

Triglycerides and cardiovascular disease: A Mendelian Randomization Study

Jingran Wang

School of Biology and Basic Medical Sciences, Soochow University, Suzhou, China

Abstract

The causal relationship between triglycerides and cardiovascular disease remains uncertain despite positive associations observed in observational studies, which are susceptible to confounding and reverse causation. This study aimed to investigate the potential causal relationship between triglycerides and cardiovascular disease using a two-sample Mendelian randomization (TSMR) approach. Instrumental variables (IVs) representing independent genetic variants associated with triglycerides were derived from the Triglyceride Genetic Epidemiology (TG-GENE) consortium, encompassing genetic data from thousands of individuals. Outcome data for cardiovascular disease, including heart disease, stroke, and atherosclerosis, were obtained from a GWAS meta-analysis. TSMR analyses were conducted using various methods, such as inverse variance weighted (IVW), MR-Egger regression, weighted median estimator (WME), to explore the causal relationship between triglycerides and cardiovascular disease. The results from the Mendelian randomization analyses suggest a potential causal association between elevated triglyceride levels and cardiovascular disease, which indicates that limit the consumption of triglyceride is possibly an effective method for the prevention of cardiovascular disease.

Keywords: Mendelian randomization; triglyceride; causal inference; cardiovascular disease

1. Introduction

Cardiovascular disease is a leading cause of death in developed countries, accounting for 50% of the population. By 2015, it is estimated that one in three deaths worldwide will be due to cardiovascular diseases. Effective prevention strategies are crucial to address this global health issue [1]. Obesity is considered as one of the significant risk factors for cardiovascular disease which leads to the development and mortality of cardiovascular disease of other cardiovascular risk factors [2].

One significant risk factor for obesity-related diseases, is the level of triglycerides in the plasma [3]. Studies have shown a strong prevalence of high triglyceride levels and low high-density lipoprotein cholesterol in individuals with high risk of cardiovascular disease [4].

Mendelian randomization (MR) is a method that uses genetic variants as instrumental variables to determine whether a risk factor causally affects a health outcome [5]. With the advancements in genome-wide association studies (GWAS) in the past decade, MR has been used to establish causal relationships between various factors and diseases [6]. However, there is limited research focused on the causal relationship between triglycerides and cardiovascular disease.

In this study, we utilized a two-sample Mendelian randomization approach to estimate the causal effect of triglycerides on cardiovascular disease. We retrieved data from the publicly available GWAS on the OpenGWAS project.

2. Material

2.1 Data Retrieval

Gene data related to exposure to triglycerides can be collected from the OpenGWAS website(<https://gwas.mrcieu.ac.uk/datasets/ieu-b-111/>). The dataset of UK Biobank includes 441,016 from European. The outcome variable data for cardiovascular disease was also from OpenGWAS website(https://gwas.mrcieu.ac.uk/datasets/finn-b-19_CVD/), which had 111,108 cardiovascular disease cases and 107,684 controls of European ancestry.

2.2 Select Instrumental Variables

After obtaining the data, we first process the exposure data by performing linkage disequilibrium (LD) pruning, with a threshold of $r^2 < 0.001$ and a distance of < 10000 kb. We then filter the SNPs that have genome-wide significance ($p < 5 \times 10^{-8}$) [7].

Next, we need to harmonize the exposure and outcome data - which means the effect estimates are always on the same allele. Then we use the MR-PRESSO package to detect the outliers and assess the level of pleiotropy in SNP measurements (Distribution = 1000, SignifThreshold = 0.05), we detected 7 SNPs as outliers and removed them from the dataset [8]. Finally, we applied 262 SNPs as instrument variables.

3. Method

All analyses were performed in RStudio using the R programming language. We utilized the TwoSampleMR, RadialMR, and MR-PRESSO packages for the analysis. These packages provide comprehensive tools and functions specifically designed for conducting Mendelian randomization analyses.

3.1 Mendelian Randomization

To estimate the causal effect of triglyceride levels on heart disease, we mainly employed the inverse-variance weighted (IVW) method [9], which provides a consistent and efficient estimation. This method combines the genetic variant-specific causal estimates using inverse-variance weighting, giving more weight to the variants with stronger instrument-exposure associations [10].

In addition to the IVW analysis, we also employed other methods such as MR-Egger and Weighted Mendelian randomization to further observe the results [11]. By comparing the results from these different methods, we aimed to gain a more comprehensive understanding of the causal relationship between the exposure and outcome variables and assess the robustness of our findings.

3.2 Sensitivity Analyses

To assess the robustness of our findings, we performed several sensitivity analyses. We also conducted a test for horizontal multicollinearity on the data [12]. Additionally, we conducted a leave-one-out analysis to examine the influence of individual genetic variants on the overall causal estimate [13].

4. Results

4.1 Mendelian Randomization results

Table 1. The Mendelian randomization analysis on the impact of elevated levels of triglycerides on the risk of heart disease. We can observe that all statistical methods demonstrate a certain level of statistical significance. And, using the IVW (Inverse Variance Weighted) method, we can find that there is a causal relationship between triglyceride levels and cardiovascular disease.

method	nsnp	b	se	pval	OR
MR Egger	262	0.06862212	0.03453709	4.798178e-02	1.071031
Weighted median	262	0.10383050	0.03390872	2.198203e-03	1.109412
Inverse variance weighted	262	0.11098038	0.02380374	3.126834e-06	1.117373
Simple mode	262	0.09991700	0.07728071	1.971855e-01	1.105079
Weighted mode	262	0.07882310	0.03001151	9.138577e-03	1.082013

(OR, odds ratio; nsnp, number of SNPs)

Figure 1. The scatterplot depicting the association between triglyceride levels and heart disease includes 95% confidence intervals for the associations between SNPs and triglyceride levels, as well as SNPs and the risk of heart disease. The x-axis represents the association between SNPs and triglyceride levels in standard deviation units, while the y-axis represents the association between SNPs and the risk of heart disease using log odds ratios.

The plot includes five regression lines, representing five different statistical regression methods. Upon observation, we can notice a high similarity among the five lines, indicating strong stability of the results. Additionally, all the lines have positive slopes. From this, we can infer that as triglyceride levels increase, the risk of heart disease also increases.

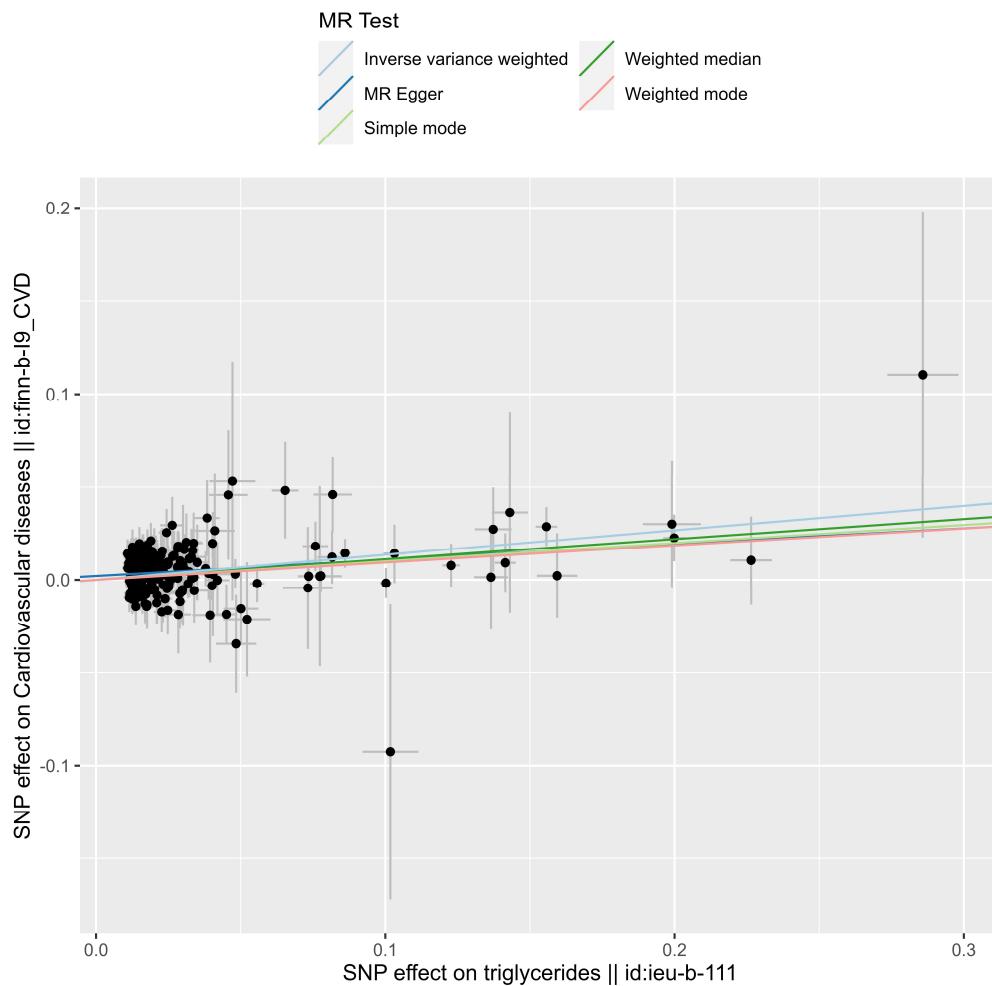
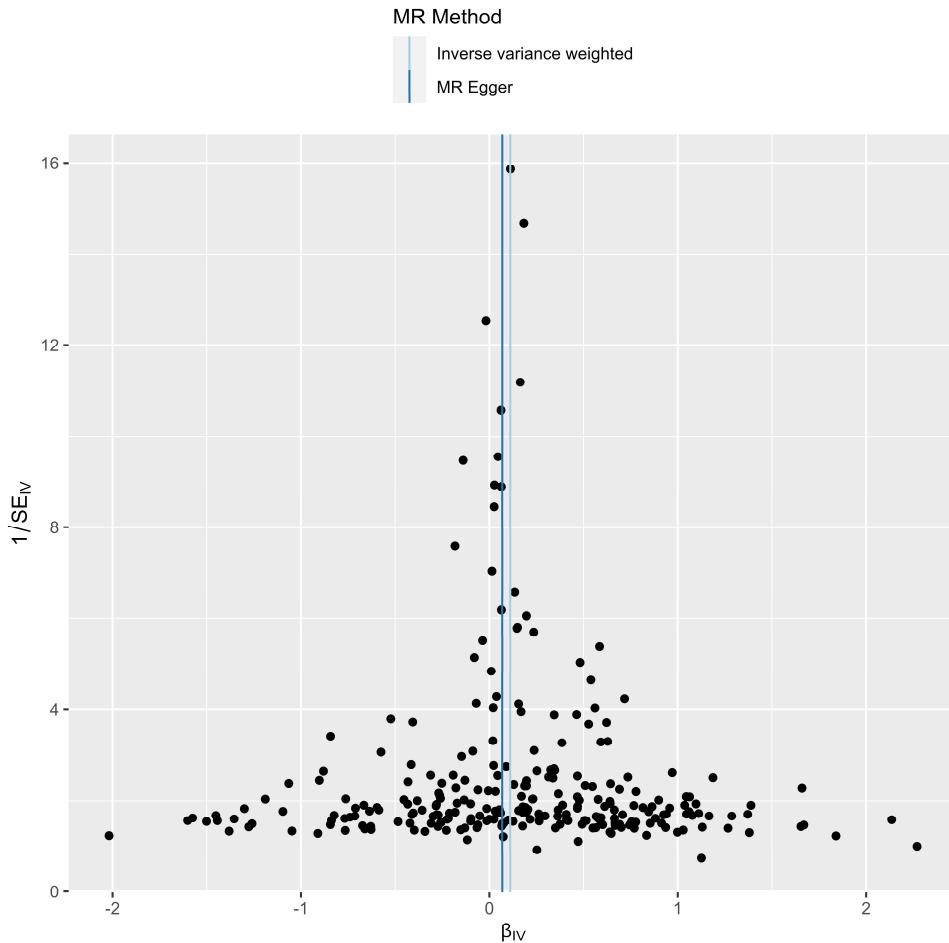


Figure 2. The funnel plot, used to evaluate Mendelian randomization analysis, displays each data point representing the results using a single SNP as an instrumental variable. The two lines represent the overall estimates obtained from the inverse variance-weighted method and MR-Egger regression. The funnel plot indicates that no single SNP has a significant impact on the statistical results. The distribution of all data points

is approximately symmetrical, suggesting that the effect of increasing triglyceride levels on the risk of cardiovascular disease follows a symmetric distribution.



4.2 Sensitivity Analyses results

We conducted a test based on Cochran's Q-statistics to assess the randomization results, and found significant heterogeneity ($p < 0.05$). Upon comparing with the database, the presence of heterogeneity may be attributed to the relatively stable population in the Finnish cohort (with minimal population mobility), resulting in genetic differences between the local population and the European population.

To address the issue of heterogeneity, we employed a random-effects model to estimate the effect size.

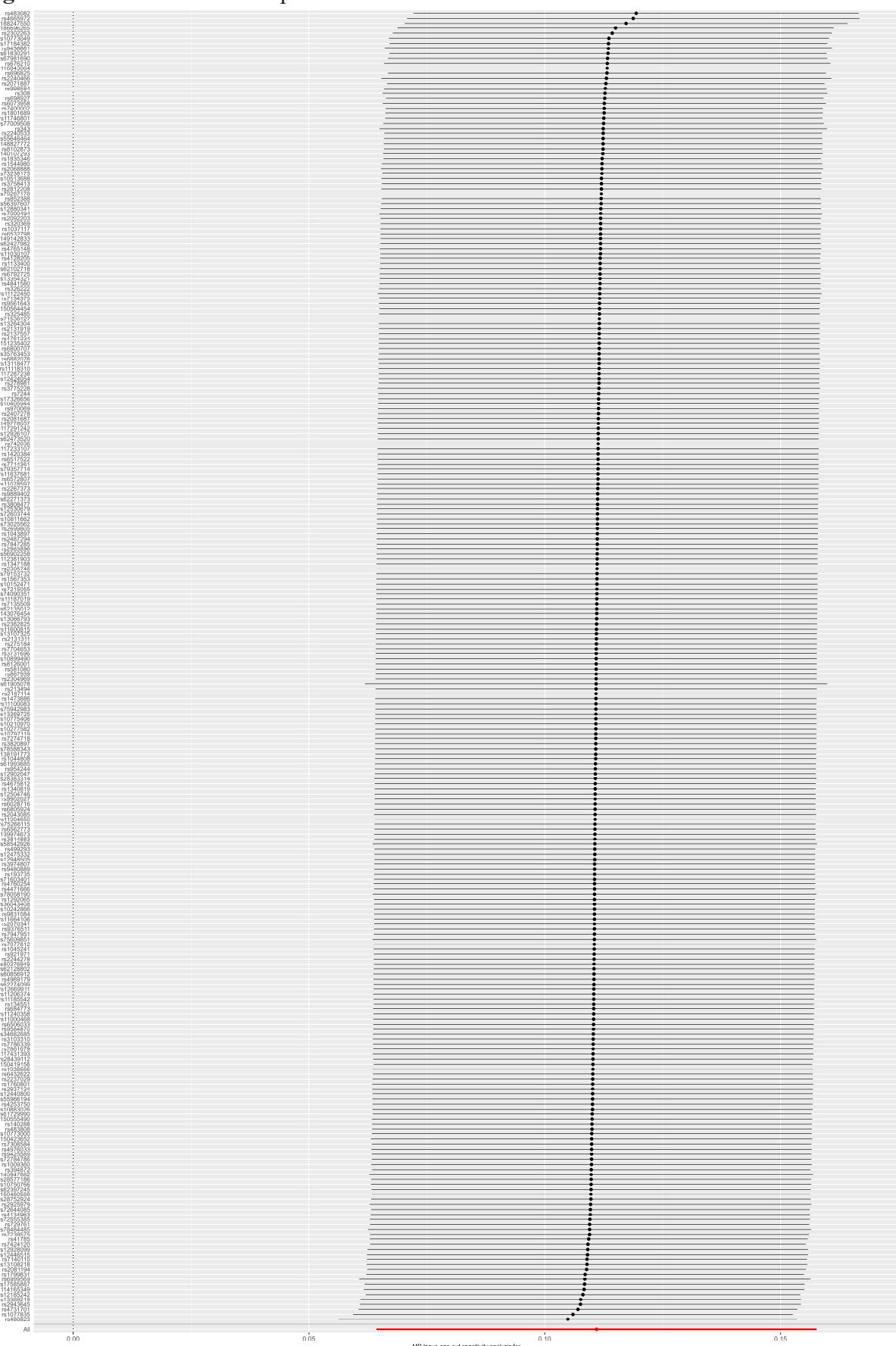
Table 2. Through the random-effects model, we can observe the positive causal relationship between triglycerides and heart disease ($p < 0.05$, $b > 0$).

method	nsnp	b	se	pval
Inverse variance weighted (multiplicative random effects)	262	0.1109804	0.02380374	3.126834e-06

We conducted a test for horizontal multicollinearity on the data, and we can conclude that there is no evidence of horizontal multicollinearity in the data ($p = 0.09$).

In addition, we performed a leave-one-out analysis as a sensitivity analysis, and the results showed consistency with the main analysis, indicating that no individual SNP had a significant impact on the MR analysis.

Figure 3. leave-one-out forest plot



5. Conclusion

We conducted a two-sample MR analysis to examine the causal relationship between triglyceride levels and heart disease using randomization analysis. We also performed sensitivity analysis on the data. Despite the presence of some heterogeneity in the data, the random-effects model revealed a causal relationship between the exposure (triglyceride levels) and the outcome (heart disease). Furthermore, the results showed good agreement with multiple statistical methods.

In conclusion, our experimental findings indicate that triglyceride levels are an important risk factor for heart disease. Reducing triglyceride intake can have a positive effect on preventing heart disease.

Reference:

- [1] Jokinen E. Obesity and cardiovascular disease. *Minerva Pediatr.* 2015 Feb;67(1):25-32. Epub 2014 Nov 11. PMID: 25387321.
- [2] Powell-Wiley TM, Poirier P, Burke LE, Després JP, Gordon-Larsen P, Lavie CJ, Lear SA, Ndumele CE, Neeland IJ, Sanders P, St-Onge MP; American Heart Association Council on Lifestyle and Cardiometabolic Health; Council on Cardiovascular and Stroke Nursing; Council on Clinical Cardiology; Council on Epidemiology and Prevention; and Stroke Council. Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Circulation.* 2021 May 25;143(21):e984-e1010. doi: 10.1161/CIR.0000000000000973. Epub 2021 Apr 22. PMID: 33882682; PMCID: PMC8493650.
- [3] Austin MA. Plasma triglyceride as a risk factor for coronary heart disease. The epidemiologic evidence and beyond. *Am J Epidemiol.* 1989 Feb;129(2):249-59. doi: 10.1093/oxfordjournals.aje.a115130. PMID: 2643302.
- [4] Esan O, Wierzbicki AS. Triglycerides and cardiovascular disease. *Curr Opin Cardiol.* 2021 Jul 1;36(4):469-477. doi: 10.1097/HCO.0000000000000862. PMID: 33797418.
- [5] Bowden J, Holmes MV. Meta-analysis and Mendelian randomization: A review. *Res Synth Methods.* 2019 Dec;10(4):486-496. doi: 10.1002/jrsm.1346. Epub 2019 Apr 23. PMID: 30861319; PMCID: PMC6973275.
- [6] Ma M, Zhi H, Yang S, Yu EY, Wang L. Body Mass Index and the Risk of Atrial Fibrillation: A Mendelian Randomization Study. *Nutrients.* 2022 Apr 29;14(9):1878. doi: 10.3390/nu14091878. PMID: 35565843; PMCID: PMC9101688.
- [7] Ma M, Zhi H, Yang S, Yu EY, Wang L. Body Mass Index and the Risk of Atrial Fibrillation: A Mendelian Randomization Study. *Nutrients.* 2022 Apr 29;14(9):1878. doi: 10.3390/nu14091878. PMID: 35565843; PMCID: PMC9101688.
- [8] Yin KJ, Huang JX, Wang P, Yang XK, Tao SS, Li HM, Ni J, Pan HF. No Genetic Causal Association Between Periodontitis and Arthritis: A Bidirectional Two-Sample Mendelian Randomization Analysis. *Front Immunol.* 2022 Jan 26;13:808832. doi: 10.3389/fimmu.2022.808832. PMID: 35154127; PMCID: PMC8825874.
- [9] Liu K, Zou J, Fan H, Hu H, You Z. Causal effects of gut microbiota on diabetic retinopathy: A Mendelian randomization study. *Front Immunol.* 2022 Sep 8;13:930318. doi: 10.3389/fimmu.2022.930318. PMID: 36159877; PMCID: PMC9496187.
- [10] Xu J, Zhang S, Tian Y, Si H, Zeng Y, Wu Y, Liu Y, Li M, Sun K, Wu L, Shen B. Genetic Causal Association between Iron Status and Osteoarthritis: A Two-Sample Mendelian Randomization. *Nutrients.* 2022 Sep 6;14(18):3683. doi: 10.3390/nu14183683. PMID: 36145059; PMCID: PMC9501024.
- [11] Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol.* 2017 May;32(5):377-389. doi: 10.1007/s10654-017-0255-x. Epub 2017 May 19. Erratum in: *Eur J Epidemiol.* 2017 Jun 29;; PMID: 28527048; PMCID: PMC5506233.
- [12] Miao R, Li J, Meng C, Li Y, Tang H, Wang J, Deng P, Lu Y. Diet-Derived Circulating Antioxidants and Risk of Stroke: A Mendelian Randomization Study. *Oxid Med Cell Longev.* 2022 Jan 17;2022:6457318. doi: 10.1155/2022/6457318. PMID: 35082970; PMCID: PMC8786473.

[13] Guan M, Yan L, Li R, Xu Y, Chen D, Li S, Ma F, Zhang X. Integration of leave-one-out method and real-time live cell reporter array system to assess the toxicity of mixtures. Environ Res. 2022 Nov;214(Pt 3):114110. doi: 10.1016/j.envres.2022.114110. Epub 2022 Aug 17. PMID: 35985486.