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GSK3β (rs334558) Promoter Polymorphism and Susceptibility to Breast Cancer: Case-Control Study in a South Indian Population with Comprehensive Statistical Analysis

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ABSTRACT

Breast cancer remains a leading cause of cancer-related morbidity in India, with its pathogenesis driven by complex molecular alterations in key signalling networks. Among these, aberrant activation of the Wnt/β-catenin pathway is a well-established contributor to tumorigenesis and disease progression. Glycogen synthase kinase-3 beta (GSK3β), a constitutively active serine/threonine kinase, serves as the catalytic core of the β-catenin destruction complex and its activity is critical for maintaining Wnt pathway suppression. The rs334558 (G>C) promoter polymorphism of the GSK3B gene is predicted to alter transcription factor binding affinity, potentially reducing basal GSK3β expression and thereby influencing oncogenic susceptibility. Despite this mechanistic relevance, population-specific data from South Indian cohorts are absent from the literature.

Material and Method: *A case-control design enrolled 50 histologically confirmed primary breast cancer patients from Nizam's Institute of Medical Sciences (NIMS), Hyderabad, and 50 age- and sex-matched controls. Genomic DNA was extracted by the salting-out method. GSK3β rs334558 genotyping was performed by PCR-RFLP using the restriction endonuclease TaqI. Statistical analysis included allele frequency estimation, Pearson chi-square test, Yates' continuity-corrected chi-square, and odds ratio (OR) calculation with 95% confidence intervals (CI) across all genotypic and clinical stratifications.*

Result: *The GSK3β rs334558 promoter polymorphism does not exhibitstatistically significant independent association with breast cancer susceptibility in this South Indian cohort. However, the moderate elevation of the GC genotype and C allele in cases, and the genotype-specific trends across ER-negative and HER2-positive subgroups, suggest a candidate role for this variant in tumour subtype biology warranting validation in larger, ethnically stratified multi-centre cohorts.*

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Introduction

Glycogen synthase kinase-3 beta (GSK3β; OMIM #605004) is one of those enzymes whose full significance in biology only became clear long after its initial discovery. Originally characterised as a regulator of glycogen metabolism phosphorylating and inactivating glycogen synthase in response to insulin it is now understood to be a constitutively active kinase operating at the convergence of the Wnt/β-catenin, PI3K/Akt, Hedgehog, and Notch pathways, all of which govern fundamental processes including proliferation, differentiation, survival, and oncogenesis [1,2]. What distinguishes GSK3β from most kinases is that it is active in resting cells and regulated through inhibition rather than activation, and that it exhibits a strong preference for substrates already phosphorylated by a priming kinase at a specified position downstream [3].In the context of Wnt signalling, GSK3β occupies a central position within the β-catenin destruction complex, alongside APC, Axin1/2, and CK1. When Wnt ligands are absent, GSK3β sequentially phosphorylates β-catenin at Ser33, Ser37, Thr41, and Ser45. This creates a phosphodegron recognised by the F-box protein β-TrCP, which targets β-catenin for ubiquitin-mediated proteasomal degradation. The result is cytoplasmic sequestration that prevents β-catenin from reaching the nucleus and activating TCF/LEF-dependent transcription of pro-proliferative targets such as cyclin D1, c-Myc, and survivin [3,4]. Activation of the Wnt pathway disrupts this complex through LRP5/6 and Dishevelled-mediated mechanisms, allowing β-catenin to accumulate and drive oncogenic transcription a sequence of events now well-documented in multiple cancer types.

The substrate spectrum of GSK3β extends considerably beyond β-catenin. Over 100 validated substrates have been identified, including Snail, c-Jun, c-Myc, NF-κB p65, MCL-1, and FOXO3a, positioning GSK3β as a regulator of EMT, apoptosis, cell cycle progression, and immune signalling simultaneously. This breadth is why its net effect on tumourigenesis depends heavily on cellular context: in some settings GSK3β acts as a tumour suppressor through β-catenin regulation, while in others it can

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promote tumour survival through NF-κB pathway engagement [5,6]. In breast cancer specifically, loss of GSK3β activity has been linked to enhanced EMT and cancer stem cell properties in triple-negative subtypes, as well as acquisition of chemoresistance, underscoring its potential as both a prognostic marker and a druggable target [7,8].The rs334558 single nucleotide polymorphism is a C-to-T transversion located within the 5' promoter region of GSK3B. Computational analyses predict that this substitution disrupts binding motifs for SP1 and related transcription factors, potentially reducing the basal transcriptional output of the gene. Given that the amount of GSK3β protein available for destruction complex assembly is a rate-limiting factor in β-catenin phosphorylation kinetics, even modest reductions in promoter-driven expression could have measurable downstream consequences for Wnt pathway suppression. Earlier reports from European and Latin American cohorts have explored this variant in different cancer types, though South Asian-specific data remain sparse [9,10].

India has one of the fastest-rising breast cancer incidence rates in South Asia, with a notably younger age at onset than is seen in Western populations. The genetic architecture of South Indian populations is distinct from that of European and East Asian reference groups, and extrapolating GWAS findings across these boundaries is not straightforward. The present study was therefore undertaken to characterise the rs334558 genotype and allele distribution in a South Indian breast cancer cohort and to evaluate its associations with disease susceptibility and a range of clinicopathological parameters.

Material and Methods

Patients and Healthy Controls

A hospital-based case–control study was carried out between February and April 2021 after obtaining approval from the Institutional Ethics Committee of Osmania University, Hyderabad, Written informed consent was secured from all participants prior to inclusion. The study included 50 histologically confirmed primary breast cancer patients recruited from the Tumour Registry of Nizam's Institute of Medical Sciences (NIMS), Hyderabad. Patients with prior chemotherapy or radiotherapy were excluded.Epidemiological information covering age, dietary habits, reproductive and lactation history, menopausal status, and family history of cancer was collected through structured interviews. Clinical parameters including tumour stage (TNM classification), tumour size, axillary lymph node status, and ER, PR, and HER2 receptor status were obtained from pathology records. Fifty age- and sex-matched healthy individuals from the local community, with no personal or three-generation family history of malignancy, served as controls.

GenomicDNAisolationandPCRamplification

5mlofbloodsamplewascollectedintoEDTAvacutainers from patients as well as control group. Genomic DNA was isolated by salting outmethod and used for PCR amplification.

PCR-RFLP Genotyping of GSK3β rs334558

A 326 bp amplicon encompassing the rs334558 locus was generated using the following primers: Forward 5′-TGAGAAGATCCTCCCTGCTG-3′ and Reverse 5′-ACTCTTCTAGGAGGGACGAC-3′. PCR was performed in a 10 µL reaction volume containing 1× buffer, 200 µM dNTPs, 0.25 µM of each primer, and 1 U Taq polymerase. Thermal cycling conditions were: initial denaturation at 95°C for 5 min; 30 cycles of 95°C/1 min, 58.3°C/40 s, and 72°C/1 min; followed by a final extension at 72°C for 10 min. Amplicons were digested overnight with TaqI (1 U per µg DNA, 37°C, 16 h) and resolved on 2.5% agarose gels with ethidium bromide staining. The C allele creates a TaqI restriction site (5′-TCGA-3′), yielding fragments of 256 and 70 bp, while the T allele remains uncut at 326 bp. Genotypes were therefore assigned as: CC (326 bp), CT (326 + 256 + 70 bp), and TT (256 + 70 bp). A random 10% subset of samples was re-genotyped for quality control, with complete concordance observed.

Statistical Analysis

Genotype and allele frequencies were estimated from observed counts. Hardy-Weinberg equilibrium (HWE) was tested by chi-square goodness-of-fit with one degree of freedom. Between-group comparisons of genotype distributions used Pearson chi-square (df = 2); Yates' continuity correction was applied for 2×2 contingency tables. Pairwise odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for TT vs CT and TT vs CC comparisons, with TT as the reference genotype. The allele frequency formula p = f(TT) + ½f(CT) was used throughout. Statistical significance was set at $p < 0.05$ (two-tailed). All analyses were performed in SPSS v20.0 (IBM Corp., Armonk, NY) and cross-checked manually.

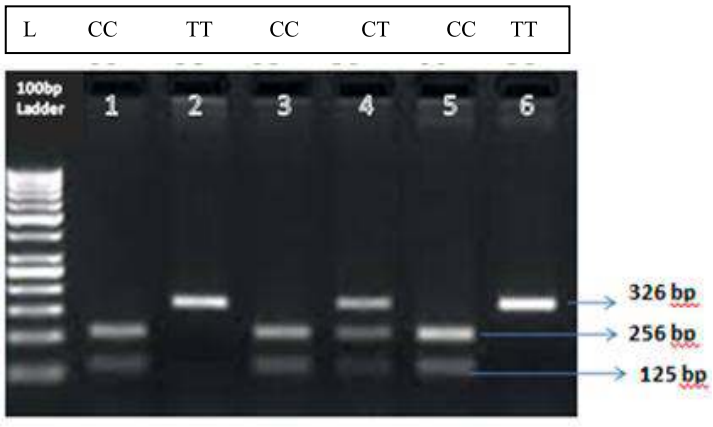

Figure1: Gel Picture of PCR-RFLP Genotypes of GSK3β polymorphism on 2.5% agarosegel

T allele: Absence of Taq1 RFLP site
C allele: Presence of Taq1 RFLP site

Table 1: Demographic data of Breast Cancer Patients

Variables	n	%
Ageat onset		
≤30	15	30
30-50	25	50
≥50	10	20
Menopausalstatus		
Pre	30	60
Post	20	40
Lactation		
Positive (+)	28	56
Negative (-)	22	44
Histologicalclassification		
Ductalcarcinoma	22	44
Lobularcarcinoma	20	40
Othertype	8	16
Stage		
Early(I&2)	28	56
Advance(III&IV)	22	44

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Estrogen receptorstatus		
Positive(+)	25	50
Negative(-)	25	50
Progesteronereceptorstatus		
Positive(+)	24	48
Negative(-)	27	54
HER2status		
Positive(+)	28	56
Negative(-)	22	44
Axillary lymph Node status		
Positive(+)	20	40
Negative(-)	30	60

Table 2 presents the genotype distribution stratified by lactation status and menopausal status. Among lactation-positive patients, the CT genotype predominated (43.0%), whereas TT was more common in lactation-negative patients (50.0%). Post-menopausal patients showed a notably higher CC frequency (35.0%) relative to pre-menopausal patients (17.0%), though neither comparison reached statistical significance (lactation: p = 0.56; menopausal status: p = 0.31)

Table 2: Association between the GSK3β rs334558 gene polymorphism and epidemiological characteristics

	Genotype (%)				Allele (%)		
Variables	**CC(n%)**	**CT (n%)**	**TT (n%)**	**χ^2p**	**C (n%)**	**T (n%)**	**χ^2p**
Earlyon-set							
≤30yrs	8 (53.3%)	5 (33.3%)	2 (13.4%)		21 (70.0%)	9 (30.0%)	
30-50 yrs	10 (40.0%)	10 (40.0%)	5 (20.0%)	0.72	30 (60.0%)	20 (40.0%)	0.63
≥50yrs	5 (50.0%)	2 (20.0%)	3 (30.0%)		12 (60.0%)	8 (40.0%)	
OR(95% CI)	1.00(Ref)	1.60 (0.38-6.62)	2.00 (0.30-13.17)		1.00 (Ref)	1.55 (0.59-4.08)	
		0.64 (0.08-4.65)	2.40 (0.29-19.78)			1.55 (0.47-5.10)	
Lactation							
Positive	10 (36.0%)	12 (43.0%)	6 (21%)	0.56	32 (57.1%)	24 (42.9%)	0.25
Negative	11 (50.0%)	8 (36.0%)	3 (14.0%)		30 (68.2%)	14 (31.8%)	
OR (95% CI)	1.00 (Ref)	0.60 [0.17-2.09]	0.45 [0.08-2.31]		1.00 (Ref)	0.62 (0.27-1.42)	
Menopausal Status							
Pre-menopausal	14 (46.0%)	11(37.0%)	5 (17.0%)		39 (65.0%)	21(35.0%)	0.21
Post-menopausal	8 (40.0%)	5 (25.0%)	7 (35.0%)	0.31	21 (52.5%)	19 (47.5%)	
OR(95%CI)	1.00 (Ref)	0.79 [0.20-3.12]	2.45 [0.58-10.3]		1.00 (Ref)	1.68 (0.74-3.80)	

3.4 Association with Clinical Parameters

Table 4 presents genotype distributions stratified by ER, PR, HER2, axillary lymph node status, and tumour stage. None of the comparisons reached statistical significance. Among ER-negative patients, TT was the predominant genotype (56.0% vs 44.0% in ER-positive). HER2-positive and HER2-negative cases showed equal TT distribution (50.0% each), though notable differences were seen in CT frequency (32.2% vs 27.3%). Among axillary lymph node-positive patients, TT predominated (55.0%) contrasted with 46.7% in node-negative cases. Tumour stage stratification revealed increasing CT genotype frequency from early to advanced stages(25.0%to41.0%).

Table 4: Association between GSK3 gene polymorphism and the clinical parameters

	Genotype(%)				Allele(%)		
Variables	CC(n%)	CT (n%)	TT (n%)	χ^2p	C(n%)	T(n%)	χ2p
EstrogenReceptor							
Positive	11(44.0%)	8(32.0%)	6(24.0%)		30(60.0%)	20(40.0%)	
Negative	14(56.0%)	7(28.0%)	4(16.0%)	0.66	35(70.0%)	15(30.0%)	
OR (95%CI)	**1.00(Ref)**	0.68[0.19-2.48]	0.52[0.12-2.32]		**1.00(Ref)**	0.64(0.28-1.47)	
Progesterone Receptor							
Positive	13 (53.0%)	7 (30.0%)	4(17.0%)		33(68.7%)	15(31.3%)	
Negative	11(42.5%)	9(34.3%)	6(23.2%)	0.69	31(59.6%)	21(40.4%)	0.34
OR(95%CI)	**1.00(Ref)**	1.52[0.42-5.42]	1.77[0.36-7.93]		**1.00(Ref)**	1.49(0.65-3.39)	
HER2Status							
Positive	14(50.0%)	9(32.2%)	5(17.8%)		37(66.1%)	19(33.9%)	
Negative	11(50.0%)	6(27.3%)	5 (22.7%)	0.88	28(63.6%)	16(36.4%)	0.80
OR(95%CI)	**1.00(Ref)**	0.84[0.23-3.11]	1.27[0.29-5.53]		**1.00(Ref)**	1.11(0.48-2.54)	
AxillaryNode							
Positive	11 (55.0%)	3(15.0%)	6(30.0%)		25(62.5%)	15(37.5%)	
Negative	14(46.7%)	12(40.0%)	4(13.3%)	0.114	40(66.7%)	20(33.3%)	0.66
OR(95%CI)	**1.00(Ref)**	0.32[0.07-1.41]	1.91[0.43-8.48]		**1.00(Ref)**	0.83(0.36-1.92)	
Tumorstage							
EarlystageI&II	15(53.0%)	7(25.0%)	6(22.0%)		37(66.1%)	19(33.9%)	
AdvancestageIII&IV	10(53.0%)	9(41.0%)	3(14.0%)	0.46	29(65.9%)	15(34.1%)	1.00
OR(95%CI)	**1.00(Ref)**	1.33[0.27-6.60]	0.52[0.14-1.84]		**1.00(Ref)**	1.00(0.43-2.31)	
Type							
Ductal	11(50.0%)	5(22.7%)	6(27.3%)		27(61.4%)	17(38.6%)	
Lobular	5(25.0%)	10(50.0%)`	5(25.0%)		20(50.0%)	20(50.0%)	
Invasive	3(30.0%)	5(50.0%)	2(20.0%)		11(55.0%)	9(45.0%)	
OR(95%CI)	**1.00(Ref)**	4.40(0.97-19.85)	1.83(0.37-8.98)		**1.00(Ref)**	1.58(0.66-3.78)	0.57
		3.66(0.61-21.73)	1.22(0.15-9.46)	0.33		1.29(0.44-3.78)	

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Discussion

This case-control study represents one of the few investigations of GSK3β rs334558 promoter polymorphism in a South Indian breast cancer cohort. While the overall genotypic and allelic distributions did not reveal statistically significant associations, several biologically plausible trends in the data merit careful consideration in the context of current molecular understanding of GSK3β biology and breast cancer pathogenesis.The modest elevation in CT heterozygote frequency in cases (38.0% vs 30.0%) and the marginal increase in C allele frequency (0.51 vs 0.49) are directionally consistent with the hypothesis that reduced GSK3β promoter activity expected under conditions of attenuated SP1 binding by the C allele would lower kinase levels within the β-catenin destruction complex, thereby facilitating Wnt pathway activation. The non-significance of these observations most likely reflects limited statistical power. Post-hoc calculations indicate that approximately 380 cases and 380 controls would be required to reliably detect an OR of 1.5 at 80% power with α = 0.05, making the present cohort underpowered by nearly four-fold for a low-penetrance variant of this expected effect size.The HER2 subgroup finding is among the more thought-provoking in the dataset. The C allele frequency was 0.80 in HER2-positive cases compared to 0.64 in HER2-negative cases. HER2 amplification drives sustained PI3K/Akt activation, and Akt is well known to phosphorylate and inactivate GSK3β at Ser9. One might initially expect lower GSK3β expression (C allele) to be enriched in HER2-positive tumours where Wnt pathway activation is already facilitated. The observed pattern T allele enrichment in HER2-positive patients could instead reflect a different mechanism: in HER2-amplified tumours where GSK3β is already post-translationally suppressed by Akt, the absolute amount of available kinase protein may matter less than in HER2-negative settings, shifting the selective pressure for genetic variants. Alternatively, there may be compensatory mechanisms at play. This observation warrants follow-up in a larger dataset, particularly given published immunohistochemical evidence linking nuclear GSK3β expression to HER2-positive breast tumours [14].

The stage-related trend CT frequency increasing from 25.0% in early-stage to 41.0% in advanced-stage disease raises an interesting possibility. GSK3β phosphorylates and targets Snail for degradation, thereby restraining EMT. A heterozygous state at the promoter, if it results in reduced kinase levels, could modestly shift the EMT threshold and confer a marginal advantage to tumour cells undergoing invasion and lymph node spread. This aligns with broader evidence that GSK3β inactivation correlates with higher-grade, more aggressive breast tumours [14], and with data from Latin American cohorts showing rs334558-associated susceptibility differences [10].

The absence of significant associations across hormone receptor strata, menopausal status, and tumour size is not unexpected for a low-penetrance promoter variant. Effects of this kind are typically only detectable in large multi-centre studies, and single-SNP candidate gene analyses often yield non-significant results that are subsequently validated through meta-analysis. Population stratification is also relevant here: the Dravidian genetic background of South Indian populations is distinct from both North Indian and European reference populations, and allele frequencies at rs334558 in this group have not previously been characterised. The main strengths of this study are its systematic multi-parameter characterisation of the variant across ten clinical and epidemiological strata and the rigorous application of both Pearson and Yates-corrected chi-square with genotype-specific ORs. The main limitations are the small sample size, single-centre design, absence of functional GSK3β mRNA or protein data, and the lack of haplotype analysis with other GSK3B variants. These limitations should guide the design of future studies.

Conclusion

The GSK3β rs334558 promoter polymorphism was not found to independently associate with breast cancer susceptibility or clinicopathological features in this South Indian cohort. The CT genotype and C allele were modestly elevated in cases, and subgroup analyses identified biologically coherent trends in HER2-positive and advanced-stage patients that are worth pursuing in a larger study. Future work should incorporate an adequately powered multi-centre design, functional GSK3β expression profiling, and haplotype-based approaches alongside interaction analyses with other established Wnt pathway susceptibility variants. Given the therapeutic tractability of GSK3β and the growing interest in Wnt pathway targeting in breast cancer treatment, its genetic epidemiology in South Asian populations represents both a scientifically important and clinically relevant research priority.

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Conflict of Interest

The authors declare no conflict of interest.

Authors Contributions

SJ has designed, executed the technical work and analysed the results; PK assisted in manuscript reviewand proof reading; RRD, clinical oncologist from NIMS hospital (Earlier), extended his support for sample and data collection from patients; VS has supplemented control and Breast cancer DNA samples; SA reviewed the analysis, results and discussion as well as mentored the work.

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