ± 7.6	0.546
BMI	35.44 ± 3.4	24.73 ± 2.4	0.004*
Triglycerides	2.76 ± 1.6	1.95 ± 1.4	0.164
HDL	1.39 ± 1.4	1.36 ± 1.1	0.446
LDL	3.89 ± 0.7	3.29 ± 0.7	0.963
Albumin	44.36 ± 5.1	42.56 ± 4.9	0.857
LH	4.30 ± 2.3	4.08 ± 1.8	0.705
SHBG	25.96 ± 10.7	33.43 ± 13.6	0.226
Testosterone total	10.62 ± 2.4	11.98 ± 3.4	0.086
Testosterone free	0.236 ± 0.6	0.24 ± 0.6	0.325
Bioavailable testosterone	5.7 ± 2.2	5.56 ± 1.9	0.132

*Statistical significance $P < 0.05$.

Genetic analysis results were obtained using TaqMan technology in real time.

The frequencies of rare alleles are similar in the patient groups and in the control group, being about 0.1 and 0.04, respectively, for rs727428 and rs10822184. There were no deviations from the Hardy-Weinberg equilibrium in any group ($p > 0.05$), which indicates the absence of obvious technical flaws in the results of genotyping.

Table 2 represents the data of the association of polymorphisms rs727428; rs10822184 with biochemical tests and sex hormones in the study groups. Analysis of the rs727428 polymorphism has shown that the TT allele (rs727428) has a lower level of albumin ($p = 0.03$), bioavailable testosterone ($p = 0.04$), and free testosterone ($p = 0.6$) than in carriers of the CC and CT genotypes. Furthermore, a decrease in total testosterone ($p = 0.001$) and an increase in SHBG levels ($p = 0.07$) in men with the TT genotype of the rs727428 gene polymorphism. The rs10822184 polymorphism showed an increase in the level of triglycerides and LDL in the TT genotype ($p \leq 0.04$), in comparison with the CC and CT genotypes.

Discussion

In this research, we studied the association of single nucleotide polymorphisms of the SHBG protein (rs727428; rs10822184) and LPL (rs754493647) in the Kazakh population with a predisposition to early development of hypogonadism.

The average age in the main group was 46 years, which is slightly lower than in the comparison group (51 years), there is also a statistically significant difference between the groups in BMI ($p = 0.004$) and an increase in lipid metabolism in the main sample. BMI in the main group is undoubtedly higher; we explain this fact by the influence of hypogonadism and impaired lipid metabolism. In this case, the trigger for obesity is a mutation of the SHBG protein, which affects the levels of total testosterone and the concentration of triglyceride and LDL. The influence of the LPL gene mutation (rs754493647 [A/G]) on lipid metabolism has

Table 2: The association between genotypes and biochemical tests and sex hormones in the population studied

	CC	CT	TT	p value
rs727428 Me (Q1–Q3)				
Triglycerides	1,9 (1,1–2,7)	1.7 (1.0–2.8)	2.1 (1.2–2.6)	0.8
HDL	1.1 (0.9–1.2)	1.2 (0.9–1.5)	1.1 (0.8–1.2)	0.06
LDL	3.3 (3.0–3.8)	3.4 (3.1–4.0)	3.3 (2.9–3.8)	0.8
Albumin	43.6 (41.6–45.6)	44.7 (42.5–47.1)	43.5 (36.7–45.4)	0.03*
LH	4.1 (2.9–5.4)	3.5 (2.5–4.4)	3.9 (3.0–5.2)	0.1
SHBG	33.1 (19.5–41.1)	27.6 (17.9–39.1	36.9 (22.6–40.7)	0.07
Testosterone total	11.7 (10.2–14.4)	10.5 (9.6–12.3)	10.3 (9.0–11.3)	0.001*
Testosterone free	0,218 (0,16–0,47)	0,217 (0,16–0,47)	0,187 (0,16–0,47)	0.6
Bioavailable testosteron	5,12 (4,0–15,5)	5,21 (4,0–15,5)	4,37 (4,0–15,5)	0.04*
rs10822184 Me (Q1–Q3)				
Triglyceride	1.8 (1.0–2.4)	1.7 (1.0–2.5)	2.2 (1.6–3.1)	0.04*
HDL	1.2 (0.9–1.3)	1.1 (1.0–1.3)	1.0 (0.9–1.3)	0.5
LDL	3.4 (3.1–3.8)	3.2 (2.8–3.8)	3.7 (3.1–4.0)	0.01*
Albumin	44.0 (41.6–46.3)	44.4 (42.6–45.8)	43.5 (38.4–45.0)	0.1
LH	3.6 (2.7–4.3)	4.0 (2.5–5.2)	4.1 (3.2–5.2)	0.1
SHBG	28.6 (19.5–41.2)	33.4 (18.2–40.9)	31.2 (22.7–37.2)	0.3
Testosterone total	11.3 (9.5–12.8)	10.2 (9.1–12.0)	11.2 (10.1–12.1)	0.9
Testosterone free	0.237 (0.16–0.47)	0.194 (0.16–0.47)	0.227 (0.16–0.47)	0.5
Bioavailable testosteron	5.68 (4.0–15.5)	4.66 (4.0–15.5)	5.31 (4.0–15.5)	0.08

*Statistical significance P < 0.05.

not been proven in our study, but such result may be associated with a small sample size and requires more detailed study.

Total testosterone levels are directly related to SHBG concentration. Due to the very high ligand binding affinity, SHBG in plasma is the main protein for testosterone transport.

Changes in blood SHBG levels affect the distribution of testosterone in plasma and its availability to tissues and target cells [14].

In our study, there was a slight decrease in the bioavailable fraction of testosterone (5.56 nmol/L) in the control group that was likely due to a decrease in albumin (42.56 ± 4.9), which was a depot for this fraction. Also in the control group, there was a high level of SHBG, which tightly bound total testosterone. No significant difference in the concentration of the free fraction of testosterone was detected in both groups. Our data suggest that the bioavailable fraction is the most stable fraction, the decrease in which occurs in the last turn against the background of age-related hypogonadism aggravated by overweight. It is known that the concentration of SHBG affects the level of total testosterone, while excess weight decreases SHBG and aging increases it.

However, it seems plausible that the use of calculated free testosterone may prevent overdiagnosis of testosterone deficiency in some men, especially in obese, in whom low serum SHBG levels may be the main cause of low total testosterone.

The role of testosterone binding protein in the human body can be two-fold. On the one hand, the binding of this hormone determines a decrease in its biological activity [15], [16]; on the other hand, it specifies the preservation of its significant concentration in the deposited form [17]. In this case, the pool of bioactive testosterone is determined by the concentration associated with blood albumin and its free fraction [18].

SNP rs727428 is located at the 17p13 locus of the SHBG gene and is associated with testosterone levels [19]. Since rs727428 affects total testosterone levels, the relationship between genotype and bioavailable testosterone levels should be expected.

The major literature sources involve the assessment of total testosterone and its free fraction. It is believed that only free testosterone in the bloodstream is absorbed by tissues and therefore biologically active. However, further studies confirmed that testosterone weakly bound to albumin freely dissociates in capillaries, thereby becoming available for absorption by cells - target of tissues [4].

As a consequence, all testosterone not associated with SHBG is considered bioavailable to tissues, and therefore bioavailable testosterone may be a better marker of testosterone bioactivity than total testosterone.

In our study, in TT genotype carriers, the calculated indicators of the bioavailable fraction of testosterone (BiT- 4.37 nmol/L, FT- 0.187 nmol/L) were lower than in the CC genotype (BiT- 5.12 nmol/L, FT- 0.218 nmol/L) and CT (BiT- 5.21 nmol/L, FT- 0.217 nmol/L). This fact of a decrease in the fraction of bioavailable testosterone is undoubtedly an alarming fact in the future development of early hypogonadism. The concentration of bioavailable testosterone is influenced by the levels of albumin, SHBG, and total testosterone. The first clinical sign of a decrease in free testosterone in serum is erectile dysfunction. The owners of this genotype (TT) are diagnosed with an increase in SHBG, which undoubtedly aggravates the picture of hypogonadism, since testosterone strongly binds to SHBG without a tendency to release into the blood, and the albumin level, on the contrary, is low, which is a reserve for free testosterone. According to the researchers, the presence of functional polymorphism, which affects the binding of testosterone to SHBG, as well as the bioavailability and action of testosterone at the target tissue level, is not excluded [20].

Together with our findings, the mechanism by which altered circulating androgen levels are influenced by the rs724828 polymorphism in 17p13 requires further functional studies.

Conventionally, SHBG is not considered a risk factor for the development of any disease (e.g., prostate cancer, type 2 diabetes, and cardiovascular disease), because it is seen as a sequestration of hormones to control their bioavailability. In recent publications, there are two opposite statements concerning the level of SHBG in hypogonadism [21]. Thus, with increasing calendar age, the level of SHBG increases, which leads to a decrease in the free fraction T, while maintaining a normal level of total T. In obesity, the concentration of SHBG decreases [22].

The results of our research prove the validity of both statements. There is a significant negative correlation between SHBG and BMI in the main group, and a positive correlation between SHBG and age in the control group.

The mechanism by which obesity is associated with decreased SHBG levels is not fully understood, but may include suppression of SHBG synthesis in the liver by increased insulin concentrations [23], [24].

Hyperinsulinemia is a predictor of overweight. In our study, rs10822184 (T > C) was not associated with serum testosterone levels, but significantly correlated with body weight and BMI.

Polymorphism of the SHBG transport protein rs10822184 demonstrated an increase in the level of triglycerides and low-density lipoproteins in the TT genotype ($p \leq 0.04$), in comparison with the CC and TT genotypes. These indicators are signs of a violation of fat metabolism, which undoubtedly has a negative effect on the production and maintenance of testosterone at the physiological level. Low levels of total testosterone and mutant SHBG are significantly associated with abdominal obesity and high triglyceride concentrations [4].

It is known that an increase in the concentration of insulin in obesity causes a rise in the concentration of triglycerides and low-density lipoproteins, and a decrease in the level of high density lipoproteins [10]. Our study has found a direct relationship between BMI and triglyceride and LDL levels, and an inverse relationship with HDL levels [25].

Lipid disorders are a predictor of obesity and a decrease in total testosterone. Changes in SHBG and total testosterone levels in obesity provoke the decrease in bioavailable testosterone levels [26].

Thus, it can be noted that the level of bioavailable testosterone is associated with symptoms of hypogonadism. Observational data may be a suitable variable for diagnosing hypogonadism in obese subjects.

We acknowledge that the present study has some limitations, for example, we realize that the sample size is limited. In the future, we are planning a more detailed study of the problem with a large number of samples.

Conclusions

Our research convincingly shows that the rs727428 (TT) polymorphism is associated with testosterone levels and therefore can determine the concentration of bioavailable testosterone, which plays an important role in male sexual performance. Decreased levels of bioavailable testosterone are a sign of male hypogonadism. The effect of rs10822184 on high LDL and triglyceride levels has been confirmed, but its association with androgen levels has not been proven in this study. SHBG polymorphism is an important factor affecting the concentration of bioavailable testosterone. Our data may be interesting for understanding the etiology of the development of early hypogonadism and lipid metabolism disorders in men. Studies on a broader sample of the Kazakh population may be required to confirm our findings.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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