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SHORT COMMUNICATION

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ASSOCIATION BETWEEN POLYMORPHISMS OF THE *BRCA2* GENE AND CLINICAL PARAMETERS IN BREAST CANCER

R. Krupa[1], T. Sliwinski[2], Z. Morawiec[3], E. Pawlowska[4], M. Zadrozny[5], J. Blasiak[2]
[1]Laboratory of DNA Repair, Department of Molecular Genetics, University of Lodz, Lodz 90-237, Poland
[2]Department of Molecular Genetics, University of Lodz, Lodz 90-237, Poland
[3]N. Copernicus Hospital, Lodz 93-510, Poland
[4]Department of Pediatric Dentistry, Medical University of Lodz, Lodz 92-216, Poland
[5]Polish Mother's Memorial Hospital, Lodz 93-338, Poland

Background: **A C/T transition — rs4987117 (the Thr1915Met polymorphism) and an A/G transition — rs11571653 (the Met784Val polymorphism) in the *BRCA2* gene were linked to breast cancer risk in Polish and Japanese populations, respectively. *Aim*: To study the association between polymorphisms of the *BRCA2* gene and clinical parameters in breast cancer. *Methods:* Both polymorphisms were evaluated by RFLP — PCR in blood samples obtained from 117 women with sporadic breast cancer. Patients were stratified by genotype, Bloom — Richardson grade, TNM stage, estrogene and progesterone receptors (PR) status and the linkages of each genotype with each stratum were calculated by logistic regression. *Results*: Variant genotypes and alleles of both polymorphisms of the *BRCA2* gene were inversely related to hormone receptor status for a group of patients with at least one positive receptor status as compared to a group with both receptors negative status (OR 0.27, 95% CI 0.07 — 0.95, $p = 0.043$ and OR 0.39, 95% CI 0.19 — 0.82, $p = 0.013$ for Met1915Met homozygote and 1915Met allele, respectively and OR 0.02, 95% CI 0.00 — 0.13, $p = 0.0005$ and OR 0.43, 95% CI 0.21 — 0.88, $p = 0.021$, for Val784Val homozygote and the 784Val allele. No association was found between both polymorphisms and Bloom — Richardson grading and TNM staging. *Conclusions*: Our results suggest that variant genotypes of the Thr1915Met and Met784Val polymorphisms of the *BRCA2* gene may be indicative factors in therapy of ductal breast cancer.**

Key Words: **breast cancer, *BRCA2*, DNA repair, gene polymorphism.**

Polymorphisms in DNA repair genes may affect genomic stability and trigger cancer transformation. Common single nucleotide polymorphisms (SNPs) in these genes, which can be associated with a difference in the ability to repair DNA damage, may be candidates for low penetrance alleles of breast cancer. Breast cancer susceptibility protein BRCA2 is involved in double strand DNA breaks (DSB) repair by homologous recombination (HRR) [1–3]. It interacts directly with the RAD51 protein in the formation of its aggregates at the site of DNA damage and regulates RAD51 binding selectivity to DNA strands [4, 5].

Our previous results suggest that the C/T transition in the *BRCA2* gene (rs 4987117) resulting in Thr → Met substitution in codon 1915 (the Thr1915Met polymorphism) in the *BRCA2* gene may be an independent marker of breast cancer risk [6]. The A/G transition (rs 11571653) resulting in Met → Val substitution in codon 784 (the Met784Val polymorphism) was found to be independent marker of breast cancer risk and also good candidate for independent prognostic factor in Japanese population [7].

In the present work we searched for an association between the Thr1915Met and the Met784Val polymorphisms of the *BRCA2* gene and clinical characterization of breast cancer patients.

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*Correspondence: Fax: 48426354484
E-mail: jblasiak@biol.uni.lodz.pl
Abbreviations used: DSB – double strand break; ER – estrogene receptor; PCR – polymerase chain reaction; PR – progesterone receptor; RFLP – restriction fragment length polymorphism; SNP – single nucleotide polymorphism; TNM – tumor nodules metastases.

Patients. Samples of blood were obtained from 117 women with breast cancer treated in 2001 and 2003 in Polish Mother's Memorial Research Institute and N. Copernicus Hospital in Lodz, Poland. All patients had histologically-confirmed ductal carcinoma. The subjects were 45 to 81 years old with median age of 65 years. Clinical data of these patients are presented in Table 1. Samples were obtained under consideration of all ethical and legal requirements. Written consents were obtained from each patient.

Genotype determination. Genomic DNA was prepared using the guanidine isothiocyanate isolation method [8]. Restriction fragments length polymorphism polymerase chain reaction (PCR-RFLP) was used to detect the genotypes of the BRCA2-Met784Val and BRCA2-Thr 1915 Met variants [6]. Each 20 μl of the PCR reaction contained 10 ng genomic DNA, 1.25 U Taq polymerase (InGen — TERPOL, Sieradz, Poland) in 1x PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 11 mM $MgCl_2$, 0.1% gelatin), 1.5 mM $MgCl_2$, 50 mM dNTPs, and 250 nM each primer. Thermal cycling conditions were as follows: initial denaturation step at 95 °C for 5 min, 30 cycles at 95 °C for 30 s and 30 s at the 55 °C annealing temperature, and at 72 °C for 30 s. The final extension step was performed at 72 °C for 5 min. The PCR was carried out in a MJ Research, INC thermal cycler, model PTC-100 (Waltham, MA, USA). The BRCA2-Met784Val polymorphism was determined using the following primers (TIB MOLBIOL, Poznan, Poland): sense, 5'-TGGAATACAGTGATACTGAC-3'; antisense, 5'-TTGGATTACTCTTAGATTTG-3'. The 346 bp PCR product was digested overnight with 5 U of the restriction enzyme *Bsp*HI. The Met allele was digested into 163 and 183 bp fragments

whereas the Val variant remained intact. The BRCA2-Thr1915Met polymorphism was determined using the following primers: sense, 5'-TTGCCAAACGAAAAT-TATGG-3; antisense, 5'-AGATTTTCCACTTGCTGTGC-3'. The 304 bp product was digested overnight with 5 U of the restriction enzyme *Sph*I. The Thr allele was digested into 95 bp and 209 bp fragments and the Met was intact. Restriction fragments were analyzed on 3% agarose gels stained with ethidium bromide.

Data analysis. Statistical analysis was performed using STATISTICA 8.0 package (Statsoft, Tulusa, USA). Distributions of genotypes and alleles between groups were tested using Fisher's exact test. A linkage between SNPs and breast cancer characteristics was accessed by the unconditional logistic regression (quasi-Newton method). For each SNP, odds ratio (OR) and 95% CI were calculated. Wild type alleles or additional homozygous variants were used as reference groups. All of the statistical tests were two-tailed and $p \leqslant 0.05$ was considered significant.

There were no differences in genotype distributions for both *BRCA2* polymorphisms — Met784Val and Thr1915Met in subjects with ductal breast cancer with different Bloom — Richardson grading (data not shown). In the case of TNM staging, genotype and allele distributions did not differ significantly between the groups either (data not shown). There were no differences in the frequencies of the Met and Val, Thr and Met alleles and relative genotypes between PR positive and PR negative patients for *BRCA2* Met784Val and *BRCA2* Thr1915Met, respectively (data not shown). We found statistically significant differences in the distribution of the genotypes of the Thr1915Met polymorphism in the group of estrogene positive breast cancer patients as compared with ER-negative ones: the Met1917Met genotype, as well as the 1917Met allele were negatively related to estrogene positive breast cancer (OR 0.24, 95% CI 0.09 — 0.66, $p = 0.0072$, statistical power 70.3% and OR 0.45, 95% CI 0.27 — 0.76, $p = 0.0028$, statistical power 90.1%, respectively). In the case of the Met784Val polymorphism we did not found any relation with ER status alone. Our results are similar to these obtained in Japanese population [7]. Negative relation was also found for variant genotypes and alleles of both gene polymorphisms and hormone receptor status for a group of patients with at least one positive (PR or ER) hormone receptor compared to a group with negative status by both receptors (OR 0.27, 95% CI 0.07 — 0.95, $p = 0.0433$, statistical power 92.7% and OR 0.39, 95% CI 0.19 — 0.82, $p = 0.0130$, statistical power 81.3% for the Met1915Met homozygote and the 1915Met allele, respectively and OR 0.02, 95% CI 0.00 — 0.13, $p = 0.0005$, statistical power 92% and OR 0.43, 95% CI 0.21 — 0.88, $p = 0.0211$, statistical power 77.5% for the Val784Val homozygote and the 784Val allele (Table 2). Hormone receptor status is a predictive factor for adjuvant systemic chemo- or endocrine therapy reducing the risk of relapse. [9].

Our results suggest that the Thr1915Met and Met784Val polymorphisms of the *BRCA2* gene may be the indicative and helpful factors for choosing of therapy of ductal breast cancer.

Table 1. Characteristic of breast cancer patients

Clinical characteristics	Patients number (n = 117) / frequency
Ductal carcinoma	117
Bloom-Richardson grading	
Grade I	24 / 0.21
Grade II	47 / 0.40
Grade III	46 / 0.39
TNM staging	
Stage IIA	2 / 0.02
Stage IIB	56 / 0.48
Stage IIIA	59 / 0.50
ER positive	58 / 0.50
PR positive	56 / 0.48

Note: ER — estrogene receptor, PR — progesterone receptor

Table 2. The frequency of alleles and genotypes and odds ratios (OR) of the Thr1915Met and Met784Val polymorphisms of the *BRCA2* gene in relation to estrogene and PR status

Genotype or allele	PR (+) ER (–) and PR(–) ER (+) and PR (+) ER (+) N = 98	PR (–) ER (–) N = 19	OR (95% CI)	*p* value[a]
	Number	Number		
Thr/Thr	30	4	1.00 ref.	
Thr/Met	46	4	1.53 (0.36 – 6.60)	0.7092
Met/Met	22	11	0.27 (0.07 – 0.95)	0.0433
Thr	106	12	1.00 ref.	
Met	90	26	0.39 (0.19 – 0.82)	0.0130
Met/Met	17	0	1.00 ref.	
Met/Val	79	14	0.26 (0.06 – 1.22)	0.1211
Val/Val	2	5	0.02 (0.00 – 0.13)	0.0005
Met	113	14	1.00 ref.	
Val	83	24	0.43 (0.21 – 0.88)	0.0211

Note: [a]Fisher's exact test

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