*Breast Cancer Research and Treatment* **80:** 145–154, 2003.


*Report*

# Genetic polymorphisms of platelet adhesive molecules: association with breast cancer risk and clinical presentation

Francisco Ayala[1], Javier Corral[2], Rocío González-Conejero[2], Ignacio Sánchez[2], José María Moraleda[1], and Vicente Vicente[1]
[1]*Department of Internal Medicine, Hematology and Medical Oncology Unit, Hospital Morales Meseguer;* [2]*Hematology and Medical Oncology Unit, Centro Regional de Hemodonación, University of Murcia, Murcia, Spain*

*Key words:* breast cancer, genetic polymorphism, glycoprotein Ibα, integrin α2, integrin β3

**Summary**

The main platelet adhesive receptors integrin α2β1, integrin αIIbβ3 and glycoprotein (GP) Ibα are also expressed in breast carcinoma cells. They play a key role in tumor cell-induced platelet aggregation and in adhesive interactions necessary for tumoral invasion and metastasis. Several polymorphisms affecting these molecules, two in integrin α2 (C807T and G1648A), one in integrin β3 (T1565C) and one in GP Ibα (VNTR), influencing their levels, structure, and possibly their function, have been previously described and associated with cardiovascular diseases. In this study, we investigated the association of these polymorphisms with breast cancer risk or clinical presentation. We studied 101 patients with invasive breast cancer. The main prognostic variables were recorded, and genomic PCR analysis of these polymorphisms was performed. A group of 101 control subjects matched on age and sex was studied and compared with patients. No association was found between VNTR (GP Ibα) polymorphism and breast cancer risk or presentation. Genotype and allele frequencies of C807T and G1648A polymorphisms of integrin α2 were not statistically different in breast cancer patients and controls, although we found an association between the 1648G/G genotype and higher disease stages (III and IV) ($p = 0.02$). Breast cancer risk was higher in carriers of β3 integrin T/T genotype (OR = 2.08, 95% CI = 1.04–4.16, $p = 0.04$). Furthermore, genotype 1565T/T was also associated with axillary nodal metastasis ($p = 0.017$) and with tumoral diameter greater than 2 cm ($p = 0.02$). Although confirmatory studies are needed, our results suggest that polymorphic genetic variation of integrins expressed in platelets and epithelial breast cells could modify the risk and the biological aggressiveness of breast carcinomas.

## Introduction

Breast cancer is one of the leading mortality causes in women from western countries. Despite great advances in its diagnosis and treatment, prognostic and predictive factors have essentially not changed in the last 20 years. Recent works have tried to identify new molecular markers of biologic behavior or response to treatment, being the *Her2neu* overexpression the best example [1]. Prevention and prophylaxis of breast cancer have also been obstructed by the lack of clear markers of individual risk. Thus, a large proportion of cases cannot be attributed to any known risk factor.

Implication of platelets in tumoral biology, specially in formation of metastasis, has been recognized previously [2]. Tumor cell induced platelet aggregation is mediated by integrin αIIbβ3 and it exerts profound effects in the main steps of metastatic cascade: adherence of tumoral cells to endothelium, extravasation and angiogenesis [3, 4]. Furthermore, some platelet receptors, specially integrins α2β1 and αIIbβ3 and glycoprotein Ibα, are also expressed by epithelial cells and their level of expression in tumoral cells is associated with motility, invasiveness and cellular differentiation [5–8]. These biologic differences might explain clinical and pathologic correlation between

expression of these two integrins and breast cancer histologic grade and axillary nodal metastases [9–11].

Genetic polymorphisms are responsible of interindividual variation and diversity. Thus, they have been recently considered as the main genetic elements involved in the development of common and complex diseases. In this context, functional polymorphisms affecting integrin α2β1, integrin αIIbβ3, GP Ibα and other platelet proteins have recently been involved in the risk of thromboembolic diseases [12]. Integrin α2 polymorphisms C807T and G1648A associate to different levels of expression of the whole α2β1 molecule in the platelet surface, determining a variable adhesion to collagen. Thus, 807T and 1648A alleles associate with increased expression of this integrin in platelets and other cells [13, 14]. Furthermore, the G1648A polymorphism induces a structural change (Glu-Lys substitution in position 505 of α2 chain) with potential relevance for its functional activity [15]. Genetic linkage between both α2 polymorphisms has been described by our group and others [14]. The β3 T1565C polymorphism determines a Leu to Pro substitution at position 33, although its functional role in platelets is controversial [16, 17]. Finally, the variable number of tandem repeats (VNTR) in the macroglycopeptide region of the GP Ibα molecule is a polymorphism with major influence in the size of the molecule, determining the distance between the ligand-binding domain and the platelet surface. Four VNTR alleles have been identified. The longest, A with four repeats, to the smallest D with one repeats [18].

Our hypothesis was that functional polymorphisms (associated with variations in the structure or expression of the molecule) affecting integrin α2β1, αIIbβ3, and GP Ibα (molecules expressed in platelets and breast cancer cells, with potential role in tumoral biology) could influence different breast cancer risk or biological behavior. In order to answer this question, we compared the prevalence of G1648A, C807T, T1565C and VNTR genotypes in a group of breast cancer patients and control subjects matched by age, sex and race. We also examined the relationship between the different genotypes and the clinical presentation of breast cancer.

## Methods

### *Subjects*

The study involved 101 consecutive Caucasian patients with histologically confirmed diagnosis of

*Table 1.* Demographic, clinical and pathological characteristics of breast cancer patients at diagnosis

| Characteristic | Breast cancer patients, No. (%) ($N = 101$) |
|---|---|
| Age at diagnosis, years, median (range) | 44 (25–76) |
| Hormonal status | |
| Premenopausal | 70 (69.3) |
| Postmenopausal | 31 (30.7) |
| Family history | |
| Positive | 14 (13.8) |
| Negative | 82 (81.2) |
| Unknown | 5 (5.0) |
| Histologic subtype | |
| Ductal | 84 (83.2) |
| Lobular | 13 (12.9) |
| Other | 5 (4.9) |
| TNM stage | |
| pT0 | 2 (2.0) |
| pT1 | 30 (29.7) |
| pT2 | 46 (45.5) |
| pT3 | 15 (14.8) |
| pT4 | 5 (5.0) |
| Tx | 3 (3.0) |
| PN0 | 15 (14.8) |
| pN1 | 60 (59.4) |
| pN2 | 23 (22.8) |
| Nx | 3 (3.0) |
| M0 | 96 (95.0) |
| M1 | 5 (5.0) |
| Stage at diagnosis | |
| I | 9 (8.9) |
| II | 51 (50.5) |
| III | 32 (31.6) |
| IV | 5 (5.0) |
| Undetermined | 4 (4.0) |
| Tumoral diameter, cm, mean (range) | 3 (0.8–13) |
| Number of axillary nodes involved, median (range) | 8 (0–44) |
| 0 | 14 (13.8) |
| 1–4 | 21 (20.8) |
| 5–9 | 24 (23.8) |
| 10 or more | 38 (37.6) |
| Undetermined | 4 (4.0) |
| Histologic grade | |
| Grade I | 12 (11.9) |
| Grade II | 34 (33.7) |
| Grade III | 33 (32.7) |
| Unknown | 22 (21.7) |

*Table 1.* (*continued*)

| Characteristic | Breast cancer patients No. (%) ($N = 101$) |
|---|---|
| Estrogen receptors | |
| Positive | 35 (34.7) |
| Negative | 16 (15.8) |
| Unknown | 50 (49.5) |

invasive breast carcinoma, who were referred for treatment to the Hematology and Medical Oncology Unit at the University General Hospital of Murcia (Spain). Characteristics of patients (Table 1) were extracted from medical records and original pathology reports. Age of patients at diagnosis ranged from 25 to 76 years (median, 44 years). Most tumors (83%) were invasive ductal carcinomas. We evaluated family history (positive if at least one first or second-degree relative affected), tumoral diameter, number of axillary nodes involved, histologic grade, and immuno-histochemically determined hormonal receptor expression. pTNM classification and stage grouping are indicated in accordance with AJCC system for breast carcinoma [19].

A group of 101 age-matched Caucasian non-related women with no history of cancer, most of them blood donors, was recruited as control group. The genotype and allele frequencies of α2, β3 and GP Ibα polymorphism in the general population of our region was evaluated in 512, 341 and 610 healthy subjects, respectively.

Patients and controls were fully informed of the aim of this study, which was performed according with the declaration of Helsinki as amended in Edinburgh 2000. Written informed consent was obtained and the study was approved by the local ethics committee.

### *DNA purification*

Blood samples were collected by venipuncture into tubes coated with EDTA. Total genomic DNA was obtained by standard procedure [20].

### *Genetic determination of the α2 C807T polymorphism*

Genomic polymerase chain reaction (PCR) of the α2 exon/intron 7 was performed essentially as described elsewhere [14]. Briefly, we used two oligonucleotide primers: 5′gatttaactttcccagctgccttc3′ and the mutagenic 5′atggtggggacctcacaaacag**g**att3′, corresponding to nucleotides 173–196 of the intron sequence, and 782–806 of the exon 7 sequence, respectively (nucleotide number according to Takada and Hemler [21]). The mutated guanine in the forward primer (bold cursive) allows the identification of the C807T polymorphism of the α2 gene by restriction of the PCR product (3 μl) with 1 U of Hinf I (New England BioLabs, Beverly, MA) at 37°C for 3 h. The restriction pattern was analyzed in a 5% acrylamide gel, run at 300 V for 15 min and stained with $AgNO_3$ as reported [22]. The 807C allele of the α2 gene corresponded to a band pattern after Hinf I restriction of 209 base pairs (bp), whereas the presence of a 231 bp band was distinctive of the 807T allele (Figure 1(A)).

### *Genetic determination of the α2 G1648A polymorphism*

Amplification of the α2 genomic 274 bp fragment, which contains position 1648, was performed as previously indicated [23], with minor modifications. The PCR product (3 μl) was digested with 1 U of Mnl I (New England BioLabs) at 37°C for 3 h, and the restriction pattern analyzed in acrylamide gels stained as indicated before. The 1648G allele displayed a pattern of four bands of 136, 97, 33 and 8 bp, whereas the presence of three bands (169, 97, and 8 bp) was distinctive of the 1648A allele (Figure 1(B)).

### *Genetic determination of the β3 T1565C polymorphism*

PCR amplification of the β3 exon 2 was performed as previously described [24]. The T1565C genotype was determined by restriction analysis of the PCR product, using 1 U of Msp I at 37°C during 5 h. Allele 1565T displayed a two bands pattern (227 and 45 bp), while allele 1565C produced three bands (50, 177 and 45 bp) (Figure 1(C)).

### *Genetic determination of the GP Ibα VNTR polymorphism*

Two nucleotides VNTR-F3: 5′cactactgaaccaacccca agc3′, and VNTR-B4: 5′cttgtggcagacaccaggatgg3′ (modified from Ishida) [25], were used for PCR amplification of the GP Ibα macroglycopeptide region. Identification of alleles B (276 bp), C (237 bp) and

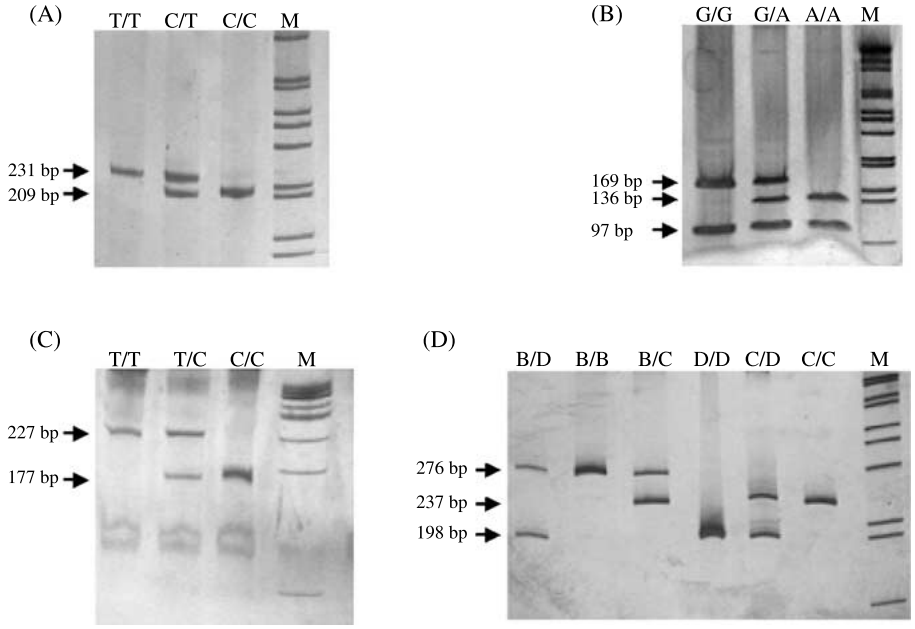


*Figure 1.* Representative genotypes of the studied polymorphisms. (A) Integrin α2 C807T. (B) Integrin α2 G1648A. (C) Integrin β3 T1565C. (D) GP Ibα VNTR. M: molecular weight marker (1 kb ladder GIBCO-BRL).

D (198 bp) was directly done after electrophoresis of the PCR product in acrylamide gels and staining with $AgNO_3$. No alleles A were observed (Figure 1(D)).

### *Statistical analyses*

Statistical analysis was made with the NCSS 6.0.9 software package. Descriptive statistics are expressed as proportions, ranges and medians. Genotype and allele frequencies were compared between cases and controls with the $\chi^2$ test. Odds ratios (OR) with 95% confidence intervals (CI) were estimated by the use of logistic regression analysis.

Regarding analysis of variables in breast cancer patients, comparison of genotype and allele proportions in $2 \times 2$ tables were made with non-parametric methods, using two-sided Fisher's exact test and/or Kruskal–Wallis $\chi^2$ statistics. Stratified analysis was performed to establish genotype influence on breast cancer characteristics by age at diagnosis (before or after the median age of the group, i.e., $<45$ or $\geq 45$ years) and by family history. Analysis of variance was used for the comparison of quantitative variables between groups of breast cancer patients with different genotypes. Differences were considered statistically significant when two-sided $p \leq 0.05$.

## Results

### *Frequency of polymorphisms in breast cancer patients and control subjects*

#### *Integrin α2*

The genotype and allele frequencies for α2 integrin C807T and G1648A polymorphisms in the 101 breast cancer patients, their age- and sex-matched controls, and normal population are shown in Table 2.

The distribution of genotypes and allele frequencies of these two polymorphisms was similar between patients and matched controls. Moreover, these results were comparable with that observed in the general population from our region (Table 2), which was in agreement with the genotype and allele frequencies described in other Caucasian populations [13, 15, 26]. Results were very similar and also not significant when patients and controls were stratified by age (data not shown). Combined C807T and G1648A analysis also demonstrated equal distribution of allele and genotype frequencies in cases and controls (Table 2).

#### *Integrin β3*

Genotype and allele frequencies of the integrin β3 T1565C polymorphism in the control group and in

*Table 2.* Integrin α2 (C807T, G1648A), integrin β3 (T1565C) and GP Ibα (VNTR) polymorphism distribution in breast cancer patients and control subjects

| | Breast cancer patients (%) | Control subjects (%) | $p^*$ | General population (%) |
|---|---|---|---|---|
| C807T polymorphism | $N = 101$ | $N = 101$ | | $N = 512$ |
| Genotype | | | | |
| C/C | 38.6 | 43.6 | 0.47 | 43.5 |
| C/T | 52.5 | 43.6 | 0.20 | 43.2 |
| T/T | 8.9 | 12.8 | 0.36 | 13.3 |
| Allele | | | | |
| C | 0.649 | 0.653 | 0.92 | 0.651 |
| T | 0.351 | 0.347 | | 0.349 |
| G1648A polymorphism | $N = 101$ | $N = 101$ | | $N = 512$ |
| Genotype | | | | |
| G/G | 73.3 | 72.2 | 0.87 | 77.9 |
| G/A | 26.7 | 24.8 | 0.75 | 20.4 |
| A/A | 0 | 3 | 0.25 | 1.7 |
| Allele | | | | |
| G | 0.866 | 0.847 | 0.57 | 0.881 |
| A | 0.134 | 0.153 | | 0.119 |
| T1565C polymorphism | $N = 100$ | $N = 100$ | | $N = 341$ |
| Genotype | | | | |
| T/T | 80.0 | 67.3 | **0.041** | 69.1 |
| T/C | 19.0 | 32.7 | **0.026** | 29.1 |
| C/C | 1.0 | 0 | – | 1.8 |
| Allele | | | | |
| T | 0.890 | 0.840 | 0.063 | 0.840 |
| C | 0.110 | 0.160 | | 0.160 |
| VNTR polymorphism | $N = 99$ | $N = 101$ | | $N = 610$ |
| Genotype | | | | |
| C/C | 52.5 | 64.3 | 0.089 | 64.4 |
| C/B | 28.3 | 17.8 | 0.078 | 14.6 |
| C/D | 15.2 | 11.9 | | 16.3 |
| B/B | 3.0 | 1.0 | | 1.4 |
| D/D | 1.0 | 2.0 | | 1.1 |
| B/D | 0 | 3.0 | | 2.2 |
| Allele | | | | |
| B | 0.171 | 0.114 | 0.115 | 0.098 |
| C | 0.742 | 0.792 | 0.350 | 0.798 |
| D | 0.086 | 0.094 | 0.774 | 0.103 |

* $\chi^2$ test was used to compare the distribution of a particular genotype or haplotype among breast cancer patients and controls.

the general population were within the range of those reported in other Caucasian populations [16, 26] (Table 2). However, the distribution of genotypes in breast cancer patients differed significantly from that observed in control subjects (Table 2). Thus, according with our results, carriers of β3 integrin 1565T/T genotype had an increased risk to develop breast cancer (OR = 2.08, 95% CI = 1.04–4.16, $p = 0.04$).

*Table 3.* Association of clinical and pathological variables of breast cancer and α2, β3 and GP Ibα genetic polymorphisms

| | *p*-Value ($\chi^2$) | | | | |
|---|---|---|---|---|---|
| | Integrin α2 C807T | Integrin α2 G1648A | Combined α2 C807T and G1648A | Integrin β3 T1565C | GP Ibα VNTR |
| Age | 0.779 | 0.269 | 0.220 | 0.680 | 0.847 |
| Menopausal status | 0.803 | 0.728 | 0.946 | 0.330 | 0.456 |
| Family history | 0.256 | 0.316 | 0.118 | 0.450 | 0.400 |
| pT | 0.773 | 0.159 | 0.475 | **0.060** | 0.312 |
| pN | 0.824 | 0.180 | 0.347 | 0.290 | 0.542 |
| Axillary node involvement | 0.700 | 0.530 | 0.881 | **0.017** | 0.454 |
| $T > 2$ cm | 0.362 | 0.174 | 0.348 | **0.002** | 0.837 |
| No. of positive axillary nodes | 0.746 | 0.376 | 0.767 | 0.230 | 0.676 |
| Histologic grade | 0.850 | 0.526 | 0.130 | 0.09 | 0.653 |
| Stage (I–II v.s. III–IV) | 0.669 | **0.064** | 0.253 | 0.850 | 0.419 |
| Estrogen receptors | 0.330 | 0.128 | 0.382 | 1 | 0.762 |

*GP Ibα*

Distribution of allele and genotype frequencies of VNTR polymorphism did not show any significant difference between breast cancer patients and control subjects (Table 2). These values were similar to those identified in the general population (Table 2).

*Polymorphisms and clinical presentation of breast cancer*

*Integrin α2*

Analyses undertaken to evaluate the association between α2 integrin genotypes and clinical characteristics of breast cancer at diagnosis were negative for the C807T polymorphism. As detailed in Table 3, we did not find any association of C807T genotypes with the main prognostic variables of breast cancer patients. Stratified analysis by family history and age at diagnosis did not demonstrate any differences between different genotype groups (data not shown).

We did not observe any difference between 1648G/G and 1648G/A patients when considering the age at diagnosis ($p = 0.27$), menopausal status ($p = 0.73$), histologic grade ($p = 0.53$), familiar history ($p = 0.32$), positive estrogen receptors ($p = 0.13$), pT ($p = 0.18$), pN ($p = 0.18$) or number of axillary nodes involved ($p = 0.37$) (Table 3). Interestingly, the percentage of patients with stages III–IV was higher among women with 1648G/G genotype than with 1648G/A genotype, although this difference did not reach statistical significance ($p = 0.07$). However, we observed significant differences in breast cancer stages according to the G1648A genotypes when patients were stratified by age at diagnosis and family history. As shown in Table 4, the 1648G/G genotype was associated with a significant higher risk of stages III–IV in women with breast cancer diagnosed after age 45 (OR = 6.5, 95% CI = 1.27–33.25, $p = 0.02$). We also observed this association in patients without a positive family history of breast cancer (OR = 4.0, 95% CI = 1.04–15.13, $p = 0.04$). As expected, the risk was higher in patients diagnosed after age 45 without familiar antecedents (OR = 6.76, 95% CI = 1.27–36.05, $p = 0.02$).

We did not observe significant differences when we analyzed the effect of combined C807T and G1648A genotype in different characteristics of breast cancer at diagnosis: age ($p = 0.22$), menopausal state ($p = 0.95$), family history ($p = 0.12$), pT ($p = 0.47$), pN ($p = 0.35$), number of axillary nodes involved ($p = 0.77$), tumoral diameter ($p = 0.34$), histologic grade ($p = 0.13$) or stage ($p = 0.25$) (Table 3).

*Integrin β3*

T1565C genotypes of β3 integrin did not show association with age ($p = 0.68$), menopausal state ($p = 0.33$), familiar history ($p = 0.45$), pN ($p = 0.29$), number of axillary nodes involved ($p = 0.23$), stage ($p = 0.85$), hormonal receptor status ($p = 0.85$), or the histologic grade ($p = 0.09$) (Table 3). However, genotype 1565T/T was more frequent among patients with axillary nodal metastasis ($p = 0.017$)

*Table 4.* Relationship between G1648A genotype and stage in breast cancer patients

| Stage[a] | No family history[b,c] | | Positive family history[c] | | All patients[c] | |
|---|---|---|---|---|---|---|
| | G/G | G/A | G/G | G/A | G/G | G/A |
| Earlier age at diagnosis (<45 years) | | | | | | |
| I–II | 21 (84%) | 4 (16%) | 1 (25%) | 3 (75%) | 23 (76.7%) | 7 (23.3%) |
| III–IV | 11 (91.7%) | 1 (8.3%) | 3 (75%) | 1 (25%) | 14 (77.8%) | 4 (22.2%) |
| | $p = 0.65$ | | $p = 0.48$ | | $p = 1$ | |
| Later age at diagnosis (≥45 years) | | | | | | |
| I–II | 13 (54.2%) | 11 (45.8%) | 3 (75%) | 1 (25%) | 15 (56.7%) | 13 (43.3%) |
| III–IV | 16 (88.9%) | 2 (11.1%) | 1 (100%) | 0 | 17 (89.5%) | 2 (10.5%) |
| | $p = 0.02$ | | $p = 1$ | | $p = 0.02$ | |
| Any age at diagnosis | | | | | | |
| I–II | 34 (69.4%) | 15 (30.6%) | 4 (50%) | 4 (50%) | 40 (66.7%) | 20 (33.3%) |
| III–IV | 27 (90%) | 3 (10%) | 4 (80%) | 1 (20%) | 31 (83.8%) | 6 (16.2%) |
| | $p = 0.04$ | | $p = 0.56$ | | $p = 0.09$ | |

[a] Stage according to American Joint Commission on Cancer classification. Stage undetermined for four patients.
[b] Family history positive if at least one first or second-degree relative affected. Family history missing for five patients.
[c] Numbers are number of patients and percents are the proportion of each stage in each genotype. Statistical comparisons are made by two-tailed Fisher's exact Test.

and patients with a tumoral diameter greater than 2 cm ($p = 0.002$) (Table 3). After stratification by age at time of diagnosis, we detected that these associations were restricted only to the group of patients ≥45 years (OR = 4.05, 95% CI = 1.21–13.60, $p = 0.01$ for nodal metastasis, and OR = 4.57, 95% CI = 1.63–12.8, $p = 0.005$ for tumoral diameter).

*GP Ibα*

We did not find any association between the previously enumerated clinical and pathological characteristics of breast cancer patients and the genotype and haplotype for the VNTR polymorphism of the GP Ibα (Table 3).

## Discussion

Several genetic polymorphisms, most of them of carcinogen or steroid metabolism genes, have been associated with breast cancer risk or prognosis [27]. Our hypothesis was that polymorphisms affecting adhesive molecules could have a role in breast cancer. This hypothesis is supported by data about participation of the main platelet adhesive molecules (integrin α2β1, integrin αIIbβ3, and GP Ibα), specially integrins, in cellular adhesion mechanisms and in tumoral progression [28, 29]. The integrin α2β1, the major integrin expressed by breast luminal cells, plays an important role in the morphogenesis of normal mammary tissue and in human mammary epithelium [30, 31]. In breast carcinomas, down-regulation of α2-integrin expression has been related to metastatic behavior and to loss of adhesive properties. Moreover, a significant decrease of α2 expression has been observed mainly in carcinomas of high-grade or associated with axillary node metastasis [7, 9, 10]. In addition, re-expression of the α2β1 integrin in a poorly differentiated breast carcinoma cell line diminishes '*in vivo*' tumorigenicity and restores both non-malignant phenotype and ability to normal morphogenesis [32]. Integrin αIIbβ3 is also expressed in breast cancer and other neoplasms. Its role in tumoral biology, specially in formation of metastasis, has been suggested to be associated with its function in platelet-mediated tumor cell adhesion to the extracellular matrix, a key step in metastatic cascade [3–5, 33]. Finally, GP Ibα also participates in tumor cell induced platelet aggregation [34], and its level of expression in breast carcinomas associates to higher stages and to nodal and distant metastasis [11]. Therefore, polymorphic genetic variation affecting the expression or function of these adhesive receptors could modify the risk of development and the biological aggressiveness of breast carcinomas.

According to our case-control study, we observed no differences in genotype or allele distribution of two α2 polymorphisms between breast cancer patients and healthy controls. Therefore, although our study was neither population-based nor stratified by other risk

factors, α2 polymorphisms do not seem to have a role in the etiology of breast cancer. However, we detected a significant association between the 1648G/G genotype and higher stages at diagnosis. This genotype has been correlated with reduced expression of this integrin in platelets and other cells [13, 14, 24]. If this polymorphism plays a similar role in breast epithelial cells, low number of α2β1 molecules in breast cancer cells might associate with decreased adhesion to collagen, and thus with increased metastatic behavior, explaining our results. However, we did not find any association between the C807T polymorphism (a polymorphism with stronger influence in platelet expression of α2β1 [13, 14]), and the biological aggressiveness of breast carcinomas. In order to explain these differences, we could speculate that both polymorphisms could have a different role in the regulation of α2-integrin expression in epithelial cells, specially in tumor cells. Certainly, regulation of α2 expression in epithelial cells could be different than in platelets, accordingly to the existence of epithelial-specific regions in the α2 gene promoter [35]. Recently, it has been described a new polymorphism (C-52T) in the core promoter of α2 integrin gene, probably implicated in transcriptional regulation [36]. Moreover, this polymorphism disrupts a Sp1 site (of paramount importance for c-erbB2 effect on α2 transcription) [37, 38]. It is very important to point out that the -52T allele seems to be in linkage disequilibrium with the 1648A allele [36]. Thus, patients with breast cancer, specially those cases expressing c-erbB2, who carry the 1648G/G genotype might have high stage tumors because of the low expression of α2β1 determined by the C-52T polymorphism. It is crucial to investigate the role of C807T and G1648A polymorphisms as determinants of α2β1 levels in normal and malignant epithelial cells, and the relevance of the new C-52T polymorphism in breast cancer. Alternatively, the role of the 1648 polymorphism in breast cancer could be explained by the structural change associated with this polymorphism. Binding of collagen to integrin α2β1 requires divalent cations ($Mg^{2-}$) and the substitution of a Glu by a Lys in position 505 of α2-chain is located between two of the three extracytoplasmic domains involved in cation binding [39]. Thus, this missense change could influence the epithelial-cell adhesion to collagen or laminin of the extracellular matrix or cell–cell adhesive interactions and, consequently, the invasive and metastatic behavior of malignant cells.

Regarding the T1565C polymorphism affecting the β3 chain, our study shows that 1565T/T genotype is significantly associated with higher breast cancer risk. Moreover, this genotype also associates with higher tumoral diameters and with axillary nodal metastasis. These results could be explained by the functional consequences of the Leu33Pro change in αIIbβ3-mediated tumor cell induced platelet aggregation. However, this is unlikely, because functional studies performed in platelets suggest that the genotype T/T has minor effect in platelet function, or results in slightly reduced platelet reactivity [11, 40, 41]. Therefore, it is possible that the importance of this polymorphism in breast cancer could be related with other roles of the αIIbβ3 integrin, specially in epithelial cells. Alternatively, this missense change affecting the β3 chain could also have relevance in the function of the αvβ3 integrin, of critical importance for angiogenesis and whose activation status has been recently associated with biological aggressiveness of breast carcinomas [42, 43].

The lack of impact of GP Ibα VNTR polymorphism in breast cancer risk or clinical presentation could be explained because this protein has a minor role in tumor cell induced platelet aggregation. Moreover, this polymorphism has minor influence in the expression of GP Ibα, at least in platelets [18].

In the interpretation of our results we also must take in account two considerations: First, the hospital-based approach of this study has lead to an overrepresentation of high-risk patients and earlier age cases. As the influence of α2-integrin 1648G/G and of β3-integrin 1565T/T genotypes was statistically significant in women with later age diagnoses, we could suppose that their influence would be larger for a group with a more representative age distribution. Second, the small size of our sample and other potential confounding environmental risk factors may have influenced our results. Therefore, further studies, population-based and including a higher number of patients, should be performed to definitely clarify the role of α2 and β3 polymorphisms in breast cancer biology.

In summary, we report the association between two integrin polymorphisms and clinical presentation of breast cancer. We also describe a higher risk of breast cancer in women carrying the integrin β3 1565T/T genotype. To the best of our knowledge, this is the first study showing the relationship between a genetic polymorphism of adhesive proteins and behavior of breast cancer or other neoplasms. Our data suggest that these polymorphisms could to be involved in the etiology and progression of breast cancer. These results

might have potential clinical and therapeutical implications, including a better prognostic characterization of patients, and the selection of higher risk groups of women for screening or prophylaxis purposes.

## Acknowledgements

We are grateful to the staff of the Unit of Hematology and Medical Oncology for their assistance in sample collection and in the preparation of this paper. The study has been supported by FIS 00/0328. JC and RG-C are Senior Scientist of the University of Murcia (Programa Ramón y Cajal).

## References

1. Hung M, Lau Y: Basic science of HER-2/*neu*: a review. Semin Oncol 26(S12): S51–S59, 1999
2. Honn KV, Tang DG, Chen YQ: Platelets and cancer metastasis: more than an epiphenomenon. Semin Thromb Hemost 18: 392–415, 1992
3. Nierodzik ML, Klepfish A, Kaspatkin S: Role of the platelets, thrombin, integrin IIb–IIIa, fibronectin and von Willebrand Factor on tumor adhesion *in vitro* and metastasis *in vivo*. Thromb Haemost 74: 282–290, 1995
4. Dardik R, Kaufmann Y, Savion N, Rosenberg N, Shenkman B, Varon D: Platelets mediate tumor cell adhesion to the subendothelium under flow conditions: involvement of platelet GPIIb–IIIa and tumor cell alpha (v) integrins. Int J Cancer 70: 201–207, 1997
5. Oleksowicz L, Mrowiec Z, Schwartz E, Khorshidi M, Dutcher JP, Puszkin E: Characterization of tumor-induced platelet aggregation: the role of immunorelated GPIb and GPIIb/IIIa expression by MCF-7 breast cancer cells. Thromb Res 79: 261–274, 1995
6. Zutter MM, Santoro SA: Widespread histologic distribution of the $\alpha_2\beta_1$ integrin cell-surface collagen receptor. Am J Pathol 137: 113–120, 1990
7. Gui GPH, Puddefoot JR, Vinson GP, Wells CA, Carpenter R: Altered cell–matrix contact: a prerequisite for breast cancer metastasis? Br J Cancer 75: 623–633, 1997
8. Grossi IM, Hatfield JS, Fitzgerald LA, Newcombe M, Taylor JD, Honn KV: Role of tumor cell glycoproteins inmunologically related to glycoproteins Ib and IIb/IIIa in tumor cell–platelet and tumor cell–matrix interactions. FASEB J 2: 2385–2395, 1988
9. Zutter MM, Krigman HR, Santoro SA: Altered integrin expression in adenocarcinoma of the breast. Analysis by *in situ* hybridization. Am J Pathol 142: 1439–1448, 1993
10. Gui GPH, Wells CA, Yeomans P, Jordan SE, Vinson GP, Carpenter R: Integrin expression in breast cancer cytology: a novel predictor of axillary metastasis. Eur J Surg Oncol 22: 254–258, 1996
11. Oleksowicz L, Bhagwati N, Fernández MD, Seno R, Etkind P: Prognostic significance of platelet inmunorelated GPIb expression in breast cancer. Cancer J Sci Am 4: 247–253, 1998
12. Bray PF: Platelet glycoprotein polymorphisms as risk factors for thrombosis. Curr Opin Hematol 7: 284–289, 2000
13. Kritzik M, Savage B, Nugent DJ, Santoso S, Ruggeri ZM, Kunicki TJ: Nucleotide polymorphisms in the $\alpha_2$ gene define multiple alleles that are associated with differences in platelet $\alpha_2\beta_1$ density. Blood 92: 2382–2388, 1998
14. Corral J, Rivera J, González-Conejero R, Vicente V: The platelet GP Ia receptor density associates with the genetically linked 807 C/T and HPA-5 polymorphisms. Transfusion 39: 372–378, 1999
15. Simsek S, Gallardo D, Ribera A, von dem Borne AEG: The human platelet alloantigens, HPA-5 (a+, b−) and HPA-5 (a−, b+), are associated with a $Glu^{505}/Lys^{505}$ polymorphism of glycoprotein Ia (the $\alpha_2$ subunit of VLA-2). Br J Hematol 86: 671–674, 1994
16. Weiss EJ, Bray PF, Tayback M, Schulman SP, Kickler TS, Becker LC, Weiss JL, Gerstenblith G, Goldschmitt-Clermont PJ: A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. N Engl J Med 334: 1090–1094, 1996
17. Di Castelnuovo A, de Gaetano G, Donati MB, Iacoviello L: Platelet glycoprotein receptor IIIa polymorphism $PI^{A1}/PI^{A2}$ and coronary risk: a meta-analysis. Thromb Haemost 85: 626–633, 2001
18. González-Conejero R, Lozano ML, Rivera J, Corral J, Iniesta JA, Moraleda JM, Vicente V: Polymorphisms of platelet membrane glycoprotein Ibα associated with arterial thrombotic disease. Blood 92: 2771–2776, 1998
19. Fleming ID, Cooper JS, Henson DE, Hutter RVP, Kennedy BJ, Murphy GP, O'Sullivan B, Sobin LH, Yarbro JW (eds): AJCC Cancer Staging Handbook. 5th edn, Lippincott, Philadelphia, 1998, pp 159–170
20. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual. 2nd edn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989
21. Takada Y, Hemler ME: The primary structure of the VLA-2/collagen receptor alpha-2 subunit (platelet GP Ia): homology to other integrins and the presence of a possible collagen-binding domain. J Cell Biol 109: 397–407, 1989
22. Corral J, Iniesta JA, González-Conejero R, Vicente V: Detection of Factor V Leiden from a drop of blood by PCR-SSCP. Thromb Haemost 76: 735–737, 1996
23. Kalb R, Santoso S, Unkelbach K, Kiefel V, Mueller Eckhardt C: Localization of the Br polymorphism on a 144 bp exon of the GP Ia gene and its application in platelet DNA typing. Thromb Haemost 71: 651–654, 1994
24. Kunicki TJ, Kritzik M, Annis DS, Nugent DJ: Hereditary variations in platelet integrin α2β1 density is associated with two silent polymorphisms in the α2 gene coding sequence. Blood 89: 1939–1943, 1997
25. Ishida F, Furihata K, Ishida K, Yan J, Kitano K, Kiyosawa K, Furuta S: The largest variant of platelet glycoprotein Ibα has four tandem repeats of 13 amino acids in the macroglycopeptide region and a genetic linkage with $methionine^{145}$. Blood 86: 1356–1360, 1995
26. Simsek S, Faber NM, Bleeker PM, Vlekke ABJ, Huiskes E, Goldschmeding R, von dern Borne AEG: Determination of human platelet antigen frequencies in the Dutch population by immunophenotyping and DNA (allele-specific restriction enzyme) analysis. Blood 81: 835–840, 1993
27. Dunning AM, Healey CS, Pharoah PDP, Teare MD, Ponder BAJ, Easton DF: A systematic review of genetic

polymorphisms and breast cancer risk. Cancer Epidem Biom Prev 8: 843–854, 1999
28. Howe A, Aplin AE, Alahari SK, Juliano RL: Integrin signaling and cell growth control. Curr Opin Cell Biol 10: 220–231, 1998
29. Tuszynski GP, Wang TN, Berger D: Adhesive proteins and the hematogenous spread of cancer. Acta Haematol 97: 29–39, 1997
30. Alford D, Pitha-Rowe P, Taylor-Papadimitriou J: Adhesion molecules in breast cancer: role of α2β1 integrin. Biochem Soc Symp 63: 245–259, 1998
31. Berdichevsky F, Alford D, D'Souza B, Taylor-Papadimitriou J: Branching morphogenesis of human mammary epithelial cells in collagen gels. J Cell Sci 107: 3557–3568, 1994
32. Zutter MM, Santoro SA, Staatz WD, Tsung YL: Re-expression of the ($\alpha_2\beta_1$ integrin abrogates the malignant phenotype of breast carcinoma cells. Proc Natl Acad Sci USA 92: 7411–7415, 1995
33. Chen YQ, Trikha M, Gao X, Bazaz R, Porter AT, Timar J, Hohn KV: Ectopic expression of platelet integrin alphaIIb beta3 in tumor cells from various species and histological origin. Int J Cancer 72: 642–648, 1997
34. Clezardin P, Drouin J, Morel-Kopp MC, Hanss M, Kehrel B, Serre CM, Kaplan C, Delmas PD: Role of platelet membrane glycoproteins Ib/IX and IIb/IIIa and of platelet α-granule proteins in platelet aggregation induced by human osteosarcoma cells. Cancer Res 53: 4695–4700, 1993
35. Zutter MM, Santoro SA, Painter AA, Tsung YL, Gafford A: The human $\alpha_2$ integrin gene promoter: identification of positive and negative regulatory elements important for cell-type and developmentally restricted gene expression. J Biol Chem 269: 463–469, 1994
36. Jacquelin B, Tarantino MD, Kritzik M, Rozenshteyn D, Koziol JA, Nurden AT, Kunicki TJ: Allele-dependent transcriptional regulation of the human integrin $\alpha_2$ gene. Blood 97: 1721–1726, 2001
37. Zutter MM, Ryan EE, Painter AD: Binding of phosphorylated Sp1 protein to tandem Sp1 binding sites regulates $\alpha_2$ integrin gene core promoter activity. Blood 90: 678–689, 1997
38. Ye J, Xu RH, Taylor-Papadimitriou J, Pitha PM: Sp1 binding plays a critical role in Erb-B2- and v-ras-mediated downregulation of $\alpha_2$-integrin expression in human mammary epithelial cells. Mol Cell Biol 16: 6178–6189, 1996
39. Santoso S, Kalb R, Walka M, Kiefel V, Mueller-Eckhardt C, Newman PJ: The human platelet alloantigens $Br^a$ and $Br^b$ are associated with a single amino acid polymorphism on glycoprotein Ia (integrin subunit $\alpha_2$). J Clin Invest 92: 2427–2432, 1993
40. Feng D, Lindpaitner K, Larson MG, Rao VS, O'Donnell CJ, Lipinska I, Schmitz C, Sutherland PA, Silbershatz H, D'Agostino RB, Muller JE, Myers RH, Levy D, Tofler GH: Increased platelet aggregability associated with platelet GPIIIa $Pl^{A2}$ polymorphism: the Framingham Offspring Study. Artherioscler Thromb Vasc Biol 19: 1142–1147, 1999
41. Michelson AD, Furman MI, Goldschmidt-Clermont P, Mascelli MA, Hendrix C, Coleman L, Hamlington J, Barnard MR, Kickler T, Christie DJ, Kundu S, Bray PF: Platelet GPIIIa Pl(A) polymorphisms display different sensitivities to agonists. Circulation 101: 1013–1018, 2000
42. Meyer T, Marshall JF, Hart IR: Expression of alphav integrins and vitronectin receptor identity in breast cancer cells. Br J Cancer 77: 530–536, 1998
43. Felding-Habermann B, O'Toole TE, Smith JW, Fransvea E, Ruggeri ZM, Ginsberg MH, Hughes PE, Pampori N, Shattil SJ, Saven A, Mueller BM: Integrin activation controls metastasis in human breast cancer. Proc Natl Acad Sci USA 98: 1853–1858, 2001

*Address for offprints and correspondence:* Vicente Vicente, MD, PhD, Professor, Head of Hematology and Medical Oncology Unit, Director of Centro Regional de Hemodonación, Department of Internal Medicine, University of Murcia, Ronda de Garay, s/n, 30001 Murcia, Spain; *Tel.:* +34-968-341990; *Fax:* +34-968-261914; *E-mail:* vvg@um.es