

IL-6R protective variant rs7529229 reduces interleukin-6 signaling and contributes to decreased ischemic stroke risk

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Research

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Abstract

Background

Interleukin-6 (IL-6) signaling is associated with the increased risk of coronary artery disease (CAD) and ischemic stroke (IS). Growing evidence shows that the minor allele of *IL-6R* rs7529229 variant could significantly increase the soluble IL-6 receptor levels and reduce CAD risk. However, it is largely unknown about the role of rs7529229 in IS, which promotes us to perform a comprehensive analysis.

Methods

In stage 1, we performed a meta-analysis of three GWAS datasets from MEGASTROKE, UK Biobank, and Million Veteran Program to evaluate the association of rs7529229 with IS. In stage 2, we conducted an expression quantitative trait loci analysis to examine the effects of rs7529229 on *IL-6R* expression in neuropathologically normal individuals from UK Brain Expression Consortium, GTEx project and eQTLGen Consortium. In stage 3, a tissue-specific gene expression analysis is used to evaluate the potential difference of *IL-6R* expression across the human tissues using gene expression data from GTEx. In stage 4, a case-control gene expression analysis is used to explore the potentially differential expression of *IL-6R* in IS and healthy controls in whole blood.

Results

Our results show that (1) rs7529229 minor allele could significantly reduce the risk of IS (OR=0.97, 95% CI 0.95-0.99, $P=2.30E-03$); (2) rs7529229 minor allele could significantly reduce *IL-6R* expression in relevant tissues especially in blood vessels and whole blood; (3) *IL-6R* is mainly expressed in skeletal muscle and whole blood; (4) expression of *IL-6R* is significantly reduced in healthy controls compared with IS cases in whole blood. Importantly, the biological senses in stage 1-4 are all convergent.

Conclusions

Collectively, these findings indicate that rs7529229 minor allele may first reduce *IL-6R* expression in relevant tissues, further reduce IL-6 signaling, and eventually reduce the IS risk. Hence, *IL-6R* may be a potential therapeutic target, and blocking IL-6 signaling might be effective in treatment of IS.

Introduction

Interleukin-6 (IL-6) is an important inflammatory cytokine, and could exert its effect via classic signaling and trans-signaling [1]. In classic signaling, IL-6 binds to membrane-bound IL-6 receptor (IL-6R), forms IL-6-IL-6R complex, and then recruits glycoprotein 130 (gp130) [2-3]. In trans-signaling, IL-6 binds to soluble IL-6R (sIL-6R), and then binds to membrane-anchored gp130 [2-3]. The observational studies have showed that IL-6 signaling is associated with the increased risk of cardiovascular diseases (CVD) including coronary artery disease (CAD) and ischemic stroke (IS) [4-8].

Until recently, Mendelian randomization (MR) studies have been conducted to evaluate the association between IL-6 signaling and CVD [9-10]. Rosa et al. applied a reduced IL-6 signaling - increased sIL-6R pattern [9]. Georgakis et al. selected the reduced IL-6 signaling - lower C-reactive protein (CRP) pattern [10]. Interestingly, both MR studies found that increased IL6 signaling was causally associated with increased risk of CAD and IS, although reported inconsistent findings for IS subtypes [9-10]. Hence, inhibition of IL-6 signaling might be effective in lowering CVD risk or treatment of CVD.

Tocilizumab, a monoclonal antibody that blocks both membrane-bound and circulating IL-6R, has been licensed for treatment of rheumatoid arthritis (RA) to reduce systemic and articular inflammation including CRP and fibrinogen concentrations [7, 11-13]. Tocilizumab could bind to the IL-6 binding site of human IL-6R and competitively inhibit IL-6 signaling [14]. Hence, patients with tocilizumab treatment had significantly increased serum IL-6 and sIL-6R levels [14]. Meanwhile, evidence from epidemiological studies indicates that RA patients have a 50% increased risk of cardiovascular-related morbidity and mortality [15]. Hence, it is important to clarify whether CVD may benefit from treatment with IL-6R blockade using tocilizumab.

Interestingly, recent studies showed that individuals with the minor allele of *IL-6R* genetic variant (rs2228145, rs7529229, and rs4129267, all three variants are in high linkage disequilibrium with each other) but without tocilizumab treatment have similar biomarker profiles to those with tocilizumab treatment, and could reduce the risk of CAD [7, 16]. In 2012, IL-6R Genetics Consortium and Emerging Risk Factors Collaboration analyzed 125,222 participants, and found that individuals with the minor allele of *IL-6R* rs2228145 variant had 34.3% increased sIL-6R levels, 14.6% increased serum IL-6 levels, 7.5% reduced CRP levels, 1.0% reduced fibrinogen levels, and 3.4% reduced CAD risk [16]. In 2012, Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium analyzed 133,449 individuals, and found that individuals with the minor allele of *IL-6R* rs7529229 variant had 9.45% increased serum IL-6 levels, 8.35% reduced CRP levels, 0.85% reduced fibrinogen levels, and 5% reduced CAD risk [7]. In 2018, Cai et al. conducted a phenome-wide association study (PheWAS) using *IL-6R* variants rs2228145 and rs4129267, and confirmed the significant association of both *IL-6R* variants with CAD [17].

Hence, the association between the minor allele of *IL-6R* genetic variant and reduced CAD risk has been well established. Importantly, randomized controlled trials (RCTs) have been performed. In 2016, Kleveland et al. selected 117 acute non-ST elevation myocardial infarction (NSTEMI) patients (placebo, n=59; tocilizumab, n=58), and conducted a double-blind, randomized, placebo-controlled phase 2 trial [18]. They demonstrated that IL-6 inhibition using tocilizumab could attenuate systemic inflammation and troponin T-release in patients with NSTEMI [18]. Meanwhile, there was no significant difference in major safety concerns between the two groups [18]. In 2020, they further found that tocilizumab treatment in NSTEMI patients could increase circulating levels of the neutrophil extracellular traps (NETs) marker citrullinated histone 3, which indicated that tocilizumab could enhance NETosis [19].

In summary, all these findings show that human genetic variants could be used to repurpose the existing targets for new therapeutic uses [7, 16, 20-21]. However, it is largely unknown about the role of rs7529229

in IS, which promotes us to perform a comprehensive analysis. In stage 1, we evaluate the association of *IL-6R* genetic variant rs7529229 with IS using three genome-wide association study (GWAS) datasets [17, 22-23]. In stage 2, we examine the effects of rs7529229 on *IL-6R gene expression* in neuropathologically normal individuals *using three independent* expression quantitative trait loci (eQTLs) dataset resources [24-26]. In stage 3, we evaluated the potential *IL-6R* expression difference across the different human tissues [27]. In stage 4, we explore the potentially differential expression of *IL-6R gene* in IS cases and controls using a gene expression dataset [28].

Materials And Methods

Study design

This study is based on large-scale GWAS summary datasets, eQTLs datasets, expression datasets, and case-control expression datasets. All participants gave informed consent in all these corresponding original studies. All relevant data, analytic methods, and study materials are within the paper. In addition, this study does not involve animal models.

GWAS datasets

We first selected three different IS GWAS dataset resources. The first IS GWAS dataset resource is from the largest multi-ancestry meta-analysis of stroke GWAS datasets conducted by MEGASTROKE [22]. Here, we limited our analysis in participants of European ancestry including 34,217 IS cases and 406,111 controls. According to the Trial of Org 10172 in Acute Stroke Treatment classification criteria, IS consisted could be mainly divided into three subtypes including large artery atherosclerotic stroke (LAS, 4,373 cases and 406,111 controls), cardioembolic stroke (CES, 7,193 cases and 406,111 controls), and small vessel stroke (SVS, 5,386 cases and 406,111 controls) [22]. The second IS GWAS dataset resource is from the UK Biobank including 1,501 IS cases with cerebral artery occlusion, and 399,017 controls [23]. More detailed information is provided in PheWeb (<http://pheweb.sph.umich.edu/SAIGE-UKB/>). The third IS GWAS dataset resource is from the Million Veteran Program (MVP) including 1,198 IS cases with cerebral artery occlusion, and 331,601 controls, as described in a recent study [17]. To be a comparison, we also selected the large-scale GWAS datasets for RA and CAD. The RA GWAS dataset is from a large-scale meta-analysis in European ancestry including 14,361 RA cases and 43,923 controls [29]. The CAD GWAS dataset is from the CARDIoGRAMplusC4D consortium ((Coronary ARtery Disease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics) including 60,801 CAD cases, and 123,504 controls, most of which are of European ancestry [30].

eQTLs datasets

The rs7529229 mutation is a no-coding variant in *IL-6R gene*, and may regulate the expression of *IL-6R gene*. Hence, we examine the association between rs7529229 variant and *IL-6R gene* expression using multiple eQTLs dataset resources. The first eQTLs dataset resource is from the UK Brain Expression Consortium (UKBEC), which is publicly available in Brain eQTL Almanac (Braineac) database [24]. The

gene expression levels were measured using the Affymetrix GeneChip Human exon 1.0 ST arrays [24]. Braineac included 10 eQTLs datasets in 10 brain tissues from 134 neuropathologically normal individuals with European descent [24].

The second eQTLs resource is from the Genotype-Tissue Expression (GTEx) project (version 8) including 49 tissues (number of samples with genotype ≥ 70), 828 donors and 15201 samples [27]. The gene expression levels were measured using the Illumina TruSeq RNA sequencing and Affymetrix Human Gene 1.1 ST Expression Array (V3; 837 samples) [27].

The third eQTLs resource is from the eQTLGen Consortium [26]. This consortium conducted a large-scale meta-analysis in 31,684 human whole blood samples from 37 cohorts and the majority individuals were of European ancestry [26]. The gene expression levels were profiled by Illumina, Affymetrix U291, Affymetrix HuEx v1.0 ST expression arrays and RNA-seq [26].

IS case-control gene expression dataset

To evaluate the potential differential expression of *IL-6R* gene, we performed a IS case-control gene expression analysis in whole blood using a gene expression dataset from the Gene Expression Omnibus (GEO) database (GSE16561). In GSE16561 dataset, gene expression profiling was measured in the peripheral whole blood of 39 IS patients (17 males and 22 females) and 24 healthy control subjects (10 males and 14 females) using Illumina microarrays [28]. All these 63 participants are of European ancestry [28].

Genetic association analysis of *IL-6R* rs7529229

We first extracted the corresponding summary statistics of rs7529229 variant in three IS GWAS dataset resources including MEGASTROKE, UK Biobank and MVP. We then conducted a meta-analysis to evaluate the association between rs7529229 variant and IS using R Package (meta: General Package for Meta-Analysis). The overall odds ratio (OR) is calculated by the fixed effect model (Mantel-Haenszel) or random-effect model (DerSimonian-Laird), which is determined by the heterogeneity among these three resources [31]. We further investigated the association of rs7529229 variant with the IS subtypes (LAS, CES, and SVS), RA, and CAD using the corresponding GWAS summary statistics. The statistical significance for the association between rs7529229 and one specific phenotype is a Bonferroni-corrected threshold $0.05/6=0.0083$. Meanwhile, the original *P* values between 0.0083 and 0.05 were considered to be suggestive association.

eQTLs analysis

In Braineac, we first downloaded the *IL-6R* gene expression data and the genotype data of generic variants with 1Mb upstream of transcription start site and 1Mb downstream of transcription end site [24]. We then evaluated the potential association between rs7529229 variant and *IL-6R* expression using a linear regression analysis under an additive model by adjusting for several critical covariates including the brain bank, gender and batch effects in Partek's Genomics Suite v6.6 [24].

In GTEx, eQTLs analysis was performed using FastQTL with following covariates: top 5 genotyping principal components, a set of covariates identified using the Probabilistic Estimation of Expression Residuals (PEER) method (the number of PEER factors was determined as function of sample size (N): 15 factors for $N < 150$, 30 factors for $150 \leq N < 250$, 45 factors for $250 \leq N < 350$, and 60 factors for $N \geq 350$), sequencing platform (Illumina HiSeq 2000 or HiSeq X), sequencing protocol (PCR-based or PCR-free), and the gender [27]. Detailed information for the laboratory methods and analysis methods was provided in the original paper and the GTEx website (<https://www.gtexportal.org/home>) [27].

In eQTLGen, a data-driven method was used to integrate the gene expression data from platforms [26]. For a given SNP, genes within 1Mb upstream and 1Mb downstream around this SNP were selected according the center position of the gene [26]. Then the eQTLs analysis was conducted by a Spearman correlation [26].

Gene expression analysis of *IL-6R* in GTEx

We conduct a gene expression analysis to investigate the potential *IL-6R* expression difference in different human tissues using the gene expression data in GTEx (version 8). The gene expression level was quantified by transcripts per million (TPM) based on the GENCODE 26 annotation, collapsed to a single transcript model for each gene using a custom isoform collapsing procedure [27]. Here, we selected the T test or analysis of variance (ANOVA) method to evaluate the potential difference of *IL-6R* expression in different human tissues. The statistical significance is $P < 0.05$.

IS case-control gene expression analysis

Here, we performed a differential expression analysis using the NCBI web application GEO2R (<http://www.ncbi.nlm.nih.gov/geo/geo2r/>) [32]. GEO2R could invoke the Bioconductor R packages to transform and analyze GEO datasets [32]. Evidence has showed the sex differences in IS epidemiology, presentations, and outcomes [33]. Hence, we further conducted a subgroup analysis in females and males, respectively. Here, we define $P < 0.05$ to be the significance level of differential expression of *IL-6R* gene in IS patients and healthy control subjects.

Results

Genetic association analysis of *IL-6R* rs7529229

We did not identify any significant heterogeneity among the three IS GWAS dataset resources with Cochran's Q test $P=0.12$. Hence, the overall OR is calculated by the fixed effect model. We found that rs7529229 variant C allele was significantly associated with 3% reduced IS risk (OR=0.97, 95% CI 0.95-0.99, $P=2.30E-03$). This finding is consistent with previous studies in RA and CAD, which reported that rs7529229 variant C allele could reduce 7% RA risk (OR=0.93, 95% CI 0.91-0.96, $P=1.90E-05$) and 4% CAD risk (OR=0.96, 95% CI 0.94-0.98, $P=2.50E-07$), respectively (Table 1). Meanwhile, rs7529229 variant C allele showed suggestive association with 4% reduced CES risk ($P=2.15E-02$), 5% reduced SVS risk

($P=4.15E-02$), but not with LAS risk ($P=3.32E-01$) using GWAS summary datasets from the MEGASTROKE, as provided in Table 1.

eQTLs analysis of *IL-6R* rs7529229

In GTEx (version 8), the eQTLs datasets in 47 of 49 tissues are available excluding two tissues kidney medulla and esophagus gastroesophageal junction. Hence, a total of 58 tissues (10 tissues in Brainiac, 47 tissues in GTEx and whole blood in eQTLGen) were selected. Here, the statistical significance for eQTLs analysis is a Bonferroni-corrected threshold of $P < 0.05/58=8.62E-04$. Meanwhile, the original P values between $8.62E-04$ and 0.05 were considered to be suggestively significant. In human brain tissues, we did not identify any significant ($P < 0.05/58=8.62E-04$) association between rs7529229 variant C allele and *IL-6R* expression including 10 brain tissues from Brainiac and 13 brain tissues from GTEx (version 8), even any suggestive association ($P < 0.05$), as provided in Table 2.

In GTEx (version 8) human non-brain tissues, we identified significant association between rs7529229 variant and *IL-6R* expression in IS relevant tissues or organs, such as blood vessels including aorta artery ($P=1.60E-05$) and tibial artery ($P=2.80E-14$), and whole blood ($P=8.5E-09$) (Table 3). Importantly, rs7529229 variant C allele could only reduce the expression of *IL-6R* in all these relevant tissues or organs ($\beta < 0$) (Table 5). Meanwhile, rs7529229 variant C allele could also significantly reduce the expression of *IL-6R* in sigmoid colon ($P=1.00E-04$), transverse colon ($P=9.80E-13$), esophagus mucosa ($P=5.60E-05$), esophagus muscularis ($P=1.50E-05$), and small intestine ($P=1.70E-05$), as provided in Table 3. In addition, there is also suggestive association between rs7529229 variant C allele and reduced *IL-6R* expression in cultured fibroblasts cells ($P=2.60E-02$), atrial appendage ($P=4.60E-03$), tibial nerve ($P=1.60E-02$), stomach ($P=1.40E-02$), testis ($P=3.80E-02$), thyroid ($P=3.50E-03$), and uterus ($P=2.60E-03$), as provided in Table 3. Interestingly, we further confirm the significant association of rs7529229 variant C allele with reduced the expression of *IL-6R* in whole blood using the eQTLGen dataset (Z score = -6.6162 and $P=3.68E-11$).

Gene expression analysis of *IL-6R* in GTEx

Gene expression analysis shows that *IL-6R* is mainly expressed in skeletal muscle (TPM median = 67.24) and whole blood (TPM median = 52.69), and shows no significant difference of *IL-6R* gene expression in both tissues. However, the expression of *IL-6R* gene in skeletal muscle and whole blood is significantly higher than other tissues ($P < 0.05$), such as the liver (TPM median = 38.03), lung (TPM median = 29.45), esophagus muscularis (TPM median = 27.04), sigmoid colon (TPM median = 25.73), esophagus gastroesophageal junction (TPM median = 25.38), spleen (TPM median = 24.00), uterus (TPM median = 23.29), and small intestine (TPM median = 22.03).

Meanwhile, *IL-6R* shows very low expression level in human brain tissues. In all GTEx (version 8) human tissues, the lowest 10 tissues with *IL-6R* expression are all brain tissues including caudate (TPM median = 3.079), hypothalamus (TPM median = 3.037), frontal cortex (TPM median = 2.753), amygdale (TPM median = 2.737), hippocampus (TPM median = 2.709), nucleus accumbens (TPM median = 2.571), anterior cingulate cortex (TPM median = 2.514), putamen (TPM median = 2.486), cerebellum (TPM

median = 1.643), cerebellar hemisphere (TPM median = 1.185). The box plots for the expression of *IL-6R* gene in different tissues are provided in Figure 1.

Case-control gene expression analysis

In human whole blood, we identified significant dysregulation of *IL-6R* expression in IS cases compared with controls ($P < 0.05$, Table 4). Importantly, we only found significantly increased *IL-6R* expression in IS cases (Table 4). In brief, two transcripts are available for *IL-6R* including ILMN_1754753 and ILMN_1696394. IS cases have 37% and 30% increased *IL-6R* expression compared with controls for transcripts ILMN_1754753 ($P = 2.33E-05$) and ILMN_1696394 ($P = 7.90E-04$), respectively. Importantly, the subgroup analysis in males and females further support these findings, as provided in Table 4.

Discussion

IL-6 signaling was associated with increased risk of CAD and stroke [4-6]. Hence, inhibiting IL-6 signaling through blockade of the IL-6R may reduce the inflammation, such as tocilizumab licensed for treatment of RA [7, 11-13]. Interestingly, some individuals with the minor allele of *IL-6R* rs7529229 variant without tocilizumab treatment have similar biomarker profiles to those with tocilizumab treatment [7, 16]. Importantly, growing evidence gradually demonstrates that the minor allele of *IL-6R* rs7529229 variant could reduce the risk of CAD, and CAD may benefit from treatment with IL-6R blockade [7, 16-17]. However, it remains currently unknown for the role of *IL-6R* genetic variant in stroke. Here, we performed a comprehensive analysis using large-scale GWAS datasets, eQTLs datasets, gene expression datasets, and IS case-control gene expression datasets. Interestingly, convergent evidence highlighted the role of *IL-6R* rs7529229 variant in IS. Here, we discussed our findings by comprehensive comparisons with previous studies.

In stage 1, we found that *IL-6R* rs7529229 variant minor allele C could significantly reduce 3% IS risk (OR=0.97, 95% CI 0.95-0.99, $P = 2.30E-03$) by a meta-analysis of three large-scale GWAS datasets from MEGASTROKE, UK Biobank and MVP including 36,916 IS and 1,136,729 controls. Importantly, our finding is consistent with previous GWAS in RA and CAD [29-30]. Meanwhile, our finding is also consistent with previous findings from candidate variant analysis and PheWAS analysis. In 2012, IL-6R Genetics Consortium and Emerging Risk Factors Collaboration found that individuals with the minor allele could reduce 3.4% CAD risk using 125,222 participants [16]. In 2012, IL6R MR Consortium found the minor allele could reduce 5% CAD risk using 133,449 individuals [7]. In 2018, Cai et al. conducted a PheWAS analysis using the MVP datasets, and found that the minor allele could reduce 5% coronary atherosclerosis risk ($P = 3.43E-12$), 5% ischemic heart disease risk ($P = 3.97E-12$), 5% other chronic ischemic heart disease risk ($P = 6.04E-12$), and 6% myocardial infarction risk ($P = 1.69E-06$) [17].

In stage 2, we demonstrated that *IL-6R* rs7529229 variant minor allele C could significantly reduce the expression of *IL-6R* in relevant tissues or organs using large-scale eQTLs dataset from Braineac, GTEx (version 8) and eQTLGen. In 2012, IL6R Genetics Consortium Emerging Risk Factors Collaboration found that rs2228145 variant minor allele was not associated with *IL-6R* mRNA levels or IL-6 production in

monocytes [16]. Here, we conducted comprehensive eQTLs analyses and found that *IL-6R* rs7529229 variant minor allele C could reduce the expression of *IL-6R* in relevant tissues or organs, such as blood vessels including aorta artery and tibial artery, and whole blood. It is reported that high expression of *IL-6R* in relevant tissues or organs is associated with increased IL-6R signaling and CAD risk [9, 34]. Hence, the directions for the effects of rs7529229 variant minor allele C from genetic association analysis and eQTLs analysis are consistent. In other words, rs7529229 variant C allele may first reduce the expression of *IL-6R* in relevant tissues or organs, further reduce IL-6 signaling, and eventually reduce the risk of CAD and stroke. We consider that this may explain why individuals with *IL-6R* variant minor allele without tocilizumab treatment still have similar biomarker profiles to those with tocilizumab treatment [7, 16].

In stage 3, we found highest expression of *IL-6R* in skeletal muscle and whole blood using the gene expression data from GTEx (version 8). The expression of *IL-6R* gene in both tissues is significantly higher than other tissues including liver, lung, esophagus muscularis, sigmoid colon, esophagus gastroesophageal junction, spleen, uterus, and small intestine. Meanwhile, we found lowest expression of *IL-6R* in human brain tissues. This may further explain why eQTLs analysis did not report any significant association between rs7529229 variant and *IL-6R* in human brain tissues.

In stage 4, we identified significantly increased *IL-6R* expression in IS cases compared with healthy controls using a whole blood IS case-control gene expression data. High expression of *IL-6R* is reported to be associated with increased IL-6 signaling [9, 34]. Hence, our findings are consistent with recent mendelian randomization studies, which identified IL-6 signaling to be causally associated with the increased risk of CAD and IS [7, 9-10]. In stage 1 and 2, we found that that rs7529229 variant C allele could reduce the risk of IS, and reduce the expression of *IL-6R* in relevant tissues or organs in normal individuals, respectively. If the reduced expression of *IL-6R* is observed in normal individuals, the biological senses in stage 1-4 will be convergent. Interestingly, we indeed observed significantly reduced expression of *IL-6R* in healthy controls.

Evidence from observational studies supported the role of tocilizumab in treating IS using the mouse models. In 2017, Hudobenko et al. found that tocilizumab could ameliorate the severity of ischemic injury, reduce behavioral deficits and infarct size in young male mice (8 week) [35]. Immunohistochemical analysis further showed that tocilizumab treatment could attenuate microglia activation [35]. In 2018, Hudobenko et al. further identified that inhibition of IL-6 signaling with tocilizumab could ameliorate IS damage and reduce behavioral deficits in aged male and female mice (18-20 month) [36].

Evidence from observational studies have demonstrated no increased risk of adverse cardiovascular events or safe cardiovascular outcomes among RA patients treated with tocilizumab compared to patients treated with other biological disease-modifying antirheumatic drugs (bDMARDs) [37-42]. In 2017, a prospective community-based clinical study evaluated the association between tocilizumab and CAD in RA population, and showed that tocilizumab could improve endothelial function [43]. In 2019, Gottenberg et al. performed a population based prospective study of 3,162 RA adults, and found no difference in major adverse cardiovascular events (MACE) using rituximab, abatacept, and tocilizumab in the

treatment of RA [37]. In 2019, Virone et al. found that tocilizumab could improve cardiovascular risk-associated biomarkers lipoprotein (a) and the leptin/adiponectin ratio than tumor necrosis factor inhibitor (TNFi) [38]. In 2019, Castagne et al. conducted a systematic review and network meta-analysis of 8 observational studies [44]. They found no evidence of MACE difference between tocilizumab and other bDMARDs including abatacept, rituximab and TNFi [44]. In 2020, Singh et al. performed a systematic review and meta-analysis of 14 observational studies in adults with RA treated with multiple anti-rheumatic drugs [41]. They found that compared to TNFi, tocilizumab was associated with a decreased risk of MACE [41]. In 2020, Best et al. selected the RA patients in US administrative claims database and conducted a real world evidence study to compare the persistence with tocilizumab to persistence with other bDMARDs [45]. Best et al. found that RA patients with tocilizumab treatment exhibited significantly better biologic persistence than those with adalimumab, certolizumab, and etanercept treatment, and similar persistence to those with abatacept and golimumab treatment [45].

Evidence from RCTs demonstrated no increased risk of adverse cardiovascular events among RA patients treated with tocilizumab compared to patients treated with other bDMARDs [42, 44, 46]. In 2019, Castagne et al. conducted a systematic review and network meta-analysis of 11 RCTs [44]. They demonstrated no difference between tocilizumab and other treatments including TNFi and abatacept in MACE, myocardial infarction, and stroke [44]. In 2020, an ENTRACTE trial evaluated the comparative cardiovascular safety of the tocilizumab and the TNFi etanercept in 3,080 patients with active seropositive RA [46]. This ENTRACTE trial found no difference in MACE risk between tocilizumab and etanercept, and ruled out a MACE occurrence risk of 1.43 or higher in patients treated with tocilizumab [46].

Conclusions

In summary, we demonstrated that (1) rs7529229 variant minor allele could significantly reduce the risk of IS; (2) rs7529229 variant minor allele could significantly reduce the expression of *IL-6R* in relevant tissues or organs especially in blood vessels and whole blood; (3) *IL-6R* is mainly expressed in skeletal muscle and whole blood; (4) the expression of *IL-6R* is significantly reduced in healthy controls compared with IS cases in whole blood. Importantly, the biological senses in above findings are convergent. Hence, *IL-6R* may be a potential therapeutic target for IS, and block IL-6 signaling such as using tocilizumab might be effective in lowering IS risk or treatment of IS, which warrants further testing in suitably powered RCTs.

Abbreviations

IS, ischemic stroke; IL-6, Interleukin-6; IL-6R, IL-6 receptor; CAD, coronary artery disease; RA, rheumatoid arthritis; CRP, C-reactive protein; GWAS, genome-wide association study; eQTLs, expression quantitative trait loci;

Declarations

Ethics approval and consent to participate

This article contains human participants collected by several studies performed by previous studies. All participants gave informed consent in all the corresponding original studies, as described in the Materials and methods. Here, our study is based on the publicly available, large-scale datasets, and not the individual-level data. Hence, ethical approval was not sought.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author contributions

GYL and XMJ conceived and initiated the project. GYL analyzed the data, and wrote the first draft of the manuscript. All authors contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript.

Data Availability

All relevant data are within the paper. The authors confirm that all data underlying the findings are either fully available without restriction through consortia websites, or may be made available from consortia upon request. Ukbiobank: <http://www.ukbiobank.ac.uk/scientists-3/genetic-data/>.

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Tables

Table 1 association of rs7529229 minor allele C with IS, RA and CAD

Traits	OR	95% CI	P value	Case #	Control #
Ischemic stroke	0.97	0.95-0.99	2.30E-03	36,916	1,136,729
Large artery atherosclerotic stroke	0.98	0.93-1.03	3.32E-01	4,373	406,111
Cardioembolic stroke	0.96	0.92-0.99	2.15E-02	7,193	406,111
Small vessel stroke	0.95	0.91-1.00	4.15E-02	5,386	406,111
Rheumatoid arthritis	0.93	0.91-0.96	1.90E-05	14,361	43,923
Coronary artery disease	0.96	0.94-0.98	2.50E-07	60,801	123,504

OR, odds ratio; CI, confidence interval;

The statistical significance for the association between rs7529229 and one specific phenotype is a Bonferroni-corrected threshold $0.05/6=0.0083$.

Table 2 rs7529229 variant and *IL-6R* expression in human brain tissues

Tissues	EA	NEA	Beta	SE	<i>P</i> value	Samples	Dataset
Cerebellar cortex	C	T	-0.003	0.024	0.89	134	Braineac
Frontal cortex	C	T	0.040	0.022	0.07	134	Braineac
Hippocampus	C	T	-0.022	0.026	0.40	134	Braineac
Medulla	C	T	-0.007	0.030	0.81	134	Braineac
Occipital cortex	C	T	0.006	0.025	0.82	134	Braineac
Putamen	C	T	0.029	0.027	0.29	134	Braineac
Substantia nigra	C	T	0.005	0.032	0.87	134	Braineac
Temporal cortex	C	T	0.022	0.025	0.38	134	Braineac
Thalamus	C	T	0.050	0.029	0.09	134	Braineac
Intralobular white matter	C	T	-0.037	0.031	0.24	134	Braineac
Amygdala	C	T	0.021	0.055	0.7	129	GTEEx
Anterior cingulate cortex	C	T	0.051	0.057	0.37	147	GTEEx
Caudate	C	T	-0.014	0.042	0.74	194	GTEEx
Cerebellar Hemisphere	C	T	-0.028	0.061	0.65	175	GTEEx
Cerebellum	C	T	-0.052	0.053	0.32	209	GTEEx
Cortex	C	T	-0.024	0.040	0.55	205	GTEEx
Frontal Cortex	C	T	0.054	0.045	0.22	175	GTEEx
Hippocampus	C	T	0.0045	0.051	0.93	165	GTEEx
Hypothalamus	C	T	0.013	0.050	0.8	170	GTEEx
Nucleus accumbens	C	T	-0.038	0.042	0.37	202	GTEEx
Putamen	C	T	-0.023	0.051	0.65	170	GTEEx
Spinal cord	C	T	-0.032	0.071	0.66	126	GTEEx
Substantia nigra	C	T	-0.028	0.080	0.73	114	GTEEx

EA, effect allele; NEA, non-effect allele; Beta is the regression coefficient based on the effect allele. Beta > 0 and Beta < 0 means that this effect allele increase and reduce disease or phenotype, respectively. The statistical significance for eQTLs analysis is a Bonferroni-corrected threshold of $P < 0.05/58 = 8.62E-04$. Meanwhile, the original *P* values between $8.77E-04$ and 0.05 were considered to be suggestively significant.

Table 3 rs7529229 variant and *IL-6R* expression in other tissues in GTEEx

Tissue	EA	NEA	Beta	SE	P value	Samples
Adipose - Subcutaneous	C	T	-0.052	0.037	1.50E-01	581
Adipose - Visceral (Omentum)	C	T	0.052	0.029	6.60E-02	469
Adrenal Gland	C	T	0.072	0.060	2.30E-01	233
Artery - Aorta	C	T	-0.13	0.030	1.60E-05	387
Artery - Coronary	C	T	-0.14	0.074	5.50E-02	213
Artery - Tibial	C	T	-0.17	0.022	2.80E-14	584
Breast - Mammary Tissue	C	T	-0.018	0.042	6.70E-01	396
Cells - Cultured fibroblasts	C	T	-0.05	0.023	2.60E-02	483
Cells - EBV-transformed lymphocytes	C	T	-0.02	0.111	8.60E-01	147
Colon - Sigmoid	C	T	-0.26	0.067	1.00E-04	318
Colon - Transverse	C	T	-0.3	0.040	9.80E-13	368
Esophagus - Mucosa	C	T	-0.16	0.039	5.60E-05	497
Esophagus - Muscularis	C	T	-0.17	0.039	1.50E-05	465
Heart - Atrial Appendage	C	T	-0.14	0.048	4.60E-03	372
Heart - Left Ventricle	C	T	-0.063	0.042	1.30E-01	386
Liver	C	T	0.0098	0.052	8.50E-01	208
Lung	C	T	-0.018	0.029	5.30E-01	515
Minor Salivary Gland	C	T	-0.076	0.048	1.20E-01	144
Muscle - Skeletal	C	T	0.017	0.019	3.60E-01	706
Nerve - Tibial	C	T	0.075	0.031	1.60E-02	532
Ovary	C	T	0.054	0.054	3.20E-01	167
Pancreas	C	T	0.019	0.034	5.80E-01	305
Pituitary	C	T	-0.024	0.040	5.50E-01	237
Prostate	C	T	-0.03	0.059	6.10E-01	221
Skin - Not Sun Exposed (Suprapubic)	C	T	-0.048	0.030	1.20E-01	517
Skin - Sun Exposed (Lower leg)	C	T	-0.048	0.032	1.30E-01	605
Small Intestine - Terminal Ileum	C	T	-0.24	0.053	1.70E-05	174
Spleen	C	T	-0.041	0.037	2.60E-01	227

Stomach	C	T	-0.089	0.036	1.40E-02	324
Testis	C	T	-0.11	0.052	3.80E-02	322
Thyroid	C	T	-0.095	0.033	3.50E-03	574
Uterus	C	T	-0.33	0.106	2.60E-03	129
Vagina	C	T	-0.067	0.084	4.20E-01	141
Whole Blood	C	T	-0.084	0.014	8.50E-09	670

EA, effect allele; NEA, non-effect allele; Beta is the regression coefficient based on the effect allele. Beta > 0 and Beta < 0 means that this effect allele increase and reduce disease or phenotype, respectively. The statistical significance for eQTLs analysis is a Bonferroni-corrected threshold of $P < 0.05/58 = 8.62E-04$. Meanwhile, the original P values between $8.77E-04$ and 0.05 were considered to be suggestively significant.

Table 4 IS case-control gene expression analysis of *IL-6R* in blood

Comparison models	ID	Fold change	P value	Dataset
IS vs. Controls	ILMN_1754753	1.37	2.33E-05	GSE16561
IS vs. Controls	ILMN_1696394	1.30	7.90E-04	GSE16561
IS vs. Controls in males	ILMN_1754753	1.52	8.39E-04	GSE16561
IS vs. Controls in males	ILMN_1696394	1.43	2.50E-03	GSE16561
IS vs. Controls in females	ILMN_1754753	1.28	5.59E-03	GSE16561
IS vs. Controls in females	ILMN_1696394	1.22	3.65E-02	GSE16561

The significance level is defined to be $P < 0.05$.

Figures

Gene expression for IL6R (ENSG00000160712.12)

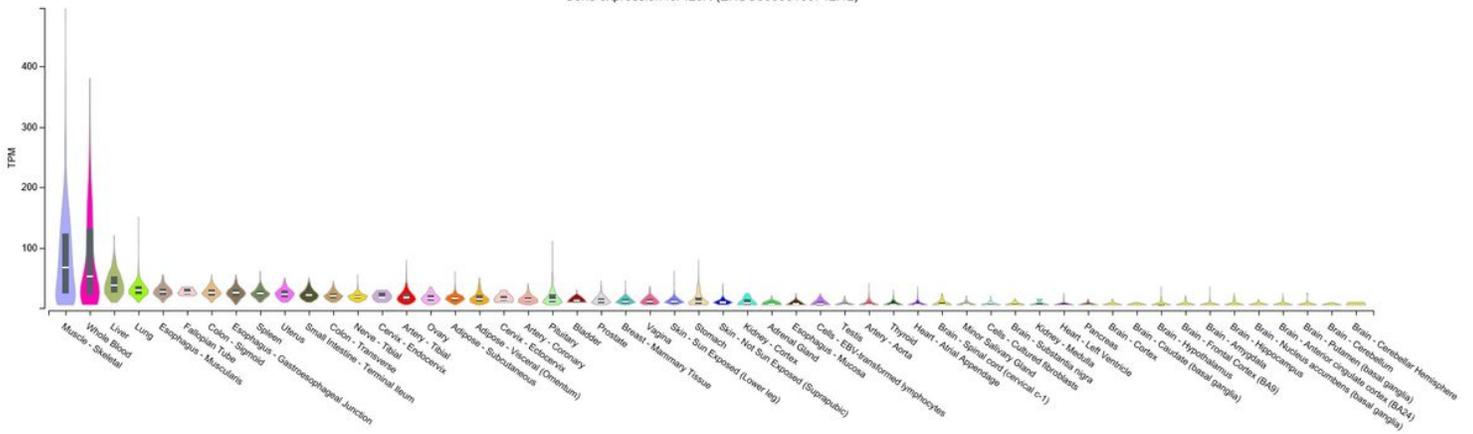


Figure 1

the box plots for the expression of IL-6R gene in different tissues in GTEx The gene expression values are shown in transcripts per million (TPM). The gene expression level was quantified by TPM based on the GENCODE 26 annotation, collapsed to a single transcript model for each gene using a custom isoform collapsing procedure [27].