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Exploring the mechanism of myrrh in treating stroke based on online pharmacology

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Abstract: The purpose of this study is to explore the effective active ingredients and specific mechanism of *myrrh* in the treatment of stroke. The research method is to obtain the main active ingredients of *myrrh* and their corresponding targets from the Traditional Chinese Medicine Systematic Pharmacology Database and Analysis Platform (TCMSP), and standardize the gene names through the Uniport database. Four databases: OMIM, GeneCards, DrugBank and TTD were used to determine stroke disease genes, and the target genes of drug active ingredients and stroke disease genes were used to identify potential therapeutic targets for *myrrh* against stroke. Protein interaction (PPI) network of potential therapeutic targets was obtained through the String database, and the topology of the PPI network was analyzed through the CytoNCA plug-in in Cytoscape software, and the core key targets were selected. The biological information annotation database DAVID was used to conduct GO analysis and KEGG pathway enrichment analysis on potential treatment targets, and visualized through R language. The research results show that after the intersection of *myrrh* drug targets and stroke disease targets, 122 potential therapeutic targets were obtained, corresponding to 30 active ingredients. The core targets are IL6, TNF, TP53, IL1B, MAPK1, AKT1, ESR1, etc. GO analysis shows that the molecular functions involved in *myrrh* are as long as DNA binding transcription factors, ubiquitin-like protein ligase binding, cytokine receptor binding, activation of nuclear receptors and transcription factor activity. KEGG pathway enrichment analysis found that myrrh's role in treating stroke involves the IL−17 signaling pathway, TNF signaling pathway, HIF-1 signaling pathway, Toll-like receptor signaling pathway, etc. The research conclusions show that *myrrh* can treat stroke through multiple channels, multiple components and multiple targets. This experiment lays a foundation for further research on the efficacy of *myrrh*.

1. Introduction

Stroke is considered to be the leading cause of death and disability globally, with multiple complications, leading to prolonged hospital stays and increased medical burdens[1]. At present, with the accelerating process of social aging and urbanization, and the prevalence of unhealthy lifestyles among residents, the incidence of cerebrovascular diseases is gradually increasing. Stroke has become the primary cause of death and disability among adults in our country. The burden of stroke disease in my country has explosive growth and shows a trend of rejuvenation[2]. Traditional Chinese medicine treatment is a common clinical method to treat stroke patients. Currently, more than 140 traditional Chinese medicine prescriptions can be used to treat cognitive impairment after stroke[3].

Myrrh is an oleogel resin exuding from the trunk bark of the *Myrrh* tree and its genus of the family *Myrrh* in the Olivaceae. It grows on hillsides at an altitude of 500 to 1,500 meters and is mainly distributed in tropical Africa and western Asia. It is irregular grains or bonded into lumps, with a diameter of about 2.5 cm[4]. The taste is spicy and bitter, and it returns to the heart, liver and spleen meridians. It has the effects of dispelling blood stasis, relieving pain, swelling and promoting muscle growth. It is used to treat chest obstruction, pain in the stomach, dysmenorrhea, amenorrhea, postpartum stasis, abdominal pain, abdominal pain, phoenix dampness arthralgia, traumatic injuries, carbuncle swelling, sores, etc[5]. The chemical components of *myrrh* are complex, mainly including resins, gums, volatile oils, salts, acids, etc[6]. At present, most research focuses on the drug properties of *myrrh*, but the research on its molecular mechanism has not been in-depth.

Traditional Chinese medicine has the characteristics of multiple components, multiple targets, and diverse regulation methods, and contains a huge amount of information. It is difficult to reflect the systematicness of traditional Chinese medicine by adopting the research ideas of single target and single component in Western medicine. However, network pharmacology is a new method developed based on the concept that drugs function through "multiple targets, multiple pathways, and multiple pathways". It can integrate various drug, protein, gene and other databases, and use bioinformatics technology to analyze and build a drug-target-disease network interaction model to explore the mechanism of drug action. Its holistic and systematic characteristics are very suitable for application to the research of traditional Chinese medicine monomers and compounds. This paper uses online pharmacology methods to discuss the mechanism of *myrrh* in the treatment of stroke, and provides a methodological basis and theoretical basis for future clinical application and scientific research.

2. Materials and Methods

2.1. Prediction of *myrrh* targets

Searching the traditional Chinese medicine systemic pharmacology database and analysis platform (TCMSP, *https://tcmsp-e.com/tcmsp2/*)[7], using oral bioavailability (OB) ≥30% and drug-like (DL) ≥0.18 as standards, screening the active ingredients of *myrrh* and corresponding targets, and standardizing target names based on the principle of species as humans through the UniProt database (*https://www.uniprot.org/*)[8].

2.2. Screening of stroke-related gene targets

Stroke-related genes were searched through the GeneCards database (*http：//www.genecards.org*) [9], the Online Mendelian Inheritance in Man database (*https://omim.org/*) [10], the TDD database (*http://db.idrblab.net/ttd/*) [11]and the DrugBank database (*https://go.drugbank.com/*)[12], and

duplicate genes were deleted after summary.

2.3. Screening of targets related to myrrh in the treatment of stroke

The obtained *myrrh* drug targets and stroke-related targets were uploaded to the Venny 2.1.0 platform (*http://bioinformatics.psb.ugent.be/webtools/Venn/*), and the intersection of the two was taken to initially obtain the target of *myrrh* in the treatment of stroke.

2.4. Construction of protein-protein interaction networks and acquisition of core goals

To investigate the targets of myrrh in the treatment of stroke, the relevant data were input into the String database (*https://string-db.org/*)[13]. The species was set to “Homo sapiens”, and the minimum interaction score was specified as “highest confidence” (0.900). Based on these parameters, a protein-protein interaction (PPI) map was constructed. The core target for *myrrh* treatment of stroke was selected through topological analysis through CytoNCA plug-in in Cytoscape software.

2.5. GO analysis and KEGG pathway analysis

The action targets of *myrrh* were uploaded to the DAVID database (*https://david.ncifcrf.gov/*)[14] for gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Species selection "Homo sapiens", screening with $P<0.05$ as the condition, and sorting according to P value, select the top 10 items of biological process (BP), cytological component (CC), molecular biological function (MF) in the GO functional annotation and the top 30 pathways in the KEGG pathway enrichment, and visualize the results using R language.

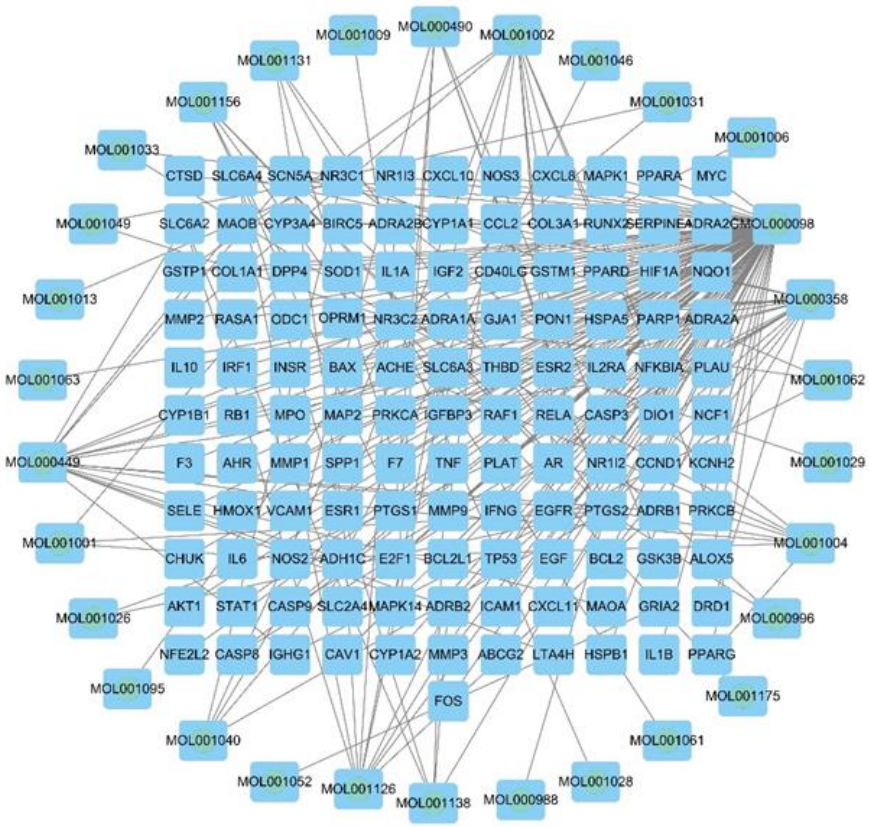

Figure 1: Active Component-target genes network diagram.

3. Results

3.1. Prediction of myrrh gene target

A total of 45 active ingredients were retrieved, corresponding to 1425 targets. The targets of ineffective ingredients were removed, and 401 potential targets of *myrrh* were obtained. Build a network diagram of the corresponding targets of *myrrh* active ingredients (Figure 1). The network consists of 152 nodes and 214 edges, among which the nodes include 173 potential targets of drugs, and 30 drug active ingredients respectively. The outer circle is the drug active ingredients, and the inner square is the potential target of drugs. The degree of connection between the drug active ingredients and the target is sorted by degree value. The top 4 ingredients are regarded as core active ingredients. They are quercetin (MOL000098), β-sitosterol (MOL000358), ellagic acid (MOL001002), and stigmasterol (MOL000449).

3.2. Screening of stroke-related gene targets

Searching with "stroke" as the search term, 2845 targets were obtained in the GeneCards database, 24 in the OMIM database, 35 in the TTD database, and 207 in the DrugBank database. After removing duplicate targets, 2915 targets were obtained for stroke disease (Figure 2A).

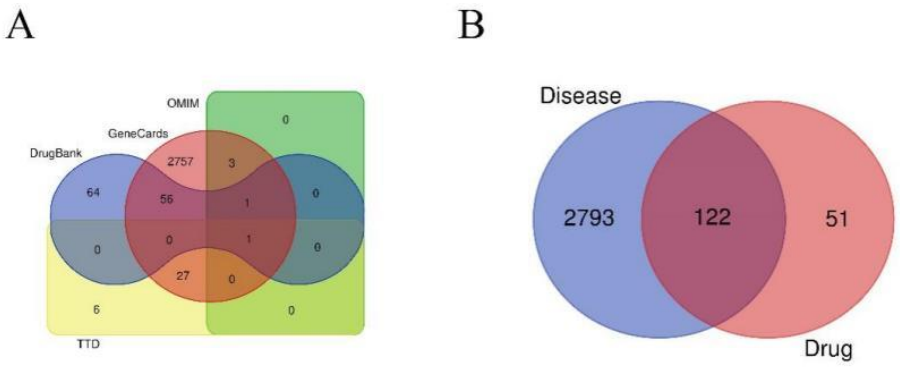

Figure 2: *myrrh*-stroke target map.

3.3. Collection of targets related to myrrh in treating stroke

By mapping and comparing the target of *myrrh* with the target genes of stroke pathogenesis, 122 common genes were found, which speculated that these genes may be related gene targets for *myrrh* in treating stroke (Figure 2B).

3.4. PPI network construction and core target analysis

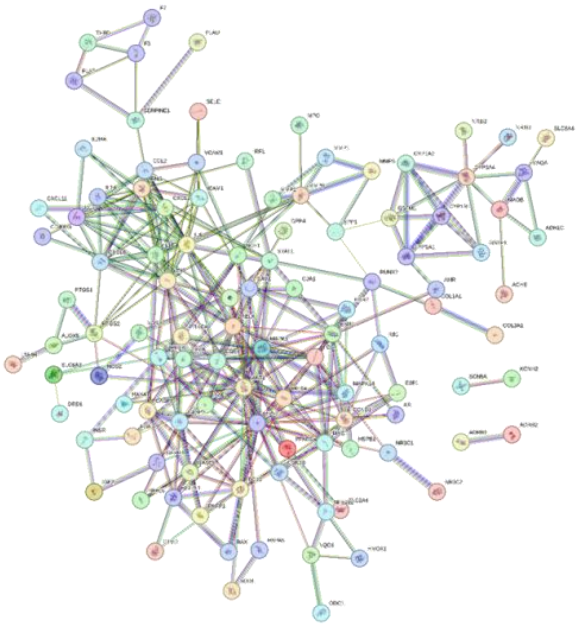

Figure 3: PPI network of *myrrh* target for Stroke

In order to further explore the function and mechanism of *myrrh* potential targets for treating stroke, the 122 potential targets screened above were imported into the String database, and a protein interaction network diagram with a total of 108 nodes and 307 edges was obtained (Figure 3). The CytoNCA plug-in was used to conduct topological analysis of the protein interaction network. Seven hub targets were screened out with BC values>9.12, CC values>0.52, DD values>8.00, EC values>0.17, LAC values>4.25, and NC values>5.53. The core targets were sorted out. It was initially concluded that the top seven targets that play an important role in *myrrh* treatment of stroke were IL6, TNF, TP53, IL1B, MAPK1, AKT1, and ESR1 (Figure 4).

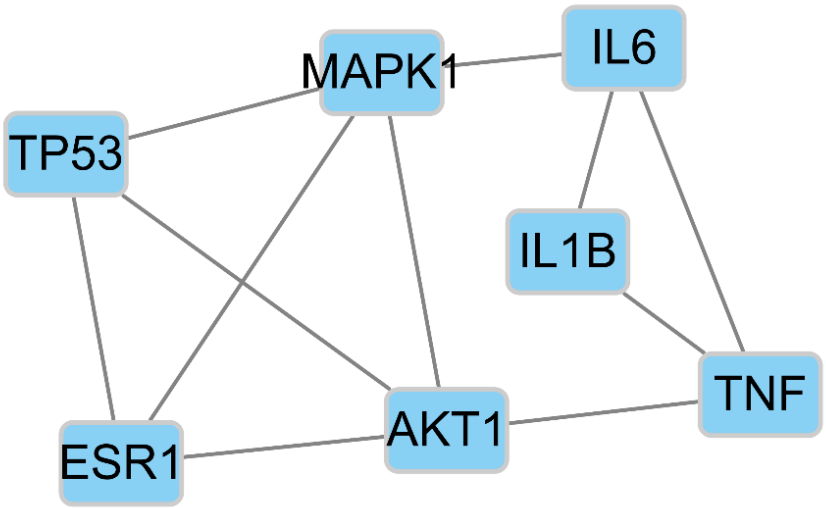

Figure 4: The PPI network construction

3.5. PPI network construction and core target analysis

The 122 effective targets of *myrrh* active ingredient in anti-stroke were imported into the DAVID online database for GO analysis and KEGG pathway enrichment analysis, and the set value was P<0.05. GO analysis received a total of 2326 entries. The top 10 entries in BP, CC, and MF was selected for visual analysis, as shown in Figure 5. Among them, 2117 biological processes were obtained, mainly involving responses to alien stimuli, responses to molecules derived from bacteria, responses to lipopolysaccharides, responses to nutritional levels, responses to oxidative stress, responses to oxygen levels, responses to reduced oxygen levels, responses to hypoxia, responses to reactive oxygen species, responses to cell responses to biological stimuli, etc. There are 41 cellular components, mainly involving membrane rafts, microdomain membranes, plasma membrane lateral, membrane pits, plasma membrane rafts, plasma membrane lateral, ficolin-1-rich particle lumens, serine peptidase complexes, Bcl−2 family protein complexes, etc.; There are 168 molecular functions, mainly involving DNA-binding transcription factor binding, ribonucleic acid polymerase II-specific DNA-binding transcription factor binding, ubiquitin-like protein ligase binding, cytokine receptor binding, transcription co-regulatory factor binding, nuclear receptor activity, ligand-activated transcription factor activity, protease binding, transcription co-activation binding, catecholamine binding, etc. The results of KEGG pathway enrichment analysis showed that a total of 182 pathways were involved. In this study, the top 30 signal pathways were selected to make bubble plots. The results are shown in Figure 6. The IL-17 signaling pathway is enriched in 20 hub genes, the TNF signaling pathway is enriched in 21 hub genes, the HIF-1 signaling pathway is enriched in 16 hub genes, the Toll-like receptor signaling pathway is enriched in 16 hub genes, and the Nf-κb signaling pathway is enriched in 15 hub genes, as shown in Figure 6.

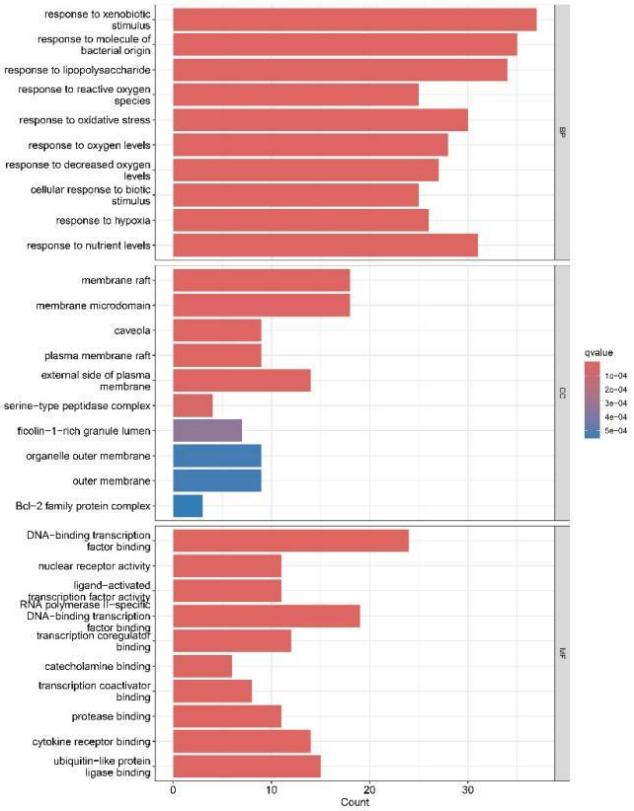

Figure 5: GO analysis of therapeutic targets of *myrrh* in the treatment of stroke

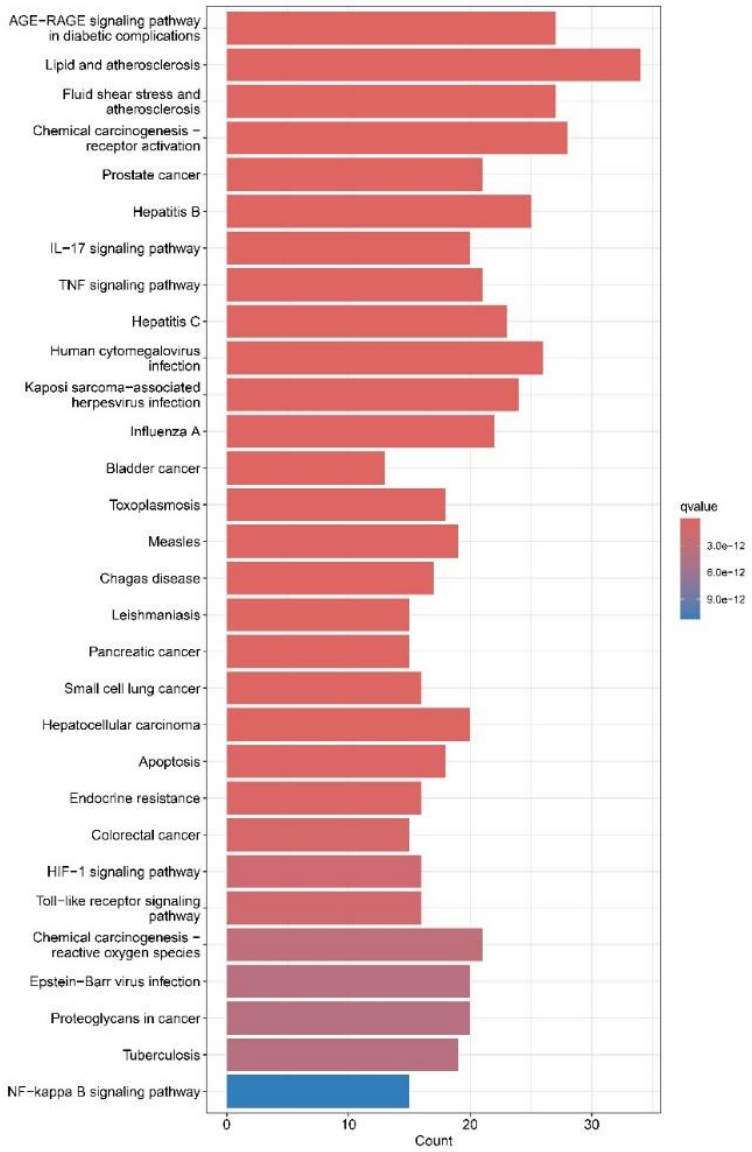

Figure 6: KEGG pathway enrichment analysis of the therapeutic targets of *myrrh* in the treatment of stroke (The top 30 signaling pathways)

4. Discussions

The etiology and pathogenesis of stroke are complex, involving neuroinflammation, energy failure, apoptosis and autophagy, etc., and each link affects each other and overlaps, resulting in the complexity of the disease process. Among them, there are many drugs that can be used in traditional Chinese medicine treatment, but there is a lack of molecular level to explore their potential effects on treating stroke[15].

This study shows that according to the statistics of shared targets, the pharmaceutical active ingredients such as quercetin and β-sitosterol have many corresponding targets, and are considered as key active ingredients for *myrrh* in the treatment of stroke. Quercetin is a polyphenol belonging to the flavonoid family. In animal and clinical studies, quercetin has been found to inhibit platelet activation by inhibiting exocytosis of dense platelet particles[16], block FeCl3-induced arterial

thrombosis formation and reduce cerebral infarction volume[17], and inhibit platelet activation by inhibiting various components of the soluble glycoprotein VI signaling pathway (such as collagen tyrosine phosphorylation)[18]. β-sitosterol has the best binding strength to the TP53 target[19]. β-sitosterol can exert an anti-inflammatory effect through the hypothalamic-pituitary-adrenal axis, and also has moderate reactive oxygen radical scavenging ability, thus playing an antioxidant role[20].

The core targets selected by PPI network topology analysis include IL6, TNF, TP53, etc. IL6 and TNF are pro-inflammatory factors. Studies have shown that the expression of IL6 and TNF is significantly increased in patients with acute ischemic stroke, mediating an acute inflammatory response dominated by leukocyte infiltration, and is related to endothelial cell damage, changes in blood-brain barrier permeability, atherosclerosis, vascular occlusion and other damages[21, 22].

Studies[23] have found that TP53 is a cancer suppressor gene that can participate in multiple metabolic pathways such as lipid metabolism, thereby inhibiting the synthesis of fatty acids. Fibronectin is an extracellular matrix that can affect the formation of thrombus by regulating platelet function. It is also an important part of the blood-brain barrier. The microvascular endothelium within the blood-brain barrier can be destroyed in ischemic stroke, suggesting that the increase of fibronectin may be an additional effect of ischemic necrosis of brain tissue[24].

KEGG pathway enrichment analysis found that *myrrh* mainly plays a role in treating stroke through the IL-17 signaling pathway, TNF signaling pathway, and HIF-1 signaling pathway. The IL-17 family is a subgroup of cytokines consisting of IL-17A-F that plays a vital role in the development of host defense against microorganisms and inflammatory diseases. It has multiple sources, from immune cells to non-immune cells, signaling through their corresponding receptors and activating downstream pathways, including NFKB, MAPKs and C/EBPs, to induce the expression of antimicrobial peptides, cytokines and chemokines[25]. After cerebral ischemia, a large amount of inflammatory cytokines such as TNF-α are secreted to accelerate the development of brain damage, and the TNF signaling pathway is an important signaling pathway that mediates the inflammatory response of the body[26]. The HIF-1 signaling pathway is a pathway that promotes vascular regeneration and maintains the integrity of the blood-brain barrier after ischemia. HIF plays an important role in regulating hypoxia response and plays an important role in the HIF-1 pathway[27]. HIF-1α is a subregulatory unit of HIF-1 and can regulate multiple pathways involved in apoptosis, autophagy and blood vessel generation in response to brain injury[28].

To sum up, this study used network pharmacology to analyze the pharmacological connection of *myrrh* in the treatment of stroke at the molecular level, and found that effective molecular targets such as IL6, TNF, and TP53 are key targets in the treatment process. These targets influence each other in series and cooperate with each other at multiple molecular levels, and jointly play a role in treating stroke through the IL-17 signaling pathway, the TNF signaling pathway, and the HIF-1 signaling pathway.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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