**Systematic Review & Meta-Analysis**

# GENETIC ASSOCIATIONS OF INTERLEUKIN-2 rs2069762 AND EARLY GROWTH RESPONSE 3 rs3750192 POLYMORPHISMS WITH SCHIZOPHRENIA

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## ABSTRACT

Although genome-wide association studies have identified numerous genes linked to schizophrenia, their specific roles remain unclear. This meta-analysis sought to explore the association of polymorphisms in the interleukin-2 (IL-2) rs2069762 and early growth response 3 (EGR3) rs3750192 with schizophrenia susceptibility. This study was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under reference ID 585910 and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The literature review on schizophrenia was conducted using databases, such as PubMed, Embase, and Google Scholar, with specific search terms. The inclusion criteria focused on studies linking IL-2, EGR3, and schizophrenia, requiring genotypic and allele frequency data. Studies utilizing animal models or those with a lack of control groups and insufficient data were excluded. The selected studies examined the IL-2 and EGR3 polymorphisms and their associations with schizophrenia risk across different populations. The data extraction included genotypic and allelic frequencies, sample size, publication details, Hardy-Weinberg equilibrium values, and ethnicities. Fixed-effect and random-effect models were employed for analyses under allele, dominant, recessive, and overdominant models. Subgroup analyses were conducted by ethnicity. No significant associations with schizophrenia risk were found for IL-2 rs2069762 across all genetic models. The allele (OR=0.94, p=0.34), dominant (OR=0.85, p=0.55), recessive (OR=0.93, p=0.76), and overdominant (OR=0.94, p=0.76) models showed non-significant results. Conversely, EGR3 rs3750192 demonstrated a significant association in the dominant (OR=0.73, p=0.012) and homozygous (OR=0.70, p=0.004) models, suggesting a protective effect. The subgroup analyses indicated ethnic differences, with Polish and Chinese showing significant protective effects for IL-2 and EGR3, respectively. While IL-2 rs2069762 lacks a consistent association with schizophrenia, EGR3 rs3750192 may confer a protective effect, particularly in certain ethnic groups. Further research is required to confirm these findings and understand the genetic mechanisms underlying schizophrenia risk across populations.

**Keywords:** Schizophrenia; interleukin-2 (IL-2); early growth response 3 (EGR3); genetic polymorphisms; mental illness

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**Highlights:**

1. The novelty of this study lies in its thorough examination of gene polymorphisms and their associations with schizophrenia risk across different populations.
2. This meta-analysis provides new insights by highlighting the lack of reliable association for interleukin-2 (IL-2) rs2069762 and revealing a substantial protective effect of early growth response 3 (EGR3) rs3750192 in specific ethnic groups, particularly among Polish and Chinese.

## INTRODUCTION

The rising incidence of mental health illnesses has become an international concern. Approximately one-eighth of the world population suffers from such conditions, highlighting a substantial global problem (Pallavi et al. 2024, Zhang et al. 2024). As reported by Kieling et al. (2024), the Global Burden of Disease Study 2019 revealed that more than one in ten people worldwide have a diagnosable mental illness, with high rates among children and youth.

Schizophrenia (SCZ) and related psychotic diseases represent severe forms of mental illness, imposing significant financial strain on patients and their families. Schizophrenia is a multifaceted disorder arising from malfunctions in brain development due to environmental, genetic, or both factors. Psychotic symptoms are partly caused by dopaminergic neurotransmission dysfunction, although evidence suggests extensive involvement of other brain circuits and regions (Owen et al. 2016, Cho et al. 2020).

The substantial heritability of schizophrenia has been recognized for a considerable time, and our understanding of its genetic basis has greatly improved. Genome-wide association studies (GWAS) have provided a wealth of genetic data, offering new insights into the etiology of this condition (Smeland et al. 2020). Over 600 genes potentially related to schizophrenia have been identified. However, no specific biochemical abnormalities have been linked to the disorder. Instead, research focuses on how genetic defects in various pathways, such as neurotransmitter metabolism, contribute to disease development (Tanrıkulu 2020).

Gene polymorphisms, as the most common form of genetic diversity in humans, have garnered significant interest in disease research and public health. Genetic susceptibility plays a crucial role in the pathophysiology of schizophrenia, with studies showing that single nucleotide polymorphisms (SNPs) contribute to unique genetic variations associated with increased risk (Năstase et al. 2022). Among the candidate genes for schizophrenia, those encoding cytokines are promising due to their roles in the central nervous system (CNS). Cytokine changes in schizophrenia patients can be classified into four groups: (1) increased cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α); (2) non-altered cytokines, such as interleukin-2 (IL-2) and interleukin-4 (IL-4); (3) cytokines with variable changes, such as interleukin-8 (IL-8); and (4) cytokines with fluctuating levels, such as interleukin-10 (IL-10) (Năstase et al. 2022).

IL-2 is crucial for producing regulatory T (Treg) cells, which maintain immune homeostasis and equilibrate innate and adaptive immune responses. This growth factor regulates the autocrine function of T cells and is deeply involved in the immune response. It has been found that IL-2 levels differ between schizophrenia patients and healthy controls, with inflammation potentially influencing this variation. Given its specific role in Treg cell development and its association with immune dysregulation, IL-2 emerges as a critical candidate for further investigation in the neurobiological underpinnings of schizophrenia (Momtazmanesh et al. 2019, Huang et al. 2022). Alterations in cytokine profiles, including IL-2, IL-6, and IL-10, suggest immunological and neurotransmission dysfunction in schizophrenia. Changes in these immune status indicators are linked to the severity of clinical symptoms and possible clinical outcomes (Morozova et al. 2021). Notably, certain negative and cognitive symptoms of schizophrenia are especially associated with IL-2. The level of IL-2 is a critical predictor of negative symptoms and cognitive impairment in outpatients with schizophrenia (Gonzalez-Blanco et al. 2019, Huang et al. 2022).

As a part of the immediate early gene (IEG) family, the early growth response (EGR) gene family encompasses EGR3, which is responsive to neuronal activity and relies on glutamate ionotropic receptor N-methyl-D-aspartate type (GRIN) receptors and calcium signaling associated with schizophrenia. EGR-family genes, including EGR1, EGR2, EGR4, and nerve growth factor-induced gene A binding protein 2 (NAB2), are mapped to the schizophrenia-related regions in genome-wide association studies (GWAS) and interact with EGR3 in regulatory feedback loops. Dysfunction in these genes impacts growth factor processes, memory, immune response, synaptic plasticity, myelination, and vascularization. According to the information presented, we hypothesized that genes regulated by EGR3 contribute to the risk of schizophrenia and other neuropsychiatric disorders involving cognition, memory, and synaptic function (Marballi et al. 2022). This meta-analysis thus focused on polymorphic variants of the cytokine gene IL-2 rs2069762 and the transcription factor EGR3 rs3750192 since the roles of these variants in schizophrenia remain unclear and require further research to elucidate their potential contributions to genetic susceptibility.

## MATERIALS AND METHODS

This study was assigned the Prospective Register of Systematic Reviews (PROSPERO) reference ID of 585910. The conduct of this study adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Sarkis-Onofre et al. 2021). The research on schizophrenia was conducted by searching through databases, such as ResearchGate, Google Scholar, Embase, PubMed, ScienceDirect, and Web of Science. We used the following phrases for the literature search: "SCZ," "early growth response protein 3," "interleukin-2," "gene polymorphisms," and "SNPs," combined with Boolean operators such as "AND" and "OR," to filter the studies. The selection of the appropriate articles began by reviewing each title and abstract. Our study was further

strengthened by reviewing citations from these pertinent publications. Relevant references were examined as part of the data evaluation and the determination of inclusion and exclusion criteria. Only articles in English were considered.

For this meta-analysis, we selected studies that met specific inclusion criteria, focusing on those examining the link between IL-2, EGR3, and schizophrenia. Included studies had to provide genotypic and allele frequency data necessary for calculating odds ratios (OR), statistical significance (p), and 95% confidence intervals (CI). The selected articles were required to present genotype frequencies for cases and controls and to employ a case-control study design. Studies were excluded for the following reasons: use of animal models, use of cell lines, absence of a control group, insufficient data, duplication of articles or results, and lack of a comparable control group (Swift & Wampold 2018).

We carefully selected data from relevant publications using predefined criteria and extracted the required information as instructed. The genotypic and allelic frequencies for both case and control groups were determined by thoroughly analyzing the collected literature. Studies were deemed ineligible if they did not provide the necessary information for patient and control groups or if they lacked the required genotypic data, including allelic frequencies. Each study included several data points: sample size, PubMed ID, year of publication, language, first author's name, Hardy-Weinberg equilibrium (HWE) value, and ethnicity (Neamatzadeh et al. 2024).

This statistical analysis used the Risk of Bias 2 (RoB2) tool (Cochrane, London, UK) to analyze the methodological quality and possible biases of the selected studies. The detected bias levels were divided into three categories: "high risk," "some concerns," and "low risk" (Sterne et al. 2019). Two techniques were used to further evaluate the validity of the chosen studies: the Newcastle-Ottawa Scale (NOS) and the Hardy-Weinberg equilibrium (HWE). A control genotype exhibiting a significance value of more than 0.05 was assigned to HWE to verify its adherence to the equilibrium. The NOS, with a maximum possible score of nine, evaluated the studies based on three main factors: exposure, comparability, and selection. Only papers with a NOS score of six or above were considered in this meta-analysis.

A systematic method incorporating certain processes and tools was necessary to thoroughly conduct this meta-analysis of genetic interactions. All genetic alterations were assessed using the MetaGenyo: Meta-Analysis of Genetic Association Studies, version 1.0 (GENYO, Granada, Spain), with a significance threshold of $p<0.05$ during the data analysis (Martorell-Marugan et al. 2017). The analysis included maximizing statistical power, evaluating genetic variations, and applying rigorous statistical inference to extensive genetic investigations. The $I^2$ metric value provided evidence for the heterogeneity assumption. A fixed effect technique was used to compute the odds ratio (OR) and its related 95% CI when the $I^2$ value was below 50. On the other hand, a random-effects method was applied for an $I^2$ value greater than 50. The HWE was analyzed using the chi-square test. In addition, a sensitivity plot was examined to evaluate the impact of excluding particular trials, especially those in which the control group diverged from the HWE. Egger's regression was employed to assess the possibility of publication bias.

A 95% CI and a significance threshold of $\alpha=0.05$ were used in a power analysis of the metadata. The statistical power for each gene was evaluated separately for every study, considering the sample sizes of the case and control groups. Power calculations were performed using G*Power, version 3.1.9.6 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) (Faul et al. 2007).

Subsequent to the power calculations, genetic interactions and gene ontology were evaluated. Pathways associated with schizophrenia were explored using the GeneMANIA web interface, focusing on the IL-2 and EGR3 genes in relation to immune response, severity, and disease susceptibility (Warde-Farley et al. 2010). This tool was employed for conducting correlation analysis to evaluate genes and gene networks functionally related to schizophrenia, with scores of $\geq 0.4$ indicating substantial relationships. The generated network revealed significant connections between genes. In addition, pathway analysis was performed using Gene Ontology data to gain a thorough knowledge of the biological processes, molecular roles, and cellular components connected to these genes, especially concerning schizophrenia.

After the analysis of the genetic interactions and gene ontology, protein-protein interactions (PPI) were assessed. The STRING online database, version 11.0 (STRING Consortium), was utilized to predict protein-protein interactions (PPIs) and functional proteins associated with the discovered SNPs in the context of schizophrenia, with a minimum score of $\geq 0.4$ (Szklarczyk et al. 2019).

## RESULTS

The literature search resulted in the selection of four publications focusing on the EGR3 gene and its

variant (rs3750192) along with five articles addressing the IL-2 gene and its variant (rs2069762). The data from the four studies on EGR3 polymorphism comprised 1,552 schizophrenia cases and 1,808 controls, whereas the data on IL-2 gene polymorphism included 1,128 people with schizophrenia and 1,116 controls. We gathered and carefully reviewed these documents to identify their relevance to our study and determine whether they contained essential information. The methods used to study IL-2 and EGR3 gene polymorphisms are shown in Figures 1a and 1b, respectively. This meta-analysis examined the relationship between the collected data and schizophrenia, as reported in the selected papers, with a particular focus on the IL-2 rs2069762 and EGR3 rs3750192 gene variants. Tables 1 and 2 provide detailed information on the characteristics of the patients and controls as well as the association between the rs2069762 polymorphism in IL-2 and the rs3750192 polymorphism in EGR3 with regard to schizophrenia susceptibility [12–20].

Table 1. Characteristics of the selected case-control studies on IL-2 gene polymorphisms and schizophrenia.

| Studies | Ethnicities | Cases | | | Controls | | | Total cases | Total controls | HWE (p) | NOS scoring |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | GG | GT | TT | GG | GT | TT | | | | |
| Ozdilli et al. (2024) | Turkish | 25 | 13 | 89 | 10 | 12 | 78 | 127 | 100 | 0 | 6 |
| Amin et al. (2023) | Javanese | 28 | 53 | 39 | 42 | 33 | 45 | 120 | 120 | 0 | 6 |
| Paul-Samojedny et al.,2013 | Polish | 6 | 90 | 19 | 17 | 116 | 2 | 115 | 135 | 0 | 8 |
| Watanabe et al. (2008) | Japanese | 232 | 251 | 53 | 225 | 228 | 57 | 536 | 510 | 0.94 | 6 |
| Schwarz et al. (2005) | Caucasian | 24 | 88 | 118 | 20 | 127 | 104 | 230 | 251 | 0.02 | 7 |

Legends: HWE=Hardy-Weinberg equilibrium; NOS=Newcastle-Ottawa Scale.

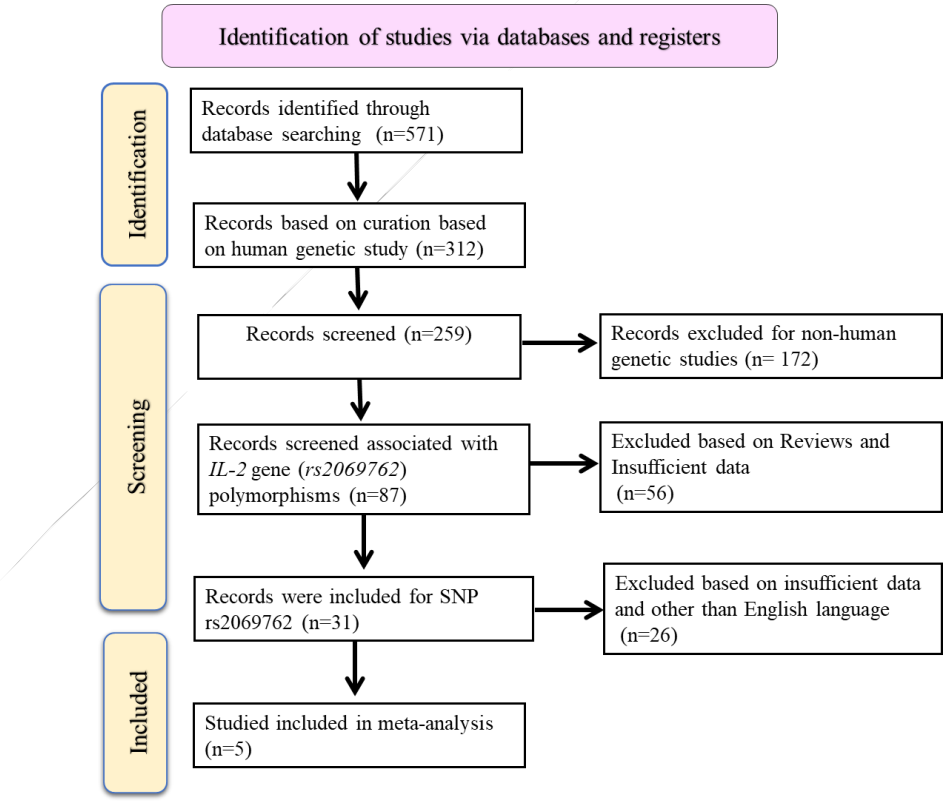


Figure 1a. A flow diagram showing the study selection of IL-2 rs2069762 polymorphism.

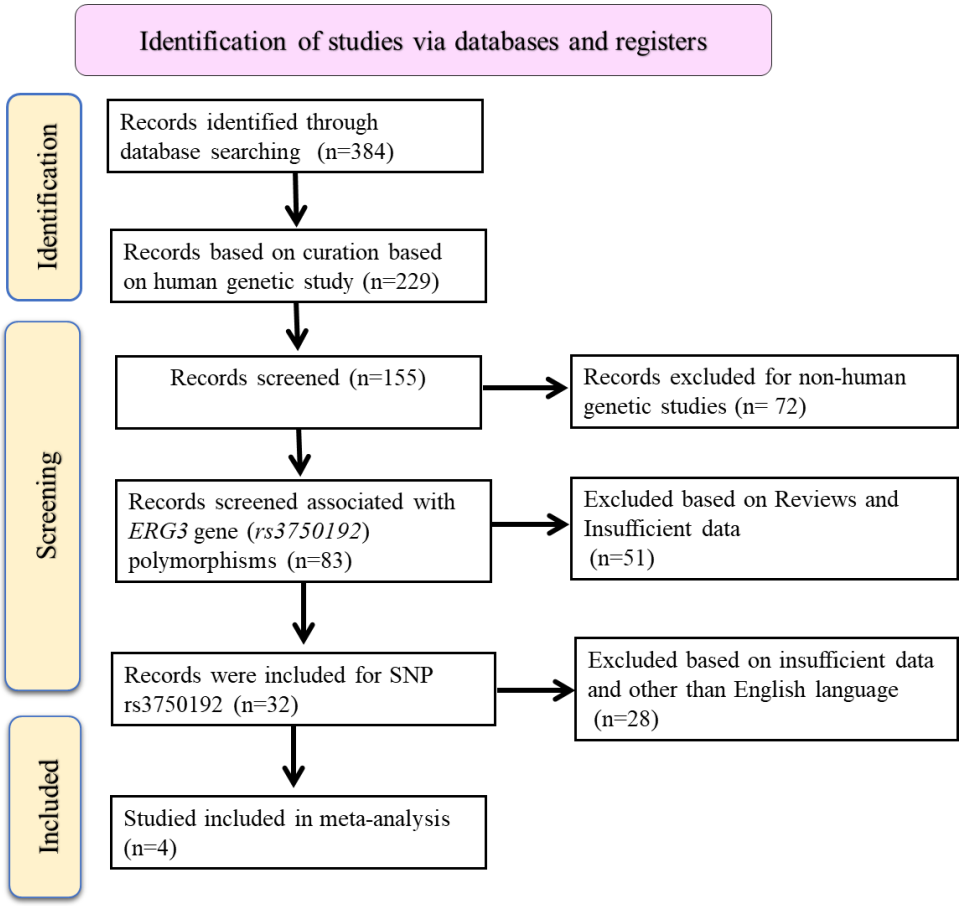


Figure 1b. A flow diagram of the study selection of EGR3 rs3750192 polymorphism.

Table 2. Characteristics of the selected case-control studies of EGR3 gene polymorphism and schizophrenia.

| Studies | Ethnicities | Cases | | | Controls | | | Total cases | Total controls | HWE (p) | NOS scoring |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | GG | GT | TT | GG | GT | TT | | | | |
| Cheng et al. (2012) | Chinese | 344 | 99 | 99 | 554 | 92 | 92 | 542 | 738 | 0 | 6 |
| Kyogoku et al. (2011) | Japanese | 159 | 122 | 15 | 143 | 84 | 13 | 296 | 240 | 0.88 | 6 |
| Kim et al. (2010) | Korean | 144 | 81 | 19 | 200 | 129 | 21 | 244 | 350 | 0.97 | 8 |
| Zhang et al. (2012) | Chinese | 338 | 115 | 17 | 318 | 144 | 18 | 470 | 480 | 0.73 | 6 |

Legends: HWE=Hardy-Weinberg equilibrium; NOS=Newcastle-Ottawa Scale.

**Assessment of the methodological quality of the studies**

The Newcastle-Ottawa Scale (NOS) and the Hardy-Weinberg equilibrium (HWE) were employed to evaluate the research quality of the selected studies. Only publications with a NOS score of six or higher were selected to guarantee high-quality research employing reliable methods and minimize the possibility of bias. Only studies with control genotype distributions that satisfied the HWE requirement ($p>0.05$) were included to maintain the integrity of the genetic data and avoid biases. These organized parameters enhanced the precision and integrity of the results obtained from this meta-analysis.

**Analyses of quantitative data on IL-2 rs2069762 and EGR3 rs3750192**

Our study reviewed a total of nine studies related to IL-2 and EGR3 gene variants and their potential association with the development of schizophrenia. Our findings showed no significant association between the risk of schizophrenia and the allelic, dominant, and recessive models of the IL-2 and EGR3 polymorphisms. Specifically, the analysis of IL-2 rs2069762 using fixed and random effect

models revealed that no associations were identified in the allele, dominant, recessive, and overdominant models. Figures 2a, 2b, 2c, and 2d show the analyzed models for IL-2 rs2069762: the comparison of G vs. T for the allele model ($I^2$=68%, OR=0.94, 95% CI=0.73–1.19, p=0.34), GG+GT vs. TT for the dominant model ($I^2$=79%, OR=0.85, 95% CI=0.50–1.45, p=0.55), GG vs. GT+TT for the recessive model ($I^2$=67%, OR=0.93, 95% CI=0.60–1.46, p=0.76), and GT vs. GG+TT for the overdominant model ($I^2$=77%, OR=0.94, 95% CI=0.61–1.44, p=0.76), respectively.

Both fixed and random effect models were utilized for EGR3 rs3750192. A significant association was detected between the dominant and homozygous groups. As shown in Figures 3a, 3b, 3c, and 3d, the models were as follows: G vs. T for the allele model ($I^2$=87%, OR=0.89, 95% CI=0.64–1.24, p=0.492), GG+GT vs. TT for the dominant model ($I^2$=0%, OR=0.73, 95% CI=0.58–0.94, p=0.012), GG vs. GT+TT for the recessive model ($I^2$=86%, OR=0.89, 95% CI=0.60–1.32, p=0.56), and GG vs. TT for the homozygous model ($I^2$=27%, OR=0.70, 95% CI=0.54–0.89, p=0.004), respectively.

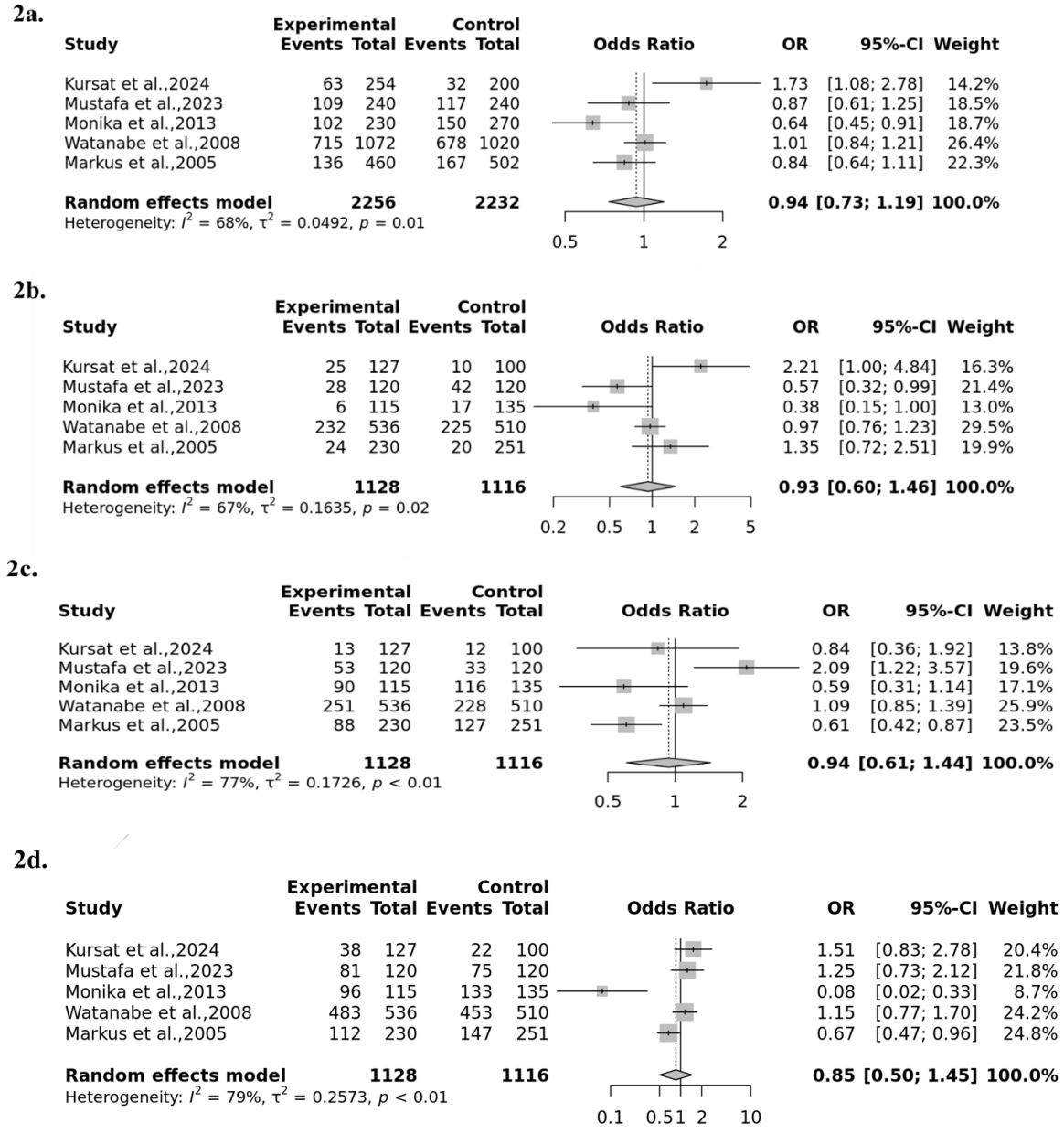


Figure 2. Forest plots showing an association between IL-2 rs2069762 polymorphism and schizophrenia in four different models: (a) allelic, (b) dominant, (c) recessive, and (d) overdominant.

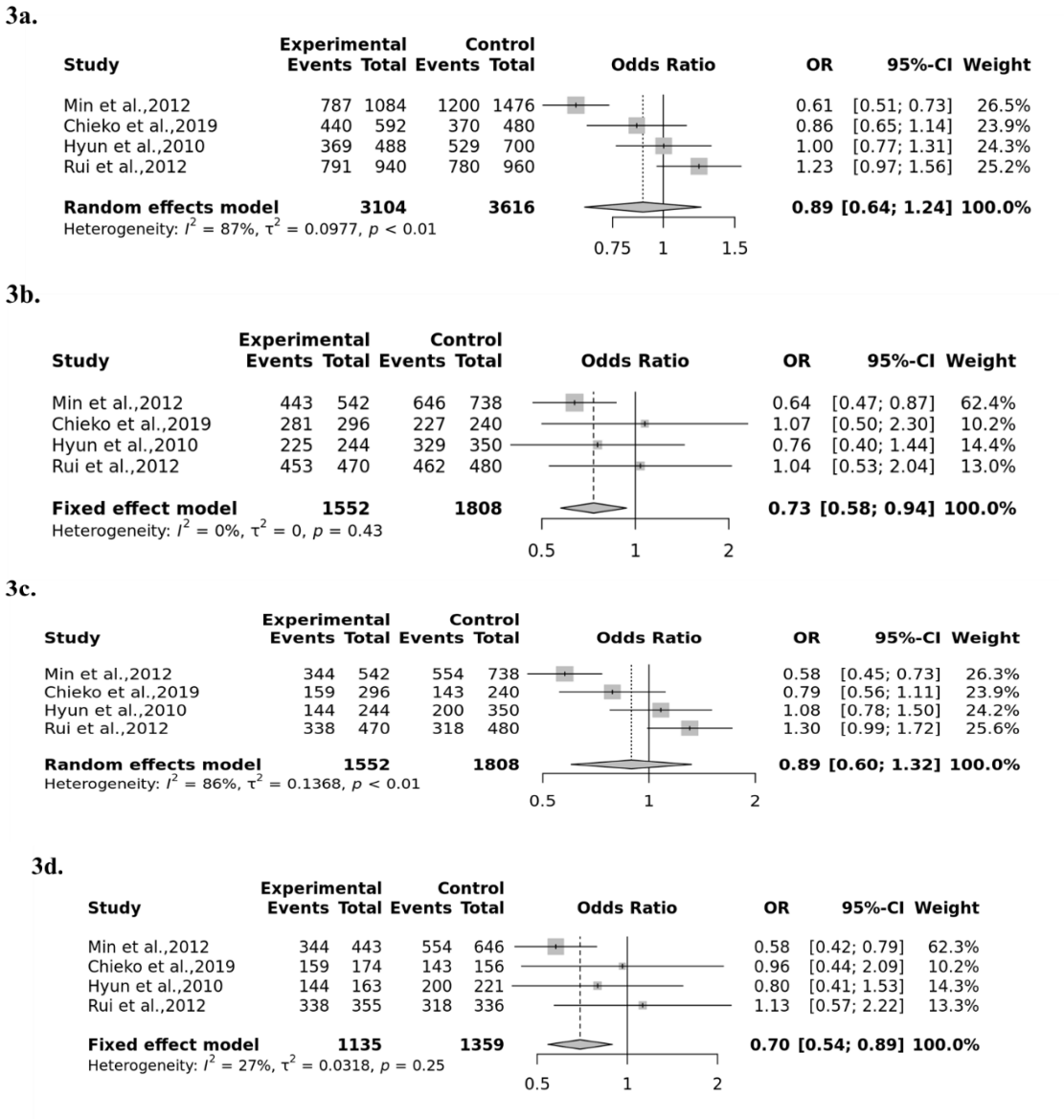


Figure 3. Forest plots displaying an association between EGR3 rs3750192 polymorphism and schizophrenia across four models: (a) allelic, (b) dominant, (c) recessive, and (d) overdominant.

### Analyses of subgroups concerning IL-2 and EGR3

The subgroup analysis of IL-2 and EGR3 revealed significant ethnic variations in genetic associations across different models, highlighting the importance of considering ethnic differences in genetic research (Table 3). The Polish group showed a strong protective effect for IL-2 in the dominant model (OR=0.076, p=0.0006) and pairwise comparisons (AA vs. aa and Aa vs. aa). In contrast, the Turkish group demonstrated an increased risk in the recessive model (OR=2.2059, p=0.0486). The Javanese group exhibited significant protective effects in the recessive (OR=0.5652, p=0.0479) and pairwise (AA vs. Aa) models. However, this group showed an increased risk in the overdominant model (OR=2.0855, p=0.0075). These results demonstrated high variability among studies, as reflected in the high overall heterogeneity ($I^2$) across models. Furthermore, the Egger's test indicated no significant publication bias in most cases.

Similar to the results for IL-2, the subgroup analysis of EGR3 revealed varying associations across different ethnic groups (Table 4). No significant association was observed in the allele contrast model, either overall or within specific ethnicities. However, the dominant model showed a significant overall protective effect (OR=0.7339, p=0.0129), particularly among the Chinese subgroup (OR=0.6933, p=0.0105), suggesting that the

presence of one or more A alleles might reduce schizophrenia risk compared to aa. The pairwise comparison between AA and aa (pairw1) also demonstrated a significant overall protective effect (OR=0.6955, p=0.0041), though this effect was inconsistent across individual ethnicities. Other models, including the recessive, overdominant, pairw2 (AA vs. Aa), and pairw3 (Aa vs. aa), did not show significant associations, either overall or within subgroups. These findings suggested a potential protective role of certain genotypes in the Chinese population, with a lack of consistent significant associations across other ethnicities and genetic models.

Table 3. Subgroup analysis of IL-2 gene polymorphism.

| Models | Ethnicities | Studies (n) | Test of association | | | Models |
|---|---|---|---|---|---|---|
| | | | OR | 95% CI | p | |
| Allele contrast (A vs. a) | Overall | 5 | 0.9358 | [0.7346; 1.1922] | 0.591384 | Random |
| | Caucasian | 1 | 0.842 | [0.6408; 1.1064] | 0.217148 | Fixed |
| | Japanese | 1 | 1.0103 | [0.8424; 1.2116] | 0.912323 | Fixed |
| | Javanese | 1 | 0.8747 | [0.6111; 1.2521] | 0.464532 | Fixed |
| | Polish | 1 | 0.6375 | [0.4475; 0.9083] | 0.012674 | Fixed |
| | Turkish | 1 | 1.7317 | [1.0788; 2.7798] | 0.022973 | Fixed |
| Recessive model (AA vs. Aa+aa) | Overall | 5 | 0.9334 | [0.5951; 1.4641] | 0.7642 | Random |
| | Caucasian | 1 | 1.3456 | [0.7221; 2.5075] | 0.349892 | Fixed |
| | Japanese | 1 | 0.9667 | [0.7570; 1.2343] | 0.785746 | Fixed |
| | Javanese | 1 | 0.5652 | [0.3211; 0.9949] | 0.047946 | Fixed |
| | Polish | 1 | 0.3821 | [0.1454; 1.0043] | 0.051034 | Fixed |
| | Turkish | 1 | 2.2059 | [1.0049; 4.8421] | 0.048588 | Fixed |
| Dominant model (AA+Aa vs. aa) | Overall | 5 | 0.8543 | [0.5045; 1.4463] | 0.557639 | Random |
| | Caucasian | 1 | 0.6715 | [0.4683; 0.9629] | 0.030358 | Fixed |
| | Japanese | 1 | 1.1467 | [0.7722; 1.7027] | 0.497395 | Fixed |
| | Javanese | 1 | 1.2462 | [0.7324; 2.1203] | 0.417092 | Fixed |
| | Polish | 1 | 0.076 | [0.0173; 0.3339] | 0.000645 | Fixed |
| | Turkish | 1 | 1.5138 | [0.8252; 2.7769] | 0.180448 | Fixed |
| Overdominant (Aa vs. AA + aa) | Overall | 5 | 0.9353 | [0.6068; 1.4417] | 0.762069 | Random |
| | Caucasian | 1 | 0.6051 | [0.4208; 0.8701] | 0.00671 | Fixed |
| | Japanese | 1 | 1.0893 | [0.8539; 1.3895] | 0.491059 | Fixed |
| | Javanese | 1 | 2.0855 | [1.2167; 3.5747] | 0.00751 | Fixed |
| | Polish | 1 | 0.5897 | [0.3057; 1.1374] | 0.115073 | Fixed |
| | Turkish | 1 | 0.8363 | [0.3638; 1.9225] | 0.673739 | Fixed |

Table 4. Subgroup analysis of EGR3 gene polymorphism.

| Models | Ethnicities | Studies (n) | Test of association | | | Models |
|---|---|---|---|---|---|---|
| | | | OR | 95% CI | p | |
| Allele contrast (A vs. a) | Overall | 4 | 0.8908 | [0.6404; 1.2390] | 0.492056 | Random |
| | Chinese | 2 | 0.8606 | [0.4342; 1.7057] | 0.667036 | Random |
| | Japanese | 1 | 0.8606 | [0.6494; 1.1405] | 0.296094 | Fixed |
| | Korean | 1 | 1.0024 | [0.7659; 1.3119] | 0.986354 | Fixed |
| Recessive model (AA vs. Aa+aa) | Overall | 4 | 0.891 | [0.6019; 1.3189] | 0.564071 | Random |
| | Chinese | 2 | 0.8651 | [0.3890; 1.9240] | 0.722382 | Random |
| | Japanese | 1 | 0.7872 | [0.5578; 1.1110] | 0.173507 | Fixed |
| | Korean | 1 | 1.0800 | [0.7753; 1.5045] | 0.649115 | Fixed |
| Dominant model (AA+Aa vs. aa) | Overall | 4 | 0.7339 | [0.5752; 0.9365] | 0.012861 | Fixed |
| | Chinese | 2 | 0.6933 | [0.5237; 0.9179] | 0.010512 | Fixed |
| | Japanese | 1 | 1.0728 | [0.5002; 2.3009] | 0.856689 | Fixed |
| | Korean | 1 | 0.7559 | [0.3972; 1.4383] | 0.393838 | Fixed |
| Overdominant (Aa vs. AA + aa) | Overall | 4 | 1.0681 | [0.7509; 1.5192] | 0.713963 | Random |
| | Chinese | 2 | 1.0866 | [0.5311; 2.2230] | 0.820117 | Random |
| | Japanese | 1 | 1.3021 | [0.9157; 1.8516] | 0.141589 | Fixed |
| | Korean | 1 | 0.8513 | [0.6037; 1.2006] | 0.358757 | Fixed |

### Analyses of sensitivity and publication bias

The sensitivity analysis was performed to evaluate the reliability of our findings while considering variations between different studies. We specifically focused on deviations from Hardy-Weinberg Equilibrium (HWE). We excluded from our analysis any studies that used alternative interventions or did not adhere to the HWE principle. Importantly, the final statistical significance (p) remained unchanged even after these exclusions. Figures 4a and 4b present the funnel plots utilized to examine the potential for publication bias. Our analysis used the Egger's test, which revealed that the significance values for IL-2 and EGR3 genes were higher than the conventional cutoff of 0.05, indicating no publication bias among the included studies.

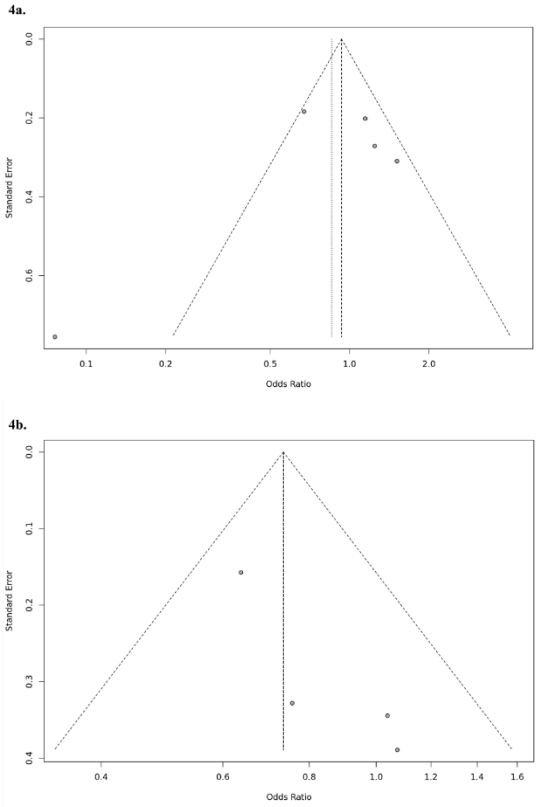


Figure 4. Publication bias regarding the association between the polymorphisms of (a) IL-2 and (b) EGR3 and schizophrenia across all models.

### Power analyses of IL-2 rs2069762 and EGR3 rs3750192 gene polymorphisms

The G*Power statistical software package was utilized to estimate the sample size and conduct a post-hoc power analysis. Our examination revealed that the sample sizes reported in the literature under review were sufficient to attain the desired significance level, with an alpha error rate set at 0.05. A two-tailed hypothesis test evaluated the probability of detecting an effect of a predetermined magnitude under specific conditions, encompassing sample size, effect size, and significance level. The number of participants included in the studies ensured a statistical power exceeding 0.8 for the IL-2 and EGR3 genes, as determined by the post-hoc power analysis. The power calculations were performed according to the alpha error probability of 0.05, the anticipated effect magnitude, and the overall number of participants. Table 5 shows that the study findings demonstrated a statistical power of 0.9975 for the IL-2 rs2069762 gene polymorphism and 0.9999 for the EGR3 rs3750192 polymorphism.

Table 5. Power analyses of IL-2 and EGR3 genes in schizophrenia.

| Genes | SNP | Studies (n) | Cases | Controls | α err prob | Power (1-β err prob) |
|---|---|---|---|---|---|---|
| IL-2 | rs2069762 | 5 | 1128 | 1116 | 0.05 | 0.9975 |
| EGR3 | rs3750192 | 4 | 1552 | 1808 | 0.05 | 0.9999 |

Legends: IL-2=interleukin-2; EGR3=early growth response 3; SNP=single nucleotide polymorphism.

### Genetic interactions and gene ontology of schizophrenia

The GeneMANIA web interface was utilized to investigate gene functions and associated networks to evaluate genetic connections. This tool offered insightful information on the genes, with an emphasis on the roles of IL-2 and EGR3 genes in relation to pathophysiological processes that might increase the risk of schizophrenia. The role of these genes in the basic processes of schizophrenia was characterized and elucidated through the integration of gene ontology. This method improved our knowledge of the intricate genetic landscape of schizophrenia and the biological mechanisms at work by providing a thorough picture of its functions and interactions throughout disease pathways, as shown in Figure 5.

### Protein-protein interactions of IL-2 and EGR3 genes

The protein-protein interaction (PPI) network for the IL-2 and EGR3 genes consisted of 21 nodes and 88 edges, as retrieved from the STRING database. A low value of p=2.33e-11 was observed for the PPI enrichment, accompanied by an average node degree of 8.38 and a clustering coefficient of 0.838. These data indicated that the proteins interacted at a level above expectation for a protein subset with a similar size and a degree of dispersion across the genome. This enrichment exhibited a significant level of protein association.

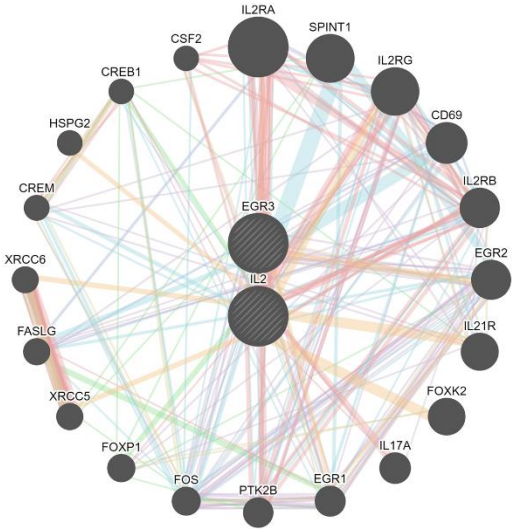


Figure 5. Protein-protein interaction network of the selected genes (IL-2 and EGR3) associated with schizophrenia.

## DISCUSSION

Schizophrenia is an extremely complex neurological disorder that causes psychotic symptoms, such as hallucinations and delusions. Epigenetic and genetic factors play a vital role in the etiology of schizophrenia. Various genes, loci, and polymorphisms have been explored to identify disease-susceptibility genes (Ahmed et al. 2017). According to research conducted by Ozdilli et al. (2024), the IL-2 rs2069762 genotype distribution of schizophrenia patients and healthy controls differed significantly. Furthermore, the frequency of homozygous GG genotype was substantially higher in the schizophrenia patient group when compared to the control group (OR=0.453, 95% CI=0.207–0.995, p=0.045). This finding suggests that persons with the GG genotype are significantly more vulnerable to the incidence of schizophrenia.

In research carried out by Amin et al. (2023), gender served as a categorical variable to compare schizophrenia patients from the Batak and Javanese ethnic groups, with most of the samples being male. According to the data, the G and T allele frequencies were found to be 43.3% and 56.7% in the Batak group and 45.4% and 54.6% in the Javanese group, respectively. A value of p=0.713 demonstrated no significant correlation between these alleles and schizophrenia. Additionally, the data showed that individuals carrying the G allele are 0.713 times more likely to develop schizophrenia compared to those who have the T allele (OR=0.919, 95% CI=0.641–1.318).

Research reported by Goldsmith et al. (2016) revealed that IL-2 levels did not differ significantly between first-episode psychosis patients and controls. This finding suggests that, unlike many other cytokines, IL-2 is not notably altered during the early stages of schizophrenia, possibly signifying a selective cytokine dysregulation. IL-2 levels did not show significant differences in patients with schizophrenia during acute exacerbation, chronic illness, or post-treatment stages compared to controls. The study additionally found that many other cytokines, such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and interleukin-1 beta (IL-1β), were significantly altered. Conversely, IL-2 levels remained stable, indicating that it may not be as strongly involved in the inflammatory processes or immune dysregulation associated with schizophrenia, unlike other cytokines that are more responsive to the disease state.

According to a study undertaken by Frydecka et al. (2013) on a Polish population, the GG, TG, and TT genotypes were not substantially linked to schizophrenia. The comparable genotype distributions of 279 controls and 151 schizophrenia patients revealed that the polymorphisms in this cohort did not substantially affect the risk of schizophrenia, as indicated by a value of p=0.72. In contrast, there was a noticeable degree of heterogeneity in the examination of IL-2 rs2069762 among various populations and models. Pairwise comparisons and the dominant model revealed a high protective impact for the Polish group (OR=0.076, p=0.0006). Conversely, the recessive model revealed an elevated risk for the Turkish group (OR=2.2059, p=0.0486). In the paired and recessive models, the Javanese group showed strong protective effects (OR=0.5652, p=0.0479). However, there was an elevated risk in the dominant model (OR=2.0855, p=0.0075). Despite considerable heterogeneity ($I^2$) among studies and no evident publication bias, the overall analysis revealed no significant relationships for IL-2 rs2069762 in most models.

The association between EGR3 and schizophrenia has been the subject of ongoing investigation. Zakharyan (2016) identified a significant association between the EGR3 rs3750192 single nucleotide polymorphism (SNP) and schizophrenia. The study revealed that the overrepresentation of the "T-G-C-G" haplotype containing rs3750192 in schizophrenia patients suggests its role in increasing susceptibility to the disorder. Our meta-analysis similarly identified a significant protective effect of EGR3 in a Chinese population. Another study conducted by Huentelman et al. (2015) also found that the EGR3 gene is significantly associated with schizophrenia in the Han Chinese population, with

two SNPs (rs1996147 and rs3750192) showing notable associations (c2>4.40, p<0.05). The linkage disequilibrium analysis further confirmed significant associations in the haplotype of two SNPs, i.e., rs3750192-rs35201266 (c2>7.10, global p<0.05). These findings highlight the genetic role of EGR3 in schizophrenia susceptibility, advocating for further research into its functional impact on the disorder.

A study conducted by Nishimura et al. (2014) on a Japanese population yielded mixed results. Contrary to the findings of other studies, theirs revealed no or weak associations between EGR3 SNPs and schizophrenia. Another study carried out by Hu et al. (2017) did not detect any significant differences in allele, genotype, or haplotype frequencies between schizophrenia patients and controls. This finding suggests that the genetic risk conferred by EGR3 may be population-specific or influenced by other factors.

Bi et al. (2024) observed an underrepresentation of a specific haplotype (i.e., haplotype 1) in schizophrenic individuals, despite finding no significant association for the rs3750192 SNP. This discrepancy could be attributed to different genetic markers examined or specific haplotype combinations that confer the risk. Additionally, Zhang et al. (2012) failed to identify a significant association between rs3750192 and schizophrenia in a Chinese population. Their finding is in contrast to ours and underscores the potential heterogeneity of the genetic effects of EGR3 across different studies and populations.

Gene association research has sought to establish a clear link between specific genes and schizophrenia, yet determining this connection is challenging due to the influence of environmental factors on psychiatric conditions. While these variables complicate efforts to pinpoint direct genetic relationships, certain genes may confer an advantage when combined with specific environmental conditions (Culej et al. 2020). This interplay underscores the importance of further genetic research, which is urgently required to deepen our understanding of gene expression and alterations in specific contexts. Such insights may lead to new approaches for managing mental illnesses by addressing unresolved questions regarding disease origins (Năstase et al. 2022). Additionally, more research is essential to identify genetic markers associated with the development of clinical features in schizophrenia. Additional research with new approaches may improve the diagnosis and treatment of schizophrenia (Poltavskaya et al. 2020).

**Strength and limitations**

This meta-analysis included diverse populations, which strengthened the findings by demonstrating the potential population-specific effects and variability in genetic risk factors. Thus, this study highlights the significance of considering ethnic diversity in genetic research and offers a novel perspective on how genetic risk factors may vary across different populations, paving the way for more targeted and informed methods to understand schizophrenia risk. This study integrated data from various ethnic groups, such as Han Chinese and Polish, revealing both protective and risk-associated effects of the analyzed genes and underscoring the complex nature of genetic susceptibility to schizophrenia.

Despite its advantages, this research has several limitations. The results of this meta-analysis are insufficient to draw firm conclusions due to a lack of studies specifically investigating the IL-2 and EGR3 genes. Schizophrenia may also be influenced by additional genetic and environmental factors. The genetic variations in IL-2 and EGR3 may contribute very little to the overall risk of schizophrenia. Additionally, these genetic variants may have different effects on various populations. The results may not be universally applicable because not all ethnic groups were included in our subgroup analysis. Furthermore, the findings should be interpreted in light of other study limitations, such as small sample sizes, confounding factors, and a focus on specific demographics. These recognized limitations highlight the necessity of conducting more comprehensive studies with larger sample sizes to enhance the understanding of the role of IL-2 and EGR3 gene variants in schizophrenia.

## CONCLUSION

Schizophrenia is a highly complex neuropsychiatric disorder with extensive genetic underpinnings. Even though our meta-analysis revealed population-specific associations for IL-2 rs2069762 and EGR3 rs3750192, these results highlight the importance of studying schizophrenia in light of genetic heterogeneity. The relationships between EGR3 and schizophrenia are still unclear across different ethnic groups, whereas the data for IL-2 indicate both protective and risk-related effects, depending on the population. These disparities underscore the need for an enhanced understanding of the etiology of schizophrenia through additional research on gene-environment interactions and the discovery of new genetic markers. Subsequent research endeavors must continue to investigate the ways in which genetic variations influence the susceptibility of the disease and its clinical presentation, with the

ultimate goal of advancing more focused diagnosis and therapy methodologies for schizophrenia.

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**Conflict of interest**

None.

**Ethical consideration**

Not applicable.

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None.

**Author contribution**

ASA contributed to the conception and design, analyzed and interpreted the data, and drafted the article. SMT analyzed and interpreted the data. RV contributed to the critical revision of the article for important intellectual content and final approval of the article.

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