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New susceptibility locus for coronary artery disease on chromosome 3q22.3

Jeanette Erdmann[1], **Anika Großhennig**[1,2], **Peter S Braund**[3], **Inke R König**[2], **Christian Hengstenberg**[4], **Alistair S Hall**[5], **Patrick Linsel-Nitschke**[1], **Sekar Kathiresan**[6], **Ben Wright**[7], **David-Alexandre Trégouët**[8], **Francois Cambien**[8], **Petra Bruse**[1], **Zouhair Aherrahrou**[1], **Arnika K Wagner**[1], **Klaus Stark**[4], **Stephen M Schwartz**[9], **Veikko Salomaa**[10], **Roberto Elosua**[11], **Olle Melander**[12], **Benjamin F Voight**[13], **Christopher J O'Donnell**[14], **Leena Peltonen**[15], **David S Siscovick**[9], **David Altshuler**[16], **Piera Angelica Merlini**[17], **Flora Peyvandi**[18], **Luisa Bernardinelli**[19,20], **Diego Ardissino**[21], **Arne Schillert**[2], **Stefan Blankenberg**[22], **Tanja Zeller**[22], **Philipp Wild**[22], **Daniel F Schwarz**[2], **Laurence Tiret**[8], **Claire Perret**[8], **Stefan Schreiber**[23], **Nour Eddine El Mokhtari**[23], **Arne Schäfer**[23], **Winfried Maärz**[24,25,26], **Wilfried Renner**[25], **Peter Bugert**[27], **Harald Klüter**[27], **Jürgen Schrezenmeir**[28], **Diana Rubin**[28], **Stephen G Ball**[5], **Anthony J Balmforth**[5], **H-Erich Wichmann**[29,30], **Thomas Meitinger**[31,32], **Marcus Fischer**[4], **Christa Meisinger**[29], **Jens Baumert**[29], **Annette Peters**[29], **Willem H Ouwehand**[33], **Italian Atherosclerosis, Thrombosis, and Vascular Biology Working Group**[34], **Myocardial Infarction Genetics Consortium**[34], **Wellcome Trust Case Control Consortium**[34], **Cardiogenics Consortium**[34], **Panos Deloukas**[15], **John R Thompson**[7], **Andreas Ziegler**[2], **Nilesh J Samani**[3], and **Heribert Schunkert**[1]

[1]Medizinische Klinik II, Universitaät zu Lübeck, 23538 Lübeck, Germany [2]Institut für Medizinische Biometrie und Statistik, Universitaät zu Lübeck, 23538 Lübeck, Germany [3]Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, LE3 9QP, UK [4]Klinik und Poliklinik für Innere Medizin II, Universitaät Regensburg, 93053 Regensburg, Germany [5]LIGHT, University of Leeds, Leeds, LS1 3EX, UK [6]Cardiovascular Research Center and Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA [7]Departments of Health Sciences and Genetics, University of Leicester, LE1 7RH Leicester, UK [8]Institut National de la Santé et de la Recherche Médicale (INSERM) Unité Mixte de Recherche UMR_S 525, Université Pierre et Marie Curie (UPMC) Univ. Paris 06, Paris, France [9]Cardiovascular Health Research Unit, Departments of Epidemiology and General Medicine, University of Washington, Seattle, Washington 98109, USA [10]Chronic Disease Epidemiology Unit, Department of Health Promotion and Chronic Disease Prevention, National Public Health Institute, FI-00271 Helsinki, Finland [11]Cardiovascular Epidemiology and Genetics

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Correspondence should be addressed to J.E. (j.erdmann@cardiogenics.eu)..
[34]A full list of members is provided in the Supplementary Note online.

AUTHOR CONTRIBUTIONS The study was designed by H.S., N.J.S., A.Z., I.R.K., J.R.T. and J.E. Subject ascertainment, recruitment and medical record review was organized and carried out by C.H., P.L.-N. and M.F. DNA material collection, handling and genotyping (Affymetrix 500K, 6.0, and TaqMan) was supervised by P.D., T.M., P. Bruse, W.H.O., P.S.B., K.S., C.P. and A. Schaäfer Statistical analysis was carried out by A.G., D.F.S., I.R.K., B.W., A. Schillert, D.-A.T., J.R.T. and A.Z. RT-PCRs were carried out by Z.A. and A.K.W., J.E., N.J.S and H.S. drafted the manuscript with substantial contributions from I.R.K, A.G. and A.Z. Principal collaborators for the case cohorts were W.M. and W.R. (LURIC), H.K. and P. Bugert (GerBS), P.L.-N. (Angio-Luebeck), N.E.M., S.S., J.S. and D.R. (PopGen), H.-E.W., T.M., C.M., A.P., and J.B. (KORA), N.J.S., A.S.H., S.G.B., P.S.B. and A.J.B. (UKMI), C.H., M.F., K.S. (GO-KARD), S.K., D. Altshuler, B.F.V., D. Ardissino, O.M., C.J.ÓD., R.E., V.S., L.P., D.S.S. and S.M.S. (MIGen), P.A.M., F.P., L.B. and D. Ardissino (IATVB), S.B., T.Z. and P.W. (Atherogene), L.T., C.P. and F.C. (ECTIM). All authors contributed to the final version of the manuscript.

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Group, Institut Municipal d'Investigació Mèdica, Barcelona 08036; Ciber Epidemiología y Salud Pública (CIBERSP), Spain [12]Department of Clinical Sciences, Hypertension and Cardiovascular Diseases, University Hospital Malmö, Lund University, SE-20502 Malmö, Sweden [13]Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts 02114, USA [14]National Heart, Lung, and Blood Institute, National Institutes of Health, Framingham, Bethesda, Maryland 20824, USA [15]The Wellcome Trust Sanger Institute, The Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK [16]Department of Molecular Biology and Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA [17]Division of Cardiology, Azienda Ospedaliera Niguarda Ca` Granda, 20162 Milan, Italy [18]Department of Internal Medicine and Medical Specialities, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico, Ospedale Maggiore, Mangiagalli e Regina Elena, University of Milan, 20162 Milan, Italy [19]Department of Applied Health Sciences, University of Pavia, 27100 Italy [20]Biostatistics Unit, Medical Research Council, CB2 0SR Cambridge, UK [21]Division of Cardiology, Azienda Ospedaliero-Universitaria di Parma, 43100 Parma, Italy [22]Department of Medicine II, Johannes Gutenberg-University 55099 Mainz, Germany [23]Institut für Klinische Molekularbiologie, Christian-Albrechts Universitaät, 24118 Kiel, Germany [24]Synlab Centre of Laboratory Diagnostics Heidelberg, 69037 Heidelberg, Germany [25]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, 8036 Graz, Austria [26]Department of Public Health, Social and Preventive Medicine, University of Heidelberg 69117 Heidelberg, Medical Faculty of Mannheim, 68131 Mannheim, Germany [27]Institute of Transfusion Medicine and Immunology, University of Heidelberg 69117 Heidelberg, Medical Faculty of Mannheim, 68131 Mannheim, Germany [28]Bundesforschungsanstalt für Ernaährung und Lebensmittel, Institut für Physiologie und Biochemie der Ernaährung, 24118 Kiel, Germany [29]Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, 85764 Neuherberg, Germany [30]Institute of Medical Information Science, Biometry and Epidemiology, LMU 80539 Munich, Germany [31]Institut für Humangenetik, Helmholtz Zentrum München, Deutsches Forschungszentrum für Umwelt und Gesundheit, 85764 Neuherberg, Germany [32]Institut für Humangenetik, Technische Universitaät München, 80333 München, Germany [33]Department of Haematology, University of Cambridge, Long Road, Cambridge, CB2 2PT, UK

Abstract

We present a three-stage analysis of genome-wide SNP data in 1,222 German individuals with myocardial infarction and 1,298 controls, *in silico* replication in three additional genome-wide datasets of coronary artery disease (CAD) and subsequent replication in ~25,000 subjects. We identified one new CAD risk locus on 3q22.3 in *MRAS* ($P = 7.44 \times 10^{-13}$; OR = 1.15, 95% CI = 1.11–1.19), and suggestive association with a locus on 12q24.31 near *HNF1A-C12orf43* ($P = 4.81 \times 10^{-7}$; OR = 1.08, 95% CI = 1.05–1.11).

Recent genome-wide association studies (GWAS) of coronary artery disease (CAD) have focused on a few chromosomal regions with strong signals1-4. We hypothesized that the application of stringent statistical thresholds may have dismissed SNPs with modest effects or low allele frequencies (Supplementary Fig. 1 and Supplementary Table 1 online). For this study, we started r by identifying SNPs meeting a less-stringent cutoff for association ($P \leq 1 \times 10^{-3}$) in a new GWAS for myocardial infarction.

In stage 1, we genotyped 869,224 autosomal SNPs from the Affymetrix Genome-Wide Human SNP Array 6.0 in 1,222 myocardial infarction cases (German MI Family Study II (GerMIFS II); Supplementary Methods online) with premature disease onset and positive family history and 1,298 population-based Germans of European descent. After quality

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control (Supplementary Fig. 2 online), 567,119 SNPs remained. The inflation factor estimated for all SNPs that passed quality control is 1.04 (s.e.m. = 9.2×10^{-5}). Of these, 694 SNPs showed association with myocardial infarction at $P \leq 1 \times 10^{-3}$ in a two-sided trend test. These SNPs were evaluated by *in silico* analysis in three additional GWAS datasets (stage 2), comprising a total of 5,768 cases and 7,657 controls (WTCCC CAD study4, GerMIFS I1 and Myocardial Infarction Genetics Consortium5 together with the Italian Atherosclerosis, Thrombosis, and Vascular Biology Working Group6; the latter two form a single data source and are grouped together (MIGen/IATVB); Supplementary Methods). SNPs that were selected for large-scale replication all met the following two criteria: (i) the same allele was associated in the same direction as in the exploratory GWAS in at least two of the three *in silico* GWAS datasets using a one-sided trend test ($P \leq 0.05$, Table 1), and (ii) the SNP had nearby correlated SNPs (within 25 kb) that also showed a signal (one-sided trend test $P \leq 0.05$). A total of 21 SNPs met these criteria. They clustered into five chromosomal regions: two regions known to be associated with CAD (9p21.3 and 1q41)1-4,7 and three previously unreported regions (3q22.3, 9p24.2 and 12q24.31; Supplementary Table 2 online).

In stage 3, we evaluated the lead SNPs of the three previously unreported loci in 12,417 cases and 12,411 controls. Two loci, represented by rs9818870 in the *MRAS* gene and rs2259816 in the *HNF1A-C12orf43* region, showed replication in this stage at $P = 2.69 \times 10^{-7}$ and $P = 0.0277$, respectively, when adjusted for study and multiple testing. Results from stages 1–3 are displayed in Table 1 and Supplementary Figure 3 online.

Our rationale for these three stages was to be liberal in terms of a broad inclusion of SNPs at stage I and conservative with respect to a stringent replication strategy in stages 2 and 3. To determine the genuineness of effects in an efficient manner and to eliminate inconsistent findings, we carried out the second stage using *in silico* replication. The final stage served as a test for significance while adjusting for multiple testing. Power analyses showed that this three-staged strategy detects relevant effects with high probability (Supplementary Methods).

SNP rs9818870 in *MRAS* on 3q22.3 showed an aggregate $P = 7.44 \times 10^{-13}$ (OR = 1.15; 95% CI = 1.11–1.19), whereas SNP rs2259816 located in the *HNF1A-C12orf43* region on 12q24.31 showed an aggregate $P = 4.81 \times 10^{-7}$ (OR = 1.08; 95% CI = 1.05–1.11) in 19,407 cases and 21,366 controls, respectively. Both SNPs met the formal criterion for genome-wide significance of 5×10^{-7} suggested by the WTCCC4 that has been applied to several GWAS. However, SNP rs2259816 in *HNF1A-C12orf43* failed to reach the more stringent threshold of 7.2×10^{-8} suggested for an infinitely dense SNP map8.

To further explore the association between SNPs in *MRAS* and *HNF1A-C12orf43*, we tested whether the lead SNPs were associated with traditional risk factors for CAD in 3,276 controls from MONICA/KORA Augsburg survey S4; however, no significant association was found (Supplementary Table 3 online). In addition, we carried out exploratory subgroup analyses to test for interaction (Supplementary Fig. 4 online).

SNP rs9818870 is located in the 3′ UTR of *MRAS* in close proximity to a cluster of miRNA binding sites. This SNP is among a cluster of four associated SNPs (rs1199338, rs2347252, rs3732837, rs9818870) that are in a block of strong LD covering the whole gene (Fig. 1a), which consists of five exons and is ~33 kb in size (MIM608435). The M-ras protein belongs to the ras superfamily of GTP-binding proteins and is widely expressed in all tissues, with a very high expression in the cardiovascular system, especially in the heart (SymAtlas). Previous work has shown that M-ras is involved in TNF-α-stimulated LFA-1 activation in

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splenocytes by using mice deficient in this process9. These findings suggest a role for M-ras in adhesion signaling, which is important in the atherosclerotic process10.

SNP rs2259816 (representing the locus on 12q24.31) is located in intron 7 of *HNF1A*. This and two further associated SNPs cluster (rs1169313 and rs2258287) are in a LD block that covers the coding region of *HNF1A* and *C12orf43* (Fig. 1b). *HNF1A* (also known as *TCF1*; MIM142410) encodes a transcription factor that binds to promoters of a variety of genes that are expressed exclusively in the liver11. Variants in *HNF1A* may cause maturity-onset diabetes of the young (MODY) (MIM600496) and affect plasma concentrations of C-reactive protein (CRP), a powerful risk marker for cardiovascular disease12. Moreover, and in parallel to the present work, a risk allele at the *HNF1A* locus (rs2258287) has been mapped to higher plasma levels of low-density lipoprotein cholesterol13. SNPs rs1169313 and rs2258287 are located within the *C12orf43* gene (alias FLJ12448, hypothetical protein LOC64897), which is located downstream of *HNF1A* in a tail-to-tail manner, with only 500 bp in between (Fig. 1b). RT-PCR studies showed that *MRAS* and *C12orf43* are ubiquitously expressed, including in mouse and human aorta and heart, tissues that are potentially involved in atherosclerosis (Supplementary Table 4 and Supplementary Fig. 5 online).

In conclusion, analysis of a new GWAS followed by *in silico* replication in three GWAS datasets and a large-scale replication study identified one new susceptibility locus for CAD on 3q22.3 with compelling statistical evidence and a second locus on 12q24.31 with suggestive evidence. Further functional work is needed to define the mechanisms by which these loci translate into a higher risk of CAD, and whether this information can be used to improve prevention, prediction or treatment of this common condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Samani NJ, et al. N. Engl J. Med. 2007; 357:443–453. [PubMed: 17634449]
2. McPherson R, et al. Science. 2007; 316:1488–1491. [PubMed: 17478681]
3. Helgadottir A, et al. Science. 2007; 316:1491–1493. [PubMed: 17478679]
4. Wellcome Trust Case Control Consortium. Nature. 2007; 447:661–678. [PubMed: 17554300]

5. Kathiresan S, Myocardial Infarction Genetics Consortium. N. Engl. J. Med. 2008; 358:2299–2300. [PubMed: 18499582]
6. Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group. Circulation. 2003; 107:111–1122.
7. Schunkert H, et al. Circulation. 2008; 117:1675–1684. [PubMed: 18362232]
8. Dudbridge F, Gusnanto A. Genet. Epidemiol. 2008; 32:227–234. [PubMed: 18300295]
9. Yoshikawa Y, et al. Mol. Biol. Cell. 2007; 18:2949–2959. [PubMed: 17538012]
10. Galkina E, Ley K. Arterioscler. ThrombVasc. Biol. 2007; 27:2292–2301.
11. Courtois G, Morgan JG, Campbell LA, Fourel G, Crabtree GR. Science. 1987; 238:688–692. [PubMed: 3499668]
12. Reiner AP, et al. Am. J. Hum. Genet. 2008; 82:1193–1201. [PubMed: 18439552]
13. Kathiresan S, et al. Nat. Genet. 2009; 41:56–65. [PubMed: 19060906]
14. McVean GA, et al. Science. 2004; 304:581–584. [PubMed: 15105499]

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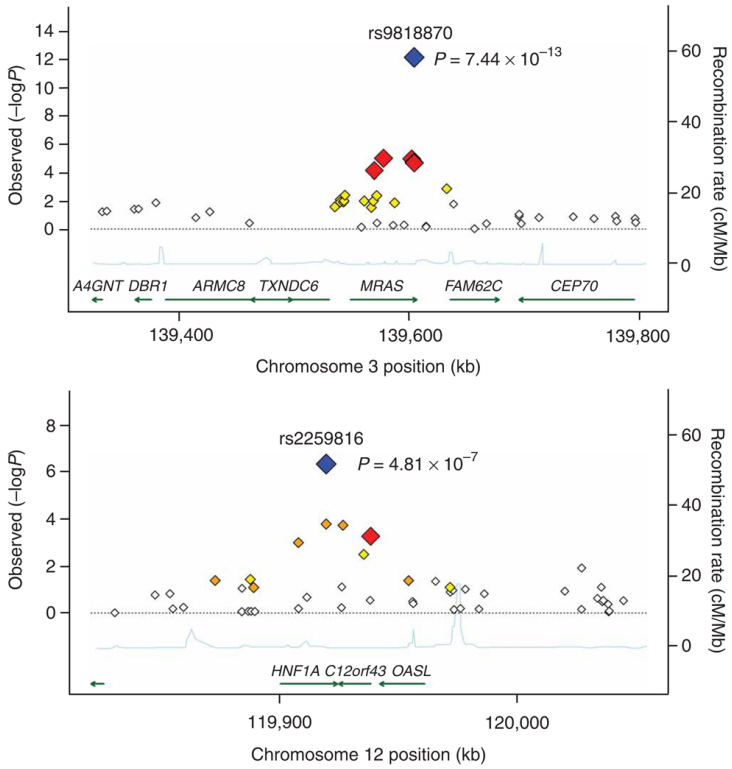

Figure 1. Association results from Stage 1

(**a**) 3q22.3 locus. (**b**) 12q24.31 locus. Genomic positions refer to the UCSC Genome Browser Human March 2006. Presented are SNPs that passed quality control in stage 1. The GWAS *P* value for the lead SNPs (rs9818870 (3q22.3), rs2259816 (12q24.31)) is denoted by a red diamond. Blue diamonds indicate *P* values for the lead SNPs in the replication sample. Proxies are indicated with diamonds of smaller size, with colors determined from their pairwise r^2 from HapMap CEU (red: high LD with lead SNP ($r^2 > 0.8$); orange: moderate LD with lead SNP ($0.5 < r^2 < 0.8$); yellow: weak LD with lead SNP ($0.2 < r^2 < 0.5$); white: no LD with the lead SNP ($r^2 < 0.2$) or no information available). The locations of the UCSC genes in the region and recombination rates and hot spots as defined previously14 are shown.

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Table 1

Association results for the three new candidate CAD risk loci identified in stage 1, represented by the corresponding lead SNPs

			rs9818870				rs7048915				rs2259816			
	Number of		MAF (%)				MAF (%)				MAF (%)			
	Cases	Controls	Cases	Controls	*P* value	OR (CI)	Cases	Controls	*P* value	OR (CI)	Cases	Controls	*P* value	OR (CI)
Stage 1[a]														
GerMIFS II	1,222	1,298	19.5	15.0	2.38×10^{-5}	1.37 (1.18,1.59)	22.8	27.1	0.0004	0.79 (0.69,0.90)	40.4	35.3	0.0002	1.25 (1.11,1.40)
Stage 2[b]														
WTCCC	1,926	2,938	17.0	15.1	0.0048	1.16 (1.05,1.27)	21.6	23.4	0.0201	0.90 (0.82,0.98)	36.3	34.3	0.0215	1.09 (1.02,1.17)
MIGen/ IATVB	2,967	3,075	14.8	13.7	0.0390	1.10 (1.01,1.19)	27.5	29.0	0.0302	0.93 (0.87,0.99)	40.2	38.0	0.0066	1.10 (1.03,1.17)
GerMIFS I	875	1,644	18.6	16.5	0.0427	1.15 (1.01,1.31)	23.4	25.9	0.0185	0.87 (0.78,0.97)	38.1	34.9	0.0118	1.15 (1.04,1.27)
Pooled	5,768	7,657	16.1	14.8	0.0002	1.13 (1.07,1.19)	25.0	26.3	0.0004	0.91 (0.87,0.95)	38.6	35.9	5.89×10^{-5}	1.10 (1.06,1.15)
Stage 3[c]														
Population -based MIs	2,737	4,469	17.4	15.0	0.0004	1.20 (1.10,1.31)	26.0	27.2	0.1520	0.94 (0.85,1.04)	37.5	35.4	0.0106	1.10 (1.03,1.17)
Hospital-based Mis	772	734	18.9	13.5	2.85×10^{-5}	1.52 (1.28,1.81)	26.4	26.3	0.4879	1.00 (0.87,1.15)	33.7	35.2	0.2002	0.94 (0.83,1.06)
Angio graphic CAD	8,908	7,208	17.7	16.3	0.0008	1.10 (1.05,1.15)	25.3	25.2	0.1915	1.02 (0.98,1.07)	36.5	36.0	0.0410	1.04 (1.00,1.08)
Pooled	12,417	12,411	17.7	15.9	2.69×10^{-7}	1.14 (1.08,1.20)	25.5	25.5	0.9999	1.01 (0.96,1.06)	36.6	35.8	0.0277	1.05 (1.00,1.09)
All stages[d]	19,407	21,366	17.3	15.4	7.44×10^{-13}	1.15 (1.11,1.19)	25.1	25.9	0.0073	0.95 (0.92,0.99)	37.4	35.8	4.81×10^{-7}	1.08 (1.05,1.11)

[a] Stage 1: *P* value, odds ratio (OR), 95% confidence interval (95% CI) from two-sided trend test (CAT).

[b] Stage 2: single study analysis: *P* value, OR, 90% CI from one-sided CAT; pooled analysis: *P* value, OR, 90% CI from one-sided Cochran-Mantel-Haenszel test (CMH).

[c] Stage 3: population-based myocardial infarction studies: *P* value, OR, 90% CI from one-sided CMH (for rs7048915 only one study: one-sided CAT); hospital-based myocardial infarction studies: *P* value, OR, 90% CI from one-sided CAT; angiographic CAD studies: *P* value, OR, 90% CI from fixed-effect logistic regression models (FELRM) with adjustments for study and multiple testing.

[d] All stages: *P* value, OR, 95% CI from (FELRM) with adjustments for study.