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IGF-1 CA repeat variant and breast cancer risk in postmenopausal women

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ABSTRACT

IGF-I is an important growth factor for the mammary gland. We evaluated the relationship of the IGF-I CA_n polymorphism with breast cancer risk in Caucasian postmenopausal women and performed a meta-analysis of published data. The IGF-I CA_n polymorphism was genotyped in 4091 from the Rotterdam Study. A disease-free survival analysis was performed along with a meta-analysis of all available data on IGF-I CA_n polymorphism and breast cancer risk. During follow-up 159 women were diagnosed with breast cancer. The disease-free survival analysis adjusted for age at entry, age at menopause, body mass index and waist hip ratio yielded a HR = 0.97 (95% CI=0.59–1.58) for CA_{19} non-carriers against carriers. The meta-analysis using the random-effects model gave a pooled OR of 1.26 (95% CI = 0.95–1.82) for IGF-I CA_{19} non-carriers versus CA_{19} homozygous carriers.

According to these results, the IGF-I CA_{19} promoter polymorphism is not likely to predict the risk of breast cancer.

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1. Introduction

Insulin-like growth factor I (IGF-I) is a paracrine and autocrine growth factor that is secreted by many tissues.[1,2] In animals and humans its expression along with its receptor is necessary for normal growth and development.[1] IGF-I has also been implicated in tumour growth and metastasis.[1] Various studies have associated elevated serum levels of IGF-I with an increased risk for colorectal, prostate and premenopausal breast cancer.[3–5]

In the breast, stromal cells of the mammary connective tissue as well as adipocytes produce IGF-I since it is important in their differentiation.[6] Furthermore, IGF-I plays an important role in the proliferation and survival of the mammary gland cells, particularly during puberty and pregnancy when proliferation occurs.[7] IGF-I is also a potent mitogen and through this pathway the genes encoding for such proteins may be involved in cell proliferation.

Twin studies have determined that about 50% of the variability of circulating levels IGF-I is genetically determined.[8] The IGF-I gene is located on chromosome 12q22-q24.1 where a cytosine adenine (CA) repeat in the gene's promoter region has been associated with plasma IGF-I levels.[9,10] The CA_n repeat polymorphism is located 1 kb upstream from the transcription start site and in our study population, homozygote carriers of 19 (CA_{19}) repeat allele have been associated with

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lower plasma IGF-I levels,[10] while in another study the opposite was found.[9] A few studies have assessed the risk of breast cancer according to carriership of the CA_{19} allele of this polymorphism[11–17] generating contradicting results. These include a meta-analysis[13] of four studies that yielded a statistically significant increased risk for carriers of the CA_{19} allele, nevertheless there have been new publications on this association. Since the association between this variant and breast cancer is still not clear, especially in postmenopausal women, a nested case-control study was performed along with a meta-analysis of published data on the risk for this disease and this polymorphism, so as to clarify the relationship between this variant and the risk of breast cancer.

2. Patients and methods

2.1. Study population

Our study population is part of the Rotterdam study,[18] a follow-up study established between 1990 and 1993. Inhabitants of a suburb of Rotterdam aged 55 or older were invited to enroll and 7983 agreed (response rate = 78.1%). All subjects signed an informed consent approved by the Medical Ethics Committee of the Erasmus Medical Center.

2.2. Measurements

Information on well-known risk factors for breast cancer such as age at menarche, age at menopause, body mass index (BMI), hormone replacement therapy (HRT), waist hip ratio (WHR), parity and number of children were retrieved at baseline through a questionnaire, the methodology of this study has been described previously.[18] BMI was calculated by dividing the weight in kilograms by the height (in meters) squared.

2.3. Cancer diagnosis

Three different databases were used for case identification. First, cases diagnosed by general practitioners in the research area (Ommoord) were collected. 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. Subsequently, breast cancer cases were validated by a physician on the basis of medical records of the general practitioner, discharge letters and pathology reports (CS). Only identified cases that had also been pathologically confirmed were considered valid and were consequently used in the analysis. The index date (date of diagnosis) was defined as the earliest date found in the pathology report.

2.4. Genotyping

Of the 4878 women participating in our study, 4686 (96%) donated DNA samples and out of these, 4091 (87.3%) were successfully genotyped for the IGF-I CA_n repeat. The genotyping procedures have been described earlier.[19] Because the CA_{19} allele was the most common allele in our population, we followed the grouping procedures performed by previous authors and joined all other alleles to be CA_{-19}.[13,15] Therefore, we had three genotype categories: CA_{19} homozygotes, CA_{19} heterozygotes and CA_{19} non-carriers.

2.5. Data analysis

We tested Hardy–Weinberg equilibrium (HWE) of the CA_n repeat polymorphism using Markov-Chain Monte-Carlo approximation of the exact test implemented in the GENEPOP package V 3.3.[20] Since this is a follow-up study, we evaluated if loss to follow-up was dependent on genotype or other risk factors for breast cancer. Categorical variables such as parity, HRT, were compared between genotype groups using the χ^2 test. Continuous variables (age at entry, age at menopause, BMI and WHR) were compared using the independent sample Mann–Whitney test. In order to calculate disease-free survival, a Cox proportional hazards model was fitted using age as the underlying time of the model and taking the CA_{19} homozygotes as the reference category since these have been associated with low levels of circulating IGF in our population.[10] Only incident cases were used in this analysis due to the fact that age at entry was used as the underlying time of the Cox proportional hazards model. We adjusted for possible confounders such as age at entry, age at menopause, WHR and BMI since these variables could be dependent on genotype.

2.6. Meta-analysis

We searched PubMed until February 2007 for all case-control studies on the association of the IGF-I CA_n repeat variant and breast cancer. Our search strategy was based on the key word 'breast cancer' combined with 'IGF' and 'polymorphism'. To verify that all studies were retrieved, the reference lists of all publications were searched for additional studies. Articles were not included if genotype frequencies were not complete. In this analysis no time dependent variable was used, instead we calculated odds ratios (OR) and 95% confidence intervals (CI) using the random-effects model of the DerSimonian and Laird method.[21] The degree of heterogeneity between the study results was tested by the inconsistency statistic (I^2). Funnel plots were used to evaluate publication bias.[22] Data were analysed using Review Manager, version 4.2 (Cochrane Collaboration, Oxford, UK).

3. Results

The distribution of the IGF-I CA_n genotypes was in Hardy–Weinberg equilibrium proportions (CA_{19} homozygous carriers = 43.8%, CA_{19} heterozygotes = 44.1% and CA_{19} non-carriers = 12.1%, p-value = 0.24). Furthermore, a total of 7.9% of the women participating in our study were lost to follow-up. Nevertheless, this loss to follow-up was independent of IGF-I genotype or risk factors for breast cancer. The distribution of the risk factors included in our study did not differ significantly between genotypes (Table 1). There were 67 women with previously diagnosed breast cancer and additionally, during follow-up, 159 were further diagnosed. Out of the 159 incident cases, we found that 70 cases were CA_{19} homozygote carriers, 53 were CA_{19} heterozygotes and 36 were the CA_{19}

Table 1 – General characteristics of the study population stratified by IGF-I CA_{19} repeat genotype

Genotype	Homozygote carriers	Heterozygote carriers	Non-carriers	Overall
Total studied % (N)	43.8 (1830)	35.2 (1473)	21 (878)	4181
Mean age of entry (SD)	70.6 (9.8)	70.5 (9.8)	71 (10.1)	70.7 (17.5)
Mean age at death	84.8 (8.8)	84.2 (8.7)	84.1 (8.6)	84.3 (8.7)
Mean age at menopause (SD)	48.9 (5.3)	48.7 (5.1)	48.9 (5.1)	48.8 (5.1)
Mean number of children	2.1 (1.7)	2.1 (1.78)	2.0 (1.6)	2.1 (1.7)
Parity (%) (⩾ 1 child)	79.8 (1362)	79.3 (1368)	78.7 (369)	79.4 (3099)
Hormone replacement therapy (%)	18.7 (247)	20.1 (275)	19.3 (73)	19.4 (595)
Waist–hip ratio	0.87 (0.09)	0.87 (0.09)	0.87 (0.09)	0.87 (0.1)
Mean body mass index (SD)	26.8 (4.1)	26.7 (3.9)	26.8 (4.1)	26.7 (4.1)

CA_{19} = CA_{19} allele carrier.

non-carriers. There were no statistically significant differences in breast cancer frequency by genotype (p-value = 0.82).

A disease-free survival analysis taking age at entry as the underlying time of the Cox proportional hazard's model and adjusting for age at menopause, BMI, and WHR yielded a HR = 0.85 (95%CI = 0.52–1.39) for CA_{19} heterozygotes versus CA_{19} homozygote carriers and a HR = 0.95 (95%CI = 0.56–1.62) for CA_{19} non-carriers against CA_{19} homozygote carriers (Fig. 1). When pooling heterozygotes and homozygotes for the CA_{19} repeat and compared them to non-carriers, we obtained an HR of 0.97 (95% CI = 0.59–1.58) for non-carriers versus CA_{19} carriers. None of the covariates included in our analyses significantly increased the risk for breast cancer in our model.

The search for articles on the relation between the IGF-I CA_n polymorphism and breast cancer risk retrieved eight studies. One study[13] had already carried out a meta-analysis but only included four publications in total, so we updated the analysis by including new available published data. Three studies were not included because genotyping frequencies were not complete.[12,17,23] For this analysis the prevalent cases in our study population were included along with the incident cases. The meta-analysis yielded a pooled OR = 1.05 (95% CI = 0.95–1.17) for CA_{19} heterozygous carriers versus CA_{19} homozygous carriers, and OR = 1.26 (95% CI = 0.87–1.82) for CA_{19} non-carriers versus CA_{19} homozygous carriers, in contrast to the results found in our study (Fig. 2). Nevertheless, there was a significantly high inter-study heterogeneity in the meta-analysis (p-value < 0.00001 for the comparison between CA_{19} non-carriers against CA_{19} homozygote carriers), which makes the interpretation of the results difficult. The evaluation of the funnel plots did not show evidence of publication bias.

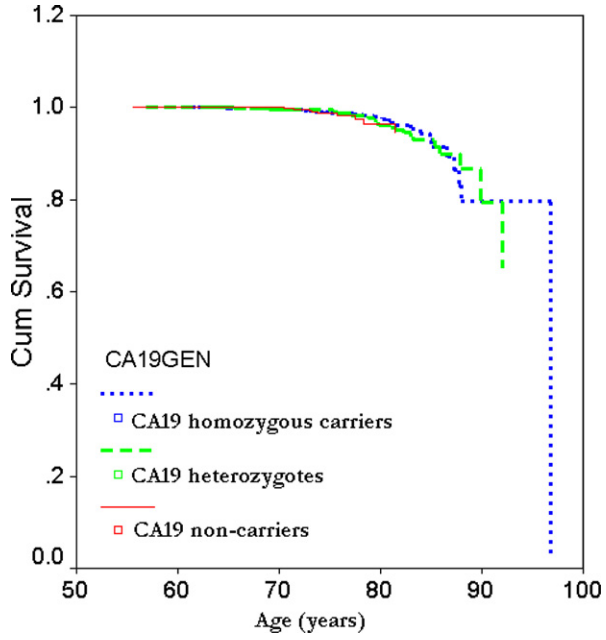

Fig. 1 – Breast cancer free survival by IGF-I genotype.

4. Discussion

We conducted a disease-free survival analysis to evaluate the role of the IGF-I CA_n polymorphism on the risk of postmenopausal breast cancer. Additionally, we performed a meta-analysis using available published data. We did not find any difference in risk of breast cancer between the different CA_n genotypes in our study population and the meta-analysis.

The results of our study yielded a non-statistically significant decreased risk for CA_{19} carriers, while the meta-analysis yielded a result in the opposite direction. However, both estimates are not significant, suggesting that this polymorphism is not associated with breast cancer risk. Nevertheless, findings in the meta-analyses including 3574 patients were also negative.

Polymorphisms that influence the level of expression of IGF-I are likely to affect lifetime exposure to this molecule by both endocrine and autocrine mechanisms.[24] The evaluation of the IGF-I promoter variant presented here allows us to evaluate lifetime exposure to circulating levels of IGF-I decrease substantially with age.[25] Earlier, we have shown that this polymorphism is associated with plasma levels of IGF-I.[10] Our findings are in accordance with those of patients with postmenopausal breast cancer showing no effect of IGF-I plasma serum levels.[24] Moreover, there is some evidence for an effect of serum IGF-I in premenopausal breast cancer, which may be explained by interaction of IGF-I with oestrogen.[26]

It should also be taken into account that the small number of cases (n = 159 incident) in the performed analysis could account for lack of power in an association analysis of such a small effect as is expected from common variants.[27] Our find-

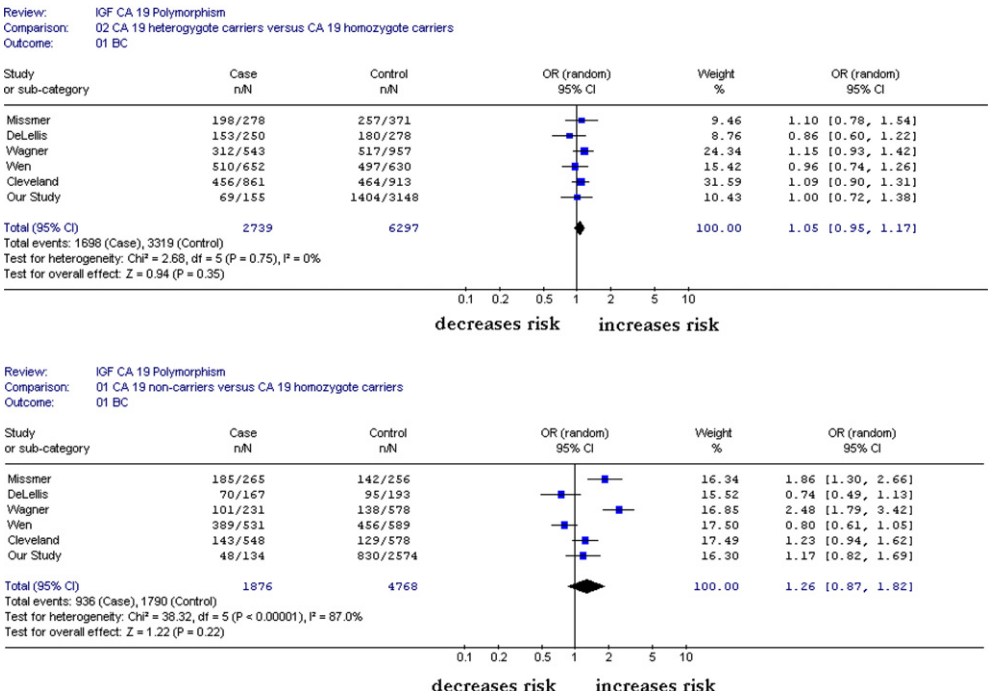

Fig. 2 – Meta-analysis.

ings suggest that genetically determined IGF-I exposure is not relevant for postmenopausal breast cancer.

Contributions

F. Liu, M.P.W.A. Houben and C.M. van Duijn participated in the design and writing of the manuscript. A. Arias Vásquez and A.C.J.W. Janssens contributed to the design of the study and also participated in the data analyses. J.W.W. Coebergh, B.H.Ch. Stricker and A. Hofman aided in the data collection and the writing of this manuscript as well. C. Siemes participated in the ascertainment of the breast cancer cases.

Conflict of interest statement

None declared.

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REFERENCES

1. Ibrahim YH, Yee D. Insulin-like growth factor-I and cancer risk. *Growth Horm IGF Res* 2004;**14**:261–9.
2. Marshman E, Streuli CH. Insulin-like growth factors and insulin-like growth factor binding proteins in mammary gland function. *Breast Cancer Res* 2002;**4**:231–9.
3. Ma J, Pollak MN, Giovannucci E, et al. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. *J Natl Cancer Inst* 1999;**91**:620–5.
4. Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet* 1998;**351**:1393–6.
5. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet* 2004;**363**:1346–53.
6. Smith PJ, Wise LS, Berkowitz R, Wan C, Rubin CS. Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes. *J Biol Chem* 1988;**263**:9402–8.
7. Richert MM, Wood TL. The insulin-like growth factors (IGF) and IGF type I receptor during postnatal growth of the murine mammary gland: sites of messenger ribonucleic acid expression and potential functions. *Endocrinology* 1999;**140**:454–61.
8. Jernstrom H, Deal C, Wilkin F, et al. Genetic and nongenetic factors associated with variation of plasma levels of insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in healthy premenopausal women. *Cancer Epidemiol Biomarkers Prev* 2001;**10**:377–84.

9. Rosen CJ, Kurland ES, Vereault D, et al. Association between serum insulin growth factor-I (IGF-I) and a simple sequence repeat in IGF-I gene: implications for genetic studies of bone mineral density. *J Clin Endocrinol Metab* 1998;**83**:2286–90.
10. Rietveld I, Janssen JA, van Rossum EF, et al. A polymorphic CA repeat in the IGF-I gene is associated with gender-specific differences in body height, but has no effect on the secular trend in body height. *Clin Endocrinol (Oxf)* 2004;**61**:195–203.
11. Missmer SA, Haiman CA, Hunter DJ, et al. A sequence repeat in the insulin-like growth factor-1 gene and risk of breast cancer. *Int J Cancer* 2002;**100**:332–6.
12. Yu H, Li BD, Smith M, Shi R, Berkel HJ, Kato I. Polymorphic CA repeats in the IGF-I gene and breast cancer. *Breast Cancer Res Treat* 2001;**70**:117–22.
13. Wen W, Gao YT, Shu XO, et al. Insulin-like growth factor-I gene polymorphism and breast cancer risk in Chinese women. *Int J Cancer* 2005;**113**:307–11.
14. DeLellis K, Ingles S, Kolonel L, et al. IGF1 genotype, mean plasma level and breast cancer risk in the Hawaii/Los Angeles multiethnic cohort. *Br J Cancer* 2003;**88**:277–82.
15. Wagner K, Hemminki K, Israelsson E, et al. Polymorphisms in the IGF-1 and IGFBP 3 promoter and the risk of breast cancer. *Breast Cancer Res Treat* 2005;**92**:133–40.
16. Cleveland RJ, Gammon MD, Edmiston SN, et al. IGF1 CA repeat polymorphisms, lifestyle factors and breast cancer risk in the Long Island Breast Cancer Study Project. *Carcinogenesis* 2006;**27**:758–65.
17. Bageman E, Ingvar C, Rose C, Jernstrom H. Absence of the common Insulin-like growth factor-1 19-repeat allele is associated with early age at breast cancer diagnosis in multiparous women. *Br J Cancer* 2007;**96**:712–7.
18. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol* 1991;**7**:403–22.
19. Vaessen N, Heutink P, Janssen JA, et al. A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. *Diabetes* 2001;**50**:637–42.
20. Raymond M. R.F. Genepop (version 1.2): population genetics software for exact tests and ecumenism. *J Heredity* 1986;**86**:248–9.
21. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;**7**:177–88.
22. Macaskill P, Walter SD, Irwig L. A comparison of methods to detect publication bias in meta-analysis. *Stat Med* 2001;**20**:641–54.
23. Figer A, Karasik YP, Baruch RG, et al. Insulin-like growth factor I polymorphism and breast cancer risk in Jewish women. *Isr Med Assoc J* 2002;**4**:759–62.
24. Fletcher O, Gibson L, Johnson N, et al. Polymorphisms and circulating levels in the insulin-like growth factor system and risk of breast cancer: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2005;**14**:2–19.
25. Augustin LS, Dal Maso L, Franceschi S, et al. Association between components of the insulin-like growth factor system and endometrial cancer risk. *Oncology* 2004;**67**:54–9.
26. Yu H, Shu XO, Li BD, et al. Joint effect of insulin-like growth factors and sex steroids on breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2003;**12**:1067–73.
27. Pharoah PD, Antoniou A, Bobrow M, Zimmern RL, Easton DF, Ponder BA. Polygenic susceptibility to breast cancer and implications for prevention. *Nat Genet* 2002;**31**:33–6.