# A case-control study and meta-analysis on the association of *NME1* rs34214448 polymorphism with breast cancer risk

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## *Abstract*

*The rs34214448 single nucleotide polymorphism (SNP) in the NME/NM23 nucleoside diphosphate kinase 1 (NME1) gene has been linked to reduced NME1 expression, thus potentially elevating cancer risk. However, its impact on the Malaysian population remains underexplored. Therefore, this study aimed to investigate the association between the NME1 rs34214448 SNP and breast cancer risk in a Malaysian population. Genotyping for this SNP was performed on 74 breast cancer cases and 158 healthy controls from the Malaysian population using a polymerase chain reaction-restriction fragment length polymorphism approach. The odds ratios with 95% confidence intervals were calculated. Additionally, a meta-analysis was conducted using the comprehensive meta-analysis software. Functional annotation of the NME1 rs34214448 SNP was performed using RegulomeDB ver.2.2. The case-control analysis indicated that this SNP did not exhibit a significant association with the risk of breast cancer development in any of the tested genetic models within the Malaysian population. The meta-analysis that involved 345 cases and 434 controls from different populations showed that there is no significant association of this polymorphism with the risk of breast cancer overall.*

*In conclusion, neither the case-control study nor the meta-analysis identified a significant association between this SNP and breast cancer risk. Functional annotation revealed that this SNP is located in a binding region and is able to alter the regulatory motif of several proteins linked to breast cancer. This study underscores the importance of considering population-specific factors in genetic associations and highlights the need for further research to comprehend the SNP's role in breast cancer susceptibility across diverse populations. The mechanisms underlying the relationship between this SNP and proteins associated with breast cancer should be empirically investigated for effective treatment.*

**Keywords:** Breast cancer, rs34214448 SNP, Malaysian population, Case-control study, Functional annotation.

## Introduction

Cancer stands as the leading global cause of mortality. In the year 2022, it gave rise to an estimated 20 million fresh instances of the disease, which resulted in approximately 9.7 million fatalities[28]. Female breast cancer is a highly heterogeneous disease presenting with various subtypes, accounting for 2.3 million new cases in the year 2020[4,22]. Interestingly, research indicates that genetic factors including genetic polymorphisms in specific genes, are linked to the development of breast cancer[3,24]. Among these, the *NME/NM23* nucleoside diphosphate kinase 1 (*NME1*) gene has emerged as a frequently associated gene in breast cancer studies[15,17,18].

The *NME1* gene is a metastasis suppressor gene. Extensive research has linked NME1 to metastasis inhibition in various types of tumors including breast cancer. This gene is mapped on chromosome 17q21 and encodes the NME1 protein, consisting of 166 amino acids and its primary role is the suppression of metastasis[20]. An investigation led by Leonard et al[12] revealed that NME1 expression facilitated the repair of UV-induced DNA damage in yeast and mammalian cells through the nucleotide excision pathway, strongly suggesting its metastasis-suppressing function. *NME1* knockout animals exhibited heightened metastasis, underscoring the pivotal role of *NME1* expression in suppressing the spread of cancer[18].

This study's focal single nucleotide polymorphism (SNP) is the rs34214448 SNP, which is located within the intron 1 of the *NME1* gene. First identified in 1991, this SNP is characterized as a bi-allelic (G/T) polymorphism, causing no alteration in the corresponding amino acids[1]. Some non-coding DNA sequences serve functional roles such as regulating gene expression[13]. As such, it is theorized that while this particular SNP may not alter the NME1 protein, it is associated with reduced *NME1* expression, a factor linked to an elevated risk of cancer, as evidenced by the observed decrease in *NME1* mRNA expression in highly metastatic cells[17]. This suggests that the rs34214448 SNP might also play a vital role in breast cancer development.

Despite numerous studies examining the relationship between this SNP and the risk of breast cancer, the findings have been rather inconclusive as well as contradictory. In a study conducted by Antar et al[1] on an Egyptian population, the minor T allele of this SNP was linked with a higher likelihood of breast cancer. However, in a different study

conducted by Iqbal et al[9] and Rubio et al[19] on an Indian and Mexican populations respectively, there was no significant association between the SNP and breast cancer risk.

Furthermore, the association of the SNP with the risk of breast cancer in the Malaysian population is unclear. Therefore, this study investigated this association in a Malaysian population using a case-control setting and the data was integrated into a meta-analysis to comprehensively assess the association between the *NME1* rs34214448 SNP and the risk of breast cancer. Subsequently, functional annotation of this SNP was performed using RegulomeDB ver. 2.2.

## Material and Methods

**Study subjects and ethics statement:** A total of 74 peripheral blood samples were taken from breast cancer patients from Queen Elizabeth Hospital, Kota Kinabalu, Sabah, with written consent. One hundred fifty-eight age-matched healthy volunteers and those without a history of breast cancer were recruited as the control group. This study was approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia [reference number NMRR-15-1783-28230 (IIR)].

**DNA extraction, PCR amplification and genotyping:** DNA was extracted from the peripheral blood samples using a modified salting-out method[2]. The isolated DNA from these samples was then used as a polymerase chain reaction (PCR) template to amplify a specific region in the *NME1* gene. For each PCR reaction, a total of 20 µL of the master mix was prepared, consisting of 1X Colorless *GoTaq*® Flexi buffer (Promega, USA), 2 mM $MgCl_2$, 0.2 mM dNTP mix and 1 unit of *GoTaq*® Flexi DNA Polymerase (Promega, USA). Additionally, 0.2 µM of forward primer (5'-CCCACCGTTTATTGGCTAG-3') and 0.2 µM of reverse primer (5'- CAACCCCCTTCATTTTACAA-3') were included in the master mix. The total volume was brought to 20 µL by adding 1 µL of DNA template (approximately 100 ng) and sterile deionized water ($sdH_2O$).

After preparing the mixture, the mixture was placed into a thermal cycler and run with predefined settings as follows: 1 cycle of initial activation at 94°C for 4 minutes followed by 35 cycles of denaturation at 94°C for 20 seconds, annealing at 57°C for 30 seconds and extension at 72°C for 15 seconds and a final extension cycle at 72°C for 2 minutes.

For genotyping, the PCR products were digested using the *EcoRI* restriction endonuclease which recognizes and cleaves the nucleic acid sequence G↓AATTC with a palindromic, complementary sequence CTTAA↓G. Each reaction comprised of 1X NEB CutSmart Buffer, 2.5 units of *EcoRI* enzyme (NEB, USA) and 5 µL of PCR products. The reaction was topped up with $sdH_2O$ to a final volume of 15 µL. The mixture was then incubated at 37°C for 16 hours. Next, gel electrophoresis was performed to analyze the digested fragments following the *EcoRI* restriction endonuclease digestion for genotype scoring. The size of the fragments presents determined the genotype present in each sample. A homozygous wild-type (G/G) had a single band at 151 bp; a heterozygous genotype (G/T) had bands at 151 bp, 82 bp and 69 bp while a homozygous variant (T/T) had bands at 82 bp and 69 bp.

**Meta-analysis:** A comprehensive search was performed on the PubMed and ScienceDirect databases up to December 2024, without imposing any language restrictions for the meta-analysis. The search terms used were: "(NME1 polymorphism)" and "(breast cancer)". Furthermore, the references were manually reviewed for the retrieved papers that were deemed relevant or related to identify any additional pertinent research papers. The studies were selected based on the following inclusion criteria: 1) studies that assessed the correlation between the *NME1* rs34214448 polymorphism and the risk of breast cancer; 2) studies designed as case-control studies and 3) studies that provided sufficient data, including detailed genotyping information in both cases and control groups, which allowed for the computation of the odds ratio (OR) and a 95% confidence interval (CI).

On the other hand, studies with the following criteria were excluded: 1) review articles, letters, comments, correspondence and conference reports and 2) studies lacking the necessary data for OR and 95% CI calculations. The following data were collected from each study: the name of the primary author, the year of publication, the country in which the study was conducted, the genotyping methodology employed and the number of genotypes observed within both the case and control groups. The quality of the included studies was scored based on the Newcastle-Ottawa Scale[26].

**Statistical analysis:** In the case-control study, the Hardy-Weinberg equilibrium (HWE) in both cases and controls was assessed using the Chi-square test. OR with 95% CI was calculated using the SPSS v.26.0 software. The association was considered statistically significant when the p-value was less than 0.05. In the meta-analysis, the pooled ORs with corresponding 95% CIs were utilized to investigate the association between the *NME1* rs34214448 SNP and the susceptibility to breast cancer. Pooled ORs were computed using various genetic models, which include the allelic model (T versus G), recessive model (TT versus TG + GG), dominant model (TT + TG versus GG), homozygous model (TT versus GG) and heterozygous model (TG versus GG). Pearson's Chi-square test was used to determine the *NME1* rs34214448 SNP genotype distribution deviation in control groups from the HWE.

Heterogeneity was quantified using the $I^2$ statistics[8], with larger $I^2$ values indicating greater heterogeneity. In cases where no significant heterogeneity was observed ($I^2 \leq 50\%$), a fixed-effects model was used to calculate the pooled ORs and 95% CIs[16]. Conversely, when heterogeneity was present

($I^2$ > 50%), a random-effects model was applied to estimate the pooled ORs[6]. A funnel plot and Egger's linear regression test were used to assess evidence for potential publication bias. Begg's test was used as an additional measure to detect publication bias[27]. A sensitivity test was also performed in order to assess the robustness of the meta-analysis results and explore potential sources of heterogeneity. The comprehensive meta-analysis software v3 was used to perform the meta-analysis.

**Functional annotation of the *NME1* rs34214448 SNP:** The possible functional annotation of the *NME1* rs34214448 SNP was performed using RegulomeDB ver.2.2 (available at https://regulomedb.org/regulome-search), consisting of data on predicted and known regulatory elements, including the binding sites for transcription factors and their motifs.

## Results

**Risk association in the case-control study:** Genotypic distributions for both cases and controls were in HWE (p = 0.180 for cases and p = 0.472 for controls). No significant difference was found with respect to genotypic (p = 0.490) and allelic (p = 0.475) frequencies between the cases and controls. Overall, there was no significant association between the *NME1* rs34214448 SNP and breast cancer risk in any of the genetic models tested in the case-control study (Table 1), with p-values greater than 0.05.

**Risk association in meta-analysis:** A total of 103 published papers were initially identified in the period from January 1991 to December 2023 (Figure 1). One duplicated study was excluded between the two databases (PubMed and ScienceDirect). After removing the irrelevant records that did not meet the inclusion criteria (n = 95), the remaining seven potentially relevant articles were examined in detail. Four articles were removed because of a lack of detailed genotyping data.

Finally, four studies listed in table 2 were selected for meta-analysis, including the data from this case-control study. These studies include a total of 345 cases and 434 controls. The studies comprised of Malaysian (one study), Indian (one study), Egyptian (one study) and Mexican (one study). The genotype distribution in the control groups of these studies was all in HWE.

Overall, the *NME1* rs34214448 SNP was not significantly associated with the risk of breast cancer development in any of the genetic models tested. However, it should be noted that the Egyptian population was excluded in two of the genetic models (recessive and homozygous) due to the lack of individuals carrying the TT genotype in the control group. Interestingly, a sensitivity analysis revealed that the Egyptian population was a significant source of the observed heterogeneity, as can be seen in the difference between the results of the allelic model, dominant model and heterozygous model in tables 3 and 4 respectively. However, the association remained non-significant in all genetic models after the removal of the Egyptian population (Figure 2).

**Table 1**
**Breast cancer risk association of the *NME1* rs34214448 SNP in the Malaysian population**

| Genetic Modelm | OR (95% CI) | *p*-value |
|---|---|---|
| Allelic (T vs. G) | 0.86 (0.57 – 1.30) | 0.475 |
| Heterozygous (TG vs. GG) | 1.00 (0.56 – 1.79) | 0.991 |
| Homozygous (TT vs. GG) | 0.51 (0.16 – 1.65) | 0.259 |
| Dominant (TT+TG vs. GG) | 0.92 (0.52 – 1.61) | 0.770 |
| Recessive (TT vs. TG+GG) | 0.51 (0.16 – 1.57) | 0.240 |

**Table 2**
**Main characteristics of studies regarding the association between the *NME1* rs34214448 SNP and breast cancer risk in meta-analysis**

| Population | Cases/ Controls | Genotyping Method | Newcastle -Ottawa Scale | Cases | | | | | Controls | | | | | HWE in Controls (*p*-value) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | G/G | G/T | T/T | G | T | G/G | G/T | T/T | G | T | |
| Malaysian* | 74/158 | PCR-RFLP | 7 | 31 | 39 | 4 | 101 | 47 | 63 | 79 | 16 | 205 | 111 | 1.489 (0.475) |
| Egyptian[1] | 75/37 | PCR-RFLP | 7 | 26 | 41 | 8 | 93 | 57 | 35 | 2 | 0 | 72 | 2 | 0.029 (0.986) |
| Indian[9] | 130/199 | PCR-RFLP | 7 | 44 | 77 | 9 | 165 | 95 | 81 | 103 | 15 | 265 | 133 | 5.295 (0.071) |
| Mexican[19] | 66/40 | PCR-RFLP | 6 | 24 | 31 | 11 | 79 | 53 | 19 | 15 | 6 | 53 | 27 | 1.042 (0.594) |

* Present study

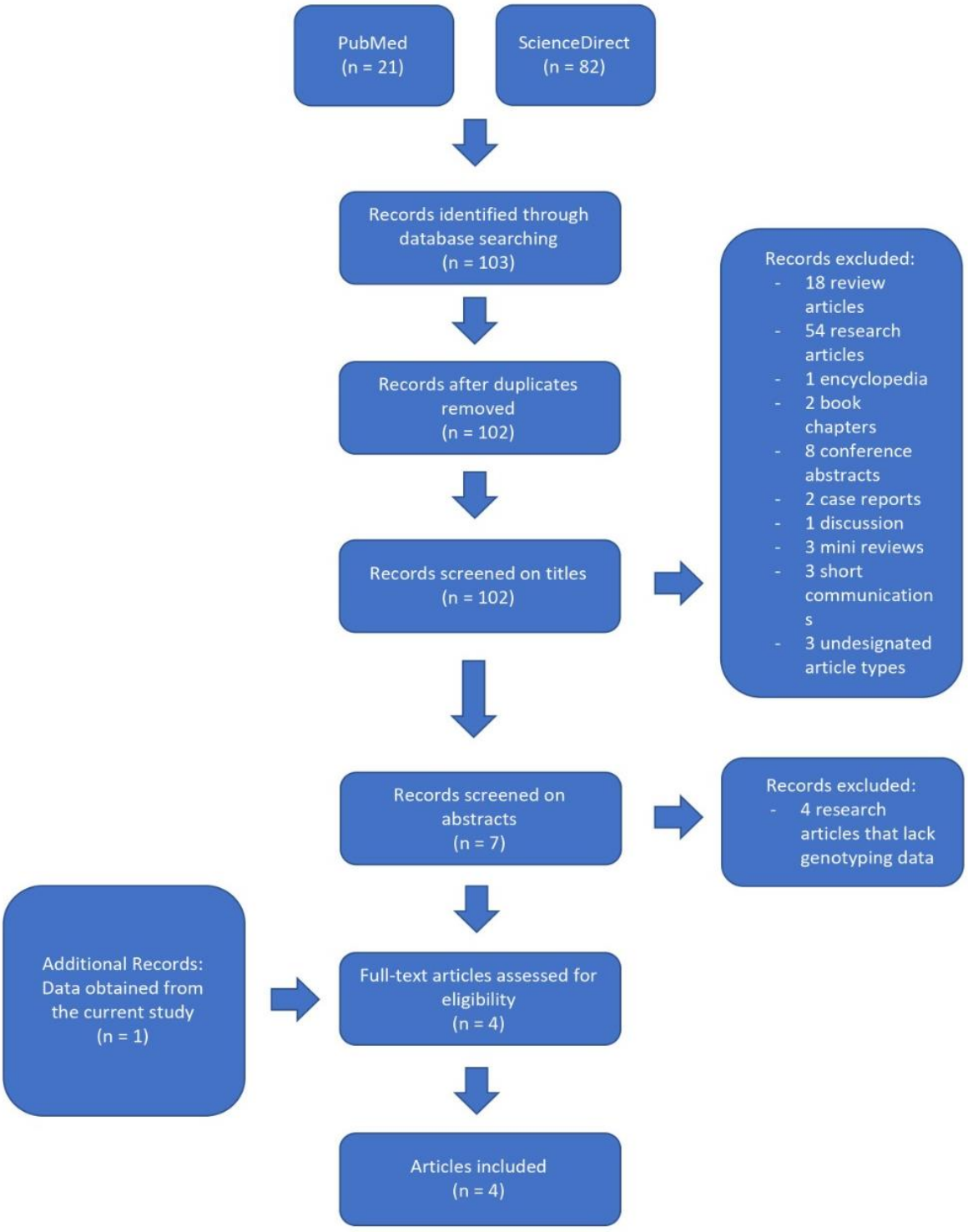


**Figure 1: Flowchart for the identification of studies used in the meta-analysis**

**Table 3**
**Overall meta-analysis results of the *NME1* rs34214448 SNP with breast cancer risk**

| Case/ Control | T vs. G | | | TT vs. TG+GG | | | TT+TG vs. GG | | | TT vs. GG | | | TG vs. GG | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **OR (95% CI)** | ***P*** | **$I^2$** | **OR (95% CI)** | ***P*** | **$I^2$** | **OR (95% CI)** | ***P*** | **$I^2$** | **OR (95% CI)** | ***P*** | **$I^2$** | **OR (95% CI)** | ***P*** | **$I^2$** |
| 345/434 | 1.60 (0.83 – 3.10) | 0. 164 | 83. 50 | 0.83[a] (0.47 – 1.49) | 0. 535 | 0. 000 | 2.16 (0.88 – 5.31) | 0. 094 | 84. 43 | 0.97[a] (0.53 – 1.78) | 0. 913 | 0. 000 | 2.15 (0.91 – 5.08) | 0. 082 | 81. 67 |

[a] Egyptian population not included due to lack of T/T genotype in control group.

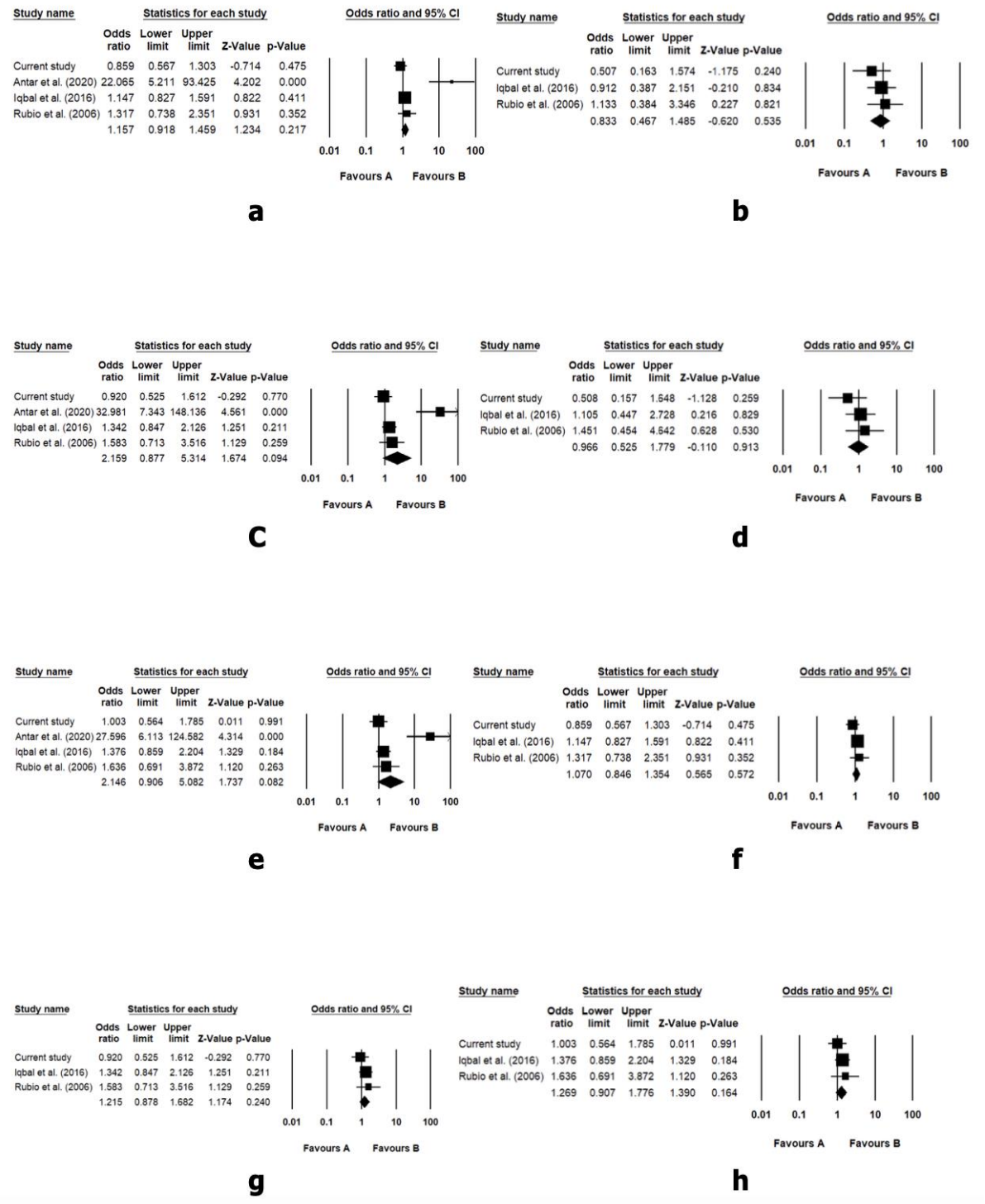


**Figure 2: Forest plots for the association of the *NME1* rs34214448 SNP with breast cancer risk. (a) Allelic model; (b) Recessive model; (c) Dominant model; (d) Homozygous model; (e) Heterozygous model; (f) Allelic model (one study removed); (g) Dominant model (one study removed); (h) Heterozygous model (one study removed)**

**Publication bias assessment in meta-analysis:** Funnel plots revealed no significant publication bias in all the comparison models (Figure 3), further supported by Egger's and Begg's tests, where the p-values were greater than 0.05 in all comparisons.

**Functional annotation analysis:** The analysis using RegulomeDB ver.2.2 predicted the *NME1* rs34214448 SNP to be located in a functional genomic location with a 2b low ranking, indicating that SNP is potentially a causal variant with a regulatory function. Based on the deoxyribonuclease-seq and formaldehyde-assisted isolation of regulatory elements-seq mapping, this SNP is strongly associated with regulatory elements linked to DNA accessibility. Further analysis showed that this SNP is located in a binding region of four proteins, including T-cell acute leukemia 1 (TAL1),

enhancer of zeste homolog 2 (EZH2), PR domain zinc finger protein 1 (PRDM1) and sine oculis homeobox homolog 4 (SIX4) (Figure 4). Additionally, this SNP is predicted to alter the regulatory motif of the TANK-binding kinase 1 (TBK1) protein.

## Discussion

The case-control study revealed no association between this SNP and breast cancer risk in all the genetic models. The absence of a significant association between the *NME1* rs34214448 SNP and breast cancer risk in the Malaysian cohort suggests that this specific SNP may not exert a substantial influence on breast cancer susceptibility within the population. These findings contrast with previous studies that reported significant associations between this SNP and cancer risk in other populations. However, the results of this case-control study underscore the variability in genetic associations across diverse populations and emphasize the importance of conducting population-specific studies to understand genetic factors contributing to the susceptibility to breast cancer.

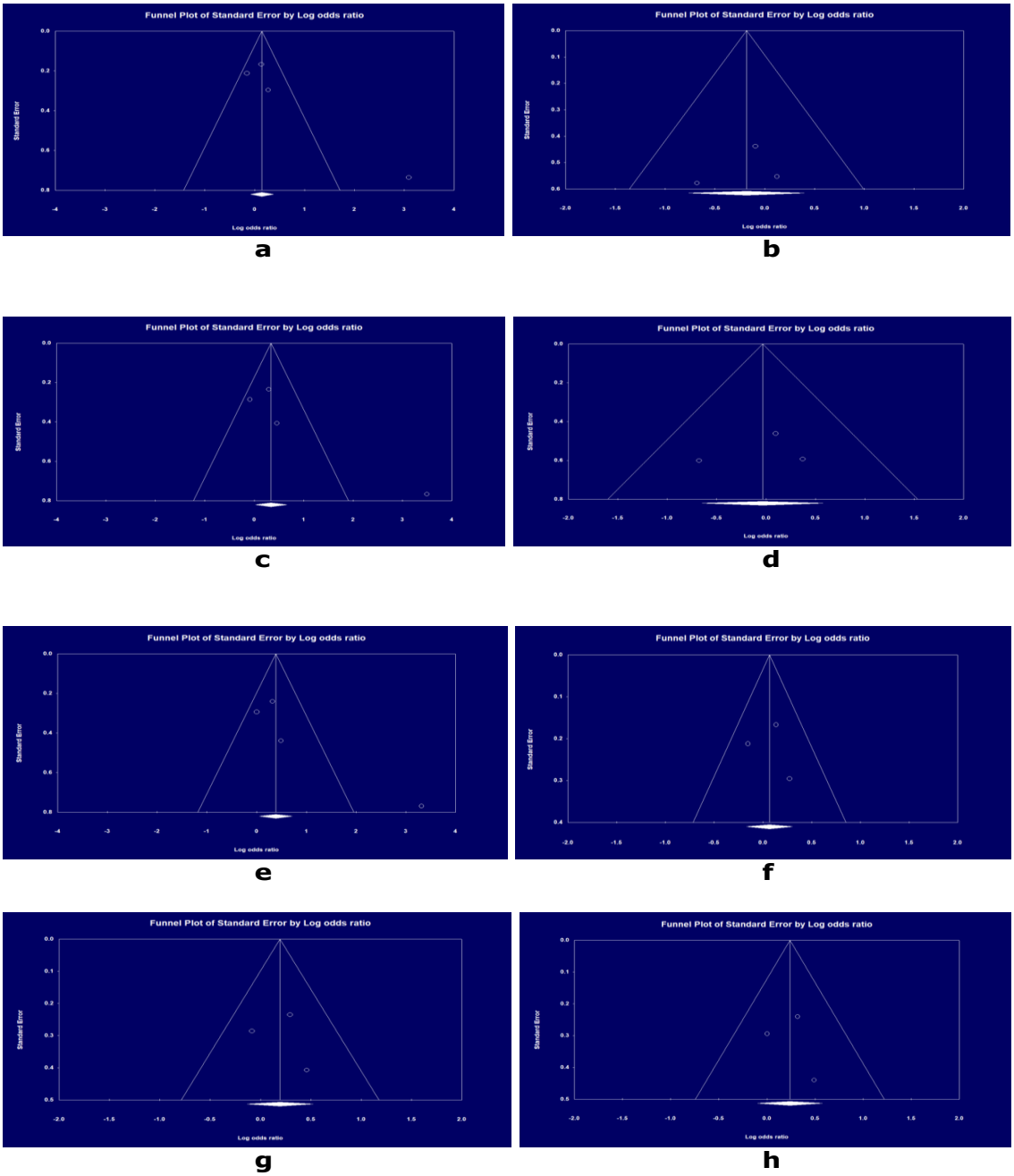


**Figure 3: Funnel plots for the assessment of publication bias. (a) Allelic model (Egger: *p* = 0.137, Begg: *p* = 0.174); (b) Recessive model (Egger: *p* = 0.732, Begg: *p* = 0.602); (c) Dominant model (Egger: *p* = 0.143, Begg: *p* = 0.174); (d) Homozygous model (Egger: *p* = 0.780, Begg: *p* = 0.602); (e) Heterozygous model (Egger: *p* = 0.152, Begg: 0.174); (f) Allelic model (one study removed) (Egger: *p* = 0.892, Begg: *p* = 0.602); (g) Dominant model (one study removed) (Egger: *p* = 0.858, Begg: *p* = 0.602); (h) Heterozygous model (one study removed) (Egger: *p* = 0.820, Begg: *p* = 0.602)**

**Table 4**
**Sensitivity test in the meta-analysis with the removal of one study**

| Case/ Control | T vs. G | | | TT vs. TG+GG | | | TT+TG vs. GG | | | TT vs. GG | | | TG vs. GG | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | OR (95% CI) | *P* | $I^2$ | OR (95% CI) | *P* | $I^2$ | OR (95% CI) | *P* | $I^2$ | OR (95% CI) | *P* | $I^2$ | OR (95% CI) | *P* | $I^2$ |
| 270/397[b] | 1.07 (0.85 – 1.35) | 0. 572 | 0. 000 | 0.83 (0.47 – 1.49) | 0. 535 | 0. 000 | 1.22 (0.88 – 1.68) | 0. 240 | 0. 000 | 0.97 (0.53 – 1.78) | 0. 913 | 0. 000 | 1.27 (0.91 – 1.78) | 0. 164 | 0. 000 |

[b] Egyptian population is removed from the statistical test.


ChIP data

| Method | Peak location | Biosample | Targets | Organ | Dataset | File | Value |
|---|---|---|---|---|---|---|---|
| ChIP-seq | chr17:49231927..49232203 | K562 | TAL1 | bodily fluid, blood | ENCSR000EHB | ENCFF482CEV | 22.04071 |
| ChIP-seq | chr17:49231580..49232530 | neural progenitor cell | EZH2 | | ENCSR069DPL | ENCFF140MVM | 16.29494 |
| ChIP-seq | chr17:49232006..49232426 | HEK293 | PRDM1 | kidney, epithelium | ENCSR098YLE | ENCFF768SBV | 62.51922 |
| ChIP-seq | chr17:49231704..49232074 | MCF-7 | SIX4 | epithelium, exocrine gland, mammary gland | ENCSR279IEM | ENCFF278ODX | 58.54611 |

**Figure 4: Functional annotation of the *NME1* rs34214448 SNP and its peak location in the binding region of four proteins**

Despite the absence of a significant relationship in the meta-analysis, the heterogeneity analysis among the included studies identified the Egyptian population as a substantial contributor to the observed variability. Identifying the Egyptian population as a source of heterogeneity signifies the existence of potential distinctive factors within that population, which subsequently influence the relationship between the SNP and breast cancer risk differently when compared to other populations, such as the Malaysian, Indian and Mexican. Identifying the Egyptian population as a significant contributor to heterogeneity in this meta-analysis prompts a deeper exploration of the potential factors underlying its divergent impact compared to other populations.

Several plausible explanations could elucidate the observed dissimilarity. First, genetic variability within the *NME1* gene may play a pivotal role. Unique allele frequencies or specific genetic variants in the Egyptian population might result in varying associations with breast cancer risk compared to other cohorts included in this meta-analysis. Moreover, genetic modifiers or interactions with other genes specific to the Egyptian population might modulate the functionality of the *NME1* gene, potentially influencing breast cancer susceptibility in distinct ways, considering how breast cancer is suggested to be a complex polygenic disease[14].

The identification of the Egyptian population as a significant source of heterogeneity, despite the lack of a significant association between the *NME1* rs34214448 SNP and breast cancer risk overall, holds critical implications for future research endeavors and clinical understanding. For instance, this divergence underscores the need for targeted research efforts specifically focused on a specific population in a large sample size. Investigating population-specific genetics is essential in elucidating the nuanced relationship between the SNP and breast cancer risk in cohorts. Such dedicated investigations might reveal insights into distinct allele

frequencies and genetic modifier exposures influencing breast cancer susceptibility[7], potentially paving the way for tailored interventions and risk stratification within a specific population.

Functional annotation of the *NME1* rs34214448 SNP has identified that the SNP is located in a binding region of four proteins. Interestingly, some of these proteins are strongly associated with breast cancer. For instance, the EZH2 protein was found to be consistently increased in invasive breast cancer compared with normal breast tissue and was significantly linked with breast cancer aggressiveness[11]. Additionally, the SIX4 protein was correlated with poor prognosis and distant metastasis of breast cancer[21]. A recent study also reported that high expression of the SIX4 gene can suppress the immune response in breast cancer patients, leading to poor survival[29].

Nevertheless, further assessment also revealed that this SNP could alter the regulatory motif of the TBK1 protein. The increased expression of this protein has been reported to enhance tamoxifen resistance in breast cancer patients and shows a high risk of relapse[25]. Therefore, it is important to understand the mechanisms underlying this SNP and these proteins in breast cancer development.

Despite providing valuable insights, this study has several limitations that warrant acknowledgment. First, the relatively modest sample size of the Malaysian cohort, consisting of 232 individuals, must have limited the statistical power to detect small effect sizes or subtle associations. Larger sample sizes are essential for robustly identifying genetic associations[23], particularly in complex diseases like breast cancer where multiple genetic and environmental factors may interact synergistically. Furthermore, this study focused exclusively on examining the association between breast cancer risk and the *NME1* rs34214448 SNP. While this SNP has been implicated in cancer susceptibility in previous studies, breast cancer is a multifactorial disease influenced by numerous genetic variants, as well as environmental and lifestyle factors[5].

This study's narrow focus may have overlooked potential contributions from other genetic variants or gene-gene interactions that could influence breast cancer risk. There is also a potential for selection bias in participant recruitment as this study relied on voluntary participation from individuals within the Malaysian population. This may have introduced biases related to access to healthcare, awareness of breast cancer risk factors, or other socioeconomic factors[10]. While efforts were made to mitigate selection bias through rigorous participant recruitment and inclusion criteria, the possibility of residual bias cannot be completely ruled out.

## Conclusion

In conclusion, the case-control study suggests that no significant association exists between the *NME1* rs34214448 polymorphism and breast cancer risk in the Malaysian population. Similarly, no significant association exists between the *NME1* rs34214448 SNP and breast cancer in any of the genetic models tested in the meta-analysis. However, identifying the Egyptian population as a significant contributor to heterogeneity in the meta-analysis emphasizes the need for targeted research in a specific population with a large sample size to provide a more holistic view of this genetic variant in relation to breast cancer susceptibility.

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