The impact of growth differentiation factor 15 on the risk of cardiovascular disease: evidence from Mendelian randomization study

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Research article

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Abstract

Background: Growth differentiation factor 15(GDF-15) concentration is apparently associated with cardiovascular disease, but whether there is a causal relationship has not been testified.

Methods: We utilized Mendelian randomization to assess the function of GDF-15 in incidence of cardiovascular disease. The single-nucleotide polymorphism- GDF-15 association evaluations came from meta-analysis of genome-wide association study (GWAS). Besides inverse-variance weighted, MR-Egger test and weighted median method were applied to examine sensitivity.

Results: Based on the instruments, GDF-15 level linked to the increasing risk of cardioembolic stroke (OR 1.09 per SD increase, 95% CI 1.01, 1.19) , atrial fibrillation (OR 1.03 per SD increase, 95% CI 1.0, 1.06), coronary artery disease (OR 0.94 per SD increase, 95% CI 0.89, 0.99) and myocardial infarction (OR 0.94 per SD increase, 95% CI 0.90, 0.98). However, the significant causal relationship between GDF-15 and the other cardiovascular diseases was not found in our study.

Conclusions: The result suggested that GDF-15 was causally associated with the risk of cardioembolic stroke,atrial fibrillation, coronary artery disease and myocardial infarction, providing us conceivable strategies to alleviate the burden of cardiovascular disease.

Introduction

Leading morbidity and worldwide mortality, cardiovascular disease(CVD) cost more than $200 billion in financial loss each year in the United States despite advanced therapy [1]. Burgeoning works that demonstrated metformin, an extraordinarily classical and first-line glucose-lowering drug, was beneficial to diabetic individuals with CVD [2]. Furthermore, Growth differentiation factor 15(GDF-15), the potential downstream of metformin, may play a crucial role in the process[3]. GDF-15 is a stress-responsive protein, involving in oxidative stress, inflammation and tissue hypoxia[4] [5], which could be an effective predictor and promising therapeutic targets for CVD. In PARADIGM-HF trial, Bouabdallaoui N, et al. manifested that GDF-15 independently provided prognostic information in patients with heart failure with reduced ejection fraction(HFrEF) in which higher baseline and incremental GDF-15 levels were obviously relevant to mortality and all cardiovascular events, even after adjusting NT-proBNP and high-sensitivity cTnT [6]. Similar results were observed in acute coronary syndrome (ACS)[7], stable coronary artery disease(CAD) [8], folks with CVD risks[9], and acute pulmonary embolism[10]. However, the argument which was regarding to the GDF15 uncorrelated to cardiometabolic outcomes was raised by a large genome-wide association study (GWAS)[11]. It remains intricate whether GDF-15 links to the pathogenesis of CVD since those observational works arduously avoid some confounding (where some factors associated with GDF-15 actually result in the disease) and reverse causality bias (where some patients with CVD may be more likely to higher GDF-15). Mendelian randomization (MR) is a robust tool for causal deduction to complement observational trials[12]. Illuminating the value of GDF-15 in CVD can feasibly provide another perspective to improve the diagnosis and prognosis of CVD.

Methods

Instrument Selection

Circulating GDF-15 level was predicted by the exposure genetically. A meta-analysis of GWAS which including 5440 individuals of European ancestry from four community-based cohorts (the mean age was 62 years and 53% were women) was utilized to obtain GDF-15 genetic associations[13], as the previous work did[14]. The selected single-nucleotide polymorphism (SNP) was associated with GDF-15 concentration at the genome-wide threshold ($p<5\times10-8$). All the SNPs were on chromosome 19 containing the PGPEP1 and MIC-1/GDF15 genes. LD-Link [15]was applied to test linkage disequilibrium between two loci in the same chromosome based on European ancestry. Every targeted SNP was searched in the PhenoScanner[16] for the known effects of restricting the potential pleiotropy.

Data for Outcomes

The summary statistics for the selected SNPs with stroke were extracted from a large-scale meta-analysis of GWAS of 446696 subjects of European ancestry (40585 cases; 406111 controls), conducted by the MESTROKE consortium[13]. Specifically, any ischemic stroke (AIS) cases were divided into three subtypes: cardioembolic stroke (CES), large artery stroke (LAS) and small vessel stroke (SVS). Genetic associations with atrial fibrillation (AF) were obtained from the largest meta-analysis of GWAS conducted by the Atrial Fibrillation consortium[17]. The study sample included 537409 individuals of European ancestry. Summary statistics of heart failure (HF) come from 47,309 cases and 930,014 controls[18] and nonischemic cardiomyopathy (NICM) were extracted from a meta-analysis of GWAS of 488010 European participants in the UK Biobank (1816 NICM cases)[19] Besides coronary artery disease (CAD) and myocardial infarction (MI) came from the meta-analysis of GWAS of 185,000 cases[20].

Statistical Analyses

Two sample MR method was performed to access the causality in genetic-predicted circulating GDF-15 concentration and stroke, AF, HF , NICM. The casual effect estimates of SNP instruments on CVD outcomes were calculated using the Wald Estimator[21], with standard error obtained using Delta method[22]. Then, odds ratios(OR) for each disease were meta-analyzed with the inverse-variance weighted (IVW) method to establish all SNPs valid or not.[23]. Sensitivity analyses were conducted with the weighted median method and the MR-Egger method. The weighted median method allows half of the information comes from invalid instrumental variables[24]. The MR-Egger method not only detects pleiotropy with regression intercept but also tests all unbalanced directional pleiotropy[25]. All statistical analyses were performed by R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) and the MR package.

Results

Among nine SNPs which identified from the GWAS, 4 SNPs were discarded for rs1054564, rs3746181, rs1363120 were high linkage disequilibrium (LD) ($r^2 \geq 0.8$) and rs16982345 that had not reach the genome-wide threshold (p value $>5 \times 10^{-8}$). The rest of SNPs (rs1227731, rs3195944, rs17725099, rs888663, rs749451) coming from chromosome 19 and containing the PGPEP1 and GDF15 genes displayed in STable 1.

Figure 1 shown the relationship between GDF-15 and the nine kinds of CVD containing AIS, CES, LAS, SVS, AF, HF, NICM, CAD and MI. Increment of GDF-15 resulted in augmenting incidence of CES (OR 1.09 per SD increase, 95% confidence interval(Cl)1.01, 1.19) and AF (OR 1.03 per SD increase, 95% CI 1.0, 1.06). However, the significant relation couldn't been reached between GDF-15 and AIS (OR 1.02 per SD increase, 95% CI 0.98, 1.07), LAS (OR 0.99 per SD increase, 95% CI 0.89, 1.11), SVS (OR 0.96 per SD increase, 95% CI 0.87, 1.06), HF (OR 0.99 per SD increase, 95% CI 0.96, 1.03), NICM (OR 1.12 per SD increase, 95% CI 0.98, 1.29), CAD (OR 0.94 per SD increase, 95% CI 0.89, 0.99) and MI (OR 0.94 per SD increase, 95% CI 0.90, 0.98).

Validation and sensitivity analyses of relation of GDF-15 concentration and outcomes conducted by IVW, MR-Egger test and weighted median method shown in Table 1. We did not find strong evidence against the hypothesis of pleiotropy of the gene of GDF-15 in patients with CES, AF, CAD or MI using MR-Egger (P=0.30, P=0.67, P=0.92 and P=0.25 respectively) while SNPs were valid proved by IVW (P=0.035, P=0.043, P=0.013 and P=0.009 respectively).

Outcome	IVW			Weighted median			MR-Egger				
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Intercept	P-value
CES	0.091	0.043	0.035	0.111	0.053	0.036	0.154	0.150	0.304	-0.017	0.660
AIS	0.024	0.022	0.268	0.022	0.027	0.409	0.104	0.077	0.175	-0.021	0.279
LAS	-0.007	0.055	0.898	-0.013	0.062	0.829	-0.033	0.189	0.862	0.007	0.886
SVS	-0.037	0.051	0.464	-0.037	0.057	0.523	0.030	0.177	0.864	-0.018	0.689
AF	0.031	0.016	0.043	0.032	0.018	0.078	0.024	0.054	0.661	0.002	0.884
HF	-0.008	0.018	0.660	0.012	0.023	0.589	0.002	0.072	0.973	-0.003	0.881
NICM	0.117	0.072	0.102	0.106	0.084	0.204	0.207	0.249	0.406	-0.024	0.708
CAD	-0.065	0.026	0.013	-0.062	0.029	0.032	-0.010	0.101	0.921	-0.015	0.569
MI	-0.063	0.024	0.009	-0.063	0.028	0.024	-0.097	0.084	0.249	0.009	0.671

Table 1 Examination of the relationship of GDF-15 and outcomes.

Examination of the relationship of GDF-15 and outcomes. Data are reported as OR and 95% CI. SE (Std Error), AIS (any ischemic stroke), CES (cardioembolic stroke), LAS (large artery stroke), SVS (small vessel stroke), AF (atrial fibrillation), HF (heart failure), NICM (nonischemic cardiomyopathy), CAD(coronary artery disease), MI(myocardial infarction).

Discussion

We used two-sample Mendelian Randomization to test the association in GDF-15 level and CVD, including AIS, CES, LAS, SVS, AF, HF, CAD and MI. The result was suggested GDF-15 level could impact on the incidence of CES , AF,CAD and MI, whereas an obvious relation could not be concluded when coming to other CVDs. Therefore, our study maybe support a causal relation in GDF-15 and the incident of CES, AF,CAD and MI and further indicated a crucial role for GDF-15 -targeted interventions to lessen the epidemic of CVD. However, the evidence was not straightforward since MR-Egger did not refuse the null hypothesis.

Our result corresponds to a slice of previous randomized and observational studies. In community-based Individuals[26], postoperative patients[27] and hypertrophic cardiomyopathy (HCM) folks[28] who had a higher GDF-15 level were more vulnerable to AF than those remaining lower level. Rienstra, M., et al. suggest that was associated with incident AF with the hazards ratios of 1.31 after adjusted for age and sex[26]. Similarly, GDF-15 was reckoned independently associated with paroxysmal AF after multivariable analyses in a study where a significantly higher serum level of GDF-15 was found in patients with paroxysmal AF than controls (1473.14 ± 628.52 vs. 1233.592 ± 262.76 pg/ml, $P < 0.05$)[29]. In contrast, Santema BT, et al. and Lamprea-Montealegre JA, et al. suggested that serum GDF-15 level was undifferentiated with AF or not in folks with HF from BIOSTAT-CHF trial[30] and people with chronic kidney disease(CKD) from CRIC study[31] respectively. Dissimilitude could have been explained for specific reasons. GDF-15, belonging to transforming growth factor β(TGF-β) cytokine superfamily, may be a sensitive but not specific biomarker. It involved in the progress of inflammatory and oxidative stress [32], which participated in the process of diverse conditions, such as HF, CAD and CDK[33-35] and may lead the course of disease to a varying degree. Besides, GDF-15 might be an early warning, a composite sign of disease and a reflection determined by various but general elements[36]. Nevertheless, in our research, MR could avoid confounding factors to clarify causality between exposure (GDF-15 level) and outcome (AF). Mechanisms association of increased GDF-15 levels with enhancive incidence of AF are unknown and need to be dug deeper. We assumed that GDF-15 expression, just similar to TGF-β, may promote ionic and structural remodeling of the atria leading vulnerability to AF by PI3K/Akt signaling and SMAD2/ 3 signaling[37, 38]. Besides, GDF-15 was demonstrated strongly related to p53[39] which induced fibrotic signaling, endothelial dysfunction and cardiac inflammation[40-42], linking to AF. However, though our result suggested the causal relation between GDF-15 and AF, it needs more genetic instruments for GDF-15 to identify the relationship.

Cardioembolic strokes were tripled in the past few decades and could triple by 2050 worldwide which AF is the most common risk [43]. Our study developed a new perspective that GDF-15 could positively correlate to cardioembolic strokes. Similar states that the incidence of any stroke could be predicted by GDF-15 in individuals with AF[44] and CVD[7, 8]. In ENGAGE AF-TIMI 48 trial, GDF-15 levels from the baseline of 1661 pg/mL to 12 months of 1711 pg/mL were independently associated with a >2-fold higher rate of stroke or systemic embolic events in patients with AF[45]. Likely, during 1.9 years of follow-up, an annual rate of stroke or systemic embolic events was 2.03% in AF patients with GDF-15 in the highest quartile (>2052 ng/L) while 0.90% in the lowest quartile (≤977 ng/L) in ARISTOTLE trial[46]. Mechanistically, on the one hand, systemic inflammation especially IL-1β, IL-6 and TNF-α was a potential mechanism promoting the formation of cerebral cardioembolism[47] and GDF-15 was proved related to them [48] [49]. On the other hand, AF, HF, CAD were one of the precondition of cardioembolic strokes which were affiliated to augment of serum GDF-15 [50].

In line with the previous works, our result suggested that there was a negative correlation between GDF-15 and CAD, though MR-Egger analyses were insignificant. Also, we reckoned that GDF-15 inversely correlated with MI. Johnen et al. believed GDF-15 may have an protective effect on atherosclerosis process for overexpression of GDF-15 in macrophages significantly attenuates atherosclerotic lesions in the ApoE(−/−) mouse model of atherosclerosis[51]. While, for most researches, experimental results tended to a higher GDF-15 associated with higher CAD and MI risk. Martinez CH, et al found that relative to the lower GDF-15 tertile, mid- and highest tertiles had 1.19-fold and 2.10-fold increases, respectively[52]. And In people with non-ST-elevation acute coronary syndrome, GDF-15 was regarded as the most promising biomarker for the risk of death or nonfatal MI at 6 months with OR 2.4[53]. Its underlying mechanism could be included that GDF-15 exhibited a complex pattern with beneficial and harmful functions. When come to the CAD and MI, beneficial function weighted the harm. Besides, GDF-15 may not involve principal process of CAD and MI. So higher expressing which tried to delay the progression of CAD and MI could not counteract the effect mayor factor made. Whereas, our result should be treated with caution for the MR-Egger analyses was insignificant.

However, our result did not support that incremental GDF-15 had a causality with HF. The arguments were prevailing that GDF-15 concentration had not only a promising value of diagnosis but also a superior prognostic biomarker [54]. Presumably, it is HF that promoted the concentration of GDF-15. Furthermore, the severer state of HF, the more comorbidities existed liking hypertension diabetes, aging, renal dysfunction, which may influence the expression of GDF-15 and needed to be eliminated.

There are certain strengths in the study. Our work provided an alternative perspective to clarify the role of GDF-15 in CVD unprecedentedly and supported an intrinsically positive relationship between GDF-15 and AF, CES, CAD, MI. Further investigations in therapies of GDF-15 control were demanded for it may be rewarding for patients with those. Besides, these selected SNPs were not associated with the other CVDs, indicating that the relationship between the SNPs of GDF15 and some related phenotypes could not confound the null association. Furthermore, it needs to remain aware of metformin employing in

individuals with high risks of AF , CES, CAD and MIsince metformin could facilitate the expression of GDF-15, possibly leading to sick and exacerbate.

Limitations were inevitable. Many of them shared common problems of Mendelian randomization[55]. Firstly, the SNPs we selected could not satisfy the demand of independence principle. However, our study found a fresh vision to the relationship between GDF-15 and CVD. Besides, The MR is not sensitive to confounders from environmental exposures and might violate exclusion restriction unless we took into consideration all influence of GDF-15. Also, our statistics based on European populations, limiting the generalizability of our work.

Conclusion

In summary, a genetic approach we utilized to represent an option to determine causality besides randomized controlled trials and suggested that GDF-15 is causally associated with risk of AF , CES, CAD and MI providing conceivable strategies to alleviate the burden of CVD.

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Availability of data and materials:

All of data included in this study could be found in the included references.

Competing interests:

The authors declare that they have no competing interests.

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Zhuo Wang and Fangkun Yang contributed equally to this work.

Authors’ contributions :

ZW and FKY contributed to the study design, the data acquisition, analysis, interpretation, the drafting, and revision of the manuscript and agreed to be accountable for all aspects of the work. MHM and QYB contributed to the supervision, data interpretation. JLS and YFM contributed to revised the manuscript. All authors read and approved the final manuscript.

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Abbreviations

GDF-15, Growth differentiation factor 15

CVD, cardiovascular disease

HFrEF, heart failure with reduced ejection fraction

ACS, acute coronary syndrome

CAD, coronary artery disease

GWAS, genome-wide association study

SNP, single-nucleotide polymorphism

AIS, any ischemic stroke

CES, cardioembolic stroke

LAS, large artery stroke

SVA, small vessel stroke

AF, atrial fibrillation

HF, heart failure

NICM, nonischemic cardiomyopathy

MI, myocardial infarction

References

1. Johnson, N.B., et al., *CDC National Health Report: leading causes of morbidity and mortality and associated behavioral risk and protective factors--United States, 2005-2013.* MMWR Suppl, 2014. **63**(4): p. 3-27.

2. Petrie, J.R., et al., *Cardiovascular and metabolic effects of metformin in patients with type 1 diabetes (REMOVAL): a double-blind, randomised, placebo-controlled trial.* Lancet Diabetes Endocrinol, 2017. **5**(8): p. 597-609.
3. Gerstein, H.C., et al., *Growth Differentiation Factor 15 as a Novel Biomarker for Metformin.* Diabetes Care, 2017. **40**(2): p. 280-283.
4. Kempf, T., et al., *The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury.* Circ Res, 2006. **98**(3): p. 351-60.
5. Kleinertz, H., et al., *Circulating growth/differentiation factor 15 is associated with human CD56(bright) natural killer cell dysfunction and nosocomial infection in severe systemic inflammation.* EBioMedicine, 2019. **43**: p. 380-391.
6. Bouabdallaoui, N., et al., *Growth differentiation factor-15 is not modified by sacubitril/valsartan and is an independent marker of risk in patients with heart failure and reduced ejection fraction: the PARADIGM-HF trial.* Eur J Heart Fail, 2018. **20**(12): p. 1701-1709.
7. Hagstrom, E., et al., *Growth differentiation factor-15 level predicts major bleeding and cardiovascular events in patients with acute coronary syndromes: results from the PLATO study.* Eur Heart J, 2016. **37**(16): p. 1325-33.
8. Hagstrom, E., et al., *Growth Differentiation Factor 15 Predicts All-Cause Morbidity and Mortality in Stable Coronary Heart Disease*. Clin Chem, 2017. **63**(1): p. 325-333.
9. Wang, T.J., et al., *Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study.* Circulation, 2012. **126**(13): p. 1596-604.
10. Lankeit, M., et al., *Growth differentiation factor-15 for prognostic assessment of patients with acute pulmonary embolism.* Am J Respir Crit Care Med, 2008. **177**(9): p. 1018-25.
11. Cheung, C.L., et al., *Evaluation of GDF15 as a therapeutic target of cardiometabolic diseases in human: A Mendelian randomization study.* EBioMedicine, 2019. **41**: p. 85-90.
12. Smith, G.D. and S. Ebrahim, *'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease?* Int J Epidemiol, 2003. **32**(1): p. 1-22.
13. Malik, R., et al., *Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes.* Nat Genet, 2018. **50**(4): p. 524-537.
14. Au Yeung, S.L., S. Luo, and C.M. Schooling, *The impact of GDF-15, a biomarker for metformin, on the risk of coronary artery disease, breast and colorectal cancer, and type 2 diabetes and metabolic traits: a Mendelian randomisation study.* Diabetologia, 2019. **62**(9): p. 1638-1646.
15. *LD-Link* Available from: https://ldlink.nci.nih.gov/.
16. *PhenoScanner* Available from: www.phenoscanner.medschl.cam.ac.uk.
17. Roselli, C., et al., *Multi-ethnic genome-wide association study for atrial fibrillation.* Nat Genet, 2018. **50**(9): p. 1225-1233.
18. Shah, S., et al., *Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure.* Nat Commun, 2020. **11**(1): p. 163.

19. Aragam, K.G., et al., *Phenotypic Refinement of Heart Failure in a National Biobank Facilitates Genetic Discovery.* Circulation, 2018.
20. Nikpay, M., et al., *A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease.* Nat Genet, 2015. **47**(10): p. 1121-1130.
21. Lawlor, D.A., et al., *Mendelian randomization: using genes as instruments for making causal inferences in epidemiology.* Stat Med, 2008. **27**(8): p. 1133-63.
22. Bautista, L.E., et al., *Estimation of bias in nongenetic observational studies using "mendelian triangulation".* Ann Epidemiol, 2006. **16**(9): p. 675-80.
23. Burgess, S., et al., *Sensitivity Analyses for Robust Causal Inference from Mendelian Randomization Analyses with Multiple Genetic Variants.* Epidemiology, 2017. **28**(1): p. 30-42.
24. Bowden, J., et al., *Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator.* Genet Epidemiol, 2016. **40**(4): p. 304-14.
25. Burgess, S. and S.G. Thompson, *Erratum to: Interpreting findings from Mendelian randomization using the MR-Egger method.* Eur J Epidemiol, 2017. **32**(5): p. 391-392.
26. Rienstra, M., et al., *Relation between soluble ST2, growth differentiation factor-15, and high-sensitivity troponin I and incident atrial fibrillation.* Am Heart J, 2014. **167**(1): p. 109-115 e2.
27. Bening, C., et al., *Atrial contractility and fibrotic biomarkers are associated with atrial fibrillation after elective coronary artery bypass grafting.* J Thorac Cardiovasc Surg, 2019.
28. Montoro-Garcia, S., et al., *Growth differentiation factor-15, a novel biomarker related with disease severity in patients with hypertrophic cardiomyopathy.* Eur J Intern Med, 2012. **23**(2): p. 169-74.
29. Shao, Q., et al., *Circulating serum levels of growth differentiation factor-15 and neuregulin-1 in patients with paroxysmal non-valvular atrial fibrillation.* Int J Cardiol, 2014. **172**(2): p. e311-3.
30. Santema, B.T., et al., *The influence of atrial fibrillation on the levels of NT-proBNP versus GDF-15 in patients with heart failure.* Clin Res Cardiol, 2019.
31. Lamprea-Montealegre, J.A., et al., *Cardiac Biomarkers and Risk of Atrial Fibrillation in Chronic Kidney Disease: The CRIC Study.* J Am Heart Assoc, 2019. **8**(15): p. e012200.
32. Bootcov, M.R., et al., *MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily.* Proc Natl Acad Sci U S A, 1997. **94**(21): p. 11514-9.
33. Zhang, Y., J. Bauersachs, and H.F. Langer, *Immune mechanisms in heart failure.* Eur J Heart Fail, 2017. **19**(11): p. 1379-1389.
34. Ferrucci, L. and E. Fabbri, *Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty.* Nat Rev Cardiol, 2018. **15**(9): p. 505-522.
35. Sato, Y. and M. Yanagita, *Immune cells and inflammation in AKI to CKD progression.* Am J Physiol Renal Physiol, 2018. **315**(6): p. F1501-F1512.
36. Natali, A., et al., *Metformin is the key factor in elevated plasma growth differentiation factor-15 levels in type 2 diabetes: A nested, case-control study.* Diabetes Obes Metab, 2019. **21**(2): p. 412-416.

37. Heger, J., et al., *Growth differentiation factor 15 acts anti-apoptotic and pro-hypertrophic in adult cardiomyocytes.* J Cell Physiol, 2010. **224**(1): p. 120-6.
38. Kunamalla, A., et al., *Constitutive Expression of a Dominant-Negative TGF-beta Type II Receptor in the Posterior Left Atrium Leads to Beneficial Remodeling of Atrial Fibrillation Substrate.* Circ Res, 2016. **119**(1): p. 69-82.
39. Tsui, K.H., et al., *Growth differentiation factor-15: a p53- and demethylation-upregulating gene represses cell proliferation, invasion, and tumorigenesis in bladder carcinoma cells.* Sci Rep, 2015. **5**: p. 12870.
40. Yoshida, Y., et al., *p53-Induced inflammation exacerbates cardiac dysfunction during pressure overload.* J Mol Cell Cardiol, 2015. **85**: p. 183-98.
41. Meyer, K., et al., *Essential Role for Premature Senescence of Myofibroblasts in Myocardial Fibrosis.* J Am Coll Cardiol, 2016. **67**(17): p. 2018-28.
42. Jesel, L., et al., *Atrial Fibrillation Progression Is Associated with Cell Senescence Burden as Determined by p53 and p16 Expression.* J Clin Med, 2019. **9**(1).
43. Yiin, G.S., et al., *Age-specific incidence, outcome, cost, and projected future burden of atrial fibrillation-related embolic vascular events: a population-based study.* Circulation, 2014. **130**(15): p. 1236-44.
44. Hijazi, Z., et al., *Growth-differentiation factor 15 and risk of major bleeding in atrial fibrillation: Insights from the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial.* Am Heart J, 2017. **190**: p. 94-103.
45. Berg, D.D., et al., *Performance of the ABC Scores for Assessing the Risk of Stroke or Systemic Embolism and Bleeding in Patients With Atrial Fibrillation in ENGAGE AF-TIMI 48.* Circulation, 2019. **139**(6): p. 760-771.
46. Wallentin, L., et al., *Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in patients with atrial fibrillation: insights from the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial.* Circulation, 2014. **130**(21): p. 1847-58.
47. Licata, G., et al., *Immuno-inflammatory activation in acute cardio-embolic strokes in comparison with other subtypes of ischaemic stroke.* Thromb Haemost, 2009. **101**(5): p. 929-37.
48. Rothenbacher, D., et al., *Association of growth differentiation factor 15 with other key biomarkers, functional parameters and mortality in community-dwelling older adults.* Age Ageing, 2019. **48**(4): p. 541-546.
49. Muralidharan, A.R., et al., *Growth Differentiation Factor-15-Induced Contractile Activity and Extracellular Matrix Production in Human Trabecular Meshwork Cells.* Invest Ophthalmol Vis Sci, 2016. **57**(15): p. 6482-6495.
50. Kamel, H. and J.S. Healey, *Cardioembolic Stroke.* Circ Res, 2017. **120**(3): p. 514-526.
51. Johnen, H., et al., *Increased expression of the TGF-b superfamily cytokine MIC-1/GDF15 protects ApoE(-/-) mice from the development of atherosclerosis.* Cardiovasc Pathol, 2012. **21**(6): p. 499-505.

52. Martinez, C.H., et al., *GDF-15 plasma levels in chronic obstructive pulmonary disease are associated with subclinical coronary artery disease.* Respir Res, 2017. **18**(1): p. 42.
53. Widera, C., et al., *Incremental prognostic value of biomarkers beyond the GRACE (Global Registry of Acute Coronary Events) score and high-sensitivity cardiac troponin T in non-ST-elevation acute coronary syndrome.* Clin Chem, 2013. **59**(10): p. 1497-505.
54. Anand, I.S., et al., *Serial measurement of growth-differentiation factor-15 in heart failure: relation to disease severity and prognosis in the Valsartan Heart Failure Trial.* Circulation, 2010. **122**(14): p. 1387-95.
55. Smith, G.D. and S. Ebrahim, *Mendelian randomization: prospects, potentials, and limitations.* Int J Epidemiol, 2004. **33**(1): p. 30-42.

Figures

Lorem ipsum

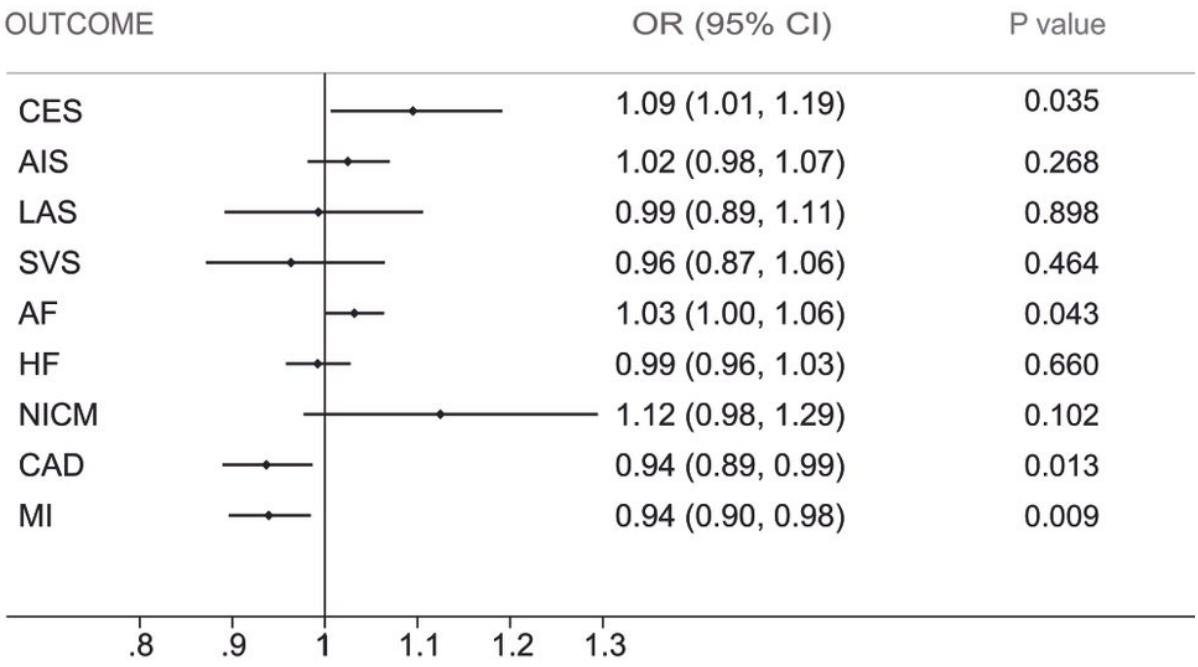

Figure 1

the relationship of GDF-15 and outcomes. Data are reported as OR(odd ratio) and 95% CI(confidence interval). AIS (any ischemic stroke), CES (cardioembolic stroke), LAS (large artery stroke), SVS (small vessel stroke), AF (atrial fibrillation), HF (heart failure), NICM (nonischemic cardiomyopathy), CAD(coronary artery disease), MI(myocardial infarction).

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