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The comparison between TP53 gene polymorphisms (c.[215G>C]) homozygotes and heterozygotes in breast cancer patients: A clinicopathological analysis

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Original Article

Abstract

Purpose: TP53 is a tumor suppressor gene which participates in regulation of cell cycle check points, DNA repair, and apoptosis. The aim of this study was to compare TP53 germ line gene polymorphisms (c.[215G>C]) wild – type homozygotes GG with heterozygotes GC according to clinicopathological factors. **Methods:** We reviewed the medical records of 87 (22% *TP53* gene homozygotes and 78% heterozygotes) breast cancer patients who were diagnosed and treated in COI in Gliwice. Polymorphism profile was assessed by RFLP-PCR technique. **Results:** The presence of lobular invasive carcinoma was observed insignificantly more often in homozygotes, especially in the group of patients at the age below 50 years (29% vs. 4%, $p = 0.095$). Patients being TP53 gene heterozygotes had larger tumor size (T > 2) than homozygotes (16% vs. 5%, $p = 0.450$). There was observed a tendency to the presence of lymph node metastases (53% vs. 34%, $p = 0.182$) and higher Ki67 (> 20%) (69% vs. 46%, $p = 0.209$) in TP53 gene homozygotes. HER2 overexpression was associated with TP53 heterozygotes, especially in the group of patients at the age above 50 years (33% vs. 8%, $p = 0.144$). A negative receptor status was reported more frequently in homozygotes (43% vs.21%, $p = 0.340$) in patients with age below 50 years. Similarly higher histological grade G3 was detected more often in homozygotes in patients at the age below 50 years (80% vs. 33%, $p = 0.130$). **Conclusion:** TP53 gene homozygotes and heterozygotes differ from each other in respect of clinicopathological factors such as: histological type, lymph node metastases, higher Ki67 (> 20%), histological grade G3, ER/PR status, tumor size (T > 2), HER2 overexpression, cancer in family history and diabetes. Patient's age was associated with the pathological characteristics of tumor.

Keywords: TP53 polymorphisms, Homozygote, Heterozygotes, Breast cancer, Clinicopathological factors

1. Introduction

p53 is a tumor suppressor gene whose product, the p53 protein, maintains DNA stability and normal cellular growth. It participates in regulation of cell cycle check points, DNA repair, and apoptosis. p53 remains the most commonly mutated gene in many human cancers and occurs in about 50% of them. In breast cancer, the

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frequency of TP53 gene mutations is approximately 20% to 30%. Somatic mutations of TP53 are more frequent at the advanced stage or in cancer subtypes with aggressive behavior (such as triple negative or HER2-amplified breast cancers).[1,2] Recent data show that breast cancer in germline TP53 mutation carriers is commonly HER2-positive (63–83%).[2] TP53 mutation is an independent marker of poor prognosis, in particular in hormone receptor-positive cases.[3]

TP53 mutation is a high risk breast cancer associated with Li Fraumeni syndrome. Patients with TP53 mutations have a 50% risk of developing any of the associated cancers (including breast cancer) by the age of 30 years and have up to 93% of lifetime cancer risk. The TP53 pathway remains largely intact in luminal A cancers but often is inactivated in the more aggressive luminal B cancers. Basal-like tumors had a high frequency of TP53 mutation (80%). Other investigations have shown significant enrichment of TP53 mutations in HER2-overexpressed or ER-negative tumors.[4]

A number of single nucleotide polymorphisms (SNPs) systematically have been identified. The most commonly studied SNP (SNP72) is a G/C variation at the second position of codon 72 in exon 4, leading to Arg72 or Pro72 protein variants. Sharp ethnic differences are observed for this SNP, the Arg72 variant being more prevalent in Caucasians, whereas the Pro72 variant is more prevalent in Chinese and African-Americans.[5,6] The Pro/Pro genotype of TP53 codon 72 appears to be an independent prognostic marker in breast cancer patients. This Pro/Pro genotype of TP53 codon 72 was shown to be associated with poorer disease-free survival (DFS) than other genotypes (p = 0.049). The association of the Pro/Pro TP53 genotype with poorer DFS was especially significant in patients who received adjuvant chemotherapy (p = 0.009). In contrast, among the patients who had received adjuvant hormonal therapy or no adjuvant systemic therapy, TP53 codon 72 genotype was not associated with DFS.[7,8]

The aim of this study was to compare TP53 germline gene polymorphisms (c.[215G>C]) wild – type homozygotes GG with heterozygotes GC according to clinicopathological factors.

2. Methods and Materials

A retrospective analysis was conducted on the medical records of 87 breast cancer patients who were diagnosed and treated with chemotherapy, hormonotherapy and /or immunotherapy at Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch in Poland (COI).

Genetic diagnostics was conducted in years 2012 – 2016. All patients gave written informed consent for genetic examination. The patients were Caucasian women from the southern part of Poland- Silesian region. 22% of patients were TP53 gene homozygotes and 78% of them were heterozygotes. The median age at diagnosis of patients was 52 years (range from 32 to 76). All of them were in good performance status (ZUBROD 0 - 1). The complete characteristics of patients with regard to demographic and clinicopathological features are presented in Table 1 and Table 2.

Chemotherapy was applied to 68% of patients. Neoadjuvant chemotherapy was administered to 21 (36%) women and adjuvant therapy to 38 (64%) of patients. Anthracycline or taxane based chemotherapy was applied to 55 (63%) and 24 (28%) of patients, respectively. 23 (26%) of them received anthracycline and taxanes together (at the same time or taxanes after anthracyclines). Trastuzumab was administered to patients with tumors HER2 (human epidermal growth factor) overexpression. All women with positive steroid receptor status (oestrogen (ER), progesterone receptor (PR)), received hormonotherapy (71% of patients). Radiotherapy was applied to 69% including all patients after breast preserving treatment (45%). Treatment strategies are presented in Table 3. The follow-up time median was 2.4 years (range from 3 months to 19 years). In case of contralateral breast cancer the follow up was counted from the date of the first diagnosis of breast cancer.

Clinical evaluation included physical examination, blood test, chest X ray, mammography, ultrasound breast exam, breast MRI and tumor core biopsy. The data on the age at onset, overweight, co- morbid conditions, menopausal status, the history of cigarette smoking, surgical procedure, disease stage according to TNM classification, histology, estrogen and progesterone receptor status, HER2 status and contralateral breast cancer were gathered from hospital records and pathology reports. The analysis of patients' medical records was performed according to the national law regulation. Hormone status, HER2 overexpression and Ki 67 were determined by routine immuno -histochemical techniques. Mutation profile was assessed by RFLP-PCR technique. We evaluated the presence of polymorphism TP53 (c.[215G>C]).

Statistical analysis was carried out using STATISTICA 7 software. The frequency of side effects appearance was counted. The qualitative features were presented as the percentage of their occurrence and evaluated with Fisher test and Chi 2 test with Yates correction. Differences were considered as significant if the p value was ≤ 0.05.

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Table 1: Clinical characteristics of the patients under study.

Risk factor		TP53		homozygotes		heterozygotes	
		n	%	n	%	n	%
Age	Median	52		51		52	
	Range	32-76		33-69		32-76	
Hypothyroidism	Yes	9	10	1	5	8	12
Cardiovascular diseases	Yes	5	6	1	5	4	6
HA	Yes	25	29	4	21	21	31
History of cancer in family:	Yes	62	71	11	58	51	75
History of breast cancer in family	Yes	28	32	5	26	23	34
Stomach cancer	Yes	6	7	1	5	5	7
Colorectal cancer	Yes	9	10	1	5	8	12
Kidney cancer	Yes	5	6	3	16	2	3
Lung cancer	Yes	4	5	0		4	6
CNS	Yes	6	7	1	5	5	7
Treatment	chemotherapy	59	68	12	63	47	69
	Radiation therapy	60	69	13	68	47	69
	Surgery	48	55	9		39	
	BCT	39	45	10	53	29	43
	hormonotherapy	62	71	13	68	49	72

Table 2: Clinicopathological characteristics of the patients according to *TP53* gene polymorphisms (c.[215G>C]).

Risk factor	Risk factor	Tp53 homozygotes		Tp53 heterozygotes		P
		n	%	n	%	
Age	Median 52	51		52		0.785
	Range 32-76	33-69		32-76		
Menopausal status	Postmenopausal	7	37	26	38	1.00
	Premenopausal	12	63	42	62	
Clinical staging nodes	N0	9	47	45	66	0.182
	N positive	10	53	23	34	
Tumor size	T3-4	1	5	11	16	0.450
	T1-2	18	95	57	84	
Grade G	G1+2	10	62	41	64	1.00
	G3	6	38	20	36	
	Missing	3		7		
Hormonal status	Negative	5	26	17	25	1.00
	Positive	14	74	51	75	
HER2 overexpression	Negative	16	84	47	69	0.568
	Positive	3	16	21	31	
Ki67	Ki67<20%	4	31	25	54	0.209
	Ki67>20%	9	69	21	46	
	Missing	6		22		
Triple negative	Yes	4	21	10	15	0.495
	No	15	79	58	85	
Luminal B type	Yes	7	37	27	40	1.00
Luminal A type	Yes	7	37	25	37	1.00

Table 3: Treatment strategy.

Features		Tp53 homozygotes		Tp53 heterozygotes		p
		n	%	n	%	
Chemotherapy	Neoadjuvant	4	33	17	36	1.00
	Adjuvant	8	67	30	64	
Immunotherapy	Yes	3	16	14	21	0.754
(Trastuzumab)	No	16	84	54	79	
Hormonotherapy	Yes	13	68	49	72	0.779
	No	6	32	19	28	
Local treatment	Mastectomy	9	47	39	57	0.449
	BCT	10	53	29	43	
Radiotherapy	Yes	13	68	47	69	1.00
	No	6	32	21	31	

3. Results

Two groups of patients were compared: 19 (22%) TP53 gene homozygotes and 68 (78%) heterozygotes, the results are presented in Table 2. The median age of patients was 52 years (range from 32 to 76). All patients were women. 38% of them had postmenopausal status, which was defined as age > 55 years. 11% of patients were younger than < 40 years. There was observed a tendency to the presence of TP53 homozygotes in younger patients (21% vs. 9%, $p = 0.215$). There was no difference according to median age (51 vs. 52 years, $p = 0.785$) and menopausal status (37% vs. 38%, $p = 1.00$) between TP53 gene homozygotes and heterozygotes. In the present analysis older (> 65) was observed in 9% of patients. (11% of homozygotes and 9% of heterozygotes, $p = 1.00$). Contralateral breast cancer was reported in 5 (6%) of patients including 1 homozygote and 4 heterozygotes.

Cancers in family history were detected in 71% of patients with TP53 polymorphisms and were observed insignificantly more often in heterozygotes than in homozygotes (75% vs. 58%, $p = 0.161$). The most frequently reported cancers in family history were: breast cancer (32%), lymphoma (17%), colorectal cancer (10%), CNS tumors (7%), gastric cancer (7%) and lung cancer (5%). Co morbid condition was reported in 52% of patients. Hypertension and cardiovascular diseases were observed in 29% and 6% of patients, respectively. Diabetes was detected in 5% of women and 10% of patients suffered from hypothyroidism. In our study, diabetes was observed insignificantly more often in TP53 heterozygotes than in TP53 homozygotes (5% vs. 0, $p = 0.572$). There was no association between TP53 polymorphisms (homozygotes vs. heterozygotes) and hypertension (21% vs. 31%, $p = 0.568$), cardiovascular diseases (5% vs. 6%, $p = 1.00$) or hypothyroidism (5% vs. 12%, $p = 0.677$).

Lobular invasive and ductale invasive carcinoma were detected in 15% and 85%, respectively. The presence of lobular invasive carcinoma was observed insignificantly more often in homozygotes (21% vs. 13%, $p = 0.468$), especially in group of patients at the age below 50 years (29% vs. 4%, $p = 0.095$). Tumor size (T3-T4) was detected in 14% of patients. Patients being TP53 gene heterozygotes had larger tumor size (T > 2) than homozygotes (16% vs. 5%, $p = 0.450$). Lymph node metastases were reported in 38% of patients. There was observed a tendency to the presence of lymph node metastases (53% vs. 34%, $p = 0.182$) in patients with TP53 gene homozygotes in comparison to heterozygotes. Ki67 > 20% was detected in 51% of tumors and was more often reported in TP53 gene homozygotes (69% vs. 46%, $p = 0.209$), especially in the group of patients at the age below 50 years (100% vs. 41%, $p = 0.090$).

Histological grade G3 was detected in 36% of tumors. There was no association between G3 grade and TP53 polymorphisms (38% vs.36%, $p = 1.00$). In contrary, in the group of patients at the age below 50 years histological grade G3 was reported more frequently in TP53 homozygotes than in heterozygotes (80% vs. 33%, $p = 0.130$). HER2 overexpression was present in 28% of women with insignificantly higher frequency in TP53 heterozygotes in comparison to homozygotes (31% vs. 16%, $p = 0.253$), especially in the group of patients at the age above 50 years (33% vs. 8%, $p = 0.144$). ER negative or PR negative tumors was observed in 26% and 29% of patients, respectively. There was detected a tendency to occur ER/PR negative tumors in TP53 homozygotes in comparison to heterozygotes but only in subgroup of the patients at the age below 50 years (43% vs. 21%, $p = 0.340$).

All patients were *TP53* polymorphisms carriers. TP53 mutation distribution was found in 76% of luminal tumors (37% of luminal A, 39% of luminal B), in 28% of HER2 amplified tumors and in 16% TNBC (triple negative breast cancer). In the younger subgroup (< 50 years) luminal B subtype was observed insignificantly more often in heterozygotes (50% vs. 29%, $p = 0.415$). TNBC subtype was reported more frequently in TP53 homozygotes than in heterozygotes (29% vs. 7%, $p = 0.171$).

Table 4: The association between clinicopathological factors and pts age.

	<50 years			>50 years		
	Tp53 homozygotes	Tp53 heterozygotes	p	Tp53 homozygotes	Tp53 heterozygotes	p
ER(-)	3 (43%)	6 (21%)	0.340	2 (17%)	12 (30%)	0.475
ER(+)	4	22		10	28	
PR (-)	3 (43%)	6 (22%)	0.340	2 (17%)	14 (35%)	0.301
PR (+)	4	22		10	26	
G 1-2	1	16	0.130	9	23	0.293
G3	4 (80%)	6 (33%)		2 (18%)	14 (38%)	

Table 4 shows the association between clinicopathological factors and patients age.

4. Discussion

In this retrospective study, we analyzed the relationship between TP53 gene polymorphisms (c.[215G>C]) homozygotes/ heterozygotes and standard clinicopathological factors such as hormone status, human epidermal growth factor, tumor size, the presence of lymph nodes metastases, co-morbid conditions, patients' age, menopausal status, history of cancer in the family and the presence of metachronous breast cancer.

The most frequent cancers observed in germline TP53 mutation carriers are premenopausal breast cancer, bone and soft-tissue sarcomas, adrenal cortical carcinomas, and CNS tumors.[9] In our analysis, cancers in family history were detected in 71% of patients with TP53 polymorphisms and were observed insignificantly more often in heterozygotes than in homozygotes (75% vs. 58%, $p = 0.161$). The most frequently reported cancer in family history was: breast cancer (32%), lymphoma (17%), colorectal cancer (10%), CNS tumors (7%), gastric cancer (7%) and lung cancer (5%).

Some study reported that the polymorphism in TP53 (rs1042522) was associated with type 2 diabetes.[10] Some data suggested that p53 is involved in stress response, metabolism and cardiovascular functioning. p53 expression in adipose tissue is associated with insulin resistance and subsequently with age-related cardiovascular disorders.[11] The C/C-genotype of rs1042522 was found to be associated with changes in diastolic blood pressure (DBP) and waist circumference over time.[12] In our study, co morbid condition was reported in 52% of patients. Most common were hypertension 29%, cardiovascular diseases 6%, diabetes 5% and hypothyroidism 10%. Diabetes was observed insignificantly more often in TP53 heterozygotes than in TP53 homozygotes (5% vs. 0, $p = 0.572$). There was no association between TP53 polymorphisms (homozygotes vs. heterozygotes) and hypertension (21% vs. 31%, $p = 0.568$), cardiovascular diseases (5% vs. 6%, $p = 1.00$) or hypothyroidism (5% vs. 12%, $p = 0.677$).

In some studies the presence of mutated p53 was associated with patient characteristics of increased age and postmenopausal status, and tumor characteristics of ductal morphology, higher grades and ER/PR negativity, in agreement with previous studies.[13,14,15] In study conducted by Hamaguchi *et al*. Pro allele of p53 codon 72 (rs1042522) were more frequent in ER-positive than ER-negative breast cancer, especially in patients less than 50-year old.[16] In our analysis there was observed a tendency to the presence of TP53 homozygotes in younger patients (21% vs. 9%, $p = 0.215$). There was no difference according to menopausal status (37% vs. 38%, $p = 1.00$) between TP53 gene homozygotes and heterozygotes. The presence of lobular invasive carcinoma was observed insignificantly more often in homozygotes (21% vs. 13%, $p = 0.468$), especially in the group of patients at the age below 50 years (29% vs. 4%, $p = 0.095$). Similarly, Ki67 > 20% (69% vs. 46%, $p = 0.209$) and the presence of lymph node metastases (53% vs. 34%, $p = 0.182$). Larger tumor size (T > 2) (16% vs. 5%, $p = 0.450$) and HER2 overexpression (31% vs. 16%, $p = 0.253$) were more often reported in heterozygotes. Similarly, ER/PR negative tumors were detected more frequently in TP53 heterozygotes but only in the subgroup of patients at the age above 50 years (ER 30% vs. 17%, $p = 0.475$; PR 35% vs. 17%, $p = 0.301$).

TP53 mutations are the most frequent genetic alterations in breast cancer, observed in 30% of breast carcinomas. Germline TP53 mutations carriers have a remarkable risk of developing cancer: 50% by age 30 years and 90% by age 60 years.[17] TP53 mutation distribution is highly linked to molecular tumor subtypes and is found in 26% of luminal tumors (17% of luminal A, 41% of luminal B), in 50% of HER2 amplified tumors, in 69% of molecular apocrine breast carcinomas and in 88% of basal-like carcinomas.[18,19] In the group of breast cancers women with germline TP53 mutations, approximately 63–83% of tumors are HER2 positive.[20] Rath *et al.* have identified a low prevalence of germline TP53 mutations among women diagnosed with HER2 positive breast cancers before 50 years of age.[21] In our study, all patients were TP53 polymorphisms carriers. TP53 mutation distribution was found in 76% of luminal tumors (37% of luminal A, 39% of luminal B), in 28% of HER2 amplified tumors and in 16% TNBC. In younger subgroup (< 50 years) TNBC were observed insignificantly more often in homozygotes (29% vs. 7%,

p = 0.170) and luminal B subtype more often in heterozygotes (50% vs. 29%, p = 0.415).

5. Conclusion

TP53 gene homozygotes were characterized by lobular invasive carcinoma subtype, lymph node metastases and higher Ki67 (> 20%). Additionally, TP53 homozygotes at the age below 50 years were associated with histological grade G3 tumors and with ER/PR negative tumors. In contrary, larger tumor size (T > 2), HER2 overexpression, cancer in family history and diabetes were mostly present in TP53 gene heterozygotes. Patients age was associated with the pathological characteristics of tumor.

Conflict of Interest

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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