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ORIGINAL ARTICLE

Serum Creatinine Protects Against Amyotrophic Lateral Sclerosis: a Mendelian Randomization Study

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Abstract

Association between serum creatinine (sCr) and amyotrophic lateral sclerosis (ALS) has been reported in previous observational studies, but results are at risk of confounding bias and reverse causation. Therefore, whether such association is casual remains unclear. Herein, we performed a two-sample Mendelian randomization study to evaluate the causal relationship between sCr and ALS in both European and East Asian populations. Our analysis was conducted using summary statistics from genome-wide association studies with 358,072 individuals for sCr and 80,610 individuals for ALS in European population, and 142,097 individuals for sCr and 4,084 individuals for ALS in East Asian population. The inverse-variance weighted method was used to estimate the casual-effect of sCr on ALS in both populations, and other MR methods were also performed as sensitivity analyses. We found evidence that genetically predicted sCr was inversely associated with risk of ALS (OR, 0.92; 95% CI, 0.85–0.99; $P = 0.028$) in European population. However, there was no strong evidence for a causal relationship between sCr and ALS in East Asian population (OR, 0.92; 95% CI, 0.84–1.01; $P = 0.084$). This study provides evidence that sCr protects against ALS in European population but not in East Asian population.

Keywords Serum creatinine · Amyotrophic lateral sclerosis · Mendelian randomization

Introduction

Amyotrophic lateral sclerosis (ALS) is a neuro-degenerative disease that is characterized by progressive motor neuron degeneration, with death from respiratory failure within 2–3 years after disease onset [1]. The standardized incidence of ALS is 1–2 per 100,000 person-years [2]. The number of ALS patients will increase by ~ 70% in the following decades, resulting in enormous social and economic burden across the globe [3]. Therefore, it is important to identify casual risk factors of ALS.

To date, no agreed environmental risk factors for ALS have been identified. Recently, several observational studies have found that serum creatinine (sCr) is reduced in ALS patients compared to controls [4–8], suggesting that sCr may protects against ALS. However, the baseline sCr in these studies were measured after disease onset, and it is unclear whether the sCr in ALS patients has been reduced before disease onset. Therefore, it still remains unclear whether sCr can reduce the risk of ALS. Given that ALS is rare in the population, it is very difficult to conduct longitudinal studies for investigating the effect of sCr on ALS. In addition, most previous studies are observational, and they are easily biased by confounding factors. Therefore, whether the causal relationship between sCr and ALS is needed to further investigate.

Mendelian randomization (MR) is a new approach using genotypes as instrumental variables to estimate the causal effect of an exposure on an outcome [9, 10]. MR can reduce the bias from confounding factors and reverse causation that are often exist in observational studies, because the genotypes

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from parents to offspring are random allocated, and are unchanged through a lifetime [10]. To our knowledge, MR approach has not been used to investigate the causal relationship between sCr and ALS. Therefore, we performed this two-sample MR study to assess the causal relationship between sCr and ALS in both European and East Asian populations.

Materials and Methods

Data Sources and Instrument Selection

All relevant ethics approval and informed consent are from original genome-wide association studies (GWASs) [11–14].

For the European population, a total of 485 independent SNPs ($r^2 < 0.1$) associated with ($P < 5 \times 10^{-8}$) sCr were selected from a GWAS of 358,072 European individuals in UK Biobank [11]. The mean (SD) sCr concentration of whole UK biobank participants is 72.4 (18.5) umol/L. In the East Asian population, 64 independent autosomal SNPs ($r^2 < 0.1$) associated with sCr were selected from a GWAS in 142,097 Japanese individuals [12]. The mean (SD) sCr concentration of Japanese participants is 0.77 (0.22) mg/dL. The proportion of variance explained by each SNP was evaluated using R^2 [15], and the F statistic was calculated to assess the instrument strength. The F statistic above 10 is considered as an indicator of strong instruments [16].

In the European population, the summary statistics for ALS were obtained from a GWAS with 80,610 European individuals (20,806 cases and 59,804 controls) [13]. For the East Asian population, we obtained the summary statistics of ALS from an East Asian GWAS in 4084 Chinese individuals (1234 cases and 2850 controls) [14].

Statistical Analysis

All MR analyses were conducted using R version 4.0.2, "TwoSampleMR" [17], and "MR-PRESSO" packages [18].

The main analyses were performed with the inverse-variance weighted method, which can provide an unbiased estimate if the MR assumptions are met and the horizontal pleiotropy is balanced. Other MR methods were also performed as sensitivity analyses, including the following: (1) Mendelian randomization robust adjusted profile score (MR-RAPS) method, which accounts for weak instruments [19], (2) weighted median method, which can provide a consistent estimate when at least 50% of instrumental variables are valid [20], and (3) MR-Egger method, which can adjust for bias from directional pleiotropy, at the cost of reduced statistical power [21], The MR-Egger is based on the "NO measurement error assumption (NOME)," and this assumption was evaluated by the regression dilution I^2_{GX} statistic [22]. If I^2_{GX} was below 90% (indicating the violation of NOME assumption), MR-Egger with simulation extrapolation correction (SIMEX) was used [22]. The Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) was also applied to identify potential pleiotropic outlier ($P < 0.1$) [18].

The pleiotropy in MR estimates were assessed using three approaches: (1) The Corchan's Q, which can assess the heterogeneity across all individual SNPs in IVW estimates. A significant Corchan's Q test ($P < 0.05$) suggests the presence of horizontal pleiotropy; (2) MR-Egger intercept, which provides an indicator of directional pleiotropy. A significant MR-Egger intercept test ($P < 0.05$) suggests the presence of directional pleiotropy, and MR-Egger estimates were used to validate the results from IVW estimates; and (3) Leave-one-out (LOO) analysis, in which IVW estimates were re-calculated removing each SNP in turn, to evaluate whether a single SNP drove the association. We evaluated whether each SNP is associated with potential risk factors for ALS ($P < 5 \times 10^{-8}$): smoking (The GWAS & Sequencing Consortium of Alcohol and Nicotine use) [23, 24]; drinking (The GWAS & Sequencing Consortium of Alcohol and Nicotine use) [23, 25]; and low-density lipoprotein (Global lipids Genetics Consortium) [26, 27]. These potential pleiotropic SNPs were removed, and the IVW estimates were re-calculated to rule out potential pleiotropic effects.

Finally, MR Steiger test was conducted to test whether the SNPs for sCr explained more variances in sCr than ALS (the opposite indicate reverse causation) [28]. Power calculations were performed with the mRnd online tool at https://shiny.cnsgenomics.com/mRnd/ [29]. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were scaled per SD unite increase in sCr concentrations. A P value < 0.05 was considered as statistically significant.

Results

In the European population, a potential SNP outlier rs34674752 identified by MR-PRESSO was excluded in the analysis. Therefore, a total of 484 independent SNPs associated with sCr, which all were available in the ALS dataset, were included in the analysis. These 484 SNPs explained 8.2% phenotypic variation of sCr concentration (Supplementary Table 1). For these SNPs, the median F statistic is 43 (ranging from 30 to 749) with an overall F statistic of 66, suggesting that weak instruments was unlikely to bias the estimates of casual effects. In the East Asian population, MR-PRESSO did not identify any outliers. A SNP rs533988 for sCr was not available in the ALS dataset, and no proxy SNP ($r^2 > 0.8$) can be used to replace it. Hence, a total of 63 SNPs were included in the primary analysis. These 63 SNPs explained 2.4% phenotypic variation of sCr concentration (Supplementary Table 2). For these SNPs, the median F statistic is 42 (ranging from 31 to 239) with an overall F statistic

of 55, indicating that weak instruments was also unlikely to bias the estimates of casual effects in the East Asian population.

sCr and ALS in the European Population

In the European population, we found evidence that genetically predicted 1-SD unit increase in sCr concentration was inversely associated with risk of ALS (IVW OR, 0.92; 95% CI, 0.85–0.99; P = 0.028; Figs. 1 and 2). Other MR estimates yield similar effect estimates (Figs. 1 and 2), although with wider CI due to lower statistical power. The I^2_{GX} for MR-Egger was 98.4%, indicating that SIMEX correction was not required to apply. The Corchan's Q statistic suggested substantial heterogeneity (Corchan's Q = 676.19, $P = 1.35\times10^{-8}$), indicating the presence of horizontal pleiotropy. However, the MR-Egger intercept test (P = 0.99) indicated no directional pleiotropy, suggesting that the horizontal pleiotropy was unlikely to bias the IVW estimate. Furthermore, the LOO analysis and forest plot indicated that no single SNP drove the association (Supplementary Figure 1-2). After removing 5 potential pleiotropic SNPs (Supplementary Table 3), the IVW estimate was not significantly changed (IVW OR, 0.924; 95% CI, 0.857–0.997; P = 0.042). The MR Steiger test for directionality indicated that using ALS as the outcome was the correct causal direction for sCr (P < 0.001).

sCr and ALS in the East Asian Population

We further evaluated the casual association between sCr and ALS in the East Asian population. The casual-effect estimate of sCr on ALS is not statistically significant in East Asian population (OR, 0.92; 95% CI, 0.84–1.01; P = 0.084; Figs. 1 and 2), although it was similar with European population in direction and magnitude, which may be due to limited power. Similar effects estimates were observed in other MR estimates (Figs. 1 and 2). The LOO analysis and forest plot indicated that no single SNP can influence the casual-effect estimate (Supplementary Figure 3-4). Furthermore, there was no evidence of substantial heterogeneity (Corchan's Q = 74.52, P = 0.132) and directional pleiotropy (MR-Egger intercept, P = 0.780).

Discussion

In this study, we investigated the causal relationship between sCr and ALS in both European and East Asian populations using a two-sample MR approach. Our findings showed that genetically predicted sCr was inversely associated with the risk of ALS in European population but not in East Asian population.

Previous observational studies have shown that sCr are decreased in ALS patients compared to controls in European population [4, 5]. Our MR study found similar inversely association between sCr and ALS in European population. These findings suggest that sCr can protect against the risk of ALS in European population. In East Asian population, some observational studies also have reported ALS cases have lower sCr compare to the controls [7, 8]. However, we did not find a statistically significant association between sCr and ALS (OR, 0.92; 95% CI, 0.84–1.01; P = 0.084), although it was similar with European population in direction and magnitude, suggesting that it is may be due to limited power. Given that the type 1 error is 5%, the sample size is 4084, the proportion of variance explained by SNPs is 2.4%, and the true casual OR of sCr on ALS is 0.90 (or equivalently 1.1), the statistical power of the two-sample MR to detect such causal effect is only 8% in the East Asian population. Therefore, whether a causal relationship between sCr and ALS in East Asian population is still needed to further investigate in larger sample.

The exact underlying mechanism linking sCr with ALS still remains unclear. Two potential mechanisms involve oxidative stress and mitochondrial dysfunction. Oxidative stress and mitochondrial dysfunction have been proved to contribute to the pathogenesis of ALS [30–35]. sCr, the end product of creatine and creatine phosphate, can reflect the change of

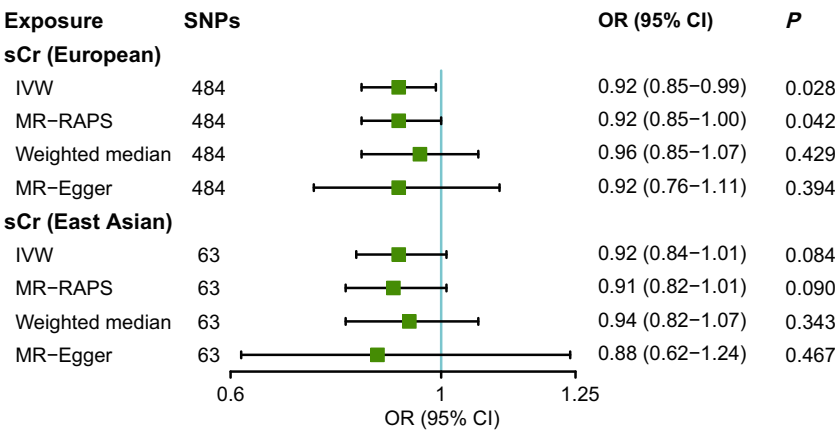

Fig. 1 Mendelian randomization estimates of serum creatinine with ALS in both European and East Asian populations

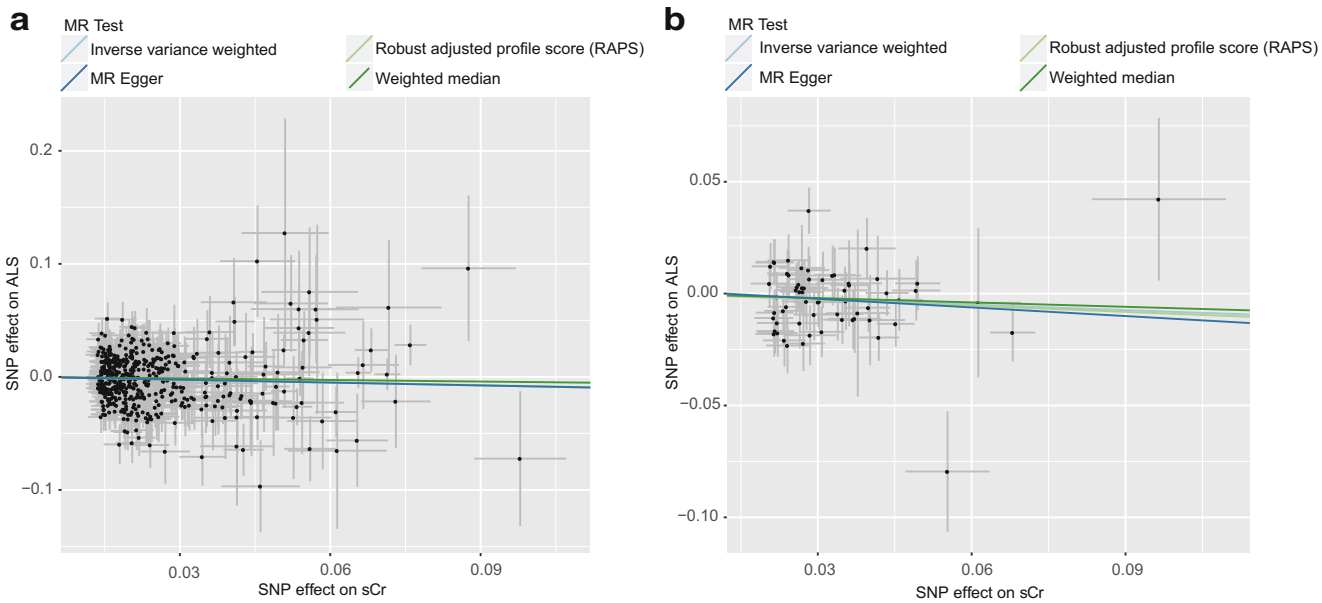

Fig. 2 **a** Scatter plots of the effect sizes for each SNP on serum creatinine and ALS in European population. **b** Scatter plots of the effect sizes for each SNP on serum creatinine and ALS in East Asian population

creatine pool, which plays a key role in mitochondrial function and has neuro-protective function in vivo and in vitro [36]. Several in vivo [37, 38] and in vitro [39–41] studies have demonstrated that creatine has antioxidant capacity. Further studies are needed to elucidate the exact mechanism linking sCr with ALS.

Our study has some strengths. First, MR study is less prone to reverse causation and confounding, providing relatively robust estimate of casual relationship between sCr and ALS. Second, we used large-scale GWASs in the European population. Our study has several limitations. First, a major limitation of MR study is pleiotropy, which may bias the IVW estimates. Hence, several approaches were performed to assess the pleiotropy. For example, MR-RAPS, weighted median, and MR-Egger estimates that are robust to pleiotropy were calculated to compare with IVW estimate. In addition, we performed the LOO analyses to evaluate whether a single SNP drove the result. All these analyses have similar results, indicating that pleiotropy is unlikely to bias the IVW estimate. Second, another limitation is that we cannot assess the correlation between sCr and other parameters of ALS (e.g., clinical phenotype, genetic background, cognitive impairment, survival, sit of onset, and age), because these parameters are not available. More studies are needed to investigate this correlation. Third, the two-sample MR assumes a linear association between exposure and outcome. Hence, we did not assess a potential nonlinear association between sCr and ALS. Fourth, the statistical power was low in the analyses of ALS in East Asian population (8%). Hence, we cannot rule out the causal relationship between sCr and ALS in East Asian population. Finally, we cannot assess sex-specific effects of sCr on ALS, because no sex-specific GWASs are available.

A previous population-based study has demonstrated that sCr may serve as a prognostic biomarker for ALS [42]. This study assessed the correlation of sCr evaluated at diagnosis with ALS outcome, and found that patients with lower sCr are significantly associated with worse clinical function at diagnosis of ALS and shorter survival [42]. In line with this study, our study found that patients with higher sCr was associated with lower risk of ALS in European population. These findings suggested that sCr is possibly involved in the pathogenesis of ALS and may play a protective role in the development of ALS. Therefore, sCr may serve as a reliable and easily accessible blood markers of the risk and outcome for ALS. Large multi-center prospective studies are still needed to investigate the association of sCr with the risk and outcome of ALS. Besides, further functional studies are warranted to investigate the mechanisms linking the association of sCr with ALS.

In conclusions, using a two-sample MR approach, our study provides evidence to support that sCr protects against the risk of ALS in European but not in East Asian population.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12035-021-02309-w.

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Data Availability The data used to perform the analyses in this study were obtained from public genome-wide association studies summary statistics.

Declarations

Ethics Approval All relevant ethics approval are from original genome-wide association studies.

Consent to Participate This study only used publicly available summary statistics from published genome-wide association studies. No individual-level data were involved, and no additional informed consent are needed in this study.

Consent for Publication No individual-level data were involved, and no consent for publication is needed for this study.

Conflict of Interest The authors declare no competing interests.

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