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The *TGFBR1**6A/9A polymorphism is not associated with differential risk of breast cancer

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Abstract A polymorphic 9-bp deletion in exon 1 of *TGFBR1* (*TGFBR1**6A) has been identified as a low-penetrance cancer susceptibility allele. The strongest association in the initial studies was with breast cancer; however, these studies included patients with different types of cancer, including colon, cervical and breast carcinomas, with only a small proportion being breast cancer patients. In subsequent case–control studies focussing on breast cancer alone, the results have been equivocal. In order to clarify whether *TGFBR1**6A is associated with breast cancer risk, we have genotyped this polymorphism in 988 breast cancer cases and 1,016 controls from the West of Ireland and also performed a meta-analysis of previously published data (5,150 cases and 6,344 controls). In our series from the West of Ireland, we found no association (genotypic odds ratio (OR) under a dominant model = 0.93, 95% confidence interval (CI) 0.73–1.19, $P = 0.57$; allelic OR = 0.93, 95% CI 0.74–1.15, $P = 0.49$). Meta-analysis showed evidence of heterogeneity among studies. Using the random effects model, it was found

that there was no evidence of an association of the *6A allele with breast cancer (genotypic OR under a dominant model = 1.10, 95% CI = 0.94–1.28, $P = 0.24$, allelic OR = 1.12, 95% CI 0.97–1.31, $P = 0.13$). In conclusion, our study shows that there is no association between *TGFBR1**6A and breast cancer risk.

Keywords Breast cancer · Genetic susceptibility · *TGFBR1* · West Ireland · Meta-analysis

Introduction

Transforming growth factor beta receptor type I (*TGFBR1*) is a serine–threonine protein kinase, which mediates the growth-inhibitory signals from *TGFB1* through a complex with *TGFBR2*. *TGFBR1* contains a common polymorphism in exon 1 resulting in a deletion of three alanines from a stretch of nine alanines (*TGFBR1**6A) [1]. This 9-bp deletion is located within the predicted signal sequence cleavage region. Functional studies have suggested that *TGFBR1**6A does not respond as well as the longer *TGFBR1**9A allele to the growth inhibitory signals of *TGFB1* [2, 3]. Several small studies have found that individuals who carry a *TGFBR1**6A allele are at increased risk of cancer [3–6]. A meta-analysis of seven published studies showed that carrying at least one copy of the *TGFBR1**6A allele increased the risk of cancer overall [colon, cervix, breast, ovarian and haematological cancers with an odds ratio (OR) of 1.26; 95% confidence interval (CI), 1.07–1.49], and in particular, increased the risk of breast cancer (OR, 1.48; 95% CI, 1.11–1.96) [5]. A further meta-analysis was then performed, which included an additional three unpublished studies and again *TGFBR1**6A was found to be a tumour susceptibility allele, increasing the risk of breast, ovarian and colorectal cancer (all

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cancers—OR 1.24, 95% CI 1.10–1.40; breast cancer—OR 1.38, 95% CI 1.14–1.67) [7]. This second meta-analysis included 7,850 individuals, of whom 1,420 were breast cancer cases. However, one of the problems with the studies in these meta-analyses was their individually small size, the mean number of cases per study being 248 (range 48–611). In 2007, Cox et al. [8] genotyped the *TGFBR1**6A/9A polymorphism in the largest cohort of breast cancer cases studied to date (1,187 cases, 1,673 controls) and found no association (OR = 0.95, 95% CI 0.76–1.17). A meta-analysis including this data and previously published data also showed no association (OR 1.10, 95% CI 0.89–1.38). Subsequently, a Swedish group [9] analysed their cohort of breast cancer cases (763 cases, 852 controls) and although they found no overall association, they did find a weak association with low-risk familial breast cancer and poorly differentiated breast cancer.

Since the association between the *TGFBR1**6A and breast cancer remains uncertain, we genotyped the 6A/9A alleles in 988 breast cancer cases and 1,016 controls from the West of Ireland. We then performed a meta-analysis with all previously published data, in order to clarify whether there is any association between *TGFBR1**6A and breast cancer risk.

Methods

About 988 breast cancer cases and 1,016 controls from the West of Ireland were collected with appropriate ethical approval, as part of the Breast cancer In Galway Genetics Study (BIGGS). This population has been subjected to few demographic movements and is consequently relatively homogenous, reducing allelic and genotypic heterogeneity. The mean age of the cases at diagnosis was 53 years (range 24–90 years). They were not selected with regard to family history of breast or ovarian cancer, personal history of ovarian cancer, the presence of a contralateral breast cancer or other second primary cancer. About 122 patients had a first degree relative with breast cancer (mean age 53.5 years), 153 patients had a second degree relative with breast cancer (mean age 50.5 years) and 40 patients had bilateral breast cancer (mean age 53.5 years). All controls were from a West of Ireland lineage (as were cases) and comprised women over the age of 60 years, with no self-reported personal

history of any cancer and no reported family history of breast or ovarian cancer.

The power of our study was 80% at alpha 0.05 to detect a genotypic OR of 1.38 (which is the level of association previously described [7]).

DNA was extracted from blood using the Chemagic Magnetic Separation Module (Chemagen, Baesweiler, Germany) using the manufacturer's reagents. The *TGFBR1**6A polymorphism was amplified using the following primers: forward GAGGCGAGGTTTGGCTGGGGTGAGG, reverse CATGTTTGAGAAAGAGCAGGAGCG. PCR products were analysed using the Genotyper programme (Applied Biosystems, CA).

Deviation of genotype frequencies from those expected under Hardy–Weinberg equilibrium (HWE) was assessed by χ^2 test. The risk associated with *TGFBR1**6A was estimated by unconditional logistic regression to give ORs under allelic, dominant and recessive models, with their associated 95% CIs.

For the meta-analysis, we undertook a Pubmed search using the following criteria: “*TGFBR1* AND breast”; “transforming growth factor beta receptor AND breast”. Studies were included in the analysis, if genotype frequencies were reported and controls were in HWE ($P > 0.05$), irrespective of sample size. Data were combined using the Mantel–Haenszel method. The data from identified studies were combined with our West of Ireland cohort to derive an overall estimate of breast cancer risk using fixed and random effects models. Cochran's Q statistic to test for heterogeneity and I^2 statistic to quantify the proportion of the total variation due to heterogeneity were calculated. All statistical analyses were undertaken using STATA 9.2 (Stata Corp, College Station, TX).

Results and discussion

Genotypes were obtained from 96% of samples and frequencies did not deviate significantly from HWE ($\chi^2 = 0.14$, $P = 0.9$; Table 1). There was no evidence of an association between *TGFBR1**6A and breast cancer under any of the tests used (Table 1); for example the OR for the allelic test of association was 0.93 (95% CI 0.74–1.15, $P = 0.49$). The OR for the *TGFBR1**6A homozygote was 0.76 (95% CI 0.33–1.75, $P = 0.53$) and that for the

Table 1 *TGFBR1* genotype results and odds ratios from BIGGS study

	9A/9A	9A/6A	6A/6A	Allelic OR	Genotypic OR (het)	Genotypic OR (hom)
Cases	796	154	10	0.93	0.93	0.76
Controls	785	160	13	95% CI 0.74–1.15 $P = 0.49$	95% CI 0.73–1.19 $P = 0.57$	95% CI 0.33–1.75 $P = 0.53$

*TGFBR1**6A heterozygote was 0.93 (95% CI 0.73–1.19, $P = 0.57$) (Table 1). We had obtained clinico-pathological data (grade, ER status, Her2 status and family history) on 795 of our cases, as described previously [10], but there was no evidence of an association between *TGFBR1* genotype and any of these variables (χ^2 test, $P > 0.2$ in all cases).

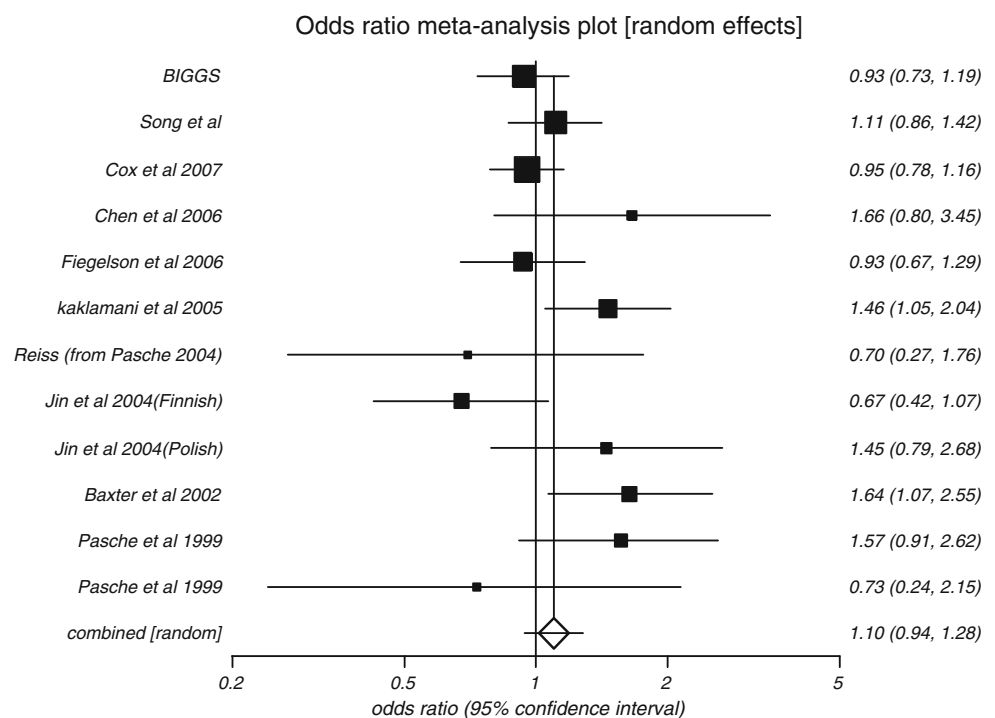
We then undertook a meta-analysis combining data from the current study and previously published studies on breast cancer and *TGFBR1**6A/9A. We identified 11 other studies that had typed the polymorphism in breast cancer cases (Table 2). All controls in these studies were in HWE and

all studies reported the genotype frequencies required for the dominant model combined OR comprising a total of 5,150 cases and 6,344 controls. For the combined allelic OR and the recessive model, only studies with data on allele frequency could be included. This resulted in the exclusion of one study, a negative study by Feigelson et al. (481 cases and 484 controls) [11], leaving 11 studies (including the current study) comprising a total of 4,669 cases and 5,860 controls (Table 2). Meta-analysis was performed using both fixed and random effects models. Under neither model was there any evidence of an

Table 2 Genotype results from the 12 studies used in meta-analysis

Study	Cases			Controls		
	9A/9A	9A/6A	6A/6A	9A/9A	9A/6A	6A/6A
BIGGS	796	154	10	785	160	13
Song et al. [9]	598	152	13	682	160	10
Cox et al. [8]	968	207	12	1,352	302	19
Chen et al. [14]	81	23	0	111	18	1
Feigelson et al. [11]	387	94 (9A/6A&6A/6A)		384	100 (9A/6A&6A/6A)	
Kaklamani et al. [15]	515	92	4	612	77	1
Reiss [7]	87	11	0	77	14	0
Jin et al.—Finnish [13]	177	38	6	171	60	3
Jin et al.—Polish [13]	140	28	2	176	26	0
Baxter et al. [4]	268	83	4	207	39	2
Pasche et al. [3]	128	24	0	654	78	0
Pasche et al. (Italy) [3]	39	8	1	38	12	0

Fig. 1 Meta-analysis of 11 studies—combined genotypic odds ratio under a dominant model



association between *TGFBR1**6A and breast cancer. There was also, however, heterogeneity between studies [heterogeneity $\chi^2 = 22.08$ (df11), $P = 0.02$; $I^2 = 50\%$], and therefore the random effects model is reported here in detail. Using a dominant model, the overall genotypic OR was 1.10 (95% CI 0.94–1.28, $P = 0.24$; Fig. 1), and using a recessive model genotypic pooled OR was 1.15 (95% CI 0.77–1.73, $P = 0.49$). The combined allelic OR was 1.12 (95% CI 0.97–1.31, $P = 0.13$; Fig. 2).

*TGFBR1**6A has been suggested as a general cancer susceptibility allele and associations with breast, colon, cervical, ovarian and haematological cancers have been demonstrated in small case–control studies [2–5, 7]. A meta-analysis of all these studies had shown an overall association with cancer for the five cancer types tested (OR 1.24, 95% CI 1.10–1.40 [5, 7].) One of the strongest associations was with breast cancer (OR 1.38, 95% CI 1.14–1.67) and on the basis of this and other studies, it has been estimated that *TGFBR1**6A accounts for up to 5% of all breast cancer cases [12]. One of the problems with these studies is their small size. Our study and the study by Cox et al. [8] are the two largest case–control studies to look for an association between *TGFBR1**6A and breast cancer, and both showed no evidence of an association. Meta-analysis by Cox et al. showed no overall association, and we confirmed these results in our own meta-analysis, which contains an additional 1,723 cases and 1,800 controls (total 5,150 cases and 6,344 controls).

Both our meta-analysis and that by Cox et al. showed considerable heterogeneity; no measures of heterogeneity were reported in the meta-analysis by Pasche et al. [7]. The

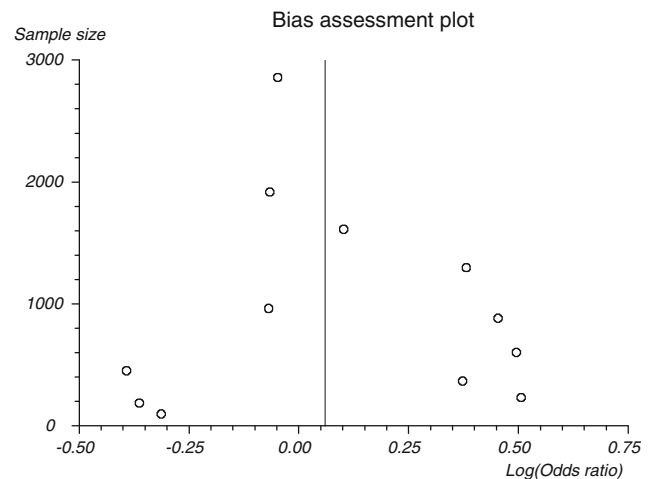


Fig. 3 Plot of sample size versus genotypic odds ratios

cause of this heterogeneity may be related to sample size and Fig. 3 shows that the studies with sample sizes <1,000 had the most extreme ORs. Another cause of heterogeneity may be due to selection of cases and controls, and ethnicity (Table 3).

The three studies [4, 9, 13] (Table 3) that selected patients on the basis of family history, young age or bilaterality showed a trend towards a positive association, although only the study by Baxter et al. [4] (355 cases) reached significance. However, subgroup analysis of the study by Song et al. [9] did show a borderline significant association in patients with ‘low-risk familial’ breast cancer (one first degree relative or second degree relative). The Polish familial study [13] selected cases using similar

Fig. 2 Meta-analysis of 12 studies—combined allelic odds ratio

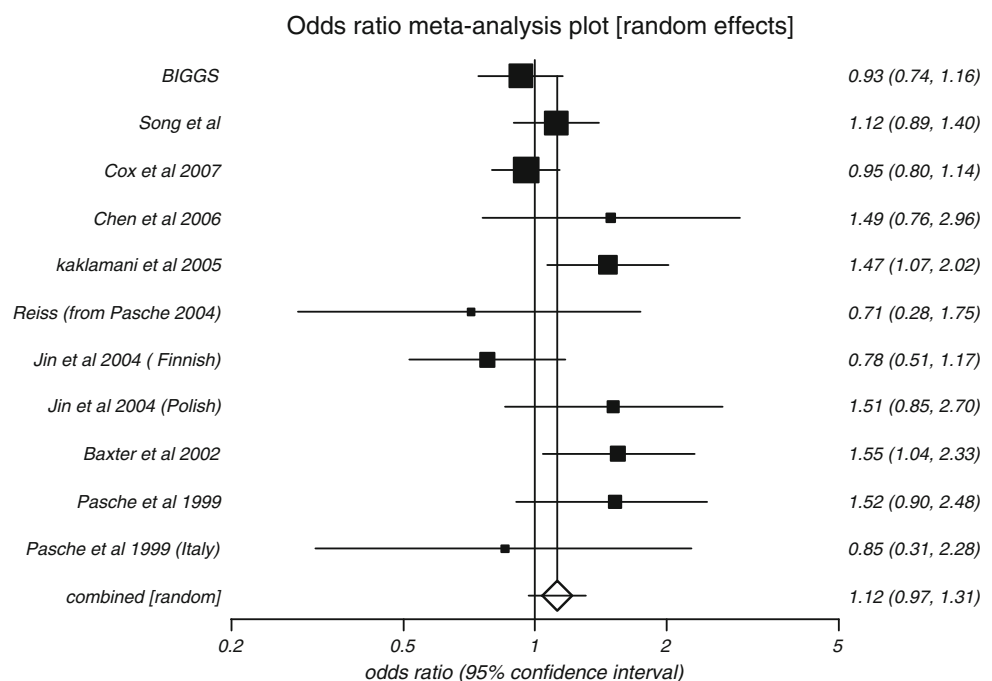


Table 3 Characteristics of studies used in meta-analysis and summary of odds ratios

Study	Ethnicity	Mean age of cases	Case selection criteria (no of cases)	Controls (no of controls)	Genotypic OR dominant model (95% CI)	Allelic OR (95% CI)
BIGGS	Irish 100% Caucasian	53	Unselected (960)	Unmatched (958)	0.93 (0.73–1.19)	0.93 (0.74–1.16)
Song et al. [9]	Swedish (no details)	54, familial 61, sporadic	Familial and unselected (763)	Unmatched (852)	1.11 (0.86–1.42)	1.12 (0.89–1.4)
Cox et al. [8]	USA (no details)	Unknown	Unselected (1,187)	Matched (1,673)	0.95 (0.78–1.16)	0.95 (0.8–1.14)
Chen et al. [14]	USA 98% Caucasian, 2% African American	62	Unselected (104)	Unmatched (130)	1.66 (0.80–3.45)	1.49 (0.76–2.96)
Feigelson et al. [11]	USA 99% Caucasian	68	Post-menopausal (481)	Matched (484)	0.93 (0.67–1.29)	No Data
Kaklamani et al. [15]	USA 83% Caucasian 7% African American 4% Hispanic	54	Unselected (611)	Unmatched (690)	1.46 (1.05–2.04)	1.47 (1.07–2.02)
Reiss [7]	USA 91% Caucasian 7% African American	Unknown	Unselected (98)	Matched (91)	0.70 (0.27–1.76)	0.71 (0.28–1.75)
Jin et al. [13]	Finnish (no details)	63	Post-menopausal (221)	Unmatched (234)	0.67 (0.42–1.07)	0.78 (0.51–1.17)
Jin et al. [13]	Polish (no details)	45	Familial/<35 years/bilateral (170)	Unmatched (202)	1.45 (0.79–2.68)	1.51 (0.85–2.67)
Baxter et al. [4]	UK (no details)	39	Familial/<40 years/bilateral (355)	Unmatched (248)	1.64 (1.07–2.55)	1.55 (1.04–2.33)
Pasche et al. [3]	USA 80% Caucasian 5% African American 10% Hispanic	56	Unselected (152)	Unmatched (732)	1.57 (0.91–2.62)	1.52 (0.9–2.48)
Pasche et al. [3]	Italian (no details)	Unknown	Unselected (48)	Unmatched (50)	0.73 (0.24–2.15)	0.85 (0.31–2.28)
Overall					1.10 (0.94–1.28)	1.12 (0.97–1.31)

criteria to Baxter et al., but contained only 170 cases and 13% of these were known BRCA1/2 mutation carriers. The two studies that selected only post-menopausal women with breast cancer [11, 13] were negative, suggesting that the association may only be present in younger cohorts enriched for genetic predisposition. We applied the same selection criteria used by Baxter et al. (age <40 or bilateral cancers or a family history of breast cancer) to our study and identified 329 such cases. However, we found no association with *TGFBR1**6A in this subgroup (allelic OR 0.83, 95% CI 0.61–1.14, $P = 0.25$) which is of similar size to that studied by Baxter et al. (355 cases).

In studies where cases were unselected, the studies showing a trend towards a positive association were from the USA and predominantly Caucasian cohorts [3, 14, 15].

Only the largest of these three studies (611 cases [15]) was statistically significant. Two of these studies [3, 15] were from the same centre, and controls were drawn from people who had attended for a non-cancer prenatal diagnosis. In both studies, the mean age of the controls was significantly younger than the cases. In the study by Kaklamani et al. [15], 50% of controls were <40 years of age, whereas only 14% of cases were under 40. There was also a significant difference in ethnicity between cases and controls—Hispanics made up 11.6% of the controls compared with 4.1% cases. These differences in control selection may account for some of the heterogeneity in the studies. The third study showing a trend towards a positive association in unselected patients was by Chen et al. [14]. In this small study of 104 cases, cases and controls were selected from a tissue

bank and genotyping was performed on DNA extracted from ‘normal’ paraffin embedded tissue, rather than DNA extracted from peripheral blood samples as in other studies. It is likely that a combination of these factors including differences in genotyping techniques has resulted in some false positive studies.

In conclusion, our study confirms that *TGFBR1*6A* is not associated with breast cancer and highlights the problems associated with small case–control studies, especially where minor allele frequencies are low. It remains to be seen whether the association with other cancer types, including colorectal cancer, can be validated in large case–control series.

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