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No association of *TGFB1* L10P genotypes and breast cancer risk in *BRCA1* and *BRCA2* mutation carriers: a multi-center cohort study

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Abstract

Background—The transforming growth factor β -1 gene (*TGFBI*) is a plausible candidate for breast cancer susceptibility. The L10P variant of *TGFBI* is associated with higher circulating levels and secretion of TGF- β , and recent large-scale studies suggest strongly that this variant is associated with breast cancer risk in the general population.

Methods—To evaluate whether *TGFBI* L10P also modifies the risk of breast cancer in *BRCA1* or *BRCA2* mutation carriers, we undertook a multi-center study of 3,442 *BRCA1* and 2,095 *BRCA2* mutation carriers.

Results—We found no evidence of association between *TGFBI* L10P and breast cancer risk in either *BRCA1* or *BRCA2* mutation carriers. The per-allele HR for the L10P variant was 1.01 (95% CI: 0.92–1.11) in *BRCA1* carriers and 0.92 (95% CI: 0.81–1.04) in *BRCA2* mutation carriers.

Conclusions—These results do not support the hypothesis that *TGFBI* L10P genotypes modify the risk of breast cancer in *BRCA1* or *BRCA2* mutation carriers.

Keywords

BRCA1; *BRCA2*; Risk modifiers; Hereditary cancer

Introduction

Transforming growth factor- β (TGF- β) is encoded by the *TGFBI* gene (OMIM 190180; Chromosome 19q13.1), and regulates normal mammary gland development and function by activating the TGF- β signaling pathway [1]. TGF- β appears to both inhibit the development of early benign breast tumors as well as promote tumor invasion and metastasis once somatic mutations have abrogated the normal TGF- β tumor suppressor function [1–3].

A number of studies have suggested that *TGFBI* L10P (rs1982073) is involved in the etiology and severity of sporadic breast cancer. L10P is associated with higher circulating levels of acid-activatable TGF- β [4] and increased TGF- β secretion in vitro [5], which led to the hypothesis that this variant plays a functional role in breast carcinogenesis. A number of epidemiological association studies have been published, but the results of these studies have not been consistent [3,5–17]. Data from large case–control series, including a recent multi-center study of 12,946 cases and 15,109 controls by the Breast Cancer Association Consortium [16], identified small but statistically significant associations with breast cancer risk, with a per-allele increase in

risk of 8% (95%CI 1.04–1.31). This study also reported some evidence of a stronger association among cases diagnosed below age 40 years. Given the biological and epidemiological evidence that *TGFB1* L10P may be associated with early onset breast cancer, we undertook a large multi-center cohort study of *BRCA1* and *BRCA2* mutation carriers to determine if the L10P variant modulates breast cancer risk on the background of these mutations.

Materials and methods

Study population

Eligibility was restricted to female carriers of pathogenic mutations in *BRCA1* or *BRCA2* who were 18 years old or older. Information collected included the year of birth, exact mutation description, family membership, country of residence, age at last follow-up, ages at breast and ovarian cancer diagnosis and information on bilateral prophylactic mastectomy. Details of the CIMBA initiative and information about the participating centers can be found elsewhere [18]. Women were included in the analysis if they carried mutations that were pathogenic according to generally recognized criteria (see also: <http://research.nhgri.nih.gov/projects/bic/>). All carriers participated in clinical and research studies at the host institutions under IRB-approved protocols.

Fifteen centers submitted data to this study. Two of those submitted data that did not meet the genotyping quality control criteria (see below), and were excluded from analysis. Thirteen centers (Table 1) submitted data that met the CIMBA inclusion criteria, as described below: BCFR (six centers in USA, Ontario, and Australia), DKFZ (Germany), EMBRACE (UK & Eire), GEMO (France and USA), GC-HBOC (Germany), HCSC (Spain), HEBCS (Finland), INHERIT (Canada), kConFab (Australia), Mayo Clinic (USA), NCI (Bethesda), OCGN (Canada), and MOD-SQUAD (USA). All centers obtained informed consent for participation in this research. Data were sent in anonymized fashion to the central data repository in accordance with approved data sharing protocols at each contributing center.

After considering genotyping quality control criteria (described below), a total of 3,442 *BRCA1* and 2,095 *BRCA2* mutation carriers yielded a *TGFB1* genotype for analysis. Six individuals carried a mutation in both *BRCA1* and *BRCA2*. For the purposes of this analysis, these six women were included in the *BRCA1* mutation group only, since the breast cancer risk in *BRCA1* mutation carriers has been noted to be higher than *BRCA2* mutation carriers at young ages [19]. Individuals were classified according to their age at diagnosis of breast cancer or their age at last follow-up. For this purpose, individuals were censored at the age of the first breast cancer diagnosis ($n = 3,113$), ovarian cancer diagnosis ($n = 486$), bilateral prophylactic mastectomy ($n = 112$) or the age at last observation ($n = 1,826$). Only individuals censored at breast cancer diagnosis were assumed to be affected.

Genotyping

Laboratory analysis to determine L10P (i.e., T29C) genotypes was undertaken in each center using a PCR-based Taqman assay. Allele specific oligonucleotide probes were added to the PCR reaction and are cleaved by the 5' exonuclease activity of Taq polymerase only if genomic DNA includes the corresponding variant of the polymorphism. The probes used were either an MGB probe or were labeled at the 5' end with the reporter dye (FAM or VIC) and at the 3' end with the quencher dye (TAMRA). The PCR primers were 5'-CCCACCACACCAGCCCTGTTC-3' in the forward direction and 5'-TTCCGCTTACCAGCTCCATGT-3' in the reverse direction. The probe for the T allele was 5'-TAGCAGCAGCAGCAGCAGCCGC-3' and for the C allele was 5'-TAGCAGCAGCGGCAGCAGCCG-3'. The annealing temperature was 60°C.

Because the assays were performed in multiple laboratories, a series of quality control measures were implemented to maximize genotype integrity. All centers duplicated genotyping on at least 2% of their samples, included no template (water) controls in every plate, and all plates were set up to have a random mixture of affected and unaffected carriers. A study was included in the analysis only if the genotype call rate was over 95%. The concordance between duplicates had to be at least 98%. We also evaluated deviation from Hardy–Weinberg equilibrium (HWE) among a subset of unrelated subjects for each study separately. The genotype frequencies among unrelated individuals for all studies were consistent with HWE at the 5% significance level. Fifteen centers initially provided data to this study. However, data from two studies were excluded because they did not meet the criteria for inclusion because their data exceeded the required discordance rate among duplicates of 2% and a failure rate >5%. Therefore, data on 13 centers were included in this study.

Statistical analysis

The data were analyzed within a survival analysis framework by modeling the retrospective likelihood of the observed genotypes, conditional on the disease phenotypes [20]. This method corrects for the likely over-sampling of affected individuals, since carriers are recruited through genetic clinics [21]. The effect of each SNP was modeled as a per-allele hazard ratio (HR). In addition, we estimated the effect of heterozygote and homozygote classes for *BRCA1* and *BRCA2* mutation carriers separately. HRs were assumed to be independent of age (i.e., by assuming a Cox proportional hazards model). Baseline age-specific incidence rates in the Cox proportional hazards model were chosen such that the overall breast cancer incidence rates, averaged over all genotypic categories, agreed with external estimates of *BRCA1* and *BRCA2* incidence rates [20]. Between-study heterogeneity was examined by comparing the models which allowed for study specific log-hazard ratios against models in which the same log-hazard ratio was assumed to apply to all studies. All analyses were stratified by study group and country of residence (where numbers were sufficiently large), and used calendar-year and cohort-specific breast cancer incidence rates for *BRCA1* and *BRCA2* [20]. Compound *BRCA1* and *BRCA2* mutation carriers were assumed to develop the disease according to the *BRCA1* incidence rates, since the penetrance of *BRCA1*-associated breast cancer is known to be earlier in age than that of *BRCA2*-associated breast cancer [19]. Therefore, on average cancers diagnosed in *BRCA1* mutation carriers would precede those diagnosed in *BRCA2* mutation carriers. A robust variance estimation approach was used to allow for the non-independence among related carriers [22,23]. Analyses were carried out using the pedigree analysis software MENDEL [24].

Results and discussion

In our data, the frequency of the 10P variant allele was 41.6% in *BRCA1* mutation carrier controls with no cancer diagnosis, LL, LP, and PP genotype frequencies of 34.4, 48.1, and 17.5%, respectively. In *BRCA2* mutation carrier controls with no prior cancer diagnosis, the 10P allele frequency was 40.2%, with LL, LP, and PP genotype frequencies of 36.5%, 47.3%, and 16.1%, respectively. The frequency of L10P variants did not differ between controls with no history of cancer between *BRCA1* and *BRCA2* mutation carriers ($\chi^2 = 1.91$, $df = 2$, $P = 0.385$). Overall and in each center, the frequency of L10P alleles did not deviate significantly from Hardy–Weinberg proportions.

There was no significant evidence of association between *TGFB1* L10P and breast cancer risk in either *BRCA1* or *BRCA2* mutation carriers (P-trend: 0.821 and 0.24, respectively; Table 1). The per-allele HR was estimated to be 1.01 (95%CI: 0.92–1.11) among *BRCA1* carriers and 0.92 (95%CI: 0.81–1.04) among *BRCA2* mutation carriers. In *BRCA1* mutation carriers, the HR associated with the heterozygote LP genotype was 1.04 (95%CI: 0.91–1.20), and the HR

associated with the homozygote PP genotype was 0.98 (95%CI: 0.81–1.17). In *BRCA2* mutation carriers the heterozygote and homozygote HRs were 0.89 (95%CI: 0.74–1.07) and 0.88 (95%CI: 0.68–1.13), respectively.

There was no evidence for heterogeneity of effects by center in *BRCA1* mutation carriers (P -value = 0.22), but there was some evidence for heterogeneity of effects across centers in *BRCA2* mutation carriers (P = 0.005). However, this appears to be in part explained by the statistically significant increased risk in the DKFZ study, which involved a sample size of only 50 *BRCA2* mutation carriers.

Our results do not support the hypothesis that *TGFBI* L10P influences breast cancer risk in *BRCA1* or *BRCA2* mutation carriers. We have reported a lack of statistical significance for such an association, and also have observed that the direction of the L10P genotype effects in most of our contributing centers, the HR estimate for the P allele is below unity, i.e. in the opposite direction to the estimated OR in the population studies. The upper bound of the 95% CI for *BRCA1* does not exclude previous estimates of *TGFBI* L10P effect including the results published by the Breast Cancer Association Consortium (BCAC) [16], which reported a per-allele OR = 1.08 (95%CI 1.04–1.11) in a sample of 15,109 cases and 12,946 controls, and a previous meta-analysis of published studies involving 4,021 cases and 8,253 controls [25], which report a per-allele OR = 1.04. However, the upper bound of the 95%CI estimate for *BRCA2* is on the lower 95% confidence limit from the BCAC study.

The differences between the risk estimates from the current study and those from the large case–control studies of women not selected for *BRCA1/2* mutations [16,17] can be interpreted in a number of ways. First, it is possible that *TGFBI* L10P does influence breast cancer risk in the general population, but that this variant does not affect the risk of breast cancer in *BRCA1* or *BRCA2* carriers (or does so to a weaker extent). The L10P proline allele, associated with increased TGF- β secretion, may be associated with a reduced risk of in situ tumors but an increased risk of invasive cancer [1,2,16]. Breast tumor characteristics in *BRCA1* mutation carriers, including histopathological features related to disease aggressiveness, differ substantially from those in the general population [26]. Similarly, there is some evidence that the phenotypic characteristics of *BRCA2* tumors may also differ from sporadic cases [27]. Therefore, the biological effect of *TGFBI* in *BRCA1*- and possibly *BRCA2*-associated tumors may be different than in tumors arising from the general population. It is also worth noting that the same relative risk would only be expected if *BRCA1/2* and *TGFBI* combine multiplicatively. It is possible that *TGFBI* L10P does increase risk in carriers, but does not “interact” multiplicatively, so that its effect is not detectable in carriers who are already at very high risk.

Second, it is possible that *TGFBI* L10P is not causally associated with breast cancer risk in the general population, and the previous reports may represent false positive associations. Although the large BCAC study found a highly statistically significant effect of L10P, with no evidence for heterogeneity of effects across studies, the level of significance (P = .00003) is not beyond reasonable doubt in the context of genetic association studies [16]. The literature contains reports of positive and inverse associations as well as null results for *TGFBI* L10P [3,5–15,28], so there is not complete consistency across studies or populations with respect to the role of this variant in breast cancer etiology. Finally, it is possible that there is a true association, but that it is weaker than previously reported. A per allele odds ratio of 1.02–1.03 would then be statistically compatible with the HR estimates seen in carriers. Further large association studies would be required to evaluate this possibility. These results emphasize that very large studies are essential to evaluate the effects of genetic polymorphisms on disease risk.

Conclusions

The evidence that *TGFBI* L10P is associated with breast cancer risk in the general population has been mixed and no strong conclusions have been reached about the role of this variant in breast cancer etiology. The present study provides no evidence that this variant is a modifier of breast cancer risk in *BRCA1* or *BRCA2* carriers.

Abbreviations

CIMBA	Consortium of Investigators of Modifiers of BRCA1/2
BCAC	Breast Cancer Association Consortium
BCFR	Breast Cancer Family Registries
DKFZ	Deutsche Krebsforschungszentrum
EMBRACE	Epidemiological Study of Familial Breast Cancer
GC-HBOC	German Consortium of Hereditary Breast and Ovarian Cancer
HEBCS	Helsinki Breast Cancer Study
HCSC	Hospital Clinico San Carlos
HR	Hazard ratio
INHERIT	Interdisciplinary Health Research International Team Breast Cancer Susceptibility
KConFab	The Kathleen Cunningham Consortium for Research into Familial Breast Cancer
MOD-Squad	Modifier study of quantitative effects on disease
NCI	National Cancer Institute Study
OCGN	Ontario Cancer Genetics Network Study
GEMO	Genetic Modifiers of cancer risk in BRCA1/2 mutation carriers study
TGF-β	Transforming growth factor- β

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Table 1

Association of *TGFB1* L10P with breast cancer by *BRCA1/2* mutation status from retrospective likelihood analysis: per-allele effects

Center	BRCA1 ^a		BRCA2			
	n	HR	Robust 95%CI	n	HR	Robust 95%CI
BCFR	137	1.24	0.88–1.73	108	0.82	0.37–1.82
DKFZ	118	0.73	0.45–1.19	50	3.04	1.83–5.05
EMBRACE	656	1.03	0.83–1.27	473	1.02	0.81–1.29
GEMO	869	0.92	0.75–1.13	384	0.74	0.54–1.00
GC-HBOC	457	1.08	0.83–1.40	249	0.94	0.64–1.36
HCSC	90	0.81	0.43–1.51	78	1.16	0.63–2.13
HEBCS	93	1.49	1.11–2.01	91	0.92	0.58–1.48
INHERIT	72	0.86	0.49–1.53	82	0.66	0.41–1.06
KConFab	385	1.13	0.86–1.49	305	0.87	0.64–1.18
Mayo	106	1.21	0.82–1.79	54	1.57	0.70–3.56
NCI	132	1.36	0.91–2.02	49	0.51	0.13–2.00
OCGN	189	0.85	0.57–1.28	137	1.02	0.64–1.61
MOD-SQUAD	138	0.85	0.52–1.40	37	0.87	0.25–3.02
Total Sample	3,442	1.01	0.92–1.11	2,095	0.92	0.81–1.04
12 df Wald χ^2 test of heterogeneity among centers (<i>P</i> -value)	15.512 (<i>P</i> = 0.22)		28.372 (<i>P</i> = 0.005)			

^aIncludes 6 women who carried both *BRCA1* and *BRCA2* mutations