

# Interleukin-6 receptor pathways in abdominal aortic aneurysm

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## 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,  $I^2 = 70%$ ,  $P = 1.1 \times 10^{-5}$  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,  $I^2 = 0%$ ,  $P = 2.7 \times 10^{-11}$ . *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

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## Introduction

Abdominal aortic aneurysm (AAA) affects ~2–5% of men and <1% of women aged 65–74 years.<sup>1–3</sup> Progressive dilatation of the aorta increases the risk of rupture and surgical intervention is indicated when the diameter reaches 5.5 cm.<sup>4</sup> 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 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.<sup>5</sup> There is also a genetic component to the disease, with twin studies reporting heritability in the region of 70%.<sup>6</sup> Two recent genome-wide association studies (GWAS) of AAA have reported robust associations with common variants in *DAB2IP* and *LRP1*<sup>7,8</sup>, while the chromosome 9p21.3 locus associated with coronary heart disease (CHD) also shows a strong association with AAA.<sup>9</sup> 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.<sup>10,11</sup>

A common non-synonymous sequence variant in *IL6R* (p.Asp358Ala, rs2228145, also annotated as rs8192284) results in an increased proteolytic cleavage of the mIL-6R.<sup>12</sup> 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,<sup>12–15</sup> 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 preventative strategy.<sup>16</sup> 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<sup>17</sup> (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  $\geq 3$  cm by ultrasound 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.<sup>7</sup> 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 Edinburgh Artery Study (EAS) is a prospective population-based cohort that included data from 62 cases of AAA and 819 AAA free controls.<sup>18</sup> In the Utrecht 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 ( $n = 1866$ ).<sup>19</sup> Studies were identified as part of established collaborative efforts to understand the genetics of AAA.<sup>8</sup> 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 KBiosciences, UK ([www.kbioscience.co.uk](http://www.kbioscience.co.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 Utrecht study, participants were genotyped using the Illumina 610k Chip.

In the SMART study, rs7529229 was genotyped and in the New Zealand study rs4129267 was genotyped. These SNPs are perfect proxies for each other ( $R^2 = 1$ ). These SNPs both tag the non-synonymous variant (p.Asp358Ala, rs2228145,  $R^2 = 0.97$ ). Linkage

disequilibrium between SNPs was assessed using the SNP Annotation and Proxy Search resources ([www.broadinstitute.org/mpg/snp/](http://www.broadinstitute.org/mpg/snp/)).

### 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 downstream target of IL-6—STAT3 signalling.<sup>20</sup>

### 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 Cochran'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 > 1.12) on disease risk with a Type 1 error rate of 1 in 1000.

## 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)<sup>21–27</sup>. 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.<sup>27,28</sup> 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 study<sup>21</sup> 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

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.<sup>26</sup> 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 =  $2 \times 10^{-8}$ ); 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<sup>22,25,29</sup> 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 population<sup>23</sup> or from surgical outpatients clinics,<sup>26</sup> 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,  $P = 0.043$ ).

### Functional analyses of the Asp358Ala (rs2228145) IL6R variant

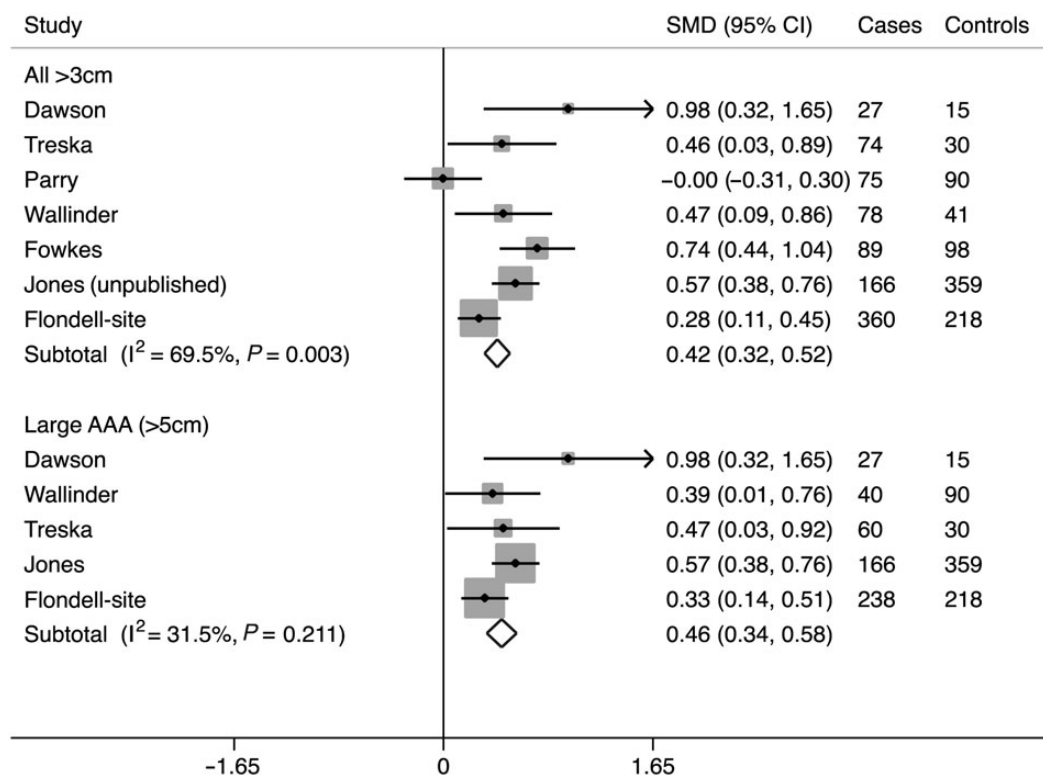
The aim of these analyses was to determine the downstream effects of the Asp358Ala variant on the expression of IL-6-mediated target genes identified in previous studies,<sup>20</sup> namely *STAT3*, *MYC*, *BCL3*, *ICAM1*, and *ATF3*.

In lymphoblastoid cells, no difference in the expression of STAT3 targets was observed at baseline (*Figure 4A*). After IL-6 stimulation, there was a reduction in the expression of *STAT3*, *MYC*, *ICAM1* in Ala358 homozygotes (CC) compared with Asp358 homozygotes (AA) (*Figure 4B*). This genotype-dependent difference was abolished by the presence of excess sIL-6R in the media (*Figure 4C*), suggesting that the effect observed with IL-6

**Table 1** Characteristics of case-control studies included in the meta-analysis of IL-6 levels in abdominal aortic aneurysms cases and controls

Author	Year	Country	Study design	Cases/controls	Case definition	Controls	Diagnosis	IL-6 cases, mean (SD)	IL-6 controls, mean (SD)
Treska	2003	Czech Republic	Case-Control	74/30	Small (<5 cm) = 30 Large (5–8 cm) = 38 Very large (>8 cm) = 22 Ruptured/ symptomatic = 16**	Matched for age and gender No history of atherosclerosis	USS	59.3 (134.8)	6.7 (5.1)
Fowkes et al. <sup>23</sup>	2006	UK	Case-Control	89/98	AP aortic diameter >3 cm, size distribution NR	Normal USS (<3 cm) Matched for age and gender. Nested case- control within the Edinburgh Artery Study <sup>18</sup>	USS	2.8 (1.62)	1.8 (1.04)
Dawson et al. <sup>21</sup>	2007	UK	Case-Control	27/15	Large AAA undergoing endovascular repair	Undergoing other vascular intervention	CT	4.94 (2.49)	2.65 (1.98)
Flondell-Site	2009	Sweden	Case-Control	360/218	Small (<4.5 cm) = 122 Medium(4.5– 5.5 cm) = 108 Large (>5.5 cm) = 130	Age and sex matched No history of Atherosclerosis/AAA Undergoing routine preventative checks	CT/USS	9.19 (31.91)	2.08 (2.90)
Jones (unpublished)	2012	New Zealand	Case-Control	166/359	All greater than 5 cm, awaiting repair	Matched for age, free from atherosclerosis and AAA. Recruited from screening programme in NZ.	CT/USS	9.1 (6.60)	6.3 (3.93)
Parry et al. <sup>26</sup>	2010	UK	Case-Control	75/90	All AAA <5.5 cm	Matched for age, gender, and race Recruited from surgical clinics	USS	3.13 (2.87)	3.14 (2.32)
Wallinder et al. <sup>25</sup>	2009	Sweden	Case-Control Case-Control	78/41	Small (<5 cm) = 38 Awaiting elective repair (>5 cm) = 40	Matched for age, gender, and smoking status Volunteers with infra-renal aortic diameter <3 cm	USS	4.02 (3.79)	2.3 (3.3)

\*\*Values from ruptures not included in the meta-analysis.



**Figure 1** Association between circulating IL-6 levels and the presence of abdominal aortic aneurysms. In pooled analysis of seven studies reporting IL-6 circulations in cases and controls (869/851) there was consistently higher concentrations in cases (SMD = 0.46, 95% CI: 0.25–0.66,  $P$  random effects model =  $1.1 \times 10^{-5}$ ,  $I^2 = 70\%$ ). Subgroup analyses, comparing IL-6 concentrations in large abdominal aortic aneurysms (>5 cm) demonstrated less heterogeneity (SMD = 0.46, 95% CI: 0.34–0.58,  $P = 2.25 \times 10^{-14}$ ,  $I^2 = 32\%$ ).

**Table 2** Demographic details of cohorts used for gene association analysis

Sample set	Controls			Cases		
	$n_{\text{controls}}$	% Female	Age (SD)	$n_{\text{cases}}$	% Female	Age (SD)
AC	5855	50	NA	1596	4	NA
SMART	6352	33	55 (12)	631	22	65 (10)
NZ	718	23	70 (6.6)	1373	20	74 (8.0)
EAS	819	47	64 (5.6)	62	66 (5.6)	74
UTRECHT	1866	56	56	862	10	68.1 (8.3)

stimulation alone is via reducing signalling through the m-IL6R is the presence of Ala358.

## Discussion

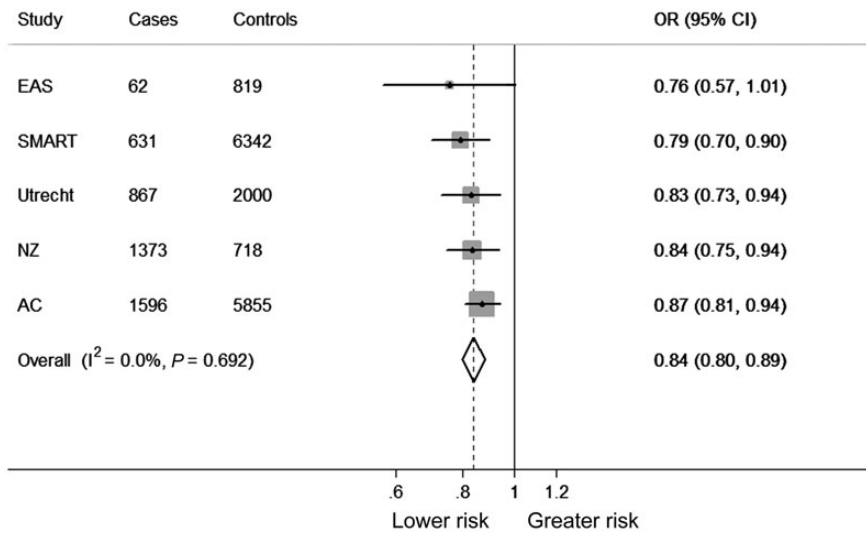
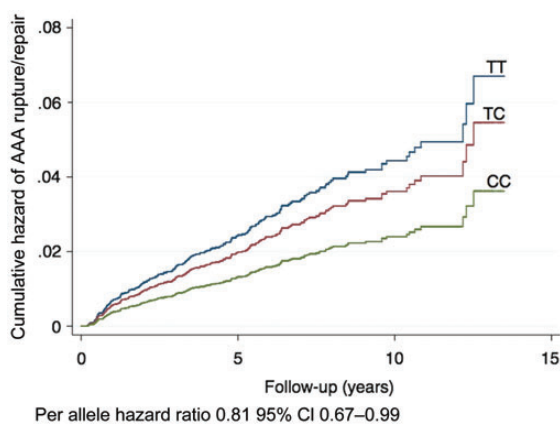
In this study, we used an extension of the Mendelian randomisation (MR) paradigm to investigate the potential utility of targeting IL-6 signalling in AAA. In the first stage, we reviewed the observational literature reporting associations between circulating IL-6 and the presence of AAA. Meta-analysis 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,<sup>21</sup> 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



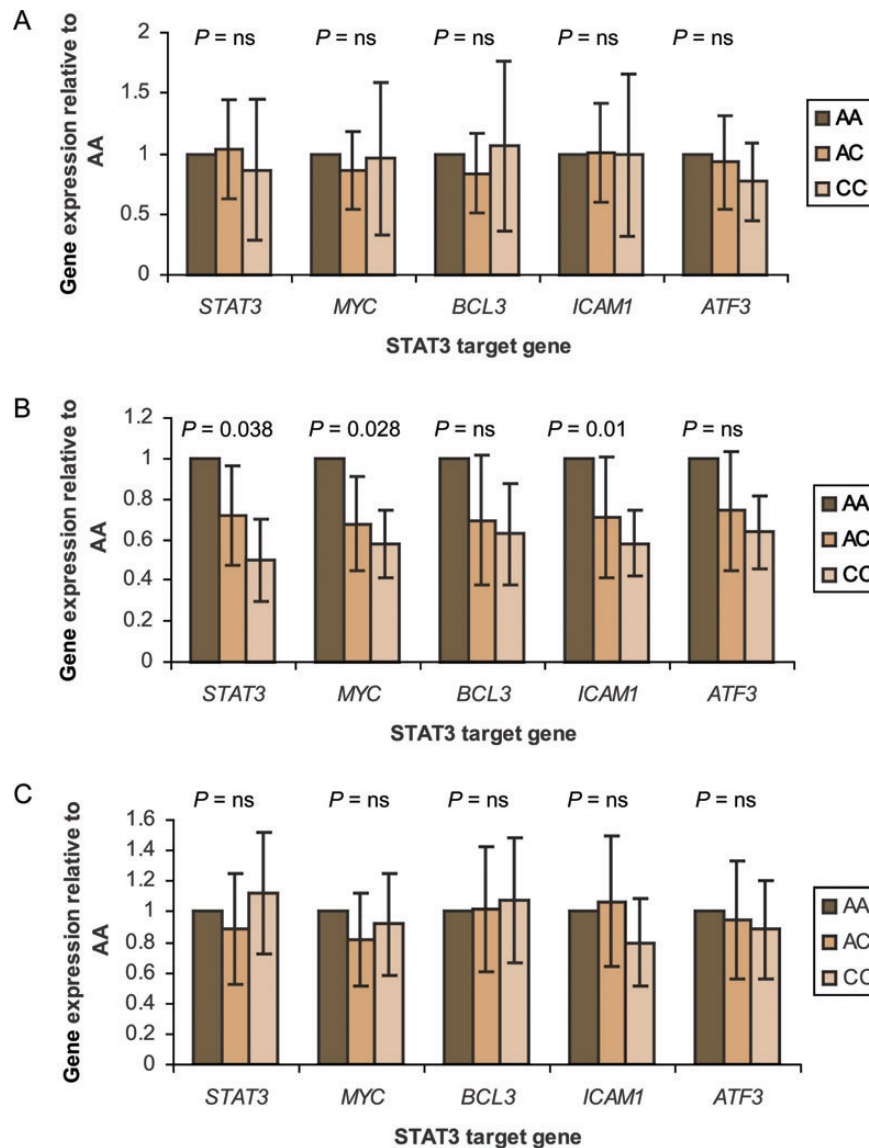
**Table 3** Genotyping metrics from each of the studies (Hardy–Weinberg equilibrium)

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

**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.

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 *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.<sup>30</sup> 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 *IL6R* variants have not been previously identified in two separate GWAS of AAA<sup>7,31</sup>; 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  $P < 1 \times 10^{-5}$ .



**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.

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<sup>30</sup>. 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

tissue specifically from AAA cases required to understand the *in vivo* mechanism of action, particularly in understating 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,<sup>32,33</sup> 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.<sup>34</sup> Furthermore, there is evidence that targeting inflammatory pathways can both prevent aneurysm formation and regress already established aneurysms<sup>35</sup> in murine models of the disease and evidence that tocilizumab acts on this pathway.<sup>36</sup> 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 *HMGCR* (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.<sup>37,38</sup> 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.<sup>13,16,30,39</sup> 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).<sup>30,39</sup> The association with CHD does, however, strengthen the case for targeting the IL-6R therapeutically, as the major non-aneurysm-related cause of death in patients with dilated aortae is cardiovascular disease.<sup>40</sup>

## Limitations

It is important to note the potential limitations of this study. The number of cases and controls identified from the published literature for the case–control analysis of IL-6 levels and AAA is relatively small, but effects were consistent and the overall result statistically robust. We did not have tissue from AAA for expression studies. The *in vitro* functional studies are in a lymphoblastoid cell-line, which may not mimic the *in vivo* immune response in the aortic wall. As there was limited phenotypic/risk factor data for some of the cohorts, we were unable to include the *IL6R* variant in a multivariate regression model including other covariates. However, no convincing effect on phenotypes such as smoking, lipids, and blood pressure has been seen in large-scale association analysis suggesting that the effects are not primarily mediated

through, or confounded by these intermediate phenotypes. Finally, we would ideally have a large data set with validated measures of aortic expansion in the context of the *IL6R* genotype and assess whether or not this could provide clinically useful information to guide surveillance programmes, but this is not available at present.

## 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.

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## References

- 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.
- Ledertle FA, Larson JC, Margolis KL, Allison MA, Freiberg MS, Cochrane BB, Graettinger WF, Curb JD. Abdominal aortic aneurysm events in the women's health initiative: cohort study. *BMJ* 2008;**337**:a1724.
- Svensjö S, Björck M, Gurtelschmid M, Djavani Gidlund K, Hellberg A, Wanhainen A. Low prevalence of abdominal aortic aneurysm among 65-year-old Swedish men indicates a change in the epidemiology of the disease. *Circulation* 2011;**124**:1118–1123.
- Mortality results for randomised controlled trial of early elective surgery or ultrasonographic surveillance for small abdominal aortic aneurysms. The UK Small Aneurysm Trial Participants. *Lancet* 1998;**352**:1649–1655.
- Forsdahl SH, Singh K, Solberg S, Jacobsen BK. Risk factors for abdominal aortic aneurysms: a 7-year prospective study: the Tromsø Study, 1994–2001. *Circulation* 2009;**119**:2202–2208.
- Wahlgren CM, Larsson E, Magnusson PK, Hultgren R, Swedenborg J. Genetic and environmental contributions to abdominal aortic aneurysm development in a twin population. *J Vasc Surg* 2010;**51**:3–7; discussion 7.
- Gretarsdottir S, Baas AF, Thorleifsson G, Holm H, den Heijer M, de Vries JP, Kranendonk SE, Zeebregts CJ, van Sterkenburg SM, Geelkerken RH, van Rij AM, Williams MJ, Boll AP, Kostic JP, Jonasdottir A, Jonasdottir A, Walters GB, Masson G, Sulem P, Saemundsdottir J, Mouy M, Magnusson KP, Tromp G, Elmore JR, Sakalihan N, Limet R, Defraigne JO, Ferrell RE, Ronkainen A, Ruigrok YM, Wijmenga C, Grobbee DE, Shah SH, Granger CB, Quyyumi AA, Vaccarino V, Patel RS, Zafari AM, Levey AI, Austin H, Girelli D, Pignatti PF, Olivieri O, Martinelli N, Malerba G, Trabetti E, Becker LC, Becker DM, Reilly MP, Rader DJ, Mueller T, Dieplinger B, Haltmayer M, Urbonavicius S, Lindblad B, Gottsater A, Gaetani E, Pola R, Wells P, Rodger M, Forgie M, Langlois N, Corral J, Vicente V, Fontcuberta J, Espana F, Grarup N, Jorgensen T, Witte DR, Hansen T, Pedersen O, Aben KK, de Graaf J, Holveijn S, Folkersen L, Franco-Cereceda A, Eriksson P, Collier DA, Stefansson H, Steinthorsdottir V, Rafnar T, Valdimarsson EM, Magnadottir HB, Sveinbjornsdottir S, Olafsson I, Magnusson MK, Palmason R, Haraldsdottir V, Andersen K, Onundarson PT, Thorgeirsson G, Kiemenev LA, Powell JT, Carey DJ, Kuivaniemi H, Lindholt JS, Jones GT, Kong A, Blankensteijn JD, Matthiasson SE, Thorsteinsdottir U, Stefansson K. Genome-wide association study identifies a sequence variant within the DAB2IP gene conferring susceptibility to abdominal aortic aneurysm. *Nat Genet* 2010;**42**:692–697.
- Bown MJ, Jones GT, Harrison SC, Wright BJ, Bumpstead S, Baas AF, Gretarsdottir S, Badger SA, Bradley DT, Burnand K, Child AH, Clough RE, Cockerill G, Hafez H, Scott DJ, Futers S, Johnson A, Sohrabi S, Smith A, Thompson MM, van Bockxmeer FM, Waltham M, Matthiasson SE, Thorleifsson G, Thorsteinsdottir U, Blankensteijn JD, Teijink JA, Wijmenga C, de Graaf J, Kiemenev LA, Assimes TL, McPherson R, Folkersen L, Franco-Cereceda A, Palmieri J, Smith AJ, Sylvius N, Wild JB, Refstrup M, Edkins S, Gwilliam R, Hunt SE, Potter S, Lindholt JS, Frikke-Schmidt R, Tybjaerg-Hansen A, Hughes AE, Gollodje J, Norman PE, van Rij A, Powell JT, Eriksson P, Stefansson K, Thompson JR, Humphries SE, Sayers RD, Deloukas P, Samani NJ. Abdominal aortic aneurysm is associated with a variant in low-density lipoprotein receptor-related protein 1. *Am J Hum Genet* 2011;**89**:619–627.
- Helgadottir A, Thorleifsson G, Magnusson KP, Gretarsdottir S, Steinthorsdottir V, Manolescu A, Jones GT, Rinkel GJE, Blankensteijn JD, Ronkainen A, Jaaskelainen JE, Kyo Y, Lenk GM, Sakalihan N, Kostulas K, Gottsater A, Flex A, Stefansson H, Hansen T, Andersen G, Weinsheimer S, Borch-Johnsen K, Jorgensen T, Shah SH, Quyyumi AA, Granger CB, Reilly MP, Austin H, Levey AI, Vaccarino V, Palsdottir E, Walters GB, Jonsson T, Snorraddottir S, Magnusdottir D, Gudmundsson G, Ferrell RE, Sveinbjornsdottir S, Hernesniemi J, Niemela M, Limet R, Andersen K, Sigurdsson G, Benediktsson R, Verhoeven ELG, Teijink JAW, Grobbee DE, Rader DJ, Collier DA, Pedersen O, Pola R, Hillert J, Lindblad B, Valdimarsson EM, Magnadottir HB, Wijmenga C, Tromp G, Baas AF, Ruigrok YM, van Rij AM, Kuivaniemi H, Powell JT, Matthiasson SE, Gulcher JR, Thorgeirsson G, Kong A, Thorsteinsdottir U, Stefansson K. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. *Nat Genet* 2008;**40**:217–224.
- Lindeman JH, Abdul-Hussien H, Schaapherder AF, Van Bockel JH, Von der Thusen JH, Roelen DL, Kleemann R. Enhanced expression and activation of pro-inflammatory transcription factors distinguish aneurysmal from atherosclerotic aorta: IL-6- and IL-8-dominated inflammatory responses prevail in the human aneurysm. *Clin Sci (Lond)* 2008;**114**:687–697.
- Liao M, Xu J, Clair AJ, Ehrman B, Graham LM, Eagleton MJ. Local and systemic alterations in signal transducers and activators of transcription (STAT) associated with human abdominal aortic aneurysms. *J Surg Res* 2011;**176**:321–8.
- Galicia JC, Tai H, Komatsu Y, Shimada Y, Akazawa K, Yoshie H. Polymorphisms in the IL-6 receptor (IL-6R) gene: strong evidence that serum levels of soluble IL-6R are genetically influenced. *Genes Immun* 2004;**5**:513–516.
- Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, Peden JF, Erdmann J, Braund P, Engert JC, Bennett D, Coin L, Ashby D, Tzoulaki I, Brown JI, Mt-Isa S, McCarthy MI, Peltonen L, Freimer NB, Farrall M, Ruokonen A, Hamsten A, Lim N, Froguel P, Waterworth DM, Vollenweider P, Waeber G, Jarvelin MR, Mooser V, Scott J, Hall AS, Schunkert H, Anand SS, Collins R, Samani NJ, Watkins H, Kooner JS. Genetic loci associated with C-reactive protein levels and risk of coronary heart disease. *JAMA* 2009;**302**:37–48.
- Danik JS, Pare G, Chasman DI, Zee RY, Kwiatkowski DJ, Parker A, Miletich JP, Ridker PM. Novel loci, including those related to Crohn disease, psoriasis, and inflammation, identified in a genome-wide association study of fibrinogen in 17 686 women: the Women's Genome Health Study. *Circ Cardiovasc Genet* 2009;**2**:134–141.
- Reich D, Patterson N, Ramesh V, De Jager PL, McDonald GJ, Tandon A, Choy E, Hu D, Tamraz B, Pawlikowska L, Wassel-Fyr C, Huntsman S, Waliszewska A, Rossin E, Li R, Garcia M, Reiner A, Ferrell R, Cummings S, Kwok PY, Harris T, Zmuda JM, Ziv E. Admixture mapping of an allele affecting interleukin 6 soluble receptor and interleukin 6 levels. *Am J Hum Genet* 2007;**80**:716–726.
- Boekholdt SM, Stroes ES. The interleukin-6 pathway and atherosclerosis. *Lancet* 2012;**379**:1176–1178.
- Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;**6**:e1000097.
- Fowkes FG, Housley E, Riemersma RA, Macintyre CC, Cawood EH, Prescott RJ, Ruckley CV. Smoking, lipids, glucose intolerance, and blood pressure as risk factors for peripheral atherosclerosis compared with ischemic heart disease in the Edinburgh Artery Study. *Am J Epidemiol* 1992;**135**:331–340.
- Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, Kuipers EJ, Nijsten TE, Stricker BH, Tiemeier H, Uitterlinden AG, Vernooij MW, Witteman JC. The Rotterdam Study: 2012 objectives and design update. *Eur J Epidemiol* 2011;**26**:657–686.
- Dauer DJ, Ferraro B, Song L, Yu B, Mora L, Buettner R, Enkemann S, Jove R, Haura EB. Stat3 regulates genes common to both wound healing and cancer. *Oncogene* 2005;**24**:3397–3408.
- Dawson J, Cockerill GW, Choke E, Belli AM, Loftus I, Thompson MM. Aortic aneurysms secrete interleukin-6 into the circulation. *J Vasc Surg* 2007;**45**:350–356.
- Flondell-Site D, Lindblad B, Kolbel T, Gottsater A. Cytokines and systemic biomarkers are related to the size of abdominal aortic aneurysms. *Cytokine* 2009;**46**:211–215.
- Fowkes FGR, Anandan CLC, Lee AJ, Smith FB, Tzoulaki I, Rumley A, Powell JT, Lowe GDO. Reduced lung function in patients with abdominal aortic aneurysm is associated with activation of inflammation and hemostasis, not smoking or cardiovascular disease. *J Vasc Surg* 2006;**43**:474–480.
- Treska V, Topolcan O, Pecan L. Cytokines as plasma markers of abdominal aortic aneurysm. *Clin Chem Lab Med* 2000;**38**:1161–1164.
- Wallinder J, Bergqvist D, Henriksson AE. Proinflammatory and anti-inflammatory cytokine balance in patients with abdominal aortic aneurysm and the impact of aneurysm size. *Vasc Endovascular Surg* 2009;**43**:258–261.
- Parry DJ, Al-Barjas HS, Chappell L, Rashid ST, Ariens RAS, Scott DJA. Markers of inflammation in men with small abdominal aortic aneurysm. *J Vasc Surg* 2010;**52**:145–151.
- Juvonen J, Surcel HM, Satta J, Teppo AM, Bloigu A, Syrjala H, Airaksinen J, Leinonen M, Saikku P, Juvonen T. Elevated circulating levels of inflammatory cytokines in patients with abdominal aortic aneurysm. *Arterioscler Thromb Vasc Biol* 1997;**17**:2843–2847.
- Oszkinis G, Kaminski J, Gabriel M, Pukacki F, Brzezinski J, Snoch M, Zielinski M, Stanisz M, Kuznar-Kaminska B, Majewski W. Value of proinflammatory cytokines determination and acute phase protein quantity alterations among patients with abdominal aortic aneurysm. <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed8&NEWS=N&AN=2008250644>.
- Jones KG, Brull DJ, Brown LC, Sian M, Greenhalgh RM, Humphries SE, Powell JT. Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. *Circulation* 2001;**103**:2260–2265.

30. Hingorani AD, Casas JP. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet* 2012;**379**: 1214–1224.
31. Bown MJ, Jones GT, Harrison SC, Wright BJ, Bumpstead S, Baas AF, Gretarsdottir S, Badger SA, Bradley DT, Burnand K, Child AH, Clough RE, Cockerill G, Hafez H, Julian ASD, Futers S, Johnson A, Sohrabi S, Smith A, Thompson MM, van Bockxmeer FM, Waltham M, Matthiasson SE, Thorleifsson G, Thorsteinsdottir U, Blankensteijn JD, Teijink JA, Wijmenga C, de Graaf J, Kiemeny LA, Assimes TL, McPherson R, Folkersen L, Franco-Cereceda A, Palmen J, Smith AJ, Sylvius N, Wild JB, Refstrup M, Edkins S, Gwilliam R, Hunt SE, Potter S, Lindholt JS, Frikke-Schmidt R, Tybjaerg-Hansen A, Hughes AE, Golledge J, Norman PE, van Rij A, Powell JT, Eriksson P, Stefansson K, Thompson JR, Humphries SE, Sayers RD, Deloukas P, Samani NJ. Abdominal aortic aneurysm is associated with a variant in low-density lipoprotein receptor-related protein 1. *Am J Hum Genet* 2011;**89**:619–27.
32. Protogerou AD, Zampeli E, Fragiadaki K, Stamatielopoulou K, Papamichael C, Sfikakis PP. A pilot study of endothelial dysfunction and aortic stiffness after interleukin-6 receptor inhibition in rheumatoid arthritis. *Atherosclerosis* 2011;**219**:734–736.
33. Kume K, Amano K, Yamada S, Hatta K, Ohta H, Kuwaba N. Tocilizumab monotherapy reduces arterial stiffness as effectively as etanercept or adalimumab monotherapy in rheumatoid arthritis: an open-label randomized controlled trial. *J Rheumatol* 2011;**38**:2169–2171.
34. Murakami M, Nishimoto N. The value of blocking IL-6 outside of rheumatoid arthritis: current perspective. *Curr Opin Rheumatol* 2011;**23**:273–277.
35. Yoshimura K, Aoki H, Ikeda Y, Fujii K, Akiyama N, Furutani A, Hoshii Y, Tanaka N, Ricci R, Ishihara T, Esato K, Hamano K, Matsuzaki M. Regression of abdominal aortic aneurysm by inhibition of c-Jun N-terminal kinase. *Nat Med* 2005;**11**: 1330–1338.
36. Kanbe K, Chen Q, Nakamura A, Hobo K. Inhibition of MAP kinase in synovium by treatment with tocilizumab in rheumatoid arthritis. *Clin Rheumatol* 2011;**30**: 1407–1413.
37. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, Simes R. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005;**366**:1267–1278.
38. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, Pirruccello JP, Ripatti S, Chasman DI, Willer CJ, Johansen CT, Fouchier SW, Isaacs A, Peloso GM, Barbalic M, Ricketts SL, Bis JC, Aulchenko YS, Thorleifsson G, Feitosa MF, Chambers J, Orho-Melanders M, Melander O, Johnson T, Li X, Guo X, Li M, Shin Cho Y, Jin Go M, Jin Kim Y, Lee JY, Park T, Kim K, Sim X, Twee-Hee Ong R, Croteau-Chonka DC, Lange LA, Smith JD, Song K, Hua Zhao J, Yuan X, Luan J, Lamina C, Ziegler A, Zhang W, Zee RY, Wright AF, Witteman JC, Wilson JF, Willemsen G, Wichmann HE, Whitfield JB, Waterworth DM, Wareham NJ, Waeber G, Vollenweider P, Voight BF, Vitart V, Uitterlinden AG, Uda M, Tuomilehto J, Thompson JR, Tanaka T, Surakka I, Stringham HM, Spector TD, Soranzo N, Smit JH, Sinisalo J, Silander K, Sijbrands EJ, Scuteri A, Scott J, Schlessinger D, Sanna S, Salomaa V, Saharinen J, Sabatti C, Ruukonen A, Rudan I, Rose LM, Roberts R, Rieder M, Psaty BM, Pramstaller PP, Pichler I, Perola M, Penninx BW, Pedersen NL, Pattaro C, Parker AN, Pare G, Oostra BA, O'Donnell CJ, Nieminen MS, Nickerson DA, Montgomery GW, Meisinger T, McPherson R, McCarthy MI, McArdle W, Masson D, Martin NG, Marroni F, Mangino M, Magnusson PK, Lucas G, Luben R, Loos RJ, Lokki ML, Lettre G, Langenberg C, Launer LJ, Lakatta EG, Laaksonen R, Kyvik KO, Kronenberg F, Konig IR, Khaw KT, Kaprio J, Kaplan LM, Johansson A, Jarvelin MR, Janssens AC, Ingelsson E, Igl W, Kees Hovingh G, Hottenga JJ, Hofman A, Hicks AA, Hengstenberg C, Heid IM, Hayward C, Havulinna AS, Hastie ND, Harris TB, Haritunians T, Hall AS, Gyllenstein U, Guiducci C, Groop LC, Gonzalez E, Gieger C, Freimer NB, Ferrucci L, Erdmann J, Elliott P, Ejebe KG, Doring A, Dominiczak AF, Demissie S, Deloukas P, de Geus EJ, de Faire U, Crawford G, Collins FS, Chen YD, Caulfield MJ, Campbell H, Burt NP, Bonnycastle LL, Boomsma DI, Boekholdt SM, Bergman RN, Barroso I, Bandinelli S, Ballantyne CM, Assimes TL, Quertermous T, Altshuler D, Seielstad M, Wong TY, Tai ES, Feranil AB, Szara CW, Adair LS, Taylor HA Jr, Borecki IB, Gabriel SB, Wilson JG, Holm H, Thorsteinsdottir U, Gudnason V, Krauss RM, Mohlke KL, Ordovas JM, Munroe PB, Kooner JS, Tall AR, Hegele RA, Kastelein JJ, Schadt EE, Rotter JJ, Boerwinkle E, Strachan DP, Mooser V, Stefansson K, Reilly MP, Samani NJ, Schunkert H, Cupples LA, Sandhu MS, Ridker KM, Rader DJ, van Duijn CM, Peltonen L, Abecasis GR, Boehnke M, Kathiresan S. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 2010;**466**:707–713.
39. Sarwar N, Butterworth AS, Freitag DF, Gregson J, Willeit P, Gorman DN, Gao P, Saleheen D, Rendon A, Nelson CP, Braund PS, Hall AS, Chasman DI, Tybjaerg-Hansen A, Chambers JC, Benjamin EJ, Franks PW, Clarke R, Wilde AA, Trip MD, Steri M, Witteman JC, Qi L, van der Schoot CE, de Faire U, Erdmann J, Stringham HM, Koenig W, Rader DJ, Melzer D, Reich D, Psaty BM, Kleber ME, Panagiotakos DB, Willeit J, Wennberg P, Woodward M, Adamovic S, Rimm EB, Meade TW, Gillum RF, Shaffer JA, Hofman A, Onat A, Sundstrom J, Wassertheil-Smoller S, Mellstrom D, Gallacher J, Cushman M, Tracy RP, Kahonen J, Karlsson M, Salonen JT, Wilhelmsen L, Amouyel P, Cantin B, Best LG, Ben-Shlomo Y, Manson JE, Davey-Smith G, de Bakker PI, O'Donnell CJ, Wilson JF, Wilson AG, Assimes TL, Jansson JO, Ohlsson C, Tivesten A, Ljunggren O, Reilly MP, Hamsten A, Ingelsson E, Cambien F, Hung J, Thomas GN, Boehnke M, Schunkert H, Asselbergs FW, Kastelein JJ, Gudnason V, Salomaa V, Harris TB, Kooner JS, Allin KH, Nordestgaard BG, Hopewell JC, Goodall AH, Ridker PM, Holm H, Watkins H, Ouwehand WH, Samani NJ, Kaptoge S, Di Angelantonio E, Harari O, Danesh J. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet* 2012;**379**:1205–1213.
40. Freiberg MS, Arnold AM, Newman AB, Edwards MS, Kraemer KL, Kuller LH. Abdominal aortic aneurysms, increasing infrarenal aortic diameter, and risk of total mortality and incident cardiovascular disease events: 10-year follow-up data from the Cardiovascular Health Study. *Circulation* 2008;**117**:1010–1017.