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Smoking and drinking influence the advancing of ischemic stroke disease by targeting PTGS2 and TNFAIP3

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Abstract. In the present study, we explored the influence of cigarette smoking and alcohol drinking on gene expression level and related functions and pathways on the development of ischemic stroke (IS) disease. The gene expression profile of E-GEOD-22255 was obtained from 20 IS samples (7 patients without smoking or drinking history and 13 patients with smoking or drinking history) and 20 controls (9 normal controls without smoking or drinking history and 11 controls with smoking or drinking history). The correlation degree between gene expression and grouping were measured by significance analysis of microarray (SAM). Smoking or drinking-related DEGs were screened. GO functional and KEGG pathway enrichment analyses were processed. Based on the KEGG database, a pathway relationship network was constructed. DEGs in significant functions and pathways were inserted and regarded as key DEGs. Gene co-expression network was constructed based on the expression value of key genes. In total, 319 IS-related DEGs, which were induced by smoking and drinking, were screened and enriched in various functions and pathways, including inflammatory response, nuclear factor-κB (NF-κB) signaling pathway and influenza A. Pathway relationship network was constructed with 44 nodes and the hub node was the MAPK signaling pathway. After merging, 87 key DEGs were obtained. The gene co-expression network with 43 node edges was constructed and the hub node was prostaglandin-endoperoxide synthase 2. In IS patients, smoking and drinking may induce different expression of many genes, including *PTGS2*, *TNFAIP3*, *ZFP36* and *NFKBIZ*. In addition, these genes participated in various pathways, such as inflammatory response.

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Introduction

Ischemic stroke (IS) occurs due to a lack of blood flow to the brain, which is induced by various risk factors, including high blood pressure, tobacco smoking, alcohol drinking, obesity, high blood cholesterol and diabetes mellitus, and results in cell death and improper functioning of part of the brain (1,2). IS patients always have symptoms including inability to move or inconvenience on one side of the body (3). Techniques, such as a neurological examination, CT scans, MRI scans and Doppler ultrasound, have been used for diagnosis and determining the type and causes of IS (4,5). Current studies found that blood tests and gene detection may help to find the potential causes of IS.

Many genes are closely associated with IS disease. For instance, phosphodiesterase 4D (PDE4D) participated in atherosclerosis, which is a primary pathological process for IS disease (6). In addition, transfer of the Kallikrein gene was confirmed to inhibit apoptosis and promote glial cell migration, and then protect against IS (7). Liu *et al* (8) confirmed that polymorphisms of heat shock protein 70 may increase the risk of IS in smoking patients. In Caucasians and northern Han Chinese, the single-nucleotide polymorphisms of interleukin-1 (IL-1) and PDE4D were also confirmed to increase the IS risk (9).

Especially in young women and adults, IS risk and cigarette smoking exhibited a strong dose-response relationship (10). In addition, heavy and light-moderate drinking exerted different effects on IS (11). Although cigarette smoking and alcohol drinking had a significant influence on IS, to the best of our knowledge, very little research has been done in the field of related molecule mechanisms. The biomarkers and related pathways of IS disease have been investigated through bioinformatics analysis (12). Based on this foundation, the aim of the present study was to focus on the special influence of cigarette smoking and alcohol drinking on gene expression in IS samples. Blood genomic expression profile was used to screen differentially expressed genes of IS specially induced by smoking and drinking history, and related functions and pathways were investigated.

Table I. Top 10 functions enriched by special DEGs.

GO ID	GO name	Diff gene counts in GO	Enrichment score	P-value	FDR
GO:0006954	Inflammatory response	24	14.50814	1.83E-20	2.39E-17
GO:0006955	Immune response	21	10.66927	2.62E-15	1.71E-12
GO:0006915	Apoptotic process	26	7.089543	1.70E-14	7.42E-12
GO:0043066	Negative regulation of apoptotic process	20	7.368978	1.20E-11	3.91E-09
GO:0008285	Negative regulation of cell proliferation	17	8.46815	5.63E-11	1.47E-08
GO:0045429	Positive regulation of nitric oxide biosynthetic process	7	41.61016	5.84E-10	1.27E-07
GO:0045087	Innate immune response	19	6.115986	9.50E-10	1.77E-07
GO:0002237	Response to molecule of bacterial origin	5	99.07182	1.32E-09	2.15E-07
GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	21	5.289427	1.54E-09	2.24E-07
GO:0006935	Chemotaxis	10	15.37321	2.48E-09	3.04E-07

Materials and methods

DEG screening of smoking or drinking induced-IS. The expression profile of E-GEOD-22255 was obtained from ArrayExpress archive (http://www.ebi.ac.uk/arrayexpress/), which was deposited by Krug *et al* (13). This profile contained 40 samples, including 20 IS samples (7 IS samples without smoking or drinking history and 13 IS samples with smoking or drinking history) and 20 controls (9 normal controls and 11 controls with smoking or drinking history). The platform of this chip was GPL570 (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 array. The expression value of probe sets was calculated by algorithm of robust multi-chip average (RMA) in three steps: Background correction, standardization and summarizing (14). According to the information of the Affymetrix official website, these processed probe sets were annotated and included with the threshold of >0.8 median NUSE <1.2 and >-0.25 median RLE <0.25. Thus, NUSE was the value of normalized unscaled standard errors, while RLE was the value of relative log expression.

Significance analysis of microarray (SAM) was used to construct a d score for each gene to measure the correlation degree between of gene expression and grouping (15). In this process, exchangeable factors were obtained by calculating the mean absolute deviation. The statistical value of each gene was calculated using the formula: $d_i = r_i/(s_i + s_0)$.

Thus, r_i reflected the difference of genes in average level, and s_i reflected overall change of samples.

Then, >1,000 permutations were applied to simulate of the distribution of d score. In this study, this method was used on samples to screen the two groups of DEGs: IS samples without smoking or drinking history compared with normal controls, IS samples with smoking or drinking history compared with controls with smoking or drinking history. Multiple test was used to calculate P-value of each gene.

To screen IS-related DEGs induced by smoking and drinking history, DEGs of samples with smoking or drinking history were deducted by DEGs of samples without smoking or drinking history based on the same gene symbols.

Functional and pathway enrichment analysis. Gene ontology (GO) analysis is a method used to select the significant functions of gene groups based on the GO database (16), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database is a strong tool for biological metabolic analysis and metabolic network research (17). In this study, these databases were used for functional and pathway enrichment analysis of smoking- or drinking-induced DEGs. To define the results more precisely, Fisher's exact test and multiple comparative test were used to calculate the P-value and FDR value, respectively.

Construction of pathway relationship network. The KEGG database was also used to construct a pathway relationship network. This network was able to show the signal transduction relationship between significant pathways, and importantly, upstream and downstream signal pathways were also identified.

Construction of gene co-expression network. Key DEGs induced by smoking and drinking history were obtained by inserting DEGs in significant functions and pathways. Based on the expression value of genes, the gene co-expression network was constructed and analyzed. Genes with mean connectedness of >1 were involved.

Results

DEGs screening. Compared with normal controls, 128 DEGs were screened in IS samples without smoking or drinking history. At the same time, 465 DEGs induced by smoking and drinking and other factors were obtained. DEGs specially induced by smoking and drinking were screened based on the same gene symbol, and 319 special DEGs were obtained.

Functional and pathway enrichment analysis. These special DEGs were enriched in different functions, including inflammatory response (FDR=2.39E-17), immune response (FDR=1.71E-12), apoptotic process (FDR=7.42E-12) and negative regulation of apoptotic process (FDR=3.91E-09) (Table I). Simultaneously, these DEGs also participated in various pathways, such as nuclear factor-κB (NF-κB) signaling pathway

Table II. Top 10 pathways enriched by special DEGs.

Pathway ID	Pathway name	Different gene counts in pathway	Gene amount in pathway	Enrichment score	P-value	FDR
4064	NF-κB signaling pathway	13	92	25.1987	1.19E-14	1.77E-12
5164	Influenza A	15	179	14.94379	2.55E-13	1.90E-11
5134	Legionellosis	10	55	32.4235	1.20E-12	5.96E-11
4621	NOD-like receptor signaling pathway	10	57	31.28584	1.75E-12	6.53E-11
5132	*Salmonella* infection	11	88	22.29116	6.03E-12	1.68E-10
4060	Cytokine-cytokine receptor interaction	16	267	10.6864	6.78E-12	1.68E-10
4380	Osteoclast differentiation	12	135	15.85149	3.72E-11	7.91E-10
4062	Chemokine signaling pathway	13	192	12.07438	1.66E-10	3.09E-09
5323	Rheumatoid arthritis	10	94	18.9712	3.06E-10	5.07E-09
5142	Chagas disease (American trypanosomiasis)	10	105	16.98374	9.26E-10	1.29E-08

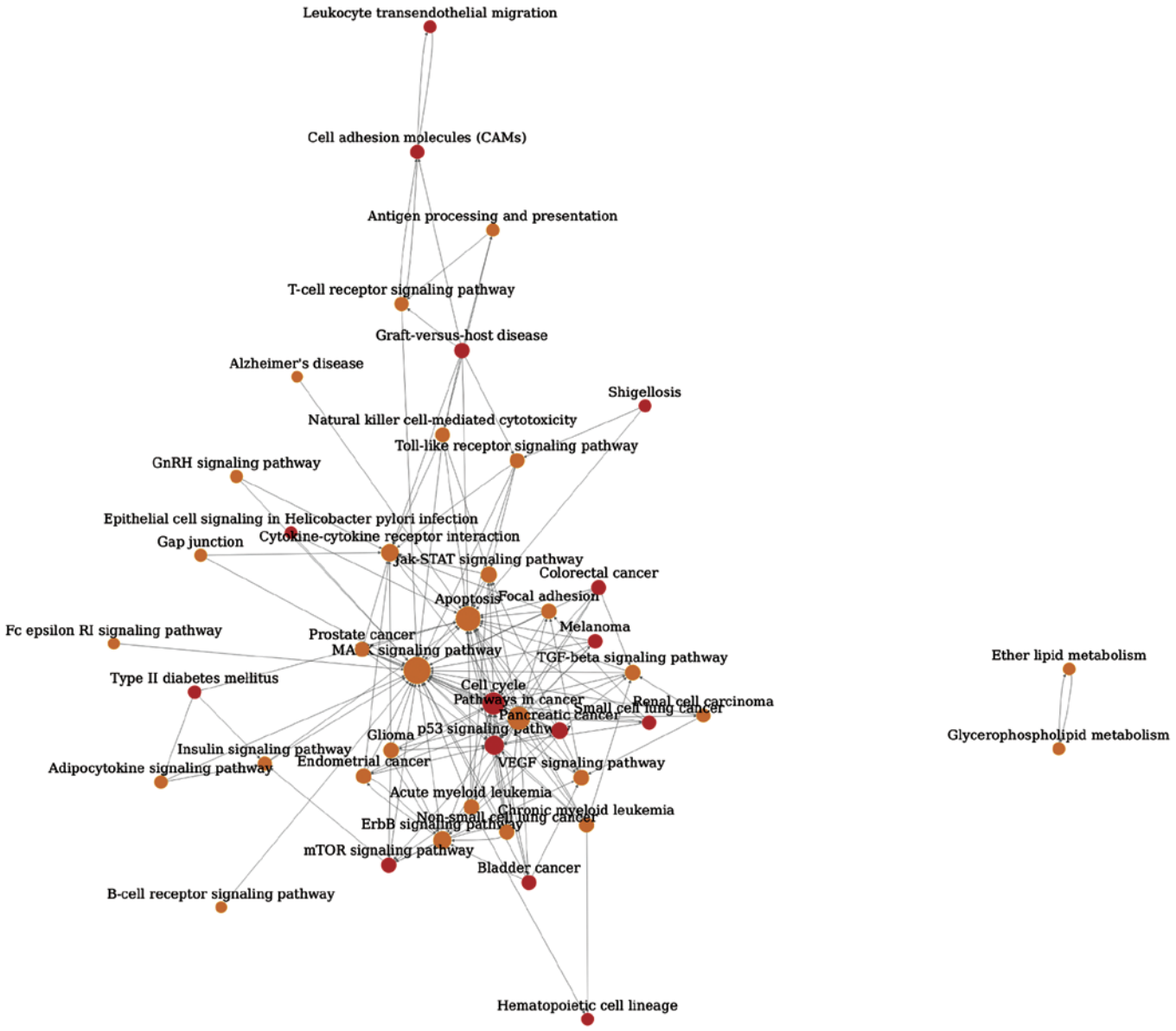

Figure 1. Pathway relationship network. The nodes represent pathways enriched by special DEGs, and the arrows represent regulatory relationship. The size of nodes was determined by degree. The red nodes represent pathways with upregulated genes, while the yellow nodes represent pathways with both upregulated and downregulated genes.

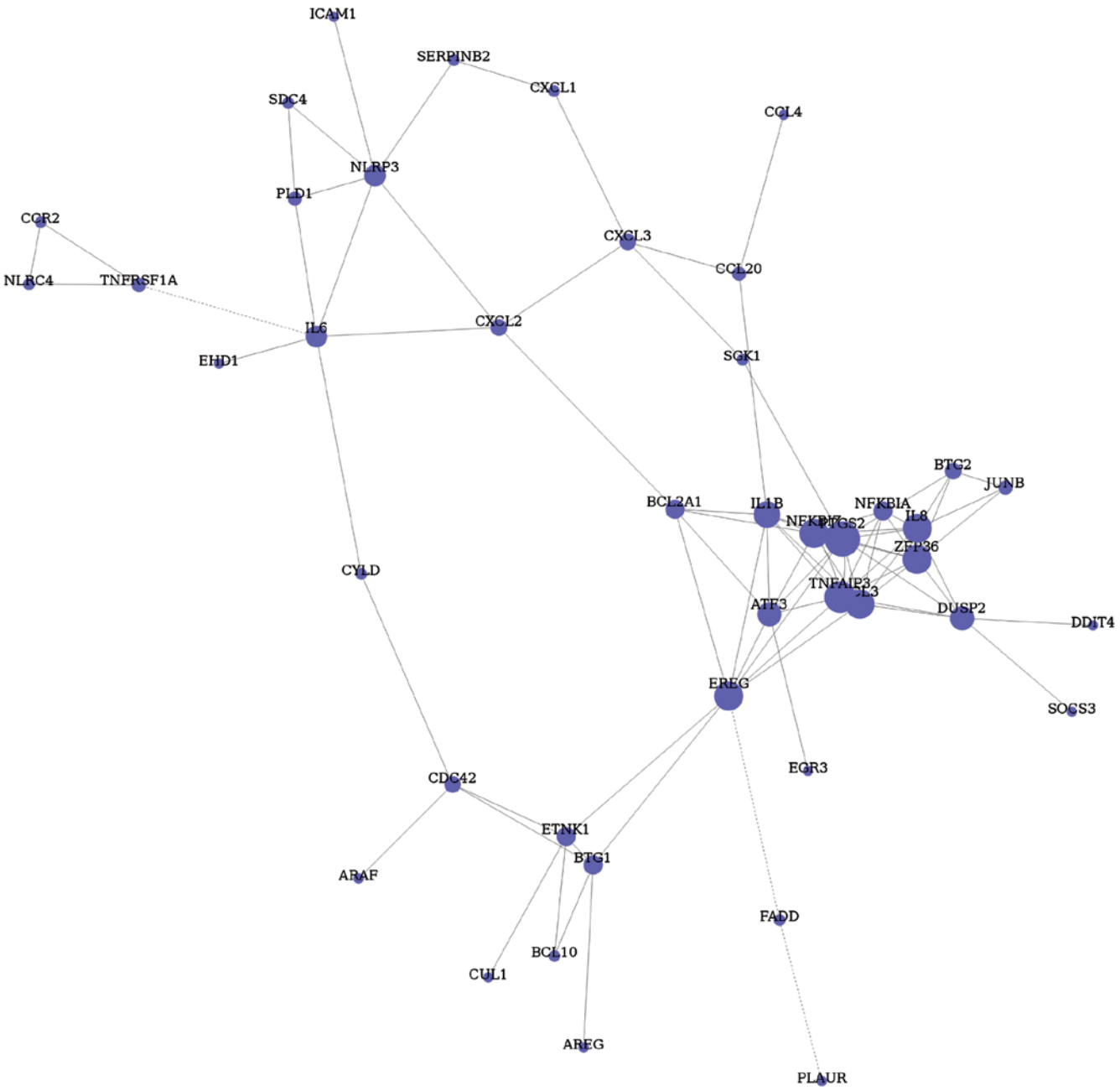

Figure 2. Gene co-expression network of key special DEGs. The nodes are key special DEGs. Solid and dotted lines show the relationship of positive and negative regulation, respectively.

(FDR=1.77E-12), influenza A (FDR=1.90E-11), legionellosis (FDR=5.96E-11) and NOD-like receptor signaling pathway (FDR=6.53E-11) (Table II).

Construction of pathway relationship network. The pathway relationship network with 44 nodes and 161 edges were constructed (Fig. 1). In this network, the hub nodes were MAPK signaling pathway (degree=32), apoptosis (degree=27) and pathways in cancer (degree=23). Of note, pathways in cancer (out-degree=23, in-degree=0) and cytokine-cytokine receptor interaction (out-degree=0, in-degree=12) were the upstream and downstream pathway, respectively.

Construction of gene co-expression network. The DEGs in GO terms and pathways were inserted and 87 key genes were obtained (Fig. 2). According to the mean connectedness of these genes, the gene co-expression network was constructed with 43 nodes and 88 edges. The hub nodes were prostaglandin-endoperoxide synthase 2 (PTGS2, degree=11), TNF-α-induced protein 3 (TNFAIP3, degree=10), ZFP36 ring finger protein (ZFP36, degree=9) and NFKB inhibitor ζ (NFKBIZ, degree=9). Importantly, these four nodes were found to have positive relationships.

Discussion

Smoking and alcohol drinking were confirmed to be risk factors for IS. However, the causes of smoking and alcohol drinking induced IS are still unknown (11,18). In this study, various factors of IS except smoking and alcohol drinking

were excluded. Importantly, several key DEGs were screened, including *PTGS2*, *TNFAIP3*, *ZFP36* and *NFKBIZ*.

PTGS2, an isozyme of prostaglandin-endoperoxide synthase (PTGS), was a critical enzyme in prostaglandin biosynthesis (19). Kunze *et al* (5) confirmed that in the environment with smoking, ΔNp63 could bind to PTGS2 promoter, and further influence the process of inflammatory response. Moreover, a case control study showed that smoking and alcohol drinking induced substantial differences between esophageal squamous cell carcinoma samples and controls (20). The overexpression of PTGS2 also played an important role in the pathogenesis of IS. In African-Americans with the variant of G-765C allele of PTGS2, IS was found with a significantly higher incidence rate (21). As shown in this study, PTGS2 was enriched in various functions and pathways, including inflammatory response, negative regulation of cell proliferation, as well as the NF-κB and VEGF signaling pathways. Moreover, the NF-κB signaling pathway was confirmed to activate the synthesis of inducible PTGS2 in the brain (22). Liu *et al* (23) found that electroacupuncture could target the NF-κB signaling pathway and inhibit inflammatory injury for IS. Thus, we inferred that PTGS2 is a potential biomarker for smoking- and drinking-induced IS disease by participating in inflammatory response and the NF-κB signaling pathway.

Furthermore, *TNFAIP3* and *PTGS2* were positively associated in this study. The expression of TNFAIPS was always induced by tumor necrosis factor. In 2010, Lodolce *et al* (24) confirmed that TNFAIP3 was a ubiquitin-modifying enzyme, and genetic polymorphisms of TNFAIP2 were also found to affect the autoimmunity regulation. Under inflammatory conditions, TNFAIP3 was a negative-feedback regulator of NF-κB activation (25). Furthermore, TNFAIP was confirmed to be a negative regulatory of Toll-like receptor signaling pathway, which could lead to inflammatory effects (26). Similarly in this study, TNFAIP3 was involved in inflammatory, innate immune response, NF-κB signaling pathway and NOD-like receptor signaling pathway.

ZFP36 was also screened with a higher degree in the gene co-expression network, and enriched in negative regulation of transcription from RNA polymerase II, response to stress and vasculogenesis. In a previous study (27) ZFP36 was found to be a critical gene for obesity-related metabolic complications by detecting the cholesterol level and omental adipose tissue ZFP36 mRNA levels. Furthermore, decreased regulation of the metabolic syndrome may significantly affect the prevalence of stroke and related disability (28). As shown in a study by Kurl *et al* who conducted an exercise stress test, an increase in systolic blood pressure was independently associated with the risk of IS and other types of stroke (29). In addition, the stress of acute ischemic cerebral insults may increase secretion of stress hyperglycemia, and further induce hyperglycemia following stroke (30). Nicotine in cigarette was also confirmed to promote vasculogenesis by affecting endothelial progenitor cells (31). Cerebral ischemic stroke could also be treated by stem cell transplantation and angiogenesis (32).

NFKBIZ, another important key DEG, is a member of the ankyrin-repeat family, induced by lipopolysaccharide (33). It is known to participate in inflammatory responses to lipopolysaccharide by interacting with NF-B proteins through ankyrin-repeat domains (34). Similarly with the results in the present study, NFKBIZ was involved in inflammatory response, transcription, DNA-dependent, and transcriptional misregulation in cancer. As known, early inflammatory response may potentiate ischemic injury, whereas late response may induce contrary function in stroke. In acute experimental stroke, phosphatidylinositol-3-kinase inhibitors were found to suppress the activation of DNA-dependent protein kinases, which played a critical role in blood-brain barrier dysfunction (35). Thus, *NFKBIZ* was inferred to be a key IS-related gene.

In conclusion, the screened DEGs, such as *PTGS2*, *TNFAIP3*, *ZFP36* and *NFKBIZ*, are potential biomarkers of smoking- and drinking-induced IS disease, participating in various functions, such as inflammatory response. Thus, giving up alcohol and tobacco, and detecting the abovementioned biomarkers is of significance for IS prevention and treatment.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZM was reponsible for DEG screening. MG, SZ and XS helped with construction of pathway relationship network. FW and HL participated in data analysis. ZM and ZC contributed to construction of gene co-expression network. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Collins P: Risk factors for cardiovascular disease and hormone therapy in women. Heart 92 (Suppl 3): iii24-iii28, 2006.
2. Urban PP, Wicht S, Vukurevic G, Fitzek C, Fitzek S, Stoeter P, Massinger C and Hopf HC: Dysarthria in acute ischemic stroke: lesion topography, clinicoradiologic correlation, and etiology. Neurology 56: 1021-1027, 2001.
3. Chen WC, Cheng HC and Datsen GW: Pharmaceutical composition for preventing/treating brain injury and enhancing recovery of sequelae and manufacture thereof. US Patent US 20150290272 A1. Filed April 9, 2015; issued October 15, 2015.

4. Wong CB and Wong JC: A novel method to quantify carotid artery stenosis by Doppler ultrasound: Using the continuity principle. Int J Angiol 19: e86-e90, 2010.
5. Kunze E, Pham M, Raslan F, Stetter C, Lee JY, Solymosi L, Ernestus RI, Vince GH and Westermaier T: Value of perfusion CT, transcranial doppler sonography, and neurological examination to detect delayed vasospasm after aneurysmal subarachnoid hemorrhage. Radiol Res Pract 2012: 231206, 2012.
6. Gretarsdottir S, Thorleifsson G, Reynisdottir ST, Manolescu A, Jonsdottir S, Jonsdottir T, Gudmundsdottir T, Bjarnadottir SM, Einarsson OB, Gudjonsdottir HM, *et al*: The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. Nat Genet 35: 131-138, 2003.
7. Xia CF, Yin H, Borlongan CV, Chao L and Chao J: Kallikrein gene transfer protects against ischemic stroke by promoting glial cell migration and inhibiting apoptosis. Hypertension 43: 452-459, 2004.
8. Liu J, Cheng J, Peng J, Han S, Yu L and Nie S: Effects of polymorphisms of heat shock protein 70 gene on ischemic stroke, and interaction with smoking in China. Clin Chim Acta 384: 64-68, 2007.
9. Li N, He Z, Xu J, Liu F, Deng S and Zhang H: Association of PDE4D and IL-1 gene polymorphism with ischemic stroke in a Han Chinese population. Brain Res Bull 81: 38-42, 2010.
10. Bhat VM, Cole JW, Sorkin JD, Wozniak MA, Malarcher AM, Giles WH, Stern BJ and Kittner SJ: Dose-response relationship between cigarette smoking and risk of ischemic stroke in young women. Stroke 39: 2439-2443, 2008.
11. Klatsky AL, Armstrong MA, Friedman GD and Sidney S: Alcohol drinking and risk of hospitalization for ischemic stroke. Am J Cardiol 88: 703-706, 2001.
12. Barr TL, Conley Y, Ding J, Dillman A, Warach S, Singleton A and Matarin M: Genomic biomarkers and cellular pathways of ischemic stroke by RNA gene expression profiling. Neurology 75: 1009-1014, 2010.
13. Krug T, Gabriel JP, Taipa R, Fonseca BV, Domingues-Montanari S, Fernandez-Cadenas I, Manso H, Gouveia LO, Sobral J, Albergaria I, *et al*: TTC7B emerges as a novel risk factor for ischemic stroke through the convergence of several genome-wide approaches. J Cereb Blood Flow Metab 32: 1061-1072, 2012.
14. Smita S, Katiyar A, Pandey DM, Chinnusamy V, Archak S and Bansal KC: Identification of conserved drought stress responsive gene-network across tissues and developmental stages in rice. Bioinformation 9: 72-78, 2013.
15. Kutalik Z, Inwald J, Gordon SV, Hewinson RG, Butcher P, Hinds J, Cho KH and Wolkenhauer O: Advanced significance analysis of microarray data based on weighted resampling: A comparative study and application to gene deletions in *Mycobacterium bovis*. Bioinformatics 20: 357-363, 2004.
16. Blake JA and Harris MA: The Gene Ontology (GO) project: Structured vocabularies for molecular biology and their application to genome and expression analysis. Curr Protoc Bioinformatics: Chapter 7, Unit 7.2, 2008.
17. Jing LS, Shah FF, Mohamad MS, Hamran NL, Salleh AH, Deris S and Alashwal H: Database and tools for metabolic network analysis. Biotechnol Bioprocess Eng 19: 568-585, 2014.
18. Shah RS and Cole JW: Smoking and stroke: The more you smoke the more you stroke. Expert Rev Cardiovasc Ther 8: 917-932, 2010.
19. Kosaka T, Miyata A, Ihara H, Hara S, Sugimoto T, Takeda O, Takahashi E and Tanabe T: Characterization of the human gene (PTGS2) encoding prostaglandin-endoperoxide synthase 2. Eur J Biochem 221: 889-897, 1994.
20. Hu HM, Kuo CH, Lee CH, Wu IC, Lee KW, Lee JM, Goan YG, Chou SH, Kao EL, Wu MT, *et al*: Polymorphism in COX-2 modifies the inverse association between *Helicobacter pylori* seropositivity and esophageal squamous cell carcinoma risk in Taiwan: A case control study. BMC Gastroenterol 9: 37, 2009.
21. Zeldin D: The G-765C promoter polymorphism in cyclooxygenase-2 (PTGS2), aspirin utilization and cardiovascular disease risk: The Atherosclerosis Risk in Communities (ARIC) study. Pharmacotherapy 26: e87, 2006.
22. Nadjar A, Tridon V, May MJ, Ghosh S, Dantzer R, Amédée T and Parnet P: NFkappaB activates in vivo the synthesis of inducible Cox-2 in the brain. J Cereb Blood Flow Metab 25: 1047-1059, 2005.
23. Liu W, Wang X, Zheng Y, Shang G, Huang J, Tao J and Chen L: Electroacupuncture inhibits inflammatory injury by targeting the miR-9-mediated NFκB signaling pathway following ischemic stroke. Mol Med Rep 13: 1618-1626, 2016.
24. Lodolce JP, Kolodziej LE, Rhee L, Kariuki SN, Franek BS, McGreal NM, Logsdon MF, Bartulis SJ, Perera MA, Ellis NA, *et al*: African-derived genetic polymorphisms in TNFAIP3 mediate risk for autoimmunity. J Immunol 184: 7001-7009, 2010.
25. Vereecke L, Beyaert R and van Loo G: Genetic relationships between A20/TNFAIP3, chronic inflammation and autoimmune disease. Biochem Soc Trans 39: 1086-1091, 2011.
26. Hung YY, Lin CC, Kang HY and Huang TL: TNFAIP3, a negative regulator of the TLR signaling pathway, is a potential predictive biomarker of response to antidepressant treatment in major depressive disorder. Brain Behav Immun 59: 265-272, 2017.
27. Bouchard L, Tchernof A, Deshaies Y, Marceau S, Lescelleur O, Biron S and Vohl MC: ZFP36: A promising candidate gene for obesity-related metabolic complications identified by converging genomics. Obes Surg 17: 372-382, 2007.
28. Seet RC, Zhang Y, Wijdicks EF and Rabinstein AA: Impact of obesity and metabolic syndrome in stroke patients undergoing intravenous thrombolysis. Stroke 43: A156, 2012.
29. Kurl S, Laukkanen JA, Rauramaa R, Lakka TA, Sivenius J and Salonen JT: Systolic blood pressure response to exercise stress test and risk of stroke. Stroke 32: 2036-2041, 2001.
30. O'Connell JE and Gray CS: The stress response to acute stroke. Stress Health 7: 239-243, 1991.
31. Heeschen C, Chang E, Aicher A and Cooke JP: Endothelial progenitor cells participate in nicotine-mediated angiogenesis. J Am Coll Cardiol 48: 2553-2560, 2006.
32. Wei L, Keogh CL, Whitaker VR, Theus MH and Yu SP: Angiogenesis and stem cell transplantation as potential treatments of cerebral ischemic stroke. Pathophysiology 12: 47-62, 2005.
33. Oonuma T, Morimatsu M, Ochiai K, Iwanaga T and Hashizume K: Role of NF-kappaB in constitutive expression of MAIL in epidermal keratinocytes. J Vet Med Sci 69: 279-284, 2007.
34. Ishiguro-Oonuma T, Ochiai K, Hashizume K, Iwanaga T and Morimatsu M: NFKBIZ regulates the proliferation and differentiation of keratinocytes. Jpn J Vet Res 63: 107-114, 2015.
35. Jin R, Song Z, Yu S, Piazza A, Nanda A, Penninger JM, Granger DN and Li G: Phosphatidylinositol-3-kinase gamma plays a central role in blood-brain barrier dysfunction in acute experimental stroke. Stroke 42: 2033-2044, 2011.