9-2-2020

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10.1089/omi.2020.0091

This is an author's accepted manuscript of:
Meng, Z., Li, C., Ding, G., Cao, W., Xu, X., Heng, Y., ... & Hou, H. (2020). Glycomics: Immunoglobulin GN-Glycosylation Associated with Mammary Gland Hyperplasia in Women. *OMICS: A Journal of Integrative Biology*, 24(9), 551-558. https://doi.org/10.1089/omi.2020.0091

Final publication is available from Mary Ann Liebert, Inc., publishers http://dx.doi.org/10.1089/omi.2020.0091

Glycomics: Immunoglobulin G *N*-glycosylation associated with mammary gland hyperplasia in women

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Short title: *IgG N-glycosylation and mammary gland hyperplasia*

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Abstract

Mammary gland hyperplasia (MGH) is very common, especially among young and middle-aged women. New diagnostics and biomarkers for MGH are needed for rational clinical management and precision medicine. We report here new findings using a glycomics approach, with a focus on immunoglobulin G (IgG) *N*-glycosylation. A cross-sectional study was conducted in a community-based population sample in Beijing, China. We recruited 387 women, aged 40-65 years for the present study. IgG *N*-glycans were characterized in the serum by ultra-performance liquid chromatography. The prevalence of MGH in our study sample was 47%. The levels of the glycan peaks (GPs) GP2, GP5, GP6 and GP7 were lower in the MGH group compared to the control group, while GP14 was significantly higher in the MGH group ($P < 0.05$). A predictive model using GP5, GP21 and age was established and a receiver operating characteristic (ROC) curve analysis was performed. The sensitivity and specificity of the model for MGH was 61.3% and 63.2%, respectively, likely owing to receptor mechanisms and/or inflammation regulation. To the best of our knowledge, this is the first study reporting on an association between IgG *N*-glycosylation and MGH. We suggest person-to-person variations in IgG *N*-glycans and their combination with multi-omics biomarker strategies offer a promising avenue to identify novel diagnostics and individuals at increased risk of MGH.

Keywords: Glycomics, breast dysplasia, mammary gland hyperplasia, immunoglobulin G, glycosylation, biomarkers

Introduction

Mammary gland hyperplasia (MGH), also called breast hyperplasia or breast dysplasia, is one of the most common disorders among women, and accounts for over 70% of all breast diseases (Li et al., 2018). Although the causes of MGH are not fully understood, it is known to be closely related to endocrine disorders (Chen et al., 2015).

Potential pathogenetic contributions to the onset of MGH include (Arendt et al., 2015; Samoli et al., 2013; Li et al., 2017):

1) an imbalance between estrogen (E_2) and progesterone (P) (i.e., decreased progesterone secretion, increased estrogen concentration;

2) aberrant expression and distribution of E_2 receptors in breast parenchyma and mesenchymal tissue; and

3) increased prolactin (PRL) related to the development of the mammary gland.

Glycosylation, a mechanism for the post-translational modification in biology, affects 90% of mammalian proteins by enabling various biological functions (Mechref et al., 2011). However, abnormal glycosylation or deglycosylation are associated with specific disorders (Zoldoš et al., 2013; Liu et al., 2018). Immunoglobulin G (IgG), a glycosylated molecule, accounting for about 75% of serum immunoglobulins, plays an important role in regulation of the inflammatory response (Masuda et al., 2007; Vučković et al., 2015; Sebastian et al., 2016). The structure of IgG consists of two parts: a fragment of antigen binding (Fab) with specific antigen-binding activity, and a crystalline fragment (Fragment crystallizable, Fc) that binds to effector molecules or effector cells. The function of IgG can be significantly interfered by aberrant *N*-

glycans at Fc segment, which can lead to inflammatory diseases, metabolic-related disorders, autoimmune diseases or carcinomas (Liu et al., 2018; Sebastian et al., 2016; Ravetch et al., 2001; Russell et al., 2017).

Omics technologies, including glycomics, can provide quantitative features of the profiles of post-translational modification of proteins, which might have a wide-range application in predictive diagnostics, targeted prevention and personalization of medical services (Lu et al., 2018). To the best of our knowledge, no studies have so far analyzed the relationship between MGH and IgG *N*-glycosylation.

This study aimed to identify the *N*-glycans profiles of IgG for individuals with MGH, and to assess the effect of IgG *N*-glycosylation with an eye to the development of MGH in a community-based population.

Materials and Methods

Study participants

Between November 2011 and February 2012, we randomly recruited 387 women participants from urban communities in Beijing, China.

Inclusion criteria were:

1) women aged 40 to 65 years,

2) no history of breast diseases, and

3) no history of medication in the past two weeks.

Exclusion criteria were:

1) pregnant and lactating women,

2) participants with severe mental disorders, or

3) individuals with other serious physical illness.

This study was approved by the ethics committee of Capital Medical University (No. 2009SY16). Each participant signed a written informed consent before enrollment.

MGH was diagnosed in accordance with the Diagnosis and Treatment of Breast Diseases of China (Chen et al., 2015): 1) Breast lumps were detected in one or two sides of breast with ultrasonography, and fibroadenoma, fat necrosis, or lipoma were excluded by clinicians; 2) For patients with nipple discharge, ductoscopy or galactography combined with cytology were used for differential diagnosis; 3) In the case of malignant lesions suspected by ultrasonic examination or molybdenum target X-ray examination, the diagnosis was confirmed by histopathological examination; 4) physiologic hyperplasia was excluded by clinicians.

Clinical measurements

Clinical measurements were carried out using standardized techniques (Yu et al., 2016; Liu et al., 2018). Body mass index (BMI) was calculated by the ratio of weight (kg) / height squared (m^2). Peripheral blood was collected from each participant after an overnight fast, and then stored in an Ethylenediaminetetraacetic acid (EDTA)-anti-coagulated tube. Fasting blood glucose (FBG), serum high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), total cholesterol (TC), urea, uric acid, creatinine (Cre), carcinoembryonic antigen (CEA), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were

assayed. All physical examinations and clinical diagnosis were performed at the Beijing Xuanwu Hospital of the Capital Medical University.

IgG *N*-glycans analyses

IgG *N*-glycans in serum samples were isolated and initially analyzed by hydrophilic interaction liquid chromatography (Waters Corp., Milford, MA, USA) with ultra high-performance liquid chromatography (HILIC-UPLC) (Menni et al., 2013). The level of each glycan was measured by a specific glycan peak (GP) detected in HILIC-UPLC assay. The detailed methods were described previously (Adua et al., 2017; Hou et al., 2019; Novokmet et al., 2014).

Statistical analyses

All statistical analyses were performed with SPSS 25.0 (IBM, Armonk, NY, USA). Kolmogorov-Smirnov test was utilized to examine whether the glycan measurements showed a statistically normal distribution. Because these data were not normally distributed, median (M) and interquartile range (IQR) were computed for descriptive statistics. A non-parametric statistical method (Mann-Whitney U test) was carried out for between-group comparisons. Other quantitative data that were normally distributed were compared by Student's t-test. Chi-squared test was used for comparisons of qualitative data. A multiple logistic regression analysis was undertaken to establish the classification model by screening the positive glycan biomarkers for MGH diagnosis. Then a receiver operating characteristic (ROC) curve was applied to evaluate the value of this model. A *P*-value <0.05 was considered statistically significant.

Results

Description of study participants

A total of 387 participants were enrolled in this study, including 194 women with MGH (cases) and 193 women who had no MGH (controls). All participants had a history of pregnancy and birth. The prevalence of MGH was 47.0%. We stratified all participants into four groups according to BMI levels.

As shown in **Table S1**, the prevalence rates of MGH were 37.5% in the underweight group, 53.1% in the normal weight group, 45.5% in the overweight group and 55.0% in the obese group. There were no significant differences between groups.

Demographic and clinical results are shown in **Table 1**. The age of individuals with MGH ranged from 40 to 64 years (mean age 46.25 years), which was statistically and slightly lower than that of the controls (range 40-65 years, mean age 47.87 years). No between-group differences were statistically significant for BMI, FBG, HDL, LDL, TG, TC, urea, uric acid, Cre, CEA, AST, and ALT.

Descriptive statistics for the glycans and derived traits

For the 24 IgG *N*-glycans detected by HILIC-UPLC, one glycan (GP3) was not include in our analyses because it did not pass the quality control standard (shown in Figure S1). Fifty-four derived glycan traits were calculated with the initial glycans (Adua et al., 2017; Hou et al., 2019).

As shown in **Table 2**, five initial glycans and five derived traits were significantly different between the MGH group and the control group ($P<0.05$). For five initial glycans, the levels of GP2, GP5, GP6 and GP7 were lower in the MGH group than in the control group, while GP14 was significantly higher in MGH group ($P<0.05$).

Among the derived traits, two derived traits ($G2^n$ and $FG1^{n\ total}/G1^n$) were higher in the MGH group, and three [FBS^{total}/FS^{total}, $G0^n$ and $BG2^n/(FG2^n+FBG2^n)$] were higher in the control group ($P < 0.05$).

Classification of MGH using IgG *N*-glycans

Using univariate logistic regression analyses, we identified whether each of IgG *N*-glycans was associated with the development MGH (**Table S2**). GP2, GP5, GP6 and GP14 were significantly associated with MGH. With regard to the derived glycan traits, FBS^{total}/FS^{total}, $G0^n$, $G2^n$, $FG2^n/(BG2^n+FBG2^n)$ and $BG2^n/(FG2^n+FBG2^n)$ were associated with MGH.

To evaluate whether IgG *N*-glycans contributed to distinguishing individuals with MGH from healthy women, we established a classification model of MGH by a multivariate logistic regression analysis. As shown in **Table 3** and **Figure S2**, two glycans (GP5 and GP21) and age were included in this model, where BMI, FBG, HDL, LDL, TG, TC, urea, uric acid, Cre, CEA, AST, and ALT were adjusted.

The ROC curve analysis was applied to evaluate the value of this model in the classification of MGH. As shown in **Figure 1**, the area under the curve (AUC) was 0.653 (95% CI: 0.598-0.707). This result indicates that the classification model could distinguish individuals with MGH from healthy persons. The cut-off value of 0.224 was used to distinguish breast hyperplasia. The sensitivity and specificity were 61.3% and 63.2%, respectively.

Discussion

Our findings revealed a high prevalence rate (47.0%) of MGH in a high-risk population. In addition, we found that IgG *N*-glycan profiles were significantly

associated with MGH. The serum levels of the five initial and seven derived traits of glycans differed between the MGH group and healthy individuals. Although the sensitivity and specificity of the predictive association is not at the level of a diagnostic for routine clinical use at this time, our findings point to the promise of glycomics, especially if it is combined as a postgenomic biomarker discovery platform with other multi-omics approaches in the future (Kunej, 2019; Liu et al. 2019; Pirih and Kunej, 2017).

MGH is a common breast disease among women (Zhao et al., 2018), which leads to significant morbidity. Clinical diagnosis of MGH would benefit from novel biomarkers (Li et al., 2017). Studies have reported 52% to 55% women of childbearing age are affected by MGH in China, which is similar to our findings in the present study (Jiang et al., 2011).

The growth and differentiation of normal mammary epithelial cells are regulated by many biological mechanisms, one of which is the hypothalamic-pituitary-gonadal axis that modulates the proliferation of mammary gland tissue and the menstrual cycle (Li et al., 2017). As a target organ of hormone action, the breast gland reacts to several hormones, including E_2, P, and PRL (O'Leary et al., 2017; Stingl et al., 2011). Endocrine disorder that causes dysregulation is one of the notable determinants of the development of MGH. E_2 and P can also indirectly regulate normal mammary gland development by paracrine signaling (Anderson et al., 2004).

E_2 and P are lipophilic steroid hormones that are synthesized periodically by ovaries under the control of pituitary gonadotropin, and transferred to target organs through circulating blood (O'Leary et al., 2017; Anderson et al., 2004). E_2 can

promote the proliferation of mammary epithelial cells and the growth of mammary ducts. P upregulates the further development and maturation of mammary acinar, and also inhibits the response of mammary gland to E_2 (Samoli et al., 2013; Arendt et al., 2015). The absolute or relative over-secretion of E_2 and lack of P leads to an imbalance between E_2 and P, resulting in excessive proliferation and sub-involution of breast parenchyma (Li et al., 2017).

PRL not only contributes to the growth, development and maintenance of breast feeding, but also affects the function of the hypothalamic-pituitary-gonad axis (Brisken et al., 1999). In addition, PRL stimulates the production of E_2 and inhibits the secretion of P in the luteal phase, thus contributing to the excessive proliferation of breast tissue (Oakes et al., 2008). PRL, together with E_2 and P, regulates the growth of mammary epithelial cells.

For healthy women, the levels of E_2 and P change periodically in accordance with the menstrual cycle, acting as nuclear transcription factors by binding with estrogen receptor (ER)-α and progesterone receptor (PR) (Stingl et al., 2011; Lee et al., 2006). The E-ER or P-PR complex enters the nucleus from cytoplasm, regulates transcription of target DNA, and modulates the growth, division and metabolism of target cells (i.e., mammary epithelial cells, ducts and acinar tissues) (Lee et al., 2006). ER and PR are proteins modified by glycosylation. *N*-glycosylation of Asn^{44} at the G protein-coupled estrogen receptor is critical for the maturation and activation of the ER receptor (Gonzalez et al., 2019). The glycosylation of ER-α at S573 is important for protein stability and nuclear localization of this receptor (Deng et al., 2018). The abnormal glycosylation of ER and PR might influence their sensitivity for coupling to

E_2 and P, which might induce the development of MGH (Li et al., 2017).

Inflammatory diseases, metabolic-related disorders, autoimmune diseases and carcinomas have also been reported to be related to aberrant glycosylation, where *N*-glycans of IgG at Fc segment play an important role in the regulation of the balance between inflammation and anti-inflammation (Russell et al., 2017). In spite of abnormal glycosylation of ER and PR, glycosylation might contribute to MGH in a pathogenetic pathway of IgG-related inflammation regulation. Mammary gland is a self-renewing tissue in which the morphology and differentiation change cyclically during menstruation, pregnancy, and lactation (Zhang et al., 2011).

Studies have found that adipocyte enhancer-binding protein-1 (AEBP-1) is a transcriptional regulator of macrophage cholesterol homeostasis and macrophage inflammatory responsiveness (He et al., 1995; Majdalawieh et al., 2010; Majdalawieh et al., 2010). AEBP-1 regulates mammary epithelial cell growth by regulation of nuclear factor (NF)-κB activity in the mammary epithelium (Holloway et al., 2012). NF-κB regulates the expression of pro-inflammatory chemokines and cytokines, ultimately promoting proliferation of mammary epithelial cells (Majdalawieh et al., 2006; Majdalawieh et al., 2007).

This inflammatory process might be regulated by IgG, as well impacted by IgG *N*-glycosylation. *N*-glycosylation on the Fc segment plays a pivotal role in the structure and function of IgG. Aberrant IgG *N*-glycans bias the anti-inflammatory and pro-inflammatory function of IgG (Ren et al., 2016). Increased terminal glycosylation of IgG Fc leads to increased binding of the antibody to FcγRIIB, thereby resulting in

upregulation of anti-inflammatory activity (de Jong et al., 2016).

We hypothesize that the inflammatory process of MGH might be related to glycosylation modification of IgG. However, the specific mechanism of action needs to be verified by experiments. Meanwhile, studies have reported that MGH can lead to inflammatory conditions, which means that the relationship between MGH and activation of inflammation is complex and needs to be explored further (Kuo et al., 2007).

Strengths and limitations

To the best of our knowledge, this is the first study reporting on an association between IgG *N*-glycosylation and MGH. The findings provide new insights into the pathogenesis of MGH. However, some limitations should be considered: First, as this was a cross-sectional study, our results cannot fully address the causal relationship between IgG glycome and MGH. Second, the moderate sample size limits the generalization of the conclusions. Third, the relationship between MGH and IgG glycome might be biased by environmental determinants that were not evaluated in this study. Therefore, the effects of IgG *N*-glycans on MGH may be underestimated or overestimated. Nevertheless, we present here a new putative pathogenetic factor of MGH based on glycomics. However, we present a new clue to investigate the etiology of MGH from the viewpoints of glycosylation of estrogen- and progesterone-receptors and inflammation regulation of IgG.

Conclusion

Person-to-person variations in IgG *N*-glycans and their combination with multi-

omics biomarker discovery strategies offer a promising avenue to identify novel diagnostics and individuals at increased risk of MGH.

Acknowledgements

This study was funded by National Natural Science Foundation of China (81673247, 81773527, 81872682 and 81903401), Australian-China Collaborative Grant (NHMRC APP1112767 – NSFC 81561128020), National Key R&D Program of China (2017YFE0118800), the Academic Promotion Programme of Shandong First Medical University (2019RC010), the Shandong Province Higher Educational Young and Innovation Technology Supporting Program (2019KJL004), and the Young Taishan Scholars Program of Shandong Province of China (No. tsqn20161046).

Author Disclosure Statement

The authors declare that no competing financial interests exist.

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Abbreviations

AEBP-1	Adipocyte enhancer-binding protein-1
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CEA	Carcinoembryonic antigen
Cre	Creatinine
E_2	Estrogen
EDTA	Ethylenediaminetetraacetic acid
ER	Estrogen receptor
Fab	Fragment of antigen binding
FBG	Fasting blood glucose
Fc	Fragment crystallizable
GP	Glycan peak
HDL	High-density lipoprotein cholesterol
HILIC	Hydrophilic interaction liquid chromatography
IgG	Immunoglobulin G
IQR	Interquartile range
LDL	Low-density lipoprotein cholesterol
M	Median
MGH	Mammary gland hyperplasia
NF	Nuclear factor
P	Progesterone
PR	Progesterone receptor
PRL	Prolactin
ROC	Receiver operating characteristic
TC	Total cholesterol
TG	Triglycerides
UPLC	Ultra high-performance liquid chromatography

Table 1 Characteristics of study participants

Variables	Cases (n=194)	Controls (n=193)	t/χ^2	*P-value*
Age	46.25±4.71	47.87±4.73	3.370	0.001
Height	159.85±5.24	159.52±5.51	0.594	0.553
Weight	61.57±8.05	61.29±8.62	0.333	0.740
BMI	24.10±3.01	24.08±3.24	0.057	0.955
Serum TC	5.05±0.83	5.18±0.98	1.399	0.163
Serum HDL	1.70±0.30	1.75±0.40	1.196	0.233
Serum LDL	2.72±0.65	2.80±0.74	1.177	0.240
Serum TG	1.21±0.79	1.26±1.17	0.582	0.561
FBG	5.18±0.55	5.18±0.74	0.027	0.978
Urea	4.96±1.03	4.84±1.16	1.094	0.275
Uric acid	218.31±54.42	220.65±55.66	0.419	0.676
Cre	49.85±6.15	50.26±6.75	0.630	0.529
CEA	1.32±0.65	1.48±1.24	1.621	0.106
AST	19.83±6.80	20.67±9.40	1.018	0.310
ALT	18.77±11.47	19.21±11.96	0.369	0.713

Data are shown as mean ± standard deviation

Mann–Whitney U-test: Age, BMI, TC, TG, HDL, LDL, FBG, Cre, CEA, AST, ALT;

BMI body mass index, TC total cholesterol, TG triglycerides, HDL high-density lipoprotein cholesterol, LDL low-density lipoprotein cholesterol, FBG fasting blood glucose, Cre Creatinine, CEA carcinoembryonic antigen, AST aspartate aminotransferase, ALT alanine aminotransferase

$P < 0.05$ was considered statistically significant

Table 2 Initial and derived glycans identified in chromatography

Glycans	Cases	Controls	Between group difference	
	M (IQR)	M (IQR)	*W*-statistic	*P*-value
GP1	0.05(0.04,0.08)	0.06(0.04,0.09)	35984.0	0.131
GP2	0.27(0.19,0.39)	0.32(0.22,0.42)	34869.0	0.012
GP4	13.92(11.76,17.01)	14.56(12.04,18.07)	35768.5	0.090
GP5	0.22(0.18,0.26)	0.24(0.20,0.28)	33921.0	0.001
GP6	3.39(2.85,3.91)	3.55(3.04,4.40)	34923.0	0.014
GP7	0.53(0.40,0.69)	0.59(0.42,0.73)	35459.5	0.048
GP8	18.44(17.13,19.40)	18.52(17.32,19.60)	36980.5	0.551
GP9	9.60(8.77,10.77)	9.65(8.86,10.37)	36756.0	0.533
GP10	4.83(4.29,5.52)	4.90(4.43,5.45)	37010.5	0.570
GP11	0.66(0.56,0.74)	0.66(0.60,0.73)	36225.5	0.200
GP12	1.00(0.71,1.39)	1.02(0.72,1.34)	37147.0	0.789
GP13	0.47(0.40,0.55)	0.49(0.41,0.59)	36671.0	0.380
GP14	19.83(17.59,21.61)	18.71(16.26,20.96)	34276.5	0.004
GP15	2.05(1.74,2.36)	1.96(1.74,2.28)	36160.0	0.244
GP16	3.08(2.71,3.38)	3.07(2.75,3.40)	37060.5	0.601
GP17	1.00(0.85,1.19)	1.05(0.87,1.23)	36865.5	0.484
GP18	13.25(11.36,14.90)	12.58(10.87,14.70)	35788.5	0.133
GP19	1.83(1.64,2.04)	1.89(1.68,2.11)	35969.0	0.130
GP20	0.33(0.29,0.41)	0.35(0.27,0.44)	36735.0	0.413
GP21	0.67(0.55,0.77)	0.69(0.59,0.79)	35981.0	0.132
GP22	0.09(0.07,0.12)	0.10(0.08,0.12)	36042.5	0.146
GP23	1.72(1.33,2.10)	1.67(1.33,2.14)	37166.0	0.802
GP24	1.50(1.25,1.84)	1.56(1.31,1.91)	36334.0	0.237
FGS/(FG+FGS)	26.93(25.02,29.56)	27.04(24.88,29.77)	37405.5	0.974
FBGS/(FBG+FBGS)	31.08(27.76,34.73)	30.73(27.96,35.03)	37163.5	0.668
FGS/(F+FG+FGS)	22.23(19.87,24.87)	22.15(19.10,24.68)	36609.0	0.449

FBGS/(FB+FBG+FBGS)	23.77(20.93,26.75)	23.15(20.44,27.08)	37080.5	0.742
FG1S1/(FG1+FG1S1)	9.93(8.61,10.90)	9.86(8.73,11.12)	37102.5	0.628
FG2S1/(FG2+FG2S1+FG2S2)	38.34(35.81,40.48)	38.61(36.13,40.56)	36691.0	0.390
FG2S2/(FG2+FG2S1+FG2S2)	5.10(4.10,6.10)	5.20(4.25,6.31)	36221.0	0.198
FBG2S1/(FBG2+FBG2S1+FBG2S2)	33.42(31.03,36.66)	34.22(31.32,37.04)	36305.5	0.227
FBG2S2/(FBG2+FBG2S1+FBG2S2)	28.03(24.42,32.18)	29.30(25.02,32.80)	36303.5	0.226
$F^{total}S1/F^{total}S2$	5.49(4.71,6.40)	5.44(4.49,6.44)	36066.5	0.211
FS1/FS2	9.37(8.01,11.00)	9.28(7.69,11.18)	36935.0	0.645
FBS1/FBS2	1.20(1.05,1.37)	1.17(1.03,1.37)	36668.0	0.482
FBS^{total}/FS^{total}	0.19(0.17,0.22)	0.19(0.17,0.23)	35438.0	0.046
FBS1/FS1	0.11(0.10,0.14)	0.12(0.10,0.15)	35555.0	0.059
FBS1/(FS1+FBS1)	0.10(0.09,0.12)	0.11(0.09,0.13)	35555.5	0.059
FBS2/FS2	0.90(0.79,1.02)	0.92(0.83,1.06)	35745.0	0.086
FBS2/(FS2+FBS2)	0.47(0.44,0.50)	0.48(0.45,0.51)	35745.0	0.086
$G0^n$	23.46(20.17,27.00)	24.65(20.83,29.52)	35216.0	0.028
$G1^n$	45.21(43.90,46.47)	45.17(43.80,46.62)	37198.0	0.824
$G2^n$	31.00(27.12,34.99)	29.64(24.60,33.60)	34974.0	0.025
$F^{n\ total}$	96.68(95.86,97.32)	96.46(95.69,97.26)	35976.5	0.183
$FG0^{n\ total}/G0n$	98.48(97.92,98.90)	98.32(97.74,98.81)	35482.5	0.075
$FG1^{n\ total}/G1^n$	98.48(98.00,98.81)	98.33(97.81,98.75)	35272.5	0.049
$FG2^{n\ total}/G2^n$	93.59(92.35,94.47)	93.09(91.94, 94.33)	35644.5	0.102
F^n	81.76(79.92,83.51)	81.56(80.08,82.79)	36057.0	0.208
$FG0^n/G0^n$	79.02(76.65,81.84)	78.99(76.65,80.86)	36704.0	0.502
$FG1^n/G1^n$	81.95(80.12,84.14)	81.92(80.25,83.43)	36775.5	0.545
$FG2^n/G2^n$	84.18(82.88,85.90)	83.86(82.26,85.26)	35385.0	0.062
FB^n	14.67(13.08,16.21)	14.78(13.72,16.18)	36282.0	0.218
$FBG0^n/G0^n$	19.21(17.09,21.44)	19.23(17.62,21.17)	37124.5	0.642

$FBG1^n/G1^n$	16.11(14.32,18.07)	16.03(14.86,18.20)	37121.5	0.640
$FBG2^n/G2^n$	9.12(7.98,10.13)	9.10(8.26,10.11)	36459.0	0.285
FB^n/F^n	0.18(0.16,0.20)	0.18(0.17,0.20)	36287.0	0.220
$FB^n/F^{n\ total}$	15.16(13.47,16.78)	15.39(14.33,16.77)	36287.0	0.220
$F^n/(B^n + FB^n)$	5.29(4.78,6.09)	5.26(4.80,5.72)	36122.0	0.230
$B^n/(F^n + FB^n)$	6.52(5.39,7.57)	6.60(5.51,8.13)	36804.0	0.450
$FBG2^n/FG2^n$	0.11(0.09,0.12)	0.11(0.10,0.12)	36353.0	0.244
$FBG2^n/(FG2^n+FBG2^n)$	9.69(8.60,10.87)	9.85(8.97,10.79)	36353.0	0.244
$FG2^n/(BG2^n+FBG2^n)$	7.61(6.72,8.44)	7.33(6.67,8.03)	35465.5	0.072
$BG2^n/(FG2^n+FBG2^n)$	21.49(19.33,25.14)	23.63(19.89,28.42)	34587.5	0.006
FGS/(FG+FGS)	26.93(25.02,29.56)	27.04(24.88,29.77)	37405.5	0.974

M, Median; IQR, interquartile range; GP, glycan peak

Table 3 Multiple logistic regression analyses of the association of glycans with MGH

Variables	B	SE	Walds	*P-value*	OR	95% CI of OR	
						LCI	UCI
Age	-0.064	0.023	7.492	0.006	0.938	0.896	0.982
GP5	-3.630	1.592	5.201	0.023	0.027	0.001	0.600
GP21	-1.318	0.669	3.874	0.049	0.268	0.072	0.995

B, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval; LCI, lower confidence interval; UCI, upper confidence interval

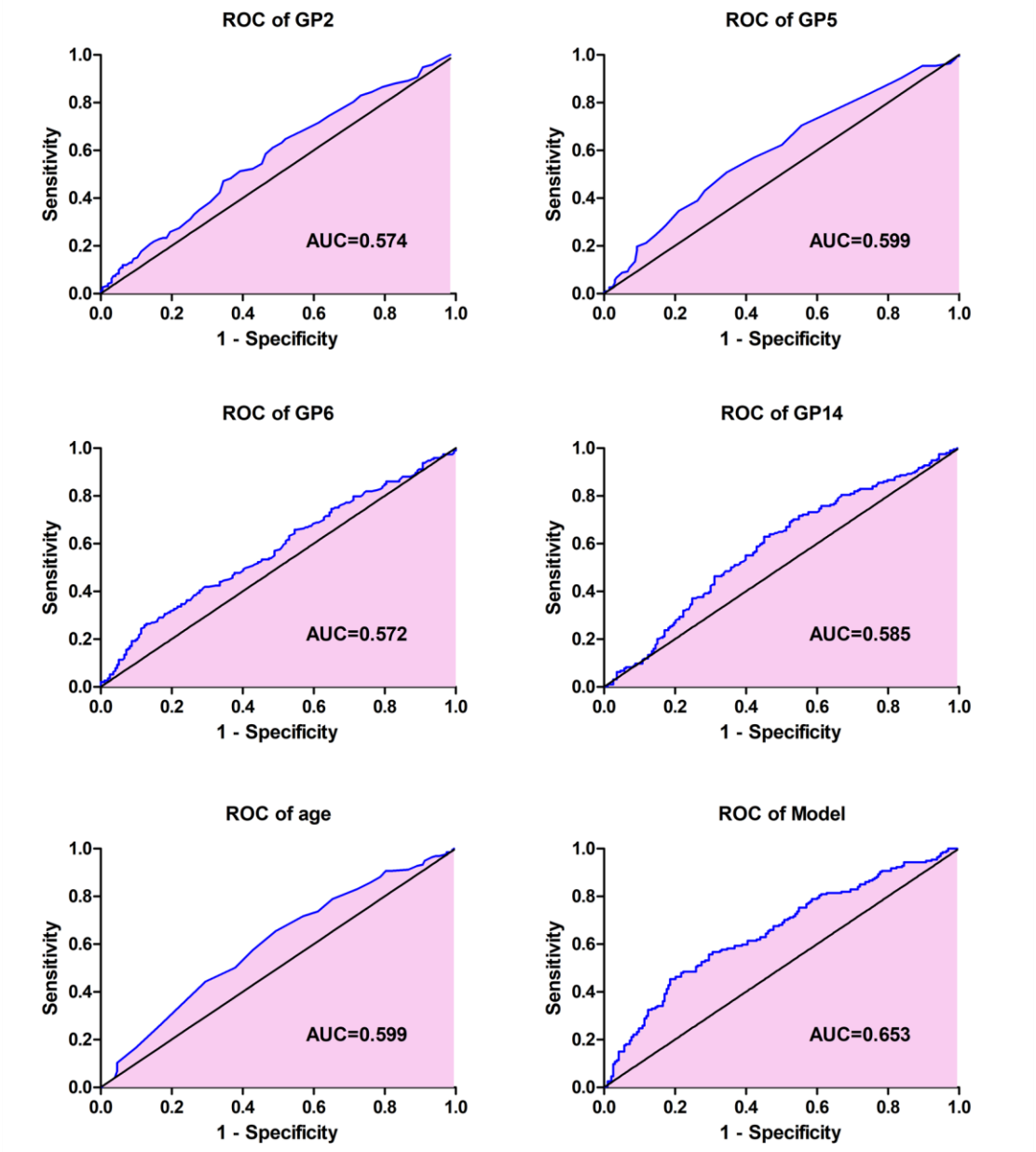

Figure 1 Receiver operating characteristic (ROC) curve analyses

AUC, area under the curve; Model, the classification model of GP5, GP21 and age