

ORIGINAL ARTICLE



Association of Chromosome 9p21 With Subsequent Coronary Heart Disease Events

A GENIUS-CHD Study of Individual Participant Data

BACKGROUND: Genetic variation at chromosome 9p21 is a recognized risk factor for coronary heart disease (CHD). However, its effect on disease progression and subsequent events is unclear, raising questions about its value for stratification of residual risk.

METHODS: A variant at chromosome 9p21 (rs1333049) was tested for association with subsequent events during follow-up in 103 357 Europeans with established CHD at baseline from the GENIUS-CHD (Genetics of Subsequent Coronary Heart Disease) Consortium (73.1% male, mean age 62.9 years). The primary outcome, subsequent CHD death or myocardial infarction (CHD death/myocardial infarction), occurred in 13 040 of the 93 115 participants with available outcome data. Effect estimates were compared with case/control risk obtained from the CARDIoGRAMplusC4D consortium (Coronary Artery Disease Genome-wide Replication and Meta-analysis [CARDIoGRAM] plus The Coronary Artery Disease [C4D] Genetics) including 47 222 CHD cases and 122 264 controls free of CHD.

RESULTS: Meta-analyses revealed no significant association between chromosome 9p21 and the primary outcome of CHD death/myocardial infarction among those with established CHD at baseline (GENIUS-CHD odds ratio, 1.02; 95% CI, 0.99–1.05). This contrasted with a strong association in CARDIoGRAMplusC4D odds ratio 1.20; 95% CI, 1.18–1.22; *P* for interaction <0.001 compared with the GENIUS-CHD estimate. Similarly, no clear associations were identified for additional subsequent outcomes, including all-cause death, although we found a modest positive association between chromosome 9p21 and subsequent revascularization (odds ratio, 1.07; 95% CI, 1.04–1.09).

CONCLUSIONS: In contrast to studies comparing individuals with CHD to disease-free controls, we found no clear association between genetic variation at chromosome 9p21 and risk of subsequent acute CHD events when all individuals had CHD at baseline. However, the association with subsequent revascularization may support the postulated mechanism of chromosome 9p21 for promoting atheroma development.

Riyaz S. Patel, MD*
Amand F. Schmidt, PhD*
Vinicius Tragante, PhD*
et al

*Drs Patel, Schmidt, and Tragante are joint first authors

†Drs Samani, Hingorani, Asselbergs contributed equally to this work.

The full author list is available on page 169.

Key Words: chromosome ■ genetic variation ■ myocardial infarction ■ risk factor ■ secondary prevention

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Using a case-control approach, a large number of common genetic variants have now been associated with coronary heart disease (CHD) through genome-wide association studies, in an effort largely led by the CARDIOGRAMplusC4D consortium (Coronary Artery Disease Genome-wide Replication and Meta-analysis [CARDIOGRAM] plus The Coronary Artery Disease [C4D] Genetics).¹ Among these variants, the chromosome 9p21 locus was the first to be discovered and the variant with the largest individual effect and is the most widely replicated genetic risk factor for CHD.^{2–4} Multiple studies including case-control and prospective cohort studies in general populations have reliably confirmed its effect on risk of CHD among otherwise healthy individuals.⁵

However, it is uncertain whether variants at the 9p21 locus also affect risk of recurrent or subsequent events, including mortality in those with established CHD. Elucidation of this hypothesis would help to better understand its mechanism and estimate its incremental value for stratification of residual risk. Prior studies have shown conflicting results, although most have been underpowered. A literature-based meta-analysis indicated a null association of chromosome 9p21 variants with subsequent CHD events but was based on summary, not individual level data, with varying outcome definitions.^{6,7}

The new collaborative GENIUS-CHD (Genetics of Subsequent Coronary Heart Disease) consortium, described in this issue of the journal, was established to investigate genetic determinants of disease progression following an index CHD event.⁸

In this article, we use the GENIUS-CHD resource to: (1) examine the association of variants at the 9p21 locus on risk of subsequent CHD events in individuals with established CHD; (2) compare these to the association between chromosome 9p21 and any CHD observed in the CARDIOGRAMplusC4D consortium; and (3) explore the potential impact on these estimates of biases that might affect genetic association studies of disease outcome and prognosis.

METHODS

In accordance with Transparency and Openness Promotion Guidelines, the data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure. Participating studies received local institutional review board approval and included patients who had provided informed consent at the time of enrollment. The central analysis sites also received waivers from their local institutional review board for collating and analysing summary level data from these individual studies. Details about the GENIUS-CHD consortium and study inclusion criteria have been published separately in this issue of the journal,⁸ whereas for this study full details about data sources, genetic variant selection, outcomes and statistical analyses are available in the [Data Supplement](#).

RESULTS

In total, 49 studies from the GENIUS-CHD consortium contributed to the federated analysis resulting in a sample size of 103 357 individuals of European descent with established CHD and available genotype data at the 9p21 locus. Of these, 93 115 individuals had available data for the primary composite outcome of subsequent CHD death/myocardial infarction (MI), of whom 13 040 experienced these events. Contributing study details are provided in [Table](#). Participant characteristics are representative for populations with established CHD with a weighted mean age of 62.9 years; 73.1% male. As expected, risk factor prevalence was high in this population, including diabetes mellitus (24.4%), hypertension (59.1%), and current smoking (25.7%). Statin use at enrollment varied by study, ranging from 5.2% to 97.3%, with a median of 61.5% ([Table](#)).

The rs1333049 single nucleotide polymorphism was genotyped in 42 studies, with the remaining 7 studies using highly correlated proxies ($R^2 > 0.90$); rs10757278 (4 studies) or rs4977574 (3 studies) when the primary single nucleotide polymorphism was unavailable. Genotyping details are provided in [Table I](#) in the [Data Supplement](#). For rs1333049, the average risk allele frequency across the participating studies was 0.518 ranging from 0.453 to 0.587 ([Figure I](#) in the [Data Supplement](#)).

From CARDIOGRAMplusC4D, after excluding 6 cohorts which had contributed data to both consortia, data were available for association with chromosome 9p21 from 41 studies, including 47 222 cases with CHD and 122 264 controls free of any CHD.

Power to detect different effect sizes, including the effect size identified in CARDIOGRAMplusC4D, using a 2-sided alpha of 0.05, are provided in [Table II](#) in the [Data Supplement](#).

Chromosome 9p21 Association With Subsequent CHD Events

Study-specific results for the association between chromosome 9p21 and risk of the primary outcome of CHD death or MI among individuals with established CHD at baseline, adjusted for age and sex are presented in [Figure II](#) in the [Data Supplement](#).

The per-allele odds ratio (OR) for the primary outcome during follow-up was 1.02 (95% CI, 0.99–1.05). The effect estimate again for the primary outcome, based on a time to event analysis and using a Cox regression model, was also similar with a hazard ratio of 1.02 (95% CI, 0.99–1.04; [Figure III](#) in the [Data Supplement](#)).

In contrast, a meta-analysis of CARDIOGRAMplusC4D data (excluding studies also contributing data to

Table. Continued

Alias	Cohort	Total N genotyped	Study Design	CHD Type	Male, %	Age, y, SD	BMI (SD)	Diabetes mellitus, %	Smoking, %	Systolic BP, SD	Total Cholesterol, SD	Statin use, %	Creatinine, SD	Prior Revasc, %	Prior MI, %	PubMED ID
GENOCOR	Genetic Mapping for Assessment of Cardiovascular Risk	497	Cohort	Mixed	86.7	65.2 (8.45)	...	13.3	64.4	129.5 (20.3)	4.70 (0.92)	72.1	94.8 (27.2)	13.7	63.2	22717531
GoDARTS incident	Genetics of Diabetes Audit and Research in Tayside Scotland (I)	1003	Cohort	CAD	62.0	71.1 (10.62)	29.7 (5.64)	77.9	...	126.7 (NA)	4.62 (1.02)	50.8	108.0 (64.6)	0.2	1.3	...
GoDARTS prevalent	Genetics of Diabetes Audit and Research in Tayside Scotland (P)	2000	Cohort	CAD	66.5	69.1 (9.20)	30.3 (5.43)	77.8	14.9	136.2 (19.7)	4.37 (0.83)	66.8	101.6 (34.5)	31.4	48.9	...
GRACE_B	Global Registry of Acute Coronary Events—Belgium	699	Cohort	ACS	75.4	65.7 (12.01)	27.0 (4.35)	81.3	49.9	138.8 (25.3)	5.33 (1.19)	79.3	102.8 (61.9)	...	80.1	20231156
GRACE_UK	Global Registry of Acute Coronary Events - UK	1086	Cohort	ACS	69.1	64.4 (12.04)	28.0 (5.15)	15.0	69.5	137.8 (27.1)	5.19 (1.29)	16.8	105.0 (40.0)	20.4	32.0	20231156
IDEAL	Incremental Decrease in End Points Through Aggressive lipid Lowering	6223	RCT	ACS	81.8	61.2 (9.32)	27.4 (3.80)	11.4	20.3	136.8 (19.8)	5.03 (0.98)	76.9	100.1 (16.7)	41.3	...	16287954
INTERMOUNTAIN	Intermountain Heart Collaborative Study	6763	Cohort	Mixed	66.7	61.2 (11.05)	29.5 (6.08)	20.3	10.2	141.8 (24.4)	4.96 (1.12)	38.7	99.6 (66.6)	...	6.6	20691829
INVEST	International Verapamil SR Trandolapril Study Genetic Substudy	2145	RCT	CAD	56.6	68.7 (9.38)	...	23.9	12.8	148.6 (18.1)	...	52.8	...	47.6	...	21372283
JUMC	Krakow-GENIUS-CHD	704	Cohort	Mixed	71.6	68.3 (10.25)	26.3 (4.46)	36.9	27.5	148.1 (23.8)	5.02 (1.06)	88.3	89.9 (37.5)	50.1	39.7	28444280
KAROLA	Karola Study	1147	Cohort	Mixed	84.6	58.6 (8.13)	27.0 (3.26)	18.5	32.4	119.9 (15.5)	4.46 (0.84)	77.4	82.4 (26.5)	42.8	21.6	24829374
LIFE-Heart	Leipzig (LIFE) Heart Study	4330	Cohort	Mixed	75.5	64.0 (11.15)	29.0 (4.68)	34.4	29.0	138.3 (21.8)	5.24 (1.18)	38.9	87.3 (34.6)	...	0.1	22216169
LURIC	The Ludwigshafen Risk and Cardiovascular Health Study	2175	Cohort	Mixed	76.5	63.8 (9.85)	27.5 (3.89)	44.3	23.9	142.3 (24.1)	4.94 (0.99)	58.9	88.7 (38.5)	48.7	57.4	11258203
NE_POLAND	North East Poland Myocardial Infarction Study	603	Cohort	ACS	75.0	62.4 (11.86)	24.8 (3.79)	22.2	48.1	138.9 (27.4)	5.04 (1.05)	80.7	91.6 (36.3)	1.3	10.6	26086777
NEAPOLIS	Neapolis Campania Italia	1380	Cohort	Mixed	74.4	67.6 (10.49)	28.0 (4.18)	43.0	26.8	129.4 (14.2)	4.57 (1.02)	82.5	101.0 (68.1)	41.5	40.8	24262617
OHGS	Ottawa Heart Genomics Study	393	Cohort	Mixed	73.0	65.3 (11.07)	28.6 (5.00)	6.9	19.5	131.9 (19.0)	5.53 (1.03)	92.4	89.8 (21.1)	28.2	21.9	...
PLATO	The Study of Platelet Inhibition and Patient Outcomes	9814	RCT	ACS	69.5	62.6 (10.95)	28.2 (4.51)	22.8	35.2	135.6 (21.8)	5.42 (1.23)	79.7	85.6 (26.3)	15.1	20.6	19332184
PMI	Post Myocardial Infarction Study	783	Cohort	ACS	78.3	62.7 (10.29)	26.5 (3.82)	12.0	28.2	117.0 (15.6)	5.98 (1.19)	46.0	87.8 (27.8)	...	17.2	12771003

(Continued)

Table. Continued

Alias	Cohort	Total N genotyped	Study Design	CHD Type	Male, %	Age, y, SD	BMI (SD)	Diabetes mellitus, %	Smoking, %	Systolic BP, SD	Total Cholesterol, SD	Statin use, %	Creatinine, SD	Prior Revasc, %	Prior MI, %	PubMED ID
POPular	The Popular study	997	RCT	ACS	74.3	63.8 (10.40)	...	18.9	27.5	145.0 (22.1)	4.25 (0.64)	80.7	92.7 (26.8)	33.1	43.7	26542508
PROSPER	Prospective Study of Pravastatin in the Elderly at Risk	439	RCT	CAD	69.9	75.4 (3.31)	26.4 (3.87)	10.3	16.2	150.0 (21.6)	5.55 (0.83)	...	109.4 (23.2)	26.0	85.9	10569329
RISCA	Recurrence and Inflammation in the Acute Coronary Syndromes Study	1052	Cohort	ACS	75.9	61.9 (11.40)	27.2 (4.43)	19.8	30.4	46.6	100.6 (28.6)	28.2	27.9	18549920
SHEEP	Stockholm Heart Epidemiology Program	1150	Cohort	ACS	70.7	59.3 (7.21)	26.8 (4.02)	18.2	50.0	131.8 (20.6)	6.28 (1.16)	17667644
SMART	Second Manifestations of Arterial Disease	2485	Cohort	Mixed	82.2	60.2 (9.26)	27.3 (3.63)	16.6	24.4	137.4 (19.8)	4.73 (0.96)	75.7	92.3 (22.7)	...	43.6	10468526
STABILITY	Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy trial	9287	RCT	Mixed	82.0	64.7 (9.10)	29.9 (4.97)	38.4	21.4	131.7 (16.1)	...	97.3	...	74.6	58.6	24678955
THI	Texgen	2729	Cohort	ACS	75.3	63.6 (10.62)	29.6 (5.59)	30.5	21.3	57.1	...	21.5	16.7	21414601
TNT	Treating to New Targets	5104	RCT	CAD	81.3	61.3 (8.73)	28.6 (4.59)	14.8	13.4	130.9 (16.8)	4.51 (0.61)	70.3	104.8 (17.3)	...	57.3	15755765
TRIUMPH	Translational Research Investigating Underlying Disparities in Acute Myocardial Infarction Patient's Health Status	1974	Cohort	ACS	72.4	59.8 (12.04)	29.5 (5.96)	28.6	37.4	117.8 (18.3)	...	89.0	112.5 (76.7)	27.0	18.3	21772003
UCORBIO	Utrecht Coronary Biobank	1073	Cohort	Mixed	75.6	65.4 (10.26)	27.2 (4.34)	21.5	23.1	...	4.76 (1.18)	64.0	91.9 (42.9)	...	28.9	...
UCP	Utrecht Cardiovascular Pharmacogenetics Study	1500	Cohort	Mixed	75.4	64.1 (9.96)	153.4 (21.4)	5.50 (1.10)	27.1	94.7 (24.8)	25652526
VHS	Verona Heart Study	907	Cohort	CAD	80.9	61.3 (9.78)	26.9 (3.57)	18.5	69.2	...	5.37 (1.10)	47.1	96.7 (32.2)	17.4	59.6	10984565
VIVIT	Voranberg Institute for Vascular Investigation and Treatment Study	1318	Cohort	CAD	73.1	64.5 (10.44)	27.4 (4.14)	30.6	18.9	137.2 (19.2)	5.45 (1.14)	49.7	88.4 (32.7)	21.1	31.0	24265174
WARSAW ACS	Warsaw ACS Genetic Registry	669	Cohort	ACS	74.5	63.6 (11.72)	28.1 (4.72)	21.9	42.0	127.8 (22.6)	4.99 (1.07)	...	93.5 (41.4)	...	18.6	...
WTCCC	WTCCC CAD Study	1924	Cohort	Mixed	79.3	60.0 (8.13)	27.6 (4.20)	11.7	12.8	143.6 (22.0)	5.28 (0.98)	71.6	...	67.1	72.0	17634449

Overview of studies contributing to chromosome 9p21 analysis and participant characteristics; alias denotes the abbreviated name of each study used in figures and tables; PubMed IDs are provided for individual study descriptions; mean (SD) with proportions (%) are provided unless otherwise stated. ACS indicates acute coronary syndrome; BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; FRISC-II, Fast Revascularization during Instability in Coronary artery disease; GENIUS-CHD, Genetics of Subsequent Coronary Heart Disease; JUMC, Jagiellonian University Medical College; KAROLA, Langzeitfolge der Kardiologischen Anschlussheilbehandlung; LIFE, The Leipzig Heart Study; MI, myocardial infarction; RCT, randomized controlled trial, and WTCCC, Wellcome Trust Case Control Consortium.

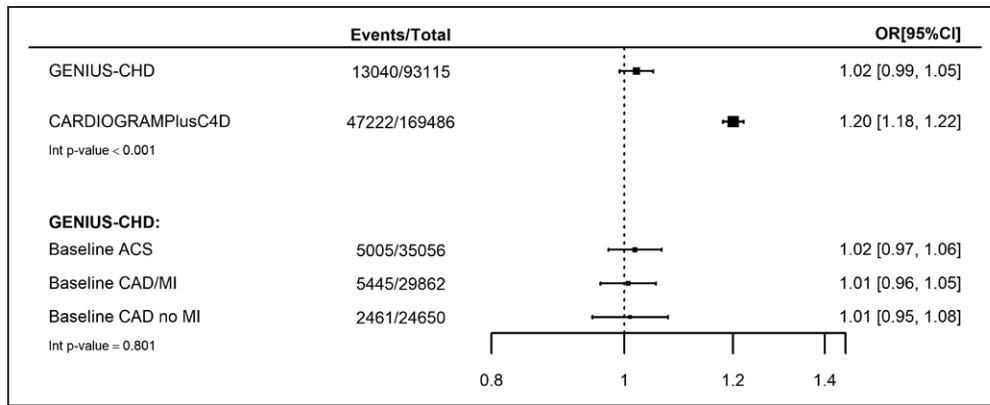


Figure 1. Association between chromosome 9p21 and subsequent coronary heart disease (CHD) events in all participants with baseline CHD (GENIUS-CHD [Genetics of Subsequent Coronary Heart Disease]) compared with association in CHD cases and CHD-free controls (CARDIoGRAM-PlusC4D).

For the CARDIoGRAMPlusC4D consortium (Coronary Artery Disease Genome wide Replication and Meta-analysis [CARDIoGRAM] plus The Coronary Artery Disease [C4D] Genetics) meta-analysis estimate, 6 studies (LURIC, LIFE-Heart [The Leipzig Heart Study], GoDARTS [Genetics of Diabetes Audit and Research in Tayside Scotland], OHGS [Ottawa Heart Genomics Study], PROSPER [Prospective Study of Pravastatin in the Elderly at Risk], WTCCC [Wellcome Trust Case Control Consortium]) were excluded as they were also included in GENIUS-CHD. Estimates for GENIUS-CHD are also presented by subtype of CHD at baseline, including acute coronary syndrome (ACS), stable coronary artery disease (CAD) without prior myocardial infarction (MI; CAD/no MI), and stable CAD with prior MI (CAD/MI). All estimates were adjusted for age and sex.

GENIUS-CHD), revealed a per-allele OR for a CHD event similar to that reported previously (OR, 1.20; 95% CI, 1.18–1.22). There was evidence of statistical heterogeneity between the estimates (interaction $P < 0.001$), Figure 1.

Subgroup Analyses

We found minimal evidence for heterogeneity in effect estimates when stratifying by CHD subtype at baseline (interaction P value 0.801), with no clear evidence for an effect of chromosome 9p21 genetic variation on subsequent CHD death or MI in individuals enrolled with acute coronary syndrome (OR, 1.02; 95% CI, 0.97–1.06), those with coronary artery disease with a prior MI (OR, 1.01; 95% CI, 0.96–1.05), and those with coronary artery disease without prior MI (OR, 1.01; 95% CI, 0.95–1.08, Figure 1).

We further examined the effect of chromosome 9p21 on the primary outcome in prespecified subgroup analyses. We noted a borderline nominally significant interaction with sex, suggesting a greater risk among women with the chromosome 9p21 risk allele, for subsequent CHD death/MI (interaction P value = 0.04), whereas nonsignificant trends were noted for greater risk in those without hypertension (P value=0.08) or without renal impairment (P value=0.17). There were minimal differences in effect estimates by other patient level characteristics including age and diabetes mellitus or according to statin or antiplatelet use or left ventricular impairment at baseline (Figure IV in the [Data Supplement](#)).

Similarly, when stratified by study level features, we observed minimal evidence for heterogeneity in effect estimates by study size, geographic region, study design, or length of follow-up (Figure V in the

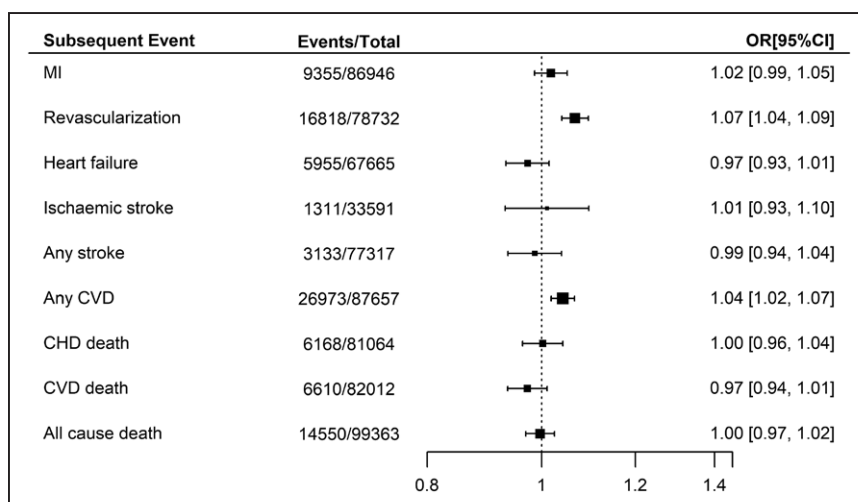


Figure 2. Association between chromosome 9p21 and secondary outcomes in participants with baseline CHD, within GENIUS-CHD (Genetics of Subsequent Coronary Heart Disease).

All meta-analysis estimates were adjusted for age and sex. CHD indicates coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction; and OR, odds ratio.

Data Supplement). However, when ordered by date of first enrollment, there was no evidence for variation in effect by time of enrollment (Figure II in the Data Supplement).

Secondary Outcomes

We additionally examined the association between chromosome 9p21 and other subsequent events available for this analysis within the GENIUS-CHD Consortium, listed in Table III in the Data Supplement, with summary estimates provided in Figure 2. Of note, the per-allele effect of risk variants at chromosome 9p21 on subsequent revascularization during follow-up was 1.07 (95% CI, 1.04–1.09). The effect on the composite outcome of any cardiovascular disease, which includes revascularization, was also significant at 1.04 (95% CI, 1.02–1.07). However, there was no clear evidence of association for the remaining secondary outcomes, with only a marginal trend to protection for both subsequent heart failure (OR, 0.97; 95% CI 0.93–1.01) and cardiovascular disease death (OR, 0.97; 95% CI, 0.94–1.01), as shown in Figure 2.

Selection Bias

To explore the potential for index event bias, we looked for differences in associations between chromosome 9p21 and known cardiovascular risk factors in the United Kingdom Biobank, among the subset of participants with established CHD, compared with the full UKB cohort (Table IV in the Data Supplement). Although there were differences between the groups in the prevalence or values of the tested risk factors, we did not find clear evidence to indicate a distortion in associations between chromosome 9p21 and age, blood pressure, diabetes mellitus, or smoking. There was, however, a small difference for body mass index, with a greater statistical association between the chromosome 9p21 risk allele and lower body mass index identified in those with established CHD than in the general population (nominal interaction *P* value 0.02, Table IV in the Data Supplement).

We also observed that the chromosome 9p21 risk allele frequency in those surviving with CHD, both in UKB (0.529) and in GENIUS-CHD (0.518, Figure I in the Data Supplement), was higher than the general population in the UKB (0.481) and European reference populations from the 1000 Genomes (Phase 3),⁹ (0.472). This difference in frequency confirms the association of chromosome 9p21 with CHD and also indicated absence of a crude survival bias with loss of large numbers of risk allele carriers to fatal events before entry into CHD cohorts. We did, however, observe a trend to an age association in those with established CHD, as well as the general population in the UKB, with lower chromosome

9p21 risk allele frequencies with advancing age, relative to younger carriers (Figure VI in the Data Supplement).

DISCUSSION

In this study, we examined the effect of genetic variation at the chromosome 9p21 locus on risk of subsequent events in 103 357 individuals with established CHD using the newly formed GENIUS-CHD consortium.⁸ We found that (1) in contrast to the known strong association with CHD observed in CARDIoGRAMPlusC4D, there was a markedly attenuated and nonsignificant association with subsequent CHD events in GENIUS-CHD; (2) effect estimates in GENIUS-CHD were broadly consistent in stratified analyses based on features related to study design, patient characteristics, and type of index CHD event; and (3) exploratory analyses suggested that selection biases were unlikely to explain the discrepancy. However, we did find evidence of an association between these variants and a secondary outcome of future revascularization events. Our findings, taken together with those from others, support the view that chromosome 9p21 promotes CHD through progressive stable atheroma rather than through development of an unstable phenotype.

The chromosome 9p21 locus is the most widely replicated genetic risk locus for CHD identified to date, with an estimated 15% to 35% increased risk in carriers of the variant allele in prospective population and case-control studies.⁵ However, studies examining the effect on subsequent CHD events in people with known CHD at baseline have reported conflicting results.^{10–14} Our group previously examined this in a literature-based meta-analysis, based on 15 studies with median sample size of 1750 individuals, accruing 25 163 cases of established CHD, and reported no clear evidence of an effect of variants at chromosome 9p21 on the risk of subsequent events.⁶ An analysis by the CHARGE consortium (The Cohorts for Heart and Aging Research in Genomic Epidemiology) of 2953 MI survivors also reported no association with subsequent mortality.⁷ However, the limited size of most prior studies and the limitations of literature meta-analyses indicate that many possible explanations, including errors in risk allele coding and selection biases, could not be adequately explored, precluding meaningful interpretations for any mechanistic or clinical implications.

The emergence of the GENIUS-CHD Consortium has now permitted a robust evaluation of the role of chromosome 9p21 in subsequent CHD event risk, revealing a clear lack of association with a common composite coronary end point. This is in marked contrast to findings from studies comparing cases to CHD-free controls, as confirmed through meta-analysis of CARDIoGRAMPlusC4D data. Furthermore, we were able to

add to previous findings by showing that the type of CHD at baseline, whether acute coronary syndrome or stable CHD with or without prior MI, does not alter this association. We also interrogated several widely proposed explanations that could account for our findings through prespecified subgroup analyses and confirmed that most of these, specifically older age, medication use at baseline (statin or antiplatelet), study size or follow-up duration, did not appreciably alter the association findings. Our finding of a possible interaction with sex, warrants further investigation but should be considered hypothesis-generating given the borderline evidence of an interaction.

Selection bias (ie, index event bias or collider-stratification bias) could potentially explain reversed or attenuated associations in disease progression studies like this, operating by inducing relationships between (otherwise independent) risk factors through the selection of individuals with disease.^{15,16} Specifically, individuals surviving a first event consequent on exposure to a particularly strong risk factor may have lower levels of exposure to other individually weaker, independent risk factors, which can then attenuate the association of the risk factor of interest with subsequent events. However, the distribution of common risk factors by chromosome 9p21 genotype did not differ when compared between the general population and the subset with CHD in the UKB, using interaction tests. The only exception was for body mass index, a potentially differential association with chromosome 9p21 in those with CHD compared with the general population was noted. However, the effect size was small in both populations and on its own is unlikely to indicate presence of substantial index event bias.

Selection bias may also theoretically occur by focusing on subjects surviving a first event, where chromosome 9p21 risk allele carriers at risk of fatal CHD events are lost before enrollment into CHD cohorts, thereby diluting the future impact of the variant on subsequent CHD events. In this scenario, we would expect a lower risk allele frequency in those surviving CHD and entering CHD cohorts, but we found no evidence for this. Among those with CHD in the UKB, and among the whole UKB cohort, we did find a progressive loss of risk allele carriers with increasing age, consistent with prior findings of a greater association with CHD, among younger individuals in case-control studies.⁵ Given patients with CHD are generally older, it is possible that a subtle survival bias may still be influencing our findings, although all analyses were adjusted for age. However, based on simulation modeling, sample size, and projected single nucleotide polymorphism effect size, we and others have previously estimated that selection biases are only minimally operating in this context and would be unlikely to account for our observed findings.^{17,18} Although our

findings potentially argue against important selection biases in the analysis for the primary outcome, they are relatively insensitive assessments and may not fully elucidate such biases.

Possible biological explanations could also exist for our findings. Pathological studies indicate differences between chronic stable atherosclerotic plaques that cause ischemia through progressive vessel occlusion and vulnerable plaques with thin caps, prone to sudden plaque rupture, unheralded MI, and coronary deaths.¹⁹ In a seminal study dissecting the phenotype of CHD, a lack of effect for chromosome 9p21 and MI was noted, when both cases and controls had underlying atherosclerosis.²⁰ Our group and others have in parallel shown that chromosome 9p21 robustly associates with atherosclerotic phenotypes,²¹ whereas functional studies have also implicated this region with molecular activity that drives atheroma.²² Furthermore, in this study, we show that the only outcome positively associated with chromosome 9p21 is incident revascularization, perhaps reflecting more severe atherosclerosis burden. Collectively, these data support the concept that chromosome 9p21 promotes progressive atheroma formation and does not confer risk via plaque rupture.

In this context, it is worth noting that chromosome 9p21 associates more robustly with CHD in case-control studies than in prospective cohort studies.⁷ The difference, as proposed by others, could hypothetically be accounted for by incidence-prevalence bias, with chromosome 9p21 carriers more likely to survive a CHD event and thus be over represented among CHD cases (the opposite to survival bias described above).⁷ This becomes more likely as stated above if chromosome 9p21 drives a more progressive and stable atheroma phenotype. If this holds true, then among survivors with established CHD, one might expect that chromosome 9p21 carriers could hold a small favorable advantage over those who experience CHD in its absence, due instead to other more dangerous or vulnerable characteristics, and despite undergoing more subsequent revascularization, these chromosome 9p21 carriers do not experience more dangerous or fatal events.

These findings have important implications. Clinically, they indicate that a degree of caution should be applied when considering or evaluating patients for chromosome 9p21 to predict disease progression or residual risk. They also highlight the need to appreciate important biases that may inflate or attenuate association findings in the setting of subsequent events for individuals with established disease. Mechanistically, these findings support existing and emerging efforts seeking to elucidate the mechanism of the most robust genetic discovery for CHD in recent decades.

There are important limitations to consider. First, among individuals in GENIUS with established CHD,

the timing of the first CHD event or age of onset was often unknown, so we could not account for this variable in our analyses. However, the lack of association in the acute coronary syndrome studies, which had documented timing of the first event, suggests this did not impact the findings. Second, we had limited information on whether subsequent revascularization events were late staged procedures, which would count as part of the index CHD event or unplanned and symptom driven and thereby a true subsequent event, which may have diluted the effect estimate. Third, although we did not observe a specific interaction for statin or aspirin use, we cannot rule out an effect of combined or additional medication usage attenuating the association signal, given the high prevalence of secondary prevention drug use in this setting compared with general population cohorts. Fourth, our analyses were restricted to participants of European descent as most of the included studies only recruited these individuals, and so we were markedly underpowered to explore associations in other ethnic groups. Unfortunately, this remains a wider problem of genetic research and global efforts are ongoing to address this imbalance. Finally, variability of follow-up duration across studies is an analytical challenge and could have impacted our findings, through misclassification. However, a sensitivity analysis stratifying on the follow-up duration of individual studies (<5 or ≥ 5 years) revealed minimal evidence ($P=0.62$) of heterogeneity in effect estimates (Figure V in the [Data Supplement](#)), suggesting that this is unlikely to have influenced our findings significantly as effect estimates were concordant across studies with different lengths of follow-up. Our major strengths, however, include the size of the study and the large number and types of subsequent events and an effort to examine for selection biases. We also sought to mitigate potential miscoding of the risk allele, given rs1333049 is a palindromic single nucleotide polymorphism, and also the risk allele C changes from being a minor allele in population cohorts to the major allele in CHD cohorts. Finally, this analysis benefitted from the collective expertise and input of over 170 investigators and analysts, many of whom have previously reported on chromosome 9p21.

In conclusion, using the newly formed GENIUS-CHD consortium, we demonstrate that variation at chromosome 9p21 shows no clear association with risk of subsequent CHD events when all individuals have established CHD at baseline. This is in marked contrast to prior case-control studies examining odds of CHD presence compared with disease-free controls. We could not account for the attenuation of effect in terms of selection biases or subgroup effects. However, we did find a greater risk for incident revascularization in those with established CHD, and although residual bias may be at play, our findings collectively support the

view that chromosome 9p21 promotes CHD through progressive stable atheroma rather than through development of an unstable phenotype.

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Authors

Riyaz S. Patel, MD*; Amand F. Schmidt, PhD*; Vinicius Tragante, PhD*; Raymond O. McCubrey, MS; Michael V. Holmes, MD, PhD; Laurence J. Howe, PhD; Kenan Direk, PhD; Axel Åkerblom, MD, PhD; Karin Leander, PhD; Salim S. Virani, MD, PhD; Karol A. Kaminski, MD, PhD; Jochen D. Muehlschlegel, MD, MMSc; Marie-Pierre Dubé, PhD, Hooman Allayee, PhD; Peter Almgren, MSc; Maris Alver, MSc; Ekaterina V. Baranova, MSc; Hassan Behlouli, PhD; Bram Boeckx, PhD; Peter S. Braund, PhD; Lutz P. Breitling, MD; Graciela Delgado, MSc; Nubia E. Duarte, PhD; Line Dufresne, MSc; Niclas Eriksson, PhD; Luisa Foco, PhD; Crystel M. Gijsberts, MD, PhD; Yan Gong, PhD; Jaana Hartiala, PhD; Mahyar Heydarpour, PhD; Jaroslav A. Hubacek, DSc; Marcus Kleber, PhD; Daniel Kofink, PhD; Pekka Kuukasjärvi, MD, PhD; Vei-Vei Lee, MS; Andreas Leihener, PhD; Petra A. Lenzini, MS; Daniel Levin, PhD; Leo-Pekka Lyytikäinen, MD; Nicola Martinelli, MD, PhD; Ute Mons, PhD, Christopher P. Nelson, PhD; Kjell Nikus, MD, PhD; Anna P. Pilbrow, PhD; Rafal Ploski, MD, PhD; Yan V. Sun, PhD; Michael W.T. Tanck, PhD; W.H. Wilson Tang, MD; Stella Trompet, PhD; Sander W. van der Laan, PhD; Jessica van Setten; Ragnar O. Vilmdarson, MSc; Chiara Viviani Anselmi, PhD; Efthymia Vlachopoulou, PhD; Eric Boerwinkle, PhD; Carlo Briguori, MD, PhD; John F. Carlquist, PhD; Kathryn F. Carruthers, MPhil; Gavino Casu, MD; John Deanfield, MD; Panos Deloukas, PhD; Frank Dudbridge, PhD; Natalie Fitzpatrick, MSc; Bruna Gigante, MD, PhD; Stefan James, MD, PhD; Marja-Liisa Lokki, PhD; Paulo A. Lotufo, MD, PhD; Nicola Marziliano, PhD; Ify R. Mordi, MD; Joseph B. Muhlestein, MD; Chris Newton Cheh, MD; Jan Pitha, PhD; Christoph H. Saely, MD; Ayman Samman-Tahhan, MD; Pratik B. Sandesara, MD; Andrej Teren, MD; Adam Timmis, MD; Frans Van de Werf, PhD; Els Wauters, PhD; Arthur A.M. Wilde, MD, PhD; Ian Ford, MD, PhD; David J. Stott, MD; Ale Algra, MD; Maria G. Andreassi, PhD; Diego Ardissino, MD; Benoit J. Arsenaux, PhD; Christie M. Ballantyne, MD; Thomas O. Bergmeijer, MD; Connie R. Bezzina, PhD; Simon C. Body, MD, MPH, MBChB; Peter Bogaty, MD; Gert J. de Borst, MD; Hermann Brenner, MD, PhD; Ralph Burkhardt, MD; Clara Carpeggiani, MD, PhD; Gianluigi Condorelli, MD, PhD; Rhonda M. Cooper-DeHoff, PharmD; Sharon Cresci, MD; Ulf de Faire, PhD; Robert N. Doughty, MD; Heinz Drexel, MD; James C. Engert, PhD; Keith A.A. Fox, MD, PhD; Domenico Girelli, MD, PhD; Emil Hagström, MD, PhD; Stanley L. Hazen, MD, PhD; Claes Held, MD, PhD; Harry Hemingway, MD, PhD; Imo E. Hoefer, MD, PhD; G. Kees Hovingh, MD, PhD; Julie A. Johnson, PharmD; Pim A. de Jong, MD; J. Wouter Jukema, MD, PhD; Marcin P. Kaczor, MD, PhD; Mika Kähönen, PhD; Jiri Kettner, PhD; Marek Kliszczek, MD, PhD; Olaf H. Klungel, PharmD, PhD; Bo Lagerqvist, MD, PhD; Diether Lambrechts, PhD; Jari O. Laurikka, MD, PhD; Terho Lehtimäki, PhD; Daniel Lindholm, MD, PhD; Bakhtawar K. Mahmoodi, MD, PhD; Anke H. Maitland-van der Zee, PharmD, PhD; Ruth McPherson, MD, PhD; Olle Melander, MD, PhD; Andres Metspalu, MD, PhD; Witold Pepinski, MD, PhD; Oliviero Olivieri, MD; Grzegorz Opolski, MD, PhD; Colin N. Palmer, PhD; Gerard Pasterkamp, MD, PhD; Carl J. Pepine, MD; Alexandre C. Pereira, MD, PhD; Louise Pilote, MD; Arshed A. Quyyumi, MD; A. Mark Richards, MD, PhD; Marek Sanak, MD, PhD; Markus Scholz, PhD; Agneta Siegbahn, MD, PhD; Juha Sinisalo, MD, PhD; J. Gustav Smith, MD, PhD; John A. Spertus, MD, MPH; Alexandre F.R. Stewart, PhD; Wojciech Szczeklik, MD, PhD; Anna Szpakowicz, MD, PhD; Jurrien M. ten Berg, MD, PhD; George Thanassoulis, MD; Joachim Thiery, MD; Yolanda van der Graaf, MD; Frank L.J. Visseren, MD; Johannes Waltenberger; CARDIOGRAMPlusC4D Consortium; Pim Van der Harst, MD, PhD; Jean-Claude Tardif, MD; Naveed Sattar, PhD; Chim C. Lang, MD; Guillaume Pare, MD; James M. Brophy, MD; Jeffrey L. Anderson, MD; Winfried März, MD; Lars Wallentin, MD, PhD; Vicky A. Cameron, PhD; Benjamin D. Horne, PhD, MPH; Nilesh J. Samani, MD, PhD†; Aroon D. Hingorani, MD, PhD†; Folkert W. Asselbergs MD, PhD†

Correspondence

Riyaz S. Patel, MD, Institute of Cardiovascular Sciences, University College London, 222 Euston Rd, London, NW1 2DA, United Kingdom. Email riyaz.patel@ucl.ac.uk or Folkert W. Asselbergs, MD, PhD, Department of Cardiology, Divi-

sion of Heart & Lungs, University Medical Center Utrecht, 3508GA, Utrecht, the Netherlands, Email F.W.Asselbergs@umcutrecht.nl

Affiliations

Institute of Cardiovascular Science, Faculty of Population Health Science (R.S.P., A.F.S., L.J.H., K.D., J.D., A.D.H., F.W.A.) and Institute of Health Informatics, Faculty of Population Health Science, University College London, United Kingdom (N.F., C.H.S., A. Timmis, H.H., F.W.A.). Bart's Heart Centre, St Bartholomew's Hospital, London, United Kingdom (R.S.P., J.D., A. Timmis). Division Heart and Lungs, Department of Cardiology (A.F.S., V.T. D.K., F.W.A.), Laboratory of Experimental Cardiology (C.M.G., B.D.H.), Department of Clinical Chemistry and Hematology (B.G., I.E.H.), Department of Clinical Chemistry, UMC Utrecht, Netherlands (G. Pasterkamp). Intermountain Heart Institute, Intermountain Medical Center, Salt Lake City, UT (R.O.M., J.F.C., J.B.M., J.L.A.). Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health (M.V.H.), Medical Research Council Population Health Research Unit (M.V.H.), and National Institute for Health Research Oxford Biomedical Research Centre (M.V.H.), University of Oxford, United Kingdom. Uppsala Clinical Research Center (A.A., N.E., S.J., E.H., C.H., B.L., D. Lindholm, A. Siegbahn, L.W.), Department of Medical Sciences, Cardiology (A.A., E.H., C.H., D. Lindholm), Department of Medical Sciences, Cardiology (S.J., B.L., L.W.), and Department of Medical Sciences, Clinical Chemistry (A. Siegbahn), Uppsala University, Sweden. Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden (K.L., U.d.F.). Section of Cardiology, Michael E. DeBakey Veterans Affairs Medical Center, Section of Cardiovascular Research, and Department of Medicine, Baylor College of Medicine, Houston, TX (S.S.V., C.M.B.). Department of Population Medicine and Civilization Disease Prevention (K.A.K.), Department of Cardiology (K.A.K., A. Szpakowicz), and Department of Forensic Medicine, Medical University of Białystok, Poland (W.P., G.T.). Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital (M.H.), Harvard Medical School, Boston, MA (J.D.M., M.H. S.C.B.). Montreal Heart Institute (J.-C.T.) and Faculty of Medicine (J.-C.T.), Université de Montréal, QC, Canada (M.-P.D.). Departments of Preventive Medicine and Biochemistry and Molecular Medicine (H.A., J.H.) and Institute for Genetic Medicine (J.H.), Keck School of Medicine of USC, Los Angeles, CA. Department of Clinical Sciences, Lund University, Malmö, Sweden (P.A., O.M.). Estonian Genome Center, Institute of Genomics (A.M.) and Department of Biotechnology, Institute of Molecular and Cell Biology, University of Tartu, Estonia (M.A., A.M.). Division of Pharmacoepidemiology and Clinical Pharmacology (E.V.B., O.H.K., A.H.M.-v.d.z.), Department of Neurology and Neurosurgery, Brain Centre Rudolf Magnus and Julius Center for Health Sciences and Primary Care (A. Algra), Department of Radiology (P.A.d.J.), and Julius Center for Health Sciences and Primary Care (Y.v.d.G.), University Medical Center Utrecht, the Netherlands. Department of Vascular Medicine, University Medical Center Utrecht and Utrecht University, the Netherlands (F.L.J.V.). Centre for Outcomes Research and Evaluation, Research Institute of the McGill University Health Centre (H.B., L.D., L.P., J.M.B.). Research Institute of the McGill University Health Centre (J.C.E.). Division of Cardiology, Department of Medicine, Royal Victoria Hospital (J.C.E., G.T.), Department of Medicine (L.P., J.M.B.), and Preventive and Genomic Cardiology, McGill University Health Centre, Montreal, QC, Canada (L.D., J.C.E., G.T.). Laboratory for Translational Genetics, Department of Human Genetics (B.B., D. Lambrechts) and Department of Cardiovascular Sciences, KU Leuven, Belgium (F.V.d.W.). Laboratory for Translational Genetics, VIB Center for Cancer Biology, VIB, Belgium (B.B., D. Lambrechts). Department of Cardiovascular Sciences (P.S.B., C.P.N., N.J.S.) and Department of Health Sciences, University of Leicester, United Kingdom. BHF Cardiovascular Research Centre (F.D.) and National Institute of Health Research (NIHR) Leicester Biomedical Research Centre (P.S.B., C.P.N.), Glenfield Hospital, Leicester, United Kingdom. Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg (L.P.B., U.M., H.B.). Vth Department of Medicine, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany (G.D., M. Kleber, W.M.). Heart Institute, University of Sao Paulo, Brazil (N.E.D., A.C.P.). Institute for Biomedicine, Eurac Research, Affiliated Institute of the University of Lübeck, Bolzano, Italy (L.F.). Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics (Y.G., R.M.C.-D., J.A.J.), Division of Cardiovascular Medicine, College of Medicine, University of Florida (J.A.J., C.J.P.). Centre for Experimental Medicine, Institut for Clinical and Experimental Medicine, Prague, Czech Republic (J.A.H., J.P.). Department of Cardio-Thoracic Surgery (P.K.), Department of Clinical Chemistry (L.-P.L., T.L.), Department of Cardiology (K.N.), Department of Clinical Physiology (M. Kähönen), and Department of Cardio-Thoracic Surgery, Finnish Cardiovascular Research Center, Faculty of Medicine and Life Sciences, University of Tampere (J.O.L.). Department of Bio-

statistics and Epidemiology, Texas Heart Institute, Houston (V.-V.L.). Voralberg Institute for Vascular Investigation and Treatment (VIVIT), Feldkirch, Austria (A.L., C.H.S., H.D.). Private University of the Principality of Liechtenstein, Triesen, Liechtenstein (A.L., C.H.S., H.D.). Medical Central Laboratories, Feldkirch, Austria (A.L.). Department of Genetics, Statistical Genomics Division (P.A.L., S.C.) and Department of Medicine, Cardiovascular Division Washington University School of Medicine, St Louis, MO (S.C.). Division of Molecular and Clinical Medicine, School of Medicine, University of Dundee, Scotland, United Kingdom (D. Levin, I.R.M., C.C.L.). Department of Clinical Chemistry, Fimlab Laboratories, Tampere, Finland (L.-P.L., T.L.). Department of Medicine, University of Verona, Italy (N. Martinelli, D.G., O.O.). Department of Cardiology, Heart Center (K.N.), Department of Clinical Physiology (M. Kähönen), Department of Cardio-Thoracic Surgery, Heart Center, Tampere University Hospital, Finland (J.O.L.). The Christchurch Heart Institute, University of Otago Christchurch, New Zealand (A.P.B., A.M.R., V.A.C.). Department of Medical Genetics (R.P.) and Department of Cardiology, Medical University of Warsaw, Poland (G.O.). Department of Epidemiology, Emory University Rollins School of Public Health (Y.V.S.), Department of Biomedical Informatics (Y.V.S.), and Division of Cardiology, Department of Medicine, Emory Clinical Cardiovascular Research Institute, Emory University School of Medicine, Atlanta, GA (A.S.-T., P.B.S., A.A.Q.). Clinical Epidemiology and Biostatistics (M.W.T.T.) and AMC Heart Center (A.A.M.W., C.R.B.), Clinical and Experimental Cardiology, Amsterdam Cardiovascular Sciences, Amsterdam UMC, Department of Respiratory Medicine, Academic Medical Center, University of Amsterdam, the Netherlands (A.H.M.-v.d.z.). Department of Cellular and Molecular Medicine, Lerner Research Institute (W.H.W.T., S.L.H.), Department of Cardiovascular Medicine, Heart and Vascular Institute and Center for Clinical Genomics (W.H.W.T.), and Department of Cardiovascular Medicine, Heart and Vascular Institute and Center for Microbiome and Human Health, Cleveland Clinic, OH (S.L.H.). Section of Gerontology and Geriatrics, Department of Internal Medicine (S.T.), and Department of Cardiology (S.T., J.W.J.), Leiden University Medical Center. Laboratory of Clinical Chemistry and Hematology, Division Laboratories, Pharmacy, and Biomedical Genetics (S.W.v.d.L.), Department of Vascular Surgery, University Medical Center Utrecht, University Utrecht, the Netherlands (G.J.d.B.). Durrer Centre of Cardiogenetic Research, ICIN-Netherlands Heart Institute, Netherlands (J.v.S., F.W.B.). Ruddy Canadian Cardiovascular Genetics Centre (R.O.V., A.F.R.S.), University of Ottawa Heart Institute (R.M.), Department of Biochemistry, Microbiology and Immunology (R.O.V., A.F.R.S.), and Departments of Medicine and Biochemistry, Microbiology and Immunology, University of Ottawa, ON, Canada (R.M.). Department of Cardiovascular Medicine, Humanitas Clinical and Research Center, Milan, Italy (C.V.A., G.C.). Transplantation Laboratory, Medicum (E.V., M.-L.L.), Heart and Lung Center, Helsinki University Hospital University of Helsinki, Finland (J.S.). University of Texas School of Public Health, Houston (E.B.). Clinica Mediterranea, Naples, Italy (C.B.). Cardiology Division, Department of Internal Medicine (J.F.C., J.B.M., J.L.A.), Department of Biomedical Informatics, University of Utah, Salt Lake City (B.D.H.). Cardiovascular Sciences (K.F.C.) and Emeritus Professor of Cardiology (K.A.A.F.), University of Edinburgh. ATS Sardegna, ASL 3, Nuoro (G. Casu, N. Marziliano). William Harvey Research Institute, Barts and the London Medical School (P.D.) and Centre for Genomic Health (P.D.), Queen Mary University of London. Centro de Pesquisa Clinica, Hospital Universitario, Universidade de Sao Paulo, Brazil (P.A.L.). Cardiovascular Research Center and Center for Human Genetic Research, Massachusetts General Hospital, Boston and Program in Medical and Population Genetics, Broad Institute, Cambridge, MA (C.N.C.). Department of Medicine and Cardiology, Academic Teaching Hospital Feldkirch, Austria. Heart Center Leipzig (A. Teren), LIFE Research Center for Civilization Diseases (A. Teren, R.B., M. Scholz, J.T.), and Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Germany (M. Scholz). Respiratory Oncology Unit, Department of Respiratory Medicine, University Hospitals KU Leuven, Belgium (E.W.). Princess Al-Jawhara Al-Brahim Centre of Excellence in Research of Hereditary Disorders, Jeddah, Saudi Arabia (A.A.M.W.). Robertson Center for Biostatistics (I.F.) and Institute of Cardiovascular and Medical Sciences, University of Glasgow, United Kingdom (D.J.S., N.S.). CNR Institute of Clinical Physiology, Pisa, Italy (M.G.A., C.C.). Cardiology Department, Parma University Hospital, Italy (D.A.). Centre de recherche de l'Institut Universitaire de cardiologie et de pneumologie de Québec (B.J.A.) and Department of Medicine, Faculty of Medicine, Université Laval, Canada (B.J.A.). St. Antonius Hospital, Department of Cardiology, Nieuwegein, the Netherlands (T.O.B., B.K.M., J.M.t.B.). Department of Anesthesia, Pain and Critical Care, Beth Israel Deaconess Medical Center, Boston, MA (S.C.B.). Service de cardiologie, Département multidisciplinaire de cardiologie, Institut universitaire de cardiologie et de pneumologie de Québec, Canada (P.B.). Unité d'évaluation cardiovasculaire, Institut national d'excellence en santé et en services sociaux (INESSS), Montreal Canada (P.B.). Institut universitaire de

cardiologie et de pneumologie de Québec, Laval University, Québec City, Canada (P.B.). Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany (R.B.). Department of Biomedical Sciences, Humanitas University, Milan, Italy (G. Condorelli). Heart Health Research Group, University of Auckland, New Zealand (R.N.D.). Drexel University College of Medicine, Philadelphia, PA (H.D.). Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands (G.K.H.). Einthoven Laboratory for Experimental Vascular Medicine, LUMC, Leiden (J.W.J.). Inter-university Cardiology Institute of the Netherlands, Utrecht, the Netherlands (J.W.J.). Department of Internal Medicine, Jagiellonian University Medical College, Kraków, Poland (M.P.K., M. Sanak, W.S.). Cardiology Centre, Institute for Clinical and Experimental Medicine, Prague, Czech Republic (J.K.). Department of Cardiology and Internal Diseases, Military Institute of Medicine, Warsaw, Poland (M. Kiliszek). Department of Internal Medicine, Skåne University Hospital, Malmö, Sweden (O.M.). Pat Macpherson Centre for Pharmacogenetics and Pharmacogenomics, Division of Molecular and Clinical Medicine, Ninewells Hospital and Medical School, Dundee (C.N.P.). Cardiovascular Research Institute, National University of Singapore (A.M.R.). Department of Cardiology, Clinical Sciences, Lund University and Skåne University Hospital (J.G.S.) and Wallenberg Center for Molecular Medicine (J.G.S.), Lund University Diabetes Center (J.G.S.), Lund University, Lund, Sweden. Saint Luke's Mid America Heart Institute and the University of Missouri-Kansas City and Saint Luke's Health System, Kansas City, MO (J.A.S.). Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital, Leipzig (J.T.). Department of Cardiovascular Medicine, University of Münster, Germany (J.V.). CARDIoGRAMPlusC4D. University of Groningen, University Medical Center, Groningen, Netherlands (P.v.d.H.). Department of Pathology and Molecular Medicine, McMaster University (G. Pare). Population Health Research Institute, Hamilton, ON, Canada (G. Pare). Synlab Academy, Synlab Holding Deutschland GmbH, Mannheim, Germany (W.M.). Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Austria (W.M.).

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