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Journal: *European Heart Journal*
Article Doi: 10.1093/eurheartj/ehab107
Article Title: Genetically determined NLRP3 inflammasome activation associates with systemic inflammation and cardiovascular mortality
First Author: Stefan J. Schunk
Corr. Author: Thimoteus Speer

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European Heart Journal (2021) **00**, 1–15
doi:10.1093/eurheartj/ehab107

TRANSLATIONAL RESEARCH

Genetically determined NLRP3 inflammasome activation associates with systemic inflammation and cardiovascular mortality

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Received 21 September 2020; revised 19 December 2020; editorial decision 9 February 2021; accepted 9 February 2021

Aims Inflammation plays an important role in cardiovascular disease (CVD) development. The NOD-like receptor protein-3 (NLRP3) inflammasome contributes to the development of atherosclerosis in animal models. Components of the NLRP3 inflammasome pathway such as interleukin-1β can therapeutically be targeted. Associations of genetically determined inflammasome-mediated systemic inflammation with CVD and mortality in humans are unknown.

Methods We explored the association of genetic *NLRP3* variants with prevalent CVD and cardiovascular mortality in 538 167 subjects on the individual participant level in an explorative gene-centric approach without performing multiple testing. Functional relevance of single-nucleotide polymorphisms on NLRP3 inflammasome activation has been evaluated in monocyte-enriched peripheral blood mononuclear cells (PBMCs).

Results Genetic analyses identified the highly prevalent (MAF 39.9%) intronic *NLRP3* variant rs10754555 to affect *NLRP3* gene expression. rs10754555 carriers showed significantly higher C-reactive protein and serum amyloid A plasma levels. Carriers of the G allele showed higher NLRP3 inflammasome activation in isolated human PBMCs. In carriers of the rs10754555 variant, the prevalence of coronary artery disease was significantly higher as compared to non-carriers with a significant interaction between rs10754555 and age. Importantly, rs10754555 carriers had significantly higher risk for cardiovascular mortality during follow-up. Inflammasome inducers (e.g. urate, triglycerides, apolipoprotein C3) modulated the association between rs10754555 and mortality.

Conclusion The *NLRP3* intronic variant rs10754555 is associated with increased systemic inflammation, inflammasome activation, prevalent coronary artery disease, and mortality. This study provides evidence for a substantial role of genetically driven systemic inflammation in CVD and highlights the NLRP3 inflammasome as a therapeutic target.

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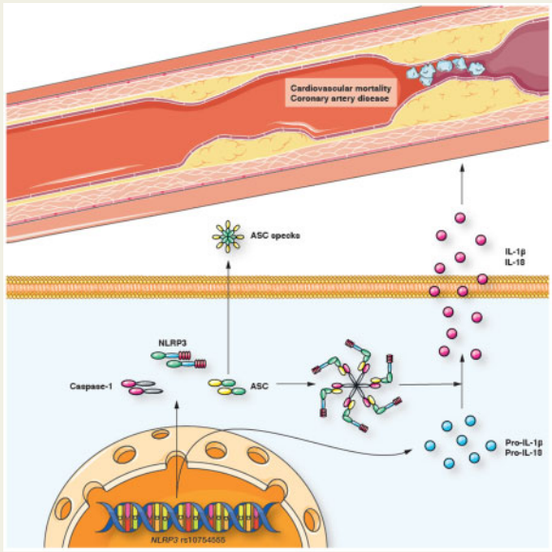
Graphical Abstract



Keywords Cardiovascular diseases • Coronary artery disease • Inflammation • Inflammasome • NLRP3

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Introduction

Vascular inflammation is important in the initiation and progression of atherosclerotic vascular diseases.[1] Inflammatory markers such as high-sensitivity C-reactive protein (hsCRP) and serum amyloid A (SAA) are associated with increased mortality in patients with manifest cardiovascular disease (CVD)[2] and healthy subjects with elevated inflammatory markers are at increased risk for the development of CVD.[3,4] Inflammation in patients with CVD is characterized by the activation of monocytes, which adhere to the endothelium and migrate into the sub-endothelial layer, where they are activated by endogenous mediators such as modified lipoproteins triggering an innate immune response.[1,5] These monocytes differentiate into tissue macrophages, acquire lipids and lipoproteins, and transform into foam cells contributing to atherosclerotic plaque formation.[6,7]

Interleukin (IL)-1β represents one of the key cytokines released by activated monocytes and macrophages leading to vascular (micro)inflammation.[1] The processing of pro-IL-1β into mature IL-1β is tightly regulated by a multimeric intracellular protein complex, the NOD-like receptor protein 3 (NLRP3) inflammasome.[8] In addition to exogenous triggers, the NLRP3 inflammasome is activated by a variety of endogenous mediators such as urate, cholesterol crystals, and oxidized low-density lipoprotein.[9] Moreover, we recently observed that lipoproteins such as the triglyceride-associated apolipoprotein C3 (ApoC3) directly mediate alternative NLRP3 activation in human monocytes leading to vascular injury *in vivo*.[10] The CANTOS trial demonstrated that inhibition of IL-1β, the effector cytokine of the NLRP3 inflammasome, with the monoclonal antibody canakinumab reduced recurrent cardiovascular (CV) events in patients with previous myocardial infarction (MI) and elevated hsCRP >2 mg/L on the top of maximally tolerated statin therapy.[11] Recently, the COLCOT trial reported that colchicine, an anti-inflammatory agent for the treatment of conditions such as gout, reduced a composite CV endpoint after MI by 23%.[12] Importantly, modulating NLRP3 inflammasome activity represents one mechanism by which colchicine reduces inflammation.[12]

Despite growing experimental evidence for the NLRP3 inflammasome being a key driver of CVD and increased understanding of its molecular regulation, the clinical relevance of inflammasome activation in patients at risk for or with prevalent CVD is incompletely understood. In the present study, we assessed the association of a gene variant affecting *NLRP3* gene expression and function with the prevalence of coronary artery disease (CAD) and CV mortality in 538 167 subjects.

Methods

Detailed description of the methods can be found in the Supplementary material online.

Genetic association validation studies

The association between single-nucleotide polymorphisms (SNPs) and all-cause as well as CV mortality was studied by genotype or in an additive genetic model. Since the current study is a gene-centric and not a

genome-wide association study (GWAS), it did not require genome-wide significance. Due to the explorative nature of the study, we did not account for the issue of multiple testing and thus report unadjusted *P*-values. Findings were validated in participants of 10 studies comprising 526 091 participants. Study details are described in the Supplementary material online.

Statistical analyses

Continuous variables are presented as mean ± standard deviation or mean ± 95% confidence intervals (CIs) for normally distributed variables, or as median and interquartile range for variables with skewed distributions. Categorical variables are presented as frequencies. Differences between continuous variables were assessed using one-way ANOVA or Kruskal–Wallis test where appropriate. Differences between categorical variables were determined using the χ^2 test. Generalized linear models were used to estimate age- and sex-adjusted marginal means of hsCRP or SAA according to rs10754555 SNP carrier status. In LURIC and GerMIFS, the association between rs10754555 genotype, CAD and severe CAD (only in LURIC) as well as mortality was assessed by logistic and Cox regression analyses. Severe CAD was defined as angiographically visualized ≥50% stenosis. To study the effect of age, an interaction term between rs10754555 and age was added to the respective models. Moreover, patients were divided into two groups at the age of 60 years corresponding to the first tertile of age in LURIC. Univariate and multivariable analyses were performed with adjustment for age, sex, diabetes mellitus, systolic blood pressure, body mass index, smoking status, estimated glomerular filtration rate, low-density lipoprotein cholesterol, hsCRP, presence of CAD, and previous MI. In the experimental studies, one-way ANOVA followed by Dunnett's *post hoc* tests were used to assess significant differences across rs10754555 genotype. Genotype distributions were tested for Hardy–Weinberg equilibrium using exact tests (https://ihg.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Meta-analysis on the association between rs10754555 SNP carrier status and CV mortality was performed by using hazard ratios (HRs) and standard errors derived from multivariable adjusted Cox regression models at individual participant level provided by each study. Standard normal random-effects weighted meta-analysis was performed using the STATA package 'metan'. Between-study heterogeneity I^2 was determined as described previously.[13] Small-study effects were excluded by using the Egger test provided within the STATA package 'metabias'. To study the effect of ApoC3, triglycerides, and urate, an interaction term with rs10754555 was introduced in the respective models. ApoC3, triglycerides, and urate were divided into two categories (Quartiles 1–3 vs. Quartile 4). All other analyses were performed using SPSS version 25 and R version 3.3.3. The significance level was set at 0.05.

Results

NLRP3 genetic variants

We used GWAS data from the LURIC study comprising 3061 patients referred for coronary angiography as cohort for SNP preselection. Prioritization of an SNP with effects on the expression of NLRP3 is shown in *Figure 1A* and identified rs10754555 as significant eQTL in the 'Blood eQTL browser' ($P = 2.32 \times 10^{-6}$) and the 'GTEX database' ($P = 9.80 \times 10^{-10}$, Supplementary material online, *Tables S1 and S2*). To validate rs10754555 as an eQTL of *NLRP3*, the association between rs10754555 and *NLRP3* mRNA expression in whole blood and peripheral blood mononuclear cells (PBMCs) was assessed in 36 cohorts comprising 31 556 samples included in the eQTLGen consortium[14] (*Figure 1B*). In these analyses, rs10754555 qualified as a significant eQTL of *NLRP3* (*Z*-score: 11.03, false discovery rate <0.05, $P = 2.73 \times 10^{-28}$). The allele and genotype frequencies of rs10754555 are consistent with Hardy–Weinberg equilibrium as shown in Supplementary material online, *Table S3*. Data from the Roadmap Epigenomics project indicate that rs10754555 maps with promoter and enhancer histone marks and DNase hypersensitivity (Supplementary material online, *Figure S1*). Importantly, heterozygous and homozygous rs10754555 carriers showed significantly higher levels of hsCRP (*Figure 1C*) and SAA (*Figure 1D*) as compared to non-carriers indicating that this variant is associated with a systemic pro-inflammatory state. AQ11

Biological relevance of rs10754555

The biological relevance of the rs10754555 variant was tested in monocyte-enriched PBMCs (*Figure 2A*, Supplementary material online, *Table S4*, and Supplementary material online, *Figure S2A*), which revealed higher *NLRP3* mRNA expression in heterozygous and homozygous carriers of the G allele as compared to PBMCs from non-carriers (*Figure 2B*). Importantly, the plasma levels of IL-18 and IL-1β as NLRP3-dependent cytokines were also significantly higher in G allele carriers (*Figure 2C and D*). To directly assess NLRP3 inflammasome activation according to the rs10754555 variant carrier status, we quantified ASC specks in plasma. Notably, the rs10754555 G allele was associated with plasma ASC specks (*Figure 2E–G*). These findings confirm that carriers of the rs10754555 *NLRP3* G allele are characterized by greater inflammasome activation.

To corroborate these results, activation of the NLRP3 inflammasome was modelled by stimulating the isolated PBMCs with known inflammasome activators [i.e. lipopolysaccharide (LPS), ATP, and nigericin] and measuring the release of IL-1β into the cell culture supernatant. Upon stimulation with LPS, LPS + ATP, and LPS + nigericin, PBMCs from heterozygous and homozygous *NLRP3* rs10754555 G allele carriers released significantly more IL-1β compared to cells from non-carriers (*Figure 3A–C*). Unstimulated monocytes did not release detectable concentrations of IL-1β. To determine the specificity of these findings, release of IL-6 and tumour necrosis factor (TNF) into cell culture supernatants was quantified (Supplementary material online, *Figure S2B–G*), which did not differ according to rs10754555 variant carrier status. To prove the relevance of rs10754555 *in vivo*, we transplanted NOD-SCID mice with human PBMCs from non-carriers and homozygous rs10754555 carriers and subjected them to perivascular carotid injury, a mouse model for re-endothelialization, which we have recently shown to be NLRP3 dependent[10] (*Figure 3D and E*). Re-endothelialization was significantly impaired in humanized mice receiving PBMCs from homozygous rs10754555 carriers, in which NLRP3 protein expression was higher as compared to non-carriers (*Figure 3F*).

Association between rs10754555 and the risk of coronary artery disease

Supplementary material online, *Tables S5 and S6* summarize the baseline characteristics of participants of the LURIC study population separated by rs10754555 genotype as well divided at age of 60 years. Minor allele frequency (G) for rs10754555 was 39.9%. The prevalence of traditional CV risk factors such as age, sex, body mass index,

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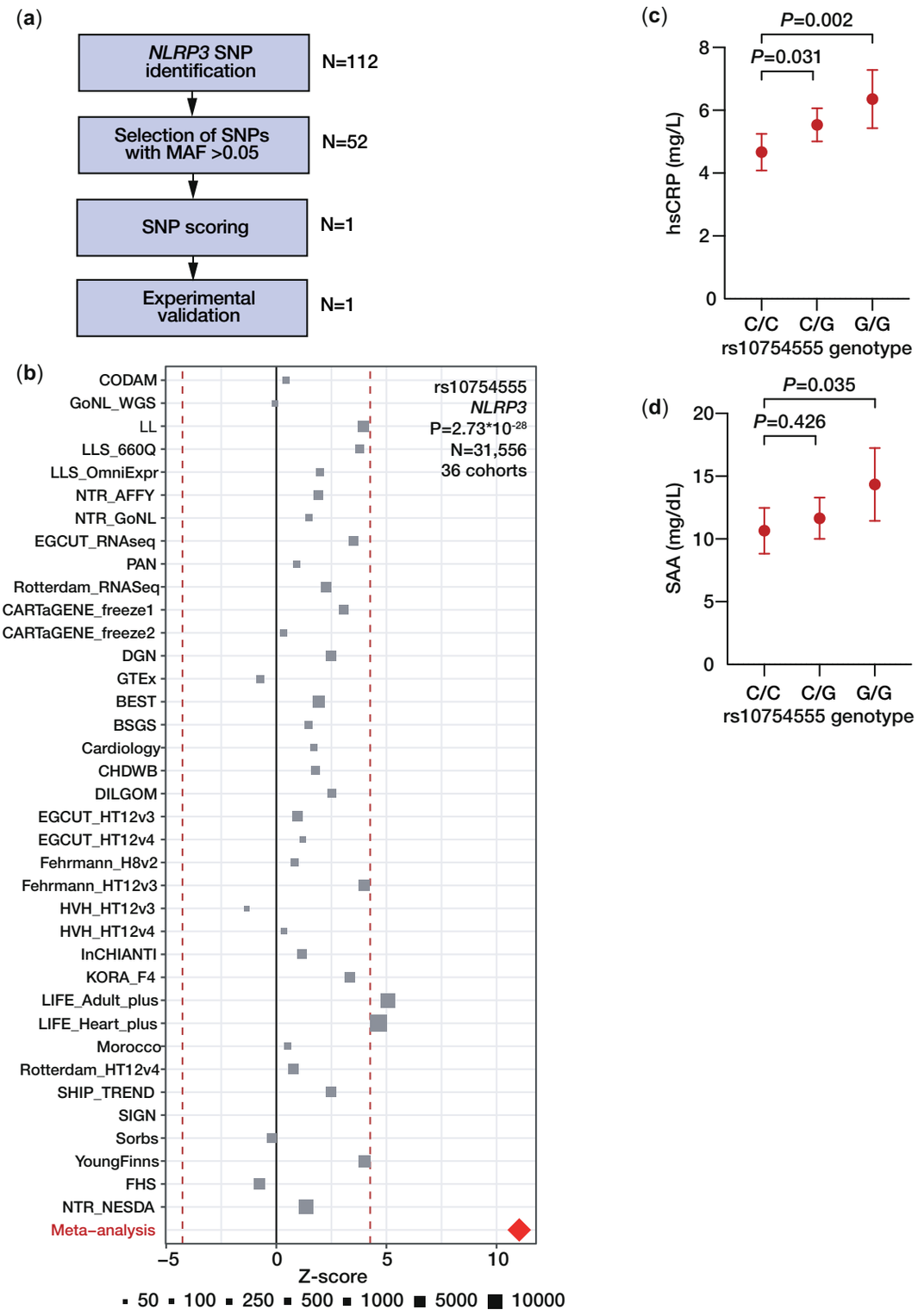

Figure 1 Identification of single-nucleotide polymorphisms regulating *NLRP3* expression. (*A*) Variant prioritization approach. (*B*) Expression quantitative trait locus meta-analysis for rs10754555 in whole blood or peripheral blood mononuclear cells in the eQTLGen consortium comprising 31 556 samples from 36 cohorts. (*C*) Age and sex-adjusted least square means of high-sensitivity C-reactive protein and (*D*) serum amyloid A in 3061 participants of the LURIC study (mean ± 95% confidence interval).

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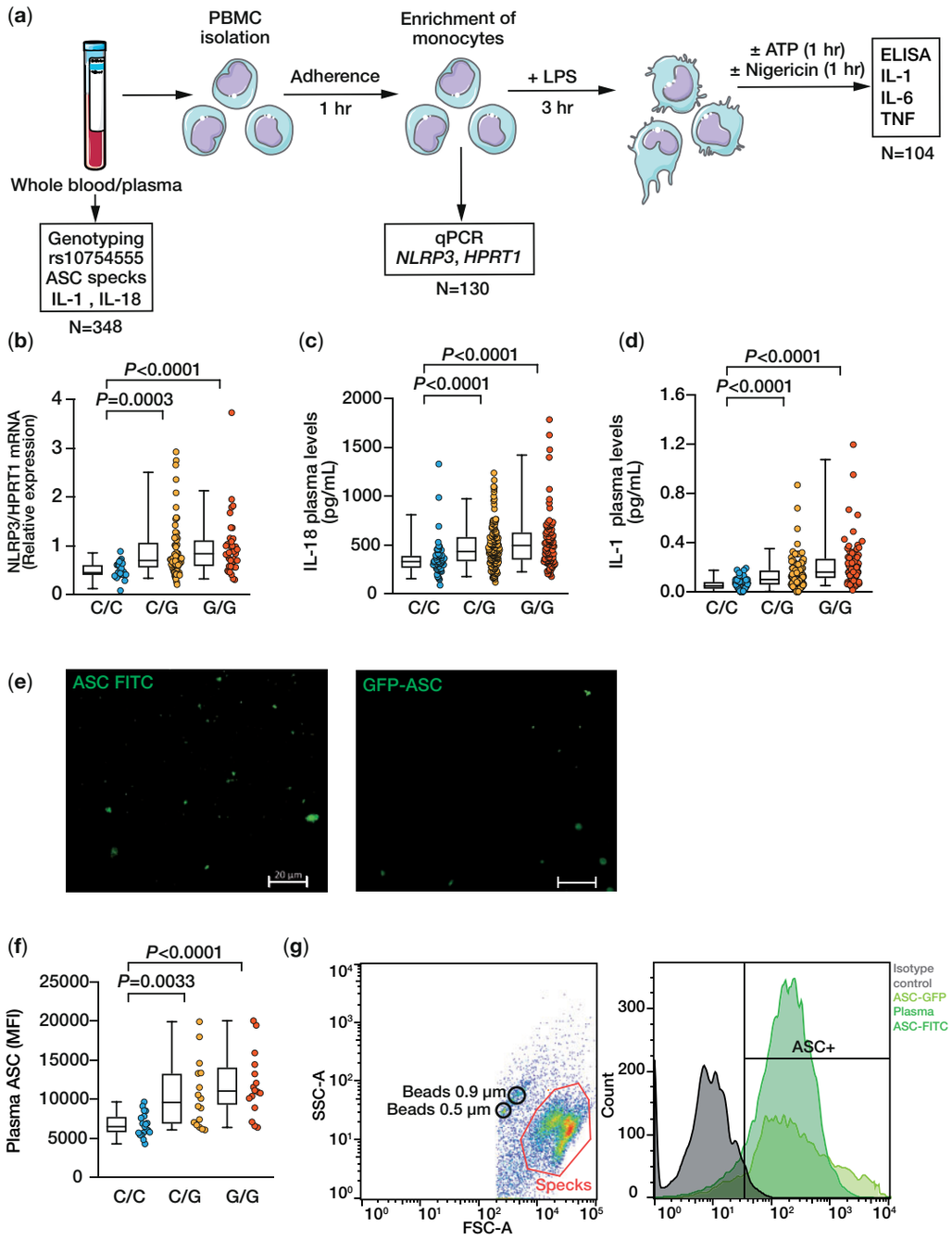

Figure 2 Functional effects of rs10754555 on expression of *NLRP3* and inflammasome activation in freshly isolated human peripheral blood mononuclear cells. (*A*) Experimental work-flow. (*B*) mRNA expression of *NLRP3* in freshly isolated peripheral blood mononuclear cells. (*C*) Plasma levels of interleukin-18 and (*D*) and interleukin-1β according to rs10754555 genotype. (*E*) Representative fluorescence microscopy of Alexa Fluor-488-labeled ASC specks from plasma and GFP-ASC in the supernatant of THP-1 cells (representative of three independent experiments). (*F*) Mean fluorescence intensity of ASC specks in plasma samples according to rs10754555 genotype. (*G*) Representative flow cytometry images of ASC speck quantification in plasma. Each dot represents an individual patient, and whiskers of the box plots represent 5 and 95 percentiles. LPS, lipopolysaccharide; qPCR, quantitative polymerase chain reaction; TNF, tumour necrosis factor.

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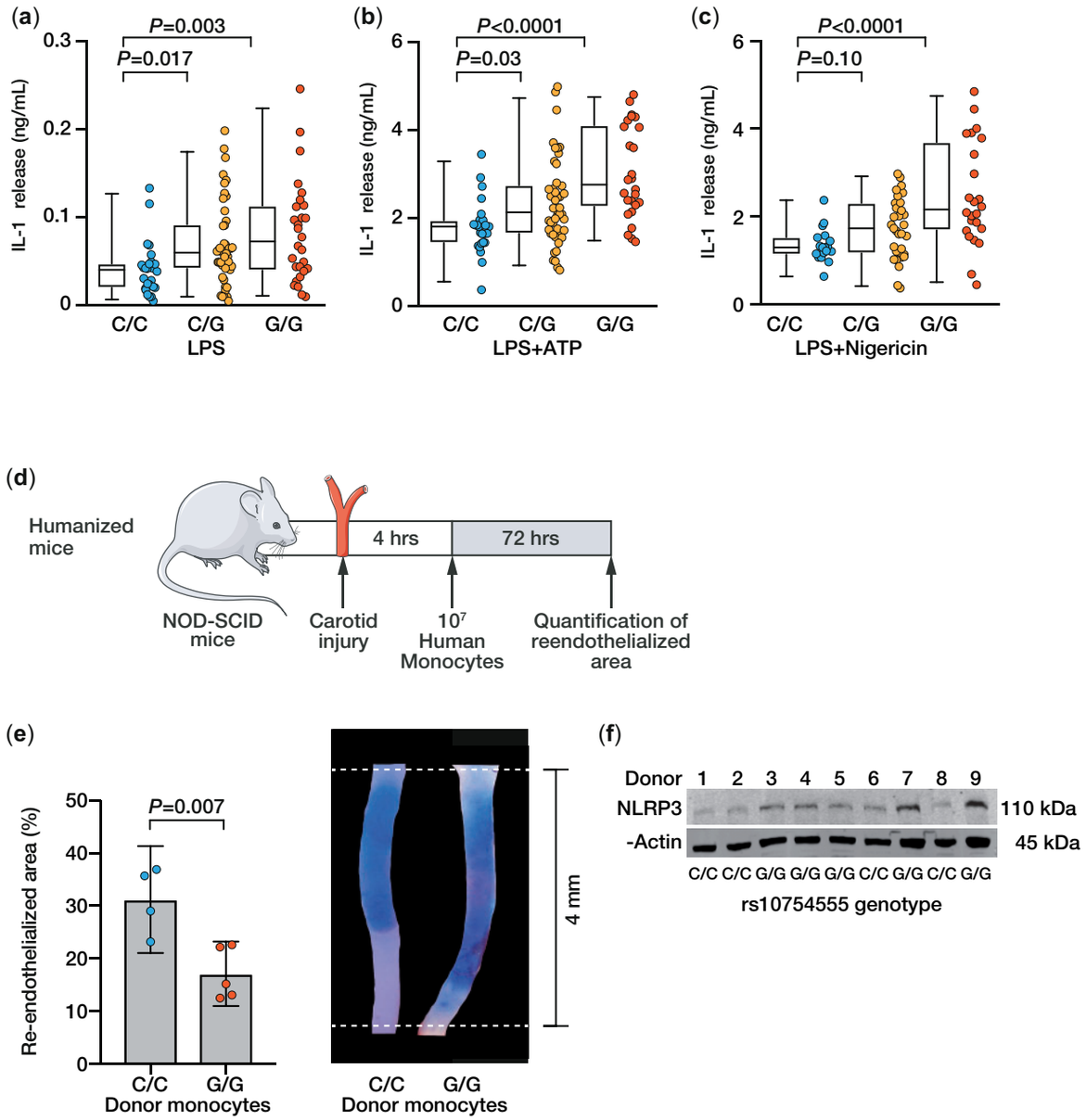

Figure 3 Modulation of NLRP3 inflammasome response by rs10754555 in freshly isolated human peripheral blood mononuclear cells and humanized mice. (*A–C*) Concentration of interleukin-1β in the supernatant of freshly isolated peripheral blood mononuclear cells stimulated with lipopolysaccharide (10 ng/mL, 3 h), lipopolysaccharide (3 h) and ATP (5 mM, 1 h), lipopolysaccharide (3 h) and nigericin (1 μM, 1 h). Each dot represents an individual patient, and whiskers of the box plots represent 5 and 95 percentiles. (*D*) Experimental outline of the murine perivascular carotid injury model in NOD-SCID mice transplanted with human peripheral blood mononuclear cells (i.e. humanized mice). (*E*) Re-endothelialized area 72 h after carotid injury in humanized mice and representative microphotographs. (*F*) Western blot of NLRP3 protein expression in transplanted peripheral blood mononuclear cells from nine individual donors. Mean ± 95% confidence interval.

smoking, hypertension, and lipid parameters did not differ between non-carriers and carriers of the rs10754555 *NLRP3* G allele. Moreover, there was no significant difference in the medication across different rs10754555 genotypes (Supplementary material online, *Table S7*). Since there was a trend towards lower prevalence of hypertension and diabetes in homozygous rs10754555 carriers in LURIC, we assessed the association between rs10754555, blood pressure, and presence of hypertension in UKBiobank, which did not differ significantly between the groups (Supplementary material online, *Table S8*), whereas the prevalence of diabetes was higher in rs10754555 G allele carriers. In homozygous rs10754555 G allele carriers, the risk for CAD and severe CAD was significantly higher as

compared to non-carriers (*Figure 4A* and Supplementary material online, *Table S9*). This association was present in participants below 60 years of age [odds ratio (OR) for prevalent CAD: 2.04, 95% CI 1.15–3.61; OR for severe CAD: 2.28, 95% CI 1.29–4.01], but not in those above 60 years (OR for prevalent CAD: 0.83, 95% CI 0.55–1.25; OR for severe CAD: 0.73, 95% CI 0.49–1.03) revealing an age-dependent association of rs10754555 with the development of atherosclerotic CVD. We confirmed these findings in the GerMIFS studies II–VII with individual patient data available. Importantly, also in GerMIFS, rs10754555 was associated with a higher risk for CAD in subjects aged below 60 years (OR 1.12, 95% CI 1.02–1.22, *Figure 4B* and Supplementary material online, *Table S10*).

Association between rs10754555 and CV mortality

In LURIC, all-cause mortality and CV mortality were significantly higher in heterozygous (HR 1.26, 95% CI 1.08–1.45 and 1.22, 95% CI 1.01–1.47) and homozygous (HR 1.31, 95% CI 1.08–1.59 and 1.35, 95% CI 1.07–1.72) rs10754555 variant carriers (Supplementary material online, *Table S11*). There was no association between rs10754555 and other clinical endpoints such as fatal cancer or fatal infection (Supplementary material online, *Table S12*). Interestingly, the percentage of rs10754555 G allele carriers decreased with increasing age (Supplementary material online, *Table S13*). Supplementary material online, *Figure S3* compares the effect of the rs10754555 genotype with other CV risk factors. Furthermore, we assessed the association between rs10754555 genotypes and CV mortality in 10 prospective clinical trials enrolling 526 091 subjects with or without pre-existing CAD. Baseline characteristics for each individual study are shown in Supplementary material online, *Tables S14–S22*. Analyses were performed at an individual participant level. Additive genetic models show that the rs10754555 genotype is associated with significantly higher CV mortality in subjects from secondary prevention studies (HR 1.14, 95% CI 1.07–1.21) and in subjects from primary prevention studies (HR 1.06, 95% CI 1.01–1.11), without significant heterogeneity (I^2 = 22.2%, P = 0.253 for secondary prevention studies and I^2 = 0.0%, P = 0.999 for primary prevention studies, *Figure 5A and B*). Small-study effects were excluded using the Egger test (P = 0.341 for meta-analysis on CV mortality in secondary prevention studies).

Known NLRP3 inflammasome activators and the association between rs10754555 and mortality

Several endogenous NLRP3 inflammasome activators have been identified, of which ApoC3, triglycerides, and urate are of particular importance in CVD. The release of IL-1β from PBMCs stratified according to the rs10754555 genotype was modulated by baseline triglyceride or urate concentrations (Supplementary material online, *Figure S4A–F* and Supplementary material online, *Tables S23 and S24*). Furthermore, PBMCs from heterozygous or homozygous rs10754555 carriers released significantly higher concentrations of IL-1β after stimulation of ApoC3 or monosodium urate (Supplementary material online, *Figure S5*). Therefore, we assessed the association between rs10754555 and CV mortality with respect to ApoC3, triglyceride, or urate plasma levels. In LURIC, rs10754555 was only associated with CV mortality in subjects with high ApoC3 and triglyceride plasma levels (i.e. in the 4th quartile, *Figure 6A and B* and Supplementary material online, *Tables S25 and S26*). This was confirmed in subjects of the UKBiobank and was independent of age and also present in subjects with elevated triglycerides due to SNPs in the *APOC3* gene locus (*Figure 6B* and Supplementary material online, *Tables S27–S30*). Vice versa, in UKBiobank, triglyceride plasma levels were associated with higher CV mortality (HR 1.17, 95% CI 1.08–1.26) in the total population, with the strongest effect in homozygous rs10754555 carriers (HR 1.57, 95% CI 1.30–1.90; Supplementary material online, *Table S31*). Similar results were obtained when participants of LURIC and UKBiobank were dichotomized according to urate plasma levels or carriers of SNPs associated with higher urate (*Figure 6C* and Supplementary material online, *Tables S32–S37*).

Discussion

The main and novel finding of this study is that genetically determined sterile inflammation mediated by a specific cellular pathway (i.e. NLRP3) associates with higher prevalence of CAD and higher CV mortality. These associations are particularly prominent in the younger population, in which the influence of genetic predisposition likely predominates over lifestyle and environmental risk factors for (premature) CVD. Moreover, these findings highlight the NLRP3 inflammasome as a pathophysiologically important pathway and a potential therapeutic target.

Sterile inflammation is a hallmark of patients with atherosclerotic CVD,[1] with experimental data showing a pivotal role of the NLRP3 inflammasome. In *NLRP3*- and *IL-1β*-deficient mice, atherosclerotic lesion formation was markedly reduced.[9,15] Nevertheless, the effect of NLRP3 on atherosclerosis is dependent on the experimental atherosclerosis model, the type of atherogenic diet, and the gender of the mice.[16] NLRP3 inflammasome activation and subsequently enhanced IL-1β production have been linked to maladaptive vascular remodelling after injury and adverse endothelial activation.[17,18] Acceleration of atherosclerosis by clonal haematopoiesis is partially mediated by NLRP3-dependent IL-1β secretion[19,20] and attenuated in subjects with genetic IL-6 signalling deficiency due to missense mutations of the IL-6 receptor.[21]

Our study links genetically driven inflammation with CVD prevalence and outcomes. An intronic variant within the *NLRP3* locus has been identified, which is not associated with other CV risk factors or alterations in lipids, but appears to specifically increase systemic (micro)inflammation. rs10754555 represents an intronic *NLRP3* variant, which is scored as *NLRP3* eQTL by the provided evidence. Moreover, rs10754555 maps with promoter and enhancer histone marks, and with DNAse I-sensitive regions. This indicates that rs10754555 might indeed associate with increased *NLRP3* mRNA transcription. Accordingly, rs10754555 was identified as *NLRP3* eQTL in whole blood and PBMCs in the eQTLGen consortium. Importantly, our experimental studies show that the rs10754555 genotype is associated with higher *NLRP3* mRNA expression, higher IL-18 plasma levels, increased ASC speck formation and inflammasome activation in human monocyte-enriched PBMCs, which represent a major inflammatory effector cell type in

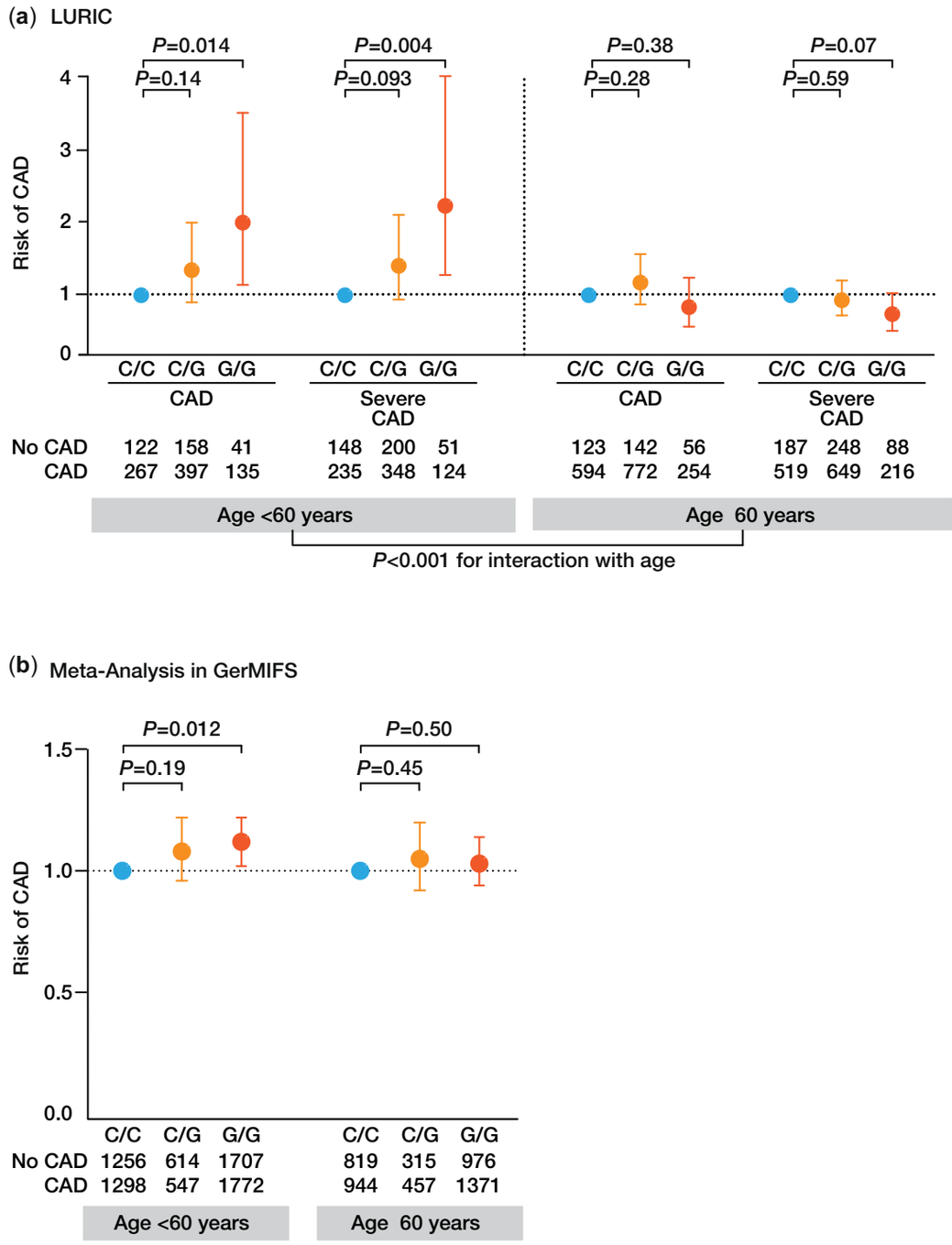

Figure 4 Risk of coronary artery disease as a function of rs10754555 genotype. (*A*) Odds ratios for prevalent coronary artery disease and severe coronary artery disease (visual stenosis ≥50% on coronary angiography) according to rs10754555 genotype in 3061 participants of the LURIC study divided into two groups at age of 60 years (first tertile of age) and (*B*) in a meta-analysis of 12 076 participants with individual patient data available included in GerMIFS. Results are adjusted for age and sex.

blood.[1] Moreover, we have shown that PBMCs from rs10754555 G allele carriers suppressed re-endothelialization in humanized mice. The release of IL-6 and TNF from monocytes treated with known NLRP3 activators was not linked to the rs10754555 carrier status. This indicates that this genetic variant is not associated with unspecific pro-inflammatory cell activation, but specifically with NLRP3 inflammasome activation.rs10754555 was only associated with higher risk for CAD in subjects aged <60 years. This SNP–age

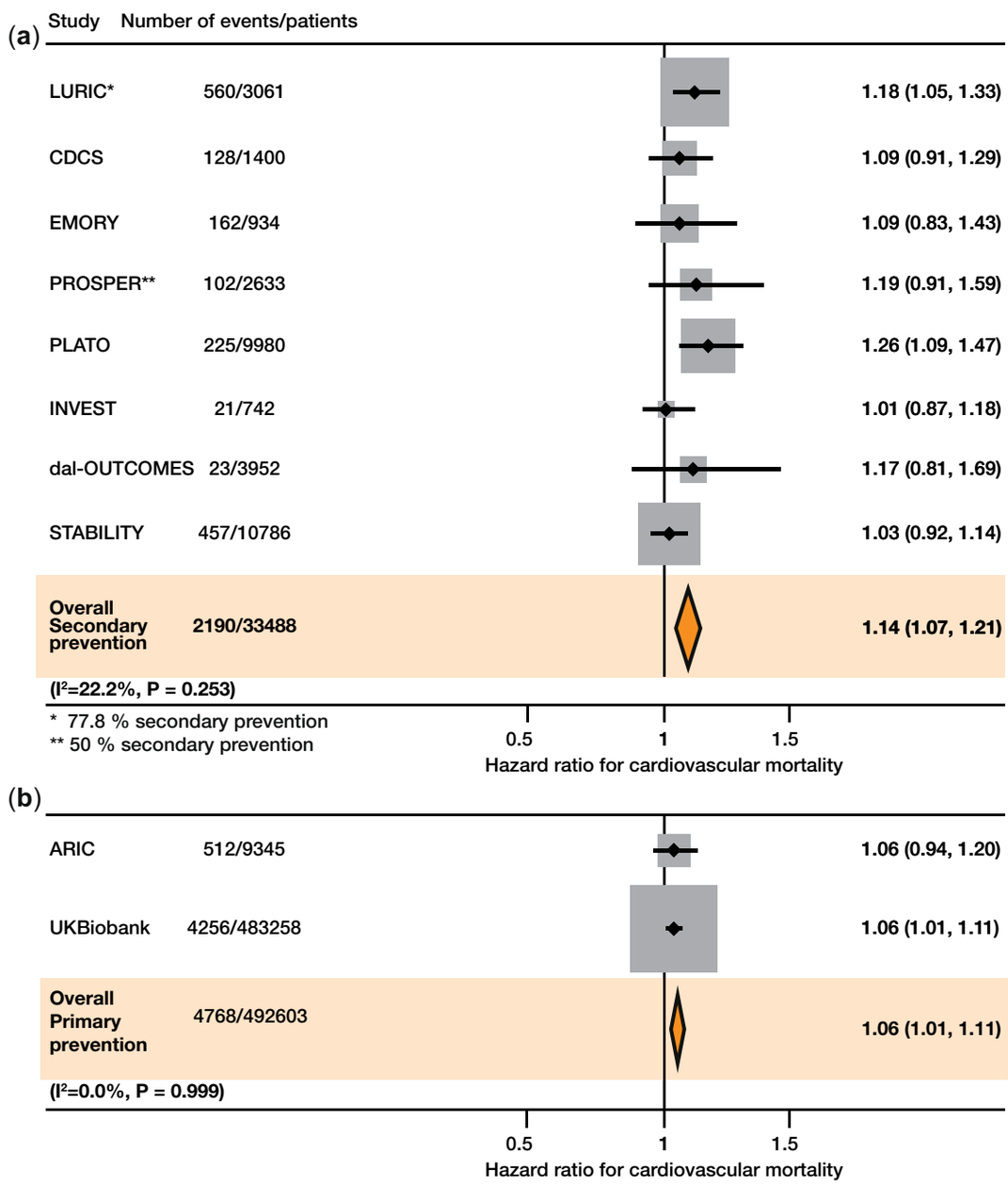

Figure 5 Risk of cardiovascular mortality as a function of rs10754555 genotype. Random-effects meta-analysis on cardiovascular mortality associated with rs10754555 genotype in (*A*) secondary and (*B*) primary prevention cohorts/studies. Shown are the hazard ratios for cardiovascular mortality associated with rs10754555 *NLRP3* variant in 33 488 participants from eight studies comprising patients with prevalent coronary artery disease (i.e. secondary prevention) and in 492 603 participants from two studies from the general population. Analyses from each individual study were adjusted for age and gender.

interaction was confirmed in the GerMIFS studies and by applying the same age cut-off. SNP–environment interactions and in particular SNP–age interactions were reported for CVD-relevant SNPs but also for SNPs in genes involved in inflammation such as *IL1RL1*.[22–25] This observation points to an interaction between age and NLRP3 activation. Although the NLRP3 inflammasome is associated with a functional decline in aging,[26,27] *NLRP3* gene expression and NLRP3 inflammasome activation have been reported to decline with age.[28,29] Moreover, we found that the percentage of heterozygous and homozygous rs10754555 carriers decreased with increasing age, which could explain the lack of association between rs10754555 and CAD in the elderly.

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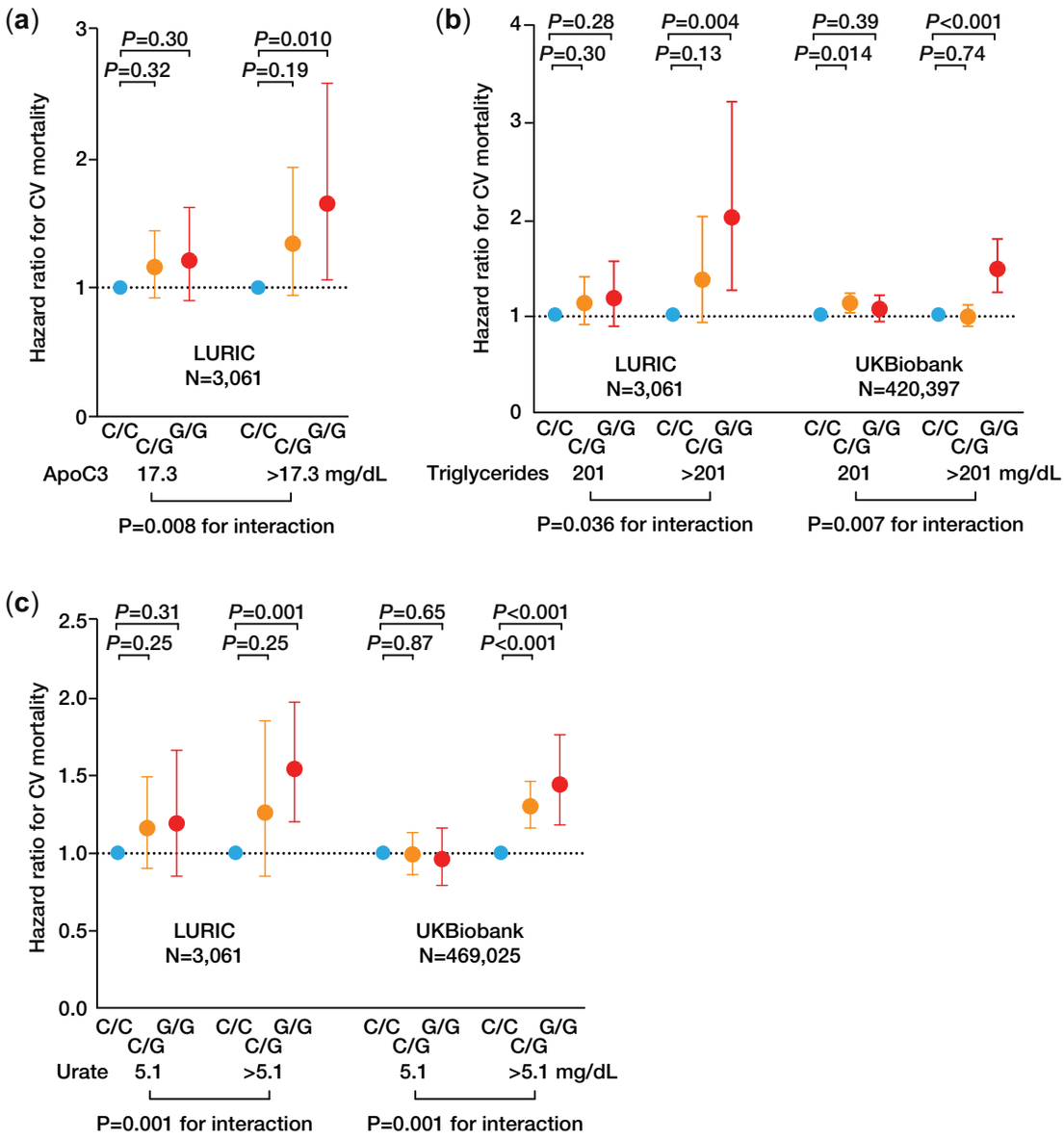

Figure 6 Apolipoprotein C3, triglycerides, and urate modulate the association between rs10754555 and cardiovascular mortality. (*A*) Association between rs10754555 genotype and cardiovascular mortality in 3061 participants of the LURIC study divided in subjects with low (≤17.3 mg/dL, Quartiles 1–3) and high (>17.3 mg/dL, Quartile 4) apolipoprotein C3 plasma levels. (*B*) Association between rs10754555 genotype and cardiovascular mortality in the LURIC study and in 483 258 participants of UKBiobank divided into subjects with low (≤201 mg/dL, Quartiles 1–3) and high (>201 mg/dL, Quartile 4) triglyceride plasma levels. (*C*) Association between rs10754555 genotype and cardiovascular mortality in LURIC and UKBiobank divided into subjects with low (≤5.1 mg/dL, Quartiles 1–3) and high (>5.1 mg/dL, Quartile 4) urate plasma levels. Interaction refers to the interaction term between apolipoprotein C3, triglycerides, or urate and rs10754555 included in the Cox regression models.

Importantly, rs10754555 is associated with CV mortality but not mortality related to infection or cancer. Our validation cohorts comprise a wide range of different patient populations including patients with prevalent CAD as well as subjects from the general population. Across these studies, the rs10754555 *NLRP3* variant was consistently associated with increased CV mortality. Moreover, the high frequency of the risk allele (MAF 39.9%) indicates that increased AQ12 NLRP3 inflammasome activity might contribute substantially to CV mortality on the population level. In agreement with the pre-clinical data on the association between NLRP3 inflammasome activation and atherosclerosis,[9] our study shows that the rs10754555-mediated increase in all-cause mortality is mainly driven by CV deaths. These

data highlight an important role of the innate immune system in the pathophysiology of CVD. Similar to NLRP3, gain-of-function mutations within the IL-6 receptor locus were found to be associated with increased risk for CAD.[30,31]

Indeed, in animal studies, inhibition of the NLRP3 inflammasome by the selective, small-molecule inhibitor MCC950 reduced experimental autoimmune encephalomyelitis and myocardial infarction.[32,33] Compelling evidence for the benefit of therapeutically targeting NLRP3-dependent pathways is provided by studies using the monoclonal, IL-1β-targeting antibody canakinumab. In patients after MI with persistently elevated hsCRP, canakinumab lowered the rate of recurrent CV events by 15%, when 150 mg of canakinumab was administered.[11] *Post hoc* analyses of the CANTOS trial revealed persistently elevated levels of the NLRP3-dependent cytokine IL-18, which are unaffected by canakinumab treatment and still associated with future CV events.[34] Therefore, inhibition of the NLRP3 inflammasome or its assembly in contrast to the specific inhibition of one effector cytokine could potentially provide a stronger reduction in CV events. Nevertheless, IL-1β release can also be induced by other inflammasome sensors such as absent in melanoma 2 (AIM2), which is activated by double-stranded DNA by exogenous pathogens and also during tissue damage.[35] In addition to canakinumab, treatment with colchicine, which modulates the NLRP3 inflammasome, reduces CV events in patients post-MI with a stronger effect as compared to canakinumab.[12] Since treatment with canakinumab or colchicine is associated with potential serious adverse events, strategies to select patients for targeted treatment are necessary. Screening for genetic variants associated with NLRP3 activation and subsequently elevated IL-1β and IL-18 may help to identify subjects with increased risk for CV events—especially at young age—as a result of sustained (micro)-inflammation particularly when plasma levels of known inflammasome activators such as ApoC3, triglycerides, or urate are elevated.

Some limitations of our study should be considered. Although our results highlight the NLRP3 inflammasome as a potential factor promoting CV mortality, further studies are needed to prove that in particular carriers of the rs10754555 *NLRP3* variant benefit from a specific anti-inflammatory treatment. In the present study, the *NLRP3* variant rs10754555 is linked to mortality. Based on the study designs, we cannot show an association between the mutant carrier status and non-fatal CV events. The age-rs10754555 on CAD risk could not have been validated in CARDIOGRAM due to limited access to individual patient data. Therefore, this interaction was validated in the GerMIFS studies ($N = 6389$ CAD cases and $N = 5687$ controls), which are part of the CARDIOGRAM consortium.

In conclusion, this is the first study to demonstrate the association between genetically driven inflammation and CVD by engaging a specific pro-inflammatory pathway (i.e. the NLRP3 inflammasome). These findings set the stage for individualized treatments in subjects with inflammation-driven high CV risk.

Supplementary material

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

Acknowledgements

This work was supported by grants of Deutsche Forschungsgemeinschaft (DFG, SFB TRR 219) for Stephen Zewinger, Stefan Wagenpfeil, Michael Böhm, Rafael Kramann, Barbara Niemeyer, Danilo Fliser, and Thimoteus Speer, CORONA Stiftung (Ulrich Laufs), Universität Leipzig (Ulrich Laufs), as well as European Uremic Toxin (EUTox) Work Group of the ERA-EDTA (Danilo Fliser, Thimoteus Speer). Support for genotyping in the LURIC cohort was provided by the seventh framework program of the European commission (AtheroRemo, grant 201668). The measurement of DNA methylation in LURIC was supported by the seventh framework program of the European commission (RiskyCAD, grant 305739) and the Competence Cluster of Nutrition and Cardiovascular Health (nutriCARD), which is funded by the German Federal Ministry of Education and Research (grant 01EA1801A). The Atherosclerosis Risk in Communities study has been funded in whole or in part with Federal funds from the National Heart, Lung, and Blood Institute, National Institutes of Health, Department of Health and Human Services (contract numbers HHSN268201700001I, HHSN268201700002I, HHSN268201700003I, HHSN268201700004I, and HHSN268201700005I), R01HL087641, R01HL59367, and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. GeneBank was supported, in part, by grants P01HL076491, R01HL103931, R01HL113452, R01HL103866, R01DK106000, R01HL126827, R01HL133169, and R01HL148110 from the National Institutes for Health (NIH). INVEST was supported by the University of Florida and grants from BASF Pharma and Abbott Laboratories and was supported by NIH grants R01HL074730 and U01GM074492. The INVEST GWAS genotyping was supported by RIKEN. Additional support for this project comes from NIH grant KL2 TR001429 (C.W.M.). LIFE-Heart was funded by the Leipzig Research Center for Civilization Diseases (LIFE). LIFE is an organizational unit affiliated to the Medical Faculty of the University of Leipzig. LIFE is funded by means of the European Union, by the European Regional Development Fund and by funds of the Free State of Saxony within the framework of the excellence initiative. The PROSPER study was supported by an investigator-initiated grant obtained from Bristol-Myers Squibb. J.W.J. is an Established Clinical Investigator of the Netherlands Heart Foundation (grant 2001 D 032). Support for genotyping was provided by the seventh framework programme of the European commission (grant 223004) and by the Netherlands Genomics Initiative (Netherlands Consortium for Healthy Aging grant 050-060-810). S.C.'s effort is supported, in part, by the National Institutes of Health (R01 NR013396). The Coronary Disease Cohort Study was funded by the New Zealand Health Research Council and the Heart Foundation of New Zealand, with funding for genotyping from the Christchurch Heart Institute Trust. TRIUMPH was sponsored by the National Institutes of Health: Washington University School of Medicine SCCOR Grant P50 HL077113. The PLATO trial was supported by Uppsala Clinical Research Center, AstraZeneca,

and the Swedish Heart-Lung Foundation. We thank the participants AQ13 of the UKBiobank.

Conflict of interest: W.M. reports other from Synlab Holding Deutschland GmbH, during the conduct of the study, grants from Siemens Healthineers, grants and personal fees from Aegerion Pharmaceuticals, grants and personal fees from AMGEN, grants from AstraZeneca, grants and personal fees from Sanofi, grants and personal fees from Amryt Pharmaceuticals, grants and personal fees from BASF, grants and personal fees from Abbott Diagnostics, grants and personal fees from Numares AG, grants and personal fees from Berlin-Chemie, grants and personal fees from Akzea Therapeutics, grants from Bayer Vital GmbH, grants from bestbion dx GmbH, grants from Boehringer Ingelheim Pharma GmbH Co KG, grants from Immundiagnostik GmbH, grants from Merck Chemicals GmbH, grants from Novartis Pharma GmbH, grants from Olink Proteomics, grants and personal fees from AMGEN, personal fees from Novartis Pharma, and personal fees from Vifor Pharma, outside the submitted work. R.M.C.-D. reports grants from NIH, during the conduct of the study. W.H.W.T. reports grants from National Institutes of Health, personal fees from Sequana Medical AG, personal fees from Owkin Inc, personal fees from Relypsa Inc, personal fees from American Board of Internal Medicine, and personal fees from Springer Nature AG, outside the submitted work. S.L.H. reports other from Procter & Gamble, other from Roche Diagnostics, and grants from NIH, outside the submitted work. In addition, S.L.H. has a patent Cleveland Heart Lab/Quest Diagnostics with royalties paid and a patent Procter & Gamble with royalties paid. L.W. reports grants from GlaxoSmithKline, during the conduct of the study, grants from AstraZeneca, grants from Boehringer Ingelheim, grants from Bristol-Myers Squibb/Pfizer, grants from Merck & Co, grants from Roche Diagnostics, and personal fees from Abbott, outside the submitted work. N.S. reports personal fees from Amgen, personal fees from AstraZeneca, grants and personal fees from Boehringer Ingelheim, personal fees from Eli Lilly, personal fees from Merck Sharp & Dohme, personal fees from Novartis, personal fees from Novo Nordisk, personal fees from Pfizer, and personal fees from Sanofi, outside the submitted work. D.J.S. reports grant Bristol-Myers Squibb, during the conduct of the study. W.K. reports personal fees from AstraZeneca, personal fees from Novartis, personal fees from Pfizer, personal fees from The Medicines Company, personal fees from DalCor, personal fees from Kowa, personal fees from Amgen, personal fees from Corvidia, personal fees from Daiichi-Sankyo, personal fees from Genentech, personal fees from Genentech, personal fees from Novo Nordisk, personal fees from Esperion, personal fees from Berlin-Chemie, personal fees from Sanofi, personal fees from Bristol-Myers Squibb, grants and non-financial support from Abbott, grants and non-financial support from Roche Diagnostics, grants and non-financial support from Beckmann, and grants and non-financial support from Singulex, outside the submitted work. E.B. reports grants from NIH, during the conduct of the study. M.S. receives funding from Pfizer Inc. for a project not related to the present work. M.E.K. reports other from SYNLAB Holding Deutschland GmbH, outside the submitted work. R.N.D. reports grants from New Zealand Heart Foundation and grants from Health Research Council of New Zealand, during the conduct of the study. M.-P.D. reports personal fees and other from Dalcor, personal fees and other from GlaxoSmithKline, other from AstraZeneca, other from Pfizer, other from Servier, and other from Sanofi, outside the submitted work. In addition, M.-P.D. has a patent Methods for Treating or Preventing Cardiovascular Disorders and Lowering Risk of Cardiovascular Events issued to Dalcor, no royalties received, a patent Genetic Markers for Predicting Responsiveness to Therapy with HDL-Raising or HDL Mimicking Agent issued to Dalcor, no royalties received, and a patent Methods for using low-dose colchicine after myocardial infarction with royalties paid to Invention assigned to the Montreal Heart Institute. H.W. reports grants and personal fees from Eli Lilly and Company, other from AstraZeneca, grants and personal fees from Omthera Pharmaceuticals, grants and personal fees from Eisai Inc., grants and personal fees from DalCor Pharma UK Inc., grants and personal fees from CSL Behring LLC, grants and personal fees from American Regent, grants and personal fees from Sanofi-Aventis Australia Pty Ltd, grants and personal fees from Esperion Therapeutics Inc., personal fees from Genentech, Inc., and grants, personal fees and other from Sanofi-Aventis, outside the submitted work. S.K.J. reports grants from AstraZeneca, outside the submitted work. V.T. reports employment from deCODE genetics/Amgen, outside the submitted work. C.H. reports grants and personal fees from GlaxoSmithKline and grants and personal fees from AstraZeneca, during the conduct of the study. H.S. reports personal fees from MSD Sharp & Dohme, personal fees from Amgen, personal fees from Bayer Vital GmbH, personal fees from Boehringer Ingelheim, personal fees from Daiichi-Sankyo, personal fees from Novartis, personal fees from Servier, personal fees from Brahms, personal fees from Bristol-Myers Squibb, personal fees from Medtronic, personal fees from Sanofi-Aventis, personal fees from Synlab, grants and personal fees from AstraZeneca, and personal fees from Pfizer and Vifor, outside the submitted work. R.K. reports grants from Chugai and personal fees from Bayer, outside the submitted work. T.S. reports personal fees from Amgen, personal fees from Sanofi-Aventis, personal fees from Bayer, and personal fees from Astellas, outside the submitted work. AQ14

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Translational perspective

Inflammation plays a crucial role in the development of atherosclerotic cardiovascular disease (CVD). Interventional studies highlight the NOD-like receptor protein 3 (NLRP3) inflammasome as an important mediator of CVD. Here, we report that a genetic variant within the *NLRP3* gene locus refers to the systemic pro-inflammatory state. This variant is associated with coronary artery disease risk and cardiovascular mortality predominately in younger subjects. Therefore, genetically determined inflammation represents an important driver of atherosclerotic CVD. Identification of subjects at high inflammation-driven cardiovascular risk sets the stage for individualized treatments.

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