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ORIGINAL RESEARCH

Immunoglobulin G N-Glycosylation and Inflammatory Factors: Analysis of Biomarkers for the Diagnosis of Moyamoya Disease

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Purpose: N-glycosylation-modified immunoglobulin G (IgG) is crucial for managing the inflammatory response balance and significantly influences the progression of many inflammatory disorders. IgG N-glycosylation has been demonstrated to correlate with many risk factors for moyamoya disease (MMD), such as hypertension, diabetes, and dyslipidemia. This research aimed to evaluate the diagnostic efficacy of IgG N-glycosylation for MMD.

Methods: Ultra-high-performance liquid chromatography (UPLC) was employed to examine the properties of IgG N-glycans in blood samples from 116 patients with MMD and 126 controls, resulting in the quantitative determination of 24 initial glycan peaks (GP). Through the Lasso algorithm and multivariate logistic regression analysis, we constructed a diagnostic model based on initial glycans and related inflammatory factors to distinguish MMD patients from healthy individuals.

Results: After adjusting for potential confounding variables, including age, fasting blood glucose (FBG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), neutrophil count (NEUT), and lymphocyte count (LYM), our study demonstrated significant differences in the characteristics of 6 initial glycans and 16 derived glycans between the MMD cohort and the healthy control group. Based on the above findings, we developed an MMD diagnostic model that combines initial glycans with related inflammatory factors. The curve of receiver operating characteristic (ROC) was utilized to evaluate the model's ability to distinguish MMD patients from healthy subjects. The findings indicated a robust area under the curve (AUC) of 0.963 (95% CI: 0.940, 0.987).

Conclusion: This study found that the occurrence and progression of MMD may be associated with decreased levels of sialylation, galactosylation, and fucosylation and increased bisecting GlcNAc. This may be involved in the occurrence of MMD by regulating the balance of inflammation. Therefore, the IgG N-glycosylation is expected to become a potential biomarker for the screening of MMD.

Keywords: moyamoya disease, immunoglobulin G, N-glycans, inflammation, biomarkers

Background

Moyamoya disease (MMD) is an uncommon, chronic cerebrovascular condition marked by the gradual narrowing or blockage of both the distal and proximal branches of the internal carotid artery, accompanied by the formation of aberrant collateral vascular networks.[1] Its clinical symptoms are diverse, including transient ischemic attack (TIA), cerebral infarction, intracranial bleeding, headache, and epilepsy.[2] Although the overall mortality of MMD is relatively low, severe cerebral infarction or intracranial hemorrhage can be life-threatening.[3] The disease was first proposed and defined by Japanese scholars Suzuki and Takaku in 1969.[4] In East Asia, such as China and Japan, the incidence is high, reaching 1.14 per hundred thousand person-years and 0.94 per hundred thousand person-years, respectively, and is increasing year

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by year. In contrast, the incidence rates in Europe and the United States are low, 0.07 per hundred thousand person-years and 0.293 per hundred thousand person-years, respectively.[5–7] However, the incidence of MMD is on the rise worldwide.

The pathophysiology of MMD is not fully elucidated, and existing treatment modalities exhibit poor effectiveness. At present, the diagnosis of MMD is still mainly based on invasive cerebral angiography, but patients often seek medical attention after experiencing typical symptoms such as cerebral ischemia or bleeding, resulting in delayed diagnosis. In addition, the disease still lacks non-invasive early biomarkers, which severely restricts screening interventions during the asymptomatic period. Numerous studies have consistently demonstrated that irregularities in immunological and inflammatory responses may be a significant component in the onset of MMD.[8–10] Inflammation is a critical regulatory factor in the physiological and biochemical processes of MMD, directly linked to the disease's origin and progression.[10,11] Patients with MMD demonstrate elevated concentrations of many inflammatory markers, including C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), interleukin-1β (IL-1β), and matrix metalloproteinase-9 (MMP9), relative to healthy persons,[12–14] but these indicators generally lack disease specificity. In addition, a large cohort study also pointed out that the disorder of autoimmune function may be associated with the incidence of MMD.[15] An increasing body of clinical evidence indicates that immune inflammatory responses may be an important trigger for MMD, and their importance is second only to genetic factors.[16] However, it is important to note that the physiological link between IgG glycosylation, which is a key part of controlling inflammation, and the development of MMD has not been well studied. Consequently, it is of great importance to investigate the molecular regulation mechanism of IgG glycosylation and the inflammatory response related to MMD and to develop specific biomarkers with clinical translational value based on this information. This will improve early diagnosis and optimise treatment strategies.

More than half of proteins contain glycosylation, a common and complex post-translational modification that widely influences their structure and function.[16] This process, governed by genetic and environmental influences, entails particular glycosyltransferases in the endoplasmic reticulum and Golgi that facilitate the attachment of oligosaccharides to protein glycosylation sites through glycopeptide bonds, thus finalizing the glycosylation modification.[17] The complex diversity of glycosylation and glycan structures provides enormous potential for the diagnosis of diseases and the prediction of biomarkers.[18]

IgG is the predominant glycoprotein in serum. It participates in both innate and adaptive immunity and modulates inflammatory responses.[19] IgG is composed of antigen-binding fragments (Fab) and crystal fragments (Fc). The former is responsible for specific antigen binding, while the latter mediates the antigen-antibody reaction by binding to effector molecules or cells, activating the complement system, and presenting antigens.[18]

The Fc region of IgG possesses two N-linked glycosylation sites at asparagine position 297, which are crucial for modulating the binding affinity to the Fcγ receptor and thus affecting the effector activities of IgG.[20,21] Although the glycosylation state of IgG is highly sensitive to physiological and pathological changes, it maintains a relatively stable level in healthy individuals.[22] Due to the conserved N-glycosylation sites in the IgG Fc domain, it is one of the ideal targets for glycomics research.[23] Changes in N-glycosylation in the Fc domain of IgG may affect its structure and function, thereby regulating the body's inflammatory responses and causing disease.[24,25] N-glycosylation of IgG in the inflammatory response has been associated with MMD risk factors, including dyslipidemia, diabetes, and hypertension.[26–28] Consequently, alterations in the IgG N-glycan profile may contribute to the pathophysiology of MMD by influencing the inflammatory response. Simultaneously, IgG N-glycosylation has provided a novel perspective for investigating associated diseases as a potential biomarker for different diseases, including chronic inflammatory diseases, cancer, autoimmune disorders, and neurological diseases.[24,29–32]

This study examines the relationship between the IgG N-glycan profile and MMD, with the objective of investigating its diagnostic potential as a biomarker for the disease by analysing the impact of IgG N-glycosylation modifications on the pathogenesis of MMD. Given that the glycosylation properties of IgG can be assessed non-invasively through blood tests, the development of a diagnostic model based on the N-glycan profile will significantly aid in the early diagnosis of MMD patients and the creation of personalised treatment strategies.

Methods

Participants

This study used a case-control study method to recruit 116 mmD patients and 126 healthy people from Liaocheng People's Hospital from January 2022 to March 2024. The criteria for participant inclusion in the study were as follows: 1) Belonging to the Chinese Han population, there is no blood relationship between the subjects; 2) The control group had no history of drug treatment within two weeks; 3) Blood samples, baseline information can be obtained. Individuals were excluded according to the following criteria: 1) Incomplete baseline information; 2) Blood samples could not be provided or were missing; 3) Pregnant or lactating women; 4) Mental illness and infectious diseases; 5) Moyamoya syndrome [combined with the following diseases]: (1) autoimmune disease (systemic lupus erythematosus, antiphospholipid syndrome, nodular polyarteritis, Sjögren's syndrome, etc) (2) meningitis (3) craniocerebral tumor (4) Down syndrome (5) neurofibromatosis type I (6) secondary cerebrovascular disease after cerebral radiation injury; 6) combined with other major diseases (leukemia, other systemic malignant tumors, and other life-threatening diseases). This study protocol was reviewed and approved by the Medical Ethics Committee of Liaocheng People's Hospital (ethical approval number: 2024372) and conducted in accordance with the Declaration of Helsinki.

Diagnosis of MMD

All patients included in this study were examined by DSA and confirmed to meet the MMD diagnostic guidelines issued by the Ministry of Health of Japan in 2021.[33] Refer to the Supplemental Materials for relevant standards. The control group selected healthy individuals with age and gender matching and no MMD history or family history.

Measurement of Demographic and Clinical Characteristics

Two 5 mL blood samples were obtained via venipuncture in the morning following an overnight fast. The first sample used a non-EDTA (ethylenediaminetetraacetic acid) vacuum negative pressure tube to collect 2 mL of blood, and the serum was separated after treatment for the analysis of blood biochemical indicators and inflammatory markers. The second sample was collected as whole blood by a vacuum negative pressure tube containing EDTA, and 2 mL of serum was isolated by centrifugation at 3000 r/min for 10 min, which was specially used for quantitative detection of n-glycosyl. All samples were processed within 8 hours of collection and maintained at −80°C to ensure the integrity of long-term preservation and eventual analysis. FBG was assessed using the glucose oxidase-peroxidase coupling technique. The demographic attributes of the individuals were gathered, encompassing age, gender, and pertinent biochemical indicators.

Identification of Serum Inflammatory Biomarkers

We used an enzyme-linked immunosorbent assay (ELISA) kit to measure the levels of inflammatory mediators in the serum, such as CRP, TNF-α, VEGF, IL-6, IL-1β, and MMP9. The detection process strictly followed the established protocols of the reported kits.[13,34,35] The specific operation is as follows: First, the standard substance and the serum sample to be tested were added to the enzyme-labeled microplate together with the enzyme-labeled anti-antigen antibody and incubated for a period of time to make the antigen fully bind to the coated antibody and the labeled enzyme. Then, the unbound free components were removed by washing the plate, and the antigen-antibody immune complexes were retained. Subsequently, the color reaction was executed, and following the reaction, the absorbance (OD value) of each well was measured using a microplate reader at a wavelength of 450 nm. Finally, according to the standard curve drawn, the specific concentrations of various inflammatory mediators in each sample were calculated.

Analysis of Serum IgG N-Glycosylation

The analysis of serum IgG N-glycosylation involves multiple steps. Firstly, IgG was extracted from serum using a protein G monolithic plate (Thermo Fisher Scientific, USA), then suitably diluted and covered with a binding buffer (1 × PBS). Subsequently, after elution with 0.1M formic acid treatment and with 1M ammonium bicarbonate neutralization, the N-glycans on IgG were effectively released and labeled with reagents such as 2-aminobenzamide for visual analysis by ultra-high performance liquid chromatography (UPLC). In the process of using ultra

performance liquid chromatography (UPLC), the specific chromatographic conditions are as follows: The chromatographic column is Phenomenex Gemini NX-C18 (3 μm, 50×2 mm), the mobile phase A is 100 mm ammonium formate (pH 4.4), the mobile phase B is acetonitrile, the linear gradient elution (75% → 62% phase B, 25 min), the flow rate is 0.4 mL/min, and the column temperature is 60 °C. Based on this method, we successfully detected 24 IgG glycan peaks, each peak representing a specific glycan structure.[36] Supplementary Table S1 shows the composition and structure of IgG N-initial glycans. For quantitative analysis, the normalised technique was employed to calculate the glycan content of each glycosyl peak as a proportion of the whole integral area, yielding 24 glycan peak (GP) values for each sample.[37] In addition, based on these directly measured glycan data, we calculated 54 derived traits, including galactosylation, sialylation, bisecting GlcNAc, and fucosylation, of which 40 key traits were selected for subsequent analysis.

Statistical methods

We utilize the Kolmogorov–Smirnov test to evaluate the distribution characteristics of the data. The mean and standard deviation (SD) represent data that follows a normal distribution, while the median and interquartile range (IQR) represent data that does not. For the variables with normal distribution, we use independent samples *t*-tests to explore the differences between groups; for non-normal distribution variables, the Mann–Whitney *U*-test is selected for analysis. The categorical variables are presented in the form of a frequency distribution and analyzed by the chi-square test. Moreover, multiple comparison correction was performed with the Benjamini–Hochberg method of false discovery rate (FDR) correction. We performed multivariate logistic regression analysis to clarify the relationship between MMD and 24 initial glycans, as well as 54 derived characteristics, while controlling for potential confounders such as age, FBG, TC, HDL, LDL, NEUT, and LYM. Furthermore, we employed canonical correlation analysis (CCA) to investigate the relationship between 24 glycans and inflammatory cytokines.

We constructed an MMD diagnosis model based on initial glycans and related inflammatory factors. Initially, the Least Absolute Shrinkage and Selection Operator (Lasso) algorithm was employed, and the optimal regularization parameter λ (lambda) was selected through 10-fold cross-validation to minimize the prediction error. This approach was used to screen for glycans and inflammatory factors closely associated with MMD, thereby reducing model complexity and avoiding overfitting. Subsequently, the salient features identified by Lasso were incorporated into a logistic regression analysis with multiple variables in order to improve the diagnostic model of MMD. We evaluated the model's predictive capability based on the ROC curve, which includes the AUC value, sensitivity (Se), and specificity (Sp).

All statistical analyses in this study were conducted using IBM SPSS Statistics 25.0 and R 4.4.1 software. A bilateral P value less than 0.05 was deemed statistically significant in all tests.

Results

Study Participant Characteristics

This study included 116 mmD patients and 126 healthy controls. All participants possessed comprehensive demographic information and IgG N-glycan-related data. Table 1 presents the demographic data, biochemical markers, and inflammatory parameters of 116 patients with MMD (71 males and 45 females) and 126 healthy controls (66 males and 60 females). The distribution of gender and age between the two groups showed no significant difference ($P > 0.05$), indicating demographic features were matched. Marked alterations in biochemical markers and inflammatory parameters were seen between the MMD group and the control group. FBG, TC, HDL, LDL, and LYM were all significantly lower in the MMD group compared to the control group. In contrast, compared to the control group, the MMD group exhibited significantly higher concentrations of total triglycerides (TG), NEUT, monocyte count (MONO), CRP, TNF-α, VEGF, IL-6, IL-1β, and MMP9.

Table 1 Characteristics of the Study Participant

Parameters	Controls (n = 126)	MMD (n = 116)	*P*-value	Adjusted *P*
Gender (male/female)	66/60	71/45	0.167	0.206
Age (years)	48.70±10.73	47.37±9.83	0.222	0.254
FBG (mmol/L)	5.31 (4.94, 5.62)	5.19 (4.65, 6.16)	0.643	0.643
TC (mmol/L)	4.70±0.67	4.21±0.96	<0.001*	<0.001*
TG (mmol/L)	1.16 (0.88, 1.51)	1.17 (0.88, 1.64)	0.620	0.643
HDL (mmol/L)	1.41 (1.21, 1.72)	1.10 (0.92, 1.30)	<0.001*	<0.001*
LDL (mmol/L)	2.67±0.59	2.28±0.76	<0.001*	<0.001*
NEUT (10^9/L)	3.22 (2.50, 3.94)	3.69 (2.69, 5.27)	0.002*	0.003*
LYM (10^9/L)	1.90 (1.65, 2.16)	1.52 (1.20, 2.03)	<0.001*	<0.001*
MONO (10^9/L)	0.41 (0.31, 0.47)	0.43 (0.32, 0.53)	0.139	0.185
CRP (mg/L)	2.11 (1.89, 2.28)	2.36 (2.18, 2.63)	<0.001*	<0.001*
TNF-α (μg/L)	0.39 (0.36, 0.41)	0.47 (0.44, 0.50)	<0.001*	<0.001*
VEGF (μg/L)	1.03 (0.91,1.17)	1.35 (1.27, 1.44)	<0.001*	<0.001*
IL-6 (ng/L)	15.81 (14.05, 19.59)	22.81 (21.64, 24.03)	<0.001*	<0.001*
IL-1β (ng/L)	33.93 (31.77, 39.70)	43.80 (40.84, 47.21)	<0.001*	<0.001*
MMP9 (mg/L)	0.88 (0.73, 1.02)	1.13 (0.97, 1.27)	<0.001*	<0.001*

Notes: *Statistically significant, P < 0.05. Adjusted P, FDR adjusted P-values using the Benjamini–Hochberg procedure.

Abbreviations: MMD, moyamoya disease; FBG, fasting blood glucose; TC, total cholesterol; TG, total triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEUT, neutrophil count; LYM, lymphocyte count; MONO, monocyte count; CRP, C-reactive protein; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor; IL-6, interleukin-6; IL-1β, interleukin-1β; MMP9, matrix metalloproteinase-9.

Inflammatory Cytokines

This investigation revealed that the serum concentrations of inflammatory factors, including CRP, TNF-α, VEGF, IL-6, IL-1β, and MMP9, in MMD patients were considerably elevated compared to the healthy control group (Table 1). Cluster analysis revealed significant disparities in serum inflammatory markers between MMD patients and healthy controls, as illustrated in Figure 1.

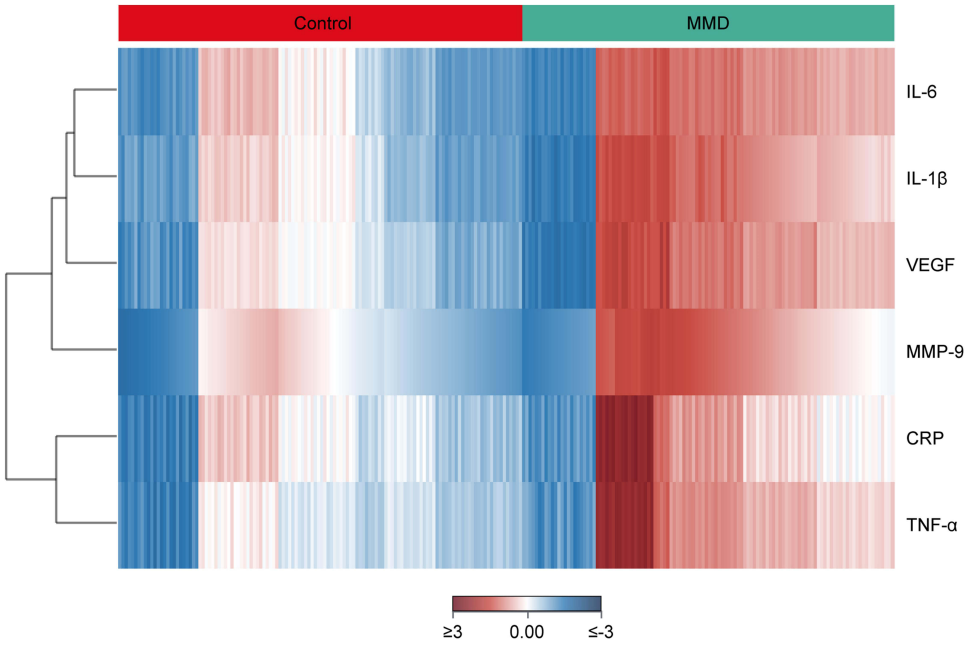

Figure 1 Cluster analysis of serum inflammatory factors in MMD patients and control group.

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Analysis of IgG N-Glycan Composition in MMD

We employed UPLC technology to identify the initial detection of 24 oligosaccharide chains that interact with the IgG Fc domain and subsequently normalized this data. To compare the initial glycan levels between MMD patients and healthy controls, we employed the Wilcoxon rank sum test for statistical analysis. Supplementary Table S2 displays the specific level differences of these 24 initial glycans. The findings indicated that the concentrations of five initial glycans (GP1, GP2, GP4, GP5, and GP6) in the MMD group were markedly elevated compared to the control group, whereas the concentrations of the other five initial glycans (GP14, GP18, GP20, GP21, and GP23) were significantly diminished relative to the control group, as illustrated in Figure 2. Additionally, to examine the possible influence of IgG N-glycans on the advancement of MMD, we calculated 54 derived glycan traits from the measured values of the initial glycans and classified them into four principal glycosylation characteristics: fucosylation, galactosylation, sialylation, and bisecting

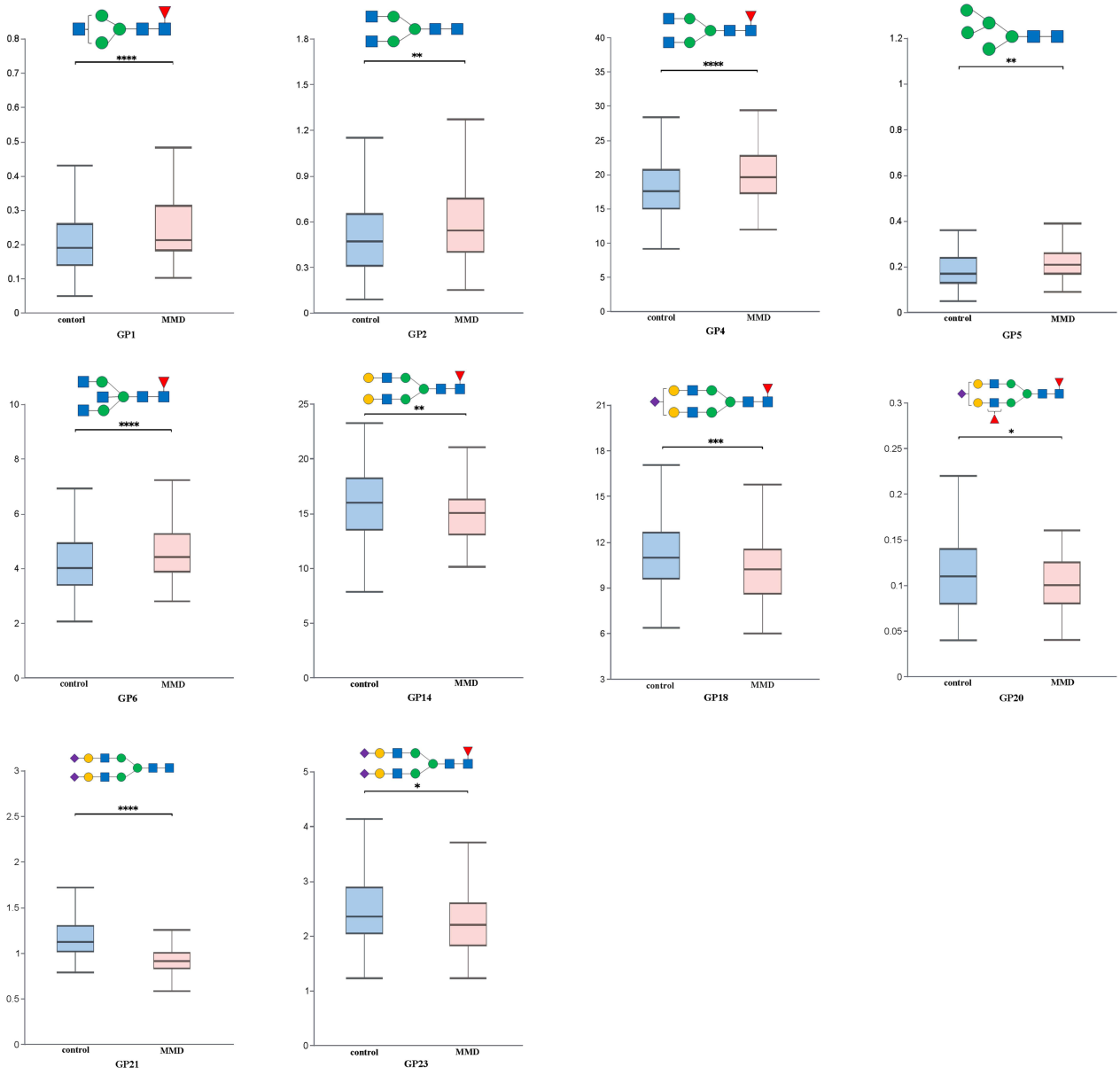

Figure 2 Differences in initial IgG glycan levels between MMD group and control group.
Notes: *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001. The box delineates the interquartile range (IQR) of the data, specifically the values between the upper quartile (Q3) and the lower quartile (Q1). The horizontal line within the box denotes the median. The lines extending outside the box denote the data range.
Abbreviations: GP, glycan peak; IgG, immunoglobulin G; MMD, moyamoya disease.

GlcNAc. A comparative study revealed that 18 of the 54 derived glycan features exhibited significant differences between the MMD group and the control group (Supplementary Table S3). These findings provide new insights and evidence about the critical role of IgG N-glycans in the advancement of MMD.

The Correlation Between IgG N-Glycan and MMD

We employed multivariate logistic regression analysis to evaluate the association between IgG N-glycans and MMD, controlling for relevant confounders such as age, FBG, TC, HDL, LDL, NEUT, and LYM. Upon correction, we identified substantial variations in the properties of 6 starting glycans and 16 derived glycans between the MMD group and the control group ($p < 0.05$) (Supplementary Tables S4 and S5). Relative to the control group, the MMD group exhibited significantly elevated levels of two initial glycans (GP4, and GP6), whereas the levels of four other initial glycans (GP14, GP18, GP20, and GP21) were markedly diminished in the MMD group (Figure 3). Regarding derivative glycan features, we noted that the levels of fucosylation (FG2n / G2n), sialylation (FGS / (F + FG + FGS)), and partial galactosylation (G1n and G2n) in the MMD group were markedly reduced compared to the control group. Conversely, the levels of an alternative sialic glycosylation (FBG2S1 / (FBG2 + FBG2S1 + FBG2S2)), galactosylation (G0n), and bisecting GlcNAc (FBS1 / FS1 and FBS1 / (FS1 + FBS1)) in the MMD group were markedly elevated compared to the control group.

The Correlation Between IgG N-Glycan and Inflammatory Cytokines

The CCA results indicate the presence of six pairs of typical sets. The first set of canonical data had a canonical correlation coefficient of 0.704 ($F = 2.56$, $p < 0.001$), which showed that there was a strong correlation between the N-glycan structure and inflammatory cytokines. There was a robust correlation between two initial glycan characteristics (GP 11 and GP 21) and the levels of TNF-α, MMP9, VEGF, CRP, IL-6, and IL-1β (Figure 4). The GP 21 level is significantly associated with canonical variables, exhibiting a load of 0.770, whereas the response variable with the highest canonical load is 0.818 (TNF-α).

Differentiating MMD From Healthy Controls by IgG N-Glycosylation Analysis

In this study, we developed three MMD diagnostic models based on IgG N-glycans and inflammatory cytokines and evaluated their performance through the ROC curve.

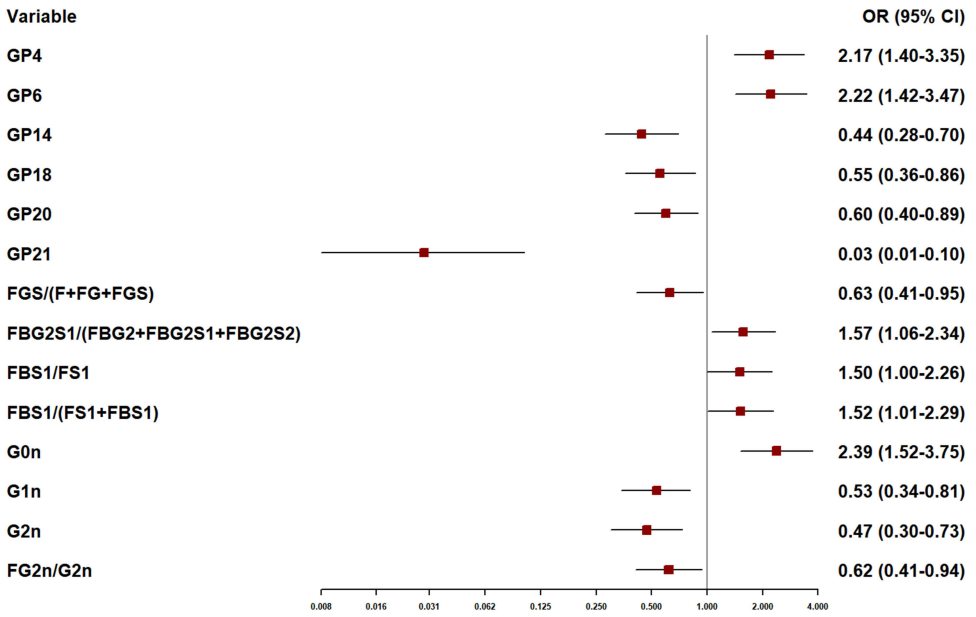

Figure 3 Differences in IgG N-glycan levels between MMD and control groups (Multifactorial).
Notes: The odds ratio (OR) and 95% confidence interval (95% CI) of IgG N-glycan levels between the MMD and control groups (adjusted for age, fasting blood glucose, total cholesterol, high-density lipoprotein, low-density lipoprotein, neutrophil count, and lymphocyte count).

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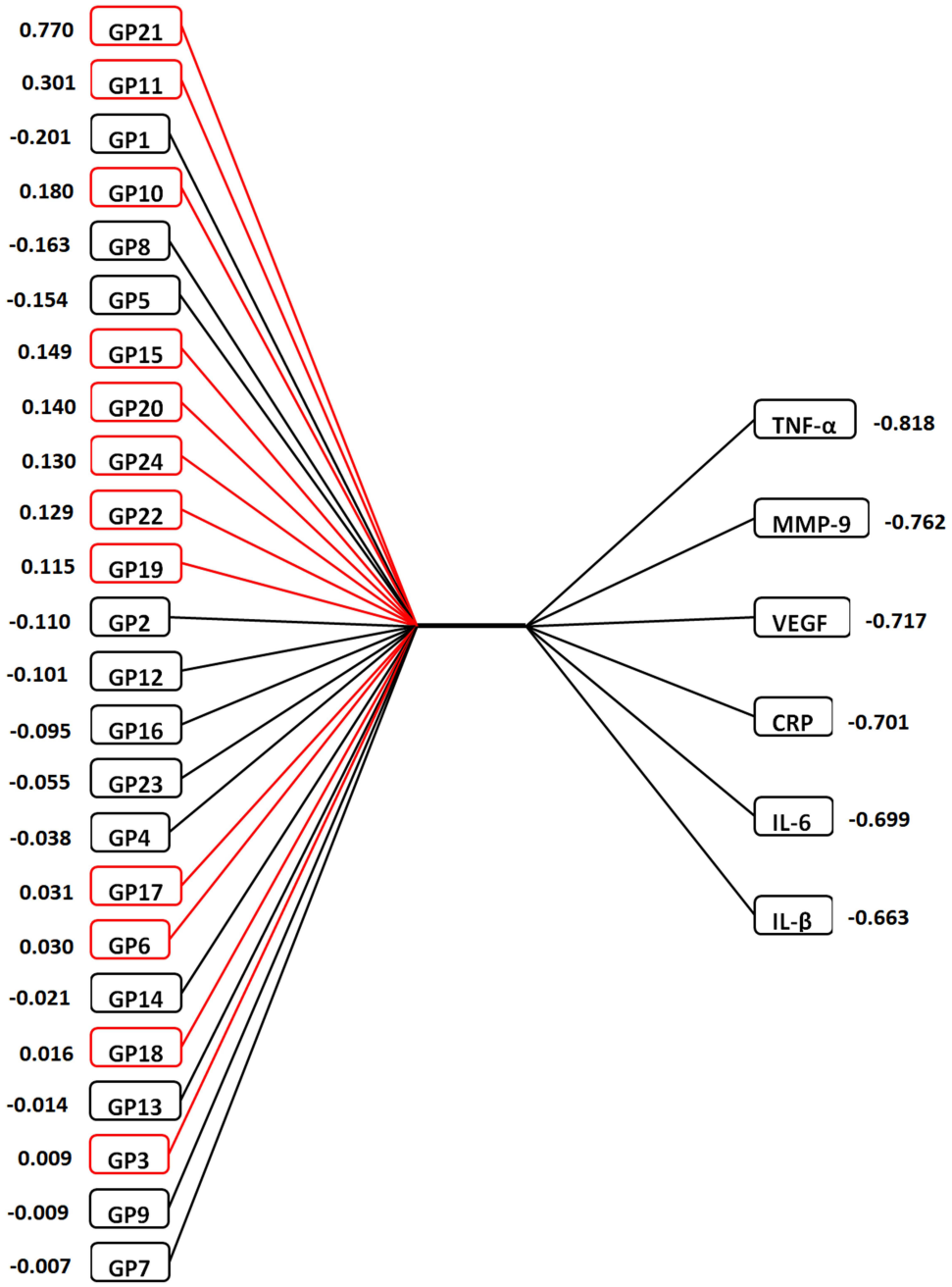

Figure 4 Canonical structures of the IgG N-glycans and inflammatory cytokines.
Notes: Significant canonical loadings were defined as those with an absolute value exceeding 0.30. The absolute value of the canonical loadings of all variables is used to rank them. Red boxes indicate positive relationships, while black squares indicate negative relationships.

Firstly, we established an MMD diagnostic model 1 (Figure 5) based on 24 initial glycans. Utilizing the Lasso algorithm for feature selection, we identified the glycan markers substantially associated with MMD, specifically GP2 and GP21. Subsequently, these indicators were incorporated into multivariate logistic regression analysis, yielding the odds ratio (OR) and 95% confidence interval (CI). ROC curve analysis revealed that the AUC of the model was 0.902, with a Se of 81.9% and a Sp of 84.1%, demonstrating that Model 1 exhibited favorable diagnostic performance.

To enhance diagnostic performance, we contemplated integrating glycans and inflammatory variables for modeling. Therefore, we established an MMD diagnostic model 2 (Figure 5) based on 24 initial glycans and 6 inflammatory factors. The Lasso algorithm is also used for feature selection, and logistic regression analysis is introduced. The results showed

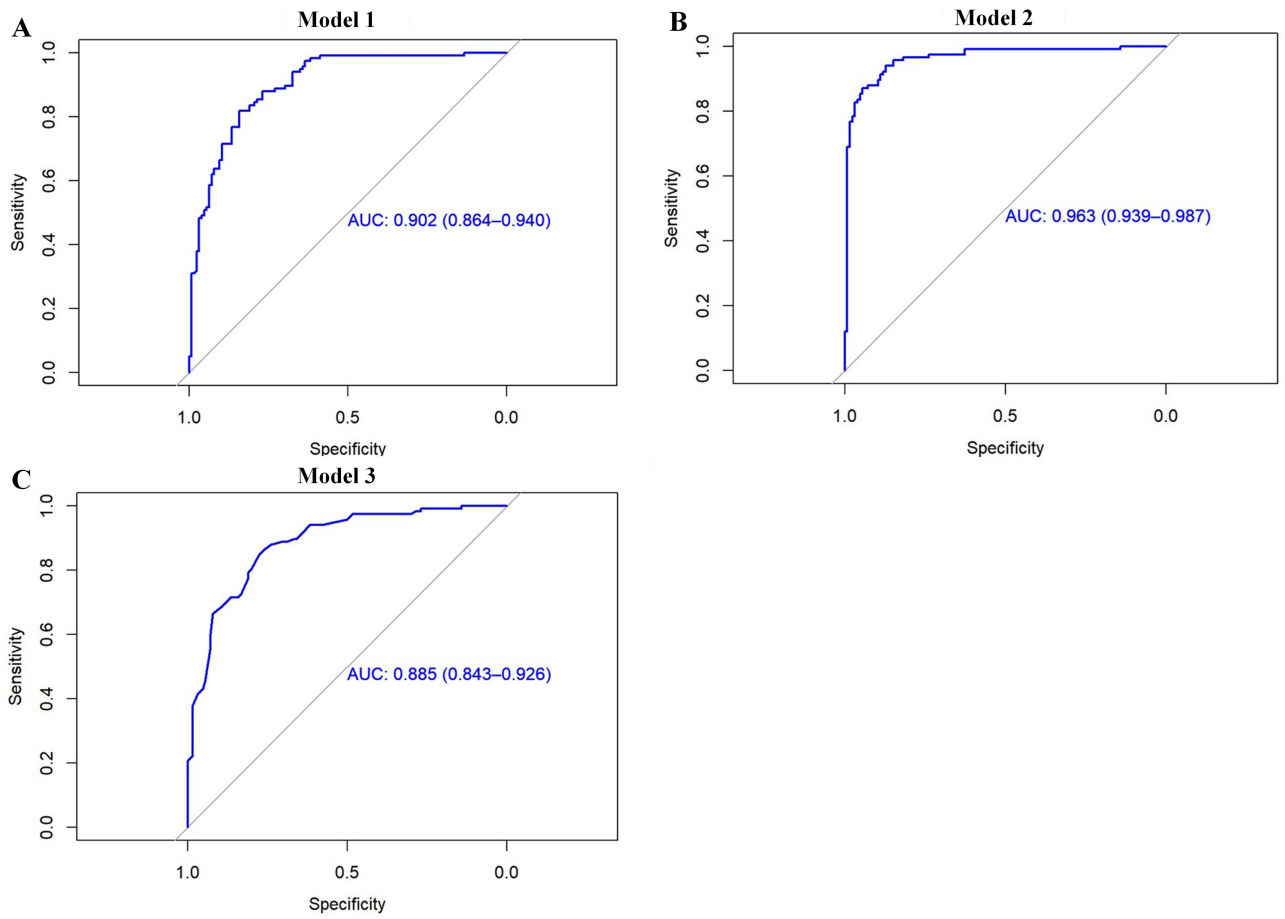

Figure 5 ROC curve of MMD diagnostic model.
Notes: (**A**) Model 1 consists of GP2 and GP21. (**B**) Model 2 consists of GP6, GP21, TNF-α, and CRP. (**C**) Model 3 consists of GP21 alone.

that GP6, GP21 glycan, TNF-α, and CRP inflammatory factors contributed significantly to the diagnosis of MMD. ROC curve analysis showed that the AUC value of Model 2 was as high as 0.963, Se was 94.0%, and Sp was 89.7%, which indicated that the predictive performance of the model was significantly improved when both glycans and inflammatory factors were considered.

In addition, we also noticed that GP21 glycan was included in both diagnostic models, suggesting its importance in MMD diagnosis. Therefore, we selected GP21 alone to establish a diagnostic model 3 (Figure 5) for MMD and analyzed its performance through the ROC curve. The results showed that the AUC value of model 3 was 0.885, Se was 84.5%, and Sp was 77.8%. Although it was lower than models 1 and 2, it still showed good diagnostic ability.

In summary, the three MMD diagnostic models we developed show good performance, especially Model 2, which combines glycans and inflammatory factors, and its predictive performance is more excellent. These models provide new ideas and methods for the early diagnosis and treatment of MMD. At the same time, the importance of GP21 glycans in the diagnosis of MMD has also been verified, providing a valuable reference for future research.

Discussion

This research employed HILIC-UPLC high-throughput technology to examine the IgG N-glycan profile in the serum of MMD patients. In comparison to the control group, the serum IgG N-glycosylation in MMD patients exhibited substantial variations, characterized by a reduction in sialylation, galactosylation, and fucosylation, alongside an elevation in bisecting GlcNAc. The alterations in glycosylation may facilitate the aberrant elevation of inflammatory cytokines (including CRP, TNF-α, VEGF, IL-6, IL-1β, and MMP9), affect the equilibrium of the inflammatory response, and

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subsequently contribute to the pathogenesis of MMD. Consequently, IgG N-glycosylation is anticipated to serve as a possible biomarker for MMD. Consequently, we advanced an early diagnosis model for MMD that integrates initial glycans and associated inflammatory markers, with the objective of facilitating early illness detection.

In comparison to the control group, MMD patients exhibited a notable elevation in the levels of two initial glycans (GP4, GP6) and a substantial reduction in the levels of four further glycans (GP14, GP18, GP20, GP21). Fucosylated glycans GP4 exhibited elevated expression in MMD patients, but the expression levels of fucosylated glycans GP14, and GP18 were diminished. Simultaneously, galactosylated glycans GP14 exhibited reduced amounts in MMD patients. Furthermore, the concentrations of sialic acid-containing glycans GP18, GP20, and GP21 were decreased in MMD patients. Specifically, GP6, a glycan including bisecting GlcNAc, exhibited elevated levels in MMD patients. Regarding derivative features, we observed that the levels of fucosylation (FG2n / G2n), sialylation (FGS / (F + FG + FGS)), and galactosylation (G1n and G2n) in the MMD group were diminished compared to the control group. Conversely, the levels of galactosylation (G0n), sialylation (FBG2S1 / (FBG2 + FBG2S1 + FBG2S2)), and bisecting GlcNAc (FBS1 / FS1 and FBS1 / (FS1 + FBS1)) were elevated compared to the control group. The alterations in these initial glycans regarding sialylation, galactosylation, fucosylation, and bisecting GlcNAc were congruent with the findings of the correlation analysis between MMD and the features of IgG N-glycan derivatives. The alterations in these derived sugar chains indicate that the reduction of sialylation, galactosylation, and fucosylation, alongside the elevation of bisecting GlcNAc, may be intricately associated with the pathogenesis of MMD.

Research indicates that IgG N-glycans significantly influence the equilibrium between pro-inflammatory and anti-inflammatory responses in inflammatory and autoimmune disorders.[21,38] Inflammation serves as a fundamental regulatory element in various physiological and biochemical processes in MMD, being intricately linked to the pathophysiology and evolution of the condition.[11] Prior research indicates that the concentrations of inflammatory markers, such as CRP, TNF-α, VEGF, IL-6, IL-1β, and MMP9, in MMD patients are markedly elevated compared to healthy control groups.[12–14] This aligns with the study's findings, which further substantiate the probable involvement of pro-inflammatory cytokines in the pathophysiology of MMD. The inflammatory regulatory role of IgG N-glycosylation is closely associated with risk factors for MMD, such as dyslipidemia, diabetes, and hypertension.[26–28] This study established the correlation between MMD-related IgG N-glycans and inflammatory cytokines, revealing a significant association between MMD and atypical alterations in IgG N-glycosylation, characterized by diminished sialylation, galactosylation, and fucosylation, alongside increased bisecting GlcNAc levels, in conjunction with the inflammatory response. This may offer a partial elucidation for the inflammatory events associated with the progression of MMD. Alterations in IgG N-glycosylation may precipitate a pathological elevation of inflammatory cytokines, thereby facilitating the progression of MMD. Consequently, IgG N-glycosylation may significantly influence the development and progression of MMD. Glycosyltransferases and glycosidases mediate IgG N-glycosylation, which has been linked to the pathogenesis of inflammatory diseases.[39] The pro-inflammatory condition associated with IgG exhibiting abnormal glycosylation alterations has been noted in numerous neurological disorders.[40] Research indicates that diminished galactosylation and sialylation, along with heightened bisecting GlcNAc, may contribute to the molecular regulatory mechanisms of inflammation, potentially linked to the chronic inflammation associated with the onset of ischemic stroke (IS).[30] Patients with dementia demonstrate analogous alterations in IgG glycosylation.[41] The findings suggest a possible association between irregularities in IgG N-glycosylation and neurological disorders through the alteration of inflammatory responses. Consequently, IgG N-glycosylation is anticipated to be crucial for elucidating the pathophysiology of MMD and may also offer novel potential targets for its diagnostics and treatment.

A substantial body of evidence demonstrates that diminished sialylation, galactosylation, and fucosylation, alongside elevated bisecting GlcNAc, correlates with an augmented risk of numerous inflammatory and chronic diseases,[27,28,30,32,39,42–44] a conclusion that aligns with our findings (Table 2). Studies have shown that galactosylation levels tend to decrease in many inflammatory diseases,[45] while their increase is usually associated with a decrease in inflammatory activity.[46] In conjunction with the summary in Table 2, we observed that a reduction in IgG galactosylation has been documented across various inflammatory and chronic diseases, suggesting that aberrant IgG glycosylation is not exclusive to any particular disease but rather a prevalent occurrence associated with diminished anti-inflammatory efficacy of circulating IgG. A decrease in the terminal galactosylation of IgG N-glycans impairs their binding affinity

Table 2 The Alteration of Glycans in Diseases

	Diseases												
Glycans	**EC**	**GC**	**CRC**	**PC**	**SLE**	**UC**	**CD**	**RA**	**CKD**	**HT**	**T2D**	**IS**	**MMD**
GP1	↑	↓	↑	↑	–	–	–	↑	–	–	–	↑	–
GP2	↑	↑	↑	↑	↑	–	–	↑	↑	–	–	–	–
GP3	↓	↓	↓	↓	–	–	–	–	–	–	–	–	–
GP4	↑	↑	↑	↑	↑	↑	↑	–	–	↑	–	–	↑
GP5	↓	↓	–	–	–	–	–	–	–	–	–	↓	–
GP6	↑	↑	↑	↑	↑	↑	↑	–	↑	↑	–	–	↑
GP7	–	–	–	–	–	–	–	–	–	–	↓	–	–
GP8	↓	↓	↓	↓	↓	–	–	–	–	–	↓	↑	–
GP9	↓	–	–	–	–	↓	↓	–	–	–	↓	–	–
GP10	↑	↑	↑	↑	↑	–	–	–	–	–	–	–	–
GP11	↓	↓	↓	–	–	–	–	–	–	–	↑	–	–
GP12	–	–	–	–	–	–	–	–	–	↓	–	–	–
GP13	↓	↓	↓	↓	–	–	–	–	–	↓	–	↓	–
GP14	↓	↓	↓	↓	↓	↓	↓	–	↓	↓	–	↓	↓
GP15	–	–	↓	↓	–	–	–	–	–	↓	–	↓	–
GP16	–	↓	–	–	–	–	–	–	–	–	–	–	–
GP17	↑	↑	↑	↑	–	–	–	–	–	–	–	↓	–
GP18	↓	↓	↓	↓	↓	↓	↓	–	↓	↓	–	↓	↓
GP19	–	–	↑	↑	–	–	↓	–	–	–	↓	–	–
GP20	↓	–	↓	↓	–	–	–	–	–	–	–	–	↓
GP21	↑	↑	↑	↑	–	–	–	–	–	–	–	↓	↓
GP22	–	↑	↑	–	–	–	–	↓	–	–	–	–	–
GP23	↓	–	↓	↓	↓	–	–	–	–	–	–	↓	–
GP24	↓	–	–	–	–	–	–	↑	–	–	–	–	–
Galactosylation	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
Sialylation	↓	↓	↓	↓	↓	–	↓	–	↓	↓	↓	↓	↓
Fucosylation	–	–	–	–	↓	–	–	↑	–	↓	–	–	↓
Bisecting GlcNA	↑	↑	↑	↑	↑	–	↑	–	↑	↑	↑	↑	↑

Notes: ↑ high level of glycans can increase the risk of disease. ↓ low level of glycans can increase the risk of disease.
Abbreviations: EC, Esophageal cancer; GC, Gastric cancer; CRC, Colorectal cancer; PC, Pancreatic cancer; SLE, Systemic lupus erythematosus; UC, Ulcerative colitis; CD, Crohn's disease; RA, Rheumatoid arthritis; CKD, Chronic kidney disease; HT, Hypertension; T2D, Type 2 diabetes; IS, Ischemic stroke; MMD, Moyamoya Disease.

to FcγRIIB and upregulates complement-dependent cytotoxicity (CDC) effects, thereby promoting inflammation.[47] Conversely, elevated galactosylation enhances the binding of IgG to the inhibitory FcγRIIB, thereby enhancing its anti-inflammatory effect.[48] In addition, decreased galactosylation also increases the binding of IgG to mannose-binding

lectins, leading to upregulation of CDC activity, which may trigger an inflammatory response.[47,49] Galactosylation is significant in the etiology of several disorders, including inflammatory bowel disease,[44] vascular cognitive impairment,[50] and Parkinson's disease,[31] with alterations in galactosylation noted in these conditions. Our observations of diminished galactosylation align with the findings of research on IS, which similarly reported reduced galactosylation.[30] Moreover, diminished galactosylation levels are significantly correlated with elevated pro-inflammatory cytokines, including TNF-α and CRP.[51,52] Our findings corroborate this, indicating that TNF-α and CRP levels are markedly higher in patients with MMD. This further substantiates the critical function of IgG galactosylation in the inflammatory mechanism of MMD. Therefore, the galactosylation of IgG plays a crucial role in regulating the inflammatory response of MMD, and its reduction may be one of the important mechanisms for the occurrence and development of various inflammatory and chronic diseases.

Sialylation significantly influences the inflammatory regulation of IgG.[51] The terminal sialic acid residue in IgG N-glycans is covalently attached to galactose, which diminishes its capacity to bind to Fcγ-RIIIa on natural killer (NK) cells, thereby decreasing inflammatory activity through the antibody-dependent cellular cytotoxicity (ADCC) pathway.[41] Simultaneously, sialylation further influences the inflammatory response by diminishing affinity for FcγR I and augmenting affinity for FcγR II, thereby initiating anti-inflammatory activity.[53] The decrease in sialic acid levels enhances the binding ability of IgG to complement C1q, activates the complement cascade reaction, shifts it from anti-inflammatory to pro-inflammatory, and exacerbates inflammation and damage to vascular endothelium.[31,54] This study unequivocally demonstrates that IgG glycosylation is pivotal in modulating antibody-mediated responses and may be one of the molecular processes that facilitate inflammation. The results of this study indicate that IgG N-glycosylation is closely related to the inflammatory state of MMD, and low levels of sialylation are associated with enhanced inflammatory response.

Fucosylation has relative stability in the human body, showing minimal variation due to age and gender, although its alterations significantly influence IgG functionality.[55,56] Fucosylation is crucial for immune control; its lowering can increase the binding of the IgG Fc region to the Fcγ-RIIIa receptor on NK cells, facilitate ADCC activation, and intensify inflammation. The core fucosylated glycoprotein in the IgG Fc region stimulates the ADCC pathway by binding to the Fcγ-RIIIa receptor on immune cells, enhances pro-inflammatory cytokine production, and initiates an inflammatory response.[56,57] A reduction in fucosylation levels has a pro-inflammatory impact.[58] Moreover, diminished fucosylation levels result in heightened synthesis of pro-inflammatory molecules, primarily IL-1β, TNF-α, and IL-6.[59] Conversely, highly fucosylated IgG can suppress the synthesis of TNF-α and IL-6, demonstrating an anti-inflammatory action.[60] This study's results corroborate previous findings, since the levels of IL-1β, TNF-α, and IL-6 in MMD patients were markedly elevated compared to the control group. Therefore, low levels of fucosylation are associated with increased inflammatory response in MMD.

Some studies have revealed that the bisecting GlcNAc of IgG can enhance its binding affinity with Fcγ-RIIIa, thereby regulating the ADCC process and promoting the pro-inflammatory effect of IgG.[61] In addition, the addition of bisecting GlcNAc glycan can significantly amplify the non-fucosylation-induced ADCC effect by several dozen times.[62] The data combined highlight the crucial role of IgG N-glycosylation in regulating inflammatory responses in persons with MMD.

The identification of biomarkers is essential for disease prediction and diagnosis. IgG N-glycosylation, a significant post-translational modification of proteins, is intricately linked to protein function and can more precisely forecast the onset and progression of illnesses.[63] Nonetheless, the implementation of glycosylation biomarkers in clinical practice continues to encounter numerous hurdles. The alterations in glycopeptides GP 21 exhibit inconsistency across several chronic illnesses (Table 2). We initially identified qualitative indicators to assess which ones elevate disease risk for the purpose of screening. Integrating prior research (Table 2), we determined that elevated levels of GP 4 and GP 6, together with diminished levels of GP 14, GP 18, and GP 20, may augment disease risk. Consequently, the findings of this study suggest that GP 4, GP 6, GP 14, GP 18, and GP 20 are anticipated to serve as significant biomarkers for chronic diseases in clinical settings in the future.

This study focuses on GP21, which was incorporated into both diagnostic models, Model 1 and Model 2. Furthermore, GP21 was independently chosen to differentiate the MMD group from the control group, and its diagnostic efficacy was evaluated by ROC curve analysis, revealing an AUC value of 0.885 (95% CI: 0.843, 0.926). The

examination of the relationship between initial glycoproteins and inflammatory indicators revealed that GP21 had a substantial negative correlation with inflammation levels. Reduced expression of GP21 correlates with an increased risk of MMD. This data aligns with the correlation between Parkinson's disease and IS but contradicts dyslipidemia.[26,30,31] A potential explanation for this is that MMD, IS, and Parkinson's disease are all neurological disorders that share a shared pathological foundation: cerebral arteriosclerosis.[64,65] GP21 is a sialylated glycan, and its decreased levels may affect the pathological process of MMD through two mechanisms. Firstly, the reduction of sialic acid enhances the affinity of IgG to the pro-inflammatory receptor Fcγ-RIIIa, thereby activating the ADCC pathway and triggering pro-inflammatory effects.[66,67] Secondly, IgG with low sialylation enhances its binding ability to complement C1q, activates the complement cascade reaction, and exacerbates endothelial inflammation and damage.[39,68] The above two mechanisms may synergistically promote the infiltration of inflammatory cells into the vascular wall, induce smooth muscle cell migration, and cause intimal hyperplasia, ultimately leading to the characteristic pathological changes of MMD. Compared to traditional inflammation related biomarkers such as MMP-9 and VEGF, IgG N-glycosylation exhibits a higher disease-specific advantage. Traditional biomarkers have significantly limited diagnostic specificity due to their widespread cross expression in various inflammatory diseases. And this study found that the predictive model constructed based on GP21 in IgG N-glycosylation features (AUC=0.885, 95% CI: 0.843, 0.926) exhibited good diagnostic efficacy. This may provide novel biomarkers for early non-invasive diagnosis of MMD. Therefore, this study suggests that GP21 has the potential to serve as a specific biomarker for early diagnosis of MMD.

At present, non-invasive IgG N-glycan testing demonstrates good precision in diagnosing inflammatory and immunological disorders,[69] and HILIC combined with fluorescence labeling and UPLC technology has been proven to be an effective detection method.[70] In the present study, by analyzing IgG N-glycosylation through high-throughput, the constructed diagnostic model showed high significance in distinguishing MMD patients from healthy control groups, indicating the potential of IgG N-glycosylation as an important biomarker for the early diagnosis of MMD.

Nonetheless, many limitations of this study warrant elucidation. Initially, while a correlation exists between the inflammatory function of IgG N-glycosylation and an elevated risk of MMD, additional validation in diverse ethnic groups and the development of standardized operating procedures are necessary prior to the application of this novel biomarker in the screening or diagnosis of MMD. Secondly, due to the case-control design of this investigation, there are constraints in establishing a causal association between IgG N-glycosylation and MMD. Although the diagnostic model demonstrated robust performance in this single-center study, further validation through large-scale, multi-center cohorts is warranted to confirm its generalizability. At the same time, based on the results of this study, further elucidate the diagnostic biomarkers of MMD and deepen the understanding of the correlation between IgG N-glycosylation and MMD.

Conclusion

This study found that the occurrence and progression of MMD may be closely related to a decrease in sialylation, galactosylation, and fucosylation, and an increase in bisecting GlcNAc. This change in IgG N-glycosylation may contribute to the pathophysiology of MMD by modulating the balance of the inflammatory response. In addition, MMD diagnostic models based on IgG N-glycans and inflammatory cytokines have shown good predictive ability. Model 1, constructed based on GP2 and GP21, has an AUC of 0.902, Se of 81.9%, and Sp of 84.1%. Model 2, constructed based on GP6, GP21, TNF-α, and CRP, with an AUC of 0.963, Se of 94.0%, and Sp of 89.7%. Both models contain GP21, and Model 3, constructed solely based on GP21, has an AUC of 0.885, Se of 84.5%, and Sp of 77.8%. This suggests that GP21 may become a specific biomarker for early non-invasive diagnosis of MMD. This provides valuable reference for early screening and clinical auxiliary diagnosis of MMD.

Data Sharing Statement

Anonymized data is available from the corresponding author on reasonable request.

Ethics Approval

This retrospective study was approved by the Medical Ethics Committee of Liaocheng People's Hospital (Ethics Approval Number: 2024372). Due to the retrospective nature of the study, the requirement for informed consent was waived. All patient data is anonymized during the processing. This study was conducted in accordance with the Helsinki Declaration.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare no conflicting interests in this work.

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