



The Significance of Erythroblast Transformation Specific (Ets) Transcription Factors in Breast Cancer Progression: A Meta-Analysis

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ABSTRACT

Numerous studies have identified members of the erythroblast transformation specific (Ets) transcription factors family showing aberrant expression in various stages of tumor formation in breast tissue. However, their use as a prognostic factor is not very clear. Therefore, a meta-analysis was performed to analyze their aberrant expression in breast tumorigenesis. A thorough literature search was performed and 73 relevant studies were identified which were further scrutinized and finally 26 studies covering 4553 subjects were included for analysis. A random effect model was applied and correlation was calculated using odds ratios (OR) at 95% confidence intervals (CI). Combined OR calculated showed a significant relation between Ets factor expression and breast cancer risk (OR=3.185, 95% CI=2.161–4.69, $p < 0.001$). In subgroup analysis, Ets-1 overexpression was found highly associated (OR=2.149, 95% CI=1.141–4.048, $p = 0.018$) with breast cancer as compared to other Ets factors. Funnel plots confirmed no publication bias. Our study suggested Ets overexpression (especially Ets-1) might indicate an increased progression rate of breast cancer. However, to make a conclusive statement further in vitro and in vivo investigations and clinical trials are needed.

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Authors' Contribution

SM literature search, data analysis, and manuscript writing. SA data analysis and manuscript writing. RUK research planning and manuscript editing. MUR concept development, manuscript editing, and finalization. NM concept development, figures development, manuscript editing, and finalization.

Key words

Meta-analysis, Ets transcription factors, Ets-1; Breast cancer, Random effect model

INTRODUCTION

Breast cancer is the most common cause of cancer-related deaths in women all over the world (Sung *et al.*, 2021). It is a multifactorial disease that has many environmental and genetic causes (Behravan *et al.*, 2020). A combination of genetic abnormalities and environmental factors decides the fate of the spreading tumor. Angiogenesis plays a critical part in metastasizing cancer as blood vessels are required for the nutrient supply to the growing tumor for stabilization and maintenance (Haibe *et al.*, 2020).

Erythroblast transformation specific (Ets) family of transcription factors has a significant role in angiogenesis and hence in cancer initiation and progression (Randi *et al.*, 2009). Several genes from this family have atypical

expression during various stages of breast cancer (Hsu *et al.*, 2004). Ets proteins, a family of mitogen-activated protein kinase (MAPK), are highly conserved proteins with a unique winged-helix turn helix DNA binding domain (Buggy *et al.*, 2006). It is divided into 12 subfamilies or subgroups based on Ets binding domain (EBD) sequence homology. These subgroups are ETS, ERG, PEA3, ETV-2, TCF, GABP, SPI, ELF, ERF, TEL, PDEF, and ESE (Macleod *et al.*, 1992).

The conversion of Ets factors from normal to oncogene is important in cancer progression. Many studies show that about three Ets proteins can bind to one eukaryotic gene at a time. So instead of an individual Ets factor, a dynamic regulatory network of multiple factors is involved in cancer progression. Ets family members are related to breast carcinoma by their increased or reduced expression. This abnormality in expression leads to migration, invasion, angiogenesis, cell growth, and adhesion of tumor cells. They interact with other transcription factors like p53, N-MYC, GATA and disturb cell homeostasis (Oikawa *et al.*, 1999). Chromosomal alterations and rearrangements like gene amplification, deletions, and translocations in Ets family members lead to abnormal expressions. Several dysregulations in breast cancer include upregulation of ETS-1, increased level of PEA-3 along with HER-2 overexpression of ESE-1 in invasive ductal carcinoma,

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and reduced expression of PDEF and FLI-1 in invasive breast cancer tissues (Watson *et al.*, 2020).

The diverse results of multiple studies related to a medical question often make clinical decisions difficult. The same is true in this case as there are quite some studies with different results which make it difficult to formulate a statement about the role of Ets factors in breast cancer. Therefore, a meta-analysis was performed to collect information from different studies and determine the relationship between ETS factors and breast cancer.

MATERIALS AND METHODS

Literature search strategy

The electronic search was performed in databases, PubMed and Google Scholar for identification of the studies related to Ets transcription factors expression in breast cancer. Keywords used were Ets transcription factors; Ets expression; Breast cancer; Breast carcinoma and Breast tumors. All Ets transcription family factors were also searched individually. Studies published before December 2020 were included while there was no lower date limit. Appropriate references of retrieved studies were also searched for data.

Selection criteria

For the selection of literature following criteria were followed; (i) only original and independent studies were included for analysis, (ii) samples used in the study were of human breast tissue or human breast cell line, (iii) patients must be diagnosed originally with breast malignancy, (iv) expression of Ets transcription factors must be checked, and (v) number or percentage for positive expression of Ets or odds ratio with 95% Confidence Interval must be mentioned. Studies lacking this information, having samples taken from other than human breast tissue like mice, rabbits, or other animals, and review articles were excluded from the analysis.

Literature retrieval and data extraction

A total of 250 articles were selected as a result of a preliminary search through databases. Out of these, 177 studies that were irrelevant to our research were excluded from further evaluation. 73 relevant studies were assessed at the abstract level. 58 retained studies were assessed for further full-text assessment. After full-text assessment 26 studies were selected for performing meta-analysis in which complete required information was given.

Data extracted from selected articles included first author name, year of publication, the country where the study was conducted, name of Ets transcription factor, mean age of patients (if mentioned), several breast tissue

samples, and positive expression of Ets transcription factor in both cancer and normal tissue, odds ratio with upper and lower limit with 95% confidence interval (CI), methods of expression analysis like immunohistochemistry, western blotting, qPCR, etc., relation or effect of Ets factor on other genes expression and type of breast cancer like HER2 positive, etc. Supplementary data were also retrieved if required information was not mentioned in the article.

Statistical analysis

The expression of Ets transcription factors was measured by calculating the odds ratio and standard error. For analyzing the significance of expression, a forest plot for the random effect model was used. The weight and residual of each study used in the meta-analysis were also calculated. A subgroup analysis was performed on the individual Ets factor family members. Bias in studies was checked by funnel plots followed by Egger's regression and the Begg-Mazumdar test. Heterogeneity within the study was estimated using I-squared (Oikawa *et al.*, 2003) and between the studies, variation was checked by the Tau² statistics (Krishnamoorthy and Lee, 2014). All calculations were done with Comprehensive Meta-Analysis Version 3.0.

RESULTS

Literature retrieval

After thoroughly analyzing 250 studies finally, 26 studies were selected for performing meta-analysis in which complete required data was given (Supplementary Fig. 1). Supplementary Table S1 summarizes the characteristics of the included studies. In 10 studies the expression of the ETS factor was analyzed at the mRNA level (Benz *et al.*, 1997; Ghadersohi and Sood, 2001; Kinoshita *et al.*, 2002; Span *et al.*, 2002; Tognon *et al.*, 2002; Bièche *et al.*, 2004; Chotteau-Lelièvre *et al.*, 2004; Katayama *et al.*, 2005; Buchwalter *et al.*, 2013; Kar and Gutierrez-Hartmann, 2017), in 14 studies it was at protein level (Behrens *et al.*, 2001; Mitas *et al.*, 2001; Fleming, 2004; Myers *et al.*, 2005, 2006; Xia *et al.*, 2006; Sood *et al.*, 2007; Turcotte *et al.*, 2007; Sood *et al.*, 2009; Zhang *et al.*, 2011; Laliotis *et al.*, 2013; Mesquita *et al.*, 2013; Puzovic *et al.*, 2014; Yuan *et al.*, 2014) while in remaining 2 studies expressions was analyzed both at mRNA and protein levels (Yuan *et al.*, 2014). Studies included in this systematic review illustrated 11 Ets transcription factors having important roles in breast cancer. These Ets transcription factors are ETS-1, ETS-2, ELK-1, ERM, ETV-4, PDEF, ELF-3, ETV-6, ETV-3, SPDEF, and ELK-4. All studies were about a single Ets transcription factor except (Myers *et al.*, 2005; Mesquita *et al.*, 2013) in which more than one

Ets factor was evaluated. The individual study sample size ranged from 13 to 364 patients. The mean patient age was 48-64 years. In two studies odds ratio was directly calculated (Turcotte *et al.*, 2007; Puzovic *et al.*, 2014). In most studies, RT-PCR was used to evaluate mRNA expression (Benz *et al.*, 1997; Ghadersohi and Sood, 2001; Kinoshita *et al.*, 2002; Span *et al.*, 2002; Tognon *et al.*, 2002; Bièche *et al.*, 2004; Chotteau-Lelièvre *et al.*, 2004; Katayama *et al.*, 2005; Buchwalter *et al.*, 2013; Kar and Gutierrez-Hartmann, 2017), while for protein analysis immunohistochemistry (Behrens *et al.*, 2001; Mitas *et al.*, 2001; Buggy *et al.*, 2004, 2006; Fleming, 2004; Myers *et al.*, 2006; Xia *et al.*, 2006; Zhang *et al.*, 2011; Laliotis *et al.*, 2013; Mesquita *et al.*, 2013; Puzovic *et al.*, 2014; Yuan *et al.*, 2014), western blotting (Behrens *et al.*, 2001; Buggy *et al.*, 2004; Sood *et al.*, 2007, 2009; Turcotte *et al.*, 2007), ELISA (Buggy *et al.*, 2004, 2006), and other methods (Benz *et al.*, 1997; Myers *et al.*, 2005) were used. The most common types of carcinoma in selected studies were ductal and lobular invasive carcinomas. One study involved a very rare subtype that is secretory breast cancer (Tognon *et al.*, 2002). Breast cancer subtypes in these studies were triple-negative (Yuan *et al.*, 2014), luminal (Buchwalter *et al.*, 2013), and HER2 +ve breast cancer. In 7 studies, the subtype was not specified (Ghadersohi and Sood, 2001; Kinoshita *et al.*, 2002; Bièche *et al.*, 2004; Myers *et al.*, 2005, 2006; Zhang *et al.*, 2011; Puzovic *et al.*, 2014).

Ets factors and breast cancer

The studies for which meta-analysis was performed, the expression of Ets transcription factors was closely related to breast cancer. In 19 studies, the increased expression of Ets factors was observed in breast cancer patients (Benz *et al.*, 1997; Kinoshita *et al.*, 2002; Mitas *et al.*, 2001; Tognon *et al.*, 2002; Bièche *et al.*, 2004; Chotteau-Lelièvre *et al.*, 2004; Fleming, 2004; Katayama *et al.*, 2005; Buggy *et al.*, 2006; Myers *et al.*, 2006; Sood *et al.*, 2007; Buchwalter *et al.*, 2013; Laliotis *et al.*, 2013; Mesquita *et al.*, 2013; Puzovic *et al.*, 2014; Yuan *et al.*, 2014; Kar and Gutierrez-Hartmann, 2017). In 9 studies the expression was almost equal in both cases (Ghadersohi and Sood, 2001; Span *et al.*, 2002; Buggy *et al.*, 2004; Myers *et al.*, 2005; Turcotte *et al.*, 2007; Sood *et al.*, 2009; Zhang *et al.*, 2011) while in 2 studies expressions of ETS factor in breast cancer samples were less than that of normal samples (Buggy *et al.*, 2004; Xia *et al.*, 2006).

A random effect model was chosen to calculate the odds ratio and heterogeneity. OR (Odds Ratio) value of individual studies and forest plot is illustrated in Supplementary Fig. 2. Combined odds ratio was OR = 3.185, 95% CI (2.161-4.693) and $P < 0.001$. This shows

a statistically significant relationship between ETS factors overexpression and breast cancer risk. The I-square statistics to measure heterogeneity between studies showed $I^2 = 86\%$ meaning that 86% of the observed variance between studies is due to the real difference in effect size and only 14% of observed variance should be expected to base on a random error. The τ^2 value to measure the variance among studies was 0.841. It was observed that this heterogeneity was due to studies (Behrens *et al.*, 2001; Span *et al.*, 2002; Tognon *et al.*, 2002; Katayama *et al.*, 2005; Xia *et al.*, 2006; Mesquita *et al.*, 2013), therefore a meta-analysis was performed excluding these studies. The combined OR after excluding these studies is OR = 2.564, 95% CI (1.88-3.48) and $P < 0.001$. After excluding these studies the I^2 and τ^2 values reduced to 76% and 0.369, respectively (Fig. 1).

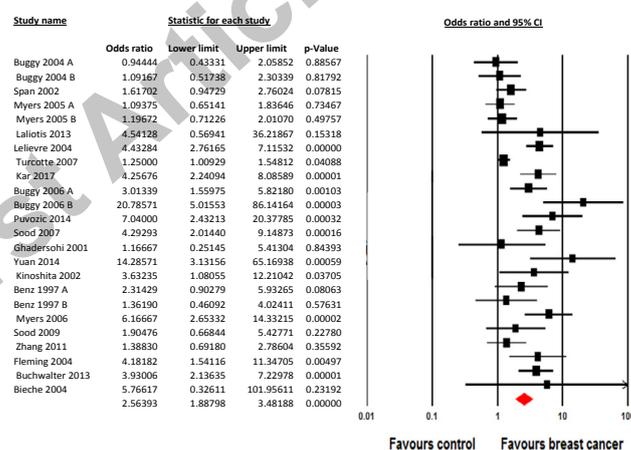


Fig. 1. Forest plot of odd ratio with a random-effects model for prognosis between increased expression of ETS factors and control in breast cancer after exclusion of some studies.

Bias in studies was calculated by funnel plot for standard error of random effect model as shown in Figure 2 and Supplementary Figure 2. The symmetrical plot indicates that there are no biases in studies included in the meta-analysis. The Egger's regression test and Begg and Mazumdar test details are shown on funnel plots. Sub-group analysis was performed for the individual Ets- family factors to minimize heterogeneity among the included studies. In each specific group, the effect of individual Ets factor was evaluated on breast cancer. The forest plots for Ets-1 (OR = 2.149, 95% CI = 1.141 – 4.048, $P = 0.018$), Ets-2 (OR = 3.06, 95% CI = 1.226 – 7.648, $P = 0.017$) and ETV-4 (OR = 2.885, 95% CI = 0.779 – 10.684, $P = 0.113$) are shown in Figure 4A, B, C. The funnel plots for publication bias were also determined and shown in

Figures 3 and 4. All reported p values were two-sided and p values < 0.05 were regarded as statistically significant.

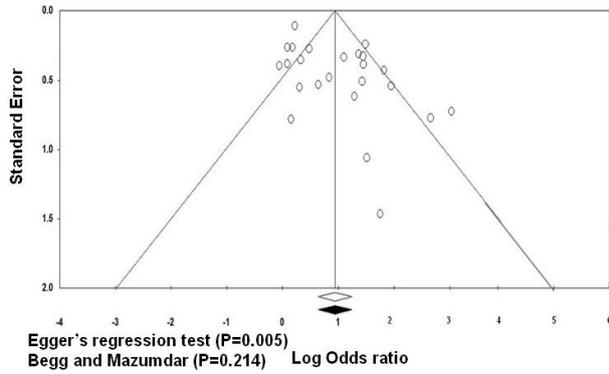


Fig. 2. Funnel plot of standard error by log odd ratio for increased expression of ETS factors in breast cancer and control group for 21 selected studies.

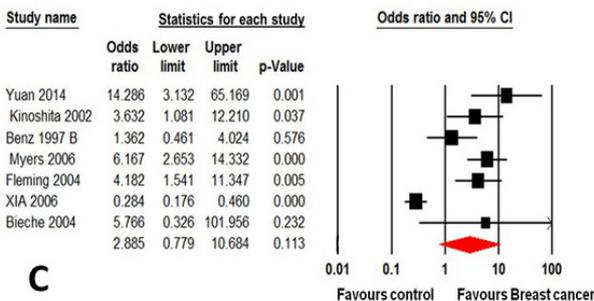
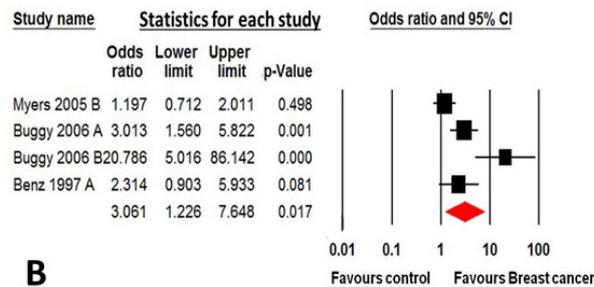
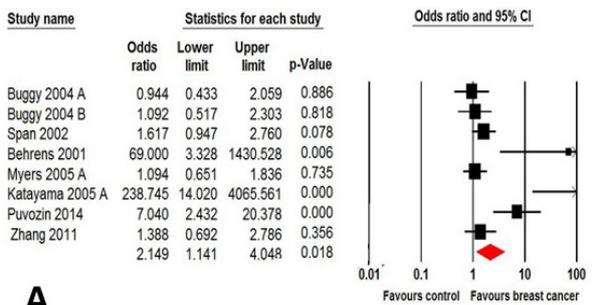


Fig. 3. Forest plot of odd ratio with a random-effects model for prognosis between increased expression of Ets-1 (A), Ets-2 (B) and ETV-4 (C) and control in breast cancer.

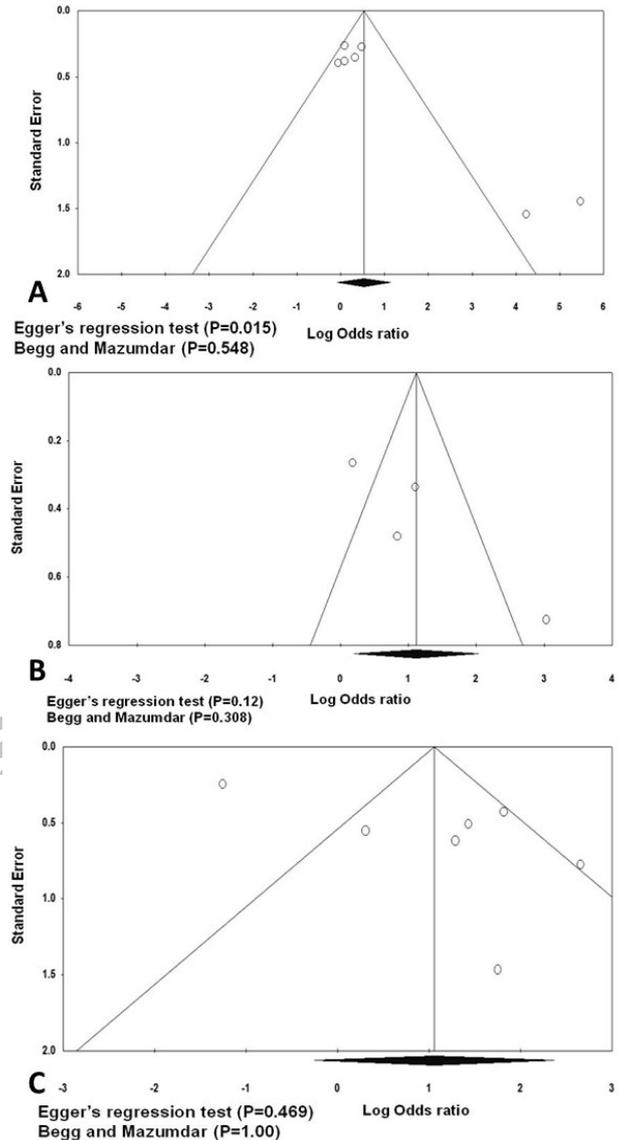


Fig. 4. Funnel plot of odd ratio with a random-effects model for prognosis between increased expression of Ets-1 (A), Ets-2 (B) and ETV-4 (C) and control in breast cancer.

DISCUSSION

The progression of breast cancer is rather unpredictable due to the greater variation and heterogeneity in the underlying causes (Kar *et al.*, 2017). It is, therefore, particularly important to identify the biomarkers that can predict the progression of the disease. Several molecular targets have been identified which can serve as potential biomarkers for breast cancer but still there is a need to find better targets for metastatic breast cancer (Polyak, 2011). Ets family of transcription factors have emerged to play a

significant role in the progression of breast malignancy. In the present study, a meta-analysis of 26 studies was performed after scrutiny to determine the relationship between Ets factors overexpression and breast cancer progression. A thorough literature survey could not find any study which has comprehensively analyzed and reviewed the Ets factors and breast cancer occurrence.

The random effect model was selected for the meta-analysis, as this model allowed examining the true variation in effect size (odds ratio) among individual studies. Effect size can be slightly higher or lower according to the characteristics or condition of subjects in a study. In combined effect size, the random effect model gives the mean effect size for all studies and more precise effect size from studies having a large number of samples or patients as compared to small sample size studies can be obtained (Li *et al.*, 2002; Walker *et al.*, 2008). The publications finalized in our meta-analysis had variable Ets factors for breast cancer patients, so the random effect model was found to be more appropriate. In contrast, the fixed-effect model was not selected because it assumes the same effect size for all studies.

The meta-analysis results showed that Ets transcription factors family overexpression increases the odd ratios of breast cancer in a significant way (combined OR= 3.262). However, the Ets family factor Ets-1 was found more closely related to breast cancer occurrence (OR= 2.149) compared to others.

Multiple pieces of evidence suggested the regulation of breast cancer metastasis by the combined action of several Ets factors affecting various pathways. Ets factors are also associated with the poor prognosis of breast cancer. The possible mechanism of their action on breast cancer progression is maybe through the angiogenesis pathway (Folkman, 1995). Among the selected 26 studies the increased expression of Ets transcription factors was observed in all types of breast cancer cases as compared to respective controls. However, there was one study (Xia *et al.*, 2006) showing the downregulation of the Ets factor, PEA3 in HER-2/neu breast metastasis and was not associated with clinicopathological features.

Ets family of transcription factors regulates the expression of numerous signaling molecules and regulators of tumor progression. The pathways of the tumor microenvironment and their interactions are also under influence of Ets factors (Haidich, 2010). It is evident from the literature that some Ets factors have coordinated functions and control tissue homeostasis for the tumor microenvironment. Ets factors altered expression in breast tumorigenesis is determined in several studies. Some Ets factors are overexpressed while others are down-regulated during breast tumorigenesis thus acting as both activators

and suppressors of the process.

Our literature search and meta-analysis results have demonstrated that Ets-1 is the most investigated factor among all Ets proteins. The subgroup analysis was performed with Ets-1, Ets-2, and ETV-4 only because for other factors number of studies was not enough (3 or less). Among these factors, the Ets-1 was most significantly related to breast cancer progression with minimum heterogeneity i.e. τ^2 was 0.54 as compared to 0.67 and 2.66 for Ets-2 and ETV-4, respectively.

Ets-1 has been found closely related to angiogenic pathways where it is involved in inducing the expression of pro-angiogenic factors (Randi *et al.*, 2009). By enhancing the angiogenic mechanism Ets-1 can contribute to invasiveness and progression of breast cancer. Several findings have shown that Ets-1 expression is related to aggressive angiogenesis and invasive phenotypes (Furlan *et al.*, 2019; Ehrenfeld *et al.*, 2019). Ets-1 has a significant correlation with several important molecules like uPA (Urokinase activator) (Madunić, 2018) and HER2/neu (a proto-oncogene) (Nazir *et al.*, 2019), therefore, its overexpression can be associated with breast tumor progression. Moreover, there is also a significant correlation between Ets-1 expression and VEGF and PAI-1 (Yuan *et al.*, 2014). Based on these observations Ets-1 can be used as a prognostic factor to determine breast cancer progression.

CONCLUSIONS

From this meta-analysis; we conclude that Ets family members can serve as the biomarkers for the progression of breast cancer. However further validation needs to be done. It is worth mentioning that instead of causative factors Ets proteins are more involved in the development of metastasis in breast cancer. Since Ets proteins appeared at various stages of breast cancer, therefore, their expression can predict the disease progression.

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None to declare

Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20220119060146>

Statement of conflict of interest

The authors have declared no conflict of interest.

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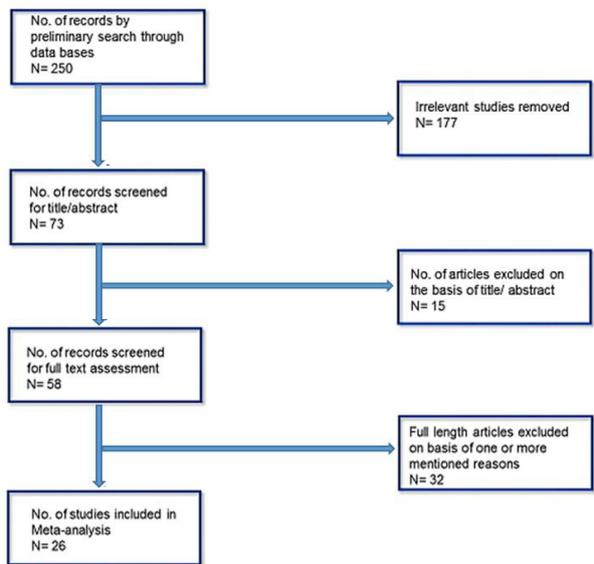
Supplementary Material

The Significance of Erythroblast Transformation Specific (Ets) Transcription Factors in Breast Cancer Progression: A Meta-Analysis

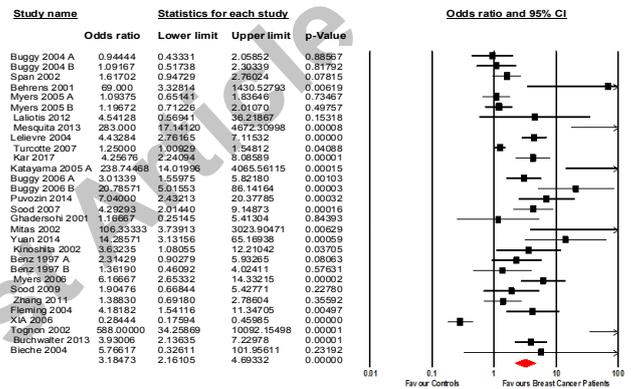
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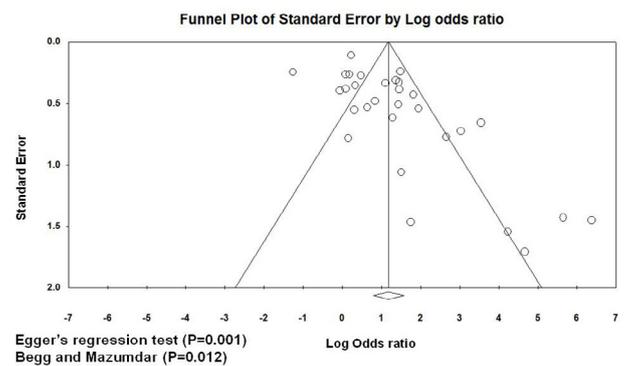
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Supplementary Fig. 1. Flowchart representing the steps of literature search and selection.



Supplementary Fig. 2. Forest plot of odd ratio with a random-effects model for prognosis between increased expression of ETS factors and control in breast cancer.



Supplementary Fig. 3. Funnel plot of standard error by log odd ratio for increased expression of ETS factors in breast cancer and control group for all 26 studies.

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Supplementary Table S1. Characteristics of all eligible studies.

Sr. #	Name	Est factor	Exp. level	Pa-tients	Positive expres-sion	Con-trol	Postive expres-sion	Odds ratio	Method of evaluation	Site of evaluation / Subtype
1A	Buggy 2004 A	ETS-1	Protein	78	42	38	21		Western Blot, IHC, ELISA	Ductal, lobular, others
1B	Buggy 2004 B	ETS-1	mRNA	179	131	42	30		PCR	Ductal, Lobular, others
2	Span 2002	ETS-1	mRNA	123	76	100	50		RT-PCR	Ductal, lobular, others
3	Behrens 2001	ETS-1	Protein	34	34	10	5		In situ hybridiza-tion, IHC,	Intralobular, Ductal Insitu, Invasive
4A	Myers 2005 A	ETS-1	Protein	134	70	100	50		Western blot, Coimmunoprecip-itation	Not specified
4B	Myers 2005 B	ETS-2	Protein	134	73	100	50		Western blot, Coimmunoprecip-itation	Not Specified
5	Laliotis 2012	ELK-1	Protein	46	45	120	109		IHC,ELISA	Ductal and lobular
6	Mesquita 2013	ETV-3, ELF3, ELK-4	Protein	141	141	100	50		IHC, Florescent In-situ Hybridization	Ductal, lobular, others
7	Lelievre 2004	ERM	mRNA	364	297	100	50		RT-PCR, ABI SEQ.	Ductal, lobular, others
8	Turcotte 2007	PDEF	Protien					1.25,(CI 95%, 1.004–1.540)	Western Blot	80% ductal, others are lobular and mixed
9	Kar 2017	ESE/ ELF-3	mRNA	186	112	61	16		PCR	Luminal B and HER-2+ subtype
10	Katayama 2005 A	ETS-1	mRNA	137	114	24	0		RT-PCR	Invasive ductal, lobular, medullary and apocrine carcinoma
11A	Buggy 2006 A	ETS-2	mRNA	181	125	47	20		RT-PCR	Ductal and lobular
11B	Buggy 2006 B	ETS-2	Protien	111	97	12	3		IHC, ELISA	Ductal and lobular
12	Puvozic 2014	ETS-1	Protien					7.04(CI 95% 2.43- 20.36)	IHC,	Not specified
13	Sood 2007	PDEF	Protien	104	50	62	11		IHC, Western blot	Intraductal, Invasive lobualr and ductal carcinoma
14	Ghadersohi 2001	PDEF	mRNA	20	14	12	8		RT-PCR	Not specified
15	Mitas 2002	PDEF	Protien	15	14	5	0		RT-PCR	Auxillary lymph nodes
16	Yuan 2014	ETV-4	Protien	77	75	58	42		IHC,	Triple negative breast cancer
17	Kinoshita 2002	ETV-4	mRNA	42	38	47	34		IHC,	Not specified
18A	Benz 1997 A	ETS-2	mRNA	33	18	41	14		In situ hybridiza-tion	Invasive breast cancer
18B	Benz 1997 B	ETV-4	Protien	33	26	41	30		In situ hybridiza-tion	Invasive breast cancer
19	Myers 2006	ETV-4	Protien	55	37	52	13		IHC, Western blot	Not Specified

Table contibued on next page.....

Sr. #	Name	Est factor	Exp. level	Pa-tients	Positive expres-sion	Con-trol	Positive expres-sion	Odds ratio	Method of evaluation	Site of evaluation / Subtype
20	Sood 2009	SPDEF	Protien	27	20	45	27		Western blot	Luminal Subtype and epithelial lineage
21	Zhang 2011	ETS-1	Protien	40	24	181	94		IHC,	Not Specified
22	Fleming 2004	ETV-4	Protien	35	24	35	12		IHC	Endocrine resistant breast cance
23	XIA 2006	ETV-4	Protien	289	64	100	50		IHC	Ductal carcinoma
24	Tognon 2002	ETV-6	mRNA	13	12	50	1		RT-PCR	Secretary breast carcinoma
25	Buchwalter 2013	PDEF	mRNA	100	77	100	46		RT-PCR	ER+ Luminal breast cancer
26	Bieche 2004	ETV-4	mRNA	130	30	9	0		PCR	Not specified

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