Meta-Analysis of Microarray-Based Expression Profiles to Identify Differentially Expressed Genes in Intracranial Aneurysms

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■ OBJECTIVE: **To gain comprehensive insight into the molecular mechanism of formation and rupture of intracranial aneurysms (IAs).**

■ METHODS: **All publicly accessible microarray-based whole-genome gene expression profiles on IAs were retrieved. The significance analysis of microarrays method was applied to identify differentially expressed genes (DEGs). Functional annotation was performed using gene ontology terms and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses. Expression of DEGs was examined using quantitative polymerase chain reaction.**

■ RESULTS: **Six data sets of 3 microarray platforms were qualified and analyzed. Comparing expression profiles between aneurysmal wall and control vessels, 5232 significant DEGs were identified among 3 platforms, and *MMP12* was shown to have the largest fold change of upregulation. In all 3 platforms, 46 DEGs were shared, and 1297 DEGs were commonly resolved in at least 2 microarray platforms. Among these 1297 concordant DEGs, the 512 upregulated genes were mainly enriched in inflammatory and immune response processes, whereas the 785 downregulated genes were primarily concentrated in smooth muscle cell contraction and development pathways. Comparison between expression profiles of ruptured and unruptured IAs revealed that a few angiogenic factors, including *HIF1A*, *VEGFA*, and *ANGPTL4*, were upregulated in ruptured aneurysms. Subsequently, the upregulation of *MMP12*, *HIF1A*, and *VEGFA* was partially confirmed using quantitative polymerase chain reaction among independent samples.**

■ CONCLUSIONS: **Inflammation, immune response, and loss of contractile vascular smooth muscle cells could potentially contribute to the formation of IAs, whereas the role of angiogenesis and vascular remodeling in IA formation and rupture needs further exploration.**

INTRODUCTION

It is reported that 1%–6% of the world's population are affected by intracranial aneurysms (IAs),[1] and approximately 85% of nontraumatic subarachnoid hemorrhages, a life-threatening emergency, are caused by rupture of IAs.[2] Furthermore, at least 50% of patients who have ruptured IAs die shortly after aneurysmal subarachnoid hemorrhage, whereas the surviving patients are at higher risk of stroke or permanent paralysis.[3]

Key words
- Contraction
- Gene expression
- Inflammation
- Intracranial aneurysm
- Meta-analysis
- Microarray
- Rupture

Abbreviations and Acronyms
DEG: Differentially expressed gene
FDR: False discovery rate
GO: Gene ontology
HIF: Hypoxia inducible factor
IA: Intracranial aneurysm
KEGG: Kyoto Encyclopedia of Genes and Genomes
MMA: Middle meningeal artery
qPCR: Quantitative polymerase chain reaction
SMC: Smooth muscle cell
STA: Superficial temporal artery

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By simultaneously analyzing the expression profiles of thousands of genes, it was found that the expression of genes involved in immune and inflammatory reactions, cell adhesion, vascular remodeling, and extracellular matrix degeneration significantly changed during formation of IAs.[2,4-6] In addition, Toll-like receptor signaling, nuclear factor κB, and hypoxia inducible factor (HIF) 1A pathways are shown to be activated in ruptured IAs compared with unruptured IAs.[7] Because of the differences in research objectives, study designs, microarray platforms, and methodologies of statistical analysis, results of previous genome-wide gene expression profiling studies are inconsistent and inconclusive, and thus the molecular mechanisms of IA pathogenesis and rupture remain elusive.

We conducted this meta-analysis to gain a panoramic view of the molecular mechanisms of IA formation and rupture. We identified the differentially expressed genes (DEGs) between IA and control vessels and compared gene expression profiles between ruptured and unruptured IAs. Moreover, functional annotation for the DEGs was carried out to investigate their roles in IA formation and rupture. We also selected a few DEGs of large fold change or that were regarded to be involved in extracellular matrix degeneration or angiogenesis and examined their expression using quantitative polymerase chain reaction (qPCR) to validate the results of the meta-analysis. Consequently, comprehensive insight into the molecular pathophysiology of IAs was obtained, shedding light on developing new therapies for IAs.

MATERIALS AND METHODS

Identification of Eligible IA Gene Expression Profiles

We adhered to reporting and conduct guidance based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (**PRISMA**). In April 2016, a thorough search for studies and data sets that examined genome-wide gene expression profiles of IAs and control vessels was carried out on the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/),[8] ArrayExpress (http://www.ebi.ac.uk/arrayexpress/), and the PubMed database. The key words "intracranial aneurysm," "microarray," and "gene expression" were applied. Two authors (Z.X. and H.L.) screened all the records with regard to full text and data availability. The inclusion criteria for this meta-analysis were 1) original articles that analyzed microarray-based whole-genome gene expression profiles, 2) case-control studies conducted using human IA walls or control vessels, and 3) explicit diagnoses for patients with IAs. The exclusion criteria were 1) studies conducted on human blood cells, cultured cells, animal tissues, or pooled materials; 2) data sets not freely accessible; 3) studies conducted using microRNA expression microarray, DNA methylation microarray, or other platforms; and 4) reviews, editorials, and repeated studies. The same 2 authors (Z.X. and H.L.) independently screened all remaining data sets and full-text articles regarding their eligibility for inclusion (**Figure 1**). All the available gene expression microarray studies and data sets fulfilling the criteria were retrieved (**Table 1**). The Gene Expression Omnibus accession, microarray platform, tissue type, number of samples, and gene

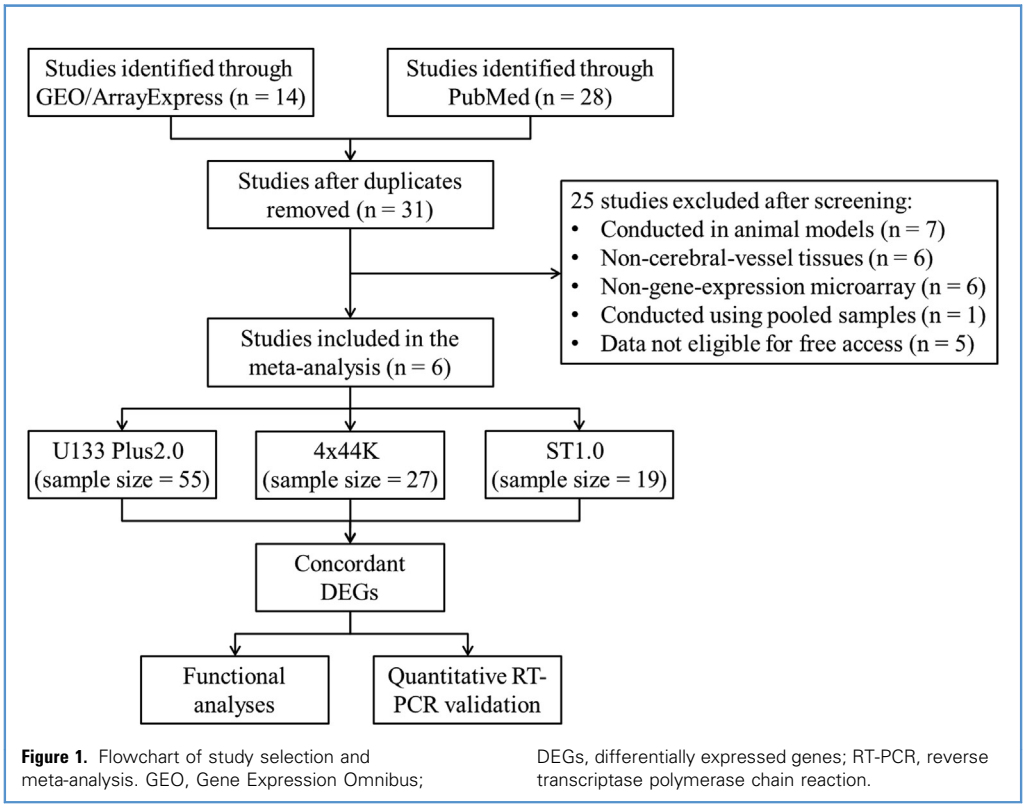

Figure 1. Flowchart of study selection and meta-analysis. GEO, Gene Expression Omnibus; DEGs, differentially expressed genes; RT-PCR, reverse transcriptase polymerase chain reaction.