Arteriosclerosis, Thrombosis, and Vascular Biology

ORIGINAL RESEARCH

Human Genetic Evidence to Inform Clinical Development of IL-6 Signaling Inhibition for Abdominal Aortic Aneurysm

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BACKGROUND: Abdominal aortic aneurysm (AAA) represents a significant cause of mortality, yet no medical therapies have proven efficacious. The aim of the current study was to leverage human genetic evidence to inform clinical development of IL-6 (interleukin-6) signaling inhibition for the treatment of AAA.

METHODS: Associations of rs2228145, a missense variant in the *IL6R* gene region, are expressed per additional copy of the C allele, corresponding to the genetically predicted effect of IL-6 signaling inhibition. We consider genetic associations with AAA risk in the AAAgen consortium (39 221 cases and 1 086 107 controls) and UK Biobank (1963 cases and 365 680 controls). To validate against known effects of IL-6 signaling inhibition, we present associations with rheumatoid arthritis, polymyalgia rheumatica, and severe COVID-19. To explore mechanism specificity, we present associations with thoracic aortic aneurysm, intracranial aneurysm, and coronary artery disease. We further explored genetic associations in clinically relevant subgroups of the population.

RESULTS: We observed strong genetic associations with AAA risk in the AAAgen consortium, UK Biobank, and FinnGen (odds ratios: 0.91 [95% CI, 0.90–0.92], $P=4\times10^{-30}$; 0.90 [95% CI, 0.84–0.96], $P=0.001$; and 0.86 [95% CI, 0.82–0.91], $P=7\times10^{-9}$, respectively). The association was similar for fatal AAA but with greater uncertainty due to the lower number of events. The association with AAA was of greater magnitude than associations with coronary artery disease and even rheumatological disorders for which IL-6 inhibitors have been approved. No strong associations were observed with thoracic aortic aneurysm or intracranial aneurysm. Associations attenuated toward the null in populations with concomitant rheumatological or connective tissue disease.

CONCLUSIONS: Inhibition of IL-6 signaling is a promising strategy for treating AAA but not other types of aneurysmal disease. These findings serve to help inform clinical development of IL-6 signaling inhibition for AAA treatment.

Key Words: aortic aneurysm, abdominal ■ aortic aneurysm, thoracic ■ arthritis, rheumatoid ■ collagen diseases ■ human genetics

Abdominal aortic aneurysm (AAA) is defined as a permanent localized dilatation of the abdominal aorta to >3 cm and has a prevalence of ≈5% in individuals aged >60 years.[1] Mortality from ruptured AAA is estimated to be ≈90%,[2,3] and other than for modifying cardiovascular risk factors, no efficacious pharmacotherapies are available for AAA treatment.[4] The mainstay of intervention is, therefore, surgical repair, despite which global AAA-related mortality remains to be at ≈150 000 to 200 000 deaths per year.[5,6]

Drug development can be slow, expensive, and inefficient,[7] with the high failure rate largely attributable to insufficient efficacy or unacceptable safety profiles.[8] As the majority of drug targets are proteins, which are coded for by genes, human genetic data offer the opportunity to dramatically improve the probability of

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Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/ATVBAHA.124.321988.
For Sources of Funding and Disclosures, see page XXX.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

AAA	abdominal aortic aneurysm
CAD	coronary artery disease
CVD	cardiovascular disease
GWAS	genome-wide association study
ICD	International Statistical Classification of Diseases and Related Health Problems
IL-6	interleukin-6
OR	odds ratio
PP-H	posterior probability hypothesis

Highlights

- Consistent with previous work, this study identified genetic evidence to support a protective effect of IL-6 (inteluekin-6) signaling inhibition on abdominal aortic aneurysms (AAAs).
- There is evidence of an additive effect for each additional rs2228145 C allele (mimicking IL-6 signaling inhibition) on AAA risk reduction, consistent with IL-6 signaling driving AAA pathophysiology.
- The magnitude of the Mendelian randomization estimate is similar for risk of fatal AAA as it is for risk of any AAA, supporting similar protective effects on risk of AAA rupture.
- The Mendelian randomization association was similar in population subgroups stratified by sex, blood pressure, and smoking status but was attenuated in individuals with AAA-related rheumatological or connective tissue disease, albeit with wider CIs in the diseased subgroups.
- The genetic evidence of effect was specific to AAA and not other types of aneurysmal disease, such as intracranial aneurysm or thoracic aortic aneurysm.

successful drug development.[9] At a population level, naturally occurring genetic variation in the gene coding for a drug target protein can be used to study the effect of pharmacologically perturbing that protein.[10] This drug target Mendelian randomization paradigm can be used to inform various aspects of clinical development,[11] including efficacy, secondary indications, adverse effects, effect heterogeneity, and biomarkers of target engagement.[12] In this way, drug target Mendelian randomization has been used to inform on the effects of various cardiovascular disease (CVD) drug targets, including for lipid-lowering, antihypertensive, antidiabetic, and anticoagulant agents.[11]

The drug target Mendelian randomization paradigm has been used extensively to study the effect of IL-6 (interleukin-6) signaling inhibition on CVD outcomes.[13–15] The genetic evidence supporting its efficacy has contributed to the pursuit of IL-6 signaling inhibition as a therapeutic target in CVD, with 1 phase 2 study (https://www.clinicaltrials.gov; unique identifier: NCT06362759) and 5 phase 3 clinical studies currently ongoing (https://www.clinicaltrials.gov; unique identifiers: NCT05021835, NCT06118281, NCT05636176, NCT05485961, and NCT06200207).[16] However, while there is also genetic support for the efficacy of IL-6 signaling inhibition in AAA,[17] this has remained limited in nature with key questions yet unanswered. The aim of this work was, therefore, to leverage the drug target Mendelian randomization paradigm to inform clinical development of IL-6 signaling inhibition for AAA. Specifically, we aimed to affirm the genetic evidence for efficacy of IL-6 signaling inhibition in AAA (including testing for a per-allele dose-response relationship) and fatal AAA, compare against established positive control outcomes and coronary artery disease (CAD), investigate specificity for different types of aneurysmal disease, and explore heterogeneity of effect across population subgroups (including subgroups defined by sex, smoking status, hypertensive status, and prevalent rheumatological or connective tissue disorders). These findings collectively serve to inform clinical development efforts of IL-6 signaling inhibition for the treatment of AAA.

MATERIALS AND METHODS

Overview

As the instrument for IL-6 signaling inhibition, we leverage a genetic variant in the *IL6R* gene region that has previously been shown to mimic pharmacological IL-6 signaling inhibition.[14] To investigate potential clinical effects of inhibiting IL-6 signaling, we present associations of this variant with traits and diseases from a variety of data sources, including publicly available summarized data sets and individual-level data from UK Biobank. The summarized data sets are larger and have more cases, resulting in more precise estimates. The individual-level data analyses allow the exploration of more specific outcomes, such as fatal AAA, and subgroup analyses in specific strata of the population. An overview of all analyses is provided in Figure 1, and a summary of the outcome data sets used in the analysis is provided in Table 1.

Genetic Instrument

We focused on rs2228145 (previously also called rs8192284), a missense variant located at chr1:154426970 on GRCh37, chr1:154454494 on GRCh38 with minor allele frequency of 42% in the European ancestry UK Biobank analytic sample considered here. It is the lead variant associated with IL-6 levels in the *IL6R* gene region (Figure S1). Associations are given per additional copy of the C allele, which is the minor allele and is associated with lower levels of C-reactive protein (0.093 units lower log-transformed C-reactive protein, $P<10^{-400}$).[18] Hence, estimates correspond to the genetically predicted effect of increasing IL-6 signaling inhibition and reflect the consequences of lifelong lower inflammation levels, with the relative magnitude of reduction in C-reactive protein in adulthood estimated at 9%. We also considered estimates based

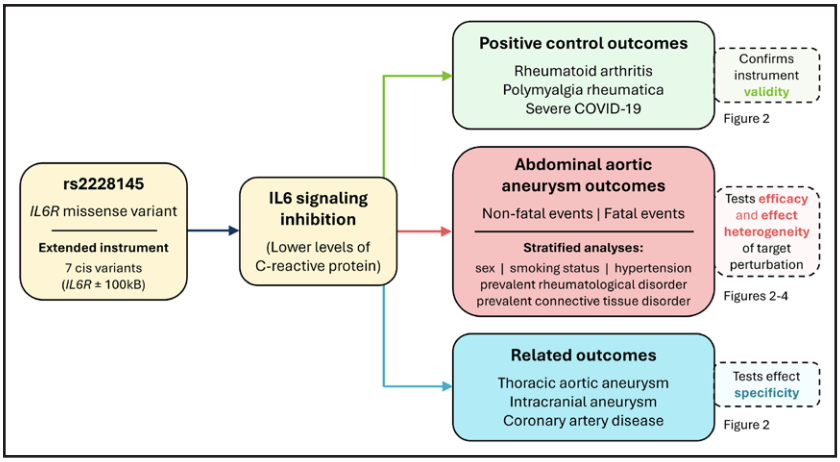

Figure 1. Overview of analyses.
The Mendelian randomization framework relies on the following core instrumental variable assumptions: (1) the genetic instrument strongly relates to the exposure; (2) there are no confounding pathways linking the variant and outcome; and (3) the genetic instrument affects the outcome only through the exposure and not through independent pathways. IL-6 indicates interleukin-6.

on an extended instrument consisting of 7 variants in the *IL6R* gene region and its neighborhood (±100 kb pairs) previously demonstrated to be conditionally associated with C-reactive protein levels (Table S1).[14]

Summarized Data Sets

The primary outcome was AAA. Associations were obtained from a meta-analysis of 17 individual genome-wide association studies (GWASs) by the AAAgen consortium including 39 221 cases and 1 086 107 controls.[19] We also validated results in FinnGen, a large GWAS in the Finnish population, including 3869 cases and 381 977 controls. FinnGen did not contribute data to the AAAgen consortium. We performed colocalization between IL-6 and AAA risk using the coloc method,[20] taking genetic associations with IL-6 from the SCALLOP consortium (N=14 242)[21] and using the default priors from the coloc package.[22] Colocalization is a statistical approach to assess whether the genetic predictors of 2 traits overlap (known as colocalization) or are distinct (known as noncolocalization). The coloc method reports 2 key outputs: the posterior probability hypothesis (PP-H) of colocalization (PP-H4) and the PP-H of noncolocalization (PP-H3). High values (close to 1) of PP-H4 indicate colocalization, which is supportive of a causal relationship; high values of PP-H3 indicate noncolocalization, which opposes a causal relationship; low values of both PP-H3 and PP-H4 indicate lack of strong evidence supporting or opposing a causal relationship.[23]

Table 1. List of Outcomes and Data Sets

Data set	Includes UK Biobank?	Outcomes considered
UK Biobank	✓	Any AAA, fatal AAA, polymyalgia rheumatica
AAAgen	✓	Any AAA
FinnGen	×	Any AAA, rheumatoid arthritis
Michigan Genomics Initiative	×	Thoracic aortic aneurysm
Aneurysm consortium	✓	Intracranial aneurysm
CAD	✓	CAD
Rheumatoid consortium	✓	Rheumatoid arthritis
COVID-19 Host Genome Initiative	✓	Severe COVID-19
Global Biobank Meta-Analysis Initiative	✓	Replication of sex-specific associations for any AAA

✓: consortium includes participants from UK Biobank; ×: consortium does not include participants from UK Biobank. AAA indicates abdominal aortic aneurysm; and CAD, coronary artery disease.

As positive controls, we present associations with rheumatoid arthritis (GWAS consortium, 35 871 cases and 240 149 controls[24]; FinnGen, 15 223 cases and 138 246 controls),[25] polymyalgia rheumatica (UK Biobank, 2460 cases and 433 511 controls),[26] and severe COVID-19 (COVID-19 Host Genome Initiative round 7, 18 152 cases and 1 145 546 controls),[27] as IL-6 signaling inhibition is known from randomized trials to offer therapeutic benefit in these diseases.[28–30]

For comparison with other aneurysmal diseases and CVDs, we present associations with thoracic aortic aneurysm (Michigan Genomics Initiative, 1351 cases and 18 295 controls),[31] intracranial aneurysm (GWAS consortium, 10 754 cases and 306 882 controls),[32] and CAD (CARDIoGRAMplusC4D, 210 842 cases and 1 167 328 controls).[33]

For replication of sex-specific findings, we present associations with AAA risk in men and women separately published by the Global Biobank Meta-Analysis Initiative.[34] We note that this is not a perfect replication, as the Global Biobank Meta-Analysis Initiative data set includes UK Biobank participants. However, we were unable to find an independent data set with sex-stratified genetic association estimates. The Global Biobank Meta-Analysis Initiative contains 515 358 women (1289 AAA cases) and 456 574 men (5589 AAA cases) from 8 cohorts: Biobank Japan, BioMe (men only), BioVU, Colorado Center for Personalized Medicine (men only), FinnGen, HUNT, Michigan Genomics Initiative, and UK Biobank.

We note that all summarized data sets include UK Biobank participants, with the exception of FinnGen and the Michigan Genomics Initiative.

Individual-Level Data in UK Biobank

The UK Biobank cohort comprises around 500 000 participants (94% of self-reported European ancestry) aged 40 to 69 years at baseline.[35] They were recruited between 2006 and 2010 in 22 assessment centers throughout the United Kingdom and followed up until November 2022 or their date of death. We performed detailed quality control procedures on UK Biobank participants and on genetic variants as described previously,[36]

restricting analyses to unrelated participants (ie, more distant than third-degree relatives) of European ancestries.

AAA in UK Biobank was defined as having an *International Statistical Classification of Diseases and Related Health Problems* (*ICD*), *Tenth Revision*, code I71.3 or I71.4 (AAA, either ruptured or without mention of rupture) or the equivalent *ICD*, *Ninth Revision*, codes (441.3 and 441.4) in their hospital episode statistics or death certificate. The secondary outcome was fatal AAA, defined as having one of the relevant *ICD* codes in their death certificate.

Stratification Variables

We estimated associations with AAA in UK Biobank in subgroups of the population, stratifying by 5 separate variables: sex (men versus women), hypertension (defined as systolic blood pressure >140 mm Hg and diastolic blood pressure >90 mm Hg at baseline or usage of hypertensive medication at baseline), smoking status (ever regular smokers versus never regular smokers), any rheumatologic disorder (present versus absent), and any connective tissue disorder (present versus absent).

A never regular smoker was defined as answering the touchscreen question "In the past, have you ever smoked tobacco?" with the response "I have never smoked" or "Just tried once or twice" as opposed to "smoked occasionally" or "smoked on most or all days" at baseline and never contradicting this answer at a future survey.

Any rheumatologic disorder was defined as ankylosing spondylitis, anti-neutrophil cytoplasmic antibody–associated vasculitis, Behçet disease, Cogan syndrome, giant cell arthritis, relapsing polychondritis, rheumatoid arthritis, sarcoidosis, systemic lupus erythematosus, or Takayasu arteritis, defined using *ICD* codes and self-reported information (Table S2).

Any connective tissue disorder was defined as Marfan syndrome, Ehlers-Danlos syndrome, or any nonspecific connective tissue disorder, defined using *ICD* codes and self-reported information (Table S2).

Statistical Analyses

All associations in UK Biobank were obtained by logistic regression (for disease outcomes) adjusted for age, sex, and 10 genomic principal components of ancestry using an additive genetic model (ie, coefficients represent log odds ratios [ORs] per additional copy of the effect allele). We repeated the primary analysis using a factorial model regarding major homozygotes as the reference group and providing separate estimates for heterozygotes and minor homozygotes. Analyses for the primary instrument are reported as genetic associations per additional C-reactive protein decreasing allele. Analyses for the extended instrument were performed using exposure data on genetic associations with C-reactive protein[18] and implemented using the inverse-variance weighted method to combine the summarized data accounting for correlation between variants and a fixed-effects model. The genetic correlation matrix was estimated in the analytic sample of UK Biobank participants. All analyses were performed in R (version 4.3.3). All *P* values are 2 sided.

Ethical Approval and Participant Consent

Individual-level analyses on data from the UK Biobank were approved by its Research Ethics Committee and Human Tissue Authority research tissue bank under application number 7493. For analysis of prior published genetic associations, summary statistics were gathered from studies that had obtained appropriate independent ethical approval and participant consent for analyses and distribution of summary-level data, as described in the original publications.

Data and Code Availability

Summary statistics used in our analyses can be accessed through the citations provided. Please also see the Major Resources Table in the Supplemental Material. Requests to access the UK Biobank data can be made by bona fide researchers from any sector. More information can be found at https://www.ukbiobank.ac.uk/enable-your-research. Statistical code for the analyses undertaken in this work are available from the corresponding author upon reasonable request. This study is reported using the Strengthening the Reporting of Observational Studies in Epidemiology-MR guidelines (STROBE-MR Checklist in the Supplemental Material).[37]

RESULTS

Demographic Information on UK Biobank Analytic Sample

A total of 367 643 unrelated UK Biobank participants of European ancestries were included in individual-level data analyses. In total, 1963 individuals had an AAA diagnosis and 131 had a fatal AAA diagnosis. A total of 198 838 individuals were women (54.1%), 120 051 individuals had hypertension (32.6%), 169 788 individuals were ever-smokers (46.2%), 11 551 had a rheumatological disorder (3.1%), and 505 had a connective tissue disorder (0.1%). Demographic information on participants is provided in Table 2.

Genetic Evidence for Efficacy of IL-6 Signaling Inhibition in AAA

The genetic association with AAA in the AAAgen consortium was an OR of 0.91 ([95% CI, 0.90–0.92] $P=4\times10^{-30}$) per additional copy of the C allele of rs2228145 (Figure 2). The genetic association in FinnGen was similar: 0.86 ([95% CI, 0.82–0.91] $P=7\times10^{-9}$). The association in the UK Biobank was also similar: 0.90 ([95% CI, 0.84–0.96] $P=0.001$). The genetic association with fatal AAA risk in the UK Biobank was similar but had wider CIs due to the lower number of events: 0.89 ([95% CI, 0.69–1.14] $P=0.34$). Associations were approximately additive considering genetic subgroups separately in a factorial model, with an OR of 0.91 ([95% CI, 0.83–1.00] $P=0.065$) for heterozygotes and 0.80 ([95% CI, 0.70–0.92] $P=0.001$) for minor homozygotes (Figure 3).

The estimate using the extended instrument of 7 variants in the *IL6R* gene region was OR of 0.93 (95% CI, 0.91–0.94) per 0.1 unit lower log-transformed C-reactive

Table 2. Demographic Information on UK Biobank Participants in the Analytic Sample Divided by Stratification Variables

Trait	Overall	Sex		Smoking status		Hypertension		RDs	CTDs
		Men	Women	Ever	Never	Yes	No		
Sample size, n	367 643	168 748	198 838	169 788	197 853	120 051	244 971	11 551	505
Age, y	57.2	57.4	57.0	58.0	56.5	59.9	55.9	59.6	56.1
Sex (women), %	54.1	0	100	48.6	58.8	44.2	59.1	62.2	68.9
AAA events, n	1963	1669	294	1622	341	1233	717	116	10
Fatal AAA, n	131	117	14	117	14	87	44	8	0
BMI, kg/m^2	27.4	27.8	27.0	27.7	27.1	29.2	26.4	28.3	26.7
LDL-c, mmol/L	3.57	3.49	3.64	3.54	3.59	3.45	3.62	3.52	3.51
ApoB, g/L	1.03	1.03	1.04	1.03	1.03	1.02	1.04	1.03	1.02
HbA1c, mmol/mol	35.9	36.3	35.6	36.4	35.5	37.6	35.1	36.6	35.4

AAA indicates abdominal aortic aneurysm; BMI, body mass index; CTD, connective tissue disorder; HbA1c, glycated hemoglobin; LDL-c, low-density lipoprotein cholesterol; and RD, rheumatological disorder.

protein (ie, per 10% lower C-reactive protein). All 7 variants provided supportive evidence of a causal effect with the exception of rs12083537, which was an outlier, and hence may be pleiotropic in terms of its genetic association with the outcome (Figure S2). On the same scale, the estimate using the rs2228145 variant only was 0.90 (95% CI, 0.89–0.92). Given limited benefit in terms of precision and the possibilities of including pleiotropic variants and overfitting using the extended instrument,[38] we performed all further analyses using the rs2228145 variant only.

There was strong evidence of colocalization for the genetic association with IL-6 levels and AAA risk at the *IL6R* gene locus, supportive of a causal relationship: PP-H of colocalization (PP-H4) of 0.996.

Genetic Evidence for Efficacy of IL-6 Signaling Inhibition on Positive Control Outcomes

The genetic association with rheumatoid arthritis was 0.94 ([95% CI, 0.92–0.96] P=2×10^{-9}) in the GWAS consortium and 0.95 ([95% CI, 0.93–0.98] P=0.0009)

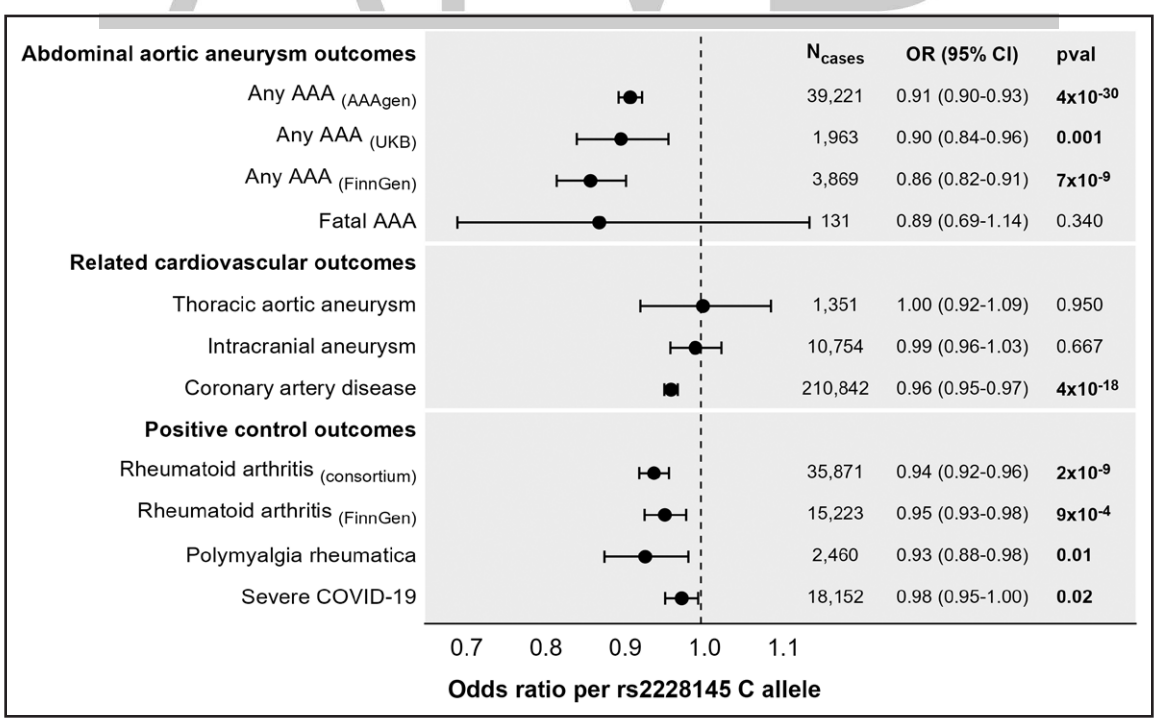

Figure 2. Genetic associations with outcomes estimated in summarized and individual-level data.
Estimates represent the odds ratio (OR) per additional copy of the C allele for rs2228145, corresponding to the genetically predicted effect of increasing interleukin-6 signaling inhibition. Positive control outcomes are outcomes for which interleukin-6 inhibitors have proven efficacious. AAA indicates abdominal aortic aneurysm; N_{cases}, number of cases; pval, P value; and UKB, UK Biobank.

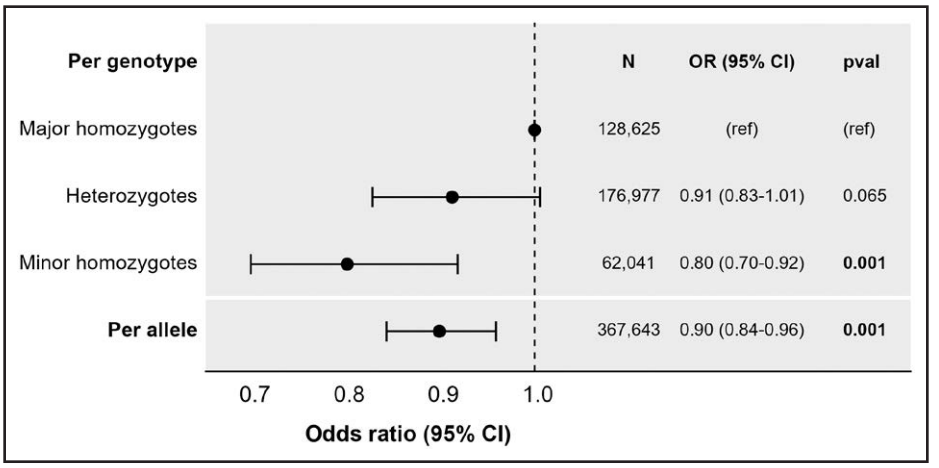

Figure 3. Genetic associations with abdominal aortic aneurysms in UK Biobank from per-allele and factorial models. Estimates represent the odds ratio (OR) of an abdominal aortic aneurysm per additional copy of the C allele for rs2228145 (per allele model) or for heterozygotes (AC genotype) and minor allele homozygotes (CC genotype) compared with major allele homozygotes (AA genotype). N indicates sample size; and pval, *P* value.

in FinnGen. The association with polymyalgia rheumatica was 0.93 ([95% CI, 0.88–0.98] *P*=0.012). The association with severe COVID-19 was 0.98 ([95% CI, 0.95–1.00] *P*=0.023). Associations with these positive controls provide evidence that the genetic associations are a reliable guide for the impact of IL-6 signaling inhibition in clinical trials.

Genetic Evidence for Efficacy of IL-6 Signaling Inhibition on Other Aneurysmal and Cardiovascular Outcomes

Associations with other aneurysmal diseases were 1.00 ([95% CI, 0.92–1.09] *P*=0.95) for thoracic aortic aneurysm and 0.99 ([95% CI, 0.96–1.03] *P*=0.67) for intracranial aneurysm. It appears that genetic evidence for benefit of IL-6 signaling inhibition on aneurysm risk is specific to AAA.

The association with CAD was 0.96 ([95% CI, 0.95–0.97] $P=4\times10^{-18}$). We note that associations with AAA are at least twice as strong as with CAD.

Stratified Analyses in UK Biobank

Stratified analyses were performed in UK Biobank participants. The genetic association with AAA risk was slightly stronger in men (0.89 [95% CI, 0.83–0.95]; *P*=0.0008) than in women (0.97 [95% CI, 0.82–1.14]; *P*=0.69), although there was no statistical evidence for a difference between estimates (*P*=0.34). Given the lower number of AAA events in women than in men (294 in women and 1669 in men), the null estimate in women may reflect low statistical power to detect an association in women rather than a genuine null finding. Similar findings were obtained regardless of hypertension or smoking status. Among individuals with rheumatological or connective diseases, estimates were attenuated toward the null although the CIs still overlapped (Figure 4).

A similar difference in estimates between men and women was observed in the Global Biobank Meta-Analysis Initiative. Sex-specific associations were available for rs12133641, a variant physically close to rs2228145, and in perfect linkage disequilibrium with rs2228145. The genetic association was OR of 0.91 ([95% CI, 0.87–0.95] $P=3\times10^{-6}$) in men and OR of 0.96 ([95% CI, 0.88–1.04] *P*=0.32) in women. Again, while the estimate was larger in magnitude in men, there was no convincing evidence for a difference in estimates (*P*=0.21). We repeat the earlier caution that this is not an independent replication of this finding, as the Global Biobank Meta-Analysis Initiative includes UK Biobank participants.

DISCUSSION

We used a genetic variant mimicking the effects of IL-6 signaling inhibition in the Mendelian randomization paradigm and identified evidence supporting protective effects on AAA risk. These findings are consistent with previous work[14,17] but make a number of important advances. First, we demonstrate an additive effect for each additional rs2228145 C allele (mimicking IL-6 signaling inhibition) on AAA risk reduction, consistent with IL-6 signaling driving AAA pathophysiology. Second, we show that the magnitude of the Mendelian randomization estimate is similar for risk of fatal AAA as it is for the risk of any AAA, supporting similar protective effects on the risk of AAA rupture. Third, we found that the Mendelian randomization association was similar in population subgroups stratified by sex, blood pressure, and smoking status but was attenuated in individuals with AAA related to rheumatological or connective tissue disease, albeit

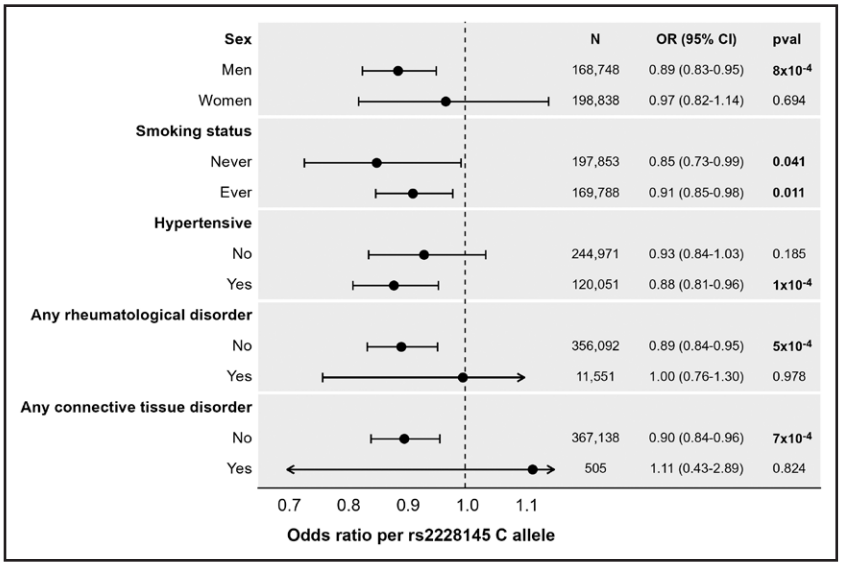

Figure 4. Stratified genetic association estimates with abdominal aortic aneurysms in UK Biobank. Estimates represent the odds ratio (OR) of an abdominal aortic aneurysm event per additional copy of the C allele for rs2228145, corresponding to the genetically predicted effect of greater interleukin-6 signaling inhibition. N indicates sample size; and pval, *P* value.

with wider CIs in the diseased subgroups. This supports that protective effects of IL-6 signaling inhibition may be specific to AAA arising secondary to atherosclerotic risk factors rather than due to preexisting rheumatological disease or connective tissue disease. Fourth, we show that the genetic evidence of effect was specific to AAA and not other types of aneurysmal disease, such as intracranial aneurysm or thoracic aortic aneurysm. The discrepancy between AAA and thoracic aortic aneurysm may relate to smooth muscle cells in the thoracic aorta originating from the neural crest and the somitic mesoderm, while smooth muscle cells in the abdominal aorta originate from the splanchnic mesoderm.[39] This distinction could in turn lead to divergent responses to injury across the 2 sites and thus a differing role for IL-6 signaling in driving pathology.

Collectively, these findings may be directly used to inform the clinical development of IL-6 signaling inhibition for the treatment of AAA. Previous work has used Mendelian randomization to study potential biomarkers and adverse effects of inhibiting IL-6 signaling.[40] Clinical trials investigating IL-6 signaling inhibition for the treatment of CVD are already underway,[16] and the insights generated in our current study may be used to inform similar endeavors for AAA. As Mendelian randomization estimates for IL-6 signaling inhibition are greater for AAA than for CAD, the beneficial effect for AAA risk may be greater on a relative scale. Indeed, IL-6 signaling may represent a disease mechanism common to both AAA and CAD, in that its relevant effects include endothelial cell activation, lymphocyte proliferation and differentiation, increased coagulation, and activation of the hypothalamic-pituitary-adrenal axis.[41] Other than addressing general CVD risk factors such as hypertension, dyslipidemia, or diabetes, there are currently no approved pharmacological therapies for the treatment of AAA. The current standard of care is based on monitoring for expansion, with the option of surgical intervention should certain size thresholds be crossed or in the case of rupture, which itself is associated with an ≈90% mortality rate.[2] Thus, the availability of efficacious pharmacological therapies for AAA treatment would represent a notable advance in patient care.

There is already a plethora of data implicating IL-6 signaling in AAA pathophysiology. Inhibition of IL-6 signaling has been shown to limit progression of AAA in animal models[42] and is also associated with improved survival.[43] In humans, IL-6 is abundantly expressed in AAA tissue[44] and may even be a source of systemic IL-6.[45] Previous Mendelian randomization analyses have supported IL-6 signaling in AAA risk,[14,43] as well as potential effects of IL-6 signaling on reducing progression on AAA, although this latter work was limited by low statistical power. Inflammation is a key driver in AAA occurring outside the background of a rheumatological or connective tissue disease,[46] with inflammatory cell infiltrates observed in the aneurysm wall,[47] and aneurysm mural thrombus.[48] It, therefore, follows that inhibition of IL-6 signaling might reduce AAA risk, progression, and rupture.

This work has a number of strengths. Using the Mendelian randomization paradigm, we were able to efficiently generate causal evidence in humans to inform clinical development efforts supporting IL-6 signaling inhibition for the treatment of AAA. Specifically, the insights generated here may be used to prioritize the specific type of aneurysmal disease and the target population. To ensure the robustness of our approach, we validated the method with established positive control outcomes where IL-6 signaling inhibition has proven efficacious, including for rheumatoid arthritis, polymyalgia rheumatica, and COVID-19. Statistically, we showed that using a biologically validated missense variant as the instrument in Mendelian randomization produced similar estimates to a polygenic *cis*-instrument, and further through

colocalization, we generated support that genetic confounding through a variant in linkage disequilibrium was unlikely to be explaining the observed associations.

There are also limitations. The Mendelian randomization paradigm used in this work considers the cumulative lifetime effect of genetic variation on risk of clinical outcomes in a select population. Caution should, therefore, be taken when extrapolating these findings to assume the effect of a clinical intervention having a larger effect at a discrete time point in life in an entirely different population. In this regard, these analyses were largely limited to European genetic ancestry populations, although previous work supports that similar associations may hold in other genetic ancestry populations.[49] Furthermore, these genetic analyses evaluated the risk of developing AAA but do not directly assess the clinical impact of reduced IL-6 signaling after AAA has already manifested, the clinical setting in which AAA therapies would actually be applied. Our analyses assume a linear model and so provide population-averaged estimates. We are not able to detect departures from linearity or to model the shape of the potential effect of IL-6 signaling on AAA risk. We did not have access to measurements of aneurysm size, and so we were not able to estimate the predicted effect of IL-6 signaling on AAA progression, limiting somewhat the clinical utility of findings for IL-6 treatment. Finally, a fundamental limitation of all Mendelian randomization analyses is that it assumes any genetic associations between the instrument and outcome are only occurring through the exposure and not some pleiotropic pathway, which can never be proven. It, therefore, remains possible that our findings may be biased by such pleiotropic effects.

In conclusion, this Mendelian randomization study finds human causal evidence to support the clinical development of IL-6 signaling inhibition for the treatment of AAA, including the specific disease subtypes and target populations to be prioritized. Five phase 3 clinical trials of IL-6 signaling inhibition for CVD are already underway,[16] and the weight of the supportive evidence for AAA coupled with the unmet need for efficacious medical therapies signposts this as a promising opportunity for clinical investigation.

ARTICLE INFORMATION

Received October 11, 2024; accepted November 21, 2024.

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Acknowledgments

This research has been conducted using the UK Biobank Resource under application number 7439. The authors acknowledge the participants and investigators of the UK Biobank and the other cohort studies incorporated in their work. They thank the cohorts and consortia that made their summary-level data publicly available. Data sources are cited throughout this article. Downloads were performed through the following repositories: IL-6 (interleukin-6), https://www.ebi.ac.uk/gwas/studies/GCST90012005; C-reactive protein, https://www.ebi.ac.uk/gwas/studies/GCST90029070; abdominal aortic aneurysm (AAA), https://csg.sph.umich.edu/willer/public/AAAgen2023/; AAA (sex stratified), https://www.globalbiobankmeta.org/resources; rheumatoid arthritis (consortium), https://www.ebi.ac.uk/gwas/studies/GCST90132222; rheumatoid arthritis and AAA (FinnGen), https://www.finngen.fi/en/access_results; polymyalgia rheumatica, https://www.ebi.ac.uk/gwas/studies/GCST90129454; severe COVID-19, https://www.covid19hg.org/results/r7/; thoracic aortic aneurysm, https://www.ebi.ac.uk/gwas/studies/GCST90027266; intracranial aneurysm, https://cd.hugeamp.org/downloads.html; and coronary artery disease, https://www.ebi.ac.uk/gwas/studies/GCST90132315.

Sources of Funding

This work was supported by Tourmaline Bio.

Disclosures

Sequoia Genetics is a private company that works with investors, pharma, biotech, and academia by performing research that leverages genetic data to help inform drug discovery and development. S. Burgess, H.T. Cronjé, and D. Gill are employees of Sequoia Genetics and were supported by Tourmaline Bio to undertake this work. Y. Chyung and E. deGoma are employees and shareholders of Tourmaline Bio. D. Gill has financial interests in several biotechnology companies.

Supplemental Material

Tables S1 and S2
Figures S1 and S2

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