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The genetic background of climacteric symptoms in women during menopause

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Summary

The subject of the study is the evaluation of the correlation between the polymorphism of candidate genes in the etiology of depression and the occurrence of the symptoms of the climacteric syndrome in women during menopause. The group subjected to the study comprised of 203 women aged between 42-65 years: 71 of them still menstruated (premenopausal group) and 132 at least one year after the last period (postmenopausal group), admitted to the Department of Gynecological Endocrinology at the University of Medical Sciences in Poznan with symptoms of the climacteric syndrome. All the examined women were evaluated according to the degree of severity of the climacteric syndrome symptoms using the Kupperman index and the concentration of FSH, LH hormones, 17β-estradiol, PRL, total testosterone, and DHEAS in peripheral blood serum. Among the candidate genes in the aetiology of depression the following were selected for the research: the serotonergic system receptor genes: 5HTR2A, 5HTR1B, 5HTR2C, TPH1, TPH2, and MAO-A; the genes of noradrenergic and dopaminergic systems (COMT, NET), the genes of the GABAergic (GABRB1) system, a gene of the estrogen receptor (ESR1), and the genes of the enzymes crucial in the methyl cycle (MTHRF, MTR, and MTHFD1). With regards to the correlation between the examined polymorphisms and the occurrence of the symptoms of the climacteric syndrome, the associations analysis indicated a connection between GABRB1.TaqI polymorphism and the occurrence of vertigo in premenopausal women (0.0198; after correction: 0.0497 CC to CA). The correlation was also found regarding the examined polymorphisms and the concentration of the examined hormones in blood serum: TPH1.MaeI polymorphism and the LH concentration in the postmenopausal group (0.004; after correction: 0.014 CC to CA), NET.Eco147I polymorphism, and the 17β-estradiol concentration in the postmenopausal group (0.0208; after correction: 0.048 GG to GA) and HTR2AMspI polymorphism and PRL concentration in all examined women (0.03; after correction: 0.038 TT to CT).

Key words: Polymorphism; Depression; Climacteric syndrome; Menopause.

Introduction

The symptoms of the climacteric syndrome are a major medical problem reported by women during menopause [1, 2] and the main factor determining the quality of this part of life [1]. In general, those symptoms last from a few months to a few years, but some women experience them for 30 years or more [1, 3, 4]. Some of the risk factors when it comes to the occurrence of the symptoms of the climacteric syndrome are: low concentration of 17β-estradiol in blood serum, African-American race, a high body mass index (BMI), nicotine smoking, and the presence of other symptoms of the climacteric syndrome in the so-called "domino effect" [3–8].

With reference to the role of the genetic factors in the etiology of the symptoms of the climacteric syndrome, individual reports connect the occurrence of the symptoms of the climacteric syndrome to specific polymorphic variants of the genes of the enzymes taking part in the metabolism of estrogens such as CYP1B1 [9, 10] and estrogen receptors [11, 12]. It seems possible that other genetic factors might also play a significant role in the etiology of the symptoms of the climacteric syndrome. This study undertakes to evaluate the correlation between the genes important in the etiology of depression and the occurrence of the symptoms of the climacteric syndrome. The selection of the candidate genes was dictated by the frequent occurrence of depression during menopause [13], as well as a positive effect of antidepressants in alleviating the symptoms of the climacteric syndrome [14].

Materials and Methods

The group subjected to the study was comprised of 203 women, aged between 42–65 years admitted to the Department of Gynecological Endocrinology at the University of Medical Sciences in Poznan with symptoms of the climacteric syndrome. Among them 71 still menstruated (premenopausal group) and 132 were at least one year after the last period (postmenopausal group). All examined women were evaluated according to the degree of severity of

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Table 1. — *Kupperman Index.*

Symptoms of the climacteric syndrome	Points*
Rush of blood to the head	4
Sweating	2
Sleep disorders	2
Anxiety	2
Depressed mood	1
Dizziness	1
General weakness	1
Arthralgia	1
Headaches	1
Heart pounding or palpitations	1
Paresthesia	1

*Kupperman Index is calculated by adding the scores for individual symptoms after multiplying them by the multiplier, the value of which depends on the degree of the severity of a given symptom. Lack of a symptom: multiplier 0; low severity of a symptom: multiplier 1; average severity of a symptom: multiplier 2; high severity of a symptom: multiplier 3.

the climacteric syndrome symptoms, using the Kupperman Index (Table 1) [15].

The BMI index was calculated using the formula of BMI = body weight/height^2. None of the examined women were on hormone replacement therapy (HRT) or on psychotropic drugs.

The concentration of the hormones important in the etiology of the symptoms of the climacteric syndrome was tested in all women participating in the research. These were: FSH, LH, 17β-estradiol, PRL, total testosterone, and DHEAS in peripheral blood serum. The concentration of the hormones: FSH, LH, 17β-estradiol, PRL, and total testosterone were carried out using the immunoenzymatic method. The ranges of intra- and inter-assay coefficient of variation (CV) amounted to 1.2–3.3% and 2.0–5.6%, respectively.

The concentration of DHEAS was determined using the radioimmunological method: the ranges of intra-assay CV amounted to 5.1%, and inter-assay CV to 11%.

For genetic research, the genes important in the etiology of depression were selected: the genes of serotonergic system: receptor genes: 5HTR2A, 5HTR1B, 5HTR2C, tryptophan hydroxylase gene TPH (isoform 1 and 2), and the gene of monoamino oxidase A (MAO-A); the genes of the noradrenergic and dopaminergic systems: a gene of catechol-O-methyltransferase (COMT) and the gene of noradrenaline transporter (NET); the gene of GABAergic system: the gene for the β1 subunit of the GABAa receptor (GABRB1); the gene of the estrogen receptor α (ESR1); the genes of the enzymes key in the methyl cycle: MTHFR, MTR, and MTHFD1.

Genetic research was conducted based on the genome DNA, isolated from peripheral blood of the patients using the salting out method. Isolated and purified DNA was used as a matrix for amplification of exons of the genes under research. DNA amplification was carried out through a chain reaction of polymerase (PCR), using specific starters (Table 2).

Amplification products were analysed in agarose gel and purified. After amplification of analysed fragments of the genes under research, five μl of reaction mixture was combined with two μl of

Table 2. — *Characteristics of the starters used for the amplification of the genes under research.*

Gene (fragment)	Polymorphism *(SNP number)*	Starter sequence	PCR product length	Temp. of annealing the starter
5HTR2A (exon 1)	c.102C>T (SNP 6313)	F: CAAGGTGAATGGTGAGCAGA R:ATGACAAGGAAACCCAGCAG	458pz	62°C
5HTR1B (exon 1)	c.861G>C (SNP 6296)	F: GTGTGGGTCTTCTCCATCTCTA R: GCAGGCATCTTTGCAGATAG	516pz	60°C
5HTR2C (exon 4)	Cys23Ser (SNP 6318)	F: AGCAGTTGTTTGCATGAGC R: CCATAAAGGATTGCCAGGAG	397pz	57°C
MAO-A (exon 14)	c.1460C>T (SNP 1137070)	F: CGAGCAGCTAGGGAGGTAAG R: GTGGCAGGAGCTTGTATTTGTA	615pz	63°C
COMT (exon 4)	c.649G>A (SNP 4680)	F: ACCAGGGAGGTGAAATACCC R: CCTTGGCAGTTTACCCAGAG	582pz	57°C
NET (exon 9)	c.1287G>A (SNP 5569)	F: TCACCCTCTCCCACGTAGTT R: GCCCCAGTTCTAAGGCTAGG	436pz	56°C
TPH2 (exon 7)	c.1077A>G (SNP 7305115)	F: CATCAGAAGCACCAAACATGAT R: CATGAACCTCCTTCACATGAAA	683pz	64°C
TPH1 (intron 7)	218C>A (SNP 1800532)	F: ACAGGTTTTTCCATCCGTCCT R: CACCACATACACACCCAAATCA	657pz	54°C
GABRB1 (exon 5)	Ile791Ile (SNP 6284)	F: CCATTTCCACCTGTCCATCT R: AAGCATAAGCCCCATGAGTG	741pz	59°C
ESR1 (intron 1)	454-351A>G (SNP 9340799) 454-397C>T (SNP 2234693)	F: TCACACATCACCATTCTCAGC R: CTTTCATTACCTCTTGCCGTC	619pz	63°C
MTHFR (exon 4)	c.677C>T (SNP 1801133)	F: AGGCTGTGCTGTGCTGTTG R: CGCTGTGCAAGTTCTGGAC	477pz	67°C
MTR (exon 26)	c.2756A>G (SNP 1805087)	F: GTTGGTGAAGGGAGAAGAAA R: CTGAAGAATGGGGGTCTGTG	583pz	56°C
MTHFD1 (exon 20)	c.1958G>A (SNP 2236225)	F: TTCTTCTCATTCTTCCTCACA R: TCTGCTCCAAATCCTGCTTC	416pz	60°C

Table 3. — *Clinical and hormonal characteristics of the examined groups.*

Analysed parameter	All patients	Premenopausal	Postmenopausal
Kupperman Index	26±13.1	27.1±13.5	25.4±12.9
BMI (kg/m^2)	26.6±5.3	26.5±4.2	26.5±5.7
FSH (IU/l)	67.1±35.8	52.7±40.5*	75.8±29.6*
LH (IU/l)	34.9±17.5	32.3±22.4	36.2±14
17β-estradiol (pg/ml)	48.1±97,4	83.4±125.8*	26.9±67.7*
Testosterone (ng/ml)	0.28±0.19	0.32±0.25	0.26±0.16
DHEAS (μg/dl)	1.36±0.82	1.6±1.03	1.25±0.66
PRL (ng/ml)	13.8±10.2	15.3±10.2	12.5±8.2

*$p < 0.05$

loading buffer and separated through electrophoresis at the voltage of 100V in 1.5% agarose gel with the addition of ethidium bromide. The products of amplification were analysed in ultraviolet light.

Clinical and hormonal parameters of the groups were compared through the Mann-Whitney test. The correlations between the occurrence of individual symptoms of depression and examined markers were evaluated via the Kruskal-Wallis test. In order to determine the correlations between the genotypes, the Wilcoxon test was used with the FDR correction (False Discovery Rate) [16]. All calculations were done using a free platform R for statistical calculations [17]. The markers were concordant with the Hardy-Weinberg principle ($p > 0.05$).

All experiments were carried out after obtaining the informed consent from all the participating women. The local Ethic Review Committee of Poznan University of Medical Sciences approved the study protocol.

Results

Clinical and hormonal characteristics are presented in Table 3. An average score in the Kupperman scale qualified an examined woman as having the climacteric syndrome of medium severity. An average value of BMI in all groups qualified a woman as overweight. Significant differences were found as regards the concentration of FSH and 17β-estradiol in blood serum between premenopausal and postmenopausal patients (as regards both hormones Mann-Whitney test $p < 0.05$). The associations found between individual symptoms of depression and the concentration of hormones in blood serum against the genotypes are presented in Table 4.

Table 4. — *The associations found between individual symptoms of the climacteric syndrome and the concentration of examined hormones in blood serum against genotypes.*

SNP	All patients	Premenopausal	Postmenopausal
GABRB1.TaqI		Dizziness (0.0198; after correction: 0.0497 CC to CA)	
HTR2AMspI	PRL (0.03; after correction: 0.038 TT to CT)		
TPH1.MaeI			LH (0.004; after correction: 0.014 CC to CA)
NET.Eco147I			17β-estradiol (0.0208; after correction: 0.048 GG to GA)

Discussion

Earlier research concerning a genetic origin of the symptoms of the climacteric syndrome covered only hot flashes and polymorphisms of the genes of the enzymes taking part in the metabolism of estrogens and estrogen receptors. It was concluded that the occurrence and the degree of severity of hot flashes is connected to polymorphisms CYP1B1 [18] and the polymorphism 3βHSD [19]. The polymorphism CYP1B1 is connected to a higher concentration of estradiol in blood serum [18]. Polymorphisms of the ER1 estrogen receptor are connected to a higher frequency of hot flashes [19].

In the present study, out of all examined polymorphisms of the genes of receptors and enzymes important in the etiology of depression (5HTR2A, 5HTR1B, 5HTR2C, TPH1, TPH2, MAO-A, COMT, NET, GABRB1, MTHFR, MTR, MTHFD1, ESR1), only one correlation was found as regards the symptoms of the climacteric syndrome. A correlation was demonstrated between the polymorphism of the gene of β1 subunit of the receptor GABAa GABRB1.TaqI and the occurrence of dizziness in premenopausal women (0.0198; after correction: 0.0497 CC to CA). Polymorphism of this gene shows a strong link with the bipolar affective disorder in women [20], with schizophrenia [21], and autism [22]. The occurrence of the correlation between the polymorphism of the receptor GABRB1 and the presence of dizziness in women during menopause has not been described before in literature, and in the opinion of the authors might be connected to the role of the GABAa receptor in the activity of steroids at the brain level [23].

This study demonstrates a connection between polymorphism of the tryptophan hydroxylase gene TPH1.MaeI and the concentration of LH in blood serum of postmenopausal women (0.004; after correction: 0.014 CC to CA). This correlation has not been described before in the literature. Polymorphism 218A>C TPH1 is attributed to a higher suicidal tendencies [24, 25]. Whereas the 779A>C polymorphic variant indicates a correlation with a greater susceptibility to addictions and the increase in the risk of committing suicide by alcoholics [26]. A high level of LH is considered one of the risk factors of hot flashes during menopause [27] and Tataryn *et al.* [28] claimed that in 85% of cases, hot flashes correlate with the occurrence of LH pulses, without any connection to FSH pulses.

This study shows a connection between the polymorphism of the noradrenaline transporter gene NET.Eco147I and the concentration of 17β-estradiol in postmenopausal women (0.0208; after correction: 0.048 GG to GA). In post mortem examination of the brains of the persons with clinically diagnosed depression, a lower level of protein NET was found [29]. An average concentration of estradiol in blood serum in the examined groups was low, which is characteristic to the menopause period [30] and is a direct cause of the occurrence of the symptoms of the climacteric syndrome [31].

This study also demonstrates a link between a polymorphism of the gene of the serotonin receptor HTR2AMspI and the PRL concentration in blood serum in all patients (0.03; after correction: 0.038 TT to CT). A decreased activity of 5HT2A receptor is one of the mechanisms of antidepressants, and in the brains of the persons suffering from depression a higher density of this receptor was found [32]. This would suggest, that 5TH2A receptor might play a role in disturbing the thermal regulation caused by the estrogen deficiency [33]. It was found, that administering estrogens increases the density of the 5HT2A receptor within the cortex [33]. A correlation of polymorphism c.102T>C (Ser34Ser) 5HTR2A with the occurrence of bipolar affective disorder type I was found [34, 35], as well as with the occurrence of suicidal tendencies in patients with depression [36]. Also demonstrated was a correlation of polymorphism 1438A>G of this gene with the occurrence of the bipolar affective disorder in the Korean population [37], with the occurrence of other mental diseases such as the seasonal affective disorder [38], anorexia, and bulimia [39], as well as the occurrence of the lowered libido in patients treated with selective serotonin reuptake inhibitors [40].

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