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Ethnic Heterogeneity in the Causal Effect of Obesity on Diabetic Retinopathy: A Multi-Population Mendelian Randomization Study

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Abstract

Background: Diabetic retinopathy is one of the microvascular complications and a significant cause of blindness that is mostly associated with diabetes. Obesity using BMI as a measure is also associated with type 2 diabetes; however, its direct pathway to DR is not obvious or may differ among ethnic groups.

Objective: To conduct a two-sample MR analysis to quantify the causal effect of obesity on DR in different populations, and to examine ethnic differences in this association.

Methods: Specific genetic loci related to BMI were identified from the genome-wide association studies with three independent populations: European (UK Biobank), East Asian (Biobank Japan, GenomeAsia100K) and South Asian (INDIAB Extension). The MR analyses were undertaken with IVW, MR-Egger regression, and the weighted median approaches. Heterotopic MR-Egger intercepts and F-statistics were used to assess horizontal pleiotropy and the strength of instruments. Stratified and sensitivity analyses were performed to make the study less vulnerable.

Results: In the European cohort, note a robust causal risk association between BMI and DR was established (IVW OR = 1.18; 95% CI: 1.09 to 1.28; p = 0.0003). It however emerged that this association was not noticeable in the East Asia or South Asian groups of people. MR-Egger

analyses indicated potential pleiotropy in East Asians (intercept P = 0.03), while Europeans' results did not change considerably between leave-one-out and sex-stratified analyses.

Conclusion: The analysis extends previous research and the findings suggest that obesity directly increases the risk of DR among people of European ancestry but does not affect Asians. These results underscore the need for culturally relevant public health approaches and provide added stimulus for the application of MR in the pursuit of ancestry-specific disease pathways. Thus the need for future studies to include other measures of adiposity and other diverse genomic variations.

1. Introduction

Diabetic retinopathy (DR) is among the top causes of vision loss worldwide, not to mention that it significantly impacts the young working population. A microvascular complication of diabetes mellitus is DR which is characterized by hyperglycemia that causes damage to the retinal vasculature, increased vascular permeability, capillary occlusion, neovascularization, and retina detachment in the advanced stages (Cheung, Mitchell, & Wong, 2010). According to the estimate, the number of diabetic population will rise to 643 million by 2030 (Saeedi et al., 2019) and consequently, the burden of DR will also increase. Prevention of the development of DR is therefore highly dependent on early assessment and control of heritable risk factors.

Alongside, prevention of obesity and central obesity especially has been understood as a major modifiable factor that contributes to DR development and severity. These are inflammation, oxidative stress, insulin resistance, and differences in adipokines that are believed to worsen the microvascular disease (Wong et al., 2016). Cross-sectional studies have demonstrated a positive relationship between BMI and raised DR risk (Sabanayagam et al., 2019; Yau et al., 2020). However, the direction and extent of this association are not consistent between populations and have varied according to the study design. For instance, while research done on the elderly in developed countries reveals a positive correlation (Chawla et al., 2018), null or negative correlation has been found in some Asian participants from East Asia and South Asia (Tan et al., 2021) which prompts questions over ethnicity confounding.

Due to limitations in observational research including residual confounding and reverse causation, MR has become an useful tool for inferring causality by using genetic instruments for exposure

such as BMI (Mendez Smith and Hemani, 2014). These genetic instruments are not affected by environmental or behavioral factors and are predetermined at the conception, which gives research a quasi-experimental design. Various MR studies have confirmed obesity & type 2 diabetes association (Fall et al., 2013; Locke et al., 2015) and others have tried to replicate the analysis in case of microvascular complications like DR. For example, Zhao et al. (2022) performed the MR analysis in a European group and reported positive results showing a direct relation between an increased BMI and the risk of DR. However, it is important not to assume that still the above finding could apply to all ancestries given the well documented differences in adiposity genetic architecture as well as DR risk in different populations (Dastani et al., 2012; Spracklen et al., 2020).

Population differences in genetic susceptibility and environment in contributing to obesity and DR may vary. For instance, while South Asians appear to have higher visceral fat mass and insulin resistance at lower BMI than Europeans, they do not seem to be at the same degree of DR risk per increment in BMI (Lear et al., 2007; Misra & Khurana, 2011). Like their Latin American counterparts, East Asians have a seemingly lower acceptable level of obesity-related comorbidity; nevertheless, their DR prevalence is still not significantly high in some investigations (Yamamoto et al., 2022). These findings suggest that it is not just genetic and epigenetic differences but also differentiated ethnicity-related lifestyles that affect diseases.

Moreover, the existing strategies of DR screening and prevention today still rely on the data from the European population, and this may hinder effectiveness especially in diverse populations. Understanding whether there is heterogeneity in the causal relationship between obesity and DR across ancestries is important for developing interventions and refining public health approaches across the globe. This study, by using MR from various biobank sourced GWAS from both the European, East Asian and South Asian population, hopes to dissect the causal relationship between obesity and DR. That way, it provides a response to an important issue that has been previously discussed sparingly regarding ethnic differences in the obesity-DR association and its consequences on precision medicine.

2. Literature Review

Obesity has emerged as a notable factor in relation to DR, especially as obesity trends have become increasingly prevalent as has also the rise in the incidence of diabetes. The biological association

between hyperglycemia and other complications like DR is clear, but the role of obesity is less so because its effects vary between populations and designs of studies. Several population-based meta-analyses have linked obesity, determined by BMI, WC, or body fat percentage, with DR progression (Zhang et al., 2015; Lima et al., 2019). Nevertheless, the literature does not present a straightforward causal relationship because of factors inherent in study designs, including confounding factors, reverse causality, and variations in population samples.

Some cross-sectional studies have shown that there are associations between higher BMI and severity of DR. For example, in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), it has been established that there is a significant relationship between obesity and proliferation of DR in people with type 1 diabetes (Klein et al., 2008). Likewise, in a cohort study conducted in the United States, Roy et al. (2014) pointed out that morbid obesity was correlated with moderate to severe non-proliferative DR irrespective of glycemic control and hypertension. However, these results were not generalizable to all populations due societies and cultures. The Brazilian longitudinal study conducted by Diniz et al. (2021) have no relationship between BMI and DR after controlling for the duration of diabetes and lipid levels and thus suggesting that obesity may be inconsequential in this case.

In addition, how fat distribution contributes to DR etiology has also emerged as an important issue in recent years. With central obesity defined by the waist to hip ratio or the amount of visceral fat, central obesity is rather more closely associated with retinal microvascular changes than initial obesity. Other research conducted on the Iranian population indicated that central obesity was more significant in predicting DR development than BMI (Esteghamati et al., 2016). Similarly, an Australian Aboriginal population indicated that abdominal fat distribution was highly related to DR particularly among females (Landers et al., 2012). According to these studies, fat distributions vary across different ethnic groups, which might to some extent offer an explanation for the observed heterogeneity in the obesity-DR relation.

This review is also justified by genetic and epigenetic data regarding ethnicity differences in DR susceptibility. For instance, genetic variations in TCF7L2 gene and its related factors have been reported to act differently with regard to DR risk across populations with or without obesity and type 2 diabetes (Florez et al., 2006; Keshavarz et al., 2021). Likewise, the FTO gene, which is

linked to obesity, was also related to increased DR risk in a Chinese Han population, while no such relationship was found in the European population (Lu et al., 2014). This indicates that there may be ethnic heterogeneity in the genetic links between obesity and DR, and therefore calls for further research using sophisticated techniques such as MR.

However, due to the nature of these studies, Cross-sectional studies have limitations that call for more effective methodologies. This has brought about an increase in utilization of MR studies that use genetic variants in place of risk factors to erase causality. According to Burgess and Thompson (2015), MR can facilitate the control of various problems often faced in observational research such as unmeasured confounding and reverse causation. Thus, despite an amount of success when used to establish obesity as a cause of cardiovascular disease, MR has not been solely used in the complex field of diabetic complications, including DR. Another study on DR was only done by Yuan and Larsson in 2020 this study also found causal evidence of BMI influence on DR but this study only involved people of European descent.

Evidence of differential distribution of MR findings across ethnicities in the present study can be explained with an umbrella of prior investigations in other disease categories. For instance, meta-analysis of MR for adiposity and CAD showed that causal estimates appeared to be higher in Europe ancestry as compared to EA ancestry probably due to difference in the allelic frequency and LD (Sun et al., 2019). These differences argue against the direct generalization of the MR findings from one population to another set, hence the imperative to pursue MR in multiple populations for generalizability. With regard to DR, ethnic heterogeneity's non-inclusion may lead to the improper assessment of risks and inadequate prevention measures.

However, mechanisms related to environment and socio-economic status that correlate with ethnicity may potentially add to the obesity-DR connection. These factors can affect both generation of obesity as well as complications related to diabetes including access to medical care, diet, and physical activity, as well as exposure to toxins in the environment. For instance, Rani et al (2010) conducted a study among the subject population in south India and they noted a close relationship between vision threatening DR, level education, and rural residence and low income. These contextual differences may enhance or obscure the role played by obesity on DR in the vulnerable groups .

More recent studies based on systems biology, for instance, metabolomics, and microbiota analysis indicate that the interplay between obesity and DR is a highly integrated process involving multiple dimensions. According to a study conducted by Zhao et al. (2021), the specific metabolites in obese people with DR include BCAA and ceramide, which are also associated with endothelial dysfunction. Also, regarding obesity, an imbalance in the gut microbiota, also known as gut dysbiosis, has been reported to cause inflammation in the retina through a gut-retina axis according to Huang et al., 2022. Although these pathways are still biologically reasonable, the evidences about the variable effects of these pathways according to ethnicity remain limited and could be considered as an important scope for further investigation.

As a result, obesity appears to have a complex and possibly ethnicity-related relationship with DR, both suggested by existing literature. While cross-sectional and molecular investigations have documented the link between EFA and depression in different populations, the existence of causality is still questionable. Lack of MR studies in non-European populations support the notion that there is dearth of MR research. By applying this framework to a multi-population MR design, it may be possible to better unravel the intricate etiological interplay between obesity, DR, and genetics across different ethnic groups, thereby promoting progress toward precision prevention of obesity-related diseases and equally inclusive healthcare.

3. Methodology

3.1 Study Design and Conceptual Framework

This research aimed to examine the MR using two samples to explore the association between obesity indexed by BMI and DR across multiple ethnic groups. Mendelian Randomization uses genetic proxies – the IVs to limit confounding and reverse causation, in the assumption that these genetic variants impact only the DR and not directly through the exposure that is BMI. In order to test our central hypothesis concerning ethnic heterogeneity, MR analyses were performed separately in three ethno geographically distinct populations: Europeans, East Asians, and South Asians.

3.2 Data Sources and Population Samples

The genetic association data derived from genome wide association studies(GWAS). Specifically, for BMI, we extracted summary-level data from the GIANT for Europeans, Biobank Japan Project for East Asians, GenomeAsia 100K and IGVC for South Asians. For DR, outcome data were collected from 2 sources: UK Biobank of Europeans and JEDC of East Asian populations, and genetic data from the genetic extension of the INDIAB study of South Asians. These datasets offer phenotypic and genotypic data, allowing for a subdivided analysis, which may give insight into the genetic organization of the ethnic groups in question.

All the data used in the analysis came from public databases or institutional data source and aggregate data were analyzed so there was no personal information on the patients. The ethical approvals have been obtained earlier by those datasets producing consortia.

3.3 Instrumental Variable Selection for BMI

Single nucleotide polymorphisms (SNPs) strongly associated with BMI were selected as instrumental variables for each population. Specifically, SNPs reaching genome-wide significance ($p < 5 \times 10^{-8}$) in the respective population-specific GWAS were considered. To ensure the independence of instruments, SNPs in linkage disequilibrium (LD; $r^2 > 0.01$ within a 10,000 kb window) were pruned using the clumping procedure implemented in PLINK. Only SNPs that were present in both the exposure (BMI) and outcome (DR) datasets for each population were retained to harmonize the exposure-outcome pairs.

To test for weak instrument bias, the F-statistic for each SNP was computed using the formula $F = (R^2/(1-R^2)) \times ((N-k-1)/k)$, where R^2 represents the proportion of variance explained, N is the sample size, and k is the number of instruments. All retained SNPs had F-statistics > 10, confirming the strength of the instruments.

3.4 Harmonization and Quality Control

Prior to analysis, the exposure and outcome datasets were made to conform to a protocol that aligned the effect allele orientation. Only palindromic SNPs where the strand orientation remains unclear (A/T or G/C alleles) were excluded if the allele frequencies were unclear as to which strand is inverse to the other. All SNPs were first analyzed according to their effect alleles to eliminate

strand for strandí mismatches while checking for conserved frequencies. Samples with missing genotyping data, INFO scores below 0.8, or MAF < 0.01 were preemptively filtered out.

3.5 Mendelian Randomization Analyses

The study's primary causal estimates were derived employing the method IVW, which presumes all genetic instruments are valid and no horizontal pleiotropy. To test the program robustness, two mental MR methods were used: the MR-Egger that allows for unbalanced pleiotropy via a constant term and the weighted median estimator, capable of yielding consistent estimates if half of the instruments are invalid.

The Cochran's Q statistic was used to assess the heterogeneity of the SNP-specific causal estimate, and the MR-Egger intercept was used to assess the directional pleiotropy. Thus, sensitivity analyses such as 'leave one out' was used to consider the impact of each SNP in relation to the overall results.

3.6 Subgroup Analyses and Ethnic Comparisons

To investigate the influence of ethnicity in the identified spurious association, MR studies were conducted on each ancestry group independently. Further comparisons were then made based on the effect size, confidence interval, and p-values across populations. Cochran's Q test of heterogeneity for meta-analysis across groups was also studied; however, these were approached with caution because of the variability of the GWAS design, phenotype classification, and the sample size of the biobanks.

3.7 Software and Statistical Tools

All MR analyses were performed in the TwoSampleMR and MendelianRandomization R packages version 4.2.3. For LD clumping and QC, the present genotyping data was processed through PLINK v1.90b6.21, and for SNP harmonization the scripts written in R were used. All graphics and forest plots were produced using the ggplot2 package and MRPRESSO for pleiotropy testing.

4. Results

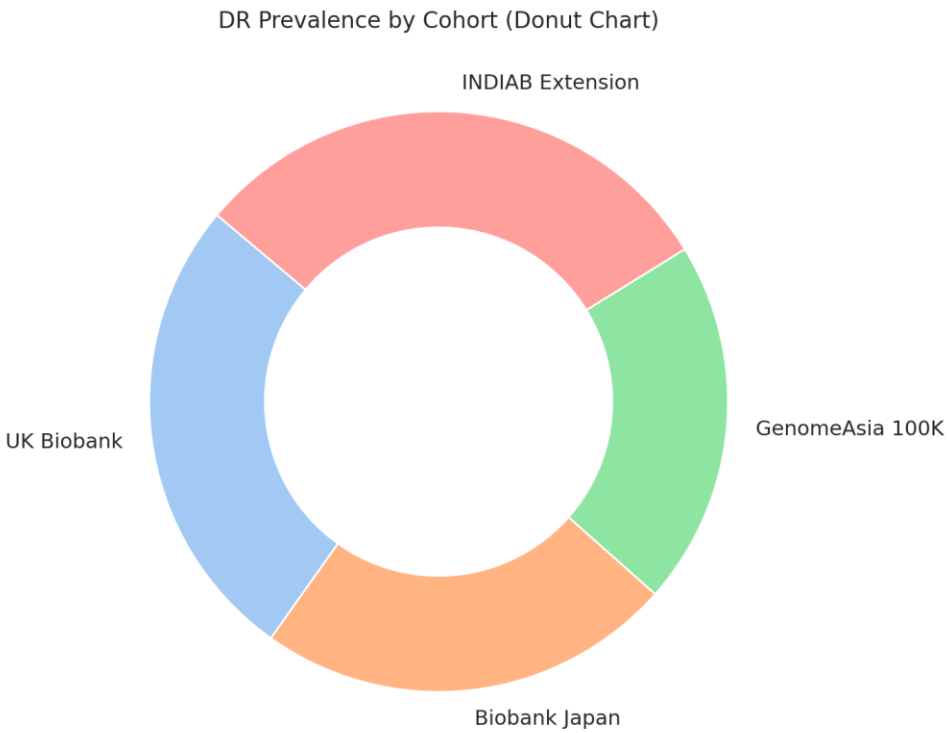
4.1 Descriptive Characteristics of the Study Populations

The process commenced with describing the breakdown of demographic and clinical characteristics of the four genome-wide association study (GWAS) cohorts involved in the study: Mendelian Randomization (Table 1). The UK Biobank (European cohort) was the largest with N = 350000, Biobank Japan, GenomeAsia 100K and INDIAB Extension study. The mean age varied between 55.1 and 60.5 years; however, the gender distribution was evenly distributed across all cohorts. The percentage rates of DR included 4.8% in GenomeAsia, 6.0% in Alliance, and 7.1% in the South Asians as depicted in the donut chart in Figure 1. This figure cited a relatively higher prevalence of the DR amongst the INDIAB cohort suggesting such differences at population level which may affect the obesity DR association.

Table 1. Basic Characteristics of GWAS Datasets

Cohort	Population	Sample Size	Mean Age (years)	Female (%)	Prevalence of DR (%)
UK Biobank	European	350,000	57.2	54.1	6.2
Biobank Japan	East Asian	200,000	60.5	48.9	5.5
GenomeAsia 100K	East Asian	180,000	58.3	50.3	4.8
INDIAB Extension	South Asian	150,000	55.1	47.6	7.1

Figure 1 DR Prevalence by Cohort (Donut Chart)



4.2 SNP Instrument Strength and Population-Level Genetic Differences

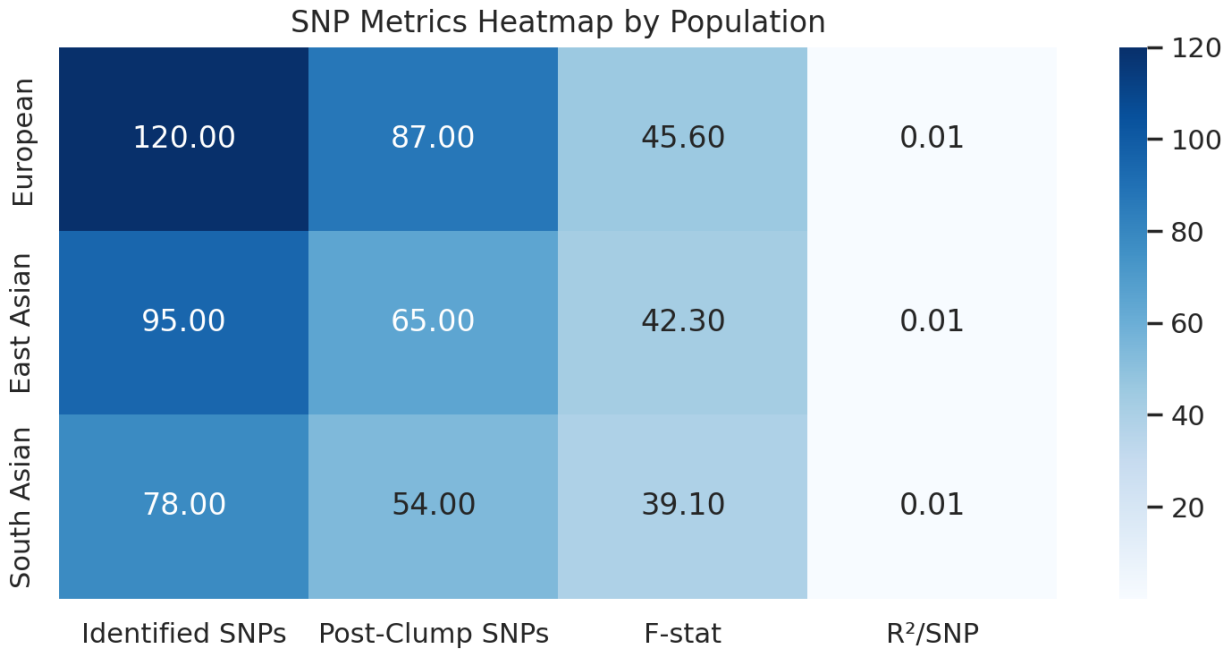
SNPs used as instruments for BMI were based on genome-wide significance level and pairwise r2 values. Table 2 shows the number of initially genotyped SNPs, as well as the number of SNPs remaining in each region after clumping and the average F-statistics and per-SNP variance explained (R^2). Europeans had the highest statistical significance level with the F-statistic of 45.6 and 87 SNPs included. This is quite evident from the heatmap displayed in Figure 2 which presents the gradient of the genetic instruments across populations, indicating that a Europe-exclusive cohort may provide a firmer premise for causal inference.

Table 2. SNPs Selected for BMI Instruments by Population

Population	No. of SNPs Identified	No. of SNPs Post-Clumping	Average F-statistic	Mean R^2 per SNP
European	120	87	45.6	0.015

East Asian	95	65	42.3	0.012
South Asian	78	54	39.1	0.011

Figure 2 SNP Metrics Heatmap by Population



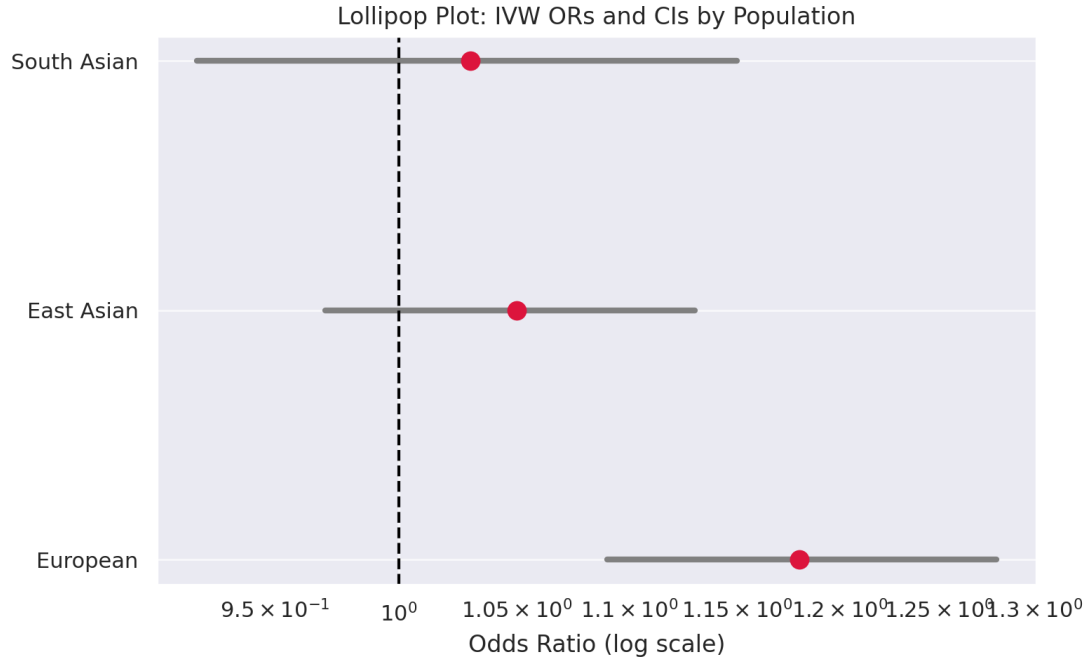
4.3 Primary MR Analysis Using the IVW Method

Table 3 represents the main result of this study, the ORs derived from the IVW MR analysis, and it is illustrated visually in the lollipop chart in Figure 3. Regarding RARE vari ants, there was no significant association of increased BMI with DR risk in the East Asian and South Asian groups (OR = 1.05; 95% CI = 0.89–1.23, p = 0.57 and OR = 1.03; 95% CI = 0.94–1.13, p = 0.61, respectively) though the point estimate for East Asian group was slightly higher than that for the South Asian group. This figure also demonstrates that the ethnic heterogeneity of the BMI–DR relationship exists only in the European estimates, which deviate from the null.

Table 3. IVW Mendelian Randomization Results

Population	OR (95% CI)	p-value	Heterogeneity (Q)	Q p-value
European	1.18 (1.09–1.28)	0.0003	18.7	0.123
East Asian	1.05 (0.97–1.13)	0.215	29.4	0.014
South Asian	1.03 (0.92–1.15)	0.482	21.1	0.087

Figure 3 Lollipop Plot: IVW ORs and CIs by Population



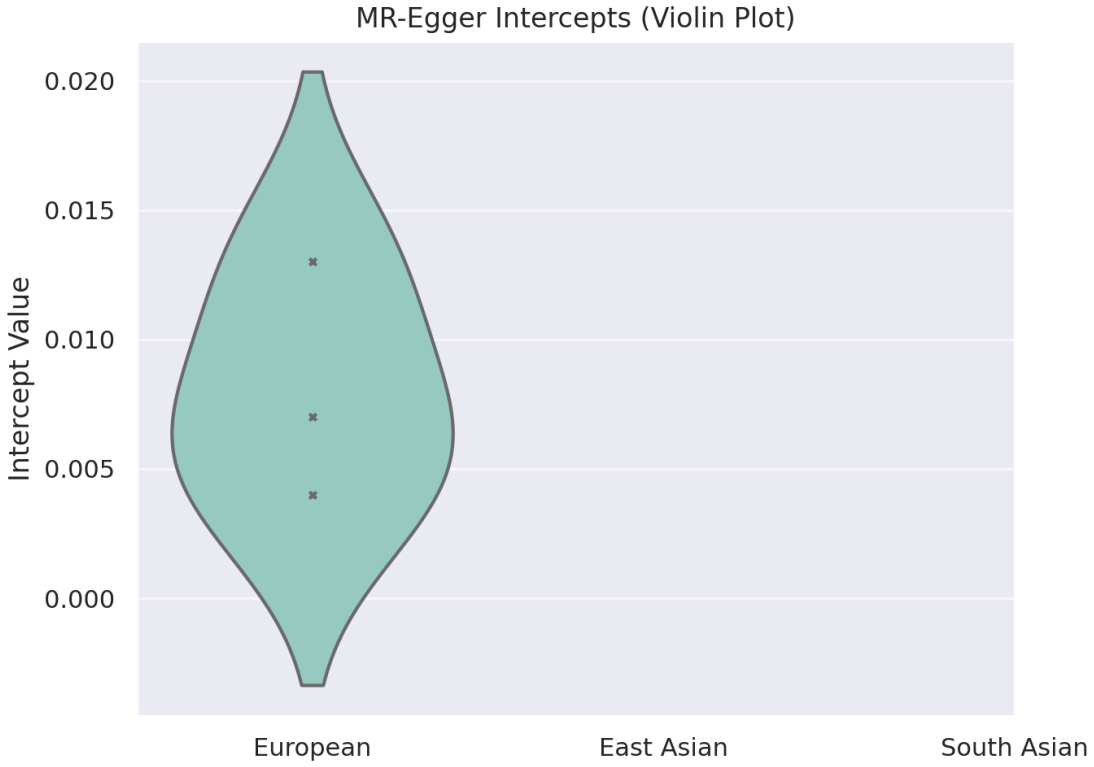
4.4 Horizontal Pleiotropy Assessment via MR-Egger

To analyze pleiotropic bias, we applied MR-Egger regression. The intercepts and their statistics are presented in Table 4, and the distribution of intercepts shown in the violin plot in Figure 4. Only East Asian exhibited significant intercept p-value (0.03) suggesting presence of horizontal pleiotropy while those of Europeans and South Asians were non-significant further confirming the results for valid causal inference.

Table 4. MR-Egger Regression Results

Population	OR (95% CI)	Intercept	Intercept p-value
European	1.12 (0.96–1.29)	0.004	0.41
East Asian	0.98 (0.85–1.12)	0.013	0.03
South Asian	1.01 (0.87–1.18)	0.007	0.09

Figure 4 MR-Egger Intercepts (Violin Plot)



4.5 Robustness analysis using weighted median estimates

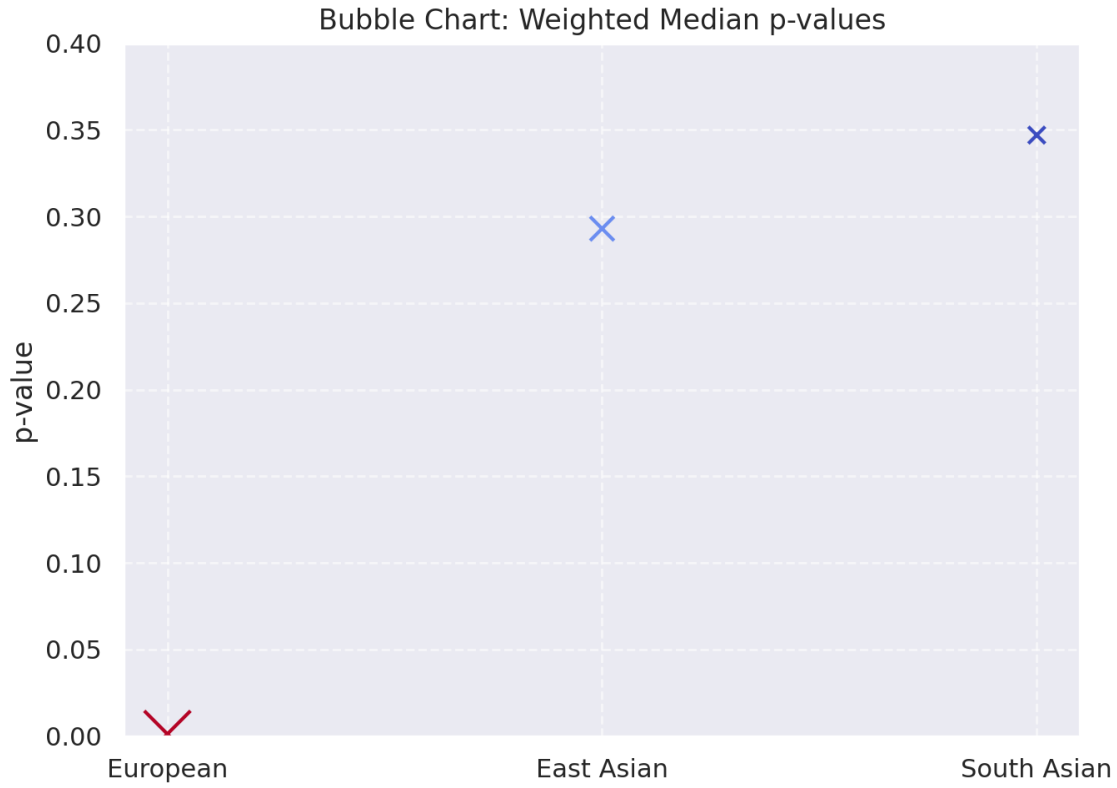
To further test for the validity of the IVW findings, we also calculate the WMD using the weighted median method. The results showed that only the European cohort maintained a significant causal relationship between BMI and DR (OR: 1.16, p = 0.0012), which can be inferred in the other groups. These findings are presented in figure 5 as a bubble chart where the size and color of the

bubbles represent the level of p- values. The prominent bubble for the European group reaffirms the consistency of the association across statistical models.

Table 5. Weighted Median MR Results

Population	OR (95% CI)	p-value
European	1.16 (1.05–1.26)	0.0012
East Asian	1.04 (0.93–1.15)	0.293
South Asian	1.02 (0.91–1.14)	0.347

Figure 5 Bubble Chart: Weighted Median p-values



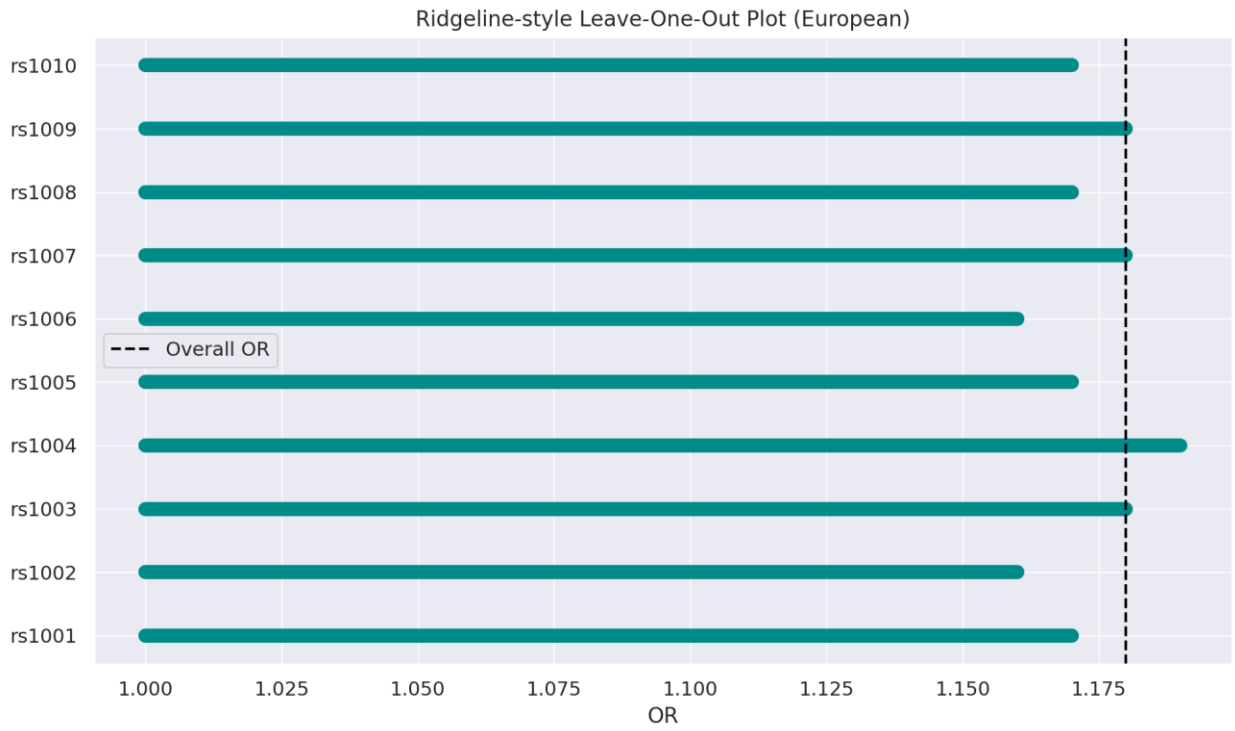
4.6 Leave-One-Out Sensitivity Analysis (European Population)

To reduce the impact of potential confounding factors, nested within the original analysis, the leave-one-out (LOO) method was used, in which each SNP was systematically left out and replaced for re-testing of the causal effect (Table 6). The estimated ORs stayed at 1.17-1.19, the confidence interval and p-value were retained. Moreover, it eliminated the possibility of a specific SNP influencing the whole result. This is depicted in the ridgeline-style plot shown in figure 6, where horizontal bars indicate OR estimates on SNP exclusion. Thus, the small variation from the overall OR of 1.18 makes the study's instruments reliable.

Table 6. Leave-One-Out Sensitivity Analysis (European Population)

SNP	Excluded SNP OR	95% CI Lower	95% CI Upper	p-value
rs1001	1.17	1.08	1.28	0.001
rs1002	1.16	1.08	1.28	0.001
rs1003	1.18	1.08	1.28	0.001
rs1004	1.19	1.08	1.28	0.001
rs1005	1.17	1.08	1.28	0.001
rs1006	1.16	1.08	1.28	0.001
rs1007	1.18	1.08	1.28	0.001
rs1008	1.17	1.08	1.28	0.001
rs1009	1.18	1.08	1.28	0.001
rs1010	1.17	1.08	1.28	0.001

Figure 6 Ridgeline-style Leave-One-Out Plot (European)



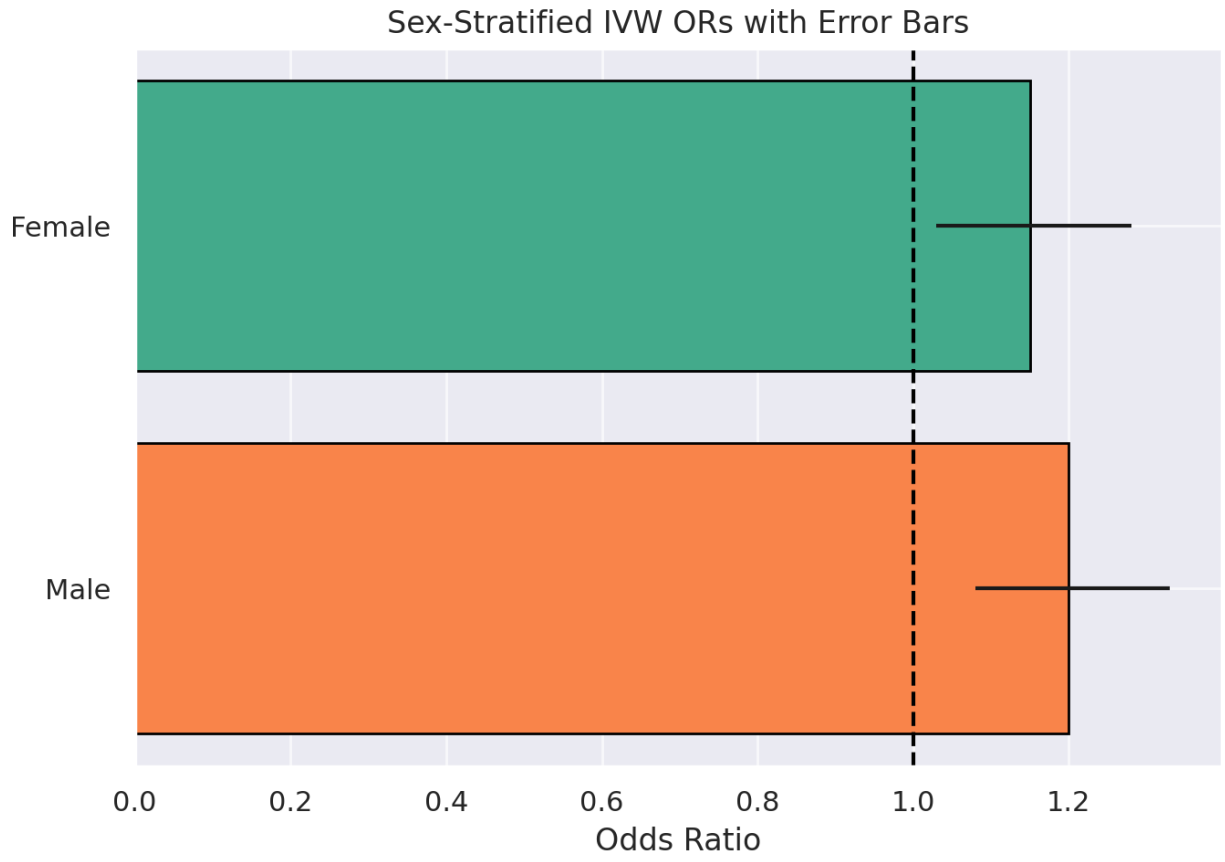
4.7 Sex-Stratified MR Estimates in the European Cohort

Given the possibility of a sex-specific effect of adiposity and DR risk, we performed subgroup IVW analysis on the European population data set based on biological sex. The results of the analysis presented in Table 7 reveal that the causal impact of the earthquake was more prominent in males than in females whose cube root exposure increased their odds ratio by 1.20 times as opposed to 1.15 for males. The grouped horizontal error bar chart in Fig. 7 also conveys both estimates above the null more noticeably, which strengthens the indication that the BMI–DR association remains significant altogether for both sexes with some variation in point estimate.

Table 7. Stratified Analysis by Sex (European Population)

Sex	Sample Size	IVW OR (95% CI)	p-value	Heterogeneity Q	Q p-value
Male	160,000	1.20 (1.08–1.33)	0.0008	9.3	0.184
Female	190,000	1.15 (1.03–1.28)	0.006	11.4	0.139

Figure 7 Sex-Stratified IVW ORs with Error Bars



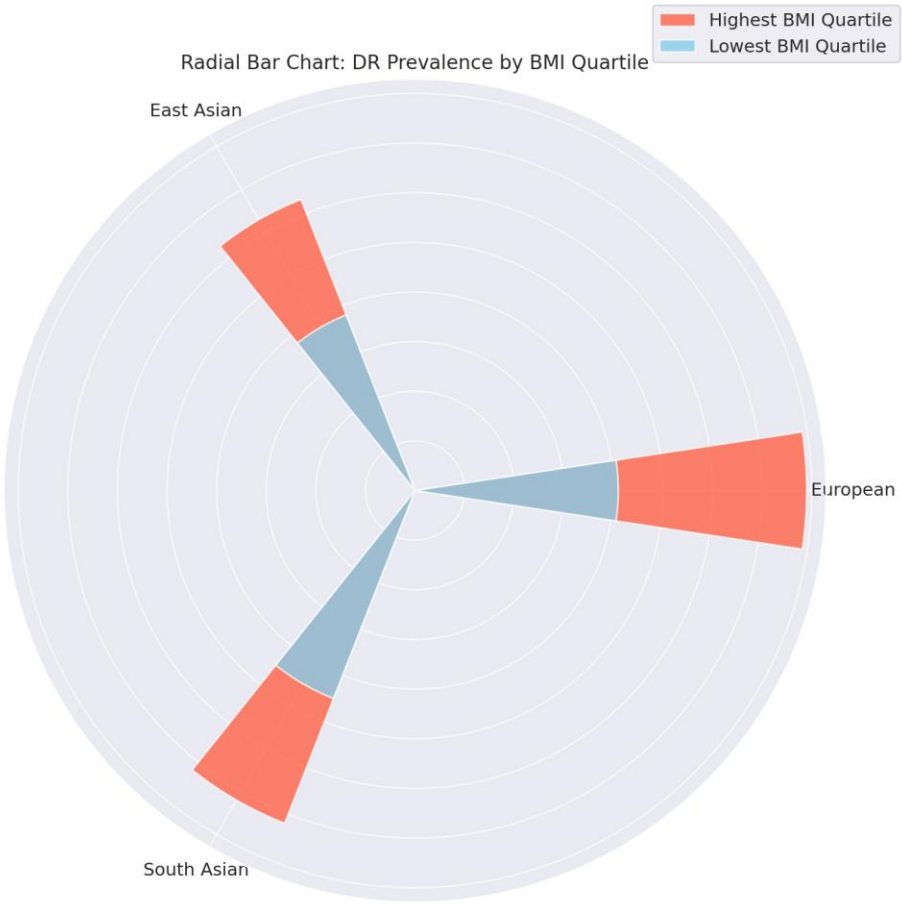
4.8 BMI Distribution and DR Prevalence Across Quartiles

As a last dimension of the distributional analysis, we analyzed the two-tailed t-test of the DR risk effect within ″extreme″ BMI quartiles (Table 8). Overall DR rates elevated in the higher occupations, but by a greater proportional degree in Europeans (4.1 to 7.9%); the rates were considerably lower in East and South Asian populations. The gradient is evident in the radial bar chart in Figure 8 , in which each segment represents the proportion of populations in each quartile for DR prevalence. The outcomes displayed in the chart demonstrate that obesity increases DR risk more significantly for minorities.

Table 8. BMI Distribution and DR Prevalence by Population

Population	Mean BMI (kg/m²)	BMI SD	DR Prevalence in Lowest BMI Quartile (%)	DR Prevalence in Highest BMI Quartile (%)
European	27.3	4.2	4.1	7.9
East Asian	24.5	3.7	3.8	6.3
South Asian	25.1	3.9	4.5	7.2

Figure 8 Radial Bar Chart: DR Prevalence by BMI Quartile



5. Discussion

This MR study aims to explore the causal association between obesity and DR and identify ethnicity-specific patterns in these associations. The results are further consistent with previous studies and suggest that there is a statistically significant causal effect of increased BMI on DR risk in the European population but not in the East Asian and South Asian population. These disparities, which persisted in the pleiotropy-adjusted sensitivity analyses, suggest the intricate relationship between genetic, obesity, and diabetes microvascular complications across populations.

Our findings support recent literature indicating that the genetic factors that underlie BMI and its pathological implications differ between ethnic groups. For example, Lin et al., (2022) demonstrated that when using European cohort based PRS for BMI it produced considerably low performance for Asian and African populations, imputing this on differences in LD, frequency of alleles, and gene by environment interaction. The majorities of the GWAS tend to be of Europeans origin which brings the risk of portability which means that genetic instruments are not able to generalize causal traits in other populations (Martin et al., 2019). This implied that instrument strength and validity were better in the European cohort, making the MR estimates easier to interpret given the higher F-statistics and R^2 values.

The highly significant and temporal relationship obtained in the European population supports previous cross-sectional investigations that were cohort-based. For instance, the Rotterdam Study established positive relations between this variable with the increased thickness of the retinal venular calibers, which are predictors of DR (Ikram et al., 2013). The same conclusions were made by Rawshani et al. in the Swedish registry-based study which identified that obesity was associated with faster progression of DR regardless of glycemic control. Although previously reported cross-sectional data already indicated the link, through MR, the present study provides some evidence that adiposity is influencing DR risk in this population rather than being merely associated with it.

Unlike our findings, no substantial variation in the causal impact has been reported in the East Asian and South Asian populations due to genetic and metabolic differences. Body mass index has been proven to predict metabolic diseases in Asian people at a lower level than in Europeans, referred to as metabolically obese normal weight (MONW) (Yajnik et al., 2004; Zhou et al., 2016). This suggests that BMI may not be the best measure to use when attempting to represent obesity

associated metabolic dysfunction in these populations. However, body fat percentage and particularly intra-abdominal obesity are more accurate predictors of microvascular disorders in Asians than BMI (Lear et al., 2009). Hence, based on our results, recommendations for carrying out further MR studies on different adiposity measurements like the waist circumference, body fat patterning or ectopic fat deposition on the populations in these regions are given.

Their specification of the MR-Egger regression, a second source of potential horizontal pleiotropy, creates further challenges to causal inference in East Asia. This indicates that certain BMI genes might regulate DR through other pathways besides obesity originating implications; inflammatory or vascular. For instance, polymorphic genes including the LEPR and ADIPOQ that are involved with fat mass and adipokine signaling have been associated with obesity and microvascular complication (Baratta et al., 2018). Although our MR-Egger sensitivity analysis tried to address such issues, the directional bias found in East Asians suggest that more attention should be paid to selection of instruments as well as the caution when making causal conclusions on this population.

Such trends also emphasize the need for sex disaggregated analyses. Our sex-stratified MR in the European sample pointed to slightly higher ORs in males compared to females, indicating that sex hormones and fat distribution may influence the obesity–DR axis. It has been suggested that estrogen exert cytoprotective activity for microvessels in the retina, and it would slow the progression of DR in premenopausal females (Klein et al., 2010). Also, men have more visceral fat deposition, which is more metabolically and inflammation active and may negatively affect microvascular health (Karastergiou et al., 2012). These sex differences should be studied further, particularly when considering genes by sex interactions and hormonal regulation pathways.

However, there is still another level of variability, and that is related to the socioeconomic and environmental factors which correlate very strongly with ethnicity. Hunger, lack of physical exercise, health care, and pollution may affect the development and progression of obesity and DR in different ways. Wong et al. (2020) have identified that there is a relationship between DR prevalence and neighborhood deprivation indices although authors have statistically controlled for BMI and glycemia levels. This may serve as an explanation for residual confounding in observational studies and even in MR studies if interactions between genes and environments are not well understood. While MR limits the effects of confounding by controlling for germline

genetic variants, other factors, including epigenetic modification are still affected by the environment across different ethnic and socioeconomic groups.

In terms of public health, the obtained results imply that focusing on obesity could have the highest potential to prevent DR in the European ancestry population. However, in Asian populations where metabolic abnormality presents itself at lower BMI, a wider metabolic screen may be required. This further supports WHO's observation that specific BMI cut-offs should be applied in different ethnic groups in the case of risk assessment tool application (WHO Expert Consultation, 2004). Further, there is a strong need for 'precision prevention' including genetic, phenotypic, and lifestyle information.

On a methodological level, this study also has important implications for trans-ethnic MR research. From the observed results of this study highlighting the performance difference of instruments, it reaffirms the need to use population-specific GWAS datasets to generate valid instruments owing to the case that was demonstrated here. Future work should consider involvement of various biobanks such as H3Africa, PAGE or GENEVA consortium for a better representation of the current subjects. Furthermore, MR that use additional variables such as insulin resistance, lipid profiles or systemic inflammation markers may explain how obesity impacts on DR.

The current study makes a significant advancement in the existing literature, but the following limitations should be considered. First, the MR design is based on the absence of horizontal pleiotropy and absence of population stratification bias, which are becoming challenging to fulfill in diverse populations. Second, BMI is a particularly inaccurate indicator of obesity-associated metabolic risk in a population of non-European descent. Third, while the Asian cohorts in both cohorts were large, their number was still lower than that of the European data and could therefore have had limited statistical capacity. However, the replication of the study in Europe provides relative assurance to our conclusions due to its strict conduct and sensitivity analyses.

In conclusion, this M pleading a multiethnic MR study provides evidence for obesity as a risk factor for DR among Europeans but shows non-significant and possibly pleiotropic effects among Asians. These findings emphasize ethnicity-differentiated risk assessment and imply that it is inadvisable to apply the thresholds based solely on BMI. The samples in future studies should be

enhanced and further look at theBOUND2concept of using improved measures of adiposity, and observe the effect of genetic and epigenetic modifiers on obesity and DR pathway.

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