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655Val and 1170Pro ERBB2 SNPs in familial breast cancer risk and BRCA1 alterations

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Abstract. Human ERBB2 presents several SNPs. One of these, Ile655Val, introduces a structural change in the transmembrane region of ERBB2 and has been the focus of debate over its potential role as a susceptibility marker for breast cancer risk. Another SNP, Ala1170Pro, introduces a structural change in the carboxyl-terminal regulatory domain of the protein, but its clinical and biological importance remains undefined. The aim of this study was to investigate the association of rare alleles of both SNPs and the risk of developing breast cancer, BRCA1 alterations and clinical-pathological features of Caucasian breast cancer patients with familial history of breast/ovarian cancer. The originality of the present paper is that it is the only specifically focusing on the relationship between ERBB2 SNPs and familiarity/BRCA1 characteristics. A consecutive series of 628 patients with first diagnosis of breast cancer and 169 healthy people had DNA analyzed for both SNPs. Genotypic or allelic frequencies of ERBB2 SNPs in breast cancer patients were similar than in controls. The variant allele 655Val was significantly associated with younger age ($p = 0.009$) particularly associated with patient family history of breast cancer ($p = 0.02$). The 655Val allele was also more commonly found in invasive, while the variant 1170Pro in estrogen receptor positive breast cancers. Furthermore, this last SNP seems to be strictly associated with the presence of BRCA1 polymorphisms. In conclusion, these findings point to the existence of an association of ERBB2 allelic variants at both loci with specific breast tumor phenotypes and to the need of deeply investigate different gene SNPs association for risk defining.

Keywords: Single nucleotide polymorphism, ERBB2, breast cancer, familiarity, risk

Abbreviations: SNPs = single nucleotide polymorphisms

1. Introduction

In 10–15% [17] of the cases breast cancer occurs according to familial clustering that may result from the transmission of breast cancer predisposition genes. Over a decade ago, alterations of *BRCA1* and *BRCA2* genes [21] (less than 20% of all hereditary breast cancers) were found to be highly penetrant variants strongly predisposing to breast cancer development. However, the search for other breast cancer predisposition genes is ongoing, and attention has focused on genes known to be involved in breast cancerogenesis.

Genomic amplification in primary breast tumors of *ERBB2* (ERBB2/*neu*) has been described with a more aggressive behavior and a poorer patient outcome [23,25,26]. Even more interesting, the *ERBB2* gene is highly polymorphic particularly in its intronic regions and its missense single nucleotide polymorphisms (SNPs) have been considered to be susceptibility markers for different pathologies [5].

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ERBB2 SNPs documented in the genome database (Single Nucleotide Polymorphism Data Base: http://www.ncbi.nlm.nih.gov/SNP) and encoding structural variations in the receptor protein include those involving amino-acids in the extracellular domain (452), the transmembrane domain (654, 655), the tyrosine kinase domain (927), and the carboxyl-terminal intracellular regulatory domain (1170). In particular, the *ERBB2* SNP (Ile655Val) has been the subject of numerous studies addressing its potential role as a susceptibility marker for breast cancer risk [1,3,14,27,28]. These studies were motivated by earlier evidence that a missense point mutation (Val664Glu) in the transmembrane region of rat *neu* proto-oncogene (*ERBB2* human homologue) is tumorigenic by constitutively activating the receptor protein [2,7]. Although *ERBB2* appears to become oncogenic in human breast epithelium only upon gene amplification and protein overexpression [15,23], detection of a missense polymorphism in the receptor's transmembrane region provides the biological rationale for its possible role in determining breast cancer risk [10,16]. In fact, computational investigation by Fleishman [10] pointed out that the presence of Val in the 655 transmembrane position stabilizes the formation of an active dimer of the protein thus predisposing to an auto-activity of the receptor.

Another *ERBB2* polymorphism recently detected, Ala1170Pro, causes a structurally significant amino-acid variation within the carboxyl-terminal regulatory domain of the receptor [4,12]. Preliminary studies suggest that the allelic frequency of the *ERBB2* 1170Pro variant differs according to race/ethnicity [18] and is associated with more advanced stages (T3-4) of breast cancer [4]. More recently, the presence of the Pro amino-acid in 1170 regulatory region may has been suggested to contribute to constitutively up-regulate the protein and subsequently activate its signal transduction pathway.

The aim of the present study is to: (1) compare the genotypic incidence of two *ERBB* SNPs, Ile655Val and Ala1170Pro, in Caucasian breast cancer patients and in a healthy population control group; and (2) investigate the genotypic and allelic frequencies of these two SNPs with the clinical-pathological features of diagnosed breast cancers and with known familial risk factors for the breast cancer including the presence of BRCA1 alterations.

2. Material and methods

2.1. Patients

A consecutive series of 628 Caucasian patients with a first diagnosis of breast cancer who underwent surgery at the Dipartimento Donna of the National Cancer Institute of Bari, Italy ($n = 508$) and at the Istituto Oncologico Romagnolo of Forlì, Italy ($n = 120$) entered the study. The control group comprised 169 Caucasian women who had undergone surgery for fibroadenomas at the same Institutions and at the Buck Institute of Novato, CA and who agreed to having their blood samples collected for study purposes. This group of healthy donors was comparable to the group of patients in terms of age and menopausal status. All the patients were characterized from the pathological and biological point of view. Each breast cancer patient was staged according to the UICC TNM classification [13], while cytohistological tumour grading in cases with infiltrating ductal carcinoma was performed according to Fisher's method [9].

ER and PgR tumor content were studied immunohistochemically on tumor samples obtained during surgery, and a cutoff of 10% of immunostained tumor cells was established for a tumor to be classified as receptor negative or positive; tumor proliferative activity was assessed by Mib1 immunostaining and a cutoff of 10% of positive cells served to differentiate between low or high proliferating tumors. All the analyses were performed in the Experimental Oncology Laboratory of the Oncology Institute of Bari – which participates in the INQAT Quality Control programs [19] and at the Buck Institute of Novato, CA.

All the breast cancer patients were involved in a genetic counseling program for familial breast/ovarian cancer. A preliminary investigation of cancer syndromes was performed by a surgeon and the patients eligible for genetic counseling were transferred to genetic counseling outpatients clinics where *ad hoc* teams updated their medical history and obtained informed consent to perform the molecular analyses. Patients were classified, modifying Eccles criteria [8] as having a family history of breast cancer if one of the following conditions was present: (1) at least 3 relatives (first or second degree) had breast or ovarian cancer; (2) two relatives <50 years had breast cancer; (3) one relative <36 years had breast cancer; (4) the patient had bilateral cancer and at least one relative with breast cancer (or a relative with bilateral cancer); (5) male breast cancer. These also the healthy

donors were assessed by expert staff members of the out-patient clinics of the above Hospitals to determine whether they exhibited a family cancer syndrome.

2.2. SNPs genotyping

The two SNPs in the ERRBB2 coding region: rs#1801200 (Ile655Val) and rs#1058808 (Ala1170Pro) from the NCBI data base (http://www.ncbi.nlm.nih.gov/SNP) were genotyped.

Blood sample has been collected from patients the day before surgery of first diagnosis. All the blood samples were obtained after patients and healthy donors provided written informed consent to be included in the study. Genomic DNA from the blood of the patients and the controls was prepared using the Wizard genomic DNA isolation Kit (Promega).

2.2.1. Ala1170Pro SNP detection

All the DNAs were genotyped by a single base primer extension method using the SnaPShot Kit (Applied Biosystems, CA). A 228 base pair (bp) nucleotide G3658C (Ala1170Pro) SNP amplicon was PCR-amplified with the forward primer 5′ CAGAGGGAGTGGCAGAGACAC 3′ and the reverse primer 5′ CCACGGCACCCCCAA 3′. Nucleotide G3658C (Ala1170Pro) SNP was detected by the sense primer 5′ (TTTTTTTTTTTTTTTTT)CTTACGATGGGATCC and confirmed by the antisense primer 5′ (TTTTTTTTTTTTTTTTTT)GGATCTCCCGGGCT 3′.

All the data were confirmed by Real Time PCR allelic discrimination analyses.

2.2.2. Ile655Val SNP detection

All the DNAs were genotyped by a TaqMan assay on an ABI 7000 Sequence Detection System (Applied Biosystems, CA) and the allelic discrimination analyses were performed as reported elsewhere [26]. Specific primers (sense: 5′-CACCCCAAACTAGCCCTCAAT-3′ and antisense: 5′-ACCAGCAGAATGCCAACCA-3′) and probes (5′-TCCATCaTCTCTGCG-3′ and 5′-TCCATCgTCTCTGC-3′) were identified using Primer Express software (Applied Biosystems, CA) in the ERBB2 transmembrane region sequence.

2.3. BRCA1 sequencing

One-hundred patients with a family history of breast cancer were selected to detect BRCA1 mutations. DNAs were screened for mutations in the entire coding region of the BRCA1 gene. Each BRCA1 coding exon was amplified using intronic primers based on those described by Friedman et al. [11] or as reported in The Breast Cancer Information Core Electronic Database website (http://research.nhgri.nih.gov/projects/bic).

BRCA1 pre-screening was performed using dHPLC analysis (Transgenomic Inc., San Jose, CA). PCR product showing narrow deviations between the sample peak and the control peak were purified and sequenced. DNA sequencing was performed on both strands of two independent PCR products by cycle sequencing on an ABI PRISM 377 sequencer (Applied Biosystems). If a mutation was identified, another DNA aliquot from the same patient was sequenced to confirm the result.

2.4. Haplotype analysis

Haplotype distribution was studied using the Phase II version 2.1 software by Stephens M. [24; www.stat.washington.edu/stephens/phase/software.html]. Reference haplotypes were 655Ile (**A**UA) 1170Ala (**G**CG): AG.

2.5. Statistical analysis

Genotype frequencies were verified to satisfy Hardy–Weinberg equilibrium by calculation of chi-square test for deviation from the expected frequencies under the assumption of Hardy–Weinberg equilibrium.

The associations between genotype frequencies and the clinical-pathological features and family history of the patients were evaluated by chi-square or Fisher's exact tests. All p values were based on two-sided testing. Case-control studies were performed by chi square for trend test or multiple logistic regression test. All the analyses were done considering for each SNP: (a) all allele combinations separately or (b) that women with Ile655Ile and Ala1170Ala present a normal risk of developing breast cancer and that women with one or two 655Val alleles and one or two 1170Pro alleles are at a greater risk or (c) that only women homozygous for the 655Val and 1170Pro alleles are at a greater risk.

All statistical analyses were carried out with SPSS statistical software (SPSS, Inc, Chicago, IL).

2.6. Databases

ERBB2: OMIM 164870,GDB 120613, GenBank NT_010647,

BRCA1: OMIM 113705, GDB 126611, GenBank U14680,

Single Nucleotide Polymorphism Data Base: http://www.ncbi.nlm.nih.gov/SNP,
Breast Cancer Information Core: http://research.nhgri.nih.gov/projects/bic,
Phase II software: www.stat.washington.edu/stephens/phase/software.html.

3. Results

3.1. ERBB2 SNPs in breast cancer patients and in healthy people

One-hundred sixty nine healthy controls and 628 breast cancer patients were evaluable for the variant 655 allele; conversely, DNA of 4 healthy people and 3 patients were not available for the variant 1170 allele study.

As reported in Table 1, the genotypic and allelic frequencies of both SNPs were similar in the breast cancer patients and in the controls. In the patients, the 655SNP genotypic frequencies were 69% for Ile homozygosity, 29% for heterozygosity and 2% for Val homozygosity. Similar percentages were found in the healthy individuals (75%, 25% and 2%, respectively). The 1170SNP genotypic frequencies were 47% for Ala homozygosity, 42% for heterozygosity and 11% for Pro homozygosity in the patients, and 39%, 49% and 13% in healthy donors. The allelic frequencies of both SNPs showed no deviation from Hardy–Weinberg equilibrium ($p = 0.8$ and $p = 0.1$ for 655SNP in healthy controls and patients and $p = 0.6$ and $p = 0.3$ for 1170SNP in healthy controls and patients). The variant 1170 SNP allele (Pro), however, was present with a much greater frequency than the variant 655 SNP allele (Val) in both the patients and normal controls (Table 1).

Table 1
Incidence of Ile655Val and Ala1170Pro ERBB2 SNPs in Caucasian healthy controls and breast cancer patients

655 SNP $_{(n)}$			1170 SNP $_{(n)}$		
	Patients $n = 628$	Controls $n = 169$		Patients $n = 625$	Controls $n = 165$
Ile/Ile	69% $_{(433)}$	74% $_{(125)}$	Ala/Ala	47% $_{(294)}$	39% $_{(64)}$
Ile/Val	29% $_{(181)}$	24% $_{(41)}$	Ala/Pro	42% $_{(264)}$	48% $_{(80)}$
Val/Val	2% $_{(14)}$	2% $_{(3)}$	Pro/Pro	11% $_{(67)}$	13% $_{(21)}$
Allele frequencies			Allele frequencies		
Ile	84% $_{(523)}$	86% $_{(145)}$	Ala	68% $_{(426)}$	63% $_{(104)}$
Val	16% $_{(104)}$	14% $_{(23)}$	Pro	32% $_{(199)}$	37% $_{(61)}$

Hardy–Weinberg equilibrium: $p = 0.8$ and $p = 0.1$ for 655SNP in healthy controls and patients and $p = 0.6$ and $p = 0.3$ for 1170SNP in healthy controls and patients.

Considering Ile655Ile or Ala1170Ala allelotypes as reference categories, the presence of other genotypes did not associate with a higher breast cancer risk. This was also true when Val655Val or Pro1170Pro allelotypes were considered with respect to the 655Ile or 1170Ala genotypes, respectively.

The 1170 SNP allele Pro variant was more frequent than the 655 SNP allele Val variant (11% vs 2%). When the 2 SNP allelotypes were analyzed in the same patient, the only interesting feature was that the Pro1170Pro and Val655Val variants were not copresent ($p = 0.03$ by chi square). Furthermore, the haplotype distribution did not differ between the patients and the controls: the most frequent haplotype AG was present in $55 \pm 0.01\%$ of cases and $54 \pm 0.01\%$ of controls and the less frequent GC was present in $4 \pm 0.01\%$ of cases and $3 \pm 0.01\%$ of controls. This haplotype distribution did not correspond to a different breast cancer risk.

3.2. ERBB2 SNPs and family history of breast cancer

When individuals were stratified according to family characteristics, the presence of 655Val carriers or of 1170Pro carriers did not associate with a different breast cancer risk in the series with a family history of breast cancer and in the one without (data not shown). However, when the patients were stratified according to age, 655Val carriers associated with an increased breast cancer risk (OR 1.8, CI = 1.1–2.8; $p = 0.009$) but only in the subgroup of women younger than 45 years. This increased risk was even higher in women with a family history of breast cancer (OR 3.6, CI = 1.1–11; $p = 0.02$) (Table 2).

3.3. ERBB2 SNPs and tumor biology

The frequencies of the variant alleles 655Val and 1170Pro were associated with different tumor biological and pathological characteristics (Table 3).

The variant allele 655Val was more frequently associated with smaller tumors (38% in T_1 vs 28% in $T_{2\text{-}4}$, $p = 0.03$) and to invasive ductal carcinoma subtypes ($p = 0.02$). Interestingly, the 655Val allele frequency in *in situ* cases was significantly low (12%).

The variant allele 1170Pro was more frequent in ER-positive patients (>10 fmol/mg cit-proteins). The median ER value was higher in the Ile655Ile cases than

Table 2
Breast cancer risk in individuals with a family history of the disease: age stratification

Overall series	655SNP frequency $_{(n)}$			1170SNP frequency $_{(n)}$		
	Ile/Ile	Ile/Val + Val/Val	OR (95%CI)	Ala/Ala	Ala/Pro + Pro/Pro	OR (95%CI)
	353	156		236	270	
Age						
>45 yrs ($n = 397$)	81% $_{(287)}$	71% $_{(110)}$	reference	79% $_{(186)}$	77% $_{(208)}$	reference
⩽45 yrs ($n = 112$)	19% $_{(66)}$	29% $_{(46)}$	1.8 (1.15÷2.87) [a]	21% $_{(50)}$	23% $_{(62)}$	1.1 (0.71÷1.73)
Family history*						
No ($n = 432$)	84% $_{(296)}$	87% $_{(136)}$	reference	86% $_{(202)}$	84% $_{(228)}$	reference
Yes ($n = 77$)	16% $_{(57)}$	13% $_{(20)}$	0.7 (0.42÷1.36)	14% $_{(34)}$	16% $_{(42)}$	1.1 (0.65÷1.84)
Family history and age at first diagnosis						
No >45 yrs ($n = 335$)	67% $_{(236)}$	63% $_{(99)}$	reference	67% $_{(158)}$	65% $_{(175)}$	reference
No ⩽ 45 yrs ($n = 97$)	17% $_{(60)}$	24% $_{(37)}$	1.5 (0.89÷2.42)	19% $_{(44)}$	20% $_{(53)}$	1.1 (0.67÷1.76)
Yes ⩽45 yrs ($n = 15$)	2% $_{(6)}$	6% $_{(9)}$	3.6 (1.12÷11.7) [b]	2% $_{(6)}$	3% $_{(9)}$	1.3 (0.43÷4.39)
Yes > 45 yrs ($n = 62$)	14% $_{(51)}$	7% $_{(11)}$	0.5 (0.24÷1.07)	12% $_{(28)}$	12% $_{(33)}$	1.1 (0.59÷1.91)

*No = absence of family history criteria (see M&M); Yes = presence of family history criteria.
[a]Val allele frequency vs Ile homozygous frequency in patients ⩽45 yrs: $p = 0.009$; [b]Val allele frequency vs Ile homozygous frequency in patients with family history and ⩽45 yrs: $p = 0.02$.

Table 3
Clinical-pathological features of 655Val carriers and 1170Pro carriers

	655 SNP frequency $_{(n)}$			1170 SNP frequency $_{(n)}$		
	Ile/Ile	Val	p	Ala/Ala	Pro	p
	$n = 353$	$n = 156$		$n = 236$	$n = 270$	
T stage						
1 ($n = 187$)	62% $_{(116)}$	38% $_{(71)}$	0.03	46% $_{(86)}$	54% $_{(101)}$	
2–4 ($n = 272$)	72% $_{(195)}$	28% $_{(77)}$		47% $_{(128)}$	53% $_{(144)}$	
Histology						
IDC ($n = 448$)	67% $_{(302)}$	33% $_{(146)}$	0.05	47% $_{(210)}$	53% $_{(238)}$	
ILC ($n = 30$)	77% $_{(23)}$	23% $_{(7)}$		43% $_{(13)}$	57% $_{(17)}$	
CIS ($n = 26$)	88% $_{(23)}$	12% $_{(3)}$		50% $_{(13)}$	50% $_{(13)}$	
Cytohistological grading						
G_1 ($n = 71$)	68% $_{(48)}$	32% $_{(23)}$		44% $_{(31)}$	56% $_{(39)}$	
G_{2+3} ($n = 362$)	67% $_{(243)}$	33% $_{(119)}$		47% $_{(168)}$	53% $_{(193)}$	
Nodal status						
N_0 ($n = 225$)	65% $_{(147)}$	35% $_{(78)}$		48% $_{(106)}$	52% $_{(117)}$	
$N_{1\text{-}2}$ ($n = 242$)	71% $_{(173)}$	29% $_{(69)}$		46% $_{(112)}$	54% $_{(130)}$	
Hormone receptor and proliferative activity						
ER ⩽10 ($n = 140$)	71% $_{(99)}$	29% $_{(41)}$		54% $_{(76)}$	46% $_{(64)}$	0.04
>10 ($n = 349$)	68% $_{(237)}$	32% $_{(112)}$		44% $_{(153)}$	56% $_{(195)}$	
PgR ⩽10 ($n = 233$)	70% $_{(163)}$	30% $_{(67)}$		49% $_{(114)}$	51% $_{(119)}$	
>10 ($n = 256$)	68% $_{(174)}$	32% $_{(82)}$		45% $_{(115)}$	55% $_{(141)}$	
Mib1 ⩽10% ($n = 102$)	70% $_{(72)}$	30% $_{(31)}$		48% $_{(49)}$	52% $_{(53)}$	
>10% ($n = 381$)	68% $_{(260)}$	32% $_{(122)}$		47% $_{(178)}$	53% $_{(203)}$	

in the 655Val carriers (184 vs 75 fmol/mg prot respectively, $p = 0.02$).

The allelic frequency of both the 655 and the 1170 SNPs did not change according to the different cyto-histological grade of the tumor and to the presence or absence of axillary metastases.

3.4. ERBB2 SNPs and BRCA1 alterations

ERBB2 SNPs were studied in relation to BRCA1 alterations in a subset of 100 consecutive patients with a family history of breast cancer whose BRCA1 gene was completely sequenced. Seven cases presented a mutated BRCA1 (all 5382insC) while 61 presented at least one missense polymorphism (E1083G, P871L, S1613G, K1183R). The frequency of different BRCA1 polymorphisms was: E1038G in 44%, P871L in 41%, S1613G in 29% and K1183R in 11%. Most cases with BRCA1 mutation resulted heterozygous for 1170SNP (71%) and homozygous for Ile655SNP (57%); conversely, in BRCA1 negative cases 1170SNP is equally distributed between heterozygous and Ala homozygous allelotypes (42% and 47% respectively) and mostly presented Ile 655SNP homozygosity (67%).

BRCA1 mutations and polymorphisms found in the present series were not associated with the presence of a particular allele of either ERBB2 SNP. The association between ERBB2 SNPs and more frequent BRCA1 alterations (mutations and missense polymorphisms) was investigated by logistic regression analysis.

Logistic regression analysis performed using alternatively 655 and 1170 SNP as dependent variables demonstrated that the 1170Pro allele was negatively associated with the less frequent 1613Gly in BRCA1 (HR $= 0.29$, $p = 0.05$) and positively associated with the 871Leu allele (HR $= 4.95$, $p = 0.02$).

4. Discussion

The present study was mainly based on some aspects concerning ERBB2 polymorphisms and breast cancer risk.

We first analyzed the potential role of 655 and 1170 SNPs as breast cancer susceptibility markers. To this purpose a large series of breast cancer and healthy Caucasian women was examined, second in size only to Wang-Gohrke [27] experience. Our results showed that only the 655 Val allele in the subgroup of early-onset breast cancer patients with a family history of the disease was significantly associated with an increased of breast cancer risk of 3.6 folds (Table 2). We utilized the same age cutoff (45 years) of other authors [20,27,28] to distinguish younger patients. Our results are in agreement with previous reports showing a positive association of 655 Val allele with younger age [22,28] and with a family history of breast/ovarian cancer [22,27].

For what concern 1170SNP, 1170Pro allele was not associated with breast cancer risk in the overall series and in specific subgroups of breast cancer patients differing for age and family history. The only previous available data on 1170SNP, as marker of breast cancer risk, come from preliminary reports [18,29] and from two recent papers which analyzed 1170SNP within a different pattern of ERBB2 SNPs haplotype analysis [4,12]. Interestingly, the frequency of different haplotypes found in our series of Caucasian people was quite similar to the frequencies reported by Benusiglio [4]; also the lack of relationship of 1170SNP with breast cancer risk was in agreement with that Author [4]. In fact, he reported a lack of difference between cases and controls in terms of frequency of different haplotypes. The originality of our study lies in the analysis of relationship between 1170SNP and family history of breast cancer. Our results showed, for the first time, that also for this aspect 1170SNP does not seem to be related to family history or early onset of the disease.

It was already defined [4,12] that 655 and 1170 SNPs belong to the same haplotype block which cover the entire ERBB2 coding region and a few kb $5'$ and $3'$-untranslated regions [12]. Analysis of the haplotypes related to the two SNPs did not show any relationship between specific haplotype and greater breast cancer risk. This evidence further supports the results presented by Benusiglio [4] and Han [12] who reported the same lack of association between the presence of different haplotypes and the disease. Interestingly, 1170Pro homozygosity was never found co-present with 655Val homozygosity. Both these observations suggest that the presence of the 1170SNP variant may have no relation with the pathogenesis of breast cancer.

The second issue of the study focused on the relationships between the two ERBB2 SNPs and breast cancer clinical-pathological features. We found that 1170Pro carriers seemed to be associated with a higher frequency (195/270 patients) of estrogen receptor tumor positivity (Table 3), data corroborated by the role that ERBB2 has in modulating the steroid pathway [6,25].

Furthermore, we analyzed the possible relationship between the two ERBB2 SNPs and mutations or mis-

sense polymorphisms of the major breast cancer susceptibility gene, BRCA1. In the subset of 100 patients sequenced for BRCA1, no association was found with mutations of this gene. However, the 1170Pro allele seemed to be significantly associated with the presence of the BRCA1 variant 871Leu allele and the BRCA1 normal 1613Ser allele. Considering that both genes are located on the long arm of chromosome 17, we decided to further investigate this aspect with a linkage disequilibrium study, currently in progress.

In conclusion, the two considered ERBB2 polymorphisms seem to be potentially involved in several pathological functions of the cell. 655SNP may play a role in the pathogenesis of breast cancer with an early onset and presence of family history of the disease. For what concern 1170SNP, the most intriguing hypothesis regards its role in determining the response to specific anti-ERBB2 drugs.

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References

[1] M. Ameyaw, N. Thornton and H.L. McLeod, Re: Population-based, case control study of HER2 genetic polymorphism and breast cancer risk, *J. Natl. Cancer Inst.* **92** (2000), 1947.

[2] C.I. Bargmann and R.A. Weinberg, Oncogenic activation of the neu-encoded receptor protein by point mutation and deletion, *EMBO J.* **7** (1988), 2043–2052.

[3] S.W. Baxter and I.G. Campbell, Re: Population-based, case control study of HER2 genetic polymorphism and breast cancer risk, *J. Natl. Cancer Inst.* **93** (2001), 557–558.

[4] P.R. Benusiglio, F. Lesueur, C. Luccarini, D.M. Conroy, M. Shah, D.F. Easton, N.E. Day, A.M. Dunning, P.D. Phaorah and B.A.J. Ponder, Common erbb2 polymorphisms and risk of breast cancer in a white British population a case-control study, *Breast Cancer Res.* **7** (2005), R204–R209.

[5] C.S. Carlson, M.A. Eberle, M.J. Rieder, Q. Yi, L. Kruglyak and D.A. Nickerson, Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium, *Am. J. Hum. Genet.* **74** (2004), 106–120.

[6] Y.L. Chung, M.L. Sheu, S.C. Yang, C.H. Lin and S.H. Yen, Resistance to tamoxifen-induced apoptosis is associated with direct interaction between Her2/neu and cell membrane estrogen receptor in breast cancer, *Int. J. Cancer* **97** (2002), 306–312.

[7] W.C. Dougall, X. Qian and M.I. Greene, Interaction of the neu/p185 and EGF receptor tyrosine kinases: implications for cellular transformation and tumor therapy, *J. Cell Biochem.* **53** (1993), 61–73.

[8] D.M. Eccles, D.G. Evans and J. Mackay, Guidelines for a genetic risk based approach to advising women with a family history of breast cancer. UK Cancer Family Study Group (UKCFSG), *J. Med. Genet.* **37** (2000), 203–209.

[9] E.R. Fisher, C. Redmond and B. Fisher, Histological grading of breast cancer, *Pathol. Ann.* **1** (1980), 239–251.

[10] S.J. Fleishman, J. Schlessinger and N. Ben-Tal, A putative molecular activation switch in the transmembrane domain of erbb2, *Proc. Natl. Acad. Sci. USA* **99** (2002), 15937–15940.

[11] L.S. Friedman, E.A. Ostermeyer, C.I. Szabo, P. Dowd, E.D. Lynch, S.E. Rowell and M.C. King, Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families, *Nat. Genet.* **8** (1994), 390–399.

[12] W. Han, D. Kang, J.E. Lee, I.A. Park, J.-Y. Choi, K.-M. Lee, J.Y. Bae, S. Kim, E.-S. Shin, J.E. Lee, H.-J. Shin, S.W. Kim, S.-W. Kim and D.-Y. Noh, A haplotype analysis of HER-2 gene polymorphisms: association with breast cancer risk, HER-2 protein expression in the tumor and disease recurrence in Korea, *Clin. Cancer Res.* **11** (2005), 4775–4782.

[13] P. Hermanek and L.H. Sobin, *UICC TNM: Classification of Malignant Tumours*, 4th edn, John Wiley & Sons, Inc, Geneva, 1988.

[14] A. Hishida, N. Hamajima, H. Iwata, K. Matsuo, K. Hirose, N. Emi and K. Tajima, Re: Population-based, case control study of HER2 genetic polymorphism and breast cancer risk, *J. Natl. Cancer Inst.* **94** (2002), 1807–1808.

[15] C.R. King, M.H. Kraus and S.A. Aaronson, Amplification of a novel v-erbB related gene in a human mammary carcinoma, *Science* **229** (1985), 974–976.

[16] J.M. Mendrola, M.B. Berger, M.C. King and M.A. Lemmon, The single transmembrane domains of ErbB receptors self-associate in cell membranes, *J. Biol. Chem.* **277** (2002), 4704–4712.

[17] A. Paradiso, M. Bruno, M. Cicoria, M. Digennaro, S. Longo, M. Rinaldi and F. Schittulli, Analysis of the reasons for accepting or declining participation in a genetic breast cancer research: an hospital-based population study, *Tumori* **90** (2004), 435–436.

[18] A. Paradiso, S. Tommasi, V. Fedele, M. Bruno, A. Crapolicchio, F. Schittulli, D. Ginzinger, G. Scott, S. Eppenberger-Castori, U. Eppenberger and C.C. Benz, ErbB2 polymorphism frequency and breast cancer risk, *Proc. AACR* "SNPs, haplotypes and cancer: applications in molecular epidemiology" **A22** (2003).

[19] A. Paradiso, S. Volpe, A. Iacobacci, E. Marubini, P. Verderio, A. Costa, M.G. Daidone, A. Marchetti, M. Mottolese, D. Amadori, F. De Paola, L. Saragoni, L. Medri, I. Nenci, P. Querzoli, M. Gion, R. Dittami, M. Plebani, C. Orlando, G. Bevilacqua and R. Silvestrini, Quality control for biomarker determination in oncology: the experience of the Italian Network for Quality Assessment of Tumor Biomarker (INQAT), *Int. J Biol. Marker* **17** (2002), 201–214.

[20] J.L. Pinto, A. Vasconcelos, S. Costa, D. Pereira, R.H. Odrigues, C. Lopes and R. Medeiros, ERBB2 polymorphism and brest cancer risk in Portugal, *J. Eur. Cancer Prev.* **13** (2004), 177–181.

[21] N.J. Risch, Searching for genetic determinants in the new millennium, *Nature* **405** (2000), 847–856.

[22] J.L. Rutter, N. Chatterjee, S. Wacholder and J. Struewing, The HER2 I655V polymorphism and breast cancer risk in Ashkenazim, *Epidemiology* **14** (2003), 694–700.

[23] D.J. Slamon, W. Godolphin, L.A. Jones, J.A. Holt, S.G. Wong, D.E. Keith, W.J. Levin, S.G. Stuart, J. Udove, A. Ullrich and M. Press, Studies of the ERBB2/neu protooncogene in human breast and ovarian cancer, *Science* **244** (1989), 707–712.

[24] M. Stephens, N.J. Smith and P. Donnelly, A new statistical method for haplotype reconstruction from population data, *Am. J. Hum. Genet.* **68** (2001), 978–989.

[25] S. Tommasi, V. Fedele, A. Crapolicchio, A. Bellizzi, A. Paradiso and S.J. Reshkin, ERBB2 and the antimetastatic nm23/NDP kinase in regulating serum induced breast cancer invasion, *Int. J. Mol. Med.* **12** (2003), 131–135.

[26] S. Tommasi, V. Fedele, R. Lacalamita, A. Crapolicchio, E. Perlino, A. Bellizzi and A. Paradiso, Molecular and functional characteristics of ERBB2 in normal and cancer breast cells, *Cancer Lett.* **209** (2004), 215–222.

[27] S. Wang-Gohrke and J. Chang-Claude, Re Population-based, case control study of HER2 genetic polymorphism and breast cancer risk, *J. Natl. Cancer Inst.* **93** (2001), 1657–1659.

[28] D. Xie, X.O. Shu, Z. Deng, W.Q. Wen, K.E. Creek, Q. Dai, Y.T. Gao, F. Jin and W. Zheng, Population-based, case control study of HER2 genetic polymorphism and breast cancer risk, *J. Natl. Cancer Inst.* **92** (2000), 412–417.

[29] F. Xu, D. Ginzinger, Y. Bauke, S. Eppenberger-Castori, U. Eppenberger, D. Albertson and C.C. Benz, A single nucleotide polymorphism (SNP), within the coding region of ERBB2 occurs with increased frequency in ErbB2-positive breast cancer, *Proc. AACR* **43** (2002), 1018, #5041.