Integrin β3 Leu33Pro Homozygosity and Risk of Cancer

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Background: Increased tumor cell expression of integrins containing the β3 subunit is associated with increased progression to invasive tumors, whereas inhibition of β3 integrin expression and/or function may reduce tumor growth and metastasis. The Leu33Pro polymorphism of the β3 subunit modulates the function of α1β3 integrin. We examined whether this polymorphism influences cancer risk. Methods: Using participants (n = 9242) from the Copenhagen City Heart Study with 24 years of follow-up and endpoints from the Danish Cancer Registry, we assessed the risk of all cancers and of 27 cancer types in individuals who carry the Leu33Pro polymorphism (heterozygotes and homozygotes) relative to those without the polymorphism (non-carriers). Relative risks (RRs) of cancer and 95% confidence intervals (CIs) were calculated by Cox proportional hazards regression analysis. Differences in cumulative cancer incidence (per 10,000 person-years) were tested using log-rank statistics. Statistical tests were two-sided. Results: Among the participants, 70.0% were non-carriers, 27.3% were heterozygotes, and 2.7% were homozygotes. We detected 1296 participants with a first cancer. Cumulative incidences in non-carriers, heterozygotes, and homozygotes were 81, 83, and 112, respectively (homozygotes versus non-carriers, P = .02). The age-adjusted RR of all cancers in homozygotes relative to non-carriers was 1.4 (95% CI = 1.1 to 1.9). Incidences in non-carriers, heterozygotes, and homozygotes were 3, 4, and 16 for breast cancer; 19, 24, and 36 for breast cancer; and 2, 3, and 7 for melanoma (homozygotes versus non-carriers; P = .002, P = .06, and P = .03, respectively). The age-adjusted RR in homozygotes relative to non-carriers was 4.7 (95% CI = 1.6 to 14) for ovarian cancer, 1.9 (95% CI = 1.0 to 3.7) for breast cancer, and 3.5 (95% CI = 1.1 to 12) for melanoma. Adjustment for other cancer risk factors did not alter these results. Heterozygotes did not differ from non-carriers with respect to cancer risk. Conclusion: Individuals homozygous for the Leu33Pro polymorphism of the β3 integrin subunit have an increased cancer risk. [J Natl Cancer Inst 2003;95:1150–7]

Integrins are transmembrane αβ heterodimers that function as key surface adhesion and cell signaling receptors influencing cell proliferation, migration, and survival (1). The 18 α and eight β subunits combine to form at least 25 different integrins, expressed in a wide variety of tissues (1,2). The β3 integrins, αvβ3 and αmβ3, are constitutively expressed by angiogenic endothelial cells and platelets, respectively (3). Increased expression of the two β3 integrins in ovarian tumors, breast tumors, and melanomas is associated with progression to invasive tumors (3–5). In mouse models, induced expression of the β3 subunit increases the metastatic potential of melanoma cells (6,7), whereas inhibition of the αvβ3 and αmβ3 integrins reduces tumor growth and metastasis through the disruption of tumor angiogenesis (8–12). The potential biologic importance of β3 integrins in cancer growth and metastases has prompted determination of the crystal structures of the extracellular segments of the αvβ3 integrin to facilitate cancer drug development (2,13,14) and the development of specific β3 integrin inhibitors, some of which are already in clinical trials (1,4).

Several polymorphisms have been identified in the β3 integrin subunit (15). The most frequent polymorphism occurs in codon 33 and results in a substitution of the wild-type amino acid leucine with proline (Leu33Pro). The allele frequency is approximately 85% for 33Leu and 15% for 33Pro (15). The polymorphism has structural and functional consequences in that it introduces a nick in the polypeptide chain just N-terminal of the hybrid domain of β3. This domain is involved in dimerization with the αv and αm subunits during the formation of αvβ3 and αmβ3 integrins. In platelets, this polymorphism increases binding of fibrinogen to the membrane-bound αmβ3 integrin (16), results in an abnormal response to stimulation with thromboxane (17) and other agonists (18), decreases bleeding time (19), enhances thrombin generation (20), increases the ability of platelets to aggregate (21), and increases activation of mitogen-activated protein kinase (22). Thus, this polymorphism seems to modify the function of cells expressing β3 integrins.

Because the polymorphism may also influence cancer progression, we therefore hypothesized that the β3 integrin subunit Leu33Pro polymorphism might influence cancer risk. To test this hypothesis, we genotyped 9242 participants of the Copenhagen City Heart Study (a prospective study of the Danish general population that has 24 years of follow-up) and 1296 participants with a first cancer and assessed the relative risks (RRs) of all cancers and of 27 specific cancer types.

Patients and Methods

Study Population

We performed a prospective population-based study. Participants aged 20 to 95 years had been recruited at random from the general population to participate in the Copenhagen City Heart Study (23,24). Of the 9242 participants, 7159 were followed from the 1976 through 1978 first examination to December 31, 2003. Of these individuals, 6127 had complete follow-up to December 31, 2003. Of these participants, 1296 had a first cancer during the follow-up period.
1999; 277 were followed from the 1981 through 1983 second examination to December 31, 1999; and 1806 were followed from the 1991 through 1994 third examination to December 31, 1999 (Fig. 1). Participation rate for the present study was 61%. More than 99% of the participants were white and of Danish descent.

All participants were interviewed between 1991 and 1994 at the third examination of the Copenhagen City Heart Study regarding medical history, family history of diseases, alcohol consumption, smoking habits, and reproductive history (women only). Body mass index was measured, and blood samples were drawn. DNA was extracted from peripheral blood lymphocytes as described (24).

Diagnoses of invasive cancer and in situ lesions for the whole cohort from 1947 through 1999 were obtained from the Danish National Cancer Registry (25,26), which identifies 97.8% of all cancers in Denmark (27). We collected information on cancers that included histology, topology, and dissemination (localized, regional metastasis, and distant metastasis) at time of diagnosis and date of initial diagnosis. Cancer diagnoses were classified according to criteria from World Health Organization International Classification of Diseases 7th edition (ICD-7) and divided into 27 different standard World Health Organization subtypes (28): oral cavity/pharynx (n = 19), esophagus (n = 13), stomach (n = 15), colon/rectum/anus (n = 144), liver/biliary tract (n = 22), pancreas (n = 27), larynx (n = 19), lung (n = 137), melanoma (n = 39), breast (n = 198), cervix uteri (n = 18), corpus uteri (n = 54), ovary (n = 36), prostate (n = 82), testis (n = 3), bladder/excretory urinary tract (n = 119), kidney (n = 18), brain/nervous tissue (n = 26), thyroid/other endocrine tumors (n = 3), non-Hodgkin’s lymphoma (n = 26), Hodgkin’s disease (n = 0), multiple myeloma (n = 7), leukemia (n = 24), non-melanoma skin (n = 322), sarcoma/other mesodermal tumors (n = 11), small intestine (n = 1), and other tumors (n = 42). Codes 175.0–5, 176.0–6, 176.9, 375.0, 475.0, 475.5, 476.0–3, 476.9, and 775.5 were classified as ovarian cancer, codes 170.0–5 and 470.0–5 were classified as breast cancer, and codes 090.0, 093.2, 176.4, 190.0–9, 192.4, and 460.0–2 were classified as melanoma. Among the study participants, 1296 had a first diagnosis of invasive cancer and 129 had invasive cancer during the follow-up period. In total, we detected 1425 cancers, of which 129 cancers occurred in participants who had previously had another cancer. Follow-up time for each participant began at entry into the study (Fig. 1) and ended at death, event, emigration, or December 31, 1999, whichever came first. The maximal and median follow-up periods were 24 and 22 years, respectively. During the study period, we had 99.98% follow-up, losing only two individuals. The date of diagnosis was not available for five individuals with primary cancer, including one participant with ovarian cancer. Accordingly, these participants were excluded from the statistical analyses of all cancers and of ovarian cancer, respectively. All participants gave written informed consent. The ethical committee of Copenhagen and Frederiksberg, Denmark, approved the study (No. 100.2039/91).

**Genotyping by Restriction Fragment Length Polymorphism**

We genotyped DNA from 9242 participants for the Leu33Pro polymorphism of the β3 integrin subunit. The 33Pro allele results from a T→C substitution in nucleotide 176 in the β3 integrin gene (GenBank accession No. NM_000212.1). This polymorphism was examined as previously described (29). Briefly, a 268-base pair (bp) polymerase chain reaction (PCR) fragment corresponding to the entire exon 3 (GenBank accession No. M32672) was amplified from genomic DNA by using intronic primers (sense, 5’-TTCTGATTGGTGACTTCTTCT-3’; antisense, 5’-TCTTCCCTCCAGGCAAGATT-3’). After thermocycling, the PCR products were digested with the restriction endonuclease MspI, subjected to electrophoresis through a 3% agarose gel, and visualized by staining the gel with ethidium bromide. Non-carrier (Leu/Leu), heterozygous (Leu/Pro), and homozygous (Pro/Pro) alleles could then be distinguished on the basis of the size of the digested fragments. Individuals with the 33Leu/Leu non-carrier genotype produced a pattern that consisted of three bands of 221 bp, 38 bp, and 8 bp; those with the 33Leu/Pro heterozygous genotype produced a pattern that consisted of five bands of 221 bp, 177 bp, 44 bp, 38 bp, and 8 bp; and those with the 33Pro/Pro homozygous genotype produced a pattern that consisted of four bands of 177 bp, 44 bp, 38 bp, and 8 bp. Because the restriction fragment length polymorphism assay included control restriction sites for MspI within exon 3 (CCGG at positions 272–275 and 278–281) that always are digested, the assay has an internal control. The migration pattern was read by an experienced laboratory technician (who was blinded to the source of the DNA) and was later re-read by a different experienced laboratory technician (who was likewise blinded to the source of the DNA). When the two laboratory technicians disagreed on the genotype or when the DNA bands were too faint for valid genotype detection, the sample from that individual was re-analyzed.

In addition to the examined Leu33Pro polymorphism, we also detected 57 individuals who were heterozygous for a T→G substitution at nucleotide position 197. This substitution results in a Leu40Arg polymorphism and is detected as an MspI restriction site. The exon 3 PCR fragment from these individuals was sequenced to confirm the genotype. These individuals were classified exclusively according to their Leu33Pro genotype for the statistical analyses, because the statistical power to detect any association with the 57 Leu40Arg heterozygotes was minimal.

**Statistical Analysis**

The primary hypothesis tested was whether an association exists between the β3 integrin Leu33Pro genotype and an increased risk of cancer. Secondary hypotheses assessed whether such an association is sex-specific, site-specific (i.e., one of 27 different cancer types), or dependent on the presence of metastases.

The statistical software package SPSS (30) or STATA (31)
was used for all analyses. All statistical tests were two-sided. For comparisons among participant characteristics, we used Student’s t test, the Mann–Whitney U test, or Pearson’s chi-square test, as appropriate. P<.05 was considered statistically significant.

We plotted cumulative cancer incidence against follow-up time using the Kaplan–Meier method and tested differences between genotypes using log-rank statistics. RRs for disease with 95% confidence intervals (CIs) were calculated using Cox proportional hazards regression analysis, adjusting for age at study entry. Individuals with events before entry were excluded from analyses. We tested for proportionality of hazards over time to ensure that this assumption of Cox proportional hazards regression was fulfilled, based on Schoenfeld residuals with left-truncated lifetime as the time scale.

Multivariable adjustment for risk of all cancers included sex (when applicable), age at entry, having one or more first-degree relatives (children not included) dead of cancer, total tobacco consumption (0, 0.01–24, or >24 pack-years), smoking habits at the time of the 1991–1994 examination (never, former, or current), body mass index (<22.8, 22.8–26.5, or >26.5 kg/m²), and alcohol consumption at the time of the 1991–1994 examination (0, 1–8, or >8 units per week; 1 unit equals 12 g of alcohol). For analyses of all cancers in women only, we also adjusted for nulliparity and number of children. Multivariable adjustment for risk of ovarian cancer included age at entry, body mass index, alcohol consumption, nulliparity, and number of children. Multivariable adjustment for risk of breast cancer included age at entry, tobacco consumption and smoking habits, body mass index, alcohol consumption, nulliparity, and number of children. Multivariable adjustment for risk of melanoma included age at entry and sex. Data were not available for other risk factors for ovarian cancer and breast cancer, including age at menarche, total exposure to exogenous estrogens, duration of breastfeeding, or for other risk factors for melanoma, including total sun exposure, history of familial melanoma, lighter pigmentation, and number of nevi. Information on covariates noted in Table 1 and used in the multivariable-adjusted Cox proportional hazards regression model was obtained at the 1991–1994 examination.

### Table 1. Characteristics of participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-carriers 33Leu/Leu</th>
<th>Heterozygotes 33Leu/Pro</th>
<th>Homozygotes 33Pro/Pro</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>6466 (70.0)</td>
<td>2525 (27.3)</td>
<td>251 (2.7)</td>
</tr>
<tr>
<td>Women, No. (%)</td>
<td>3579 (55.4)</td>
<td>1392 (55.1)</td>
<td>140 (55.8)</td>
</tr>
<tr>
<td>Age at entry, y</td>
<td>45.3 ± 0.1</td>
<td>45.5 ± 0.2</td>
<td>45.9 ± 0.7</td>
</tr>
<tr>
<td>Proportion of first-degree relatives dead of cancer, %</td>
<td>10.2 ± 0.2</td>
<td>10.3 ± 0.3</td>
<td>10.8 ± 1.2</td>
</tr>
<tr>
<td>Total tobacco consumption, pack-years</td>
<td>20.6 ± 0.3</td>
<td>21.6 ± 0.5</td>
<td>21.8 ± 1.7</td>
</tr>
<tr>
<td>Current or former smokers, %</td>
<td>74.1</td>
<td>74.8</td>
<td>74.1</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.6 ± 0.1</td>
<td>25.7 ± 0.1</td>
<td>26.0 ± 0.3</td>
</tr>
<tr>
<td>Alcohol, units per wk</td>
<td>9.5 ± 0.2</td>
<td>9.4 ± 0.2</td>
<td>8.2 ± 0.6</td>
</tr>
<tr>
<td>Nulliparity, % (women only)</td>
<td>24.8</td>
<td>25.7</td>
<td>19.4</td>
</tr>
<tr>
<td>No. of children born (women only)</td>
<td>1.6 ± 0.02</td>
<td>1.6 ± 0.03</td>
<td>1.8 ± 0.12</td>
</tr>
</tbody>
</table>

*Participants were genotyped for a polymorphism in the β3 integrin. Non-carriers refers to individuals without the polymorphism (33Leu/Leu). Heterozygotes carry one polymorphic allele (33Leu/Pro), and homozygotes carry two polymorphic alleles (33Pro/Pro). Values are means ± standard errors or frequencies, as appropriate. Statistical comparisons between heterozygotes or homozygotes and non-carriers were made using the two-sided Mann–Whitney U test, Pearson’s chi-square test, or Student’s t test on untransformed or log-transformed parameters, as appropriate. No parameter differed statistically significantly among genotypes.

### Table 2. Incidence and relative risk of cancer according to Leu33Pro genotype in β3 integrin by Cox regression

<table>
<thead>
<tr>
<th>Stratification</th>
<th>No. of participants</th>
<th>No. of incident events</th>
<th>Incidence/10,000 person-years (95% CI)</th>
<th>Relative risk (95% CI)</th>
<th>Age only</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women and men combined†</td>
<td>6339</td>
<td>890</td>
<td>81 (75 to 86)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
<td></td>
</tr>
<tr>
<td>Non-carriers</td>
<td>2453</td>
<td>359</td>
<td>83 (74 to 92)</td>
<td>1.0 (0.9 to 1.1)</td>
<td>1.0 (0.9 to 1.1)</td>
<td></td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>244</td>
<td>47</td>
<td>112 (82 to 149)</td>
<td>1.4 (1.1 to 1.9)</td>
<td>1.4 (1.0 to 1.9)</td>
<td></td>
</tr>
<tr>
<td>Women‡</td>
<td>3484</td>
<td>501</td>
<td>81 (74 to 89)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
<td></td>
</tr>
<tr>
<td>Non-carriers</td>
<td>1347</td>
<td>188</td>
<td>77 (66 to 88)</td>
<td>0.9 (0.8 to 1.1)</td>
<td>0.9 (0.8 to 1.1)</td>
<td></td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>135</td>
<td>29</td>
<td>126 (85 to 182)</td>
<td>1.6 (1.1 to 2.3)</td>
<td>1.6 (1.1 to 2.3)</td>
<td></td>
</tr>
<tr>
<td>Homozygotes</td>
<td>2855</td>
<td>389</td>
<td>80 (72 to 88)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
<td></td>
</tr>
<tr>
<td>Men§</td>
<td>1106</td>
<td>171</td>
<td>91 (77 to 105)</td>
<td>1.1 (0.9 to 1.3)</td>
<td>1.1 (0.9 to 1.3)</td>
<td></td>
</tr>
<tr>
<td>Non-carriers</td>
<td>109</td>
<td>18</td>
<td>95 (56 to 150)</td>
<td>1.2 (0.7 to 1.9)</td>
<td>1.1 (0.7 to 1.8)</td>
<td></td>
</tr>
</tbody>
</table>

*Participants were genotyped for a polymorphism in the β3 integrin. Non-carriers refers to individuals without the polymorphism (33Leu/Leu). Heterozygotes carry one polymorphic allele (33Leu/Pro), and homozygotes carry two polymorphic alleles (33Pro/Pro). CI = confidence interval.
†We excluded 199 (122 non-carrier, 70 heterozygous, and seven homozygous) participants with cancer before entry, two (non-carrier) participants who were lost during follow-up, and five (three non-carrier and two heterozygous) participants because of a lack of a date of diagnosis of cancer.
‡We excluded 140 (91 non-carrier, 44 heterozygous, five homozygous) female participants with cancer before entry, two (non-carrier) participants who were lost during follow-up, and three (two non-carrier and one heterozygous) participants because of a lack of date of diagnosis of cancer.
§We excluded 59 (31 non-carrier, 26 heterozygous, two homozygous) male participants with cancer before entry and two (one non-carrier, one heterozygous) participants because of a lack of date of diagnosis of cancer.
RESULTS

We genotyped 9242 participants selected from the Danish general population. Of those, 70.0% were non-carriers, 27.3% were heterozygous, and 2.7% were homozygous for the Leu33Pro polymorphism. This distribution did not differ from the Hardy–Weinberg equilibrium ($P = .81$, chi-square test). Frequencies of the 33Leu and 33Pro alleles were 83.6% and 16.4%, respectively. There was no statistically significant difference in any of the established risk factors for cancer among individuals, regardless of genotype (Table 1).

During 24 years of follow-up, we detected 1296 participants with a first cancer. The incidence of cancer in non-carriers, heterozygotes, and homozygotes was 81, 83, and 112 per 10,000 person-years, respectively, in women and men combined; 81, 77, and 126, respectively, in women alone; and 80, 91, and 95, respectively, in men alone (Table 2). The cumulative incidence of cancer was higher in homozygotes than in non-carriers among women and men combined (Fig. 2, upper panel; log-rank $P = .02$) and in women alone (Fig. 2, middle panel; log-rank $P = .01$), but not in men alone (Fig. 2, lower panel; log-rank $P = .14$).

In age-adjusted Cox proportional hazards regression models, the RR of cancer in homozygotes relative to non-carriers was 1.4 ($95\%$ CI = 1.1 to 1.9) in men and women combined, 1.6 ($95\%$ CI = 1.1 to 2.3) in women alone, and 1.2 ($95\%$ CI = 0.7 to 1.9) in men alone (Table 2). However, sex did not interact with the Leu33Pro genotype in predicting cancer risk ($P = .15$). Male and female heterozygotes considered separately or together did not differ from non-carriers with regard to risk of cancer (Table 2). These results were similar when a multivariable-adjusted model that included established cancer risk factors was used to calculate the RR for cancer in homozygotes or heterozygotes compared with non-carriers (Table 2).

We next assessed the risk of cancer stratified for presence or absence of metastasis among the study participants. At the time of a first cancer diagnosis, 324 participants presented with metastases and 798 participants presented with localized disease only (Table 3). For 174 participants with cancer, the stage of cancer with regard to metastasis at the time of diagnosis was unknown (Table 3). In age-adjusted Cox proportional hazards regression analyses among those participants without metastasis, the RR of cancer in homozygotes relative to non-carriers was 1.6 ($95\%$ CI = 1.1 to 2.3) in women and men combined, 1.7 ($95\%$ CI = 1.0 to 3.6) in women alone, and 1.5 ($95\%$ CI = 0.8 to 2.6) in men alone (Table 3). Similar RRs were determined for those participants with metastases: 1.2 ($95\%$ CI = 0.7 to 2.3) in women and men combined, 1.7 ($95\%$ CI = 0.8 to 3.6) in women alone, and 0.8 ($95\%$ CI = 0.3 to 2.5) in men alone.

We next assessed whether there was an association between specific cancers and the Leu33Pro polymorphism. Of the 27 types of cancer examined separately, only ovarian cancer, breast cancer, and melanoma showed an association with the Leu33Pro polymorphism (Fig. 3 and Table 4). During follow-up, 36 participants had ovarian cancer, 195 participants had breast cancer, and 39 participants had melanoma. The incidences of ovarian cancer were 3, 4, and 16 per 10,000 person-years for non-carriers, heterozygotes, and homozygotes, respectively (Table 4 and Fig. 3, upper panel; log-rank for homozygotes versus non-carriers, $P = .002$). The incidences of breast cancer were 19, 24, and 36 per 10,000 person-years for non-carriers, heterozygotes, and homozygotes, respectively (Table 4 and Fig. 3, middle panel; log-rank for homozygotes versus non-carriers, $P = .06$). The incidences of melanoma were 2, 3, and 7 per 10,000 person-years for non-carriers, heterozygotes, and homozygotes, respectively (Table 4 and Fig. 3, lower panel; log-rank for homozygotes versus non-carriers, $P = .03$).
participants because of a lack of date of diagnosis of cancer. During follow-up, and five (three non-carrier and two heterozygous) participants because of a lack of a date of diagnosis of cancer.

The RRs of ovarian cancer, breast cancer, and melanoma in homozygous individuals might contribute to metastatic spread, we found that an increased cancer risk for 33Pro/Pro homozygotes could be related to a lack of a date of diagnosis of cancer.

In age-adjusted Cox proportional hazards regression models, the RRs of ovarian cancer, breast cancer, and melanoma in homozygous relative to non-carriers were 4.7 (95% CI = 1.6 to 14), 1.9 (95% CI = 1.0 to 3.7), and 3.5 (95% CI = 1.1 to 12), respectively (Table 4). However, when homozygotes were compared without non-carriers, there was no increase in the RRs of ovarian cancer, breast cancer, or melanoma. Multivariable adjustment for established risk factors did not change these results.

We next assessed whether the basic assumption for Cox proportional hazards regression analyses of similar RRs (that is, proportion of hazards) over time for the three genotypes was fulfilled in the different analyses. The curves for homozygotes and non-carriers of the Kaplan–Meier plots (Figs. 2 and 3) seemed to cross, indicating that the RR might change over follow-up time. To determine whether the risks did change over time, we performed a test for proportional hazards over time for the three genotypes in multivariable-adjusted models and obtained the following results: all cancer, all men combined, P = .16; all cancer, women only, P = .83; all cancer, men only, P = .02; ovarian cancer, women only, P = .63; breast cancer, women only, P = .95; melanoma, women and men combined, P = .58. The P value for all cancer, men only, was statistically significant, suggesting that only for this category might the RR for homozygotes versus non-carriers change over time. However, after correcting for multiple comparisons, this finding in men was no longer statistically significant, suggesting that this observation probably represents a chance finding rather than a real phenomenon.

**DISCUSSION**

This prospective study among individuals from the general population enrolled in the Copenhagen City Heart Study demonstrates that individuals who are homozygous for the Leu33Pro polymorphism of the β3 integrin subunit, a component of αvβ3 and α1β3 integrins, have an increased risk of cancer. After evaluating 27 individual types of cancer, we determined that the elevated overall cancer risk partly originates from an increased risk of ovarian cancer, breast cancer, and melanoma. In accordance with this, ovarian cancer, breast cancer, and melanoma are among the cancers most frequently reported to express β3 integrins (3–5, 12, 32–36).

β3 integrins are likely involved in tumor growth and metastasis (6–12). The increased expression of cell surface integrins on cancer cells is associated with the development of invasive properties, i.e., altered migration on and adhesion to the extracellular matrix (4). Moreover, increased integrin expression has also been noted on angiogenic non-neoplastic vascular endothelial cells, providing the necessary vascular supply for the tumor (37–39). Induced expression in melanoma cells of the αvβ3, and α1β3 integrins promotes a more malignant phenotype, whereas inhibition of β3 integrins reduces progression of tumors (4,7,40).

These potential roles of β3 integrins in cancer has led to clinical trials of function-blocking antibodies or small synthetic peptides against β3 integrins (1,4).

The molecular mechanism behind the observed increased risk of cancer in 33Pro/Pro homozygotes is not known; however, the mechanism could involve the function of 1) the α1β3 integrin on platelets, 2) the αvβ3 integrin on neangiogenic endothelial cells (41–43), 3) ectopic expression of both β3 integrins (αvβ3 and α1β3) by the tumor cells themselves (9,22), or 4) a combination of all three. Of these possibilities, most experimental data describe the modulating effect of the Leu33Pro polymorphism on the function of platelets (18–21,44–48).

The interaction between cancer cells and platelets is well known (49,50). Platelets from individuals homozygous for the Leu33Pro polymorphism (33Pro/Pro) have increased reactivity and aggregability relative to those from non-carriers (18,21,48). Although this observation would suggest that platelets from homozygous individuals might contribute to metastatic spread, we found that an increased cancer risk for 33Pro/Pro homozygotes...
ERK2 is a mitogen-activated protein kinase involved in the stimulation of transcription factors, promotion of cell-cycle progression, and increased proliferation of tumor cells. These observations suggest that the increased cancer risk associated with 33Pro/Pro homozygosity of $\beta_3$ integrins might be the result of a more malignant phenotype, rather than of increased metastasis-promoting properties of 33Pro/Pro homozygous platelets.

Although the increased risk of cancer in 33Pro/Pro homozygotes may be partly the result of an increased risk of ovarian cancer, breast cancer, and melanoma, we cannot totally exclude the possibility that the increased overall risk of cancer may represent a chance finding. Indeed, after adjusting for multiple comparisons among the 27 different cancers, only the risk for ovarian cancer was statistically significant. However, of the 27 different cancers examined in our study, these three tumor types (ovarian cancer, breast cancer, and melanoma) are among the most frequently reported to express $\beta_3$ integrins, either in primary tumor tissue or cell cultures. Thus, the probability that this concurrence results from chance is small, and we therefore believe that the associations between 33Pro/Pro homozygosity and increased risk of ovarian cancer, breast cancer, and melanoma reported in this study are based on reasonable biologic mechanisms.

Our study has several limitations. First, the study sample had relatively few cancer events for most cancer types. Although we found statistically significant associations for ovarian cancer, breast cancer, and melanoma, the small number of events for some other cancer types limits our ability to make inferences about the role of this gene polymorphism for most cancer sites. Evidence supporting this hypothesis is given by the RR estimates. Only breast cancer, which occurred in a relatively large number of women, was associated with an RR of less than 2. By contrast, the frequencies of melanoma and ovarian cancer were much lower, and the associations were found with much higher RRs. This suggests that the study could have been underpowered to detect associations with smaller magnitudes of effect for other cancers.

Second, confounding resulting from close linkage with other causative genetic factors is possible in association studies. The $\beta_3$ integrin gene is located on chromosome 17q21, which also contains the ovarian and breast cancer susceptibility gene BRCA1. Whether variation in BRCA1 influenced the associations investigated in this study is unknown. However, the $\beta_3$ integrin gene and BRCA1 gene are not linked.

Third, the cohort was selected from individuals enrolled in a study evaluating cardiovascular risk factors, not cancer risk factors. Therefore, knowledge on some established cancer risk factors is limited. For example, given the importance of variables such as sun exposure in the etiology of melanoma, substantial additional analyses may be warranted to fully understand the relationship of the $\beta_3$ integrin subunit Leu33Pro polymorphism with other etiologic factors on risk of melanoma. Our knowledge of melanoma risk factors (such as sun exposure, familial melanoma, lighter pigmentation, number of nevi) and hormonal history of women participating in the study was incomplete. However, our knowledge of age, sex, smoking history and status, family history of fatal cancer, body mass index, alcohol con-

![Fig. 3. Kaplan–Meier curves showing 24-year cumulative incidence of ovarian cancer, breast cancer, and melanoma according to Leu33Pro genotype in $\beta_3$ integrin. Numbers at risk at the beginning of the study vary resulting from exclusion of participants with ovarian cancer, breast cancer, or melanoma before study entry, and because only women are included in the analyses of risk of ovarian cancer and breast cancer. Log-rank tests are two-sided.](image-url)
Follow-up, and one (non-carrier) participant because of a lack of date of diagnosis of ovarian cancer. Carriers carry one polymorphic allele (33Leu/Pro), and homozygotes carry two polymorphic alleles (33Pro/Pro). CI confidence interval.

Conservative estimate concerning associations between the homozygous 33Pro/Pro genotype with cancer and thus cannot exclude certain individuals from being genotyped. However, two observations make substantial selection bias against genotypes less likely: 1) Age percentiles for non-carriers and homozygotes display a linear relationship, as would be expected if no selection occurred against homozygotes; and 2) The genotype distribution does not differ from a Hardy–Weinberg equilibrium (chi-square test, \( P = .81 \)). Thus, we do not consider selection bias against homozygosity likely, but if this nevertheless does apply to our study, selection bias would tend to result in a conservative estimate concerning associations between the homozygous 33Pro/Pro genotype with cancer and thus cannot explain our results.

Fifth, systematic misclassification of genotypes in this study could have occurred. However, this possibility is unlikely for several reasons: 1) agreement with the Hardy–Weinberg equilibrium; 2) the fact that the PCR assay included a control restriction enzyme site in each person assayed; 3) the fact that sequencing of exon 3 of the \( \beta_3 \) integrin subunit have

In conclusion, we demonstrated that individuals homozygous for the 33Pro/Pro polymorphism of the \( \beta_3 \) integrin subunit have an increased risk of cancer. The main strengths of this study include its size, the high (almost 100%) follow-up rate, the prospective nature of the study, and the fact that individuals in the general population were studied. However, despite these strengths, this paper should be considered as a hypothesis-generating exploratory epidemiologic study implying that a common \( \beta_3 \) integrin polymorphism is a genetic risk factor for cancer.

### REFERENCES

8. Varner JA, Nakada MT, Jordan RE, Coller BS. Inhibition of angiogenesis and tumor growth by murine \( \beta_3 \)E3, the parent antibody of c7E3 (abiximab; ReoPro™). Angiogenesis 1999;3:53–60.


NOTES
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