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Transforming-growth factor β_1 Leu10Pro polymorphism and breast cancer morbidity ☆

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ABSTRACT

TGF-β_1 has a dual role in carcinogenesis. In this gene, a leucine to proline substitution in codon 10 leads to higher circulating levels of TGF-β_1. This variant has been studied in relationship to the risk for breast cancer yielding contradicting results. We aim to unravel the relationship of this polymorphism and the risk of breast cancer. Women participating in the Rotterdam Study including 143 patients with incident breast cancer were genotyped for this polymorphism. We carried out a logistic regression and a survival analysis using age as the time variable. The logistic regression analysis showed an increased risk of breast cancer for Proline carriers (OR = 1.4; 95% confidence interval (CI) = 1.1–2.0) *versus* non-carriers. The survival analysis showed that carriers of the same allele had an increased risk of breast cancer (HR = 1.4, 95% CI = 1.1–2.0) against non-carriers.

Our data suggest that the TGF-β_1 Leu10Pro polymorphism might play a role in breast cancer risk.

1. Introduction

The proliferation of cancerous breast epithelial cells is regulated by different stimuli including cytokines and growth factors,[1] such as the transforming-growth factor β (TGF-β). TGF-β has three isoforms TGF-β_1, TGF-β_2 and TGF-β_3. TGF-β_1 is the most abundant and universally expressed isoform.[2] It is known to be expressed in endothelial tissue[3] and has an effect on the growth of mammary epithelium.[4] Furthermore, it has recently been suggested that TGF-β_1 has a dual role in tumour growth. It acts as a tumour suppressor inhibiting epithelial cell proliferation in early stages and as a tumour promoter in later stages of carcinogenesis.[5] Both activities of TGF-β have been clearly demonstrated in genetically modified mouse lines in which the TGF-β signalling pathway is ablated or modified.[6] These studies imply that TGF-β isoforms inhibit the development of early, benign lesions but enhance invasion and metastasis when the tumour suppressor activity is overridden by oncogenic mutations in other pathways.[7]

The gene encoding for TGF-β_1 is located on chromosome 19q13.1. A T29C transition that results in a Leu10Pro substitution in the signal peptide sequence in this gene has been associated with higher circulating levels of TGF-β_1. Proline homozygotes have been found to have increased serum levels of TGF-β_1.[8,9] This variant has been studied in relationship to the risk for breast cancer but these studies have been

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inconclusive.[10–17] The aim of this study is to examine the relationship of the Leu10Pro polymorphism and the risk of breast cancer in an association study.

2. Materials and methods

2.1. Study population

Our study population is part of the Rotterdam study[18] where inhabitants of Ommoord, a suburb in Rotterdam, aged 55 or older were invited to participate and 7983 agreed to do so (response rates = 78.1%). Participants' informed consent was obtained and the Medical Ethics Committee of the Erasmus Medical Center approved the study. Our study group comprised 4878 postmenopausal women.

2.2. Measurements

Information on risk factors such as age at menarche, age at menopause and hormone replacement therapy use (HRT) was retrieved at baseline.[19] Body mass index (BMI) was calculated by dividing the weight in kilograms by the height (in m) squared.

2.3. Case identification and validation

Three different databases were used for case identification. First, cases diagnosed by general practitioners in the research area (Ommoord) were collected (International Classification of Primary Care (X76)). Second, the Dutch National Registry of all hospital admissions (LMR) was consulted to detect all malignancy related hospital admissions for study participants. Finally, regional pathology databases were linked to the Rotterdam Study to identify cases.[20] Subsequently, breast cancer cases were validated by a physician on the basis of medical records of the general practitioner, discharge letters and pathology reports. Only pathologically confirmed cases were considered in the analysis. The index date was defined as the earliest date found in the pathology report.

2.4. Genotyping

Of the 4878 women participating in our study, there were 3905 DNA samples available for genotyping. Of these, 3646 (93.4%) were successfully genotyped. The genotyping procedures have been previously described.[21]

2.5. Data analysis

We tested Hardy–Weinberg equilibrium (HWE) of the TGF-β_1 Leu10Pro polymorphism using Markov–Chain Monte-Carlo approximation of the exact test implemented in the GENEPOP package V 3.3.[22] Categorical variables, such as parity and hormone replacement therapy (HRT), were compared between genotype groups using the χ^2-test. Continuous variables (age at entry, age at menopause, BMI and waist hip ratio (WHR)) were compared between genotypes using the independent sample Mann–Whitney test. We used logistic regression to study the risk of breast cancer by TGF-β_1 genotype. We adjusted for possible confounders such as age at entry, age at menopause, HRT, WHR and BMI. Then, we performed a Cox proportional hazards model to assess breast cancer free survival by TGF-β_1 genotype. The logistic regression was performed in SPSS version 11 and the disease free survival was done in S-plus version 6.

3. Results

The frequencies of the Leu10Pro genotypes of the TGF β_1 gene were in Hardy–Weinberg equilibrium proportions ($p = 0.98$). The descriptive statistics of our study population are shown in Table 1. The distribution of these risk factors was not significantly different among genotype groups.

At baseline there were 66 prevalent postmenopausal breast cancer cases, while another 143 were diagnosed during follow-up. The prevalent cases were not included in our analyses. We did not find any statistically significant differences between the distribution on risk factors in women who were and women who were not successfully genotyped (data not shown).

The distribution of breast cancer in our population stratified by the TGF β_1 genotype is shown in Fig. 1. The figure shows that the incidence of breast cancer in carriers of at least one proline allele was statistically higher ($p = 0.04$) than non-carrier. Since the distribution for homozygotes carriers of proline was similar to that of heterozygotes, we pooled heterozygous and homozygous carriers in the logistic regression model, which we used to adjust for known risk factors. The odds ratio was 1.4 (95% CI = 1.1–2.0, $p = 0.04$). According to our power calculations our number of cases was sufficient to find an effect of this size.

Table 1 – General characteristics of the study population stratified by TGF-β_1 genotype

Genotype	Leu/Leu	Leu/Pro	Pro/Pro	Total
Total studied (%)	1488 (40.8)	1679 (46.1)	479 (13.2)	3646
Mean age of entry (standard deviation, SD)	70.2 (9.5)	70.4 (9.5)	69.6 (9.4)	70.2 (9.5)
Mean age at death	84.3 (8.8)	83.5 (8.9)	83.9 (8.6)	83.9 (8.8)
Mean age at menopause (SD)	52 (13.5)	51.7 (12.6)	51.5 (18.1)	51.8 (12.8)
Mean number of children	2.1 (1.7)	2.1 (1.7)	2.2 (1.8)	2.1 (1.7)
Parity (%) ($\geqslant$1 child)	1135 (79.3)	1278 (79)	373 (81)	2786 (79.4)
Hormone replacement therapy (%)	272 (19.7)	248 (19.3)	63 (18.4)	533 (19.4)
Mean body mass index (SD)	26.81 (4.1)	26.71 (4.1)	26.47 (3.8)	26.72 (4)
Mean waist–hip ratio (SD)	0.87 (0.1)	0.87 (0.1)	0.86 (0.1)	0.88 (0.1)

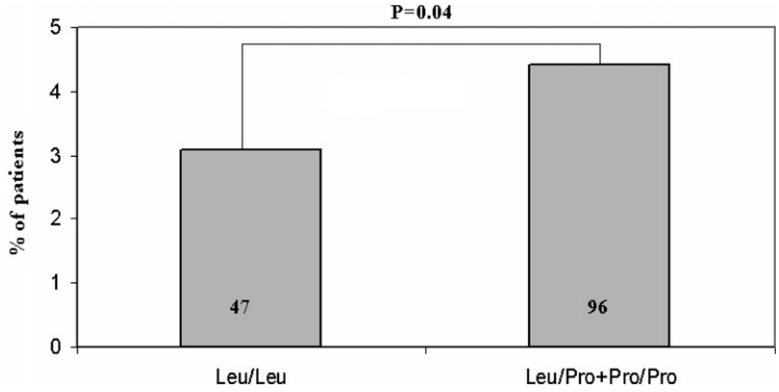

Fig. 1 – Breast cancer cases by genotype.

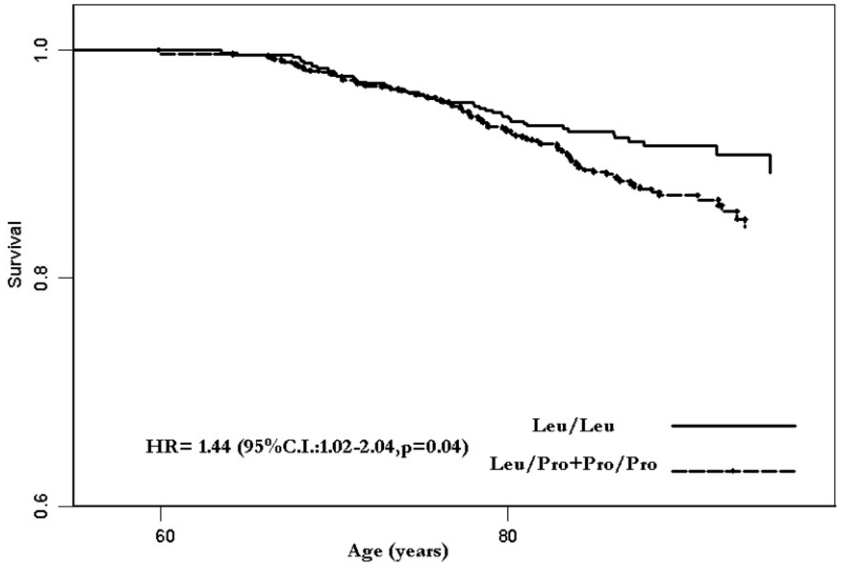

Fig. 2 – Disease free survival by genotype.

Additionally, we performed a disease free survival analysis. We found that carriers of the proline allele had a HR of 1.4 (95% CI = 1.2-2.0, $p = 0.04$) compared to non-carriers (Fig. 2). This effect was independent of well-known risk factors such as HRT and BMI.

4. Discussion

In this association study, we show a statistically significant increase in risk of breast cancer for carriers of at least one copy of the proline allele of the Leu10Pro polymorphism in the TGF-β_1 gene, when compared to non-carriers in Caucasian postmenopausal women. Our research is part of the Rotterdam Study, a population based cohort study for disease determinants in the elderly. The strength of our study is based on its prospective basis but although we did find significant evidence for an association between genotype and disease, our study had some limitations. The first one is that only a few number of breast cancer cases were diagnosed during follow-up. Nevertheless, this number is sufficient to detect a moderately increased risk as the one we do, according to our power calculations. The second one is that 21% of the women entering the study did not give a DNA sample. These women were older at entry, at death and at menopause and they were also less likely to have children or receive HRT. These women were less likely to develop breast cancer, and including them in our analysis could have driven our results towards the null.

TGF-β_1 is a cytokine that has been linked to both tumour inhibition[3,23] and promotion[5] at different stages of carcinogenesis in the breast tissue. *A priori* it is therefore difficult to predict the effect of the protein as well as the gene encoding for it. The leu10Pro polymorphism has been related to higher serum levels of TGF-β_1.[9] It has been hypothesised that polymorphisms that affect the level of expression of this cytokine may alter an individual's susceptibility to cancers including breast.[24] We found that women with the allele associated with higher levels of TGF-β_1 have an increased risk for breast cancer. According to these findings, the tumour suppressor

properties of TGF-β_1 would be rapidly exceeded by breast epithelial cells prone to oncogenesis.

While the majority of studies could not elucidate a clear relationship between TGF-β_1 and breast cancer risk,[12–14] in two studies, an increased risk for proline allele carriers was found.[1,11] Three other studies did not find a difference in risk[12–14] and one found an inverse association between the proline allele and breast cancer.[10] The latter was conducted in women over 65 years old.

In conclusion, our results suggest that the proline allele of the Leu10Pro polymorphism in the TGF-β_1 gene may play a role in the predisposition to breast cancer in Caucasian postmenopausal women.

Conflict of interest statement

None declared.

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