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Cancer Epidemiology, Biomarkers & Prevention

Research Article

Validation of Plasma Proneurotensin as a Novel Biomarker for the Prediction of Incident Breast Cancer

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Abstract

Background: High fasting plasma proneurotensin concentration was associated with the development of breast cancer in the Malmö Diet and Cancer Study (MDCS). Here, we aimed at replicating the initial finding in an independent second cohort.

Methods: The Malmö Preventive Project (MPP) is a population study and comprised 18,240 subjects when examined in 2002–2006. Of women without history of breast cancer at examination, we included all who developed breast cancer during follow-up ($n = 130$) until December 31, 2010, and a random sample of women without breast cancer until the end of follow-up ($n = 1{,}439$) for baseline plasma proneurotensin assessment (mean age, 70.0 ± 4.4 years). Proneurotensin was measured in fasting plasma samples and was related to the risk of later breast cancer development using multivariate logistic regression.

Results: Proneurotensin [odds ratio (OR) per standard deviation (SD) increment of LN-transformed proneurotensin] was significantly related to incident breast cancer [OR, 2.09; 95% confidence interval (CI), 1.79–2.44; $P < 0.001$; adjusted for age, body mass index (BMI), smoking, and hormone replacement therapy]. The effect estimate in the MPP was larger than in the discovery cohort (MDCS), with the main difference between the two cohorts being that women of the MPP study were on the average about 10 years older and follow-up time was shorter than that of the MDCS.

Conclusion: As initially found in the MDCS, fasting plasma proneurotensin was significantly associated with the development of breast cancer in the MPP study as well.

Impact: Measurement of plasma proneurotensin warrants further investigation as a blood-based marker for early breast cancer detection. *Cancer Epidemiol Biomarkers Prev; 23(8); 1672–6. ©2014 AACR.*

Introduction

Neurotensin is a 13–amino acid peptide primarily expressed in the central nervous system and gastrointestinal tract (1–3). Neurotensin binds to three different receptors: neurotensin receptor 1 and 2 (Ntsr1 and Ntsr2), which are G-protein–coupled receptors, and neurotensin receptor 3 (Ntsr3), which is non–G-protein–coupled and also known as Sortilin-1 (SORT1; refs. 4, 5). Interestingly, neurotensin has trophic effects on both normal and neoplastic tissue, and neurotensin and Ntsr1 have been suggested to be prognostic tumor biomarkers (6, 7). Neurotensin and Ntsr1 expression is common in human malignant ductal breast cancer tumors, and in mice xenografted with a malignant human breast cancer cell line, pharmacologic blockade or RNA silencing of the Ntsr1 reduces tumor growth (8, 9). The peripheral secretion of neurotensin is stimulated by food intake, especially of fat, and is known to regulate gastrointestinal motility and pancreatic and biliary secretion (10). Both central (intracerebroventricular) and peripheral (intraperitoneal) injection of neurotensin acutely reduces food intake in rats, an effect mediated through the Ntsr1 (11, 12), and has therefore been implicated in obesity as a satiety hormone.

Experimental data suggest neurotensin to have a role in breast tumor growth, and there is epidemiologic evidence that obesity increases the risk of breast cancer (6, 9, 13). Thus, we recently tested whether fasting plasma concentration of a stable 117–amino acid fragment from the N-terminal part of the prepro-neurotensin/neuromedin precursor hormone, referred to as proneurotensin, which is produced in stoichiometric amounts relative to the mature neurotensin, predicts the development of breast cancer during long-term follow-up in the Malmö Diet and Cancer Study (MDCS), a population-based prospective cohort study from Southern Sweden. We found that there was a strong and graded positive relationship between

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fasting plasma concentration of proneurotensin and later development of breast cancer with the top versus the bottom quartile of proneurotensin having an approximately 2.4-fold increased risk (14). Here, we attempted to replicate the finding in an independent cohort of women from the Malmö Preventive Project (MPP).

Materials and Methods

Study subjects

The study subjects are from the MPP. The MPP is a Swedish single-center prospective population-based study. Between 1974 and 1992, a total of 33,346 men and women of the homogenous ethnic background from the Malmö city area were recruited and screened for traditional risk factors of all-cause mortality and cardiovascular disease (CVD). A detailed description of baseline procedures may be found elsewhere (15, 16). In the years 2002–2006, all survivors from the original MPP cohort were invited for a reexamination. Of these, 18,240 participants ($n = 6{,}682$ women) responded to the invitation and were reexamined including blood sampling and immediate −80°C storage of EDTA plasma aliquotes. The reexamination in 2002–2006 represents the baseline time point in the current study. Of women participating in the 2002–2006 examination who had not participated in the MDCS discovery study (14) and were without history of breast cancer at this baseline examination, we included all who developed breast cancer during follow-up ($n = 130$) until December 31, 2010. As control group, we selected 1,500 women randomly among those participating in the 2002–2006 examination of the MPP who fulfilled the following criteria: (i) were without history of breast cancer at the 2002–2006 examination, (ii) remained free from breast cancer until the end of follow-up (December 31, 2010), and (iii) did not participate in the MDCS discovery study (14). Of these 1,500 women, fasting plasma samples for measurement of proneurotensin were available in 1,439. Thus, a total of 1,569 women were included using a case–control design. The baseline characteristics of the study population are shown in Table 1.

Table 1. Clinical characteristics of the study population

	Women in MPP ($n = 1{,}569$)
Age, y	70.0 ± 4.4
BMI, kg/m^2	27.1 ± 4.8
Current smokers, *n* (%)	307 (19.6)
HRT, *n* (%)	186 (11.9)

NOTE: Data are given as mean ± SD for normally distributed variables. Categorical data are presented as numbers (percentages).

Clinical examination, assays, endpoint assessment, and statistics

Body mass index (BMI) was defined as the weight in kilograms divided by the square of the height in meters. The use of hormone replacement therapy (HRT) and current cigarette smoking was assessed using a questionnaire. Proneurotensin was measured in stored fasting plasma specimens that were frozen at −80°C immediately at the MPP examination 2002–2006 using a recent chemiluminometric sandwich immunoassay to detect a proneurotensin precursor fragment (pro-NT 1–117) as described previously (17).

Breast cancer events were retrieved by record linkage with the Swedish Cancer Registry (SCR) using the unique 10-digit personal identification number. We included cancer *in situ* of the breast in our definition. Approximately 99% of all tumors diagnosed at Swedish Hospitals are registered in the SCR and 98% are morphologically verified (18, 19). Tumor site was registered according to ICD-7 and the International Classification of Diseases (ICD) version used at diagnosis.

We calculated odds ratios (OR) and 95% confidence intervals (CI) for the risk of incident breast cancer in a model adjusted for age, BMI, smoking, and use of HRT. Crude differences in proneurotensin concentration between cases of breast cancer and controls were calculated using the Mann–Whitney test. Because of a skewed distribution, proneurotensin was transformed with the natural logarithm (LN) and its OR is expressed per 1 standard deviation (SD) increase of the LN-transformed value in continuous analyses. We also analyzed quartiles of proneurotensin in relation to breast cancer risk.

Results

The mean age of the women in the study was 70 years (Table 1), and among the 130 women who developed breast cancer during follow-up, the mean time from the baseline examination until diagnosis of breast cancer was 3.1 ± 1.8 years. The median fasting plasma concentration ranged from 57.1 pmol/L in the lowest quartile of proneurotensin to 172 pmol/L in the highest (Table 2). The median (interquartile range) of proneurotensin in women who developed breast cancer as compared with those who did not was 123 (83.1–218) pmol/L versus 79.1 (63.0–110) pmol/L ($P < 0.001$). Each SD increment of LN-transformed proneurotensin was associated with a highly significant doubling of the OR for risk of incident breast cancer (Table 2). Additional adjustment for the use of antihypertensive medication, systolic blood pressure, diabetes mellitus, prevalent cardiovascular disease, and fasting concentrations of high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) resulted in an OR of 2.11 and 95% CI of 1.80–2.47, with $P < 0.001$. Thus, such additional adjustment did not lead to any attenuation of the strength of the results. There was no correlation between proneurotensin and the time from baseline to breast cancer diagnosis in women who developed breast cancer ($r = 0.04$; $P = 0.66$). Analysis of quartiles of

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Table 2. Fasting plasma concentration of pro-neurotensin (pro-NT) in relation to future risk of breast cancer in the MPP

	All women	*P*	Quartile 1 (lowest pro-NT)	Quartile 2	Quartile 3	Quartile 4	P_{trend}
N/N events[a]	1,569/130		391/9	394/21	392/30	392/70	
pro-NT, pmol/L[b]	81.4 (34.5–1,100)		57.1 (34.5–63.6)	71.2 (63.7–81.4)	94.0 (81.5–115)	172 (116–1,100)	
OR (95% CI)[c]	2.09 (1.79–2.44)	<0.001	1.0 (ref)	2.34 (1.06–5.18)	3.46 (1.62–7.40)	9.11 (4.47–18.6)	<0.001

Abbreviation: pro-NT, fasting plasma concentration of pro-neurotensin.

[a]*N/N* events refer to number of participants/number of first breast cancer events.

[b]pro-NT is given as median (range).

[c]OR (95% CI) are expressed per 1 SD increment of LN-transformed pro-NT and in analyses of quartiles the lowest quartile of pro-NT (quartile 1) was defined as the reference category and the OR (95% CI) for each of quartiles 2, 3, and 4 were compared with the reference quartile. Analyses were adjusted for age, BMI, current smoking, and the use of HRT.

proneurotensin indicated a graded positive relationship over quartiles 1–4 and women belonging to the highest quartile of proneurotensin had a 9-fold increased risk of developing breast cancer as compared with the lowest quartile of proneurotensin.

To test whether the effect size of the proneurotensin association with incident breast cancer was similar over different age ranges within the MPP, fasting proneurotensin was related to risk of incident breast cancer within each quartile of baseline age. The effect size of each SD increment of LN-transformed proneurotensin was associated with a significantly increased risk in all four strata of baseline age in the MPP. The OR (95% CI) for risk of incident breast cancer in the first quartile of age (57–67 years; $P < 0.001$) was 1.97 (1.48–2.62), whereas it was 1.73 (1.29–2.33) for the second age quartile (age 67–70 years), 2.94 (2.06–4.21) for the third age quartile (70–73 years), and 2.31 (1.57–3.39) for the fourth age quartile (73–80 years; all $P < 0.001$).

To test whether the original discovery study cutoff levels that defined quartiles in the MDCS (14) were applicable in the MPP, we divided the current study sample into four categories defined by proneurotensin cutoff levels derived from quartile boundaries of the MDCS discovery study (≤77.5, >77.5–108, >108–150, >150 pmol/L, $Q1_{MDCS}$–$Q4_{MDCS}$; ref. 14). As compared with the referent group ($Q1_{MDCS}$, defined as proneurotensin ≤77.5 pmol/L; ref. 14), increasing concentration of proneurotensin concentration ($Q2_{MDCS}$, $Q3_{MDCS}$, and $Q4_{MDCS}$) was associated with an OR (95% CI) of 2.89 (1.66–5.01), 3.87 (2.09–7.16), and 8.15 (4.81–13.8; $P < 0.001$).

Discussion

Being a satiety hormone implicated in breast cancer pathogenesis, we recently hypothesized that neurotensin may underlie part of the epidemiologic relationship between obesity and breast cancer development. We found a strong positive and graded relationship between fasting plasma concentration of a stable fragment of the precursor hormone, proneurotensin, and incident breast cancer among women in the MDCS (14). Here, we replicate the original finding in an independent sample of women from the MPP and demonstrate an even stronger relationship than in the discovery cohort. There was a 9-fold increased risk of developing breast cancer among women belonging to the top as compared with the bottom quartile of proneurotensin.

There are differences between the discovery cohort of MDCS (14) and the current study. One of those differences, which may in part explain the higher effect sizes observed in the current study, is that the follow-up time was shorter. In the current study, the average follow-up time was 3.1 ± 1.8 years from baseline to event in women who developed breast cancer compared with a follow-up time of 15.7 years in the MDCS (14). Another difference between the discovery and replication study samples is that the women of the replication study were on the average 70 years of age, suggesting that they were all postmenopausal at baseline, as compared with the discovery cohort, which had a mean age of 58 ± 6 years, and thus also included some premenopausal women. Thus, it can be speculated that the relationship between proneurotensin and breast cancer risk is stronger in postmenopausal women than in premenopausal women. Finally, we cannot exclude the possibility of bias caused by unmeasured factors, which influence both proneurotensin and the incidence of breast cancer. One such factor could be that high proneurotensin is associated with an intensive health-seeking behavior, which in turn could make early diagnosis of breast cancer more likely. However, the fact that there was no relationship between proneurotensin and time from baseline to breast cancer diagnosis, argues against such a bias.

We noted that proneurotensin concentration was nominally lower in the older MPP women than in the MDCS. One possible explanation of this is that neurotensin production declines with age in postmenopausal women. Importantly, however, the relationship between proneurotensin and incidence of breast cancer

was independent of age and was equally strong and highly significant across increasing strata of age, suggesting that despite a slightly lower proneurotensin concentration with increasing age, its relationship with breast cancer risk was similar in all age strata studied.

Continuous analyses and quartile analyses within the discovery study of MDCS as well as within the current replication sample of the MPP clearly indicated that the relationship between proneurotensin and breast cancer risk was direct and linear. To test the robustness of the association and the linearity, we applied discovery study–derived quartile cutoff levels ($Q1_{MDCS}$–$Q4_{MDCS}$) on the current study and found that the association remained highly significant with a seemingly linear relationship with ORs comparable with quartile analysis based on the actual MPP data.

In summary, this study replicates the previous findings in an independent cohort, showing that proneurotensin strongly predicts future incidence of breast cancer among women with no history of breast cancer. There are several potential clinical implications for healthy women having a high proneurotensin level. One would be to be considered for more frequent screening by mammography and possibly by regular magnetic resonance examination of the breasts. Another one, given the widespread and sometimes well-indicated use of HRT among postmenopausal women, would be to not prescribe this treatment to women with a high proneurotensin level as their increased breast cancer risk may override the beneficial effects of HRT.

We conclude that, as initially found in the MDCS study, fasting plasma proneurotensin was significantly and independently associated with the development of breast cancer in the MPP study as well. The replication suggests that proneurotensin may be of value for the identification of women at high risk of breast cancer development.

Disclosure of Potential Conflicts of Interest

G. Engström was formerly employed as an epidemiologist at AstraZeneca R&D. A. Bergmann is CEO of Sphingotec and has ownership interest (including patents) in the same. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: O. Melander, G. Engström, J. Struck, A. Bergmann, M. Orho-Melander
Development of methodology: O. Melander, A. Bergmann
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