

Potential Molecular Pathway Linking Caffeine Exposure to Trigeminal Neuralgia

An *In Silico* Study

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Abstract

Trigeminal neuralgia is a debilitating neuropathic pain disorder characterized by recurrent, brief, electric shock-like facial pain. Although caffeine is widely consumed and has neuroinflammatory modulation ability, its potential molecular association with trigeminal neuralgia remains unclear. This study aimed to investigate potential molecular pathways linking caffeine exposure to trigeminal neuralgia-related biological processes. Compound-disease datasets were obtained from CTD database. Gene-gene interaction network was constructed with GeneMANIA. GO and KEGG pathway enrichment analysis were performed using DAVID bioinformatics (p -value set to 0.05; FDR-correction applied). Cell-type specific gene-expression were assessed using single-cell data from HPA server. Eight overlapping genes —FOS, GFAP, GRIN1, IL1B, MAPK1, MAPK3, MAPK9, and TNF— were recognized in caffeine-trigeminal neuralgia datasets. Gene-gene interaction network showed predominance predicted and physical interaction. Biological processes, cellular components, molecular functions, and pathway enrichment analysis were obtained. HPA analysis demonstrated gene expression across vascular and immune cell populations. Molecular overlaps, biological frameworks and pathways, showed potential trigeminal sensitization through neuroinflammation, glial response, MAPK signaling, immune-cell interaction and neural excitability. These findings provide computational evidence of potential molecular links between caffeine and mechanism underlying trigeminal neuralgia.

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Introduction

Trigeminal neuralgia (TN), formerly known as *tic douloureux*, is a chronic pain disorder characterized by recurrent, brief, electric shock-like episodes affecting structures innervated by the trigeminal nerve [1]. The trigeminal nerve supplies innervation of the forehead, cheek, and lower jaw; TN is predominantly unilateral and may involve one or more of its divisions [2]. TN has multifactorial pathophysiology involving genetic, anatomical, and neurophysiological factors that promote neuronal

hyperexcitability, central sensitization, and neural plasticity. Neurovascular compression and focal demyelination of the trigeminal root are well-established common etiological factors in trigeminal neuralgia, while the specific modulation of disease development remains incompletely understood [1,3,4].

Caffeine is one of the most widely consumed substances worldwide, approximately 80% of the global population [5]. Caffeine is known for its beneficial effect including decreased cardiovascular disease and diabetes risk with maximum recommendation of 400-600mg/day [6].

However, caffeine exposure has also been implicated in the onset or exacerbation of specific pain disorders, including migraine and caffeine-withdrawal headache [7]. Chronic caffeine intake may modulate pathophysiological processes relevant to neurological disorder, including neuroinflammation, based on the experimental models. It suggests a potential role in neural pain processing mechanism [8].

A recent study investigated the association between oral caffeine intake and trigeminal neuralgia (TN). However, experimental factors such as co-exposure to other substances, may

have influenced the findings, leaving both the potential association and its underlying mechanism remain insufficiently elucidated [7]. Therefore, this study aimed to investigate the potential molecular pathways linking the caffeine and TN-related biological mechanisms through the identification of altered gene and protein expression, functional enrichment and cell specific association.

Material and Methods

Comparative Toxicogenomics Database (CTD, <http://CTD.mdibl.org>) is a literature-based resource that provides information about the association between chemical compound, gene product, disease and environmental exposure publicly [9]. In CTD database, the gene interaction includes the increase and decrease of protein activity, mRNA expression, and protein expression. Gene interactions quantification was based on the published literature curation using a hierarchical interaction type vocabulary which characterizes general biochemical, regulatory, and physical chemical-genes/protein interactions. CTD was used to disclose the information about the caffeine and trigeminal neuralgia association. The caffeine-TN related genes were retrieved from the disease data cards. The report of chemical-disease-gene analysis was collected in July 2026. Subsequent interactions analysis among related genes was conducted through GeneMANIA online (<https://genemania.org>) server. GeneMANIA website was used to create hypothesis or prediction of the gene list, gene function, and prioritized genes for functional test [10]. The information about predicted networks, physical interactions, co-expression, shared protein domains, pathway and genetic information were retrieved. The *Homo sapiens* was chosen as the identified species.

GO and KEGG functional annotation pathway enrichment analysis were performed in DAVID Bioinformatics website (<https://davidbioinformatics.nih.gov/>) in order to explore the molecular and biological functions [11]. The biological process, molecular function and cellular component analyses were conducted; FDR (False Discovery Rate) correction and $p < 0.05$ were chosen for the analysis. The retrieved data were subsequently visualized using Scientific and Research plot tool (SRplot; <http://www.bioinformatics.com.cn/srplot>). SRplot was chosen to comprehensively visualize most influenced biological process, molecular function, cellular component and pathway enrichment [12].

The evaluation of potential tissue-specific effects were performed using human gene expression diagrams from the Human Protein Atlas (HPA) website (<https://www.proteinatlas.org/>) [13,14]. The single cell type-

vasculature was chosen to identify the caffeine effect related to trigeminal neuralgia. The selected genes were the caffeine-induced upregulated genes in the development of TN. The values of averaged raw gene counts across all cells within a cluster were normalized using Trimmed Mean of M-values (TMM) normalization, referred to as normalized counts per million (nCPM). The nCPM of each cell was visualized using SRplot.

Results

Trigeminal neuralgia was chosen among several disease cards in mouth disease category of CTD database. There were eight caffeine-TN overlapping genes obtained. Those are FOS (Fos proto-oncogene, AP-1 transcription factor subunit), GFAP (glial fibrillary acidic protein), GRIN1 (glutamate ionotropic receptor NMDA type subunit 1), IL1B (interleukin 1 beta), MAPK1 (mitogen-activated protein kinase 1), MAPK3 (mitogen-activated protein kinase 3), MAPK9 (mitogen-activated protein kinase 9), and TNF (tumor necrosis factor).

Caffeine was shown to increase MAPK1, MAPK3 and TNF protein activity (Table 1). Additionally, caffeine was shown increased mRNA expression of FOS and TNF as well as decreased mRNA expression of GRIN1 and IL1B. There was an increased level of protein expression in FOS, GFAP, MAPK1, MAPK3 and TNF.

Type of the network construction and genes interactions were determined using GeneMANIA. Caffeine-TN intersected genes were loaded into the GeneMANIA web plug-in, resulting a link network (Figure 1). The result showed a predominance of predicted and physical interactions, indicating that those genes are predicted to interact when their orthologs are known to interact in another organism, and linked when their encoded protein interactions had been identified in protein-protein interaction studies. Additionally, the following interactions obtained were co-expression (24.99%), shared protein domains (5.31%), pathway (0.78%), and genetic interactions (0.05%).

GO and KEGG functional annotation pathway enrichment analysis were performed with the selection of *Homo sapiens* annotation. Result of the analysis showed involvement of 86 biological process terms, including positive regulation of neuroinflammatory response, Bergmann glial cell differentiation, response to ethanol, positive regulation of macrophage derived foam cell differentiation, and positive regulation of glial cell proliferation. There were 9 cellular component terms, including pseudopodium, cytosol, plasma membrane, caveola, and ciliary tip. There were 11 molecular function terms including MAP kinase activity, identical protein binding protein serine kinase

activity, protein serine/threonine kinase activity, and protein kinase activity (Figure 2).

The result of KEGG pathway enrichment analysis showed that the most common terms were related to Pertussis, IL-17 signaling pathway, Chagas disease, Toll-like receptor signaling pathway, TNF signaling pathway, Yersinia infection, Osteoclast differentiation, Pathogenic *Escherichia coli* infection, Lipid and atherosclerosis, and Leishmaniasis (Figure 3). Further discussion would focus on three main pathways which were the IL-17 signaling pathway, Toll-like receptor signaling pathway, and TNF signaling pathway that related to inflammation process.

The potential tissue-specific effects for the single cell-vasculature through caffeine-TN related genes normalized counts per million (nCPM) analysis are presented in Figure 4. These results showed potential affected single cell of the vasculature in the development of trigeminal neuralgia including Schwann cell, vascular endothelial cell, vascular smooth muscle cell, mast cell, neutrophil, monocyte, macrophage, T-cell, and NK-cell due to the upregulated caffeine-induced genes.

Discussion

The pathophysiology of trigeminal neuralgia involves complex network-level dysfunctions that amplify, integrate, and sustain nociceptive signaling across multiple brain regions. Rather than following a linear pathway, TN pain involves interconnected parallel circuits, ascending facilitation, descending modulation, and positive-feedback mechanisms [15].

TN is a disorder which related to neuronal hyperexcitability, neuroinflammatory, glial, and signaling mechanisms [3,15,16]. This suggests convergence among neuronal, glial, intracellular signaling, and inflammatory mechanisms rather than independent causal effects of all eight genes in our findings.

Recent study disclosed a connection between c-Fos expression and trigeminal pain-state activation [17]. In animal models, c-Fos expression marks trigeminal pain-related neuronal activation, however it does not establish FOS as a primary causal mediator [18]. Glial fibrillary acidic protein (GFAP) encodes one of the major intermediate filament proteins of mature astrocytes. GFAP is widely used as a marker to identify and distinguish astrocytes from other glial cell types during development and is also regarded as an important biomarker of neuroinflammatory conditions [19]. Recent studies indicate that GFAP-marked satellite glial cell activation in the trigeminal ganglion is associated with trigeminal neuropathic pain. Nerve injury increases GFAP expression, while reducing TN-associated pain also decreases

GFAP, supporting a link between satellite glial cell activation, inflammation, and neuropathic pain [20,21].

Our findings showed increased MAPK1/MAPK3 activity and protein expression, with MAPK9 also appearing in the overlapping-gene set. Recent studies implicate MAPK signaling in TN-related inflammation and neuropathic pain, with increased ERK1/2, p38, and JNK activity and reduced pain following pathway inhibition [22,23]. Moreover, phosphoproteomic evidence identified MAPK signaling, as a shared mechanism in trigeminal neuropathic pain [24].

TNF showed increase at mRNA, protein and activity levels. The production of the pro-inflammatory cytokine TNF- α is increased by various cell types, including astrocytes and microglia, in response to stress, injury, or inflammatory conditions [25]. Recent review showed TN patients' elevated TNF- α , which may contribute to glial activation and neuroinflammation [22]. Experimental evidence further indicates that macrophage-derived TNF- α enhances neuronal excitability, sustaining neuroinflammation and mechanical allodynia [26].

The result of pathway enrichment analysis showed that TNF, IL-17, and Toll-like receptor signaling pathways were plausibly associated with trigeminal neuralgia through inflammatory and immune mechanisms. In contrast, enrichment of infection-related pathways should not be interpreted as direct evidence that these infections cause TN. They may appear in enrichment analyses for KEGG pathways share inflammatory or innate-immune genes. TNF and Toll-like receptor signaling have relatively strong evidence linking them to trigeminal neuropathic pain through inflammatory and neuronal sensitization mechanisms [22,27,28]. In addition, IL-17 signaling is biologically plausible yet has more limited direct evidence in TN. However, it is known for its involvement in the nerve injury pathological development [29–31].

The HPA results further indicated that caffeine-associated genes are expressed in vascular and immune cell populations, as descriptive expression data, suggesting that interactions among neural, vascular, and immune compartments may be relevant to the proposed mechanism. Directly relevant to our cell list, latest study demonstrated that macrophages in the trigeminal ganglion contribute to TN by releasing TNF- α and increasing neuronal excitability, establishing a neuron-macrophage feedback loop [26].

The increased expression or activity of induced-overlapped genes, altogether with the identification of related pathways, provided a biologically coherent framework in which caffeine-associated molecular modulation could

potentially influence mechanisms involved in trigeminal sensitization. This interpretation is consistent with recent TN studies demonstrating important roles for satellite glial cells, macrophages, inflammatory cytokines, MAPK signaling, and neuron-immune interactions in the initiation and maintenance of trigeminal neuropathic pain.

Nevertheless, these findings should be interpreted as a mechanistic hypothesis rather than evidence of causality. In particular, the computational analyses do not establish that caffeine directly activates these pathways in trigeminal tissue, nor do they demonstrate that caffeine produces neurovascular compression or directly induces TN. The bioinformatic-based analyses of biological response also may be context, cell, or time-dependent and requires experimental validation. Accordingly, the present study supports the hypothesis that caffeine may modulate molecular pathways capable of altering trigeminal neuronal-glial-immune signaling and potentially lowering the threshold for pain attacks in susceptible individuals, rather than establishing caffeine as a direct cause of trigeminal neuralgia. Further studies employing *in vitro* or *in vivo* approaches are warranted to elucidate the dosage and time-dependent effects of caffeine on trigeminal neuralgia.

Conclusion

Bioinformatic analysis revealed eight overlapping genes between caffeine and trigeminal neuralgia. Increased FOS and TNF mRNA expression; as well as MAPK1, MAPK3, TNF activity; and FOS, GFAP, MAPK1, MAPK3 and TNF protein expression emphasized potential biological process related to pathophysiology of trigeminal neuralgia. Overlapped genes were connected predominantly through predicted physical interactions. These findings suggest that caffeine may modulate molecular pathways involving neuroinflammation, glial response, MAPK signaling, and neural excitability based on the computational-based analysis.

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Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

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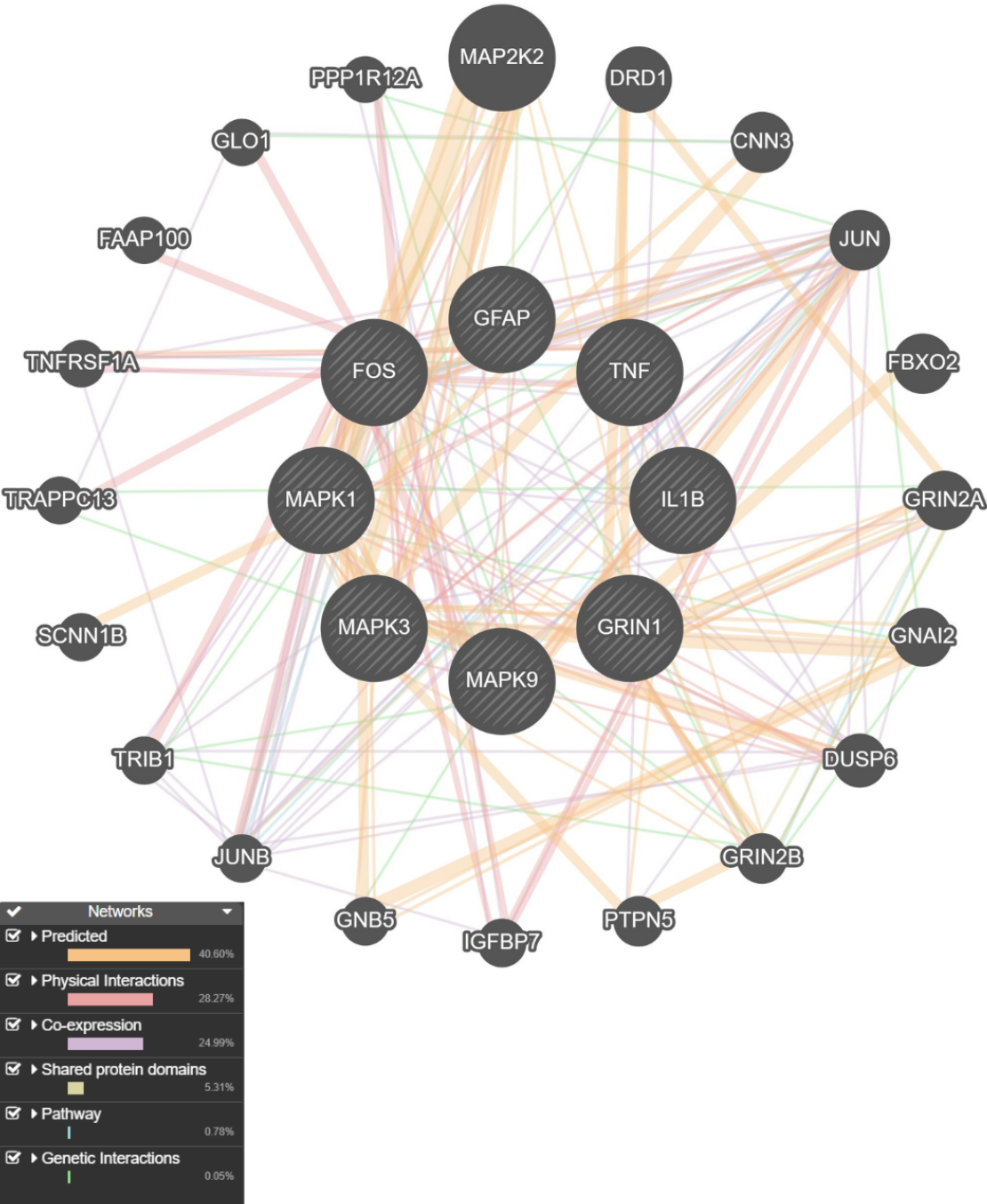


Figure 1. Gene-gene interaction network.

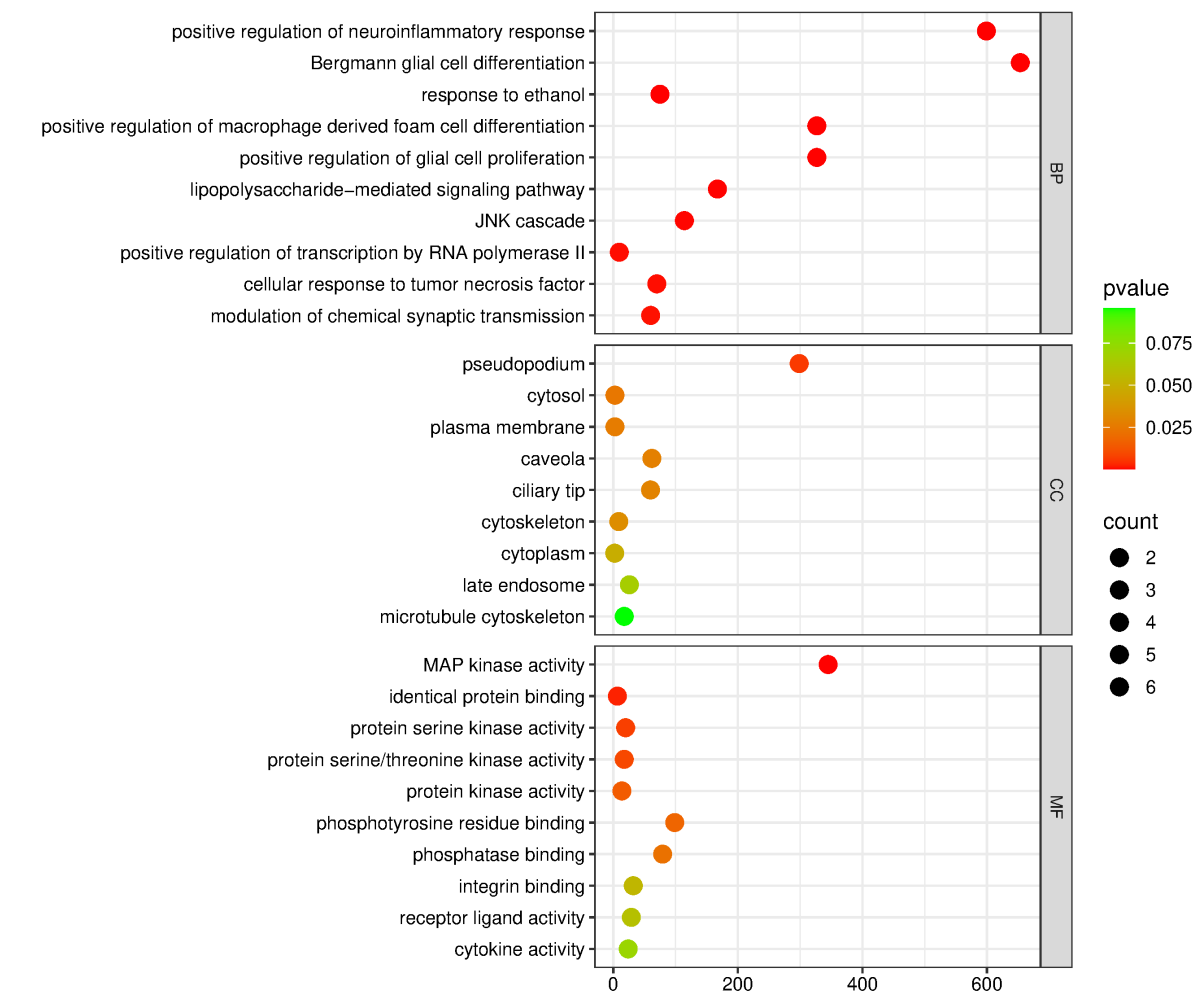


Figure 2. Gene Ontology (GO) analysis including biological process (BP), cellular components (CC), and molecular function (MF).

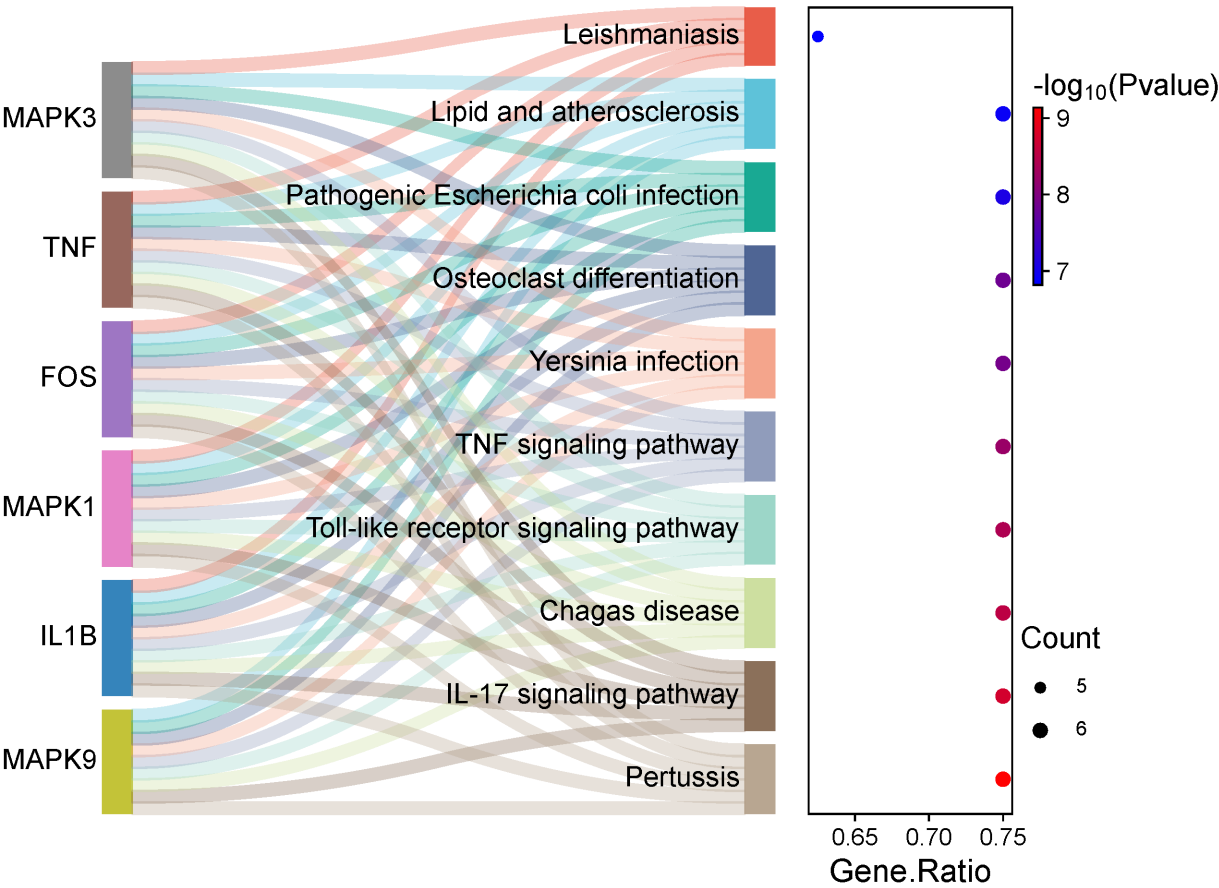


Figure 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) top 10 pathways enrichment analysis.

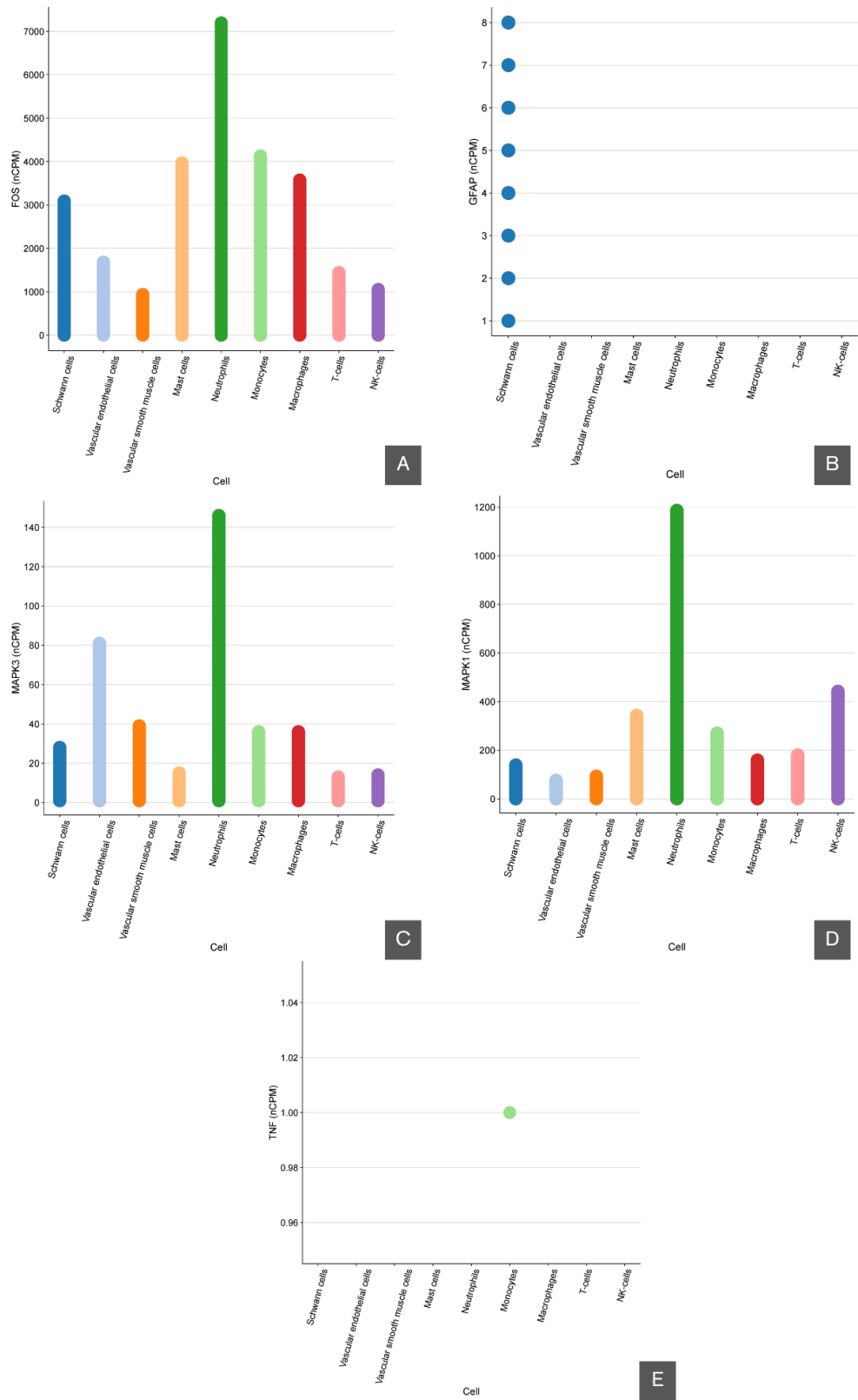


Figure 4. Single cell-vasculature effect due to upregulated caffeine-induced genes. FOS (A). GFAP (B). MAPK3 (C). MAPK1 (D). TNF (E).

Table 1. The interaction between caffeine and annotated genes.

Gene	Protein Activity	mRNA Expression	Protein Expression
FOS	/	+	+
GFAP	/	/	+
GRIN1	/	-	/
IL1B	/	-	/
MAPK1	+	/	+
MAPK3	+	/	+
MAPK9	-	/	/
TNF	+	+	+

+ increase; - decrease.