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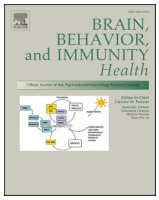

# Exploring the role of inflammatory biomarkers in trigeminal neuralgia

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ABSTRACT

*Background:* Trigeminal neuralgia (TN) is a severe facial pain disorder with complex etiology. Inflammation has been suggested as a contributing factor to TN pathogenesis. This study investigates the causal relationship between inflammatory biomarkers, including 41 circulating inflammatory cytokines, C-reactive protein (CRP), and procalcitonin (PCT), and TN using Mendelian randomization (MR) analysis.

*Methods:* A two-sample MR approach was employed using genome-wide association study (GWAS) data from 8293 Finnish individuals for inflammatory cytokines and data from the FinnGen database for TN. Instrumental variables (IVs) were selected based on genome-wide significance and clumping thresholds to avoid linkage disequilibrium. Inverse variance weighting (IVW) was used as the primary method, complemented by MR Egger regression, weighted median, simple mode, and weighted mode methods. Additionally, Bayesian Weighted MR (BWMR) and Multivariable MR (MVMR) were utilized to validate the findings and explore potential confounders.

*Results:* The present MR analysis identified significant causal associations for three inflammatory cytokines with TN. Stem cell growth factor beta (SCGF-β) (OR = 1.362, 95% CI = 1.049–1.770, p = 0.021) and Interleukin-4 (IL-4) (OR = 1.533, 95% CI = 1.014–2.316, p = 0.043) were positively associated with TN, while Interleukin-16 (IL-16) (OR = 0.720, 95% CI = 0.563–0.921, p = 0.009) had a protective effect. CRP levels were also linked to TN risk (OR = 0.751, 95% CI = 0.593–0.951, p = 0.017). No significant causal effect of PCT on TN was observed. Sensitivity analyses confirmed the robustness of these findings, showing no evidence of horizontal pleiotropy or heterogeneity.

*Conclusion:* This study highlights specific inflammatory biomarkers that may play pivotal roles in TN pathogenesis. SCGF-β and IL-4 are potential therapeutic targets due to their facilitative effects on TN, while IL-16 could offer protective benefits. CRP's association with TN further supports the involvement of systemic inflammation in this condition. These findings provide novel insights into TN's inflammatory mechanisms, suggesting new avenues for targeted interventions.

## 1. Introduction

Trigeminal neuralgia (TN) is a chronic pain disorder that causes intense and recurrent episodes of facial pain, significantly impacting the quality of life for those affected (Araya et al., 2020). It affects approximately 4–29 per 100,000 individuals annually, with a higher prevalence in women and those over 50 years of age (De Toledo et al., 2016; Lambru et al., 2021; Torpy et al., 2013). TN has a multifactorial etiology, with distinct classifications including classical, secondary, and idiopathic forms, each contributing to the understanding of this condition (Cruccu et al., 2020). The mechanisms behind TN involve peripheral alterations, like ectopic action potentials in demyelinated axons, and central changes, including synaptic reorganization in the trigeminal nucleus and thalamus (Finnerup et al., 2021; Ashina et al., 2024). The genetic basis of TN has been a subject of interest, with several studies suggesting a heritable component to the condition. While the exact genetic mechanisms are not fully elucidated, a number of genetic associations have been reported (Mannerak et al., 2021). For instance, genetic variations in the SCN10A gene (Stefano et al., 2020), which encodes a voltage-gated sodium channel, have been associated with an increased risk of TN in some populations. Additionally, other genes such as CACNA1A and KCNQ2 have been implicated in the pathogenesis of TN (Gambeta et al., 2021; Ling et al., 2017), suggesting a complex genetic architecture. These genetic findings underscore the potential for targeted therapies and the importance of understanding the interplay between genetic predisposition and environmental factors in TN. Despite

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current understanding, the precise molecular pathways of TN's onset and progression remain unclear, necessitating further research.

The primary objective of treating TN is to effectively manage pain and enhance the overall well-being of patients, thereby improving their quality of life. First-line therapies for the pharmacological management of TN often include anticonvulsant medications like carbamazepine and oxcarbazepine, which are commonly prescribed to alleviate symptoms (Gambeta et al., 2020). When medications fail to provide adequate relief or cause intolerable side effects, surgical interventions may be considered. Microvascular decompression, radiofrequency rhizotomy, and gamma knife radiosurgery are among the surgical procedures used to relieve neurovascular compression or disrupt pain transmission pathways (Diana et al., 2021). Despite these treatments, a significant number of patients experience persistent or recurrent pain (Maarbjerg et al., 2014; Di et al., 2018), highlighting the need for novel therapeutic approaches. Understanding the precise mechanisms of TN development and progression remains a critical area of research. Investigating the role of inflammatory biomarkers in the pathogenesis of TN through Mendelian randomization studies may provide new insights into potential causal relationships and therapeutic targets. Further studies are essential to elucidate these mechanisms and improve the management of TN.

Circulating inflammatory cytokines, which are small proteins released by immune cells, have essential functions in regulating both inflammatory and immune responses, playing pivotal roles in these processes (Gupta et al., 2020). Influential cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), have been identified as key players in the modulation of chronic pain conditions. These cytokines operate by influencing pain signaling pathways and sensitizing nociceptive neurons (Verrico et al., 2020; Pinto et al., 2021). Emerging evidence suggests that these cytokines may also be involved in TN's pathogenesis (Zhao et al., 2021; Lin et al., 2022). Evidence suggests a potential association between TN and systemic inflammation, as indicated by the presence of elevated pro-inflammatory cytokines in the cerebrospinal fluid and serum of TN patients (Ostertag et al., 2023). However, the exact mechanisms by which these cytokines contribute to TN are not fully understood, highlighting the need for further investigation.

C-reactive protein (CRP) and procalcitonin (PCT) are essential inflammatory biomarkers employed extensively in clinical settings to evaluate the existence and intensity of inflammation as well as infection. These biomarkers serve as valuable markers in assessing the inflammatory response and aiding in diagnostic and prognostic evaluations (Hausfater et al., 2021). CRP, an inflammation-induced protein produced by the liver, serves as a vital component in the innate immune response. In clinical settings, a CRP level greater than 10 mg/L is often considered elevated, and increased CRP levels are often observed in various acute and chronic inflammatory conditions, encompassing infections, autoimmune disorders, and cardiovascular diseases. This association with elevated CRP levels helps in diagnosing and monitoring patients with such inflammatory and immune-related conditions (Herwald and Egesten, 2021). PCT, a hormone precursor, is synthesized by different tissues in the body as a response to bacterial infections and systemic inflammation. Elevated PCT levels are generally defined as greater than 0.5 ng/ml. In cases of severe bacterial infections and sepsis, PCT levels exhibit a significant increase, rendering it an invaluable biomarker for differentiating bacterial infections from viral ones and assisting in the appropriate administration of antibiotic therapy (Schuetz et al., 2019). The clinical significance of CRP and PCT extends beyond their diagnostic utility. Elevated CRP levels are linked to an increased risk of cardiovascular diseases, diabetes, and certain cancers (Ridker et al., 2023; Mohammadi et al., 2023; Wang et al., 2020). PCT, on the other hand, serves as a prognostic marker in sepsis, with higher levels indicating a poorer outcome. Both biomarkers are crucial in monitoring disease progression and treatment response, thereby aiding in clinical decision-making (Pierrakos et al., 2020). The impact of CRP and PCT on various human diseases has been extensively studied; however, their role in the pathogenesis of TN remains underexplored. TN is a debilitating condition characterized by severe facial pain, and its etiology involves complex interactions between peripheral and central nervous system changes. Recent evidence suggests that inflammation may play a role in the development and progression of TN (Yao et al., 2020). Elevated levels of inflammatory cytokines have been observed in patients with TN, indicating a potential link between systemic inflammation and the condition (R, 2020). Understanding the mechanisms by which CRP and PCT influence the onset and progression of TN is crucial for developing targeted therapies. Current research on CRP and PCT in TN is limited, highlighting the need for further studies to elucidate their role in this condition.

Observational studies, often used to examine the relationship between an exposure and an outcome, are prone to confounding and bias, which can lead to inaccurate conclusions (X and X, 2020). While Randomized Controlled Trials (RCTs) are considered the ideal method for establishing causal relationships between exposures and outcomes. However, conducting RCTs requires significant investments in terms of human and material resources (Zabor et al., 2020). In order to bridge this gap, we have employed Mendelian randomization(MR) to investigate the causal association between inflammatory biomarkers and TN. MR leverages genetic variants as instrumental variables to assess causality, thereby mitigating confounding factors and the issue of reverse causation. This approach allows for a more robust examination of the relationship between inflammatory biomarkers and TN (Bowden and Holmes, 2019). By integrating genetic data from genome-wide association studies (GWAS) with inflammatory biomarkers and TN incidence, we aim to determine if genetically predicted inflammatory biomarkers are associated with TN risk. This study seeks to clarify the causal pathways and mechanisms underlying TN, potentially leading to novel therapeutic targets and improved patient outcomes.

## 2. Materials and methods

### 2.1. Study design

A MR design was utilized in this study to explore the causal relationship between inflammatory biomarkers and TN. Fig. 1A illustrates the overall design of our two-sample MR study. To ensure the robustness of our MR findings, we adhered to three key assumptions: First, the genetic instrumental variables (IVs) must have a strong association with 41 circulating inflammatory cytokines, CRP and PCT. Second, the selected genetic IVs should be independent of potential confounding factors. Third, the chosen IVs should influence the occurrence of TN only through their effect on cytokines, without any direct effect. Following the initial analysis, we substantiated our findings using Bayesian Weighted MR (BWMR) and Multivariable Mendelian Randomization (MR) techniques. Subsequently, to explore the potential reverse causal relationships, we conducted a directional MR analysis with TN as the exposure and various inflammatory biomarkers as the outcomes (Fig. 1B).

### 2.2. Data source

Genomic variants from a total of 8293 individuals of Finnish origin were included in a GWAS meta-analysis to acquire data on inflammatory cytokines. This dataset served as the foundation for investigating the role of these cytokines in the context of our study on TN (Ahola-Olli et al., 2017). The GWAS data for CRP levels and PCT were extracted from the European Bioinformatics Institute (EBI) database and the UniProt database, respectively. The summary-level data for TN included genotype information from a cohort of 800 TN patients and 195,047 controls, sourced from the FinnGen database. Both GWAS analyses utilized summary data obtained from the IEU Open GWAS Project (https://gwas.mrcieu.ac.uk). All data used in this MR analysis are publicly accessible, eliminating the need for ethical approval and participant

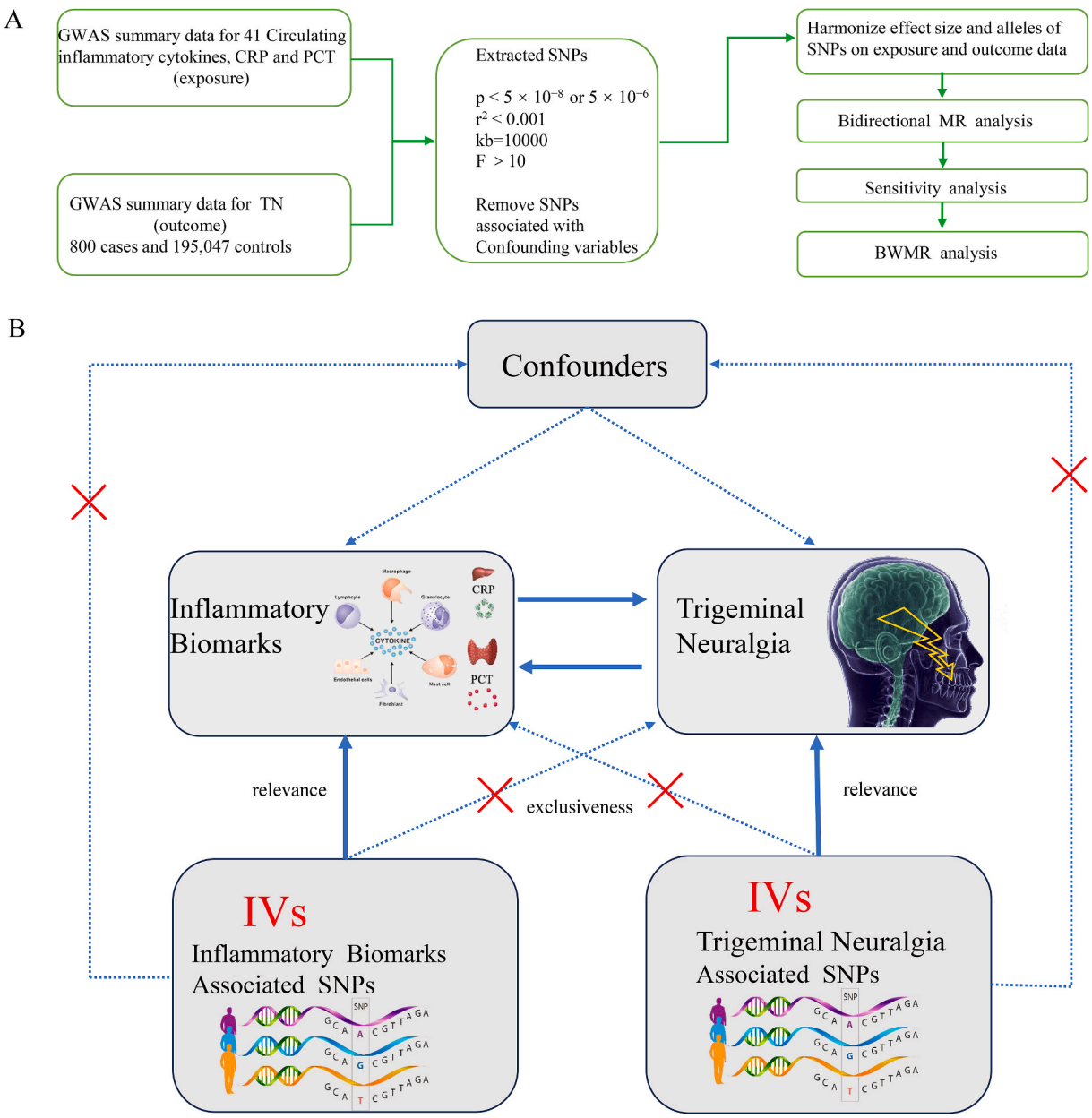


**Fig. 1.** (A) Overall flow chart of this study. (B) Assumptions and study design of the MR study of the associations between inflammatory biomarkers and TN. GWAS, Genome-Wide Association Study; MR, mendelian randomization; BWMR, bayesian weighted mendelian randomization; SNPs, single-nucleotide polymorphisms; TN, trigeminal neuralgia.

consent.

### 2.3. Instrumental variable selection

First, to ensure the selection of single nucleotide polymorphisms (SNPs) strongly associated with inflammatory cytokines, CRP, and PCT, we implemented a genome-wide significance (GS) threshold of $p < 5 \times 10^{-8}$. This stringent filtering criterion helped to identify the most relevant SNPs for our analysis. However, some inflammatory cytokines as well as PCT did not have enough SNPs under this criterion to perform MR analysis, prompting us to use a higher cutoff value of $p < 5 \times 10^{-6}$. Second, we adopted specific strategies to handle potential issues related to linkage disequilibrium (LD) and weak instrument bias. For the exposure to inflammatory cytokines, SNPs within a 10,000 kb window and with an $r^2$ threshold of 0.001 were clumped together to remove any closely correlated SNPs. Similarly, for TN as the exposure, we applied a genome-wide significance threshold of $p < 5 \times 10^{-6}$ and a strict criterion of 10,000 kb and $r^2 = 0.001$ to exclude SNPs in LD. Subsequently, the $r^2$ value was estimated for each SNP to determine the proportion of exposure explained by the IVs. In order to minimize weak instrument bias, we evaluated the strength of the IVs using the F-statistic, and only included SNPs with an F-statistic greater than 10 in the MR analysis. As the number of IVs exceeded expectations for TN as the exposure, we further implemented stringent screening criteria for the correlation hypothesis, setting a threshold of $P < 5 \times 10^{-6}$. These rigorous steps were taken to enhance the reliability of our MR analysis.

### 2.4. Methodology of MR

To ensure robustness and reliability in our analysis, we employed various methods including inverse variance weighting (IVW), MR Egger regression, weighted median, simple mode, and weighted mode. The

IVW method weighted the SNPs by their inverse variance, assuming their validity as instrumental variables and independence from each other. This method excluded the intercept term in the regression. MR Egger regression, on the other hand, considered the intercept term and assumed independence between instrument-exposure and instrument-outcome associations, allowing for a consideration of instrument strength independent of the direct effect assumption. The weighted median method provided a robust estimate by tolerating a certain number of invalid instruments, as long as at least 50% of the instrumental variables were deemed valid. These different approaches were implemented to enhance the credibility and robustness of the present MR analysis.

### 2.5. BWMR

To address pleiotropy and improve the reliability of causal estimates, we employed BWMR, a method that integrates prior knowledge and adjusts the weights of genetic instruments. By utilizing this approach, we obtained more precise and dependable outcomes in evaluating the impact of inflammatory biomarkers on TN.

### 2.6. MVMR

In this study, we used MVMR to evaluate the causal relationship between inflammatory biomarkers and TN. We employed genetic variants associated with dietary factors as IVs while controlling for potential confounders, such as multiple sclerosis (MS) (GWAS ID: ieu-b-18) and brain tumor (GWAS ID: ebi-a-GCST90018800). This method allows for the estimation of the direct effects of inflammatory biomarkers on TN taking into account these confounding factors.

### 2.7. Sensitivity analysis

To evaluate the reliability and robustness of the present MR results, we implemented three sensitivity analysis methods: heterogeneity test, pleiotropy test, and leave-one-out sensitivity test. Heterogeneity was assessed using Cochran's Q test and Rucker's Q test, where a p-value greater than 0.05 indicated no significant heterogeneity. The presence of horizontal pleiotropy was examined using the intercept from the MR Egger analysis, with a p-value above 0.05 suggesting no pleiotropy. Furthermore, we utilized the MR pleiotropy residual sum and outlier (PRESSO) method to identify outliers and detect potential horizontal pleiotropy. For simulations, we set the NbDistribution parameter to 1000. The leave-one-out sensitivity test was conducted to explore the influence of individual SNPs on the causal association. It is important to note that this study was not pre-registered on any platform.

### 2.8. Statistical analysis

The MR analysis was performed using the "TwoSampleMR" R package (Version 0.6.1) within the RStudio environment (Version 4.4.0), employing five distinct MR methods. Inflammatory cytokines that exhibited statistical significance were assessed based on the following criteria: the IVW results presented 95% confidence intervals (CI) that did not encompass 1 (or 0), a significance level of $p < 0.05$, and consistency among all five MR methods employed. This comprehensive approach ensured the reliability and consistency of the results obtained.

## 3. Results

In this MR study, we aim to examine the association between TN and a range of inflammatory biomarkers. Specifically, we investigated 41 circulating inflammatory cytokines, as well as CRP and PCT. The findings of this analysis provided compelling evidence for a causal relationship between these inflammatory biomarkers and TN.

### 3.1. Bidirectional interactions between 41 circulating inflammatory cytokines and TN

GS SNPs ($P < 5x10^{-8}$) for 5 circulating inflammatory cytokines represent robust instruments, whereas the remaining 36 cytokines did not have enough SNPs under this criterion to perform MR analysis, prompting us to use a higher cutoff value of $p < 5 \times 10^{-6}$, and the F-statistics were all surpassed 10. Fig. 2 illustrates the results of the MR analysis examining the impact of inflammatory cytokines on TN. Out of the 41 circulating inflammatory cytokines evaluated as exposures, our analysis revealed three factors that were statistically associated with the development of TN. Stem cell growth factor beta (SCGF-β) levels demonstrated a significant association (OR = 1.362, 95% CI = 1.049–1.770, p = 0.021) with a cutoff value of $p < 5 \times 10^{-8}$, while Interleukin-4 (IL-4) levels (OR = 1.533, 95% CI = 1.014–2.316, p = 0.043) and Interleukin-16 (IL-16) levels (OR = 0.720, 95% CI = 0.563–0.921, p = 0.009) exhibited associations with TN at a cutoff value of $p < 5 \times 10^{-6}$, serving as facilitators and inhibitors, respectively. To further illustrate the trends of inflammatory cytokines, scatter plots were utilized for each of the five MR methods (Fig. 3). Among the 41 cytokines evaluated, the MR-Egger intercept revealed no significant evidence of horizontal pleiotropy, and the MR-PRESSO method did not identify any outliers (Supplementary Tables S1 and S2).

When TN was employed as an exposure, the MR analysis involved the remaining 38 single nucleotide polymorphisms (SNPs), and the results are presented in Supplementary Table S3. Notably, no significant associations were observed between TN and the evaluated inflammatory cytokines.

### 3.2. Bidirectional interactions between CRP and TN

A total of 264 SNPs associated with CRP were identified using a stringent threshold of $P < 1x10^{-8}$. The relationship between CRP levels and the risk of TN was assessed using a MR approach, specifically the IVW analysis. The analysis revealed a significant inverse association between CRP levels and TN risk [OR = 0.751, 95% CI = 0.593–0.951, p = 0.017], as depicted in Fig. 4. No indications of horizontal pleiotropy or heterogeneity were observed in the causal impact of CRP on TN (PQ = 0.69, PMR-PRESSO = 0.14), emphasizing the robustness and reliability of the initial findings (Supplementary Tables S1, S2, S5).

However, when reversing the exposure and outcome, no evidence was found for a causal influence of TN on CRP using GS SNPs under the IVW method [OR = 1.000, 95% CI = 0.995–1.006, p = 0.798] (Supplementary Table S3).

### 3.3. Bidirectional interactions between PCT and TN

A total of 16 SNPs associated with PCT were selected for analysis. However, genetically predicted PCT levels did not exhibit a significant association with the risk of TN when assessed using GS SNPs under the IVW method [OR = 1.148, 95%CI: 0.908–1.451; p = 0.250], as well as the weighted median [OR = 1.047, 95% CI: 0.772–1.419; p = 0.768], weighted mode [OR = 0.951, 95% CI: 0.646–1.400; p = 0.802], and MR-Egger [OR = 0.906, 95% CI: 0.537–1.527; p = 0.716] methods (Supplementary Table S4).

Moreover, when reversing the exposure and outcome, no evidence was found for an influence of TN on PCT using the selected 7 SNPs (with a p-value $< 1x10^{-6}$) under the IVW method [OR = 0.990, 95% CI: 0.946–1.037; p = 0.675]. Similar non-significant results were observed with other MR methods (Supplementary Table S3). Furthermore, no horizontal pleiotropy or heterogeneity was observed in the causal influence of TN on PCT (PQ = 0.572, PMR-PRESSO = 0.433), as indicated by Supplementary Tables S1, S2, and S5.

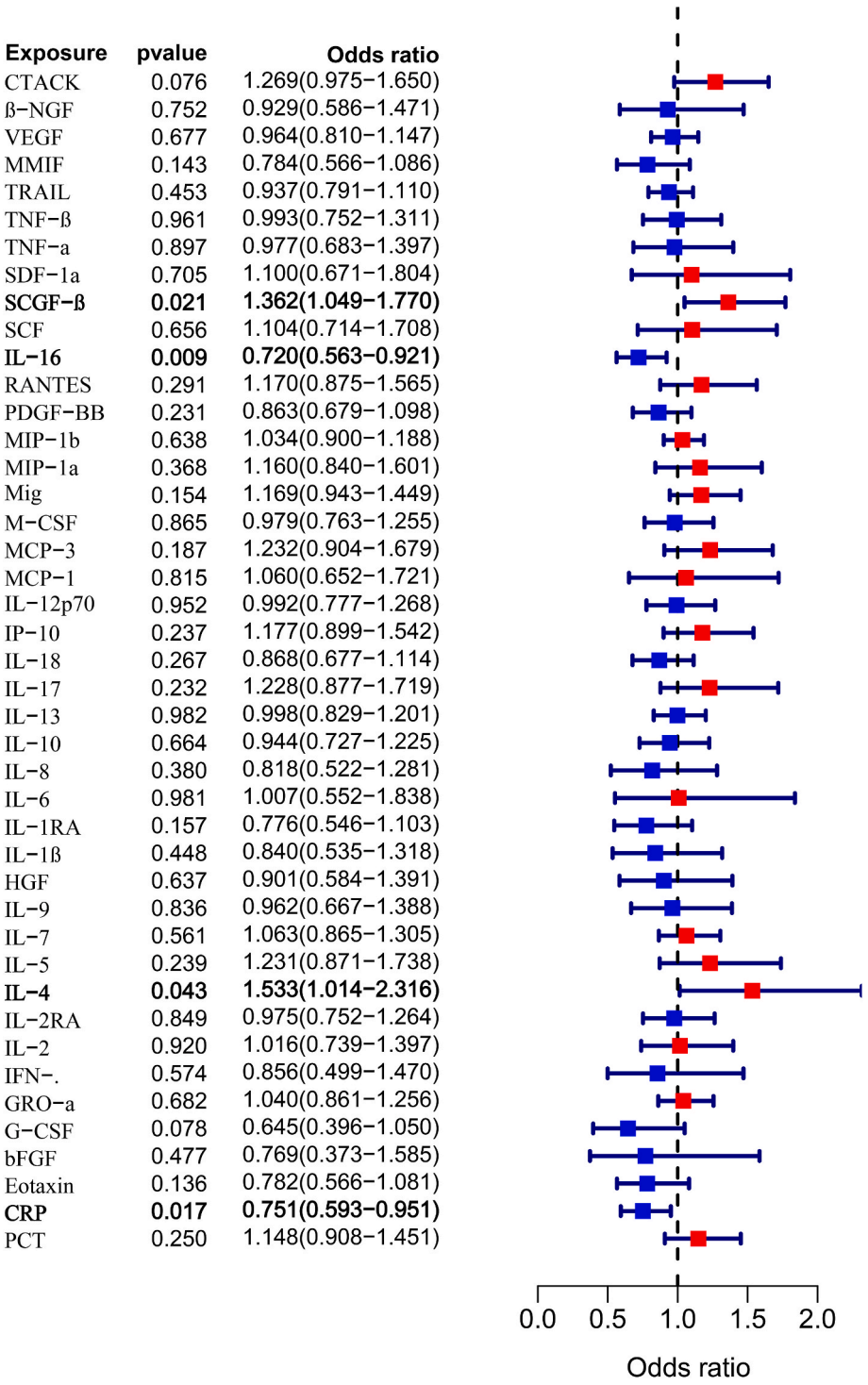


**Fig. 2.** Forest plots of the MR results (IVW method) to present the causal associations of inflammatory biomarkers on TN risk.

### 3.4. BWMR

The BWMR results differ somewhat from previous studies. Positive associations were found for SCGF-β (OR = 1.387, 95% CI = 1.037–1.855, P = 0.027), IL-16 (OR = 0.777, 95% CI = 0.609–0.991, P = 0.042), and CRP (OR = 0.729, 95% CI = 0.581–0.916, P = 0.007). All other results were consistent with previous findings, except for IL-4, which showed a negative result (OR = 1.302, 95% CI = 0.881–1.925, P = 0.186) (Fig. 5).

### 3.5. MVMR

Our Multivariable MR analysis incorporated positive factors such as SCGF-β levels, IL-4 levels, IL-16 levels, CRP, as well as potential confounding variables including MS and brain tumor. The MVMR analysis did not yield any significant findings: SCGF-β (OR = 1.118, 95% CI = 0.879–1.421, P = 0.365), IL-4 (OR = 0.787, 95% CI = 0.543–1.141, P = 0.206), IL-16 (OR = 0.744, 95% CI = 0.590–0.938, P = 0.013), CRP (OR = 0.744, 95% CI = 0.576–0.960, P = 0.023), MS (OR = 1.026, 95% CI = 0.793–1.327, P = 0.843), and brain tumor (OR = 0.975, 95% CI =

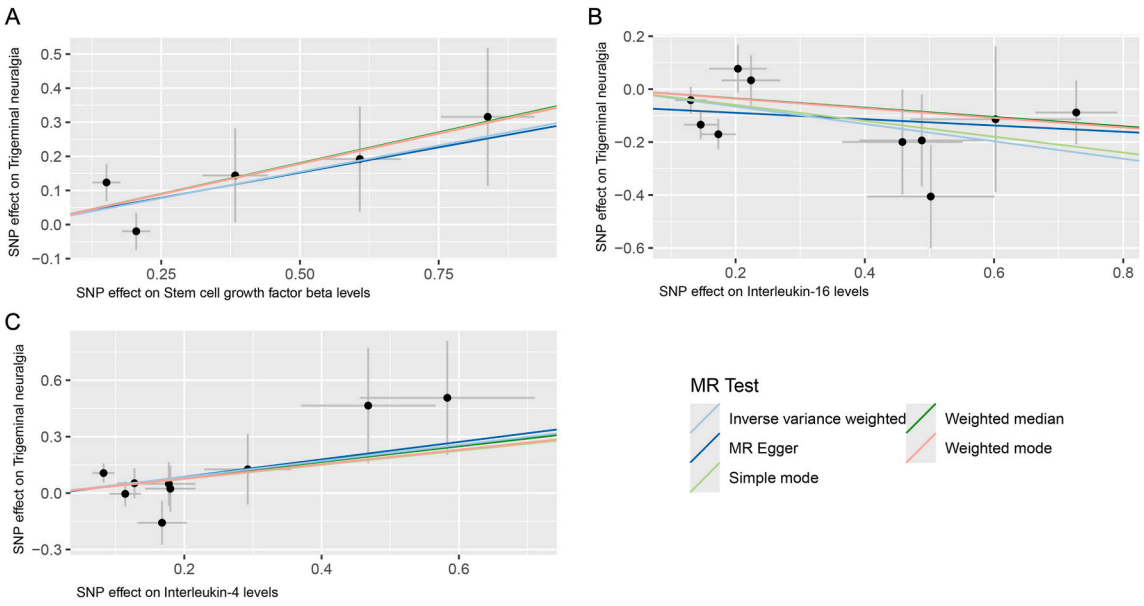


**Fig. 3.** The MR test for inflammatory cytokines on TN. (A) Scatter plot of MR analysis for stem cell growth factor beta (SCGF-β) levels on TN; (B) Scatter plot of MR analysis for interleukin-16 (IL-16) levels on TN. (C) Scatter plot of MR analysis for interleukin-4 (IL-4) levels on TN. SNPs, single-nucleotide polymorphisms.

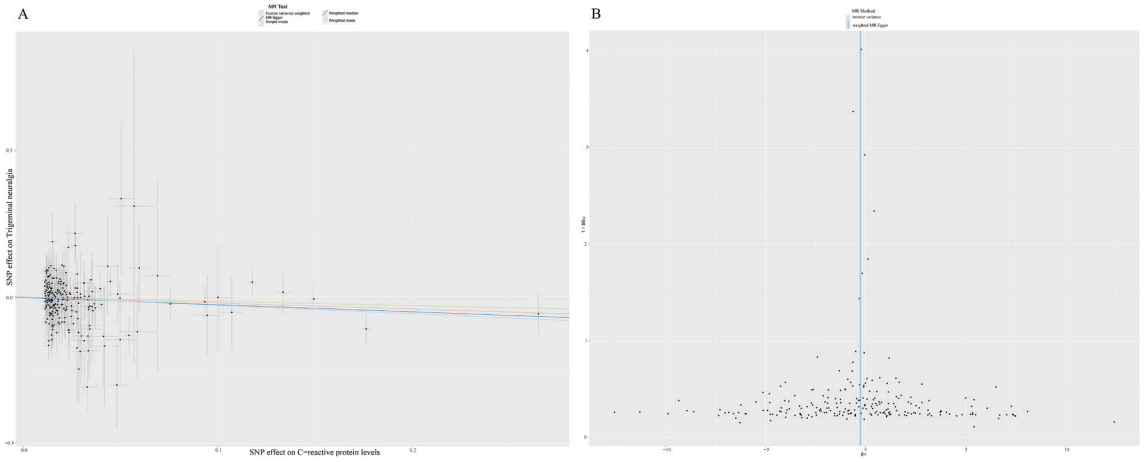


**Fig. 4.** The MR test for CRP on TN. (A) Scatter plot of MR analysis for CRP on TN; (B) Funnel plot from single SNP analyses for CRP on TN. MR, mendelian randomization; SNPs, single-nucleotide polymorphisms; CRP, C-reactive protein; TN, trigeminal neuralgia.

0.833–1.142, P = 0.756). The results showed in Supplementary Fig. S5.

### 3.6. Sensitivity analysis

In addition, all outcomes assessed in the study exhibited no significant heterogeneity according to Cochran's Q tests ($p > 0.05$ for all outcomes). The MR-Egger intercept test yielded no statistically significant evidence of horizontal pleiotropy within the study. Leave-one-out analyses demonstrated that no individual SNP had a significant impact on the overall MR findings. These comprehensive sensitivity analyses collectively enhance the reliability and robustness of our results. Supplementary Tables S1, S2, S5 and Supplementary Figs. S1, S3, S4 provide detailed information supporting these conclusions.

## 4. Discussion

Our MR study has identified 3 circulating inflammatory cytokines out of a panel of 41 that play significant roles in the development and progression of TN. These cytokines are SCGF-β, IL-4, and IL-16. Notably, SCGF-β and IL-4 were found to promote the onset and progression of TN, whereas IL-16 exhibited a protective effect against the disease.

Circulating inflammatory cytokines play vital roles in immune system signaling, orchestrating inflammation and immune responses (Collier et al., 2021). SCGF-β is involved in hematopoiesis and immune regulation (Hiraoka et al., 2001). Elevated levels of SCGF-β have been associated with various inflammatory and autoimmune conditions, indicating its role in promoting inflammation and immune activation (Fujita et al., 2023). IL-4 is a key cytokine in the regulation of allergic responses and the differentiation of naive T-helper cells into Th2 cells (Liebold et al., 2024). It is involved in the modulation of immune responses and has been linked to various inflammatory diseases (Bernstein et al., 2023). IL-16, on the other hand, functions as a chemoattractant for $CD4^+$ T cells and plays a role in modulating immune responses and inflammation (González-Rodríguez et al., 2022).

In the context of TN, our findings suggest that SCGF-β and IL-4 contribute to the disease's pathogenesis by promoting inflammatory processes and immune activation. SCGF-β may enhance the proliferation and differentiation of immune cells that produce pro-inflammatory

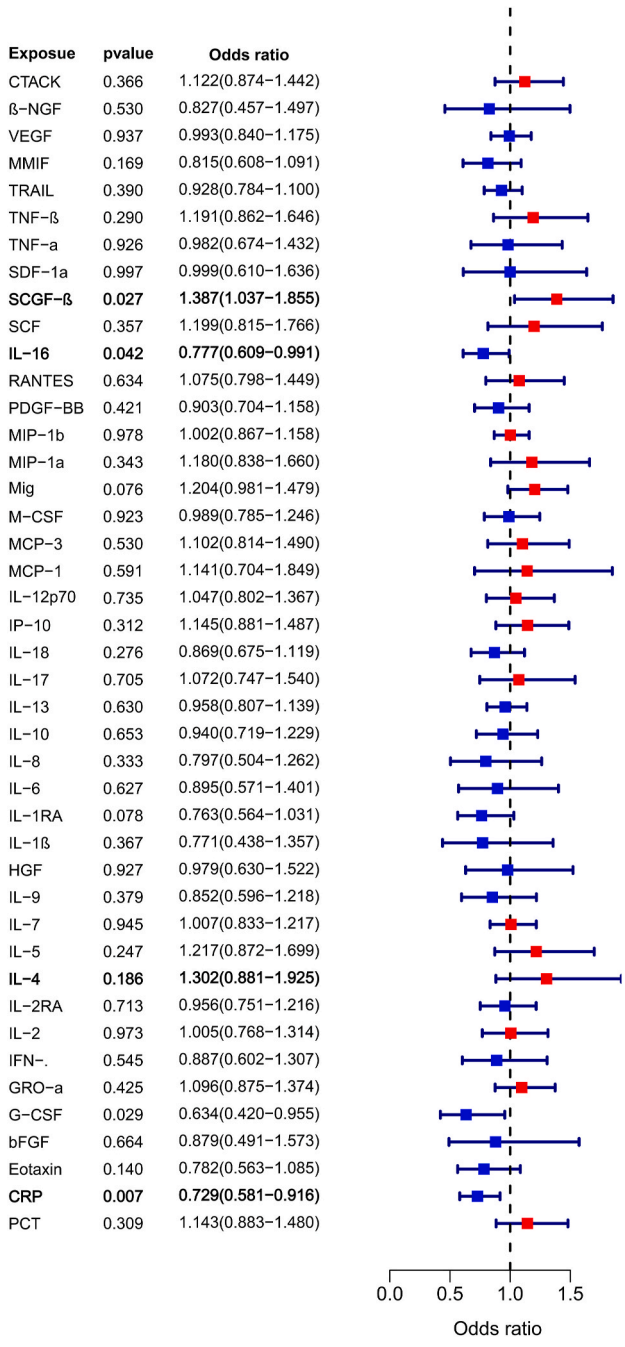


**Fig. 5.** Forest plots of the BWMR analysis for inflammatory biomarkers on TN risk.

cytokines (Nees et al., 2019), leading to an exacerbated inflammatory response in the trigeminal nerve. IL-4, by promoting Th2 cell differentiation (Iwaszko et al., 2021), may contribute to a skewed immune response that favors inflammation and tissue damage, further aggravating the condition. These mechanisms underline the potential of SCGF-β and IL-4 as therapeutic targets for TN, as their modulation could help in reducing inflammation and slowing disease progression. In our analysis for IL-4 on TN, the IVW method showed significant associations with p-values less than 0.05. However, when using the BWMR method, the associations were not significant, with p-values greater than 0.05. This discrepancy suggests that while the IVW method indicates potential causal relationships, the BWMR method, which accounts for pleiotropy and potential biases more robustly, does not confirm these findings. Therefore, the results should be interpreted with caution, and further studies are needed to validate the observed associations.

Conversely, IL-16 appears to play a protective role in TN. Its chemoattractant properties for $CD4^+$ T cells suggest that it may help in regulating the immune response and mitigating excessive inflammation (Hridi et al., 2021). By attracting regulatory T cells or other immune cells that dampen inflammatory responses, IL-16 might help in controlling the extent of inflammation and protecting against nerve damage. This protective mechanism of IL-16 highlights its potential as a therapeutic target for boosting the body's natural anti-inflammatory responses in TN.

The identification of these cytokines provides new insights into the inflammatory mechanisms underlying TN and underscores the complex interplay between various immune factors in the disease's pathophysiology. The dual role of these cytokines—where some promote and others inhibit TN progression—illustrates the intricacy of the immune system's involvement in this condition. This complexity necessitates further research to fully elucidate the precise pathways through which these cytokines affect TN and to explore their potential as biomarkers for disease severity and treatment response.

Furthermore, our MR study has revealed that CRP acts as a protective factor in the development and progression of TN. CRP is a well-known acute-phase protein produced by the liver in response to inflammation (Herwald and Egesten, 2021). It is commonly used as a biomarker for systemic inflammation and is elevated in various inflammatory and infectious conditions (Rizo-Téllez et al., 2023). CRP plays a crucial role in the body's immune response by binding to the surface of dead or dying cells and some types of bacteria, which enhances their clearance by phagocytosis and activates the complement system (Sheriff et al., 2021). In the context of TN, our findings suggest that CRP may help mitigate the inflammatory processes that contribute to the disease. The protective mechanism of CRP in TN could be attributed to its role in modulating the immune response and reducing excessive inflammation. By enhancing the clearance of inflammatory cells and debris, CRP might prevent the sustained inflammatory state that exacerbates nerve damage and pain in TN. Additionally, CRP's activation of the complement system could help in resolving inflammation more efficiently, thereby protecting the trigeminal nerve from ongoing inflammatory insults. These insights into CRP's protective role offer a new perspective on managing TN, highlighting the potential of targeting inflammatory pathways to develop more effective treatments. Further research is needed to explore the therapeutic implications of CRP modulation in TN and to fully understand the mechanisms through which it exerts its protective effects.

PCT is another significant inflammatory biomarker, primarily produced in response to bacterial infections and systemic inflammation. It is widely used in clinical settings to differentiate between bacterial and viral infections and to guide antibiotic therapy (Schuetz et al., 2019). In our Mendelian randomization study, we did not find evidence of a causal relationship between PCT levels and TN. This finding contrasts with the observed protective role of CRP in TN. Previous research suggests that while both CRP and PCT are markers of inflammation, they are regulated differently and respond to distinct inflammatory stimuli. CRP is broadly associated with chronic inflammatory conditions, which may play a role in the pathogenesis of TN. In contrast, PCT is more specific to acute bacterial infections (Tang et al., 2018), which might not have a direct impact on the chronic inflammatory processes underlying TN. This difference in response to inflammation could explain why CRP, but not PCT, is relevant to TN's development and progression.

Despite the valuable insights gained from our MR study on the causal relationships between inflammatory biomarkers and TN, several limitations need to be acknowledged. Firstly, the accuracy of our findings relies heavily on the quality and comprehensiveness of the genetic data used. While MR is a powerful tool for inferring causality, it is dependent on the availability of robust genetic instruments. In this study, we utilized genetic variants associated with the inflammatory biomarkers, but the strength and specificity of these instruments can vary. Weak genetic instruments could lead to biased estimates and reduced power to detect

true causal relationships. Additionally, pleiotropy, where a single genetic variant influences multiple traits, could confound our results, despite the application of methods to minimize its impact. Secondly, our study is limited by the availability of data on inflammatory biomarkers and TN from diverse populations. Most genetic data come from populations of European descent, which limits the generalizability of our findings to other ethnic groups. Genetic and environmental factors contributing to TN may differ across populations, and thus our results might not be fully applicable to non-European populations. Future studies should aim to include more diverse cohorts to validate our findings and ensure broader applicability. Thirdly, while our study identifies associations between specific inflammatory biomarkers and TN, it does not provide detailed mechanistic insights into how these biomarkers influence the disease process. The observed associations, particularly with CRP, suggest a protective role, but the underlying biological mechanisms remain unclear. Further experimental studies are needed to elucidate these pathways and understand how CRP and other biomarkers interact with the nervous system in the context of TN. Another limitation is the potential for residual confounding. While MR reduces confounding compared to observational studies, it cannot eliminate it entirely. Unmeasured confounders, such as other genetic or environmental factors, could influence our results. Additionally, our study focuses on a limited number of inflammatory biomarkers, and other relevant biomarkers not included in our analysis might also play significant roles in TN. Lastly, the cross-sectional nature of our genetic data does not account for changes in biomarker levels over time or during different stages of TN. Longitudinal data would provide a more comprehensive understanding of how inflammatory biomarkers fluctuate with disease progression and treatment response, offering deeper insights into their roles in TN. In conclusion, while our study provides important insights into the role of inflammatory biomarkers in TN, these limitations highlight the need for further research. Addressing these challenges through more comprehensive, diverse, and longitudinal studies will enhance our understanding of the complex interplay between inflammatory biomarkers and TN and guide the development of more effective therapeutic strategies.

## 5. Conclusion

The MR study advances the understanding of TN by highlighting the significant roles of SCGF-β, IL-4, IL-16 and CRP in its pathogenesis. These findings pave the way for future research aimed at developing targeted therapies that modulate these cytokines, potentially leading to more effective treatments for patients suffering from TN. The identification of specific inflammatory pathways involved in TN also opens up new avenues for personalized medicine approaches, where treatments can be tailored based on an individual's cytokine profile.

## CRediT authorship contribution statement

**Shenglong Lai:** Writing – original draft. **Haiyang Li:** Writing – review & editing. **Yazhou Xing:** Writing – review & editing. **Du Wu:** Software, Writing – review & editing. **Lin Wang:** Writing – review & editing. **Qinghua Liang:** Writing – review & editing.

## Ethics approval and consent to participate

The GWAS data used in this study are publicly available and have been de-identified to ensure confidentiality. Ethical approval for each GWAS was obtained and documented in their respective original publications.

## Data Sharing Statement

The study relied on openly accessible datasets, which can be accessed through the IEU Open GWAS Project (https://gwas.mrcieu.ac.uk/).

## Declaration of competing interest

The authors affirm that they do not have any competing financial interests or personal relationships that may have influenced the work presented in this paper.

## Funding

None.

## Declaration of competing interest

The authors declare no conflicts of interest. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. All data analyzed during this study are publicly available, and ethical approval and participant consent were not required as per the guidelines of the institution and national regulations.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2024.100930.

## Glossary

| MR | Mendelian randomization |
|---|---|
| **IVs** | Instrumental variables |
| **GWAS** | Genome-wide association study |
| **IVW** | Inverse-variance weighted |
| **BWMR** | Bayesian weighted mendelian randomization |
| **MVMR** | Multivariable MR |
| **LD** | Linkage disequilibrium |
| **PRESSO** | Pleiotropy residual sum and outlier |
| **ID** | Identification |
| **OR** | Odds ratio |
| **CI** | Confidence interval |

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| MR | Mendelian randomization |
|---|---|
| **P** | P-value |
| **RCTs** | Randomized Controlled Trials |
| **EBI** | European Bioinformatics Institute |
| **TN** | Trigeminal neuralgia |
| **CRP** | C-reactive protein |
| **PCT** | Procalcitonin |
| **IL-1β** | Interleukin-1β |
| **IL-6** | Interleukin-6 |
| **IL-4** | Interleukin-4 |
| **IL-16** | Interleukin-16 |
| **TNF-α** | Tumor necrosis factor-alpha |
| **SCGF-β** | Stem cell growth factor beta |
| **MS** | Multiple sclerosis |

## Data availability

The data that has been used is confidential.

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