BACKGROUND: The Asp358Ala variant (rs2228145; A>C) in the IL (interleukin)-6 receptor (IL6R) gene has been implicated in the development of abdominal aortic aneurysms (AAAs), but its effect on AAA growth over time is not known. We aimed to investigate the clinical association between the IL6R-Asp358Ala variant and AAA growth and to assess the effect of blocking the IL-6 signaling pathway in mouse models of aortic aneurysm rupture or dissection.

METHODS: Using data from 2863 participants with AAA from 9 prospective cohorts, age- and sex-adjusted mixed-effects linear regression models were used to estimate the association between the IL6R-Asp358Ala variant and annual change in AAA diameter (mm/y). In a series of complementary randomized trials in mice, the effect of blocking the IL-6 signaling pathways was assessed on plasma biomarkers, systolic blood pressure, aneurysm diameter, and time to aortic rupture and death.

RESULTS: After adjusting for age and sex, baseline aneurysm size was 0.55 mm (95% CI, 0.13–0.98 mm) smaller per copy of the minor allele [C] of the Asp358Ala variant. Change in AAA growth was −0.06 mm per year (−0.18 to 0.06) per copy of the minor allele; a result that was not statistically significant. Although all available worldwide data were used, the genetic analyses were not powered for an effect size as small as that observed. In 2 mouse models of AAA, selective blockage of the IL-6 trans-signaling pathway, but not combined blockage of both, the classical and trans-signaling pathways, was associated with improved survival (P<0.05).

CONCLUSIONS: Our proof-of-principle data are compatible with the concept that IL-6 trans-signaling is relevant to AAA growth, encouraging larger-scale evaluation of this hypothesis.

Ellie Paige, PhD*
Marc Clément, PhD*
et al

*Paige and Clément are joint first authors.
†Freitag, Paul, and Mallat are joint senior authors.
The full author list is available on page 92.

Key Words: alleles ▪ aortic aneurysm ▪ genetics ▪ inflammation ▪ interleukins

© 2019 The Authors. Circulation: Genomic and Precision Medicine is published on behalf of the American Heart Association, Inc., by Wolters Kluwer Health, Inc. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution, and reproduction in any medium, provided that the original work is properly cited.

https://www.ahajournals.org/journal/circgen
Abo...
Similar results were observed when the analysis was restricted to those with a small aneurysm at baseline (growth = −0.10 mm/y [−0.23 to 0.02] per copy of the minor allele; Figure IV in the Data Supplement) or medium aneurysm at baseline (growth = −0.08 mm/y [−0.21 to 0.05] per copy of the minor allele; Figure V in the Data Supplement). We did observe an association between the Asp358Ala variant and time to surgery threshold after adjusting for age and sex (hazards ratio = 0.85 [0.73–0.98] per copy of the minor allele; Figure VIA in the Data Supplement). The hazards ratio was in the same direction but became statistically nonsignificant in the subset of studies for which we were able to additionally adjust for current smoking, diabetes mellitus status, body mass index, and measurement method (hazards ratio = 0.91 [0.77–1.06]; Figure VIB in the Data Supplement). The overall change in AAA growth remained the same when individuals with only a single measure of aneurysm size were included in the model (n=2691, growth = −0.06 mm/y [−0.18 to 0.06]; Figure VII in the Data Supplement).

**Inhibition of IL-6 Signaling Pathway in Angiotensin II + Anti-TGF-β Mouse Model**

We next tested the effect of blocking the IL-6 pathway in 2 distinct, previously characterized mouse models of AAA (Methods). In the Ang II (angiotensin II) + anti-TGF (transforming growth factor)-β model, mice infused with anti-IL-6R (blocking both classical and trans-signaling pathways) demonstrated a significant increase of plasma concentration of IL-6, as compared to isotype-treated mice, and this difference
was sustained over the course of the experiment (Figure 2A). We observed a reduction in plasma concentrations of serum amyloid A, a protein expressed in response to inflammation, after blocking IL-6R compared with the control mice (Figure 2B). Blocking IL-6R significantly reduced plasma concentration of IL-2 before and after the infusion and reduced concentration of IL-5 and CXCL1 (chemokine ligand 1) after the infusion (Figure 2A). After anti-IL-6R treatment, systolic blood pressure was significantly lower after the infusion compared with control treatment (Figure 2C). However, there was no significant difference in rate of aneurysm rupture between the anti-IL-6R treated and control groups (Figure 2D). Because there was no observed association with AAA rupture, we did not further assess the effect of blocking the IL-6R pathway on AAA growth.

Selectively blocking the IL-6 trans-signaling pathway using sgp130Fc did not change the concentration of IL-6 (Figure 3A) or serum amyloid A (Figure 3B), but significantly induced IL-5 and reduced TNF (tumor necrosis factor)-α plasma concentration (Figure 3A). Although we observed no difference in systolic blood pressure between the mice treated with sgp130Fc and the control mice (Figure 3C), there was a significant reduction in aneurysm rupture after sgp130Fc treatment compared with control treatment (Figure 3D).

### Inhibition of IL-6 Signaling Pathway in Elastase + Anti-TGF-β Mouse Model

Using the elastase + anti-TGF-β model, we found that blockage of the IL-6R pathway using anti-IL-6R resulted in significantly increased mortality (Figure 4A) induced by aortic rupture (Figure 4B) but there was no change in the diameter of the aneurysm at the end of the experiment (Figure 4C). α-smooth muscle actin (α-SMA+) density in the media was similar in the 2 groups of mice (data not shown). There was also no change in the collagen content of the aortic wall (Figure 4D) or the recruitment of myeloperoxidase positive (MPO+) cells (Figure 4E), but treatment with anti-IL-6R significantly enhanced the recruitment of CD3+ T cells in the aortic wall (Figure 4F).

Blocking only the IL-6 trans-signaling pathway using sgp130Fc significantly increased survival (Figure 5A) by reducing aortic ruptures (Figure 5B), although at the end of the experiment there was no change in the aortic diameter between the treated and control mice (Figure 5C). Histological analysis of aortic samples revealed a significant increase in the collagen content of the arterial wall (Figure 5D) but no differences in α-SMA+ density (data not shown), Ly6G+ (Figure 5E) and CD3+ T-cell accumulation (Figure 5F) after sgp130Fc infusion, as compared to the control mice. Table 2 summarises the results of the different mouse models with a comparison to the human genetic data.
Evaluation of the Effect of *IL6R*-rs2228145 on a Range of Cardiovascular Markers

As would be expected, the minor allele of *IL6R*-rs2228145 was associated with increased plasma concentrations of IL-6 and sIL-6R. The variant was also associated with increased monocyte count, after correcting for multiple comparisons (*P* < 1.389 × 10⁻³). At a nominal significance level (*P* < 0.05), rs2228145-C was associated with reduced lymphocyte count, increased levels of the cytokines CXCL10, CXCL11, and IL1-α, as well as CD6 (an important regulator of T cells), and reduced levels of MMP-3 (a matrix metalloproteinase), TIMP-4 (a metalloproteinase inhibitor), OPG (osteoprotegerin), and IGFBP1 (Figure VIII in the Data Supplement). In our analysis, the effects on plasma IL-2, IL-5, and CXCL1 levels, as well as on blood pressure, were not statistically significant.

**DISCUSSION**

In a combined analysis of the available worldwide clinical genetic data on AAA growth, we observed no statistically significant decrease in annual AAA growth rates for carriers of the minor allele of the Asp358Ala variant (rs2228145) in the *IL6R* gene. While we did observe a 15% decrease in the rate of reaching the surgery threshold of ≥55 mm (hazards ratio = 0.85 [0.73–0.98] per copy of the minor allele), people with copies of the *IL6R*-Asp358Ala variant also had, on average, smaller baseline aneurysm diameters. Although we tried to account for this by allowing baseline hazards to vary depending on initial aneurysm size, some residual confounding is possible and could explain the observed results. In experimental data from mouse models, we found that selective blockade of the IL-6 trans-signaling pathway was associated with decreased aortic rupture and death. In exploratory analyses of cardiovascular and inflammatory biomarkers in healthy participants, we found that rs2228145-C was inversely associated with plasma levels of OPG, MMP-3, and TIMP-4 (*P* < 0.05). OPG has previously been shown to promote MMP release from monocytes and vascular smooth muscle cells, and aberrant aortic ECM (extracellular matrix) remodeling has been suggested to play a key role in the pathogenesis of AAA. However, we note that further studies are needed to validate our biomarker data. Taken together, these human genetic, biomarker, and experimental murine findings are compatible with the concept that IL-6 trans-signaling is relevant to AAA growth, encouraging larger-scale evaluation of this hypothesis.

If increased availability of sIL-6R results in a dampening of the IL-6 trans-signaling pathway, this may explain potential protective effects in AAA and is con-
consistent with previously observed protective effects in mouse models of sepsis and pancreatic and lung failure. As we found a consistent pattern of results when the trans-signaling pathway was selectively blocked in the mouse models, it suggests that this pathway could have a detrimental effect on AAA growth (Figure 6).

For example, the minor allele of the rs2228145 variant may result in a local reduction of IL-6 trans-signaling in the abdominal vasculature, reducing AAA risk and, perhaps, AAA growth rates. Our observation of only a small, but statistically insignificant, decrease in annual AAA growth rates does not preclude meaningful clinical effects, because the growth rate reduction estimated from natural genetic variation does not necessarily relate to the magnitude of the benefit that might result from pharmacological treatment directed at the IL-6 trans-signaling pathway. Selective blocking of the IL-6 trans-signaling pathway using sgp130Fc is being investigated in phase II clinical trials in patients with inflammatory bowel disease.

Nevertheless, apparent inconsistencies in our findings require further elucidation. For example, blockade of both the classical and trans-signaling IL-6 pathways using the animal-equivalent (MR16-1) of tocilizumab had no effect on AAA rupture in the Ang II + anti-TGF-β model, but it was associated with decreased survival in the elastase + anti-TGF-β and isotype or anti-IL-6R, at day 16. Note that the aneurysm from the isotype treated mouse was not ruptured. Analysis of the aortic diameter (µm) based on the perimeter obtained from aortic cross sections. Quantification and representative images of myeloperoxidase (MPO) and CD3 immunofluorescent stainings on aortic cross section. *P<0.05 isotype vs anti-IL-6R; Mann-Whitney test. All data for the generation of the graphs shown in Figure 4 were generated in one independent experiment.
that the blocking of both the classical signaling cascade (considered to have protective and regenerative cellular effects) and trans-signaling cascade (considered to have proinflammatory effects) canceled each other out, leading to no detectable effect on AAA rupture. Further studies are needed to replicate and further characterize our findings.

We undertook a range of sensitivity analyses to test assumptions underlying our longitudinal human genetic studies. We studied complementary murine models of AAA, including the elastase + anti-TGF-β mouse model that has been shown to more closely mimic the AAA growth and rupture patterns seen in humans. To generate new mechanistic hypotheses, we conducted exploratory studies of the IL6R-Asp358Ala variant in relation to cardiovascular and inflammatory plasma biomarkers recorded in healthy participants. The experimental mouse studies were conducted under severe conditions in which TGF-β was blocked. Although any effect of IL-6R signaling might have been easier to observe.

Table 2. Comparison of Results for the Association Between IL-6R and Abdominal Aortic Aneurysm From Human Genetic Analysis and Mouse Experimental Models

<table>
<thead>
<tr>
<th>Model</th>
<th>Inhibition of IL-6 Signaling</th>
<th>Effect on AAA Growth/ Survival</th>
<th>P Value of Effect</th>
<th>Other Cardiovascular Markers (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humans stratified for IL6R-Asp358Ala</td>
<td>Minor allele associated with dampening of classical signaling. Effects on trans-signaling not clear.</td>
<td>Nonsignificant change in AAA growth (0.06 mm/y)*</td>
<td>0.136</td>
<td>Increased IL-6, sIL-6R, CXCL10, CXCL11, IL-1α, and CD6; Reduced MMP-3, OPG, IGFBP1, and TIMP-4</td>
</tr>
<tr>
<td>Angiotensin II + anti-TGF-β mouse model</td>
<td>Both classical and trans-signaling</td>
<td>No difference in survival</td>
<td>1.000</td>
<td>Increased IL-6, Reduced SAA, IL-2, IL-5, CXCL1, and SBP</td>
</tr>
<tr>
<td>Elastase + anti-TGF-β mouse model</td>
<td>Both classical and trans-signaling</td>
<td>Decreased survival</td>
<td>0.029</td>
<td>Increased recruitment of CD3+ T cells</td>
</tr>
</tbody>
</table>

AAA indicates abdominal aortic aneurysm; CD, cluster of differentiation; CXCL, chemokine ligand; IGFBP1, insulin-like growth factor-binding protein 1; IL, interleukin; MMP, matrix metalloproteinase; OPG, osteoprotegerin; SAA, serum amyloid A; SBP, systolic blood pressure; sIL-6R, soluble IL-6 receptor; TIMP, metalloproteinase inhibitor; and TNF, tumor necrosis factor.

*Effect indicated per copy of the IL-6R minor allele.
serve in a less severe model (aortic dilatation without rupture), a protective effect of the intervention in that setting would not provide assurance that the intervention will also be protective in a more severe model (aortic rupture). A treatment that limits aortic dilatation but does not reduce the risk of aortic rupture would have limited clinical relevance.

Our study had potential limitations. It was powered to detect reductions in aneurysm growth of $\approx 0.21$ mm per year or larger, much greater than the observed non-significant decrease of 0.06 mm per year. Future studies powered to see an effect the same size as that observed in the current study would need to recruit an additional $\approx 21,500$ participants (total participants needed $= 24,444$, Material in the Data Supplement). This is unlikely to be achievable in the near future; alternative study methods using a composite phenotype for disease progression may be needed. Index event and survival bias, in which participants are selected into the study based on both having and surviving an event, may have biased the results towards the null. However, this bias is likely to be small ($<10\%)$. Further, ultrasound, the primary method used to assess AAA diameter in the included studies, has a margin of error of 2 to 3 mm, greater than the annual rate of aneurysm growth, making changes in growth difficult to detect. This might be why we observed an association between the $IL6R$-Asp358Ala variant and time to surgery threshold of $\geq 55$ mm but not when looking at continuous change in AAA size. Although we examined rupture rather than aortic diameter as the outcome in the mouse experimental models, our published data indicate that aortas that rupture have larger diameters or faster diameter progression than the ones that do not rupture.

It is also uncertain how well the results of our animal models translate to clinical disease. For example, an important difference is that IL-6 blockage is initiated before or at the time of disease development in the mice models of AAA, thereby not truly mimicking the treatment effects expected in humans, in which drugs to block IL-6 pathway would be started after disease onset. Blockade of the IL-6 signaling pathways in the Ang II + anti-TGF-β mouse model resulted in reproducible reductions in systolic blood pressure. Although tocilizumab has been anecdotally reported to improve pulmonary hypertension in Castleman disease, the $rs2228145$ variant was not associated with changes in systemic blood pressure in healthy participants in a genome-wide association study. A large-scale randomized trial found no difference in the number of hypertension events reported in those using tocilizumab compared to placebo. Thus, the acute responses to pharmacological doses of Ang II in the mouse model may not faithfully reproduce the human setting of AAA.

Our study may have clinical implications. Tocilizumab is currently indicated in a few disease settings, including rheumatoid arthritis and giant cell arteritis, both of which are associated with an increased risk of aortic aneurysm. The development of coronary artery aneurysms has also been reported in a nonplacebo-controlled pilot study of tocilizumab in children with Kawasaki Disease. Findings from this pilot study in children, combined with our finding that blocking the IL-6 pathway using the animal-equivalent of tocilizumab was associ-
ated with decreased survival in the elastase + anti-TGF-β model, suggests that patients treated with tocilizumab for conditions associated with aortic aneurysm development should possibly be monitored for AAA.

In conclusion, our proof-of-principle data are potentially compatible with the concept that IL-6 trans-signal-

ing is relevant to AAA growth, encouraging larger-scale evaluation of this hypothesis.

**ARTICLE INFORMATION**

Received November 12, 2018; accepted January 15, 2019.

The Data Supplement is available at https://www.ahajournals.org/doi/suppl/10.1161/CIRCGEN.118.002413.

**Authors**

Elie Paige, PhD*; Marc Clément, PhD*; Fabien Lareyre, MD, PhD; Michael Swee	tling, PhD; Juliette Raffort, MD, PhD; Céline Grenier, PhD; Alison Finigan; James Harrison, BA; James E. Peters, MBChB, PhD; Benjamin B. Sun, PhD; Adam S. Butterworth, PhD; Seamus C. Harrison, PhD; Matthew J. Bown, MBChB, PhD; Jess S. Lindholt, MD, PhD, DMSc; Stephen A. Badger, MD, MCh; Rftkhar I. Kullo, MD; Janet Powell, MD, PhD; Paul E. Norman, DS; D. Julian A. Scott, MD, MBChB; Marc A. Bailey, MBChB, PhD; Stefan Rose-John, PhD; John Danesh, MBChB, DPhil; Daniel F. Freitag, PhD†; Dirk S. Paul, PhD†; Ziad Mallat, MD, PhD†

**Correspondence**

Ziad Mallat, MD, PhD, Department of Medicine, University of Cambridge, Cambridge, CB2 0QQ, United Kingdom. Email zm255@medschl.cam.ac.uk

**Affiliations**

National Centre for Epidemiology and Population Health, Research School of Population Health, The Australian National University, Canberra, Australia (E.P.). Division of Cardiovascular Medicine (M.C., F.L., J.R., C.G., A.F., J.H., Z.M.) and BHF Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care (E.P., M.S., J.E.P., B.B.S., A.S.B., J.D., D.F.F., D.S.P.) University of Cambridge, Cambridge, United Kingdom. Université Côté d’Azur, Institut National de la Sante et de la Recherche Medecale, Centre Méditerranéen de Recherche Moléculaire (F.L., J.R.). University Hospital of Nice, France (F.L., J.R.). Department of Health Sciences (M.S.) and Department of Cardiovascular Sciences, NRHR Leicester Biomedical Research Centre (S.C.H., M.J.B.), University of Leicester. British Heart Foundation Centre of Excellence, Division of Cardiovascular Medicine, Addenbrooke’s Hospital, Cambridge, UK (J.E.P., A.S.B., S.C.H., J.D., D.F.F., D.S.P., Z.M.). NRHR Blood and Transplant Research Unit in Donor Health and Genomics, Cambridge, United Kingdom (A.S.B., J.D.). Department of Cardiovascular and Thoracic Surgery, Eltary Research Centre of Individualised Medicine in Arterial Disease, Odense University Hospital, Denmark (J.S.L.). Regional Vascular Surgery Unit, Belfast Health and Social Care Trust, United Kingdom (S.A.B.). Department of Cardiovascular Medicine, Gonda Vascular Center, Mayo Clinic, Rochester, MN (E.P.). Faculty of Medicine, Department of Surgery and Cancer, Imperial College London, United Kingdom (J.D.). Medical School, University of Western Australia, Perth, Australia (P.E.N.). Leeds Vascular Institute, Leeds General Infirmary (D.J.A.S., M.A.B.). Department of Biochemistry, Christian-Albrechts-Uni-

versity, Kiel, Germany (S.R.-J.). Department of Human Genetics, Wellcome Sanger Institute, Hinxton, United Kingdom (J.D.). Institut National de la Santé et de la Recherche Médicale, Paris Cardiovascular Research Center, France (Z.M.).

**Acknowledgments**

We thank Chugai Pharmaceutical Co, Ltd for providing the MR16-1. We thank Tao Jiang and Praveen Surendran (Cardiovascular Epidemiology Unit, University of Cambridge) for providing access to genomic datasets.

**Sources of Funding**

The Cardiovascular Epidemiology Unit is supported by the UK Medical Research Council (MR/L003120/1), British Heart Foundation (RG/13/13/30194), and National Institute for Health Research (Cambridge Biomedical Research Centre at the Cambridge University Hospitals NHS Foundation Trust). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care. Dr Rose-John was supported by grants of the Deutsche Forschungsgemeinschaft (CRC877, project A1) and the German Cluster of Excellence 306 Inflammation at Interfaces. The Leeds Aneurysm Development Study was funded by the Garfield Weston Foundation and Dr Bailey is supported by the British Heart Foundation. Dr Mallat is supported by the British Heart Foundation (RG/79120 and RG/79915).

**Disclosures**

Dr Freitag has been a full-time employee of Bayer AG, Germany, since October 2015. The other authors report no conflicts.

**REFERENCES**


44. Surendran P, et al.; CHARGE-Heart Failure Consortium; EchoGen Consortium; METASTROKE Consortium; GIANT Consortium; EPIC-InterAct Consortium; Lifelines Cohort Study; Wellcome Trust Case Control Consortium; Understanding Society Scientific Group; EPIC-CVD Consortium; CHARGE+ Exome CHIP Blood Pressure Consortium; T2D-GENES Consortium; GoT2DGenes Consortium; ExomeBP Consortium; CHD Exome+ Consortium. Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. Nat Genet. 2016;48:1151–1161. doi: 10.1038/ng.3654.

