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Multi-omics research on moyamoya disease: current perspectives and future directions

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Abstract

Moyamoya disease (MMD) is a rare, progressive cerebrovascular disorder characterized by internal carotid artery stenosis and compensatory vascular network formation. While its pathogenesis remains unclear, multi-omics approaches provide crucial molecular insights. Genomic studies identify significant associations with the RNF213 p.R4810K variant and other susceptibility loci like HLA-DQA2 and GUCY1A3. Transcriptomics reveals dysregulation in extracellular matrix organization and mitochondrial oxidative phosphorylation, with specific markers such as AQP4 and non-coding RNAs (e.g., miR-107). Proteomic analyses highlight alterations in proteins including VEGF, apolipoproteins (APOC1, APOD), and ferroptosis-related pathways. Metabolomics identifies diagnostic amino acid markers (L-lysine, L-glutamate) and altered lysophosphatidylcholine (LPC 16:1) levels. Epigenomics implicates DNA methylation changes in genes like SOX6 and KCNMA1. Integrated multi-omics facilitates the development of multifaceted treatments, including revascularization surgery, targeted molecular therapies, and personalized interventions based on individual omics profiles, advancing precision medicine for MMD. This article outlines the omics techniques' application progress in MMD, discussing their pros and cons in disease analysis, prevention, and treatment, aiming to guide future research and inform clinical decisions.

Keywords Moyamoya disease, Genomics, Transcriptomics, Proteomics, Metabolomics, Epigenomics, Multi-omics integration

Introduction

Moyamoya disease (MMD) is a rare cerebrovascular disorder characterized by the gradual occlusion of the terminal portion of the internal carotid arteries and the formation of a distinctive compensatory collateral vascular network at the base of the brain. The disease was first described in Japan where the term "moyamoya," meaning "a puff of smoke" in Japanese, refers to the smoke-like appearance of the tangle of tiny vessels seen on cerebral angiograms [1]. MMD shows a significant geographical disparity in incidence and prevalence worldwide; it has an annual incidence rate of 0.5–1.5 per 100,000 in East Asian countries but as low as 0.1/100,000 in other regions, including North America [2]. Two age groups show peak incidence: children around 5 years of age and adults in their 40 s [3]. Female patients are almost twice as likely to be affected as male patients [4].

Clinically, MMD presents a variety of manifestations ranging from transient ischemic attacks, seizures, ischemic and hemorrhagic strokes, to cognitive deficits, with symptoms progressively worsening over time, leading to permanent neurological damage [5]. Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) scans are instrumental in diagnosing ischemic or hemorrhagic

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strokes in MMD and have been enhanced in recent years with advances in imaging technology for the diagnosis of MMD. Nonetheless, the gold standard for MMD diagnosis remains digital subtraction angiography (DSA) [6].

Research into MMD presents numerous challenges. Pathological specimens for the disease are hard to come by, and a lack of viable animal models constitutes a significant obstacle to understanding its pathogenesis and developing effective treatments. Current treatment strategies are primarily surgical, focusing on revascularization to improve cerebral blood flow. However, the highly heterogeneous progression of the disease, coupled with limited predictive tools for treatment outcomes, makes clinical decision-making highly challenging [7]. Given these challenges, omics-based studies could provide substantial support in unraveling the mysteries of MMD. These technologies can provide comprehensive insights into the genetic, transcriptomic, proteomic, metabolomic, and epigenetic characteristics that contribute to the disease phenotype [8]. Omics might reveal underlying molecular pathways, identify biomarkers for early diagnosis, measure the severity of the disease, and uncover new therapeutic targets [9]. Moreover, they can provide personalized insights into patients and facilitate individualized clinical interventions. Integration of omics data in MMD research holds the potential to fill current gaps in our understanding of the pathophysiology of the disease. By associating genotypic data with phenotypic expression, researchers can gain more refined insights into etiology, biomarkers, and potential molecular targets suitable for drug development. Exploring the interplay between omics data and various biological systems could revolutionize the diagnostic and therapeutic landscape of MMD, offering improved treatments, counseling, and prognoses for patients and families affected by MMD in the future.

Overview of multi-omics

Multi-omics approaches represent an integrative strategy in modern biology aimed at systematically analyzing and interpreting complex biological datasets from distinct molecular layers [10]. This article introduces the main fields of multi-omics - genomics, transcriptomics, proteomics, metabolomics and epigenomics, discusses the significance of integrating multi-omics data, and introduces common techniques and methods in this field.

Genomics is the study of the entire genome of organisms, providing insights into the functional implications of DNA sequences and structures and genetic variations. Second-generation sequencing (NGS) promotes the identification of the genetic basis of diseases like MMD by quickly and high-throughput sequencing the entire genome, thereby fundamentally changing genomics research [11]. Transcriptomics studies the entire set of RNA transcripts produced by the genome, reflecting gene expression levels under various conditions. RNA sequencing (RNA-seq) allows for the quantification of gene expression changes and the discovery of new transcripts, isoforms, and non-coding RNAs [12]. Proteomics studies the protein landscape, exploring the structure, function, and modifications of proteins in specific biological backgrounds. Techniques such as mass spectrometry (MS) provide detailed proteomics maps, which are invaluable for understanding disease mechanisms and potential therapeutic targets [13]. Metabolomics analyzes small molecule metabolites in cells, biological fluids or tissues, directly reflecting physiological status. Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) for metabolite identification and quantification can elucidate metabolic changes associated with diseases [14]. Epigenomics focuses on the study of epigenetic modifications, such as DNA methylation and histone modifications, which regulate gene expression without changing the genomic sequences. Techniques like Whole Genome Bisulfite Sequencing (WGBS) and Chromatin Immunoprecipitation Sequencing (ChIP-seq) assess epigenomic landscapes related to different disease states [15]. The integration of multi-omics data is crucial, as it provides a comprehensive understanding of the biological system by capturing the interactions between various molecular components. Integration strategies often involve advanced computational methods and bioinformatics tools for constructing comprehensive networks and pathways that can enhance our comprehensive and systematic understanding of complex diseases like MMD [16]. Multi-omics approaches include, but are not limited to, high-throughput sequencing of genomics and transcriptomics, mass spectrometry and chromatography of proteomics and metabolomics, bisulfite sequencing in epigenomics, and various bioinformatics analysis techniques for data processing and integration as well as visualization [17]. In fact, multi-omics approaches represent an integrative strategy that combines multi-layer biological information, helping to more fully understand the pathogenesis and progression of diseases, advancing our understanding of complex diseases like MMD, and promoting the development of precision medicine.

Genomics research on MMD

Familial aggregation in MMD is well recognized, indicating the importance of genetic factors [18]. Previous studies have explored genetic factors and revealed several loci associated with MMD; 3p24-p26 [19], 6Q25 [20], 8Q23 [21] and 17Q25 [22]. Recently, the RNF213 gene of the 17q25-ter region was identified as a novel susceptible gene for MMD in East Asians [23]. Functional studies using Rnf213-knockout (Rnf213-KO) and Rnf213-knockin (Rnf213-KI) mouse models have demonstrated

that dysregulation of RNF213 impairs antigen uptake, processing, and presentation in dendritic cells, leading to aberrant T-cell responses and disrupted inflammatory pathways [24]. Additionally, RNF213 deficiency has been shown to compromise endothelial barrier function by downregulating caveolin-1 and platelet endothelial cell adhesion molecule-1 (PECAM-1), thereby enhancing vascular permeability and promoting leukocyte infiltration [25]. These findings suggest that RNF213 mutations contribute to MMD pathogenesis through both vascular and immune mechanisms.

Beyond the well-characterized RNF213 p.R4810K variant, recent genome-wide association studies (GWAS) have identified additional SNPs linked to MMD susceptibility. For instance, studies in Chinese Han populations have revealed associations between MMD and SNPs in HLA-DQA2 and HLA-B, implicating immune-related pathways in disease etiology [26]. Moreover, comparative genomic analyses have highlighted population-specific SNPs, such as the rare RNF213 p.Thr554Ile variant (rs766831703) identified in an Indian patient, which expands the genetic spectrum of MMD beyond East Asian populations [27]. These SNPs not only contribute to individualized risk stratification but also offer insights into the ethnic heterogeneity of MMD.

Moreover, variations in genes like GUCY1A3 and MMP3 have also been identified, even if it was in smaller patient cohorts, suggesting that MMD involves multiple genetic pathways [28]. Genome-wide association studies (GWAS) have discovered multiple genome areas related to the risk of MMD, expanding the range of research, beyond single candidate genes [29]. Our team has previously also identified several new MMD susceptible genes, including homocysteine metabolism (MTHFR) and immune system (enriched susceptible genes in TCN2), and therapeutic intervention with these genes may be an effective method for treating MMD [30]. GWAS studies have also found the polygenic nature of MMD and put forward other genetic loci of potential pathophysiological importance. In addition, whole-genome and exome sequencing (WGS and WES) technologies can also discover rare variations that may lead to the risk of MMD. By sequencing the entire genome or protein coding areas, researchers can capture rare gene variations that may be missed in GWAS due to low frequency. Therefore, WGS and WES provide a more comprehensive assessment of the genetic contribution to MMD, revealing new candidate genes and rare mutations that may play a role in specific patient groups or family cases [31]. Comparative genomic studies, by exploring genetic variations in MMD in different populations and races, further enrich the genetic background of MMD. These studies help to describe the genetic structure of MMD, revealing common and unique genetic risk factors between populations. Furthermore, comparison with other vascular diseases may identify common disease pathways and diagnostic markers [32].

With these multidimensional genomics approaches, the field is closer to understanding the genetic landscape of MMD. These findings are not only critical to decoding the pathogenic pathways of the disease, but also lay the groundwork for the development of diagnostic, prognostic, and therapeutic strategies. Genomic studies may revolutionize the clinical management of MMD by paving the way for personalized medication approaches tailor-made for each patient's genetic characteristics. In summary, genomics has had a profound impact on MMD research, providing valuable insights into its genetic basis. The continued efforts of genomic research are crucial as it helps to unravel the complexity of MMD and enhance our predictive, diagnostic, and therapeutic abilities for this complex disease.

The integration of genomic data with other omics may pave new avenues for therapeutic intervention and a more comprehensive understanding of MMD. While genomics provides the fundamental blueprint of MMD susceptibility, transcriptomics offers a dynamic view into how these genetic alterations dysregulate gene expression programs, ultimately contributing to the disease phenotype.

Transcriptomics research on MMD

The transcriptome is a complete set of RNA transcripts produced by the genome under specific conditions. In MMD research, transcriptomics analysis has become a valuable method for revealing the molecular mechanisms of this cerebrovascular disease pathology. RNA sequencing is a powerful high-throughput technology that allows for the quantitative analysis of gene expression levels across the entire genome. It can also precisely analyze sequence and structural variations such as cSNPs (coding sequence single nucleotide polymorphisms), variable splicing, etc., of transcripts using the sequenced information. Additionally, it has unique advantages in detecting low abundance transcripts and discovering new transcripts, making it an ideal tool for analyzing complex gene expression variations related to MMD [14]. Previous RNA sequencing revealed the gene expression profile of intracranial arteries in MMD. Compared to arteriosclerosis-related intracranial arterial stenosis/occlusion (ASICASO), 556 MMD differentially expressed genes (DEGs) were found, of which 449 were upregulated and 107 were downregulated. The upregulated genes in MMD mainly participate in extracellular matrix (ECM) organization, while downregulated genes are mainly related to mitochondrial function and oxidative phosphorylation. Further gender subgroup analysis discovered more DEGs (n = 1022), and identified 133 and 439 gender-specific DEGs

in MMD males and females, respectively. About 95.6% of gender-specific DEGs are protein-coding genes, and 3% of genes are long non-coding RNAs (lncRNAs). Gender-specific DEGs were observed on all chromosomes, of which 95.49% and 96.59% in males and females are autosomal genes, respectively. Gender-specific DEGs, such as AQP4, SOD3, and NR4A1, may lead to the gender differences in MMD. This transcriptomics study emphasized that ECM and mitochondrial functions are the core molecular mechanisms of MMD, and revealed the gender difference in intracranial arterial gene expression, providing new insights into the pathogenesis of MMD [33]. The range of RNA sequencing applications in MMD research extends from differentially expressed genes in MMD vascular tissues to discovering new transcripts that may contribute to disease onset and progression.

Apart from identifying differentially expressed genes, pathway and network analysis aims to understand complex interactions and biological pathways involved in MMD. A total of 94 miRNAs, 3649 lncRNAs, and 2294 mRNAs were differentially expressed between MMD patients and controls. A collaborative ceRNA lncRNA-miRNA-mRNA regulation network was constructed. It identified the core regulatory miRNAs (miR-107 and miR-423-5p) and key mRNAs (STAT5B, FOSL2, CEBPB, and CXCL16) involved in the ceRNA network. GO and KEGG analyses showed that DEmRNAs are involved in the regulation of the MMD immune system and inflammation. Finally, CMap identified two potential small molecule drugs, CAY-10,415 and Indigo, as candidate drugs for MMD treatment. Network analysis also facilitated the identification of central hub genes that might serve as potential therapeutic targets [34].

Recent transcriptomics studies have also revealed the role of non-coding RNAs (ncRNAs) in MMD. ncRNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have been proven to regulate gene expression at multiple levels. Their involvement in MMD has become a hot topic. For instance, differential expression of specific miRNAs in MMD patients suggests their potential role in regulating endothelial cell function and angiogenesis, two key aspects in the pathogenesis of MMD [35].

Collectively, these transcriptomic findings deepen the mechanistic understanding of MMD pathogenesis by linking differential gene expression to pathways essential for vascular differentiation, maturation, and stability. However, mRNA levels alone cannot fully predict functional protein abundance or activity; thus, direct profiling of the proteome is essential to bridge this gap and uncover the final executors of pathological processes.

Proteomics research on MMD

Proteomics is the large-scale study of proteins, providing important insights into the pathophysiology of diseases by analyzing protein expression patterns and identifying post-translational modifications (PTMs). In MMD, proteomics has become an important research tool, revealing the molecular mechanisms of disease progression and pointing out potential biomarkers and therapeutic targets. A thorough understanding of MMD requires studying proteomic changes that occur as the disease progresses. Compared to a healthy control group, proteomics can clarify different protein expression patterns in the vascular tissue of MMD. Several proteins involved in processes such as angiogenesis, inflammation, and thrombosis are reported to be dysregulated in MMD [36]. For example, higher concentrations of angiogenesis factors such as vascular endothelial growth factor (VEGF) were found in MMD patients, consistent with pathological angiogenesis observed in this disease [37]. PTMs such as phosphorylation, glycosylation, and proteolytic cleavage play crucial roles in regulating protein function. In MMD, PTMs can affect protein stability, location, activity, and interactions, which is significant for cellular signaling and vascular pathobiology [38]. Proteomic studies focusing on PTMs can gain a better understanding of the abnormal signaling pathways causing the vascular remodeling characteristics of MMD. Unfortunately, in MMD research, PTMs still remain unexplored.

The cerebrospinal fluid (CSF) and blood of MMD patients are valuable sources for proteomic analysis. By analyzing the proteomes of CSF and plasma, researchers can identify biomarkers indicating the presence or progression of a disease. Possible biomarkers may include proteins reflecting abnormal vessels and inflammation in MMD that cannot be evaluated by imaging or other non-invasive techniques. Decreased apolipoprotein C-I (APOC1), apolipoprotein D (APOD), and apolipoprotein A-IV (APOA4) were found in previous serological proteomic studies of MMD [39]. In differential protein analysis of ischemic and hemorrhagic MMD, both glutathione S-transferase omega-1 (GSTO1) and peptidyl-prolyl cis-trans isomerase A (PPIA) were upregulated, while ADAMTSL4 was downregulated. Afamin (AFM) and transforming growth factor-beta induced protein ig-h3 (TGFBI) were elevated in ischemic patients but lowered in hemorrhagic ones [40]. Recently, our proteomics study has revealed that MMD is closely associated with ferroptosis-related proteins [41]. Several novel biomarker candidate proteins with differential expression in MMD patients and controls were also identified in the cerebrospinal fluid proteome, however, due to the small sample size, the predictability needs to be further assessed.

The progress of mass spectrometry (MS) technology has paved the way for proteomic research, providing sensitive and accurate means for protein detection and quantitation. MS-based proteomics can simultaneously generate detailed maps of thousands of proteins, particularly suitable for discovering disease-related protein changes in MMD [42]. The high throughput and the ability to recognize low-abundance proteins make MS an indispensable tool for depicting the proteomic landscape of MMD.

Proteomics plays an indispensable role in MMD research, providing a window into the complex mechanisms involved in the disease. By examining the protein expression patterns, PTMs, and proteomic maps of various biofluids, significant progress can be made in understanding the pathological mechanism of MMD. Especially MS-based proteomics provides comprehensive data, which can promote the discovery of biomarkers and the development of targeted treatments.

The continued exploration of the MMD proteome is anticipated to improve patient diagnosis, management, and treatment outcomes. Moving beyond the proteome, the study of downstream metabolic alterations provides a unique window into the functional biochemical consequences of these genomic, transcriptomic, and proteomic disturbances, reflecting the real-time physiological state of the diseased vasculature.

Metabolomics research on MMD

Metabolomics is a comprehensive analysis of small molecule metabolites in biological systems, providing a powerful perspective that can observe the changes in metabolic status under disease conditions [43]. In MMD, metabolomics is increasingly recognized for its ability to elucidate pathophysiological changes and support the discovery of new biomarkers, which help understand disease progression and monitor therapeutic responses. The characteristic of MMD is that the internal carotid artery gradually narrows and eventually occludes, forming an abnormal vascular network. This pathology inevitably leads to changes in the brain's metabolic environment. Changes in energy metabolism, oxidative stress, lipid metabolism, and amino acid spectra have been discovered in MMD, each revealing a complex pathological process. For example, targeted metabolomics studies have found significant changes in MMD serum amino acids, and identified 4 amino acids (L- lysine, L- glutamate, β-alanine, and threonine phosphate) as MMD diagnostic markers [44]. Untargeted metabolomics analysis revealed differences in the expression of lysophosphatidylcholine (LPC) between MMD patients and healthy control (HC) Specifically, the expression of LPC 16:1–2 in ischemic MMD patients was significantly lower than in HC, suggesting that it may be a candidate biomarker for determining different subtypes of MMD. Supplementing LPC can inhibit the abnormal cell activity and cell proliferation of human brain vascular smooth muscle cells (HBVSMCs), as well as the angiogenic function of human brain microvascular endothelial cells (HBMECs), which may provide a new treatment method for MMD [45]. Understanding these metabolic changes can not only reveal the potential pathophysiology of MMD but also guide the development of targeted treatments.

The metabolic spectrum of MMD patients not only reveals the disease state but can also provide clues to the patients' prognosis and response to intervention. Surgical treatments such as direct and indirect revascularization procedures aim to restore blood flow to the affected brain regions and are expected to improve metabolism. By tracking changes in metabolic product levels before and after surgery, metabolomics can deeply understand the efficacy of these interventions and help adjust according to individual patients' metabolic phenotypes, thereby enhancing personalized drug applications for MMD.

Additionally, liquid chromatography-mass spectrometry (LC-MS) is the most advanced method for metabolomics analysis. Its high sensitivity and selectivity make it an ideal platform for detecting and quantifying a wide range of metabolites in MMD patient biological samples. LC-MS-based metabolomics studies can differentiate between MMD patients and healthy individuals based on their metabolic fingerprints, allowing researchers to map biochemical pathways affected by disease [46]. Integrated proteomic and metabolomic analyses have revealed an association between MMD and dysregulation of the ferroptosis pathway [41].

Metabolomic analysis provides a new perspective on interpreting the complex biochemical changes in MMD. The use of LC-MS has driven the understanding of the metabolomic landscape and the discovery of meaningful biomarkers.

Continuous development in metabolomics research is expected to greatly contribute to revealing disease progression of MMD, guiding clinical management, and optimizing treatment responses. Complementing this multi-omics framework, epigenomics research seeks to elucidate the regulatory mechanisms that integrate genetic predisposition with environmental cues to modulate gene expression without altering the DNA sequence itself, offering another layer of understanding for MMD's complex etiology.

Epigenomics research on MMD

Epigenomics is a study of heritable changes in gene function that do not involve changes in the DNA sequence itself, which has become increasingly prominent in the study of complex diseases such as MMD [47]. Through mechanisms such as DNA methylation and histone

modification, epigenetics provides another regulatory mechanism, regulating gene expression in response to environmental cues and cellular environment. DNA methylation, mainly by adding methyl groups at the cytosine base of CpG dinucleotide, is associated with gene silencing when present in the gene promoter. In MMD, aberrant DNA methylation patterns may lead to dysregulation of genes crucial for vascular development and stability. Low expression of epigenetic regulatory genes such as SOX6 and RBM in MMD epigenetic studies may be associated with vascular occlusion in MMD, while overexpression of KCNMA1 and GALNT2 may be associated with angiogenesis. The results confirmed the involvement of DNA methylation in the pathogenesis of MMD and identified new pathogenic genes as biomarkers [48]. In addition, a study based on dizygotic twins with inconsistent MMD conditions confirmed a new circulating microRNA feature in MMD, which as a potential diagnostic biomarker, is minimally confounded by genetic heterogeneity. This new type of circulating microRNA feature will aid future functional microRNA analysis to find new diagnostic and therapeutic targets for MMD [49]. Histone modification, such as acetylation, methylation, phosphorylation and ubiquitination, adds another layer of epigenetic regulation [50]. These post-translational modifications of histones alter chromatin structure, thereby altering gene expression. Although research on histone modifications in MMD is in its early stages, aberrant patterns of acetylation, methylation, phosphorylation, and ubiquitination are postulated to impair vascular integrity and promote pathological angiogenesis by altering the chromatin structure of key genes governing vascular smooth muscle cell proliferation, endothelial function, and inflammatory responses [51]. Furthermore, in other cerebrovascular diseases—such as intracranial aneurysms and arteriovenous malformations—histone modifications including H3K27ac and H3K4me3 have been mechanistically implicated in endothelial dysfunction and vascular remodeling, processes that are highly pertinent to MMD. This connection offers a plausible mechanistic bridge and highlights the critical need for future MMD-specific investigations in this field [52].

Epigenetic mechanisms can influence the genes involved in vascular system development and function. Endothelial cells, vascular smooth muscle cells, and other components of the vascular wall are subject to epigenetic regulation, which affects their proliferation, migration, and differentiation. Dysfunctions of epigenetic regulation in these cell types are associated with various vascular diseases, although they have yet to be studied in MMD and may be key to understanding the pathogenesis of MMD. For example: Arteriovenous malformations (AVMs) are caused by a series of changes in DNA methylation and histone modification in genes related to vascular development. Abnormal epigenetic modifications in the endothelial cell (EC) genome may result in aberrant phenotypes in arteries or veins [53]. Other studies have confirmed that endothelial cells have a plasticity in arterial or venous phenotype that is not only genetically controlled, but also adapts to local environmental cues to express arterial or venous-specific genes [54, 55]. Using time-lapse videomicroscopy system, arterial-venous differentiation was examined in the yolk sac of developing chick embryos. They observed that prior to the formation of blood flow, EC expressing arterial and venous-specific markers were located at the posterior artery and anterior venous pole. By ligating one artery with a metal clip, lifting the artery and blocking arterial blood flow distal to the ligation site, the artery can be physically transformed into a vein. When arterial blood flow is restored by removing the vascular clip, the arterial-specific markers are re-expressed, further confirming that the genetic fate of arterial EC is plastic and influenced by hemodynamics [56]. Endothelial-mesenchymal transition (EndMT) is a key process in vascular development that may be dysregulated in epigenetics. This highlights the importance of balanced epigenetic landscape in maintaining vascular homeostasis and illustrates how epigenetic disorders can lead to disease [57].

Advanced molecular techniques have facilitated the exploration of the epigenome, which allow for high-resolution analysis of epigenetic markers across the entire genome. Bisulfite sequencing (BS-Seq) is the gold standard for detecting DNA methylation, capable of mapping methylation patterns in detail at single base resolution. Similarly, chromatin immunoprecipitation followed by sequencing (ChIP-Seq) allows researchers to discover whole-genome histone modification patterns and chromatin binding sites of epigenetic regulatory factors. The latest advancements in sequencing technology, such as the third generation sequencing platform, offer long-read sequencing capabilities that can span larger genomic regions. This enables us to more comprehensively understand the epigenetic features within complex genomic landscapes. In addition, integrative approaches combining transcriptomics, genetics and epigenomics have extended our ability to associate epigenetic changes with changes in gene expression and genetic variation.

As our understanding of the epigenetic landscape deepens, new opportunities emerge for identifying biomarkers and developing therapeutic interventions capable of preventing or reversing the pathogenic processes underlying MMD. The mapping of the epigenome represents a promising frontier for advancing personalized treatment strategies in vascular diseases. In conclusion, the integration of data across genomic, transcriptomic, proteomic, metabolomic, and epigenomic layers is constructing a more holistic and multi-dimensional model of

MMD pathogenesis, which is crucial for developing targeted diagnostics and therapies.

Integrative Multi-omics studies: comprehensive Understanding of MMD

MMD is a chronic progressive cerebrovascular disease, the cause of which is complex and may be related to genetics, immunity, inflammation, and environmental factors. This complex disease requires integrative analysis of multi-omics data for overall understanding. The integration of multi-omics data from genomics, transcriptomics, proteomics, metabolomics, and epigenomics promises to provide a comprehensive map of molecular changes in MMD. This integration poses huge challenges for MMD research, but also reveals unprecedented opportunities in the development of big data and systems biology. Herein, we discuss the complexity of integrating multi-omics datasets and its potential in MMD research.

The fusion of multi-omics data faces several obstacles, including the diversity of data types, large quantity, and their complexity. Coordination of different data sets requires careful preprocessing, including normalization, annotation, and parsing of batch effects. In addition, high-dimensional omics data with thousands of features need robust statistical models to avoid overfitting and extract meaningful biological insights. The strategy to meet these challenges is to adopt an incremental approach, gradually integrating layers of multi-omics information, creating composite models. Moreover, dimensionality reduction techniques, such as principal component analysis (PCA) and t-distributed Stochastic Neighbor Embedding (t-SNE), can help visualize high-dimensional omics data. The application of integrated bioinformatics platforms enables datasets to be merged into a single analytical framework.

Multi-omics data fusion uses a variety of computational technologies to integrate different omic information layers into a unified space for data analysis [58]. Machine learning (ML), in particular supervised learning and unsupervised learning methods, have become effective tools in this field. Supervised learning can predict disease status based on omics features, creating classification models to enhance diagnostic accuracy. Unsupervised learning, such as clustering algorithms, groups omics features of similar patterns, revealing patient subtypes or disease pathways [59]. The advent of deep learning architectures, with their ability to simulate complex interactions in massive data, has profoundly impacted the integration of multi-omics. Neural networks can be designed to learn advanced representations from original omics data, providing an end-to-end mechanism for revealing MMD's underlying structure and potential biomarkers [60]. The intricate regulatory network among multi-omics molecules is detailed in Fig. 1.

Systems biology and network medicine create a framework for understanding the interconnections of biological systems. Systems biological approaches to MMD investigate the disease at the system level rather than

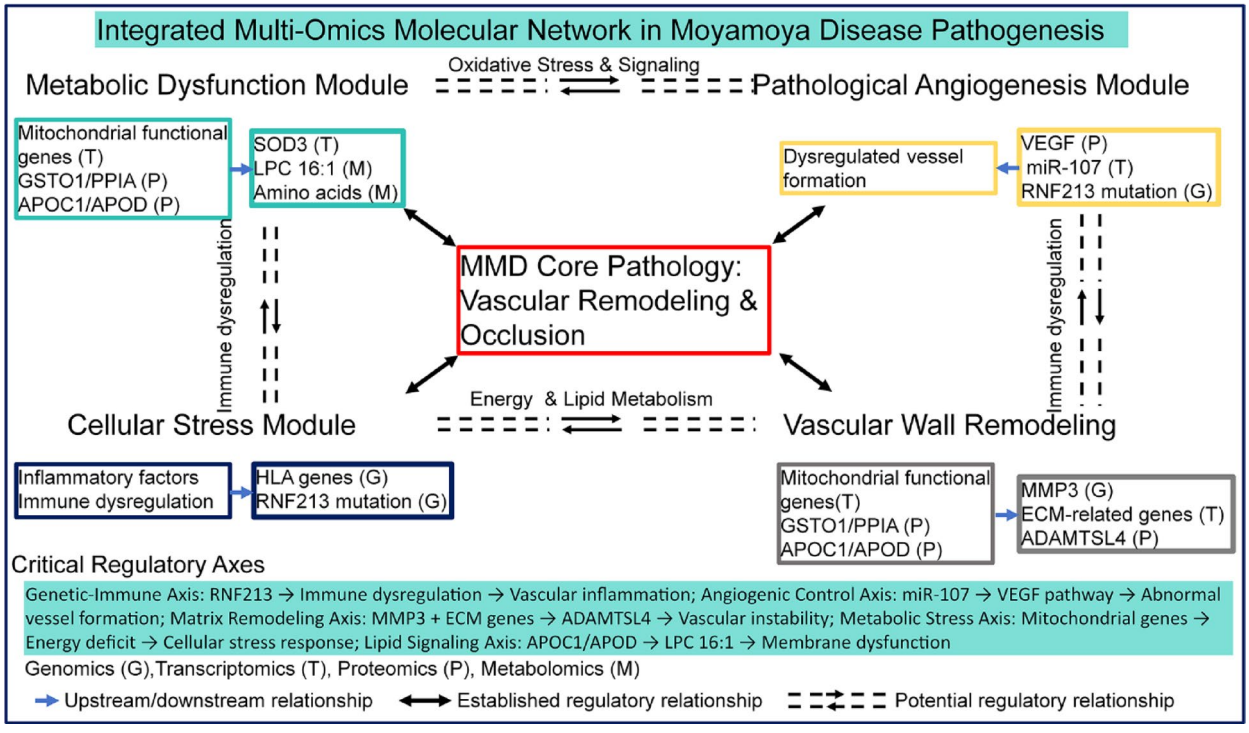

Fig. 1 Integrated Multi-Omics Molecular Network in Moyamoya Disease Pathogenesis

isolated components. Constructions such as gene regulatory networks, protein interaction networks and metabolic pathways provide insights into the cooperative nature of molecular components. Network medicine expands systems biology by interweaving disease-centered networks with patient-centered data, nurturing a more personalized view of disease etiology and treatment. By using network-based methods, multi-omics data can be converted into actionable knowledge, providing a roadmap for determining therapeutic targets and tailor-made interventions for MMD patients.

The integration of multi-omics data in MMD research has deepened our understanding of the molecular complexity of the disease. It requires complex computational methods and system-level analysis. As we transition into a new era of precision medicine, the multi-faceted discoveries brought by multi-omics integration bring new hope for significantly improving the treatment and prognosis of MMD.

Clinical significance and future directions of Multi-Omics in MMD research

The application in multi-omics research has completely changed the clinical management of cancer patients [61]. The integration of multi-omics approaches—encompassing genomics, transcriptomics, proteomics, metabolomics, and epigenomics—has provided a comprehensive understanding of the pathophysiology of MMD. These insights have not only shed light on the molecular dysregulations underlying MMD, as summarized in Table 1, but have also facilitated the translation of basic research findings into potential clinical applications, paving the way for personalized treatment strategies and informing future research directions.

Inclusion of multi-omics research into clinical practice is crucial to the transformation of precision medicine [62]. Identification of specific genetic variations, gene expression profiles, protein biomarkers, metabolic product characteristics, and epigenetic patterns in MMD patients provides a comprehensive approach to diagnosis and prognosis. For example, identification of gene mutations that confer higher risk for MMD can provide targeted genetic counseling and preventative strategies for high-risk individuals. In addition, multi-omics data reveal molecular mechanisms that can be used for therapeutic intervention. Treatment may target specific dysregulated pathways related to abnormal angiogenesis in MMD, such as those identified through proteomic and transcriptomic analyses. These integrative techniques can aid in the development of novel therapeutic drugs that target the precise molecular pathways altered in MMD patients.

Table 1 Summary of key dysregulated molecules in Moyamoya disease (MMD) across Multi-Omics studies

Omics layer	Molecule name/gene	Specific change/Variant	Function/Biological Process	References
Genomics	RNF213	p.R4810K (rs112735431); p.Thr554Ile (rs766831703)	Vascular integrity; Immune regulation; Angiogenesis	[23, 27]
	HLA genes	SNPs in HLA-DQA2, HLA-B	Immune response regulation; Antigen presentation	[26]
	GUCY1A3	Variations (specific SNPs not detailed)	Nitric oxide signaling; Vascular tone regulation	[28]
	MMP3	rs3025058	ECM remodeling	[28]
Transcriptomics	mRNAs (Up)	ECM-related genes	ECM organization	[33]
	mRNAs (Down)	Mitochondrial function genes	Oxidative phosphorylation; Energy metabolism	[33]
	AQP4	Gender-specific DE	Water transport; May contribute to gender differences	[33]
	SOD3	Gender-specific DE	Antioxidant defense	[33]
	NR4A1	Gender-specific DE	Regulation of cell proliferation & differentiation	[33]
	lncRNAs	3649 DE lncRNAs	Regulating gene expression; Constructing ceRNA networks	[34]
	miRNAs	miR-107; miR-423-5p	Post-transcriptional regulation; Endothelial cell function; Angiogenesis	[34]
Proteomics	VEGF	Increased expression	Angiogenesis	[37]
	APOC1, APOD, APOA4	Decreased levels in serum	Lipid metabolism; Potential biomarkers	[39]
	GSTO1, PPIA	Upregulated	Cellular stress response; Protein folding	[40]
	ADAMTSL4	Downregulated	ECM organization	[40]
	AFM, TGFBI	Elevated in ischemic, lowered in hemorrhagic	Vitamin E transport; Cell adhesion	[40]
Metabolomics	LPC 16:1–2	Significantly lower in ischemic MMD	Lipid metabolism; Cell signaling; Potential biomarker & therapeutic target	[44]
	L-lysine, L-glutamate, β-alanine, Threonine phosphate	Altered levels	Potential diagnostic markers	[43]

Personalized medicine in MMD aims to tailor treatment strategies according to the patient's individual circumstances based on the unique multi-omics landscape [63]. By understanding each patient's specific molecular features, clinicians can provide treatment and monitoring in a more personalized manner [64]. For instance, drug therapy can take into account individual genetic backgrounds, and the setup of surgical strategies may also consider protein expression patterns related to vascular fragility or rebleeding tendency. Predictive modeling based on multi-omics data can also provide information for clinical decision making. Comprehensive multi-omics analysis can help divide patients into subgroups with different prognoses or treatment responses, enabling clinicians to determine the most effective treatment modality for each subgroup -whether it be surgical revascularization, medical management of related complications, or meticulous follow-up plans.

The field of multi-omics is constantly expanding, and future research directions may focus on enhancing data integration methods to better understand complex diseases like MMD. It is hoped to develop a comprehensive multi-omics database specifically for MMD to collect and store data from global research work, promoting meta-analysis and big data mining work. In addition, future research may utilize cutting-edge techniques such as single-cell omics [65] to reveal cellular heterogeneity within MMD lesions. These single-cell methods can provide resolution at the individual cell level, exploring multiple cellular contributions to the pathogenesis of MMD. Another promising approach is the implementation of longitudinal multi-omics research. These studies can track MMD progression over time, providing dynamic insights into molecular changes accompanying disease evolution or therapeutic intervention. These insights could reveal temporal biomarkers indicating disease milestones or treatment responses.

While these future research directions offer exciting potential for unprecedented mechanistic discovery, translating such multi-omics discoveries into clinical practice for MMD faces several substantive challenges that warrant careful consideration. First, the sensitivity, specificity, and overall clinical validity of proposed biomarkers—such as LPC 16:1 for disease subtyping—require rigorous validation in large, independent, and ethnically diverse cohorts. Second, reproducibility of multi-omics signatures across different centers and platforms remains a critical hurdle, necessitating standardized operating procedures for sample processing, data generation, and bioinformatic analysis. Furthermore, the practical integration of omics-based tools into clinical workflows demands the development of robust, cost-effective, and scalable assays, alongside formally validating their additive value beyond current diagnostic and monitoring strategies. Finally, regulatory approval, clinician acceptance, and health-economic evaluation represent essential steps before such tools can be adopted into routine practice. Addressing these challenges through collaborative, multidisciplinary efforts will be crucial for fulfilling the promising yet unproven clinical potential of omics technologies in MMD.

Limitations

It is important to acknowledge several limitations inherent in the current multi-omics studies of MMD. First, significant sample heterogeneity—including variations in disease stages (e.g., Suzuki angiographic grades) (Fig. 2), clinical subtypes (ischemic vs. hemorrhagic presentation), and demographic factors—may confound integrative analyses and affect the reproducibility of findings. Second, batch effects and platform-specific variations during multi-omic data generation and integration pose challenges in harmonizing datasets across studies, potentially introducing technical artifacts. Most importantly, the associative nature of omics data limits causal inference regarding molecular mechanisms; although bioinformatic predictions and correlation networks suggest functional interactions, these hypotheses require validation through mechanistic experiments in suitable disease models. Additionally, issues such as statistical power due to limited cohort sizes, potential overfitting in high-dimensional data analyses, and the absence of orthogonal validation in independent populations further underscore the need for cautious interpretation. Future studies should aim for larger, prospectively designed cohorts with standardized sampling and profiling protocols, alongside functional studies to establish causality.

Conclusion

Multi-omics approaches have systematically delineated the molecular architecture of MMD, identifying stage-specific biomarkers and mechanisms crucial for its pathogenesis. Genomics has established the foundational genetic susceptibility, primarily through the RNF213 p.R4810K variant, which predisposes to initial vascular vulnerability. As the disease progresses, transcriptomics reveals dysregulation in extracellular matrix organization (e.g., AQP4) and mitochondrial oxidative phosphorylation, potentially driving vascular wall instability. Proteomics further captures the dynamic disease phase, highlighting VEGF-mediated pathological angiogenesis and apolipoprotein alterations (APOC1, APOD) linked to ongoing vascular remodeling, alongside emerging roles of ferroptosis. Metabolomics identifies circulating markers like L-lysine and LPC 16:1, which may reflect real-time ischemic stress and aid in subtyping advanced disease. Finally, epigenomics elucidates how DNA methylation of genes like SOX6 and KCNMA1

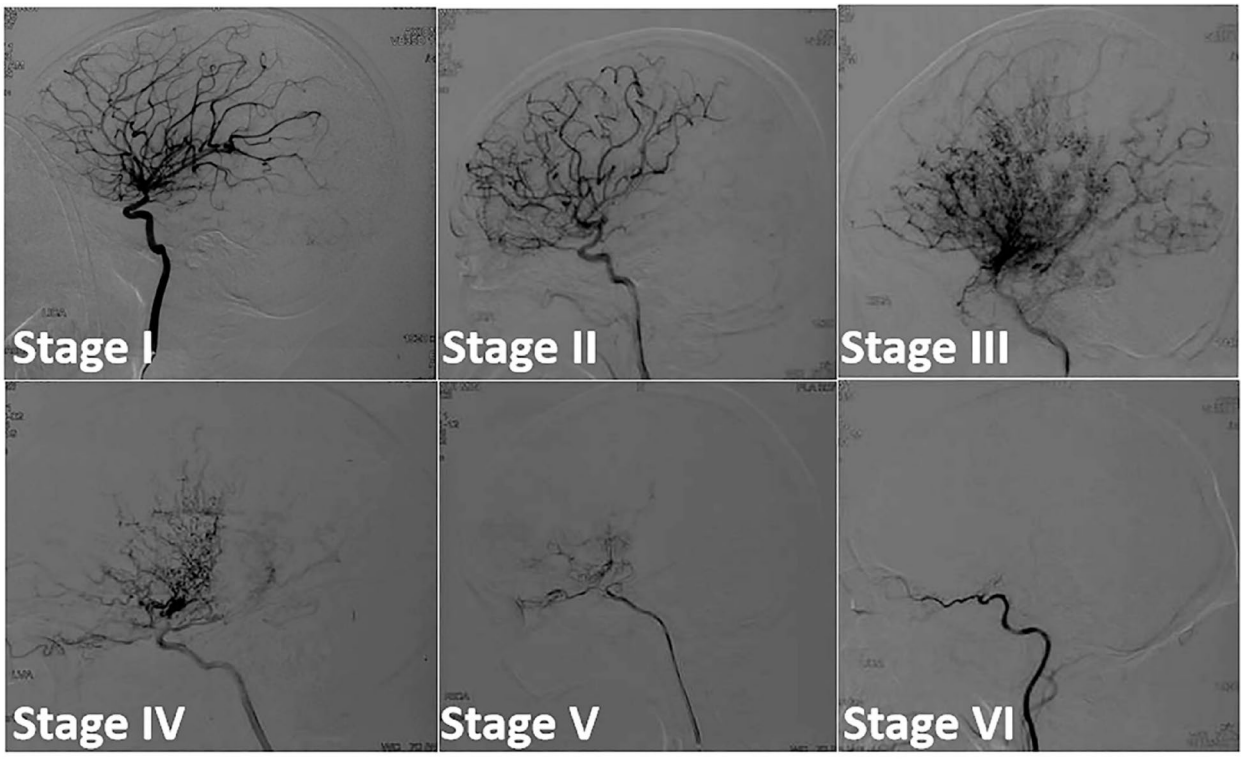

Fig. 2 Representative Cerebral Angiograms and Suzuki Staging in Moyamoya Disease

may persistently modulate vascular tone and integrity throughout disease evolution. The integration of these omics layers is thus pivotal, not only for unraveling the spatiotemporal dynamics of MMD but also for translating these findings into stratified diagnostics, targeted molecular therapies, and personalized revascularization strategies, ultimately advancing precision medicine for this complex vasculopathy.

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Author contributions
Qingbao Guo designed the study. Qingbao Guo, and Na Li wrote the manuscript. Qingbao Guo contributed to the manuscript discussion.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All the authors contributed to the manuscript and approved the submitted version.

Competing interests
The authors declare no competing interests.

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