Interleukin-1 receptor antagonist, interleukin-2 receptor alpha subunit and amyotrophic lateral sclerosis

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Keywords: amyotrophic lateral sclerosis, immunological prevention, interleukin-1 receptor antagonist, interleukin-2 receptor α subunit, Mendelian randomization analysis

Background and purpose: To clarify the causal associations of interleukin-1 receptor antagonist (IL-1ra) and interleukin-2 receptor alpha subunit (IL-2rα) with the risk of amyotrophic lateral sclerosis (ALS).

Methods: A two-sample Mendelian randomization study design was employed. Single-nucleotide polymorphisms associated with IL-1ra (n = 2) and IL-2rα (n = 1) at the genome-wide significance level were used as unbiased instrumental variables. Summary-level data for ALS were obtained from Project MinE, an international collaboration consortium with 12 577 ALS cases and 23 475 controls of European descent.

Results: Genetic predisposition to higher levels of IL-1ra was significantly associated with lower odds of ALS. For a 1-SD increase of circulating IL-1ra levels, the odds ratio of ALS was 0.64 (95% confidence intervals, 0.46–0.88; P = 0.005). There was a borderline inverse association between IL-2rα levels and ALS (odds ratio, 0.91; 95% confidence intervals, 0.83–1.00; P = 0.058).

Conclusions: Interleukin-1 receptor antagonist levels were inversely associated with ALS, suggesting that interleukin-1 inhibitors may lower the risk of this always fatal disease. The role of IL-2rα levels in ALS needs further verification in causal inference studies with larger sample sizes.

Introduction

Amyotrophic lateral sclerosis (ALS) is an always fatal progressive neurodegenerative disorder that leads to muscle weakness and paralysis [1]. Despite the low incidence of ALS of 2–3 in 100 000 individuals [1], the serious outcome of the disease has prompted several studies of ALS etiology and pathology. Inflammation responses mediated by interleukin-1 and interleukin-2 have been proposed to play a role in the development of ALS [2]. However, the causal inference on these associations is limited because available evidence originates from observational studies prone to confounding and reverse causality.

Mendelian randomization (MR) studies can strengthen the causal inference on exposure–disease associations by exploiting genetic variants as unbiased proxies (i.e. instrumental variables) of the exposure [3]. This approach can effectively diminish unobserved confounding as genetic variants are randomly allocated at meiosis, which is similar to the random assignment of participants to experimental and control groups in a randomized controlled trial [3]. It can also overcome reverse causality as the development and progression of diseases cannot modify genotype. This method has been proved to be as effective as randomized controlled trials [4]. We conducted a two-sample MR study to explore the potential causal associations of interleukin-1 receptor antagonist (IL-1ra), an endogenous inhibitor of both IL-1α and IL-1β, and interleukin 2 receptor α subunit (IL-2rα), with ALS risk.

Methods

Study design overview

This two-sample MR study was based on summary-level data from published genome-wide association
studies (GWASs) on IL-1ra, IL-2rα and ALS, which obtained appropriate patient consent and ethical approval. Information on the assumptions of the MR study design and the data sources used for the analyses are presented in Fig. 1. The analyses were approved by the Swedish Ethical Review Authority.

**Single-nucleotide polymorphism selection and data source**

As instrumental variables, we selected two and one single-nucleotide polymorphisms (SNPs) associated with circulating IL-1ra and IL-2rα at genome-wide significance ($P < 5 \times 10^{-8}$) in two GWASs with up to 13,955 and 8,293 individuals of European ancestry, respectively (Table 1) [5,6]. The mean levels of IL-1ra ranged from 251.4 to 433.9 pg/mL across the 11 included studies. For IL-2rα, the mean levels ranged from 81.7 to 278 pg/mL across the six included studies. The two SNPs for IL-1ra and the SNP for IL-2rα explained 2.0% and 4.4%, respectively, of the variation in circulating levels [5,6].

Summary-level data for ALS were obtained from Project MinE, an international collaboration consortium, with 12,577 ALS cases and 23,475 controls of European descent [7]. All patients with ALS and controls were recruited through clinics affiliated to the research groups and genetic information was extracted from blood samples.

**Statistical analysis**

The associations of IL-1ra and IL-2rα with ALS were estimated under the Wald assumption by dividing the beta coefficient for the SNP–ALS association by the beta coefficient for the SNP–interleukin association [8]. The inverse-variance-weighted approach was used to combine the Wald estimates for the two IL-1ra SNPs [8]. Considering a moderate linkage disequilibrium between two SNPs used for IL-1ra ($R^2 = 0.11$), we additionally used the inverse-variance-weighted method adjusting for the correlations across SNPs [9] to reassess the association between IL-1ra and ALS risk. The odds ratios (ORs) with 95% confidence intervals (CI) for ALS were scaled to a 1-SD increment of genetically predicted circulating levels of IL-1ra and IL-2rα. We calculated power using a web-based tool [10] and searched PhenoScanner V2 [11] to evaluate if the selected instrumental variables were associated with any other trait at genome-wide significance level. All statistical analyses were two-sided and performed in Stata/SE 15.0 (StataCorp, College Station, TX, USA).

**Results**

Genetic predisposition to higher levels of IL-1ra was significantly associated with lower odds of ALS (Fig. 2). For a 1-SD increase in circulating IL-1ra levels, the OR of ALS was 0.64 (95% CI, 0.46–0.88; $P = 0.005$). In the analysis considering the correlations of two used SNPs, the causal association between IL-1ra levels and ALS risk persisted (OR, 0.64; 95% CI, 0.44–0.93; $P = 0.019$). There was a borderline inverse association between IL-2rα levels and ALS (OR, 0.91; 95% CI, 0.83–1.00; $P = 0.058$) (Fig. 2). We detected no possible pleiotropic traits driving the observed associations. Only rs4251961 for IL-1ra was associated

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**Figure 1** Three assumptions of the Mendelian randomization design and summary of data sources used in the present study. GWAS, genome-wide association study; IL-1ra, interleukin-1 receptor antagonist; IL-2rα, interleukin 2 receptor alpha subunit; SNP, single-nucleotide polymorphism. [Colour figure can be viewed at wileyonlinelibrary.com]
with certain immune cell counts (Table 2). We had 100% power to detect an OR of 0.6 in analyses of IL-1ra. However, the power to detect an OR of 0.9 was only 50% in analyses of IL-2rα.

**Discussion**

This MR study based on data from 12,577 ALS cases and 23,475 controls showed a statistically significant 36% lower odds of ALS per 1-SD increment in circulating IL-1ra levels. The association between IL-1ra levels and ALS was assessed in a previous smaller MR study, including 4,240 ALS cases and 5,104 controls from the International Consortium on ALS Genetics [11]. In that MR study, the OR of ALS per additional IL-1ra-raising allele was 0.96 (95% CI, 0.91–1.00; \( P = 0.08 \)) with significant heterogeneity (\( I^2 = 79\% \); \( P = 0.029 \)) between the SNPs (rs6759676 and rs1542176 on chromosome 2) used as instrumental variables [12]. To the best of our knowledge, no previous study has examined the role of circulating IL-2rα levels in ALS. Our study provides suggestive evidence of a possible causal inverse association of IL-2rα levels with ALS.

Although rs4251961 is an intron gene (removed during maturation of the final RNA product), it is in linkage with coding SNPs in or near the *IL1RN* region, which has been proved to play a role in coding interleukin 1 cytokine family protein. It is likely that the genetic variants correlated with rs4251961 alter the structure of IL-1ra and change the capacity to bind interleukin 1, thereby influencing the risk of ALS. In addition, the original GWAS for IL-1ra showed that the two SNPs, possibly representing coding SNPs, also influenced the levels of IL-1ra [4]. Therefore, whether the causal effect of IL-1ra on ALS comes from the alteration of IL-1ra structure or its level needs further investigation. Although we detected several associations of overall and individual white blood cells with rs4251961 at the genome-wide threshold, these related traits were more likely to be mediators (known as the vertical pleiotropy, which does not violate the assumptions of MR analysis and bias the results) linking IL-1ra to ALS as the genetic variants selected for IL-1ra are located close to the gene coding IL-1ra itself.

To reduce population stratification bias, we restricted the study population to those of European descent. This restriction reduced the generalizability of our results to individuals of non-European ancestry. A limitation is that, with only two and one instrumental variables for IL-1ra and IL-2rα, respectively, we were unable to use complementary MR approaches such as the weighted median and MR–Egger methods.

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**Table 1** Characteristics of the single-nucleotide polymorphisms (SNPs) associated with interleukin 1 receptor antagonist (IL-1ra) and interleukin 2 receptor alpha subunit (IL-2rα) and their associations with amyotrophic lateral sclerosis (ALS)

| Trait | SNP       | Gene  | Chr | Position | EA    | NEA   | EAF   | MAF    | Betaa | SE   | P-value |
|-------|-----------|-------|-----|----------|-------|-------|-------|--------|-------|------|---------|
| IL-1ra| rs4251961 | IL1RN | 2   | T        | 0.64  | 0.36  | 0.082 | 0.009  | 2.76 × 10^{-21} | -0.032 | 0.018 | 0.080   |
| IL-1ra| rs6759676 | IL1F10| 2   | C        | 0.40  | 0.40  | 0.075 | 0.009  | 1.73 × 10^{-17} | -0.039 | 0.018 | 0.028   |
| IL-2rα| rs12722497| IL2RA | 10  | A        | 0.14  | 0.14  | 0.628 | 0.049  | 1.57 × 10^{-38} | -0.057 | 0.030 | 0.058   |

Chr, chromosome; EA, effect allele; EAF, effect allele frequency; MAF, minor allele frequency; NEA, none effect allele; SE, standard error.

*Beta coefficients represent the change of interleukin levels in SD units for each additional effect allele. *β*Beta coefficients represent the log odds ratio of ALS for each additional effect allele.
to adjust for pleiotropy. However, we detected no related pleiotropic traits, driving the associations, for the SNPs selected in this study.

In conclusion, the present two-sample MR study provides support for a causal inverse association between circulating IL-1ra levels and ALS risk. Interleukin-1 inhibitors such as anakinra have been shown to reduce the levels of cytokines in patients with ALS [13]. The association between IL-2ra levels and ALS needs further verification in causal inference studies with larger sample sizes.

Acknowledgements

Summary-level data for ALS were contributed by the Project MinE GWAS Consortium. The authors thank the investigators for sharing these data. S. C. Larsson is supported by the Swedish Research Council for Health, Working Life and Welfare, FORTE (Forskningsrådet om Hälsa, Arbetsliv och Välstånd, grant no. 2018-00123) and the Swedish Research Council (Vetenskapsrådet, grant no. 2019-00977).

Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

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| Table 2 | Related traits of the single-nucleotide polymorphisms associated with rs4251961 at genome-wide significance level |
|------------------|---------------------------------|
| **Biomarker SNP** | **Nearby gene** | **Chr EA Trait** | **Trait** | **Beta** | **P-value** |
| IL-1ra rs4251961  | ILRN                | 2 T Sum basophil neutrophil counts | −0.032 | 3.93 × 10⁻⁸ |
|                  |                     | 9 T Neutrophil count               | −0.022 | 5.14 × 10⁻⁸ |
|                  |                     | 10 T Granulocyte count             | −0.022 | 6.13 × 10⁻⁸ |
| IL-2ra rs4251961  | ILRN                | 2 T Myeloid white cell count       | −0.022 | 3.19 × 10⁻¹⁷ |
|                  |                     | 9 T Neutrophil percentage of white cells | −0.029 | 5.52 × 10⁻⁸ |

Chr, chromosome; EA, effect allele. Web of PhenoScanner V2: http://www.phenoscanner.medschl.cam.ac.uk/
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