Association of Fibroblast Growth Factor 23 With Ischemic Stroke and Its Subtypes: A Mendelian Randomization Study

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Fibroblast growth factor 23 (FGF23), which is involved in the regulation of vitamin D, is an emerging independent risk factor for cardiovascular diseases. Previous studies have demonstrated a positive association between FGF23 and stroke. In this study, we aimed to assess the association of FGF23 with ischemic stroke and its subtypes by applying a Mendelian randomization (MR) framework. Five genetic variants obtained from a genome-wide association study involving 16,624 European subjects were used as valid instruments of circulating FGF23 levels. MR was applied to infer the causality of FGF23 levels and the risk of ischemic stroke using data from the MEGASTROKE consortium. Subsequently, several MR analyses, including inverse-variance weighted meta-analysis, MR-Egger, weighted median estimate (WME), MR Pleiotropy Residual Sum and Outlier were performed. The heterogeneity test analysis, including Cochran's Q, I^2 test and leave-one-out analysis were also applied. Furthermore, potential horizontal/vertical pleiotropy was assessed. Lastly, the power of MR analysis was tested. Three validated variants were found to be associated with circulating FGF23 levels and were used for further investigation. We found that high expression level of FGF23 was not associated with any ischemic stroke. However, a causal association between genetically predicted FGF23 levels and the risk of large-artery atherosclerotic stroke (LAS) was significant, with an odds ratio of 1.74 (95% confidence interval = 1.08–2.81) per standard deviation increase in circulating FGF23 levels. Our findings provide support for the causal association between FGF23 and LAS, and therefore, offer potential therapeutic targets for LAS. The specific roles of FGF23 in LAS and associated molecules require further investigation.

Keywords: ischemic stroke, large-artery atherosclerotic stroke, Mendelian randomization, fibroblast growth factor 23, MEGASTROKE consortium, vitamin D regulation

Abbreviations: AIS, any ischemic stroke; CES, cardioembolic stroke; CI, confidence interval; FGF23, fibroblast growth factor 23; GWAS, whole-genome association studies; IS, ischemic stroke; IVW, inverse-variance weighted; LAS, large-artery atherosclerotic stroke; MR, Mendelian randomization; MR-PRESSO, MR pleiotropy residual sum and outlier; OR, odds ratio; SNPs, single-nucleotide polymorphisms; SVS, small-vessel stroke; WME, weighted median estimate.

INTRODUCTION

Stroke is one of the major causes of death and long-term disability worldwide (GBD 2016 Stroke Collaborators, 2019). Approximately 70% of strokes are ischemic stroke (IS), which is usually caused by the occlusion of the middle cerebral artery (The GBD 2016 Lifetime Risk of Stroke Collaborators et al., 2018). The increasing global burden and limited therapy options for stroke have led to urgent demands for more effective preventive and therapeutic measures (Avan et al., 2019).

Fibroblast growth factor 23 (FGF23), a bone-derived hormone, plays an important role in the regulation of calcium, phosphate, and active vitamin D levels (Vervloet, 2019). Recently, increasing evidence has indicated a strong relationship between FGF23 and cardiovascular diseases (Panwar et al., 2018). Several studies have demonstrated that an increased circulating FGF23 level was correlated with a higher risk (Wright et al., 2014) and a poorer outcome (Seiler et al., 2010) for stroke. Other studies have indicated that plasma FGF23 was associated with carotid atherosclerosis in patients who suffered from stroke as well as in the normal population (Shah et al., 2015; Yan et al., 2017; Chang et al., 2020). Meanwhile, higher FGF23 level also correlated with increased instability of carotid plaques (Biscetti et al., 2015). However, a case–cohort study indicated that there was a graded association of FGF23 with the risk of cardioembolic stroke, but there was no significant association between FGF23 and other IS subtypes or with hemorrhagic strokes in community-dwelling adults (Panwar et al., 2015). In addition, a Multi-Ethnic Study of Atherosclerosis (MESA) showed that FGF-23 was not associated with carotid intima-media thickness or stroke (Kestenbaum et al., 2014). Until now, it is unclear whether FGF23 levels are causally associated with risk of IS. Therefore, in this study, we aimed to investigate the possible causal relationships of FGF23 with IS and its subtypes and the potential research value of FGF23.

Recently, with the development of whole-genome association studies (GWAS), an increasing number of single-nucleotide polymorphisms (SNPs) related to human diseases have been identified (Pei et al., 2019; Liu et al., 2020a,b). Meanwhile, Mendelian randomization (MR) has been widely used for causal inference (Davies et al., 2018; Larsson et al., 2019). Since genetic variants such as SNPs are randomly allocated during conception and the genotypes are determined in the zygote stage, the MR framework can detect causality by minimizing the impacts of confounders and reverse causality (Davies et al., 2018). In this study, an MR design was used to investigate the association of circulating FGF23 levels with IS and its subtypes.

MATERIALS AND METHODS

Study Design

MR was performed based on three primary assumptions as described previously (Yang et al., 2019; He et al., 2020). The first assumption was that the SNPs identified to be the instrumental variables (IVs) should be significantly related to the exposure (FGF23) (**Figure 1**). The second assumption was that genetic variants should be unrelated to the confounding factors of an outcome (IS) (Liu et al., 2018). The third assumption was that the genetic variants must only affect the risk of the disease (IS) through the exposure (FGF23) but not via other routes. Meanwhile, both the second and third assumptions were identified to be independent of pleiotropic effects. As the large-scale datasets from the published genome-wide meta-analysis were publicly available, no additional ethical approval was required.

Selection of SNPs and Validation

The circulating FGF23-associated variants were collected from a meta-analysis comprising 16,624 individuals of European-descent after excluding those whose estimated glomerular filtration rate was less than 30 mL/min/1.73 m^2 (Robinson-Cohen et al., 2018). The selected genetic instruments from the GWAS of FGF23 were composed of top five significant ($P < 5 \times 10^{-8}$) SNPs near *CYP24A1*, *ABO*, *RGS14*, *LINC01506*, and *LINC01229* genes, and were located in five genomic regions, accounting for approximately 3% of FGF23 variation. Detailed information is provided in **Supplementary Table 1**. The strength of the IVs was evaluated using the mean F-statistic, defined as the ratio of the mean square of effect size to the mean square of standard error for each genetic instrument (Bowden et al., 2016b). The rule of thumb threshold of F value is greater than 10 to avoid potential bias from weak instruments (Burgess and Thompson, 2011). The F statistics for each of the five instruments was greater than 10 (**Supplementary Table 1**). Subsequently, we verified the independence among these SNPs by linkage disequilibrium ($R^2 < 0.1$) through the 1000 Genomes Phase 3 (European) reference panel.
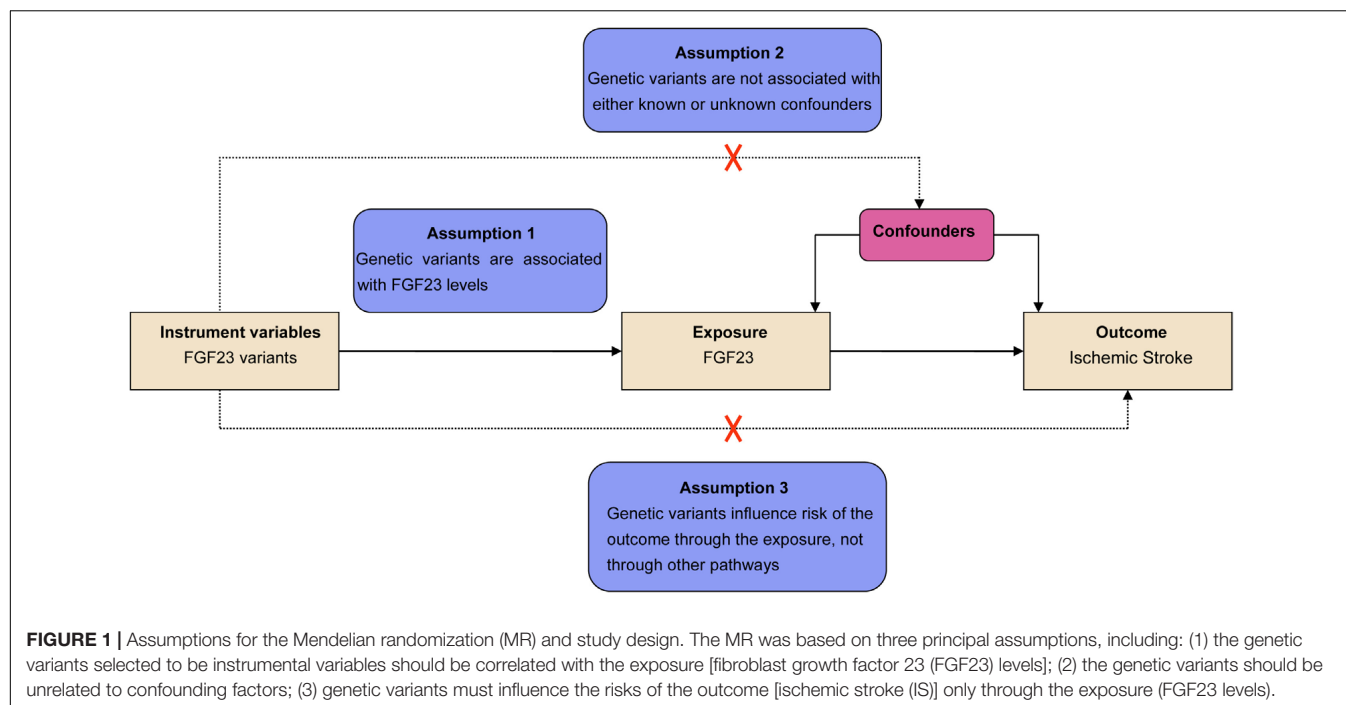
Data Sources

The summary-level data for IS and its subtypes were obtained from the MEGASTROKE consortium. Any ischemic stroke (AIS) group ($n = 34,217$), regardless of the subtype of European ancestry, was selected and compared with 406,111 control subjects. The three main subtypes of IS were acquired mainly on the basis of the Trial of ORG 10172 in Acute Stroke Treatment criteria, including LAS ($n = 4,373$), cardioembolic stroke (CES; $n = 7,193$), and small-vessel stroke (SVS; $n = 5,386$) (Malik et al., 2018). As all the five genetic instruments associated with FGF23 levels were available in the MEGASTROKE consortium, no proxy variant was needed. The MEGASTROKE-matched data are shown in **Supplementary Table 2**.

Statistical Analysis

The principal analyses assessing the causal associations of FGF23 with IS and its subtypes were performed using the inverse-variance-weighted (IVW) method (Davies et al., 2018). For each of the five SNPs, we computed an Wald's ratio estimates by dividing the beta-coefficients (log odds ratio) for the SNP–stroke association by the beta coefficient for the SNP–FGF23 association. Moreover, to improve the reliability of causal effect estimates, we also carried out the MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) test (Verbanck et al., 2018).

To further evaluate the impact of potential pleiotropy on causal estimates, we performed sensitivity analyses using several

FIGURE 1 | Assumptions for the Mendelian randomization (MR) and study design. The MR was based on three principal assumptions, including: (1) the genetic variants selected to be instrumental variables should be correlated with the exposure [fibroblast growth factor 23 (FGF23) levels]; (2) the genetic variants should be unrelated to confounding factors; (3) genetic variants must influence the risks of the outcome [ischemic stroke (IS)] only through the exposure (FGF23 levels).

other methods. First, we used the MR-Egger regression to assess the presence of directional pleiotropy (Bowden et al., 2015). A statistically significant intercept term from the MR-Egger regression suggests the possibility that genetic variants may not affect the outcome via the exposure of interest. We also conducted the weighted median estimate (WME) (Bowden et al., 2016a), which provides an effective estimate of causality when at least 50% of genetic IVs is valid. Furthermore, to evaluate the potential heterogeneity due to pleiotropy or other causes, we conducted the Cochran's Q-test (together with the I^2 statistic), as reported in a previous study (Liu et al., 2013). In addition, we selected the leave-one-out sensitivity method to sequentially remove each SNP from the MR analysis and assess the impact of single-gene variants on the causal estimates (He et al., 2020). Moreover, vertical pleiotropy was assessed using the Steiger test to verify the causal direction between FGF23 and stroke (Hemani et al., 2017).

Lastly, we excluded those SNPs associated with potential confounders (Bonferroni correction, $P < 0.05/5$ SNPs) by using PhenoScanner V2 in March 2020 (Staley et al., 2016; Kamat et al., 2019), and repeated the MR analysis using the IVW, MR-Egger regression, and weighted-median estimate. To correct for potential pleiotropic bias, we performed multivariable MR following Sanderson's method (Sanderson et al., 2020). We also calculated the power of MR estimates using the mRnd platform[1] and the effect size based on a 5% type 1 error rate and enough power ($>80\%$). Statistical analyses were performed using Mendelian Randomization (version 0.4.1) (Yavorska and Burgess, 2017) and TwoSampleMR (version 0.5.1) (Hemani, 2019) on R 3.6.2 (The R Foundation for Statistical Computing, Vienna,

Austria). All statistical tests were two-sided and the statistical significance was set at the level of $P < 0.05$.

RESULTS

Primary MR Analysis of the Association of FGF23 With IS and Its Subtypes

As listed in **Supplementary Table 1**, five SNPs were used as the IVs for FGF23 levels. We identified significant association of high FGF23 levels with increased LAS risk (OR = 1.94, 95% CI 1.35–2.27; $p = 3.04E{-}04$) but not with the other IS subtypes or AIS using the IVW method (**Supplementary Table 4**). However, a potential heterogeneity was identified using the Cochran's Q test and I^2 for causal estimates of five SNPs in the conventional IVW model for AIS (14.34, $p = 0.0063$, $I^2 = 72.10\%$), LAS (14.12, $p = 0.0069$, $I^2 = 71.70\%$), and CES (16.79, $p = 0.0021$, $I^2 = 76.20\%$) (**Supplementary Table 4**), suggesting the possibility that the obtained effect estimates of these associations from the IVW method may be biased by outlier SNPs.

Sensitivity Analysis of the Association of FGF23 With IS and Its Subtypes

To assess the robustness of the causal effect of FGF23 on IS and its subtypes, we performed several sensitivity analyses as follows. First, WME suggested significant association between FGF23 levels and LAS risk with an odds ratio of 1.75 (95% CI 1.06–2.90; $p = 0.029$), but not with the other IS subtypes or AIS (**Supplementary Table 4**). Second, the intercept term from MR-Egger analysis revealed no evidence of directional pleiotropy in the analysis of LAS ($p = 0.81$), SVS ($p = 0.97$), CES ($p = 0.22$), or AIS ($p = 0.26$).

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[1]https://shiny.cnsgenomics.com/mRnd/

However, MR-PRESSO test identified horizontal pleiotropic outliers in AIS ($p = 0.0066$), LAS ($p = 0.0134$), and CES ($p = 0.0034$). The leave-one-out permutation analysis further indicated that the direction and precision of the genetics estimates between increased FGF23 levels and risk of IS and its subtypes changed largely with the deletion of *rs2769071* (**Supplementary Table 7**).

We next searched the PhenoScanner V2 database (Kamat et al., 2019) for possible pleiotropic associations of individual SNPs with risk factors for IS. Among the FGF23-associated SNPs, associations were observed for the *rs2769071* variant with low-density lipoprotein ($P = 3.06E{-}10$), total cholesterol ($P = 7.48E{-}13$), diastolic blood pressure ($P = 2.80E{-}10$), and type 2 diabetes ($P = 2.30E{-}05$). The *rs11741640* variant was significantly related to self-reported hypertension ($P = 2.22E{-}04$) and alcohol intake frequency ($P = 3.74E{-}03$). Detailed information is provided in **Supplementary Table 3**.

In total, we excluded two SNPs (*rs2769071* near the *ABO* gene and *rs11741640* near the *RGS14* gene) that were potentially associated with at least one secondary phenotype and repeated the MR analyses. Based on the remaining three effective SNPs, FGF23 levels were significantly associated with LAS but not with the other IS subtypes or AIS (**Figure 2**). In the standard MR analysis-IVW method, the odds ratios per standard deviation of the genetically predicted increase in FGF23 levels was 1.74 (95% CI 1.08–2.81; $p = 0.023$) for LAS. Importantly, the results obtained for LAS were similar in the WME analysis (OR = 1.76, 95% CI = 1.04–2.99; $p = 0.036$), while the Egger estimate was

less precise despite having the same direction and a similar size (OR = 1.80, 95% CI = 0.26–12.46; $P = 0.549$). The single variant causal ratio and results of all three variants for the association of FGF23 and LAS are shown in **Supplementary Table 6**. No heterogeneity among these three instruments was found using Cochran's Q analysis ($Q = 0.02$, $P = 0.992$, $I^2 = 0.00\%$) in LAS (**Supplementary Table 5**). The leave-one-out sensitivity analysis also showed the same direction and estimates between the increased FGF23 levels and the risk of LAS, although the deletion of IV *rs17216707* near *CYP24A1* gene was not statistically significant (**Supplementary Table 8**). No directional pleiotropy in LAS was found according to the Egger intercept test (–0.003, 95% CI = –0.150 to 0.145; $P = 0.970$). Considered the potential effects of obesity and smoking-the two most important confounders for both heart disease and circulating metabolites, we then applied the multivariable MR analysis. The BMI or smoke adjusted data by three validated instruments also verifies our results (**Supplementary Table 9**).

Besides, the direction of causality inferred by the Steiger test showed that the SNPs–FGF23 association ($r^2 = 1.04E{-}02$) was more significantly correlated ($p_{\text{Steiger}} = 3.20 \times 10^{-14}$) than the SNPs-LAS association ($r^2 = 1.26E{-}05$), suggesting that higher FGF23 levels leads to the increased risk of LAS, consistent with expectation. We had enough power ($>80\%$) to detect 1.59 OR of LAS risk per SD increased log FGF23 levels (cases $n = 4,373$; non-cases $n = 406,111$); and the power of causal estimate for FGF23 to LAS here was 94%.
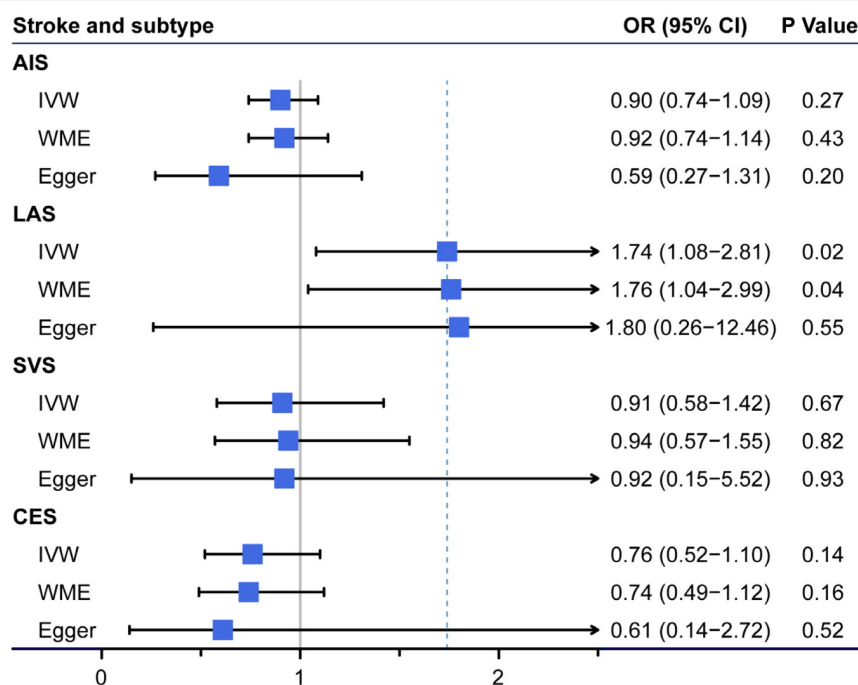
FIGURE 2 | Association of genetically predicted circulating fibroblast growth factor 23 (FGF23) levels with ischemic stroke (IS) and other stroke subtypes. The odds ratio (OR) represented at the center of each box was the risk of genetically predicted one standard deviation increase in FGF23 levels. AIS, any ischemic stroke; LAS, large-artery atherosclerotic stroke; SVS, small-vessel stroke; CES, cardioembolic stroke; CI, confidence interval; IVW, Mendelian randomization (MR) inverse-variance weighted method; WME, weighted median estimate; Egger, the MR-Egger method.

DISCUSSION

Until now, it has remained unclear whether the circulating FGF23 levels is genetically associated with risk of IS. In this study, we found a potential association of genetically predicted high levels of FGF23 and the increased risk of LAS. The risk of LAS increased by 74% with a 23 pg/mL per SD increase in circulating FGF23 levels. This effect size was similar to previously reported sizes of low-density lipoprotein cholesterol (OR = 1.28, 95% CI = 1.07–1.53) (Hindy et al., 2018), fasting blood glucose (OR = 1.42, 95% CI = 1.08–1.85) (Larsson et al., 2017), systolic blood pressure (OR = 1.56, 95% CI = 1.37–1.78) (Parish et al., 2019), and waist-to-hip ratio (OR = 1.75, 95% CI = 1.44–2.13) (Marini et al., 2020).

Our results are consistent with those of previous epidemiological studies (Seiler et al., 2010; Shah et al., 2015; Yan et al., 2017; Chang et al., 2020). In patients with acute IS, the plasma FGF23 concentration was positively correlated with the presence and burden of intracranial carotid atherosclerosis (Chang et al., 2020). FGF23 seems to be mainly involved in vessel calcification, vascular stiffness, and inflammation (Mirza et al., 2009; Kim et al., 2011; Libby et al., 2019; Vervloet, 2019). In mice, excessive plasma FGF23 directly stimulates the production of inflammatory factors such as interleukin-6 (Singh et al., 2016). Meanwhile, inflammatory factors in turn promote the production of FGF23 and exacerbate LAS progression (Feger et al., 2017; Durlacher-Betzer et al., 2018; Egli-Spichtig et al., 2019; McKnight et al., 2020). Our analysis implied that reducing FGF23 levels may be a potential therapeutic strategy for IS, especially for LAS. However, the potential mechanisms that correlate FGF23 with LAS still require further investigations.

Considering the role of FGF23 in regulation of vitamin D levels, some previous studies argued that the pathophysiological effects of FGF23 were partially through decreasing the level of active vitamin D. FGF23 inhibits the functions of vitamin D by promoting its degradation via 24-hydroxylase encoded by the *CYP24A1* gene and inhibiting its production by 1α-hydroxylase encoded by the *CYP27B1* gene (Vervloet, 2019). The physiological roles of vitamin D, including anti-inflammation and inhibition of artery calcification, are contrary to the effects of FGF23 (Han et al., 2016; Wang et al., 2018). In addition, vitamin D receptor activation enables the recovery of αKlotho, an anti-aging protein, while this recovery is inhibited in an inflammatory environment (Lim et al., 2012). FGF23 induces vessel damage and inflammation through an αKlotho-independent pathway when αKlotho is insufficient (Komaba and Fukagawa, 2012; Navarro-González et al., 2014; Krick et al., 2018). The aforementioned studies collectively suggest that proper calcitriol supplements might reduce the risk of LAS in people or those with intracranial atherosclerosis. The effects of calcitriol supplements involved in the process of vasomotion and immune modulation have been reported by several studies (Chitalia et al., 2014; Ojeda López et al., 2018). In this study, the validated genetic variant (*rs17216707*) near the *CYP24A1* gene showed a strong association with LAS (**Supplementary Table 6**), which supports the critical role of active vitamin D in the regulation of FGF23 level and the risk of LAS.

To our knowledge, this is the first MR study to clarify the genetic causalities between FGF23 levels and IS with MR methods. Considering the ethical care of patients and the high cost of randomized controlled trials, the MR framework is effective in the discovery of potential targets of intervention and can indicate potential therapeutic strategies. In addition, our findings in this study were especially prospective, as analyzed data were extracted from the database with the largest number of participants currently known.

However, this study also has several limitations. The different methods for FGF23 measurement could have potentially caused bias in the results. The FGF23 levels were detected in two forms: intact and C-terminal FGF23 (Robinson-Cohen et al., 2018). In patients with chronic kidney diseases, the production of FGF23 (intact FGF23) was separated from its cleaved form (C-terminal FGF23) (Edmonston and Wolf, 2020). Meanwhile, the FGF23-associated GWAS data were obtained from individuals whose estimated glomerular filtration rate was above 30 mL/min/1.73 m^2. In addition, log-transformed FGF23 levels, applied in each cohort and the following meta-analysis, could reflect the relative change in circulating FGF23 levels.

In our study, only three SNPs accounting for 1.13% of the total variation in FGF23 levels were identified as genetic instruments, causing a possible limitation in the results. Thus, additional influential loci are necessary as IVs in the future if new GWAS data are available. As this limited number of IVs restricted the application of PRESSO, the sensitive analysis of potential horizontal pleiotropy could not be performed completely. However, similar results were obtained from WME and IVW estimate, while no signs of heterogeneity (Cochran's Q test) and directional pleiotropy (MR-Egger intercept analysis) were discovered. Therefore, the above results indicated that confounders are unlikely to explain the observed associations.

Population stratification also potentially restricted the accuracy of this study. The MR inference depended on three instrumental assumptions that rely on the same genetic backgrounds in the exposure and outcome data. In this study, we used European-descent genotypes to assess the association between FGF23 levels and IS. This result may be altered in different populations due to different genetic backgrounds, such as linkage disequilibrium. Moreover, the MR framework was not able to infer the association during specific periods of the life cycle or conditions. Thus, further animal experiments and possible intervention trials are needed.

In summary, our results provide support for a suggestive causal association between higher circulating FGF23 levels and an increased risk of LAS. Our findings may offer new therapeutic targets for LAS. Further studies are necessary to investigate whether genetic variants at or near the *CYP24A1* gene influence the risk of LAS through downstream effects or pathways related to vitamin D.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and

accession number(s) can be found in the article/**Supplementary Material**.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene. 2020.608517/full#supplementary-material

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Supplementary Table 1. Circulating FGF23 associated genetic variants.

SNP	Near Gene	Chr: Pos	A1/A2	EAF	Beta	SE	*P*-value	N	F-statistics
rs17216707	CYP24A1	20: 52732362	T/C	0.8	0.054	0.005	3.00×10^{-24}	16624	116.64
rs2769071	ABO	9: 136145974	G/A	0.37	0.037	0.005	6.10×10^{-17}	16624	54.76
rs11741640	RGS14	5: 176792743	G/A	0.73	0.039	0.005	1.60×10^{-16}	16624	60.84
rs17479566	LINC01506	9: 71198013	T/C	0.22	0.031	0.005	2.00×10^{-09}	16624	38.44
rs9925837	LINC01229	16: 79927303	G/A	0.13	0.035	0.006	5.10×10^{-09}	16624	34.03

Five FGF23 (Fibroblast growth factor 23) associated SNPs (single nucleotide polymorphisms) were derived from a European-descent GWAS (Genome-wide association study) meta-analysis study (N = 16,624). β-estimates are interpreted as the relative difference in FGF23 concentration per minor allele. Abbreviation Chr: Pos means the chromosome and location of SNPs; EAF = the frequency of A1 allele in European ancestry (hg19 reference panel); SE = standard error.

Supplementary Table 2. FGF23 associated genetic variants mapped associations in ischemic stroke.

Trait	SNP	Near Gene	Chr: Pos	A1/A2	Beta†	SE	*P*-value
AIS	rs17216707	CYP24A1	20: 52732362	T/C	-0.0198	0.0129	1.23×10^{-01}
	rs2769071	ABO	9: 136145974	G/A	0.0412	0.0116	3.84×10^{-04}
	rs11741640	RGS14	5: 176792743	G/A	0.0016	0.0110	8.83×10^{-01}
	rs17479566	LINC01506	9: 71198013	T/C	0.0012	0.0133	9.30×10^{-01}
	rs9925837	LINC01229	16: 79927303	G/A	0.0014	0.0139	9.20×10^{-01}
LAS	rs17216707	CYP24A1	20: 52732362	T/C	0.0569	0.0323	7.80×10^{-02}
	rs2769071	ABO	9: 136145974	G/A	0.1333	0.0285	2.79×10^{-06}
	rs11741640	RGS14	5: 176792743	G/A	-0.0088	0.0270	7.45×10^{-01}
	rs17479566	LINC01506	9: 71198013	T/C	0.0332	0.0301	2.70×10^{-01}
	rs9925837	LINC01229	16: 79927303	G/A	0.0323	0.0348	3.53×10^{-01}
SVS	rs17216707	CYP24A1	20: 52732362	T/C	-0.0061	0.0298	8.38×10^{-01}
	rs2769071	ABO	9: 136145974	G/A	-0.0068	0.0267	8.00×10^{-01}
	rs11741640	RGS14	5: 176792743	G/A	-0.0137	0.0254	5.89×10^{-01}
	rs17479566	LINC01506	9: 71198013	T/C	0.0076	0.0280	7.85×10^{-01}
	rs9925837	LINC01229	16: 79927303	G/A	-0.0286	0.0327	3.82×10^{-01}
CES	rs17216707	CYP24A1	20: 52732362	T/C	-0.0303	0.0249	2.24×10^{-01}
	rs2769071	ABO	9: 136145974	G/A	0.0866	0.0227	1.34×10^{-04}
	rs11741640	RGS14	5: 176792743	G/A	0.0048	0.0212	8.20×10^{-01}
	rs17479566	LINC01506	9: 71198013	T/C	-0.0028	0.0234	9.07×10^{-01}
	rs9925837	LINC01229	16: 79927303	G/A	-0.0288	0.0272	2.90×10^{-01}

The mapped associations were adjusted into increased FGF23 (Fibroblast growth factor 23) direction (†). AIS (any ischemic stroke), LAS (large atherosclerotic stroke), SVS (small vessel stroke), and CES (cardioembolic stroke) associated data are derived from the MEGASTROKE consortium. SNP = single nucleotide polymorphism; SE = standard error.

Supplementary Table 3. The pleiotropic associations with detected confounders for FGF23 associated SNPs.

SNP	Trait	Beta	SE	*P*-value	Study	PMID	year	N
rs2769071	High density lipoprotein	-0.0199	0.0050	2.61×10^{-04}	GLGC	24097068	2013	187,167
A/G†	Low density lipoprotein	-0.0353	0.0054	3.06×10^{-10}	GLGC	24097068	2013	173,082
(ABO)‡	Total cholesterol	-0.0383	0.0051	7.48×10^{-13}	GLGC	24097068	2013	187,365
	Type 2 diabetes adjusted for BMI	-0.0500	0.0150	1.20×10^{-03}	DIAGRAM	28566273	2017	159,208
	Type 2 diabetes	-0.0540	0.0130	2.30×10^{-05}	DIAGRAM	28566273	2017	159,208
	Self-reported high cholesterol	-0.0053	0.0009	4.26×10^{-10}	Neale B	UKBB	2017	337,159
	Diabetes diagnosed by doctor	-0.0020	0.0006	4.54×10^{-04}	Neale B	UKBB	2017	336,473
	Self-reported diabetes	-0.0017	0.0005	8.69×10^{-04}	Neale B	UKBB	2017	337,159
	Diastolic blood pressure	0.0167	0.0026	2.80×10^{-10}	Neale B	UKBB	2017	317,756
	Self-reported hypertension	0.0059	0.0011	2.85×10^{-07}	Neale B	UKBB	2017	337,159
	Vascular or heart problems diagnosed by doctor: high blood pressure	0.0065	0.0012	2.48×10^{-08}	Neale B	UKBB	2017	336,683
rs11741640	Self-reported hypertension	0.0045	0.0012	2.22×10^{-04}	Neale B	UKBB	2017	337,159
A/G†	Vascular or heart problems diagnosed by doctor: high blood pressure	0.0042	0.0012	5.66×10^{-04}	Neale B	UKBB	2017	336,683
(RGS14)‡	Alcohol intake frequency	0.0117	0.0040	3.74×10^{-03}	Neale B	UKBB	2017	336,965

All pleiotropic associations were detected on the European-descent data. Abbreviation FGF23 = SE = standard error; SNPs = single nucleotide polymorphisms; PMID = Pubmed identifier; GLGC = Global Lipids Genetics Consortium; DIAGRAM = Diabetes Genetics Replication and Meta-analysis; Neale B = Neale Biobank. The mark † means effect allele with the other allele and ‡ means nearby gene.

Supplementary Table 4. MR analyses of FGF23 and IS and subtypes of five genetic variants.

Methods		Number of SNP	OR	95% lower	95% upper	P-value	Heterogeneity	Hete P†
AIS	IVW	5	1.0627	0.92	1.23	4.15E-01		
	WME	5	1.0217	0.83	1.26	8.39E-01		
	Egger	5	0.4724	0.11	1.97	3.04E-01		
	Egger intercept	5	0.0614	-0.04	0.17	2.58E-01		
	PRESSO	5	1.0627	1.03	1.09	6.89E-01		6.60E-03
	Cochran's Q	5					14.34	6.27E-03
	I^2	5					72.10%	
LAS	IVW	5	1.9367	1.35	2.77	3.04E-04		
	WME	5	1.7513	1.06	2.90	2.90E-02		
	Egger	5	1.1948	0.02	72.70	9.32E-01		
	Egger intercept	5	0.0363	-0.27	0.34	8.14E-01		
	PRESSO	5	1.9369	1.44	2.60	1.27E-01		1.34E-02
	Cochran's Q	5					14.12	6.93E-03
	I^2	5					71.70%	
SVS	IVW	5	0.8883	0.64	1.24	4.87E-01		
	WME	5	0.9126	0.62	1.35	6.49E-01		
	Egger	5	0.9470	0.84	1.07	3.77E-01		
	Egger intercept	5	-0.0028	-0.13	0.13	9.66E-01		
	PRESSO	5	0.8883	0.94	0.84	1.85E-01		9.58E-01
	Cochran's Q	5					0.75	9.44E-01
	I^2	5					0.00%	
CES	IVW	5	1.0986	0.83	1.45	5.11E-01		
	WME	5	0.9408	0.64	1.38	7.54E-01		
	Egger	5	0.3965	0.01	10.67	5.82E-01		
	Egger intercept	5	0.1208	-0.07	0.31	2.18E-01		
	PRESSO	5	1.0986	1.05	1.15	7.64E-01		3.40E-03
	Cochran's Q	5					16.79	2.12E-03
	I^2	5					76.20%	

Note: † Hete P represents the P value of heterogeneity.

Supplementary Table 5. MR analyses of FGF23 and IS and subtypes of three genetic variants.

| Methods		SNP	OR	95% lower	95% upper	*P*-value	Cochran's Q	Hete_P†	I²
AIS	IVW	3	0.8963	0.74	1.09	2.71E-01			
	WME	3	0.9181	0.74	1.14	4.31E-01			
	Egger	3	0.5920	0.27	1.31	1.96E-01			
	Egger intercept	3	0.0332	-0.03	0.09	2.91E-01			
	Cochran's Q test	3					1.16	5.60E-01	0.00%
LAS	IVW	3	1.7407	1.08	2.81	2.30E-02			
	WME	3	1.7630	1.04	2.99	3.56E-02			
	Egger	3	1.8043	0.26	12.46	5.49E-01			
	Egger intercept	3	-0.0028	-0.15	0.14	9.70E-01			
	Cochran's Q test	3					0.02	9.92E-01	0.00%
SVS	IVW	3	0.9087	0.58	1.42	6.72E-01			
	WME	3	0.9443	0.57	1.55	8.21E-01			
	Egger	3	0.9219	0.15	5.52	9.29E-01			
	Egger intercept	3	-0.0011	-0.14	0.14	9.87E-01			
	Cochran's Q test	3					0.70	7.04E-01	0.00%
CES	IVW	3	0.7589	0.52	1.10	1.44E-01			
	WME	3	0.7423	0.49	1.12	1.59E-01			
	Egger	3	0.6112	0.14	2.72	5.19E-01			
	Egger intercept	3	0.0171	-0.10	0.13	7.70E-01			
	Cochran's Q test	3					0.48	7.86E-01	0.00%

Note: † Hete_P represents the P value of Cochran's Q.

Supplementary Table 6. Associations of genetically predicted FGF23 levels with large atherosclerotic stroke (LAS).

Method	SNP	Near Gene	OR	95% lower	95% upper	P-value
	rs17216707	CYP24A1	1.76	0.94	3.31	7.81E-02
	rs17479566	LINC01506	1.78	0.64	4.95	2.70E-01
	rs9925837	LINC01229	1.64	0.58	4.69	3.53E-01
IVW	All		1.74	1.08	2.81	2.30E-02
WME	All		1.76	1.04	2.99	3.56E-02
Egger	All		1.80	0.26	12.46	5.49E-01

Associations of genetically predicted FGF23 levels with large atherosclerotic stroke (LAS).
The odds ratio (OR) represents the risk of LAS per genetically predicted 1 standard
deviation increase of FGF23 levels, which equals to the center of each box, whereas the
size of each box represents the weight of each SNP (single nucleotide polymorphism).

Method	SNP	Nearby Gene	OR (95% CI)	p-value
	rs17216707	CYP24A1	1.76 (0.94−3.31)	7.81×10^{-2}
	rs17479566	LINC01506	1.78 (0.64−4.95)	2.70×10^{-1}
	rs9925837	LINC01229	1.64 (0.58−4.69)	3.53×10^{-1}
IVW	All		**1.74 (1.08−2.81)**	**2.30×10^{-2}**
WME	All		**1.76 (1.04−2.99)**	**3.56×10^{-2}**
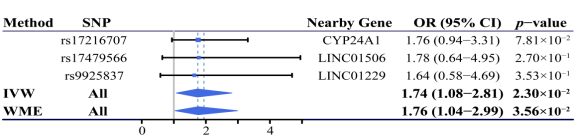
Supplementary Table 7. Leave-one-out sensitivity two-sample MR analysis based on the inverse variance weighted (IVW) model for FGF23 on ischemic stroke and its subtypes

Outcome	SNP	Beta	SE	*P*- value
AIS	rs11741640	0.073158794	0.186639645	0.695073839
	rs17216707	0.191827308	0.151948921	0.206788346
	rs17479566	0.065475832	0.172065399	0.703552957
	rs2769071	-0.06996831	0.083130051	0.399971076
	rs9925837	0.066280927	0.173757012	0.702863755
	All	0.060821024	0.141195397	0.666644458
LAS	rs11741640	0.910058378	0.349445918	0.009206492
	rs17216707	0.706103952	0.48047692	0.1416729
	rs17479566	0.672836572	0.423347974	0.111987542
	rs2769071	0.351490053	0.203981654	0.084862231
	rs9925837	0.682892503	0.420873774	0.104683725
	All	0.661008938	0.343778789	0.054508431
CES	rs11741640	0.102797347	0.387559982	0.790822719
	rs17216707	0.291049515	0.363832481	0.423737026
	rs17479566	0.11421582	0.359796088	0.750905227
	rs2769071	-0.175160369	0.158629033	0.269500403
	rs9925837	0.165186173	0.33932415	0.626393435
	All	0.094050993	0.292891259	0.748125272
SVS	rs11741640	-0.096568883	0.195418257	0.621189584
	rs17216707	-0.147011184	0.208595067	0.48095446
	rs17479566	-0.153702612	0.182266412	0.399068821
	rs2769071	-0.123207423	0.190025326	0.516743281
	rs9925837	-0.076624847	0.181459066	0.67282744
	All	-0.118494406	0.170664275	0.487486006

Supplementary Table 8. Leave-one-out sensitivity two-sample MR analysis based on the inverse variance weighted (IVW) model for FGF23 on LAS.

Near Gene	SNP	Beta	SE	*P*-value
CYP24A1	rs17216707	0.537184374	0.3736803	0.1505616
LINC01506	rs17479566	0.547968322	0.2756445	0.0468169
LINC01229	rs9925837	0.569386889	0.2739588	0.0376756
All	All	0.55415465	0.2438058	0.0230301

Supplementary Table 9. Multivariable MR Results adjust for the effects of obesity and smoking.

Confounder	Outcome	SNPs	OR	95%CI		Conditional F	Q_A	Hete_P†
BMI	LAS	5	1.77	1.00	3.13	14.16	3.37	0.185
Smoke	LAS	5	2.4	0.19	6.29	21.52	6.45	0.040
BMI	LAS	3	1.75	1.64	2.16	3.54	0.01	0.000
Smoke	LAS	3	1.74	1.64	1.78	62.51	0.00	0.000

The five FGF23 (Fibroblast growth factor 23) associated instruments and three effect instruments were tested the multivariable Mendelian randomization test[14]. The Q_A refers to Cochran's Q minimization, a heterogeneity test robust for weak instruments, with *Hete_P* as its statistics result. And the *Conditional F* is the value of conditional F statistics. BMI (Body mass index) and Smoke (Current Smoke) associated data were obtained from UK Biobank. The symbol † refers that the *Jackknife* method were used if large heterogeneity exists according to Sanderson's Process[14].