Interleukin-6 receptor pathways in abdominal aortic aneurysm

Seamus C. Harrison1,2,*, Andrew J.P. Smith1, Gregory T. Jones3, Daniel I. Swerdlow4, Riaz Rampuri1, Matthew J. Bown5, on behalf of the Aneurysm Consortium, Lasse Folkesen6, Annette F. Baas7, Gert Jan de Borst8, Jan D. Blankensteijn9, Jacqueline F. Price10, Yolanda van der Graaf7, Stela McLachlan10, Obi Agu11, Albert Hofman12, Andre G. Uitterlinden12, Anders Franco-Cereceda13, Ynte M. Ruigrok14, F.N. van’t Hof14, Janet T. Powell15, Andre M. van Rij3, Juan P. Casas16, Per Eriksson5, Michael V. Holmes4, Folkert W. Asselbergs17,18,19, Aroon D. Hingorani4, and Steve E. Humphries1

1Department of Cardiovascular Genetics, Institute of Cardiovascular Science, University College London, London WC1E 6JJ, UK; 2BHF Laboratories, Centre for Cardiovascular Genetics, Institute of Cardiovascular Science, University College London (UCL), The Rayne Building, 5 University Street, London WC1E 6JJ, UK; 3University of Otago, Dunedin 9054, New Zealand; 4Genetic Epidemiology, University College London, London WC1E 6JJ, UK; 5Department of Cardiovascular Sciences, Leicester University, Leicester LE2 7UX, UK; 6Atherosclerosis Research Unit, Department of Medicine Solna, Karolinska Institute, Stockholm 171 76, Sweden; 7Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht 3584CG, The Netherlands; 8Department of Surgery, Vascular Surgery, VU Medical Center, PO Box 85500, G04.129, Utrecht 3508GA, The Netherlands; 9Department of Cardiovascular Genetics, Institute of Cardiovascular Science, University College London, Rayne Building, University Street, London WC1E 6JJ, UK; 10Department of Cardiovascular Sciences, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, UK; 11Vascular Surgery, University College London Hospital, London NW1 2BU, UK; 12Department of Epidemiology, Erasmus Medical Center, Rotterdam 3000CA, The Netherlands; 13Cardiothoracic Surgery Unit, Karolinska Institutet, Stockholm 171 76, Sweden; 14Department of Neurology and Neurosurgery, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht 3508 GA, The Netherlands; 15Vascular Surgery Research Group, Imperial College Charing Cross Hospital, 4th Floor, Fulham Palace Road, London W6 8RF, UK; 16London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK; 17Division Heart and Lungs, Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands; 18Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands; and 19Department of Medical Genetics, Biomedical Genetics, University Medical Center, Utrecht, The Netherlands

Received 3 June 2012; revised 24 August 2012; accepted 10 September 2012; online publish-ahead-of-print 30 October 2012

Methods

We conducted a systematic review and meta-analysis of studies reporting circulating IL-6 in AAA, and new investigations of the association between a common non-synonymous functional variant (Asp358Ala) in the IL-6R gene (IL6R) and AAA, followed the analysis of the variant both in vitro and in vivo.

Inflammation may play a role in the development of abdominal aortic aneurysms (AAA). Interleukin-6 (IL-6) signalling through its receptor (IL-6R) is one pathway that could be exploited pharmacologically. We investigated this using a Mendelian randomization approach.

Results

Up to October 2011, we identified seven studies (869 cases, 851 controls). Meta-analysis demonstrated that AAA cases had higher levels of IL-6 than controls [standardized mean difference (SMD) = 0.46 SD, 95% CI = 0.25–0.66, I² = 70%, P = 1.1 × 10−5 random effects]. Meta-analysis of five studies (4524 cases/15 710 controls) demonstrated that rs7529229 (which tags the non-synonymous variant Asp358Ala, rs2228145) was associated with a lower risk of AAA, per Ala358 allele odds ratio 0.84, 95% CI: 0.80–0.89, I² = 0%, P = 2.7 × 10−11. In vitro analyses in lymphoblastoid cell lines demonstrated a reduction in the expression of downstream targets (STAT3, MYC and ICAM1) in response to IL-6 stimulation in Ala358 carriers.

Conclusions

A Mendelian randomization approach provides robust evidence that signalling via the IL-6R is likely to be a causal pathway in AAA. Drugs that inhibit IL-6R may play a role in AAA management.

Keywords

Abdominal aortic aneurysm • Mendelian randomization • Interleukin-6 • Polymorphism

* Corresponding author. Tel: +44 20 7679 6969, Fax: +44 20 7679 6211, Email: seamus.harrison@ucl.ac.uk

© The Author 2012. Published by Oxford University Press on behalf of European Society of Cardiology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by-nc/3.0/), which permits non-commercial reuse, distribution, and reproduction in any medium, provided that the original authorship is properly and fully attributed; the Journal, Learned Society and Oxford University Press are attributed as the original place of publication with correct citation details given; if an article is subsequently reproduced or disseminated not in its entirety but only in part or as a derivative work this must be clearly indicated. For commercial re-use, please contact journals.permissions@oup.com.
**Introduction**

Abdominal aortic aneurysm (AAA) affects ~2–5% of men and <1% of women aged 65–74 years. Progressive dilatation of the aorta increases the risk of rupture and surgical intervention is indicated when the diameter reaches 5.5 cm. Currently there are no recognized treatments to diminish aneurysm progression at an early stage and a pharmacological treatment that targeted the processes underlying aortic dilatation, as well as other vascular disease in general would provide an attractive adjunct to the surveillance and surgery treatment paradigm. However, few if any therapeutic targets have been validated in humans to date.

The major environmental exposures associated with AAA development include male sex, advancing age, cigarette smoking, dyslipidaemia, and hypertension. There is also a genetic component to the disease, with twin studies reporting heritability in the region of 70%. Two recent genome-wide association studies (GWAS) of AAA have reported robust associations with common variants in \( \text{DAB2IP} \) and \( \text{LRP1} \), while the chromosome 9p21.3 locus associated with coronary heart disease (CHD) also shows a strong association with AAA. These variants, however, explain only a small proportion of the observed heritability suggesting other contributory genetic mechanisms remain to be discovered.

Interleukin-6 (IL-6) signalling is initiated by binding of IL-6 to its receptor (IL-6R), which forms a dimer with the ubiquitously expressed signal transducer glycoprotein-130 (gp-130). This, in turn, leads to the activation of the intracellular receptor-associated kinases and downstream effects via the transcription factor STAT3. Two forms of IL-6 signalling have been described, namely classical and trans-signalling. In classical signalling, IL-6 binds the membrane bound IL-6R (mIL-6R), which is expressed in hepatocytes and cells of the innate immune system. In trans-signalling, IL-6 binds to the circulating soluble IL-6R (sIL-6R), and this complex is capable of binding to gp130 in a wide range of cell types. It has previously been shown that the expression of IL-6 and downstream mediators of IL-6 signalling, such as STAT3, are greater in AAA than in non-aneurysmal aortic tissue.

A common non-synonymous sequence variant in \( \text{IL6R} \) (p.Asp358Ala, also annotated as rs8192284) results in an increased proteolytic cleavage of the mIL-6R. This variant is strongly associated with higher levels of circulating IL-6 levels, but importantly, lower levels of downstream products of IL-6 signalling, such as C-reactive protein and fibrinogen, suggesting that it acts by the attenuation of signalling via the IL-6 receptor. Furthermore, it was shown that this variant conferred protection against CHD, prompting speculation that targeting the IL-6R in CHD is a potentially novel preventive strategy. Whether or not this is the case for AAA has not yet been evaluated.

In the present study, the primary hypothesis is that pro-inflammatory signalling via the IL-6R plays a causal role in AAA development. This was investigated using a Mendelian randomization approach. First, a systematic review and meta-analysis of the published literature was performed in order to establish the relationship between circulating IL-6 levels and AAA. Secondly, novel analysis of the association between the functional Asp358Ala IL6R variant and AAA was performed. Finally, *in vitro* analyses were used to investigate the mechanism by which this variant could protect from cardiovascular disease.

**Methods**

**Observational association between interleukin-6 and abdominal aortic aneurysms**

Studies were identified using a two-stage search strategy following PRISMA guidelines (Supplementary material online). In the first stage, two electronic databases (MEDLINE and EMBASE) were searched and in the second stage articles were identified by manually searching references of articles identified in the first stage and review articles. Authors of large epidemiological studies of AAA were also approached to determine whether they had measures of IL-6 in cases and controls. The MEDLINE database was searched from January 1966 to January 2012, and the EMBASE from 1980 to January 2012. Inclusion criteria were decided by consensus (SCH, RR, MVH, and SEH), and the studies were screened and abstracted in duplicate by SCH and RR. The search strategy is described in detail in the Supplementary material online.

**Study populations**

Detailed descriptions of the study cohorts and demographic details are presented in the Supplementary material online. Briefly, for the genetic association analyses, we used data from five case–control studies of AAA that have access to genetic information, and are part of an existing collaborative group investigating the genetics of AAA. All studies defined AAA as an infra-renal aortic diameter ≥3 cm by ultrasonound or computed tomography imaging, or previous AAA rupture/repair. The Aneurysm Consortium (AC) Genome Wide association Study of Abdominal Aortic Aneurysm recruited 1596 cases of AAA from centres across the UK and Western Australia. Control data were taken from the Wellcome Trust Case Control Consortium (n = 5855). The New Zealand study included 1373 individuals with AAA and 718 controls with no previous history of vascular disease. The Secondary Manifestations of ARterial disease (SMART) study included data from 631 cases of AAA and 6342 AAA free controls recruited from University Medical Center Utrecht, the Netherlands. The Edinburough Artery Study (EAS) is a prospective population-based cohort that included data from 62 cases of AAA and 819 AAA free controls. In the Utrectht Study (separate from the SMART study), 862 individuals with AAA were recruited to the ‘genetics AAA’ study and compared with controls from the ERGO/Rotterdam study (n = 1866). Studies were identified as part of established collaborative efforts to understand the genetics of AAA. All studies had full ethical approval.

**Genotyping**

Participants in the AC AAA GWAS were genotyped using the Illumina 660 k gene-chip, with previously described quality control filters. Individuals in the SMART study were genotyped using KASpar at K Biosciences, UK. For the New Zealand study, samples were genotyped using the TaqMan allelic discrimination method (using a pre-designed, functionally tested assay Applied Biosystems, assay C_26292282_10). For the Utrectht study, participants were genotyped using the Illumina 610k Chip. In the SMART study, rs7529229 was genotyped and in the New Zealand study rs41929267 was genotyped. These SNPs are perfect proxies for each other (\( R^2 = 1 \)). These SNPs both tag the non-synonymous variant (p.Asp358Ala, rs2228145, \( R^2 = 0.97 \).)
disequilibrium between SNPs was assessed using the SNP Annotation and Proxy Search resources (www.broadinstitute.org/mpg/snap).

**Functional analysis of the p.Asp358Ala (rs2228145) variant**

Epstein Barr Virus-transformed lymphoblastoid cell lines were used based on the genotype, confirmed using Taqman allelic discrimination (Applied Biosystems). After RNA extraction, gene expression was analysed using Taqman Gene Expression Assays designed for targeting STAT3, MYC, ATF3, ICAM1, and BCL3 (Applied Biosystems, Carlsbad, USA) using a 384-well plate format, following the standard protocol provided by Applied Biosystems. Full details of the in vitro methodology are described in the Supplementary material online. These target genes were chosen because they have been shown to be the major down-stream target of IL-6—STAT3 signalling.20

**Statistical analysis and power**

To estimate the association between circulating IL-6 and AAA, we performed meta-analysis of summary statistics identified by our systematic review. For each study, we used mean concentrations of IL-6 in cases and controls to determine a standardized mean difference (SMD) and standard error. For studies that reported median concentration levels and inter-quartile ranges (IQR), we took the median to be the mean value and divided the IQR by 1.35 in order to obtain the SDs. Mean differences in each study were pooled using inverse-weighted-fixed-effect meta-analysis. Heterogeneity was assessed using the $I^2$ statistic and Cochrane’s Q. Owing to heterogeneity in units used to measure IL-6, and baseline levels, a SMD was calculated preferentially to a weighted mean difference.

SNP-disease associations in each cohort were determined using logistic regression adjusted for age and gender, under an additive genetic model. Effects sizes are reported as odds ratios (OR) and 95% confidence intervals. Meta-analysis was performed using fixed and random-effects modelling and we measured between study heterogeneity using the $I^2$ statistic. Gene expression was analysed using the Rest Expression Software Tool by MW Pfaffl utilizing a Pair-Wise Fixed Reallocation Randomization Test. With 4523 case and 15 406 controls, there was over 80% to detect modest effects (OR 0.80–0.89, $P = 2.7 \times 10^{-11}$, $I^2 = 0%$ fixed-effect meta-analysis) but only one study matched for smoking status, but none specifically adjusted for these factors in the primary analyses. An assessment of the quality of the case–control studies is provided in Supplementary material online, Table S1.

In meta-analysis, pooling data from 869 cases and 851 controls, individuals with AAA had higher circulating IL-6 concentrations than controls; SMD = 0.46, 95% CI: 0.25–0.66, $P$ random effects model $= 1.1 \times 10^{-5}$, $I^2 = 70\%$. Concentrations of IL-6 were higher in cases than in controls in all but one of the studies in which no difference was observed.26 This study included only small AAA in the case group (3–5.5 cm), and reported different measurement units to all the other studies (ng/mL vs. pg/mL). Exclusion of this study reduced overall heterogeneity ($I^2 = 56\%$) and increased the statistical significance of the association ($P$ random effects $= 2 \times 10^{-3}$); however, this study did not overly influence the overall effect estimate greatly (sensitivity analysis presented in the Supplementary material online, Figure S2). Possible reasons for heterogeneity may be related to the selection of cases and controls. For example, there is evidence that IL-6 concentrations are correlated with the AAA size,22,25,29 and subgroups analyses demonstrated that when the analysis was restricted to studies that compared IL-6 in large AAA compared with controls, there was less heterogeneity ($I^2 = 31.5\%$, Figure 1). Furthermore, there was variation in how controls were selected, e.g. from distinct cohorts such as the general population23 or from surgical outpatients clinics,26 which is likely to contribute to heterogeneity. There was no evidence of publication bias using Begg’s adjusted rank correlation test ($P = 0.7$).

**Association of IL6R variants with abdominal aortic aneurysms**

Demographic details of the five case–controls studies are shown in Table 2 and genotyping quality control measures are shown in Table 3. There was a consistent association between the rare allele of rs7529229 (tagging Ala358) and reduced risk of AAA in all cohorts. Meta-analysis, pooling data from 4524 cases and 15 710 controls demonstrated a per-allele OR of 0.84 (95% CI: 0.80–0.89, $P = 2.7 \times 10^{-11}$, $I^2 = 0%$ fixed-effect meta-analysis) (Figure 2). In time-to-event analyses of prospective data from the SMART study, using Cox proportional hazard models, adjusted for age and gender, the rare Ala358 allele was also associated with a reduction in the risk of AAA clinical endpoints (AAA rupture, repair; $n_{\text{events}} = 223$) (Figure 3, per allele HR: 0.81, 95% CI: 0.67–0.99, $P = 0.043$).

**Results**

**Systematic review and meta-analysis of IL-6 and abdominal aortic aneurysms**

Seven studies reporting circulating levels of plasma IL-6 in AAA cases and controls were identified (Supplementary material online, Figure S1), including one previous unpublished study (the NZ study)21–27. Two studies that reported higher IL-6 in cases than controls, but did not report summary measures of IL-6 (mean and SD or median and IQR) were not included in the meta-analysis.27,28 Details of the studies included in the meta-analysis are provided in Table 1. All identified studies utilized a case–control design and used recognized methods to diagnose and define the presence of AAA and to measure IL-6 levels. No prospective population studies were identified. All but one study21 selected controls that were matched for age and gender, but only one study matched for smoking status, but none specifically adjusted for these factors in the primary analyses. An
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Study design</th>
<th>Cases/controls</th>
<th>Case definition</th>
<th>Controls</th>
<th>Diagnosis</th>
<th>IL-6 cases, mean (SD)</th>
<th>IL-6 controls, mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treska</td>
<td>2003</td>
<td>Czech Republic</td>
<td>Case–Control</td>
<td>74/30</td>
<td>Small (&lt;5 cm) = 30 Large (5–8 cm) = 38 Very large (&gt;8 cm) = 22 Ruptured/ symptomatic = 16**</td>
<td>Matched for age and gender No history of atherosclerosis</td>
<td>USS</td>
<td>59.3 (134.8)</td>
<td>6.7 (5.1)</td>
</tr>
<tr>
<td>Fowkes et al.</td>
<td>2006</td>
<td>UK</td>
<td>Case–Control</td>
<td>89/98</td>
<td>AP aortic diameter &gt; 3 cm, size distribution NR</td>
<td>Normal USS (&lt;3 cm) Matched for age and gender. Nested case– control within the Edinburgh Artery Study18</td>
<td>USS</td>
<td>2.8 (1.62)</td>
<td>1.8 (1.04)</td>
</tr>
<tr>
<td>Dawson et al.</td>
<td>2007</td>
<td>UK</td>
<td>Case-Control</td>
<td>27/15</td>
<td>Large AAA undergoing endovascular repair</td>
<td>Undergoing other vascular intervention</td>
<td>CT</td>
<td>4.94 (2.49)</td>
<td>2.65 (1.98)</td>
</tr>
<tr>
<td>Flondell-Site</td>
<td>2009</td>
<td>Sweden</td>
<td>Case–Control</td>
<td>360/218</td>
<td>Small (&lt;4.5 cm) = 122 Medium(4.5–5.5 cm) = 108 Large (&gt;5.5 cm) = 130</td>
<td>Age and sex matched No history of Atherosclerosis/AAA Undergoing routine preventative checks</td>
<td>CT/USS</td>
<td>9.19 (31.91)</td>
<td>2.08 (2.90)</td>
</tr>
<tr>
<td>Jones (unpublished)</td>
<td>2012</td>
<td>New Zealand</td>
<td>Case–Control</td>
<td>166/359</td>
<td>All greater than 5 cm, awaiting repair</td>
<td>Matched for age, free from atherosclerosis and AAA. Recruited from screening programme in NZ.</td>
<td>CT/USS</td>
<td>9.1 (6.60)</td>
<td>6.3 (3.93)</td>
</tr>
<tr>
<td>Parry et al.</td>
<td>2010</td>
<td>UK</td>
<td>Case–Control</td>
<td>75/90</td>
<td>All AAA &lt;5.5 cm</td>
<td>Matched for age, gender, and race Recruited from surgical clinics</td>
<td>USS</td>
<td>3.13 (2.87)</td>
<td>3.14 (2.32)</td>
</tr>
<tr>
<td>Wallinder et al.</td>
<td>2009</td>
<td>Sweden</td>
<td>Case–Control</td>
<td>78/41</td>
<td>Small (&lt;5 cm) = 38 Awaiting elective repair (&gt;5 cm) = 40</td>
<td>Matched for age, gender, and smoking status Volunteers with infra-renal aortic diameter &lt;3 cm</td>
<td>USS</td>
<td>4.02 (3.79)</td>
<td>2.3 (3.3)</td>
</tr>
</tbody>
</table>

**Values from ruptures not included in the meta-analysis.
stimulation alone is via reducing signalling through the m-IL6R is the presence of Ala358.

**Discussion**

In this study, we used an extension of the Mendelian randomisation (MR) paradigm to investigate the potential utility of targeting IL-6 signalling in AAA. In the first stage, we reviewed the observational literature reporting associations between circulating IL-6 and the presence of AAA. Meta-analysis of case–control studies demonstrated that IL-6 was significantly higher in subjects with AAA compared with those without. These data support a hypothesis that IL-6 and associated signalling pathways may play a role in the development of AAA, but it is not possible to directly infer a causal relationship, from observational data associations alone, owing to limitations inherent in such analyses. For example, many of the studies did not adjust for potential confounders such as smoking status, and previously published reports have found that AAAs are a source of circulating IL-6, suggesting that the observed association could at least in part be the result of reverse causation. Furthermore, none of the identified studies was prospective population cohorts, considered the gold standard.
in epidemiological studies and there was some heterogeneity in how cases and controls were selected in the papers identified by the literature search.

To address this, we applied the principles of MR for drug target validation by studying the association between a functional non-synonymous variant in $\text{IL6R}$ and AAA. Genotype is randomly allocated at conception and not subject to reverse causality and therefore represents a useful tool to examine causal relationships in complex diseases phenotypes. The Asp358Ala variant (or close proxy) was selected because it has previously been shown that it has concordant effects as tocilizumab on a range of inflammatory and cardiovascular biomarkers and, therefore, may be considered a useful genetic instrument to investigate the potential for targeting the IL-6R pharmacologically. There was a consistent association between the Ala358 variant and a reduced risk of AAA, a finding that suggests targeting the IL-6R is a plausible strategy in AAA. This was a statistically robust association, surpassing threshold levels of significance commonly used in genome-wide studies.

The $\text{IL6R}$ variants have not been previously identified in two separate GWAS of AAA; however, the largest of the discovery cohorts ($n_{\text{cases}} = 1866$) had only 20% power to detect a variant of this effect size at the significance threshold of $P < 1 \times 10^{-5}$. 

<table>
<thead>
<tr>
<th>Study</th>
<th>Genotyping platform</th>
<th>Minor allele frequency</th>
<th>Call rate (%)</th>
<th>HWE, P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>Illumina 670 k Beadchip</td>
<td>0.39</td>
<td>&gt;99</td>
<td>0.28</td>
</tr>
<tr>
<td>New Zealand</td>
<td>ABI Taqman</td>
<td>0.40</td>
<td>&gt;98</td>
<td>0.89</td>
</tr>
<tr>
<td>SMART</td>
<td>Kaspar</td>
<td>0.39</td>
<td>&gt;97</td>
<td>0.77</td>
</tr>
<tr>
<td>EAS</td>
<td>ABI Taqman</td>
<td>0.42</td>
<td>&gt;97</td>
<td>0.08</td>
</tr>
<tr>
<td>UTRECHT</td>
<td>Illumina 610 K</td>
<td>0.38</td>
<td>100</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Figure 2  Association between rs7529229 and abdominal aortic aneurysms following fixed-effect meta-analysis of four case–control studies (4524 cases/15 710 controls). Per allele odds ratio = 0.84 (95% CI: 0.80–0.89; $I^2 = 0, P = 2.7 \times 10^{-11}$).

Figure 3  Time to event analysis curves showing the probability of abdominal aortic aneurysm-related endpoint (Rupture/Endovascular Repair/Open Repair) by genotype at rs7529229 (CC is the rare homozygote) in 7139 individuals from the SMART study, in who there were 223 events. The hazard ratio per C-allele is 0.81, 95% CI = 0.67–0.99, $P = 0.043$.) The P-value was calculated using Cox regression, adjusting for age and gender.
used to triage those SNPs to be submitted for analysis in replication studies.

Given the higher levels of circulating IL-6 in patients with AAA, it may seem paradoxical that a variant associated with higher circulating IL-6 is also associated with a lower risk of AAA. However, this variant has previously been associated with reduced concentrations of C-reactive protein and fibrinogen (two downstream markers of IL6R activation), a pattern consistent with a pharmacological blockade of the IL-6 receptor with tocilizumab. Furthermore, it is important to note that this study is not an MR study of circulating IL-6 and AAA, but uses the MR concept to evaluate the potential utility of targeting the IL-6R in AAA disease.

The functional analysis suggests that the Ala358 variant is associated with a reduction in the sensitivity of immune cells to IL-6 in vitro. One possible interpretation of these data is that this variant reduces inflammation in the aortic wall in response to injury, via reduced signalling through the mIL-6R in immune cells recruited to the injury, resulting in a lower risk of AAA development. Although the lymphoblastoid cell line is useful to understand the functional consequences of this variant in vitro, further work on

**Figure 4** Gene expression in STAT3 target genes in lymphoblast cell lines of different IL6R rs2228145 (AA = common homozygote; CC, rare homozygote). (A) Basal gene expression in lymphoblast cell lines shows no difference in gene expression between IL6R genotypes. (B) Gene expression following IL-6 stimulation; lower gene expression was observed in all STAT3 target genes in lymphoblasts possessing the C allele. (C) Gene expression following IL-6 stimulation and addition of excess sIL-6R. To examine if the effect seen in (B) was due to receptor shedding, excess soluble IL-6R was added to the stimulated cells, suggesting that the mechanism is of genotype-specific reduction in STAT3 targets is the result of increased shedding of the mIL-6R.
tissue specifically from AAA cases required to understand the in vivo mechanism of action, particularly in understanding the balance of pro- and anti-inflammatory effects on remodelling of the arterial wall in response to damaging environmental exposures that promote vascular injury.

These findings have potentially important translational implications as they support a hypothesis that targeting the IL-6R pharmacologically is a strategy that merits evaluation in AAA. No randomized trials of tocilizumab (a monoclonal antibody to the IL6R used to treat rheumatoid arthritis) have yet examined AAA as an outcome, although two recent open-label studies have reported that in patients with rheumatoid arthritis, tocilizumab reduces arterial stiffness, which suggests that the IL6R blockade has effects on the vasculature. Indeed, a role for tocilizumab in the treatment of other forms of inflammatory vascular disease such as Takayasu’s arteritis has been postulated, although randomized trials in these conditions have not yet been reported. Furthermore, there is evidence that targeting inflammatory pathways can both prevent aneurysm formation and regress already established aneurysms in murine models of the disease and evidence that tocilizumab acts on this pathway. Tocilizumab does, however, have a number of side effects (such as hypersensitivity and respiratory tract infections) and the potential for benefit in patients with AAA would of course need to be balanced against the potential risks.

Although the effect of the IL6R Asp358Ala variant on AAA risk is modest, the potential for clinical benefit from pharmacological intervention at the IL-6R should not be discounted. For example, SNPs in HMGCGR (the target for statins) show only a modest (but highly significant) effect on CHD risk, but the role of statins in the prevention of CHD is well established. The fact that tocilizumab has been shown to be safe in large trials in humans adds further weight to the translational potential of the present findings.

In common with two of the three previously identified well-validated loci associated with risk of AAA the IL6R locus is also associated with CHD. The magnitude of the effect identified in the present analysis (OR: 0.86, 95% CI: 0.80–0.89) is, however, greater than that reported for the association with CHD risk (OR: 0.95). The association with CHD does, however, strengthen the case for targeting the IL-6R therapeutic- ally, as the major non-aneurysm-related cause of death in patients with AAA would of course need to be balanced against the potential risks.

Conclusion
This study has confirmed that AAA cases have higher concentrations of circulating IL-6 than controls, and provided novel evidence that a common functional variant in IL6R is associated with risk of AAA. This provides evidence that targeting the IL-6R may be a useful and novel strategy in AAA, and supports development of clinical trials in this regard.

Supplementary material
Supplementary material is available at European Heart Journal online.

Funding
S.C.H is funded by a BHF clinical training fellowship (FS/11/16/28696). S.E.H, & A.J.S are supported by the BHF (RG2008/08). This study makes use of data generated by the Wellcome Trust Case-Control Consortium. A full list of the investigators who contributed to the generation of the data is available from www.wtccc.org.uk. Funding for the project was provided by the Wellcome Trust under award 076113 and 085475. M.J.B is supported by a Senior Lectureship from the Higher Education Funding Council for England and the Leicester NIHR Biomedical Research Unit in Cardiovascular Disease. The Edinburgh Artery Study was supported by the British Heart Foundation and genotyping on this project was funded by a project grant from the Chief Scientist Office for Scotland. For the SMART study, research was financially supported by BBMRI-NL, a Research Infrastructure financed by the Dutch government (NWO 184.021.007). Folkert W. Asselbergs is supported by a clinical fellowship from the Netherlands Organisation for Health Research and Development (ZonMw grant 90700342). A.F. B. was supported by a E. Dekker grant from the Netherlands Heart Foundation (2009T001). The New Zealand AAA data was provided by the Vascular Research Consortium of New Zealand, and was supported by programme grant funding from the Health Research Council of New Zealand. MVH is funded as an MRC population health scientist (G0802432). A.D.H is supported by the BHF as a Senior Research Fellow (FS 05/125). DIS is supported by a UK Medical Council Doctoral Training Award. The generation and management of GWAS genotype data for the Rotterdam Study is supported by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGL)/Netherlands Organisation for Scientific Research (NWO) project nr. 050-060-810. We thank Pascal Arp, Mira Jhamai, Marijn Verkerk, Lizbeth Herrera and Marjolein Peters for their help in creating the GWAS database, and Karol Estrada and Maksim V. Struchalin for their support in creation and analysis of imputed data. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of
Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists.

Conflict of interest: none declared.

References

1. Multicentre aneurysm screening study (MASS), cost effectiveness analysis of screening for abdominal aortic aneurysms based on four year results from randomised controlled trial. BMJ 2002;325:1135.


