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Influence of genomic variation in FTO at 16q12.2, MC4R at 18q22 and NRXN3 at 14q31 genes on breast cancer risk

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Abstract Breast cancer is a major cause of cancer-related deaths in women. It is known that obesity is one of the risk factors of breast cancer. The subject of our interest was genes: *FTO*, *MC4R* and *NRXN3*–associated with obesity. In this study we have analyzed frequencies of genomic variants in *FTO*, *MC4R* and *NRXN3* in the group of 134 breast cancer patients. We genotyped two polymorphic sites located in *FTO* gene (rs993909 and rs9930506), one polymorphic site of *MC4R* gene (rs17782313) and one polymorphic site of *NRXN3* gene (rs10146997). Our hypothesis was that above mentioned SNPs could participate in carcinogenesis. Our research has showed that only rs10146997 was significantly ($P = 0.0445$) associated with higher risk of breast cancer development (OR = 0.66 (95% CI 0.44–0.99)). Moreover, G allele carriers in rs10146997 of the *NRXN3* gene were the youngest patients at onset of breast cancer. On the basis of our research we suggest that further functional may elucidate the role of genomic variation in breast cancer development.

Keywords Breast cancer risk · *FTO* · *MC4R* · *NRXN3*

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Introduction

Approximately 20% of deaths worldwide are due to complications of the overweight and obesity which are risk factors for type 2 diabetes and cardiovascular diseases as well as for cancer and cancer-related mortality [1]. Since breast cancer is one of major causes of cancer deaths in women, the relative influence of obesity-mediated hormones, proinflammatory mediators, and adipokines on tumor development and progression as well as its genetic background should be precisely assessed [2].

Increased production of estrogens in adipose tissue is considered to be one of the most crucial mechanisms potentially contributing to the risk of developing breast cancer as well as to worse prognosis in obese patients [3]. In obese women, adipose tissue is an active endocrine and metabolic tissue and produces an excess of estrogens due to increased tissue mass and up-regulation of aromatase. Furthermore, obesity is also associated with a lower level of sex hormone–binding globulin that restricts the biologic activity of estrogens [4]. Another factor that promotes breast cancer development and progression in patients with overweight or obesity is insulin resistance [5]. Insulin is involved not only in growth of primary tumor because of mitogenic, antiapoptotic, and proangiogenic properties but also in progression of metastasis due to increased level of IGF-1 [5]. Finally, in obese patients carcinogenesis is probably modulated indirectly by adipose tissue hormones such as leptin and adiponectin [6]. The first one stimulates transcription of aromatase, which results in increased production of estrogens. Overexpression of leptin in breast cancer is associated with the development of metastases and shorter survival. In contrast, lower level of adiponectin contributes to insulin resistance.

Genome-wide association (GWA) studies carried out in the last years revealed that common low-penetrance susceptibility alleles of *FTO, MC4R* and *NRXN3* genes are associated with obesity in general population [7]. In the current study we aim to explore whether the susceptibility for breast cancer in obese woman is also partially promoted by genomic variation at 16q12.2/*FTO*, 18q22/*MC4R* and 14q31/*NRXN3* loci previously considered as obesity susceptibility regions.

Materials and methods

Patients

Specimens were consecutively obtained from 134 women with operable invasive ductal carcinomas not otherwise specified (NOS) at a time of routine surgery at the Oncology Department of Copernicus Memorial Hospital in Lodz, Poland, between 1998 and 2001. In all cases, surgical procedure was a radical mastectomy with axillary lymph node dissection. The primary pathologic diagnosis was confirmed in H&E staining.

Methods

Fresh tumor specimens were frozen immediately after excision at −80°C. All specimens were homogenized by means TissueRuptor (Qiagen, Germany). Genomic DNA was extracted with the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The concentrations of extracted DNAs were measured by NanoDrop 8000 Spectrophotometer (Thermo Scientific, USA). Each DNA sample was adjusted to 5 ng/μl concentration. All assayed polymorphic sites were analyzed by TaqMan allelic discrimination assay (Applied Biosystems, USA). All polymerase chain reactions (PCRs) were done in a volume of 5 μl containing TaqMan universal PCR Master Mix, specific TaqMan SNP Genotyping Assays (Applied Biosystems, USA) and 10 ng of genomic DNA, according to the manufacturer's instructions. Thermal cycling conditions were 10 min at 95°C, and 42 cycles each of 95°C for 15 s and 60°C for 1 min. The 7900HT Real-Time PCR System (Applied Biosystems, USA) was used for genotyping.

Statistical analysis

Univariate comparisons were performed using Pearson's or Yates' corrected χ^2 test depending on number of degrees of freedom and sample size. These tests evaluated the differences of observed allele frequencies between the groups in comparison to those expected purely by chance. Distributions of alleles within controls were tested against the Hardy–Weinberg equilibrium. This comparison evaluated whether the control group is a representative sample of the population with terms of expected allele distribution. Continuous variables were compared using analysis of variance or Student's *t*-test depending on the number of groups and presented as means with standard deviations (SD)/95% confidence intervals. Analysis of variance was used when more than two groups were compared, while the Student's *t*-test compared means and SD between two groups. Odds ratios (OR) with 95% Confidence intervals (95% CI) were calculated where possible. An effect with $OR < 1$ signified a protective effect, while one with an $OR > 1$ represented an increased likelihood of developing cancer in individuals with a given genotype. Statistical significance was declared when the *P* value was lower than the 0.05 threshold or the 95% CI for OR did not overlap the value of 1 representing a neutral or ambiguous effect.

Results

Age of diagnosis and other characteristics of the study group are listed in Table 1. The sample size consisted of 134 cases. The mean age of the group was 57.45 but twice more women were over 50 years of age then 50 years or younger. Less than one-third had the first stage of disease. Number of patients with lymph node metastases was almost equal to the number of non-metastatic ones.

Frequencies of genotypes at respective loci are given in Table 2. No deviations from the H–W equilibrium were observed. The most frequent genotypes of *FTO* gene were TA (65 cases) and AG (64 cases) in rs9939609 and rs9930506 respectively. In case of rs1778231 of *MC4R*

Table 1 Patient characteristics

Factor	Number of patients
Number of patients	134
Age (years, mean)	
≤57.45	74
>57.45	60
Stage	
I	27
II–IV	107
Tumor	
T1	37
T2–4	97
Nodal status	
Positive	70
Negative	64

Table 2 Genotype frequencies in breast cancer and controls

Group	*FTO rs9939609*			
	TT	TA	AA	*P* for HWE
Controls	110	180	67	0.66
Breast cancer	40	65	29	
Group	*FTO rs9930506*			
	AA	AG	GG	*P* for HWE
Controls	102	189	76	0.50
Breast cancer	37	64	33	
Group	*MCR4R rs1778231*			
	TT	TC	CC	*P* for HWE
Controls	212	128	27	0.21
Breast cancer	77	49	8	
Group	*NRXN3 rs10146997*			
	AA	AG	GG	*P* for HWE
Controls	188	145	35	0.37
Breast cancer	82	45	7	

HWE Hardy–Weinberg equilibrium

Table 3 Comparisons of genotype frequencies between breast cancer patients and controls

	Breast cancer vs. controls (*P* value)
FTO rs9939609	
All genotypes	0.7739
A allele carriage	0.8368
OR	1.05 (95% CI 0.68–1.61)
FTO rs9930506	
All genotypes	0.6171
G allele carriage	0.9681
OR	1.01 (95% CI 0.65–1.57)
MCR4R rs1778231	
All genotypes	0.8390
C allele carriage	0.9516
OR	1.01 (95% CI 0.68–1.51)
NRXN3 rs10146997	
All genotypes	0.0862
G allele carriage	0.0445
OR	0.66 (95% CI 0.44–0.99)

OR odds ratio

Table 4 Age of diagnosis of breast cancer depending on genotype

	Mean	SD	−95.00%	+95.00%	*N*	*P*
FTO rs 9939609						
TT	56.87500	12.39559	53.05101	60.69899	40	0.6411
TA	56.95385	12.19174	53.95406	59.95363	65	
AA	59.34483	12.06315	54.85378	63.83588	29	0.7266
FTO rs9930506						
AA	57.27027	12.67028	53.31707	61.22347	37	0.3019
AG	56.14063	11.88953	53.13482	59.14643	64	
GG	60.18182	12.08164	55.99588	64.36776	33	0.9175
MC4R rs17782313						
TT	56.07792	12.20630	53.35509	58.80076	77	0.1304
TC	58.42857	11.39444	55.01532	61.84183	49	
CC	64.62500	14.89907	56.17763	73.07237	8	0.1306
NRXN3 rs10146997						
AA	58.58537	12.48896	55.92941	61.24132	82	0.3090
AG	56.13333	11.82178	52.54807	59.71860	45	
GG	52.57143	9.82950	43.48113	61.66173	7	0.1753

gene and rs10146997 of *NRXN3* gene there were the largest number of homozygotes (TT and AA respectively).

Comparative analysis of genotype frequencies between the control and breast cancer group are summarized in Table 3. Among analyzed genotypes only the presence of G allel in polymorphic site rs10146997 in *NRXN3* was significantly ($P = 0.0445$) associated with higher risk of breast cancer development [OR = 0.66 (95% CI 0.44–0.99)].

The effect of genotype on age at onset of breast cancer is shown in Table 4. The youngest age at onset of breast cancer had women who were G allele carriers in rs10146997 of the *NRXN3* gene, the oldest age at onset–C allele carriage in rs177782313 of the *MC4R* gene, but none of them achieved statistical significance.

Discussion

Obesity and overweight are important risk factors for developing a breast cancer in postmenopausal women. Insulin resistance, chronic inflammation and altered adipokines secretion are common phenomena in obesity and they promote cancer progression. However, the role of genomic variation significantly linked with the excess of body mass in the development of breast cancer is still poorly understood. In the current study we analyzed the frequency of genomic variants in *FTO, MC4R* and *NRXN3,* previously indicated by the GWA studies as the obesity susceptibility genes, in population of women with breast cancer.

We genotyped two polymorphic sites located in *FTO* gene (16q12.2) which encodes the protein involved in DNA dealkylation, demethylation and repair [8]. FTO mRNA is most abundant in the brain and pancreatic islets [8]. Frayling et al. identified a common variant in the *FTO*

gene as a risk factor for diabetes and obesity [9]. Almost 16% of white Europeans are homozygous for the A allele of rs9939609 and these carriers are 1.67 times likely to be obese in comparison with homozygous for the T allele [9].

Subsequent polymorphic site, which was taken under consideration was rs17782313 located in *MC4R* gene. The protein encoded by this gene is a member of the melanocortin receptor family—membrane-bound receptor which is mediated by G proteins and interacts with adrenocorticotropic and MSH hormones [10]. The correlation between the signaling properties of these mutant receptor and energy intake emphasizes the key role of this receptor in the control of eating behavior in humans. Melanocortin 4 receptor (MC4R) deficiency is the commonest monogenic form of obesity. Mutations in *MC4R* gene are noted in about 6% of subjects with severe obesity of early onset (before 10 years of age) [11]. Moreover, adults homozygous for rs17782313 (CC) had 0.44 BMI units more in comparison to carriers of other genotypes [12].

Based on well-assessed influence of above mentioned SNPs on obesity development and the elevated frequency of postmenopausal breast cancer in obese women, we hypothesized that these polymorphic sites may be also involved in carcinogenesis.

However, no significant association between polymorphic sites rs9939609 and rs9930506 in *FTO* and rs17782313 in *MC4R* and risk of breast cancer was observed in the study group.

In contrast, carriers of G allele in rs10146997 of *NRXN3* gene had higher risk for breast cancer development [OR = 0.66 (95% CI 0.44–0.99)] in comparison with other genomic variants in this locus. *NRXN3* and *NRXN1* are among the largest human genes, they encode neurexins—polymorphic cell surface proteins expressed mainly in neurons [13]. Three of the genes (*NRXN1-3*) utilize two alternate promoters and include numerous alternatively spliced exons to generate thousands of distinct mRNA transcripts and protein isoforms [14]. Heard-Costa et al. identified a SNP (rs10146997) as a novel locus in the *NRXN3* gene associated with waist circumference (WC) They observed that mean waist circumference was higher among G allele carriers in rs10146997 of *NRXN3* gene, and this variant was also associated with elevated BMI and the risk of obesity [7]. *NRXN3* has been previously implicated in addictions (alcohol dependence, cocaine addiction, and illegal substance abuse) [15]. However, up to date the association between *NRXN3* genotype and breast cancer incidence has not been validated.

An additional analysis of possible impact of genotype on the age of breast cancer onset revealed that the youngest age at presentation of breast cancer had women who were G allele carriers in rs10146997 of the *NRXN3* gene, the oldest age at onset—C allele carriage in rs177782313 of the *MC4R* gene, but none of them achieved statistical significance.

In summary, influence of adipose tissue on cancer development is extremely complex with poorly understood role of its genetic background. Probably particular polymorphisms responsible for promoting obesity have a mild influence on carcinogenesis. However, further functional analyses are required to elucidate the role of genomic variation in both developments of obesity and breast cancer.

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