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ORIGINAL ARTICLE

Causal associations of specific immunoglobulin G N-glycosylation subtypes with osteoporosis: A two-sample Mendelian randomization

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Osteoporosis is a global health burden which affects over 200 million individuals worldwide.[1,2] Age-related bone mass loss leads to fractures in one-third of women and one-fifth of men over 50 years.[3,4] Osteoporosis constitutes a major global economic burden, with estimated annual direct medical costs reaching €37 billion in the European Union and projected to surpass $25 billion in the United States of America by 2025, largely due to the high cost of treating fractures.[5] The disease disrupts bone homeostasis through complex

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ABSTRACT

Objectives: This study aims to examine whether genetically predicted immunoglobulin G (IgG) N-glycosylation patterns (IGPs) affect osteoporosis risk using a two-sample Mendelian randomization (MR) method.

Materials and methods: In a collaborative effort involving the Medical Research Council (MRC) Human Genetics Unit and the FinnGen consortium, we conducted genome-wide association studies (GWAS) to explore the relationship between 77 IGPs (8,090 samples) and osteoporosis (438,872 samples). Utilizing the inverse-variance weighted (IVW) method as our primary analytical tool, we delved into these complex genetic associations. To further substantiate our findings, we employed additional complementary methods such as MR-Egger, weighted median, and weighted mode. Sensitivity analyses, including MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO), MR-Egger, Cochran's Q, and leave-one-out methods, were used to test the core MR assumptions and validate the robustness of the results. This multi-faceted approach allowed us to detect underlying causal relationships with greater confidence.

Results: The IGP4 exhibited a protective effect against osteoporosis with an odds ratio (OR) of 0.77 (95% confidence interval [CI]: 0.63-0.95, p=0.012). In contrast, IGP45 demonstrated a modest risk increase with an OR of 1.10 (95% CI: 1.01-1.19, p=0.021). Similarly, the results of the present MR study suggest that IGP56 also showed a protective trend, with an OR of 0.86 (95% CI: 0.78-0.96, p=0.006). To confirm our findings, we conducted rigorous sensitivity analyses utilizing MR-PRESSO, MR-Egger, Cochran's Q, and leave-one-out methods. These analyses revealed no evidence of heterogeneity or horizontal pleiotropy, thereby reinforcing the robustness and reliability of our findings.

Conclusion: Our study results indicate that IgG45 contributes positively to osteoporosis, whereas IgG4 and IgG56 exhibit a negative correlation. Nonetheless, additional research is crucial to understand their mechanisms and devise broader preventive strategies for osteoporosis.

Keywords: Causal association, genome-wide association study, immunoglobulin G N-glycosylation, instrumental variables, Mendelian randomization, osteoporosis.

hormonal and cytokine interactions, although its exact mechanisms remain unclear.[6] Emerging evidence suggests that immune regulation and inflammation, including immunoglobulin G (IgG), may play a previously overlooked role in bone metabolism, potentially offering new therapeutic targets.

Immunoglobulin G N-glycosylation patterns (IGPs) have recently emerged as promising biomarkers for osteoporosis.[7] Specific IgG glycosylation alterations, including hypogalactosylation and hyperglucosaminidation, have been associated with disease progression in various chronic inflammatory conditions.[8] These modifications significantly affect the immunomodulatory functions of IgGs by altering their binding affinity to Fc gamma receptors (FcγR) and complement C1q through structural changes in the Asn297-linked N-glycans.[9,10] Notably, IgG may exert protective effects against glucocorticoid-induced osteoporosis via FcγRI interactions, suggesting a potential mechanistic link between IgG glycosylation and bone metabolism regulation.[7] These findings highlight the critical role of IgG glycosylation in bone homeostasis and provide new insights into osteoporosis pathogenesis from immunological and inflammatory perspectives. Furthermore, humanized monoclonal antibodies of the IgG2 isotype and subtype have emerged as effective and safe options for preventing fractures in patients with osteoporosis.[11] These findings underline the potential therapeutic implications of targeting IGPs in the management of bone health.

However, not all changes in IGPs are beneficial. In patients with multiple myeloma, high serum IgG levels can induce osteoclast activation, leading to bone loss.[12] It suggests a complex interplay between IgG glycosylation, inflammation, and bone metabolism. Indeed, inflammatory factors have been closely linked to IgG N-glycosylation and osteoporosis.[13] Changes in IgG subclasses and Fc glycosylation are associated with inflammatory diseases and hint at possible associations between inflammatory factors and osteoporosis.[14,15] Still, the number of observational studies is limited in establishing causality due to potential confounding and reverse causation. Further research is needed to clarify the underlying mechanisms and evaluate the therapeutic potential of targeting IGPs for osteoporosis prevention and treatment.

In the present study, the Mendelian randomization (MR) methodology was used to explore the potential causal links between various exposures and health outcomes.[16] It is a powerful method for uncovering causal relationships that are difficult to isolate in traditional studies due to confounders and reverse causality.[17] Following this approach, Sun et al.[18] recently demonstrated a causal link between human IgG N-glycosylation and aging. Yet, to the best of our knowledge, no MR study has investigated the connection between IGPs and osteoporosis. We, therefore, applied a two-sample MR analysis to test for a causal link between IGPs and osteoporosis, as well as between inflammatory cytokines and osteoporosis.

MATERIALS AND METHODS

Study design

This MR study was conducted at Zhejiang Provincial People's Hospital (Affiliated People's Hospital), Hangzhou Medical College, Department of Endocrinology, Rheumatology and Immunology, and the Information Center between November 26, 2024, and January 26, 2025 and reported following the Strengthening the Reporting of Observational Studies in Epidemiology using-MR (STROBE-MR) guidelines.[19] The analysis relied on the three core MR assumptions:[20] *(i)* the instrumental variables (IVs) are strongly associated with the exposure, *(ii)* the IVs are not associated with any confounding factors, and *(iii)* the IVs affect the outcome only through the exposure (Figure 1).

All data used in this Mendelian randomization study were obtained from publicly available genome-wide association study (GWAS) summary statistics, published literature, and other public databases. The study was conducted in accordance with the GWAS data access policies and ethical guidelines, and did not require additional ethical approval or written informed consent.

Data source

Summary-level data for osteoporosis were obtained from the FinnGen consortium (Release R11). The analysis included 9046 cases of osteoporosis and 429,826 controls, all of European ancestry. The osteoporosis phenotype was defined as a composite endpoint including cases diagnosed under the International Classification of Diseases, 10th Revision (ICD-10) codes M80 (osteoporosis with pathological fracture), M81 (osteoporosis without pathological fracture), and M82 (osteoporosis in diseases classified elsewhere).

Data on 41 inflammation cytokine traits were sourced from a GWAS published in the American

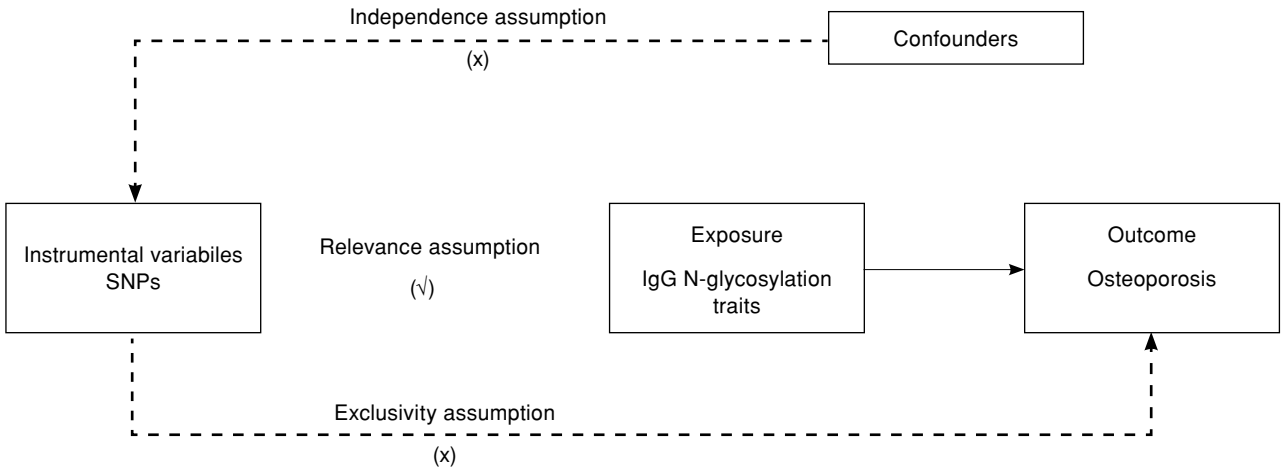

FIGURE 1. Overview of Mendelian randomization.
SNPs: Single-nucleotide polymorphisms; IgG: N-glycosylation patterns.

Journal of Human Genetics.[21] In addition, GWAS results for 77 IGPs from 8090 samples originating from the Medical Research Council (MRC) Human Genetics Unit were used, focusing on European ancestry (accessible via https://datashare.ed.ac.uk/handle/10283/3238).[22]

All GWAS data were sourced from European ancestry to avoid confounding caused by racial factors.

Instrumental variable selection

Single-nucleotide polymorphisms (SNPs) were selected as IVs for the exposures based on the following four criteria. First, SNPs associated with each exposure at a p-value threshold of $p<5\times10^{-6}$ were identified.[23] Second, to ensure independence, the SNPs were pruned for linkage disequilibrium (LD) using a strict cutoff ($r^2<0.001$, window size=10,000 kb).[24] Third, SNPs with a minor allele frequency (MAF) below 0.01 were excluded.[25] When selected IVs were not available in the outcome GWAS data, proxy SNPs with high LD ($r^2>0.8$) were used.[26] Finally, the F-statistic was calculated for each SNP to assess instrument strength, excluding any with an F-statistic <10 to minimize weak instrument bias.[27] The formula used was $F=R^2 * (N-2) / (1-R^2)$, where R^2 is the variance in the exposure explained by the SNP and N is the sample size.

Mendelian randomization analysis

The inverse-variance weighted (IVW) method was used as the primary approach to estimate the causal effect of IGPs on osteoporosis. The results were reported as odds ratios (ORs) with 95% confidence intervals (CIs).[28] Three additional MR analyses were also performed to support the robustness of the results: MR-Egger, weighted median (WM), and weighted mode.[29] The MR-Egger method is particularly advantageous in accounting for pleiotropic biases by considering potential intercepts, ensuring accurate causal effect estimates.[28,30] A *p* value of <0.05 was considered statistically significant. All analyses were performed using the "TwoSampleMR" package in R 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria).

Sensitivity analysis

Several sensitivity analyses were performed to test the robustness of the MR results. Cochran's Q test was used to quantify heterogeneity among IVs. A Q-test p value of >0.05 suggests no significant heterogeneity.[31] The MR-Egger regression intercept was used to test for horizontal pleiotropy; an intercept not significantly different from zero (p>0.05) indicates no evidence of pleiotropy.[32,33] The MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method was used to identify outlier SNPs (global p<0.05), and the causal associations were reassessed after their removal to correct for pleiotropy.[33] Funnel plots were generated for visual inspection of heterogeneity. A leave-one-out analysis was performed to check if any single SNP was driving the overall causal estimate.

RESULTS

Instrumental variable selection

In this study, 1,048 IVs related to 77 IGPs were screened (the mean F statistic was 43.14, with a

TABLE I
Relationship between IgG N-glycosylation traits and osteoporosis (Positive results)

Exposure	Outcome	N SNP	Method	OR	95% CI	*p*
IGP4	Osteoporosis	3	IVW	0.77	0.63-0.95	0.012
IGP4	Osteoporosis	3	MR Egger	1.09	0.65-1.83	0.794
IGP4	Osteoporosis	3	Weighted median	0.81	0.65-1.03	0.082
IGP4	Osteoporosis	3	Weighted mode	0.83	0.65-1.06	0.281
IGP45	Osteoporosis	15	IVW	1.10	1.01-1.19	0.021
IGP45	Osteoporosis	15	MR Egger	0.92	0.75-1.11	0.392
IGP45	Osteoporosis	15	Weighted median	1.11	1.00-1.24	0.061
IGP45	Osteoporosis	15	Weighted mode	1.12	0.95-1.32	0.191
IGP56	Osteoporosis	13	IVW	0.86	0.78-0.96	0.006
IGP56	Osteoporosis	13	MR Egger	0.95	0.59-1.52	0.831
IGP56	Osteoporosis	13	Weighted median	0.84	0.72-0.98	0.023
IGP56	Osteoporosis	13	Weighted mode	0.82	0.66-1.02	0.105

IGP: IgG N-glycosylation patterns; N SNP: Number of single-nucleotide polymorphisms included in the analysis; OR: Odds ratio; CI: Confidence interval; IVW: Inverse variance weighted; MR: Mendelian randomization.

minimum of 20.77 and a maximum of 1165.43) (Supplementary Table I). A total of 452 IVs related to 41 cytokines were screened. The F statistics of all identified SNPs were over 10, suggesting no indication of weak instrument bias in our analysis. All SNPs in the summary data are shown in Supplementary Table II and Table III.

Causal association between IGPs and osteoporosis

The results of the MR-Egger regression indicated the potential presence of horizontal pleiotropy while analyzing IGP45 as the exposure (Supplementary Table IV). Sensitivity analyses, including leave-one-out plots and the MR-PRESSO test, subsequently identified SNP rs909674 as an influential outlier (Supplementary Table V). After removing this outlier SNP, the re-analysis revealed a statistically significant positive association between genetically predicted IGP45 and osteoporosis (Table I). For some other IGPs where initial IVW analyses did not show a significant association, the MR-PRESSO test also detected potential outliers (Supplementary Table V). However, after removing these identified outliers, the associations for these traits remained non-significant or negative (Supplementary Table VI).

After eliminating outliers, we re-analyzed the results. The IVW results showed that IGP4 (OR (95% CI): 0.77 (0.63-0.95), p=0.012), IGP45 (OR: 1.1 (1.01-1.19), p=0.021) and IGP56 (OR: 0.86 (0.78-0.96), p=0.006) were causally associated with osteoporosis (Table I). Supplementary Table VII provides a comprehensive summary, including detailed descriptions of each IGP as well as their potential candidate genes, which were primarily extracted from the source study.[22] The scatter and forest plots of positive results are shown in Figure 2 and 3. The scatter plots showed that the slope of the trend line was less or more than 0, indicating that the protective or risk of an outcome decreases as the level of exposure increases (Figure 2). Similarly, the forest plots composite line lies to the left or right of 0 and the confidence interval does not contain 0, indicating a significant negative or positive causal relationship between IGPs and osteoporosis (Figure 3). However, no causal association between other IGPs and osteoporosis was found using IVW methods, and the other three methods (MR-Egger, WM, and weighted mode) were analyzed with similar results (Supplementary Table VIII).

In addition, in analyzing the correlation between 41 cytokines and osteoporosis, the IVW results show that interleukin (IL)-8 (OR=0.78, 0.63-0.98, p=0.0303) and monocyte chemoattractant protein-3 (MCP3) (OR=1.22, 1.08-1.38, p=0.0017) were causally associated with osteoporosis (Supplementary Table IX).

Sensitivity analysis

Furthermore, the results of MR-Egger regression indicated that this analysis was not influenced by horizontal pleiotropy and remained robust, as depicted in Table II. Cochran's Q test

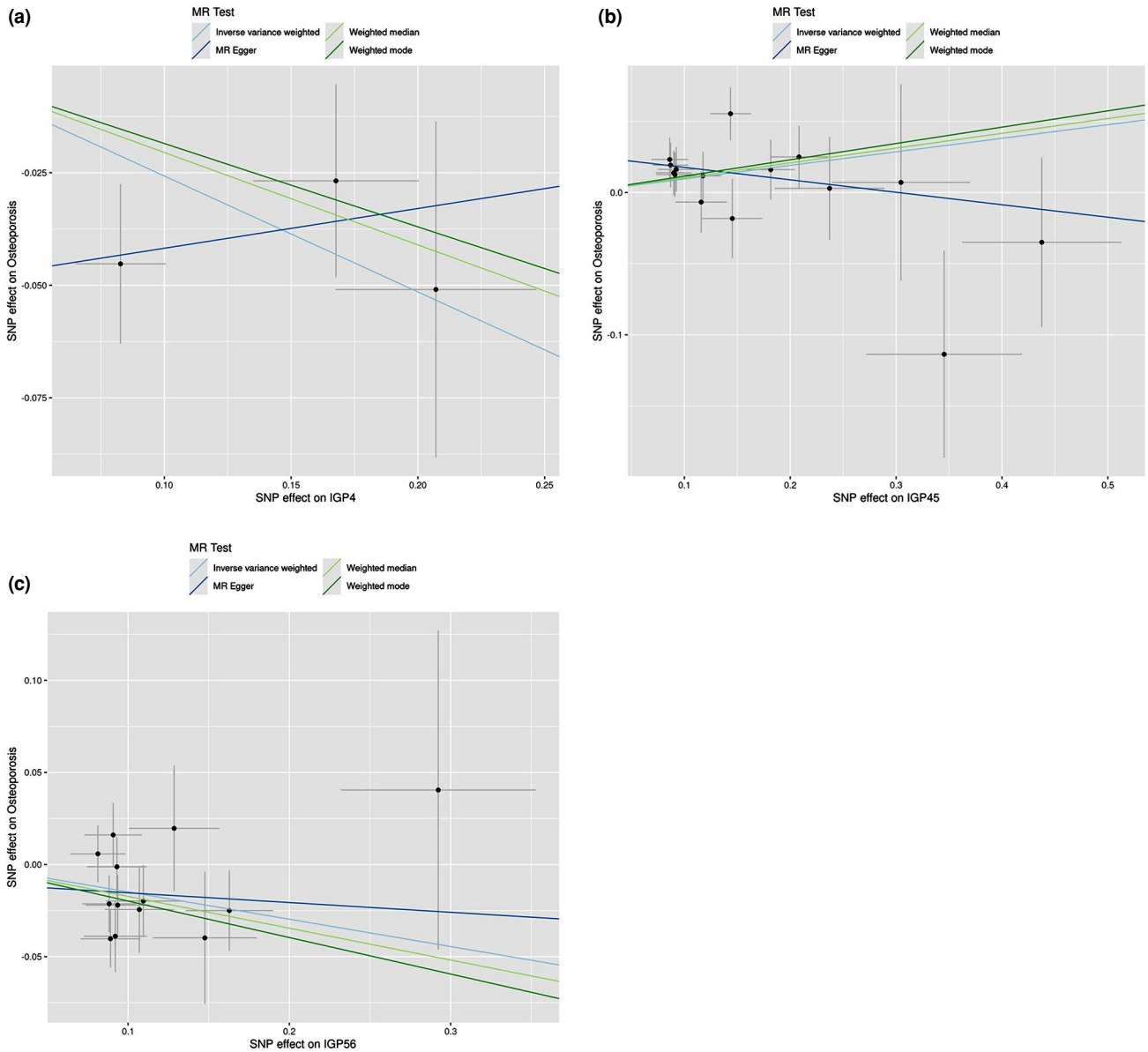

FIGURE 2. The Scatter plots between IgG N-glycosylation patterns and Osteoporosis (Positive results). The Scatter plots of **(a)** IGP4, **(b)** IGP45 and **(c)** IGP56 on osteoporosis.
MR: Mendelian randomization; SNP: Single-nucleotide polymorphism; IgG: N-glycosylation patterns.

results showed significant heterogeneity in the analysis of IGP15, IGP23, IGP24, IGP28, IGP31, IGP38, and IGP77 with osteoporosis (Table II). After eliminating outliers, the funnel plots suggested that there was no observable horizontal pleiotropy for the results of this analysis (Supplementary Figure 1). And the leave-one-out sensitivity analysis plots demonstrated that no single SNP was likely to have influenced the causal association and that our conclusions were, therefore, robust (Supplementary Figure 2). Similarly, the sensitivity analysis of cytokines on osteoporosis was robust (Supplementary Table X and Table XI).

DISCUSSION

In the present study, we used random-effects IVW to provide strong evidence for a causal relationship between IGPs and osteoporosis. We provide evidence that individuals with high levels of IGP45 have a higher risk of developing osteoporosis, whereas those with high levels of IGP4 and IGP56

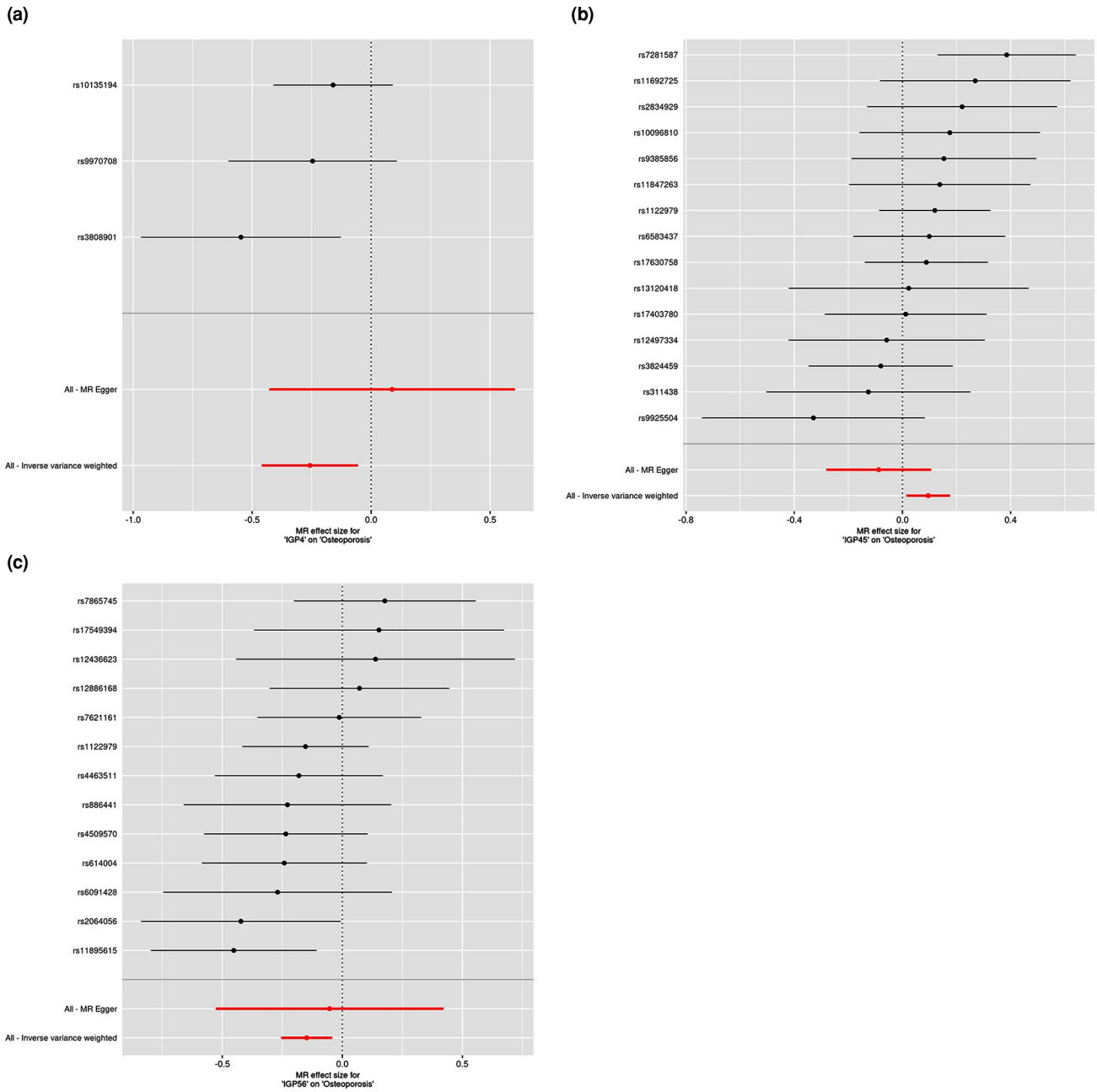

FIGURE 3. The Forest plots between IgG N-glycosylation patterns and Osteoporosis (Positive results). The Forest plots of **(a)** IGP4, **(b)** IGP45 and **(c)** IGP56 on osteoporosis.
MR: Mendelian randomization; IgG: N-glycosylation patterns.

have a lower risk of developing osteoporosis. But this association is not present in other IGPs and osteoporosis. These findings not only reveal the potential regulatory role of IgG glycoconjugate structure in bone metabolism, but also provide new molecular perspectives for understanding the immune-metabolic interaction mechanism of osteoporosis. By using genetic variants as IVs in our study, we believe that the MR approach avoids the major confounding factors of observational studies and can offer clearer insights into the causes of osteoporosis.

Immunoglobulin G N-glycosylated patterns are specific glycosylations in IgG's N-linked oligosaccharides, impacting IgG function,

TABLE II

Eliminated tests for horizontal pleiotropy and heterogeneity of IgG N-glycosylation traits and osteoporosis

		Heterogeneity		Pleiotropy	
Exposure	Outcome	Q statistic (IVW)	*p*	MR-Egger Intercept	*p*
IGP1	Osteoporosis	5.423	0.366	0.006	0.872
IGP10	Osteoporosis	7.815	0.855	0.012	0.391
IGP11	Osteoporosis	9.676	0.469	–0.009	0.589
IGP12	Osteoporosis	13.618	0.191	0.009	0.702
IGP13	Osteoporosis	7.794	0.649	–0.008	0.697
IGP14	Osteoporosis	10.926	0.692	0.003	0.874
IGP15	Osteoporosis	34.423	0.005	–0.008	0.508
IGP16	Osteoporosis	11.391	0.181	0.013	0.518
IGP17	Osteoporosis	7.604	0.574	–0.003	0.855
IGP18	Osteoporosis	15.214	0.173	–0.017	0.304
IGP19	Osteoporosis	11.354	0.124	0.071	0.147
IGP2	Osteoporosis	14.820	0.464	–0.006	0.398
IGP20	Osteoporosis	4.326	0.228	0.009	0.878
IGP21	Osteoporosis	3.842	0.798	0.008	0.803
IGP22	Osteoporosis	10.549	0.721	0	0.974
IGP23	Osteoporosis	11.269	0.024	0.014	0.721
IGP24	Osteoporosis	22.125	0.023	0.011	0.452
IGP25	Osteoporosis	20.997	0.179	–0.016	0.244
IGP26	Osteoporosis	12.474	0.489	0.006	0.613
IGP27	Osteoporosis	7.236	0.405	–0.037	0.373
IGP28	Osteoporosis	24.989	0.035	0.01	0.417
IGP29	Osteoporosis	25.805	0.104	0.009	0.273
IGP3	Osteoporosis	5.610	0.346	0.036	0.342
IGP30	Osteoporosis	25.700	0.058	0.019	0.319
IGP31	Osteoporosis	32.449	0.009	–0.002	0.898
IGP32	Osteoporosis	10.246	0.331	0.01	0.405
IGP33	Osteoporosis	11.535	0.317	0.03	0.255
IGP34	Osteoporosis	14.067	0.445	0.006	0.666
IGP35	Osteoporosis	12.511	0.406	–0.012	0.237
IGP36	Osteoporosis	14.899	0.136	–0.036	0.222
IGP37	Osteoporosis	22.137	0.104	–0.016	0.326
IGP38	Osteoporosis	33.378	0.010	–0.022	0.216
IGP39	Osteoporosis	21.643	0.086	0.005	0.663
IGP4	Osteoporosis	2.414	0.299	–0.051	0.394
IGP40	Osteoporosis	18.705	0.133	0	0.998
IGP41	Osteoporosis	4.105	0.128	–0.011	0.918
IGP42	Osteoporosis	15.220	0.363	–0.009	0.423
IGP43	Osteoporosis	5.904	0.434	0.03	0.386
IGP44	Osteoporosis	0.680	0.878	0.013	0.699
IGP45	Osteoporosis	14.972	0.380	0.026	0.064
IGP46	Osteoporosis	9.633	0.381	0.006	0.752
IGP47	Osteoporosis	24.033	0.195	–0.017	0.303

TABLE II
Continued

		Heterogeneity		Pleiotropy	
Exposure	Outcome	Q statistic (IVW)	*p*	MR-Egger Intercept	*p*
IGP48	Osteoporosis	20.962	0.051	0.026	0.152
IGP49	Osteoporosis	19.753	0.410	−0.014	0.207
IGP5	Osteoporosis	21.800	0.193	0.018	0.192
IGP50	Osteoporosis	10.299	0.415	−0.022	0.119
IGP51	Osteoporosis	6.909	0.547	−0.017	0.336
IGP52	Osteoporosis	13.391	0.269	−0.006	0.749
IGP53	Osteoporosis	6.849	0.445	0.022	0.479
IGP54	Osteoporosis	13.629	0.478	−0.037	0.081
IGP55	Osteoporosis	4.717	0.787	0.018	0.265
IGP56	Osteoporosis	12.583	0.400	−0.01	0.693
IGP57	Osteoporosis	11.988	0.214	−0.013	0.661
IGP58	Osteoporosis	13.932	0.176	0.013	0.497
IGP59	Osteoporosis	8.427	0.587	−0.005	0.699
IGP6	Osteoporosis	7.020	0.635	0.001	0.961
IGP60	Osteoporosis	12.463	0.569	0.001	0.921
IGP61	Osteoporosis	10.081	0.523	−0.002	0.899
IGP62	Osteoporosis	11.197	0.738	−0.003	0.672
IGP63	Osteoporosis	12.746	0.388	0.004	0.818
IGP64	Osteoporosis	20.755	0.237	0.001	0.839
IGP65	Osteoporosis	8.681	0.851	−0.002	0.917
IGP66	Osteoporosis	22.050	0.282	0.001	0.910
IGP67	Osteoporosis	11.956	0.610	0.001	0.941
IGP68	Osteoporosis	19.749	0.474	−0.005	0.679
IGP69	Osteoporosis	16.293	0.503	−0.01	0.445
IGP7	Osteoporosis	15.144	0.585	0.012	0.319
IGP70	Osteoporosis	22.365	0.216	0.001	0.874
IGP71	Osteoporosis	22.353	0.217	0.001	0.880
IGP72	Osteoporosis	23.573	0.132	0.010	0.404
IGP73	Osteoporosis	16.288	0.131	−0.002	0.919
IGP74	Osteoporosis	16.791	0.604	−0.007	0.605
IGP75	Osteoporosis	16.790	0.604	−0.007	0.607
IGP76	Osteoporosis	28.344	0.077	0.015	0.333
IGP77	Osteoporosis	29.569	0.009	0.031	0.100
IGP8	Osteoporosis	14.996	0.183	0.011	0.579
IGP9	Osteoporosis	19.975	0.173	−0.008	0.648

IGP: IgG N-glycosylation patterns; IVW: inverse variance weighted.

FcγR binding, immunomodulation, and inflammation.[34,35] Of note, IGP45 represents the proportion of G0FN-type glycans in the total neutral IgG glycan chain, and its core modification is characterized by the absence of galactose residues but the presence of fucose.[22] The present MR study suggests that IGP45 may be a risk factor for osteoporosis. The proportion of IGP45 is lower in chronic inflammatory and autoimmune diseases, suggesting a potential role in immune responses.[36,37] Different IGPs and their proportions alter IgG function and their

binding to FcγR.[38] Hence, the different IGPs and their proportions affect inflammation signaling.[39] In addition, IGP45-related glycosylation changes IgG's structure, interactions, and immune response nature, directly affecting IgG functions like ADCC and CDC, increasing susceptibility to infections, and indirectly influencing bone metabolism.[40,41]

However, findings from MR suggest that IGP4 and IGP56 may be protective factors against osteoporosis. The IGP4 and IGP56 are specific IGPs, and their glycosylated forms may have an important impact on IgG function and activity. IGP4 represents the proportion of mannose pentasaccharide (M5) in the total IgG glycan chain, which is a high-mannose-type glycan chain usually associated with immature B-cell-derived IgG and has low pro-inflammatory activity, while IGP56 reflects the proportion of all monogalactosylated structures in the neutral IgG glycan chain, which is suggestive of low overall glycan chain maturity.[22] On the one hand, IGP4 and IGP56 may be involved in the promotion of bone formation and the inhibition of bone resorption. IGP4 and IGP56 may have a direct role in promoting osteoblast activity, thereby increasing the synthesis and mineralization of bone matrix. This helps to maintain or increase bone mass and reduce the risk of osteoporosis. In contrast to IGP45, IGP4 and IGP56 may reduce bone resorption by inhibiting osteoclast activity or reducing the number of osteoclasts, helping to maintain bone stability and strength. On the other hand, it may be involved in regulating the endocrine system. There is a close interaction between the bone and endocrine system.[42] IGP4 and IGP56 may indirectly affect bone health by influencing the secretion or activity of hormones related to bone metabolism (e.g., estrogen, parathyroid hormone, vitamin D, etc.).[43] Furthermore, IGP4 and IGP56 may have synergistic effects with other known protective factors (e.g., appropriate physical activity, balanced diet, adequate sun exposure, etc.). Together, these protective factors act on the skeletal system to help maintain bone health and stability.

In view of the causal relationship between IGPs and osteoporosis described above, we believe that the levels of these IGPs can, on the one hand, serve as potential markers of osteoporosis and can be used for early diagnosis and risk assessment, and on the other hand, the understanding of the protective/risk mechanism of IGPs in osteoporosis can provide new targets for the development of new therapeutic approaches; In addition, personalized therapeutic regimens can be developed based on the level of IGPs in patients. In the future, further basic research is needed to explore the specific mechanisms of IGPs in the pathogenesis of osteoporosis, including how they affect inflammatory responses, osteogenesis, immunomodulation, and anti-oxidative stress. There is still a need to validate the relationship between IGPs and osteoporosis risk in larger cohorts and to explore their applicability in different populations. Intervention strategies targeting IGPs should also be developed to find new therapeutic approaches.

The N-glycosylation patterns of IgG not only affect its immune function, but also interact with inflammatory factors through a variety of mechanisms which, in turn, jointly affect bone metabolism and the development of osteoporosis. Inflammatory factors can alter the glycosylation pattern of IgG by regulating the activity or expression of glycosylation enzymes;[44] altered glycosylation affects the immunoreactivity of IgG, including its affinity for the FcγR, which in turn regulates the intensity of the inflammatory response.[13] Previous MR studies have shown that inflammatory factors may have a causal relationship with osteoporosis.[45] The current MR results disagree with prior studies on IL-8 as an osteoporosis protector. Multiple MR studies support IL-8's protective role, suggesting it reduces the risk of bone loss. IL-8's anti-apoptotic effect and angiogenesis promotion may be key mechanisms. The complexity and varying inflammatory factor roles of osteoporosis, in addition to external factors, contribute to diverse findings. Several studies have demonstrated that different patterns of N-glycosylation can significantly alter the function of IgG, giving it anti-inflammatory or pro-inflammatory properties.[44] To illustrate, complex glycan chains containing fucose (e.g., IGP4 and IGP56) usually exhibit anti-inflammatory properties.[46] and are able to inhibit FcγR-mediated immune responses.[47] and reduce the release of inflammatory cytokines. High mannose-type glycan chains without fucose (like IGP45) tend to elicit stronger immune responses and boost inflammatory cytokines. Inflammatory factors, in turn, affect IgG glycosylation, which is altered by metabolic changes during chronic inflammation. The IL-6 and tumor necrosis factor-alpha (TNF-α) specifically contribute to osteoporosis by activating osteoclasts and inhibiting osteoblasts.[48] The IgG glycosylation modifications may affect the level of inflammation *in vivo* by modulating the

anti-inflammatory or pro-inflammatory activity of IgG. This regulation may indirectly affect the onset and progression of osteoporosis. Overall, the roles of IgG glycosylation and inflammatory factors in osteoporosis may be combined. While inflammatory factors may directly affect bone metabolism and remodeling, IgG glycosylation may indirectly affect bone health by regulating the activity of inflammatory factors.

There are several strengths of this study. This is the first two-sample MR study to investigate the causality between IGPs and osteoporosis, which is the closest approximation to a randomized-controlled study and allows for random allocation based on the genotype. This study design can prevent some limitations of conventional observational studies, including reverse causation and potential confounding factors. The large sample sizes of included studies and IVs robustly associated with IGPs (F statistics ≥10) are used in our MR study. Nevertheless, certain limitations should also be considered. Although this study used stringent screening criteria for IVs, the threshold for some IVs for this screening was 10-6, which was larger than the usual 10-8 and not the conventional threshold, and this more lenient criterion may have had an impact on disease outcome. Moreover, all participants included in the study were European, which may limit the generalizability of our findings to other ethnic populations. So, more studies should be conducted to confirm the applicability of our findings to other populations. On the other hand, the specific mechanism of action of IGPs in osteoporosis has not been thoroughly investigated. Therefore, there is a lack of sufficient experimental evidence to support a direct link between the protein and osteoporosis. Finally, there is still a need to evaluate the potential of IGPs as diagnostic and prognostic biomarkers for osteoporosis.

In conclusion, our study performed MR analysis of the putative causal relationship between IGPs and osteoporosis. The results highlight the strong association between IGPs and osteoporosis. These findings emphasize the potential role of early screening and prevention of IGPs in patients with osteoporosis. Nonetheless, further well-designed studies are warranted to establish more reliable conclusions on this subject.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Carried out the studies, participated in collecting data, and drafted the manuscript: Y.Z., Y.M.Z.; Performed the statistical analysis and participated in its design: C.Y.Y., C.Y.Y.; Participated in acquisition, analysis, or interpretation of data and draft the manuscript: W.Z., L.J.W.; Designed the study, supervised the research and revised the manuscript: L.J.W. All authors read and approved the final manuscript.

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