

# A decade of genome-wide association studies for coronary artery disease: the challenges ahead

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## Abstract

In this review, we summarize current knowledge on the genetics of coronary artery disease, based on 10 years of genome-wide association studies. The discoveries began with individual studies using 200K single nucleotide polymorphism arrays and progressed to large-scale collaborative efforts, involving more than a 100 000 people and up to 40 Mio genetic variants. We discuss the challenges ahead, including those involved in identifying causal genes and deciphering the links between risk variants and disease pathology. We also describe novel insights into disease biology based on the findings of genome-wide association studies. Moreover, we discuss the potential for discovery of novel treatment targets through the integration of different layers of 'omics' data and the application of systems genetics approaches. Finally, we provide a brief outlook on the potential for precision medicine to be enhanced by genome-wide association study findings in the cardiovascular field.

## Keywords

Coronary artery disease • Atherosclerosis • Genome-wide association studies • Genetics • Post-GWAS

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## 1. Introduction

Coronary artery disease (CAD) is a leading cause of death worldwide.<sup>1</sup> It represents the manifestation of atherosclerosis in the coronary arteries, which supply the myocardium with oxygen and other nutrients.

Atherosclerosis is an inflammatory process that is strongly driven by lipid accumulation during which the coronary artery wall thickens, forming a plaque, eventually leading to reduced blood flow and myocardial ischaemia.<sup>2</sup> The major sequelae of CAD include angina, myocardial infarction (MI), arrhythmias, heart failure, and sudden cardiac death. CAD has a complex aetiology, and various environmental factors, including cigarette smoking, sedentary lifestyle, unhealthy diet and obesity, and disease predisposition.<sup>3</sup> These lifestyle-related factors can lead to type 2 diabetes, hypercholesterolaemia and arterial hypertension, which are key risk factors for CAD. In addition, the influence of heritability on CAD susceptibility has been recognized for many years<sup>4</sup> and accounts for 40–50% of cases.<sup>5</sup> Although the inherited CAD risk is particularly evident in large families with multiple affected members,<sup>6</sup> decade ago only mutations in the *LDL receptor* gene were reproducibly linked to the disease.

However, with the emergence of genome-wide association studies (GWAS), large number of common variants displayed strongly reproducible yet small effects and substantially broadened the spectrum of genetic factors recognized as contributing to CAD aetiology.<sup>3,7</sup>

## 2. In retrospect: 10 years of CAD GWAS

### 2.1 Laying the foundation for GWAS

During the period when candidate gene studies on small case–control samples often produced controversial findings, novel chip designs and decreasing costs facilitated the development of genome-wide genotyping. In parallel, statistical methods (e.g. imputation and haplotype tagging) were improving and international consortia were established to conduct large-scale GWAS on CAD.

One imperative technological breakthrough that aided the rapid success of GWAS was the completion of the International HapMap (short

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for haplotype map) Project in 2007, and the public availability of the resulting data,<sup>8–10</sup> which allowed the mapping of haplotype landscapes of SNPs in three continental populations. Eight years later, in 2015, the 1000 Genomes Project<sup>11</sup> took advantage of more affordable sequencing technologies and released freely available human genetic variation data, based on low-coverage whole-genome sequencing, reaching a pinnacle with the 1000GP3 reference panel. Recently, the Haplotype Reference Consortium (HRC)<sup>12,13</sup> combined all whole-genome sequencing data sets into a single haplotype reference panel to facilitate genotype imputation. Promisingly, the HRC reference panel markedly improved the concordance between assayed and imputed genotypes for low-frequency variants. This advance has permitted significant clarification of association signals, particularly for suggestive variants,<sup>12</sup> thereby outperforming 1000GP-based imputation concordance and final *P*-value results.

## 2.2 From single GWAS to large-scale collaborative research

GWAS for CAD/MI have generated numerous successful outcomes (Figure 1), beginning in 2007 with the discovery of the chromosome 9p21 risk locus by four independent research groups.<sup>14–17</sup> By 2009, twelve additional genetic risk variants had been discovered by several individual GWAS.<sup>18–20</sup> Subsequently, large GWAS consortia were formed and eventually merged, ultimately analysing hundreds of thousands of individuals, for example, the Coronary ARtery Disease Genome-wide Replication and Meta-analysis (CARDIoGRAM) Consortium,<sup>21</sup> the MI Gen Consortium,<sup>18</sup> the Coronary Artery Disease (CAD) Genetics Consortium,<sup>22</sup> and, more recently, the UK Biobank (UKBB).<sup>23</sup> In 2011 and 2013, the CARDIoGRAM (plus C4D) consortia reported 25 and 46 loci associated with CAD, respectively, both confirming previously published variants and identifying new associations.<sup>24,25</sup> Interestingly, earlier this year, three studies that analysed CAD data from the first UKBB data release, CARDIoGRAMplusC4D and other smaller studies<sup>26–28</sup> each found 13, 14, and 15 new loci associated with CAD, of which seven overlapped between the three studies. Subtle differences in study design (mainly phenotype definition) may explain the moderate overlap between studies. Very recently, by analysing the full UKBB data set (34 541 CAD cases and 261 984 controls) and using CARDIoGRAMplusC4D 1000 G data for replication an additional 64 novel CAD risk loci were reported.<sup>29</sup> Altogether, 163 loci have now been associated with CAD at a genome-wide level of significance (Table 1; Supplementary material online, Table S1; Figure 2). Most CAD risk variants have been discovered under an additive model of inheritance; however, for 25% of all CAD risk variants it seems that a dominant (26 SNPs) or a recessive model (12 SNPs) might offer a better fit.<sup>29</sup> In another analysis, only one CAD risk variant (rs11830157 tagging *KSR2* gene) showed genome-wide significance in a recessive model.<sup>30</sup> A more comprehensive study about the recessive component of the inheritance of CAD has been performed recently by studying runs of homozygosity (ROH). Christofidou et al.<sup>31</sup> reported an excess of homozygosity in CAD and suggested an important role of ROH in the pathogenesis of atherosclerosis.

Although most of the 163 loci represent the effects of common alleles, studies with large sample sizes also have the potential to identify low-frequency variants that are associated at the required level of significance. Nevertheless, a previous large-scale exome-wide study including more than 120 000 participants only had 80% power to detect an OR of  $\pm 2.0$  for CAD-associated variants with a minor allele frequency (MAF) of 0.1%.<sup>32</sup> Nevertheless, it did identify two low-frequency non-synonymous genetic variants associated with CAD risk.<sup>33</sup>

Modern GWAS arrays and subsequent imputation cover the human autosomal genome quite well, but the X- and Y-chromosomes have been somewhat neglected due to specific analytical challenges, such as unequal gene dosage in males and females and X-chromosome inactivation.<sup>34,35</sup> Recommendations how to include the X-chromosome have recently been published<sup>36</sup> but a first GWAS for CAD for X-chromosomal variants in more than 43 000 CAD cases and 58 000 controls from 35 international study cohorts revealed no genome-wide significant association for any variant<sup>37</sup> which may change when larger samples are being studied.<sup>38</sup> Lately, a study on the human Y-chromosome has shown that genetic variation within the male-specific region of the Y-chromosome confers risk for CAD, confirming the notion that the increased risk for CAD in men cannot be fully explained through common autosomal genetic risk factors.<sup>39</sup>

## 2.3 Knowledge revealed by GWAS to date

### 2.3.1 Contribution of common genetic variants to CAD

As stated earlier, GWAS have identified 163 genetic loci associated with CAD risk at a genome-wide significance level after Bonferroni correction (Table 1; Supplementary material online, Table S1). Over 300 additional loci are suggestive for CAD risk, as they have false discovery rate values below 5%.<sup>25,28</sup> These loci may be useful for improved prediction of CAD risk and understanding of the biology underlying the disease.<sup>28</sup> The majority of these loci is represented by common variants with an MAF of >5% and is associated with modest increases in CAD risk.<sup>37</sup> Combined, these loci explain roughly 30–40% of CAD heritability, which is estimated to account for approximately 40% of all cases.<sup>28</sup> Therefore, together, common variants appear to explain a far greater proportion of CAD heritability than can be attributed to rare variants.

Loci associated with CAD at the genome-wide significance level have been assigned to several pathophysiological pathways with known functions in atherosclerosis or CAD (see Section 2.3.5 for more details, Figure 2); however, despite intriguing hypotheses regarding many genes and pathways at most CAD risk loci the exact mechanisms leading to the disease remain unknown. Even the assignment of these loci to genes is primarily based on proximity only. The link between genetic variants and downstream mechanisms is especially uncertain for SNPs outside protein-coding regions. In fact, the majority of common lead SNPs (approximately 75%) is located outside of coding or classical promoter regions and is rather found to cluster in regulatory elements, such as those annotated by the ENCODE project.<sup>40</sup> The ENCODE project also revealed that only ~27% of distal regulatory elements tend to interact with their nearest promoter, implying that the nearest gene might often not be the target of an identified GWAS variant.<sup>41</sup> The challenges related to gene annotation in post-GWAS analysis are discussed in more detail in Section 3.1.

### 2.3.2 Multiple independent signals at CAD risk loci

Traditionally, the variants with the strongest *P*-values have been reported as the lead SNPs representing a risk locus. However, fine-mapping approaches demonstrate that most of the loci show intra-locus allelic heterogeneity, i.e. a particular risk locus may contain multiple independent risk variants. For CAD, the most complete fine-mapping analysis was performed in a recent study by van de Harst and Verweij.<sup>29</sup> Interestingly, in their study, it was only possible to define for 28 out of the known 163 CAD risk loci a single CAD variant as the most likely causal variant.

**Table 1** Summary of genome-wide significant CAD risk loci (January 2018)

No	Position	Lead SNP	EAf	OR	Gene(s) at locus	References
1	chr1: 2252205	rs36096196	T (0.15)	1.05	<i>MORN1, SKI</i>	29
2	chr1: 3325912	rs2493298	A (0.14)	1.06	<i>PRDM16, PEX10, PLCH2, RER1</i>	29
3	chr1: 38461319	rs61776719	A (0.53)	1.04	<i>FHL3, UTP11, SF3A3, MANEAL, INPP5B</i>	29
4	chr1: 55496039	rs11206510	T (0.82)	1.08	<i>PCSK9</i>	18, 90, 103, 104
5	chr1: 56962821	rs17114036	A (0.91)	1.17	<i>PPAP2B</i>	24, 90
6	chr1: 109822166	rs599839	A (0.78)	1.11	<i>SORT1, PSCR1, CELSR2</i>	14, 24, 90
7	chr1: 115753482	rs11806316	G (0.66)	1.04	<i>NGF, CASQ2</i>	29
8	chr1: 151762308	rs11810571	G (0.79)	1.07	<i>TDRKH, RP11-98D18.9</i>	27, 28
9	chr1: 154422067	rs4845625	T (0.47)	1.06	<i>IL6R, AQP10, ATP8B2, CHTOP, UBAP2L</i>	25
10	chr1: 169094459	rs1892094	C (0.50)	1.04	<i>ATP1B1, BLZF1, CCDC181, F5, NME7, SELP, SLC19A2</i>	105
11	chr1: 200646073	rs6700559	C (0.53)	1.04	<i>DDX59, CAMSAP2, KIF14</i>	105
12	chr1: 201872264	rs2820315	T (0.30)	1.05	<i>LMOD1, IPO9, NAV1, SHISA4, TIMM17A</i>	105
13	chr1: 210468999	rs60154123	T (0.15)	1.05	<i>HHAT, SERTAD4, DIEXF</i>	29
14	chr1: 222823529	rs17465637	C (0.74)	1.14	<i>MIA3, AIDA, C1orf58</i>	14, 24
15	chr1: 230845794	rs699	G (0.42)	1.04	<i>AGT, CAPN9, GNPAT</i>	29
16	chr2: 21286057	rs515135	C (0.83)	1.07	<i>APOB</i>	25, 90
17	chr2: 44073881	rs6544713	T (0.30)	1.06	<i>ABCG5, ABCG8</i>	24, 90, 106
18	chr2: 45896437	rs582384	A (0.53)	1.03	<i>PRKCE, TMEM247</i>	29
19	chr2: 85809989	rs1561198	T (0.45)	1.06	<i>VAMP5, VAMP8, GGCX</i>	25
20	chr2: 145801461	rs2252641	C (0.46)	1.06	<i>ZEB2, TEX41</i>	25
21	chr2: 164957251	rs12999907	A (0.82)	1.06	<i>FIGN</i>	29
22	chr2: 188196469	rs840616	C (0.65)	1.04	<i>CALCRL, TFPI</i>	29
23	chr2: 203745885	rs6725887	C (0.15)	1.14	<i>WDR12, CARF, FAM117B, ICA1L, NBEAL1</i>	18, 24
24	chr2: 216304384	rs1250229	T (0.26)	1.07	<i>FN1, ATIC, LOC102724849, ABCA12, LINC00607</i>	26, 28
25	chr2: 218683154	rs2571445	A (0.39)	1.04	<i>TNS1, CXCR2, RUFY4</i>	105
26	chr2: 227100698	rs2972146	T (0.65)	1.07	<i>LOC646736, IRS1, MIR5702</i>	26
27	chr2: 233633460	rs1801251	A (0.35)	1.05	<i>KCNJ13, GIGYF2</i>	55
28	chr2: 238223955	rs11677932	G (0.68)	1.03	<i>COL6A3</i>	29
29	chr3: 14928077	rs748431	G (0.36)	1.04	<i>FGD5</i>	26
30	chr3: 46688562	rs7633770	A (0.41)	1.03	<i>ALS2CL, RTP3</i>	29
31	chr3: 48193515	rs7617773	T (0.67)	1.04	<i>CDC25A, SPINK8, MAP4, ZNF589</i>	29
32	chr3: 49448566	rs7623687	A (0.86)	1.07	<i>RHOA, AMT, TCTA, CDHRA, KLHDC8B, and others</i>	26–28, 105
33	chr3: 124475201	rs142695226	G (0.14)	1.08	<i>UMPS, ITGB5</i>	26–28
34	chr3: 132257961	rs10512861	G (0.86)	1.04	<i>DNAJC13, NPHP3, ACAD11, UBA5</i>	29
35	chr3: 136069472	rs667920	T (0.78)	1.05	<i>STAG1, MSL2, NCK1, PPP2R3A</i>	29
36	chr3: 138119952	rs2306374	C (0.18)	1.12	<i>MRAS, CEP70</i>	19, 24
37	chr3: 153839866	rs12493885	C (0.85)	1.07	<i>ARHGEF26</i>	26–28
38	chr3: 156852592	rs4266144	G (0.32)	1.03	<i>CCNL1, TIPARP</i>	29
39	chr3: 172115902	rs12897	G (0.41)	1.04	<i>FNDC3B</i>	29
40	chr4: 3449652	rs16844401	A (0.07)	1.07	<i>HGFAC, RGS12, MSANTD1</i>	29
41	chr4: 57838583	rs17087335	T (0.21)	1.06	<i>REST, NOA1</i>	30
42	chr4: 77416627	rs12500824	A (0.36)	1.04	<i>SHROOM3, SEPT11, FAM47E, STBD1</i>	29
43	chr4: 81181072	rs10857147	T (0.27)	1.06	<i>PRDM8, FGF5</i>	26–28
44	chr4: 82587050	rs11099493	A (0.69)	1.04	<i>HNRNPd, RASGEF1B</i>	29
45	chr4: 96117371	rs3775058	A (0.23)	1.04	<i>UNC5C</i>	29
46	chr4: 120901336	rs11723436	G (0.31)	1.05	<i>MAD2L1, PDE5A</i>	26–28
47	chr4: 146782837	rs35879803	C (0.70)	1.05	<i>ZNF827</i>	27
48	chr4: 147472512	rs1878406	T (0.15)	1.10	<i>EDNRA</i>	25
49	chr4: 156635309	rs7692387	G (0.81)	1.08	<i>GUCY1A1<sup>a</sup></i>	6, 25, 107
50	chr4: 169687725	rs7696431	T (0.51)	1.04	<i>PALLD, DDX60L</i>	29
51	chr5: 9556694	rs1508798	T (0.81)	1.05	<i>SEMA5A, TAS2R1</i>	29
52	chr5: 55860781	rs3936511	G (0.18)	1.04	<i>MAP3K1, MIER3</i>	29
53	chr5: 121413208	rs1800449	T (0.17)	1.09	<i>LOX</i>	26

Continued

**Table 1** Continued

No	Position	Lead SNP	EAF	OR	Gene(s) at locus	References
54	chr5: 131667353	rs273909	G (0.14)	1.07	<b>SLC22A4</b>	25
55	chr5: 131867702	rs2706399	G (0.51)	1.07	<b>IL5, RAD50</b>	106
56	chr5: 142516897	rs246600	T (0.48)	1.05	<b>ARHGAP26</b>	105
57	chr6: 1617143	rs9501744	C (0.87)	1.05	<b>FOXC1</b>	29
58	chr6: 12927544	rs12526453	C (0.67)	1.10	<b>PHACTR1, EDN1</b>	18, 24
59	chr6: 22583878	rs35541991	C (0.31)	1.05	<b>HDGFL1</b>	27, 28
60	chr6: 31888367	rs3130683	T (0.86)	1.09	<b>C2, C4A, and others</b>	55
61	chr6: 35034800	rs17609940	G (0.75)	1.07	<b>ANKS1A, UHRF1BP1</b>	24
62	chr6: 36638636	rs1321309	A (0.49)	1.03	<b>CDKN1A, PI16</b>	29
63	chr6: 39174922	rs10947789	T (0.76)	1.07	<b>KCNK5</b>	25
64	chr6: 43758873	rs6905288	A (0.57)	1.05	<b>VEGFA, MRPL14, TMEM63B</b>	29
65	chr6: 57160572	rs9367716	G (0.68)	1.04	<b>PRIM2, RAB23, DST, BEND6</b>	29
66	chr6: 82612271	rs4613862	A (0.53)	1.03	<b>FAM46A</b>	29
67	chr6: 126717064	rs1591805	A (0.49)	1.04	<b>CENPW</b>	29
68	chr6: 134214525	rs12190287	C (0.62)	1.08	<b>TCF21, TARID (EYA4-AS1)</b>	24
69	chr6: 150997401	rs17080091	C (0.92)	1.05	<b>PLEKHG1, IYD</b>	29
70	chr6: 160961137	rs3798220	C (0.02)	1.51	<b>LPA, SLC22A3, LPAL2</b>	20, 24, 90
71	chr6: 161143608	rs4252120	T (0.73)	1.07	<b>PLG, LPAL2</b>	25
72	chr7: 1937261	rs10267593	G (0.8)	1.04	<b>MAD1L1</b>	29
73	chr7: 6486067	rs7797644	C (0.77)	1.04	<b>DAGLB, RAC1, FAM220A, KDELR2</b>	29
74	chr7: 12261911	rs11509880	A (0.36)	1.04	<b>TMEM106B, THSD7A</b>	29
75	chr7: 19036775	rs2023938	C (0.10)	1.08	<b>HDAC9</b>	25
76	chr7: 45077978	rs2107732	G (0.91)	1.06	<b>CCM2, MYO1G</b>	29
77	chr7: 107244545	rs10953541	C (0.80)	1.08	<b>BCAP29, GPR22</b>	22
78	chr7: 117332914	rs975722	G (0.4)	1.03	<b>CTTNBP2, CFTR, ASZ1</b>	29
79	chr7: 129663496	rs11556924	C (0.62)	1.09	<b>ZC3HC1, KLHDC10</b>	24
80	chr7: 139757136	rs10237377	G (0.65)	1.05	<b>PARP12, TBXAS1</b>	105
81	chr7: 150690176	rs3918226	T (0.06)	1.14	<b>NOS3</b>	30
82	chr8: 18286997	rs6997340	T (0.31)	1.04	<b>NAT2</b>	29
83	chr8: 19813180	rs264	G (0.86)	1.11	<b>LPL</b>	25, 33, 90
84	chr8: 22033615	rs6984210	G (0.06)	1.08	<b>BMP1, SFTPC, DMTN, PHYHIP, DOK2, XPO7</b>	29
85	chr8: 106565414	rs10093110	G (0.58)	1.03	<b>ZFPM2</b>	29
86	chr8: 126490972	rs2954029	A (0.55)	1.06	<b>TRIB1</b>	25, 90, 106
87	chr9: 22125503	rs1333049	C (0.46)	1.29	<b>ANRIL, CDKN2B-AS</b>	14-16, 24, 62
88	chr9: 110517794	rs944172	C (0.28)	1.04	<b>KLF4</b>	29
89	chr9: 113169775	rs111245230	C (0.04)	1.14	<b>SVEP1</b>	33
90	chr9: 124420173	rs885150	C (0.27)	1.03	<b>DAB2IP</b>	29
91	chr9: 136154168	rs579459	C (0.21)	1.10	<b>ABO, SURF6, GBGT1</b>	24, 90, 108
92	chr10: 12303813	rs61848342	C (0.36)	1.04	<b>CDC123, NUDT5, OPTN</b>	29
93	chr10: 30335122	rs2505083	C (0.38)	1.07	<b>KIAA1462</b>	22, 109
94	chr10: 44775824	rs1746048	C (0.87)	1.09	<b>CXCL12</b>	14, 24
95	chr10: 82251514	rs17680741	T (0.72)	1.05	<b>TSPAN14, MAT1A, FAM213A</b>	29
96	chr10: 91002927	rs1412444	T (0.42)	1.09	<b>LIPA</b>	22
97	chr10: 104719096	rs12413409	G (0.89)	1.12	<b>CYP17A1, CNM2, NT5C2</b>	24, 110, 111
98	chr10: 105693644	rs4918072	A (0.27)	1.04	<b>STN1, SH3PXD2A</b>	29
99	chr10: 124237612	rs4752700	G (0.45)	1.03	<b>HTRA1, PLEKHA1</b>	29
100	chr11: 5701074	rs11601507	A (0.07)	1.09	<b>TRIM5, TRIM22, TRIM6, OR52N1, OR52B6</b>	29
101	chr11: 9751196	rs10840293	A (0.55)	1.06	<b>SWAP70</b>	30
102	chr11: 10745394	rs11042937	T (0.49)	1.03	<b>MRV1, CTR9</b>	55
103	chr11: 13301548	rs1351525	T (0.67)	1.05	<b>ARNTL</b>	27, 28
104	chr11: 43696917	rs7116641	G (0.31)	1.03	<b>HSD17B12</b>	29
105	chr11: 65391317	rs12801636	G (0.77)	1.05	<b>PCNX3, POLA2, RELA, SIPA1, and others</b>	105
106	chr11: 75274150	rs590121	T (0.30)	1.05	<b>SERPINH1</b>	105
107	chr11: 100624599	rs7947761	G (0.28)	1.04	<b>ARHGAP42</b>	29

Continued

**Table 1** Continued

No	Position	Lead SNP	EAF	OR	Gene(s) at locus	References
108	chr11: 103660567	rs974819	T (0.32)	1.07	<b>PDGFD</b>	22
109	chr11: 116648917	rs964184	G (0.13)	1.13	<b>APOA1-C3-A4-A5</b>	24, 112
110	chr12: 7175872	rs11838267	T (0.87)	1.05	<b>C1S</b>	29
111	chr12: 20220033	rs10841443	G (0.67)	1.06	<b>RP11-664H17.1</b>	26
112	chr12: 54513915	rs11170820	G (0.08)	1.10	<b>HOXC4</b>	27
113	chr12: 57527283	rs11172113	C (0.41)	1.06	<b>LRP1, STAT6</b>	55
114	chr12: 95355541	rs7306455	G (0.9)	1.05	<b>NDUFA12, FGD6</b>	29
115	chr12: 111884608	rs3184504	T (0.44)	1.07	<b>SH2B3, FLJ21127, ATXN2, and others</b>	24, 90, 110, 111, 113
116	chr12: 118265441	rs11830157	G (0.36)	1.12	<b>KSR2</b>	30
117	chr12: 121416988	rs2244608	G (0.35)	1.06	<b>HNF1A, OASL, C12orf43, and others</b>	26–28, 105
118	chr12: 124427306	rs11057401	T (0.69)	1.08	<b>CCDC92</b>	26
119	chr12: 125307053	rs11057830	A (0.15)	1.07	<b>SCARB1</b>	55, 105
120	chr13: 28973621	rs9319428	A (0.32)	1.06	<b>FLT1</b>	25
121	chr13: 33058333	rs9591012	G (0.66)	1.04	<b>N4BP2L2, PDS5B</b>	29
122	chr13: 110960712	rs4773144	G (0.44)	1.07	<b>COL4A1, COL4A2</b>	24
123	chr13: 113631780	rs1317507	A (0.26)	1.04	<b>MCF2L, PCID2, CUL4A</b>	29
124	chr14: 58794001	rs2145598	G (0.42)	1.03	<b>ARID4A, PSMA3</b>	29
125	chr14: 75147552	rs3832966	I (0.46)	1.05	<b>TMED10, ZC2HC1C, RPS6KL1, NEK9, EIF2B2e, ACYP1</b>	27
126	chr14: 94838142	rs112635299	G (0.92)	1.13	<b>SERPINA2, SERPINA1</b>	29
127	chr14: 100133942	rs2895811	C (0.43)	1.07	<b>HHIPL1, YY1</b>	24
128	chr15: 65024204	rs6494488	A (0.82)	1.05	<b>OAZ2, RBPMS2, TRIP4, and others</b>	105
129	chr15: 67455630	rs56062135	C (0.79)	1.07	<b>SMAD3</b>	30
130	chr15: 79089111	rs3825807	A (0.57)	1.08	<b>ADAMTS7</b>	22, 24, 108
131	chr15: 89574218	rs8042271	G (0.9)	1.10	<b>MFGE8, RP11-326A19.4, ABHD2</b>	30
132	chr15: 91416550	rs17514846	A (0.44)	1.07	<b>FURIN, FES</b>	25, 107
133	chr15: 96146414	rs17581137	A (0.75)	1.04	gene desert	29
134	chr16: 56961074	rs1800775	C (0.51)	1.03	<b>CETP</b>	55
135	chr16: 72096666	rs1050362	A (0.38)	1.04	<b>DHX38, HP, DHODH</b>	105
136	chr16: 75387533	rs3851738	C (0.60)	1.07	<b>CFDP1, BCAR1</b>	26, 27
137	chr16: 81906423	rs7199941	A (0.4)	1.04	<b>PLCG2, CENPN</b>	29
138	chr16: 83045790	rs7500448	A (0.77)	1.07	<b>CDH13</b>	26–28
139	chr17: 2126504	rs216172	C (0.37)	1.07	<b>SMG6, SRR</b>	24
140	chr17: 17543722	rs12936587	G (0.56)	1.07	<b>Ral1, PEMT, RASD1, SMCR3, TOM1L2</b>	24
141	chr17: 27941886	rs13723	G (0.49)	1.04	<b>CORO6, BLMH, ANKRD13B, GIT1, SSH2, EFCAB5</b>	29
142	chr17: 30033514	rs76954792	T (0.22)	1.04	<b>COPRS, RAB11FIP4</b>	29
143	chr17: 40257163	rs2074158	C (0.18)	1.05	<b>DHX58, KAT2A, RAB5, NKIRAS2, DNAJC7, KCN4, HCRT, GHDC</b>	29
144	chr17: 45013271	rs17608766	C (0.14)	1.07	<b>GOSR2, MYL4, ARL17A, and others</b>	105
145	chr17: 46988597	rs46522	T (0.53)	1.06	<b>UBE2Z, GIP, ATP5G1</b>	24
146	chr17: 59013488	rs7212798	C (0.15)	1.08	<b>BCAS3</b>	30
147	chr17: 62387091	rs1867624	T (0.61)	1.04	<b>PECAM1, DDX5, TEX2</b>	105
148	chr18: 47229717	rs9964304	C (0.38)	1.04	<b>ACAA2, RPL17</b>	29
149	chr18: 57838401	rs663129	A (0.26)	1.06	<b>PMAIP1, MC4R</b>	30
150	chr19: 8429323	rs116843064	G (0.98)	1.14	<b>ANGTPL4</b>	33, 90
151	chr19: 11163601	rs1122608	G (0.77)	1.14	<b>LDLR, SMARCA4</b>	18, 24, 90, 112
152	chr19: 17855763	rs73015714	G (0.2)	1.06	<b>FCHO1, COLGALT1</b>	29
153	chr19: 32882020	rs12976411	A (0.91)	1.33	<b>ZNF507, LOC400684</b>	30
154	chr19: 41854534	rs8108632 <sup>a</sup>	T (0.48)	1.05	<b>HNRNPUL1, CCDC97, TGFB1, B9D2</b>	26–28
155	chr19: 45395619	rs2075650	G (0.14)	1.14	<b>APOE, APOC1, TOMM40, PVRL2, COTL1</b>	90, 106
156	chr19: 46190268	rs1964272	G (0.51)	1.04	<b>SNRPD2, GIPR</b>	28
157	chr20: 33764554	rs867186	A (0.89)	1.07	<b>PROCR, ASIP, NCOA6, ITGB4BP/EIF6 and others</b>	105
158	chr20: 39924279	rs6102343	A (0.25)	1.04	<b>ZHX3, PLCG1, TOP1</b>	29
159	chr20: 44586023	rs3827066	T (0.14)	1.04	<b>PCIF1, ZNF335, NEURL2, PLTP, MMP9</b>	29, 114
160	chr20: 57714025	rs260020	T (0.13)	1.04	<b>ZNF831</b>	29

Continued

**Table 1 Continued**

No	Position	Lead SNP	EAF	OR	Gene(s) at locus	References
161	chr21: 30533076	rs2832227	G (0.18)	1.04	<i>MAP3K7CL</i> , <b><i>BACH1</i></b>	29
162	chr21: 35599128	rs9982601	T (0.15)	1.18	<i>MRPS6</i> , <i>SLC5A3</i> , <b><i>KCNE2</i></b>	18
163	chr22: 24262640	rs180803	G (0.97)	1.20	<b><i>ADORA2A</i></b>	30

The most likely causal gene(s) at each locus are formatted as bold text. Evidence for respective causal gene(s) is based on literature searches and/or data from the GTEx portal (V6) and SNIpA v3.2<sup>102</sup> (accessed January 2018).

<sup>a</sup>*GUCY1A3* is now designated *GUCY1A1*, according to the HUGO Gene Nomenclature Committee.

### 2.3.3 From rare to common variants: allelic series for CAD

For many years, it has been contended that the genetic component reflected by multiple common genetic variants identified in GWAS cannot explain the familial clustering of CAD, as indicated by a positive family history. As stated earlier, the risk variants identified in GWAS are characterized by high MAF and small effect size. In a diploid genome, every individual of Western European descent carries on average between 130 and 160 risk alleles at the 163 loci detected so far. Hence, common risk alleles appear to explain the widespread predisposition of humans to atherosclerosis, rather than the genetic signal indicated by a positive family history.<sup>6,42</sup> In contrast, a positive family history appears to be mediated by rare deleterious genetic variants<sup>6</sup> that segregate according to Mendelian inheritance or by specific interactions of more common genetic variants (epistasis).<sup>43,44</sup> Remarkably, a recent study by Gormley et al.<sup>45</sup> demonstrated a substantial contribution of common polygenic variation to familial aggregation in migraine, adding to the discussion of the origin of familial clustering in complex diseases. Similar results have been observed for familial dyslipidaemia.<sup>46</sup> Nevertheless, in a few families with multiple affected individuals, rare causal variants have been identified by exome sequencing.<sup>6,47–49</sup> Interestingly, there is a marked overlap between genes that exhibit co-segregation in family-based studies and those identified in GWAS. Indeed, almost all genes causing monogenic forms of CAD or MI also generate a signal in GWAS [i.e. *GUCY1A3* (now designated *GUCY1A1*, according to the HUGO Gene Nomenclature Committee), *PDE5A*, *LDLR*, *PCSK9*, *APOB*, and *LPA*], implicating the same pathways in CAD aetiology, including those regulating vascular tone, blood pressure, LDL-C, triglyceride-rich lipoproteins, inflammation, trans-endothelial migration, smooth muscle proliferation, and lipoprotein(a).<sup>6,48–50</sup>

### 2.3.4 Pleiotropy: a common characteristic of CAD risk variants

Pleiotropy describes the phenomenon whereby one gene affects more than one phenotype. It has been suspected for years that pleiotropy is a common phenomenon in the human genome;<sup>51,52</sup> however, it is only since the availability of comprehensive data sets, such as the GWAS catalog,<sup>53</sup> that a systematic analysis of pleiotropy in the human genome has been feasible.<sup>54</sup> Using this resource, Chesmore et al. discovered that the degree of pleiotropy scales positively with the average effect size of a gene variant and negatively with the variance of the effect sizes of genes with a given number of associated phenotypes. Based on this and prior data, it is becoming increasingly evident that pleiotropy is a common, if not ubiquitous, phenomenon. In general, these results have implications for the understanding of disease aetiologies, with the potential for common biological mechanisms to underlie even disparate diseases, and for the elucidation of the genotype–phenotype landscape. In the context of CAD specifically, a recent study by Webb et al.<sup>55</sup> systematically evaluated the degree of

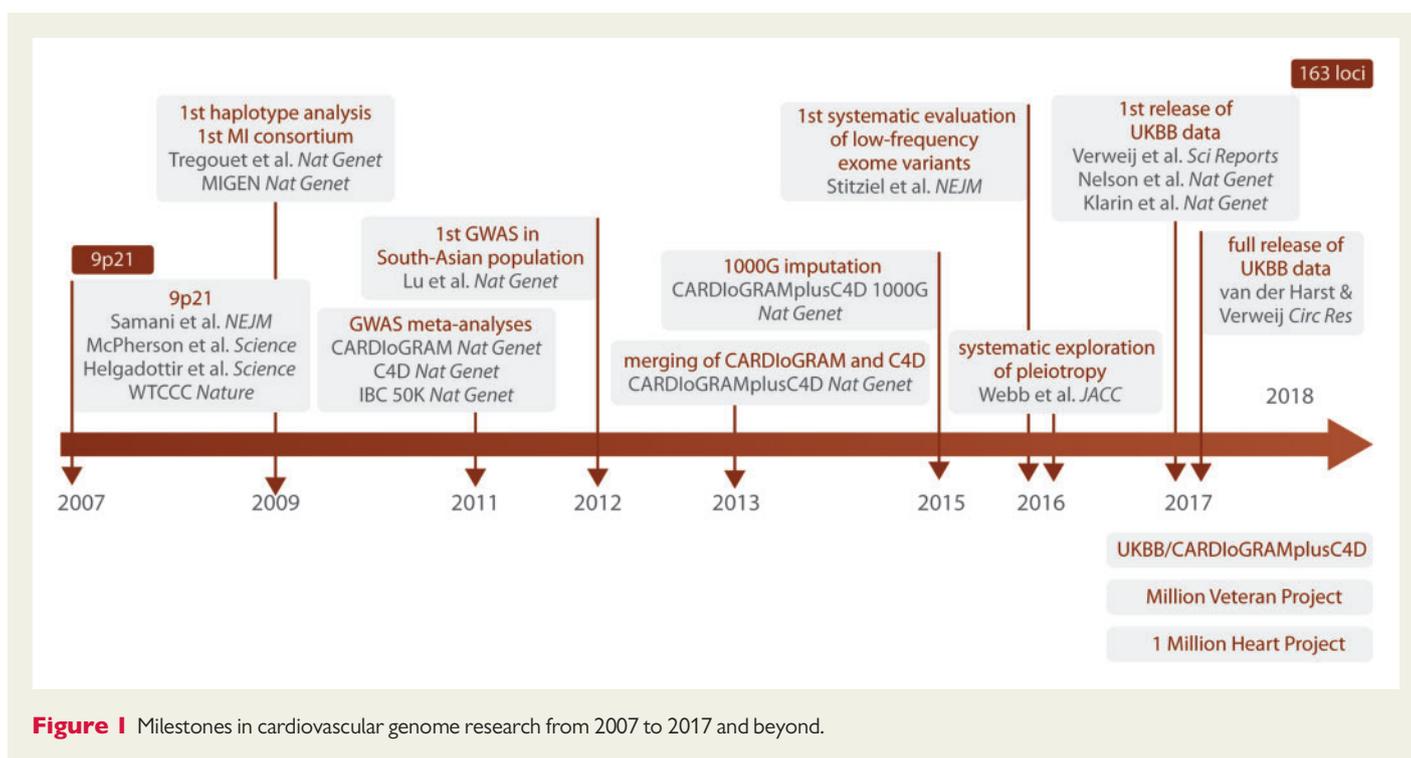
pleiotropy by testing all known CAD loci for association with cardiovascular risk factors (lipid traits, blood pressure phenotypes, body mass index, diabetes, and smoking behaviour), as well as with other diseases/traits, through interrogation of the currently available GWAS catalogue.<sup>53</sup> Of the 62 CAD risk loci studied by Webb and colleagues, 24 (38.7%) were significantly associated with a traditional cardiovascular risk factor, with some exhibiting multiple associations. Consistent with the findings of Chesmore et al., half of CAD risk loci exhibited associations with a range of other diseases/traits (such as migraine, cancer, and height) (Figure 3).

### 2.3.5 Biological insights from GWAS

Experimental and *in silico* studies have both confirmed previous findings and revealed novel insights into the biology of atherosclerosis. In the following sections, we discuss the effects of SNPs at the chromosome 9p21 locus and in the vicinity of genes affecting nitric oxide (NO)/cGMP signalling. For a more detailed discussion of biological insights into lipid, as well as non-lipid, mechanisms underlying CAD derived from GWAS, we refer to Khera and Kathiresan.<sup>7</sup>

**2.3.5.1 Chromosome 9p21.** The strongest effects on coronary atherosclerosis risk are conferred by risk alleles at chromosomal locus 9p21, which are carried by almost 75% of the global population (excluding black Africans).<sup>56</sup> This locus is an archetype for the challenges that arise in attempts to unravel the precise mechanisms underlying disease.<sup>57–60</sup>

The 9p21 locus appears to be associated specifically with the risk of a first CHD event [hazard ratio (HR) of first event = 1.19; 95% confidence interval (CI): 1.17–1.22], rather than subsequent CHD events (HR = 1.01; 95% CI: 0.97–1.06), suggesting that the effect of the functional variant(s) at 9p21.3 may be to stimulate coronary atherosclerosis (i.e. CAD) rather than MI.<sup>61</sup> Recently, it was suggested that the cardiovascular disease-associated region on 9p21 is located in the last exons of a long non-coding RNA, specifically the antisense non-coding RNA in the *INK4* locus (*ANRIL*; also known as *CDKN2BAS*).<sup>62</sup> Interestingly, Holdt et al.<sup>63</sup> reported that a linear form of *ANRIL* confers risk, whereas its circular (circANRIL) counterpart confers atheroprotection, by controlling ribosomal RNA maturation and modulating pathways of atherogenesis. Remarkably, the observed high stability of circRNAs appears to be a common phenomenon and is therefore an appealing potential novel therapeutic target for human diseases more generally.<sup>63</sup> The closest protein-coding (candidate) genes include the cyclin-dependent kinase inhibitors *CDKN2A* and *CDKN2B*. As covered in a review by Hannou et al.,<sup>57</sup> several studies failed to decipher the exact mechanism through which the *CDKN2A/B* gene products might function in relation to CAD. Using unbiased genomic techniques based on chromosome conformation capture, Harismendy et al.<sup>64</sup> detected long-distance interactions between the enhancer interval containing the CAD locus and *CDKN2A/B*. Considering the effects of interactions across large distances, this



observation tentatively (and excitingly) points to the possibility that 9p21.3 disease-associated SNPs could interact with and modify other distant genes. Nevertheless, the strongest data at present time suggest that CAD risk relates to the ratio of circular to linear ANRIL which affects basic cellular mechanisms, including transcriptional activity, proliferation, and apoptosis in vascular smooth muscle cells.<sup>63</sup>

**2.3.5.2 NO/cGMP signalling.** There is strong evidence that NO/cGMP signalling has an important role in atherosclerosis, as several SNPs tagging key genes in the pathway, including *NOS3*, *GUCY1A1* (formerly *GUCY1A3*), *PDE5A*, *PDE3A*, and *MRV11* are associated with CAD with genome-wide significance (Figure 4).<sup>62,25,26,30</sup> Moreover, a genetic risk score (GRS) using common variants in *NOS3* (rs3918226) and *GUCY1A1* (rs7692387) is associated with risk for coronary heart disease, peripheral arterial disease, and stroke.<sup>65</sup> Kessler et al.<sup>66</sup> elucidated the molecular link between rs7692387, which tags the *GUCY1A1* locus, and CAD risk; the SNP is located in an intronic region and modulates *GUCY1A1* promoter activity, as shown by luciferase assays. Interestingly, the transcription factor ZEB1 binds preferentially to the non-risk allele, leading to increased *GUCY1A1* expression, higher sGC levels and higher sGC activity upon stimulation with NO. In conjunction with mouse data, Kessler and colleagues subsequently linked augmented sGC expression to lower risk of CAD. Additionally, the importance of the pathway was confirmed by the fact that a loss-of-function mutation in *GUCY1A1* caused premature CAD and MI in an extended family.<sup>6</sup> Unlike *GUCY1A1*, the precise molecular mechanisms linking the lead SNPs at *NOS3*, *PDE5A*, *PDE3A*, and *MRV11* with CAD risk are largely unknown.

Emdin et al.<sup>65</sup> confirmed that rare variants that inactivate the *GUCY1A1* or *NOS3* genes are associated with higher systolic blood pressure and an up to three-fold higher risk of coronary heart disease. Based on the results of these genetic studies, pharmacologic stimulation of NO signalling may prove useful in the prevention or treatment of cardiovascular disease. Indeed, Riociguat (BAY 63-2521), an sGC stimulator, is already in clinical use. In the PATENT trial, the drug had proved efficacy in patients with

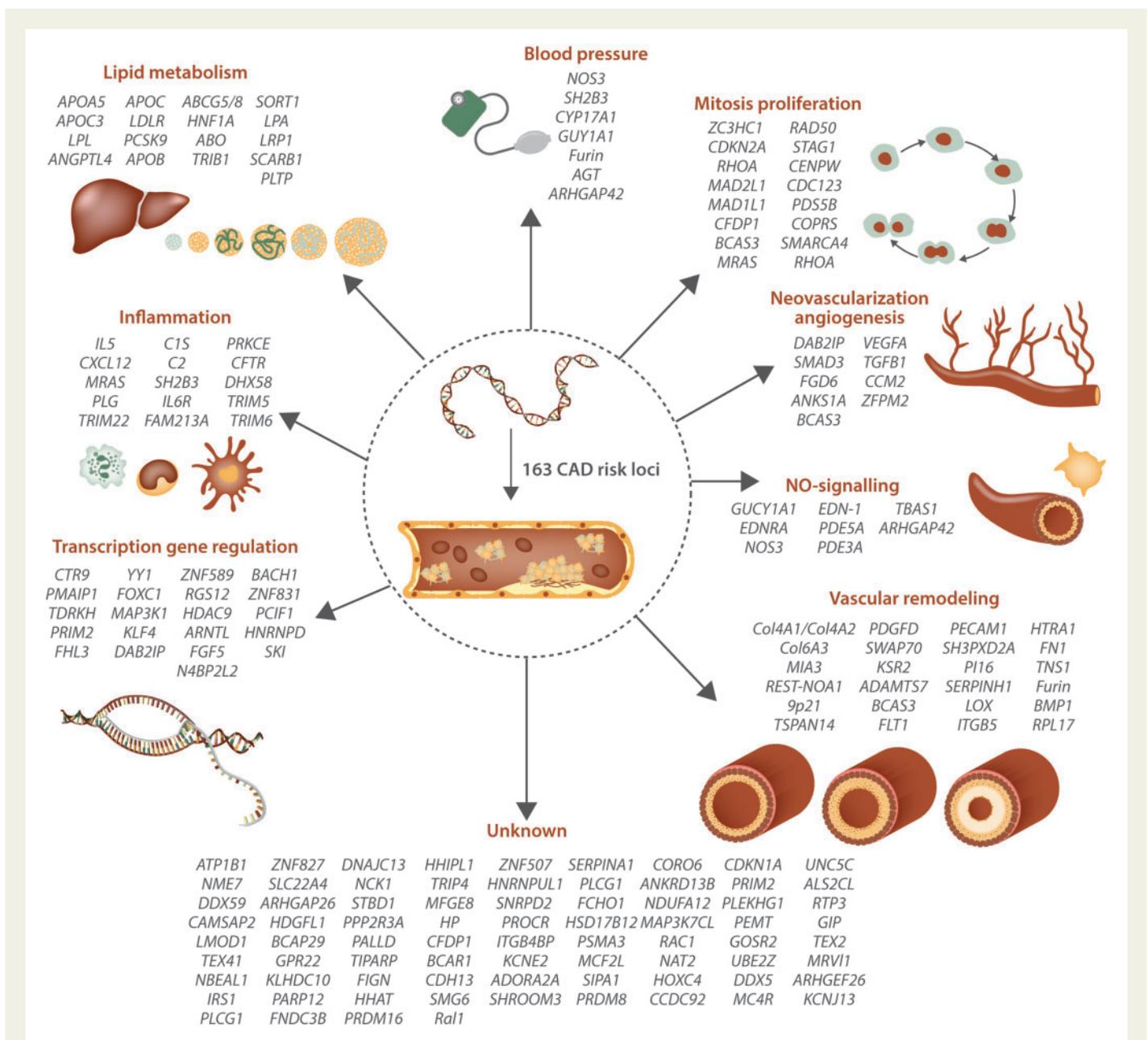
pulmonary hypertension.<sup>67</sup> Riociguat is of particular interest because it acts synergistically with NO,<sup>68</sup> i.e. reduced sGC activity or expression could hypothetically be compensated for by the presence of pharmacologic modulators of sGC activity. Future clinical studies are needed to define the effect of sGC stimulators on atherosclerosis phenotypes.

### 3. The challenges involved in moving from genetic associations to therapeutic targets and beyond

Although genetic associations provide a strong foundation for the identification of therapeutic targets, a number of challenges can arise. First, the majority of lead associated variants lies within non-coding regions, making it difficult to predict their functions and identify specific targets/genes. Secondly, almost all loci harbour multiple genes, and the likely causal gene often needs to be defined by detailed investigations. Thirdly, only few loci encompass candidate genes that unequivocally explain the association signal (e.g. *LDLR* and *PCSK9*); at all other loci, the gene(s) responsible must be determined and the underlying pathomechanisms elucidated. Finally, even where a validated gene/pathway is clear, it is necessary to demonstrate that the target is druggable. Ultimately, success in defining targets for pharmaceutical exploitation will depend not only on the identification of causal genes but also on the elucidation of the downstream pathways and biological networks, as the best druggable target may not be the causal gene, but rather some other node in the network. The following sections will discuss in more detail some of the challenges in moving from genetic association to therapeutic targets (see also Figure 5).

#### 3.1 Annotation of causal genes at GWAS loci

As stated earlier, an inherent limitation of GWAS is that they do not provide information about the biological meaning of the causal variants that



**Figure 2** Genes mapped to 163 CAD risk loci and pathophysiological pathways in atherosclerosis.

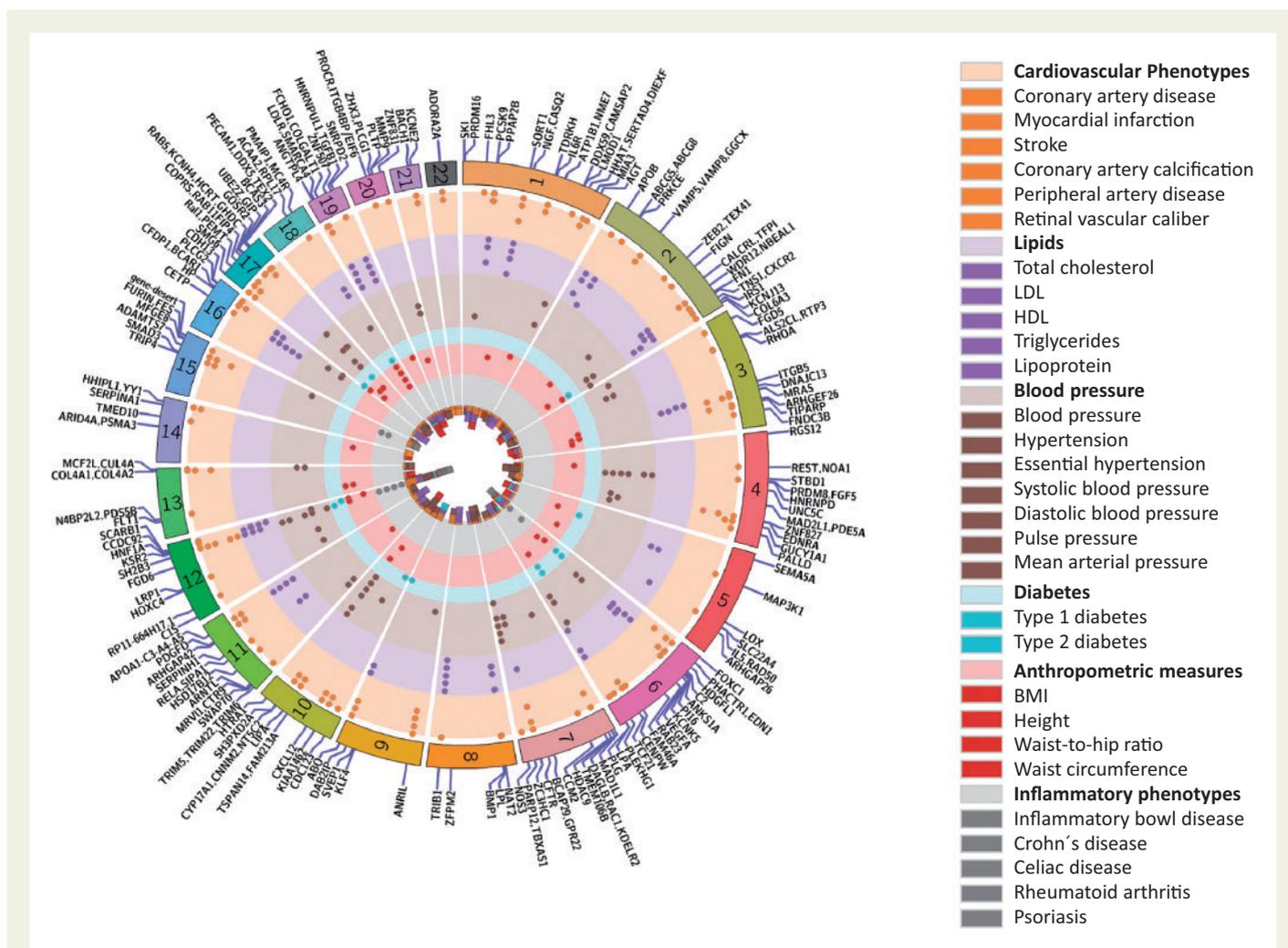
are tagged by genome-wide significant SNPs (tagSNPs). To translate GWAS results to biological function, post-GWAS analysis is necessary. Usually, such analyses start with inexpensive *in silico* (bioinformatics) studies of tagSNPs. Several tools and statistical techniques are used to extract meaning from, and prioritize, association signals, which are subsequently investigated experimentally (a selection of available tools, methods, and platforms is presented in [Supplementary material online, Table S2](#)). In the sections below, we briefly describe some of the most strongly validated and recently developed methods.

### 3.1.1 Expression quantitative trait loci

Over the last decade, studies to unravel the genetics underpinning the regulation of gene expression have progressed substantially. Gene transcript levels, which are heritable and therefore amenable to GWAS,

were the first expression phenotypes to be studied. SNPs that modulate transcript levels are referred to as expression quantitative trait loci (eQTL) or eSNPs, and can be associated with gene expression levels either locally (*cis*) or at a distance (*trans*, i.e. more than 5 Mb away from the associated variant).<sup>69</sup> Recently, the study of eSNPs has been extended to other molecular QTL that regulate gene expression at various levels, from chromatin state to cellular response. The importance of regulatory SNPs has recently been documented by a study from Nikpay *et al.*<sup>70</sup> showing that the heritability of CAD is mainly attributed to SNPs located in epigenetic sites associated with transcriptional activity.

Original GWAS papers reporting novel SNPs usually explore publicly available multi-tissue transcriptome data sets for differential expression levels related to the risk alleles. Such signals are used to provide additional evidence that the associated SNP has a functional effect and to



**Figure 3** Circosplot showing 163 risk loci for CAD under the chromosomes, where they are located. The dots represent conditions which are likewise significantly associated with CAD risk alleles indicating pleiotropy. Some loci, e.g. the SH2B3 locus at chromosome 10, show signals for multiple diseases (January 2018).

choose candidate target genes for experimental follow-up. A variety of tissue and disease-specific databases are available, including ENCODE,<sup>40</sup> GTEx,<sup>71</sup> Epigenome RoadMap,<sup>72</sup> STARNET,<sup>73</sup> and chromatin interaction information,<sup>74</sup> some of which have been used to investigate CAD-relevant tissues.<sup>75</sup> For example, a study using STARNET data showed that *cis*- and *trans*-acting loci could contribute to a mechanism by which multiple loci affect the heritability of risk for cardio-metabolic diseases (precursors of CAD).<sup>73</sup> Clearly, eQTL analyses can facilitate annotation of the most likely causal gene at a locus identified by GWAS; however, prioritization of genes within a locus is sometimes difficult, because variants at a single GWAS locus are often eQTL for multiple genes, as observed by Braenne *et al.*<sup>76</sup>

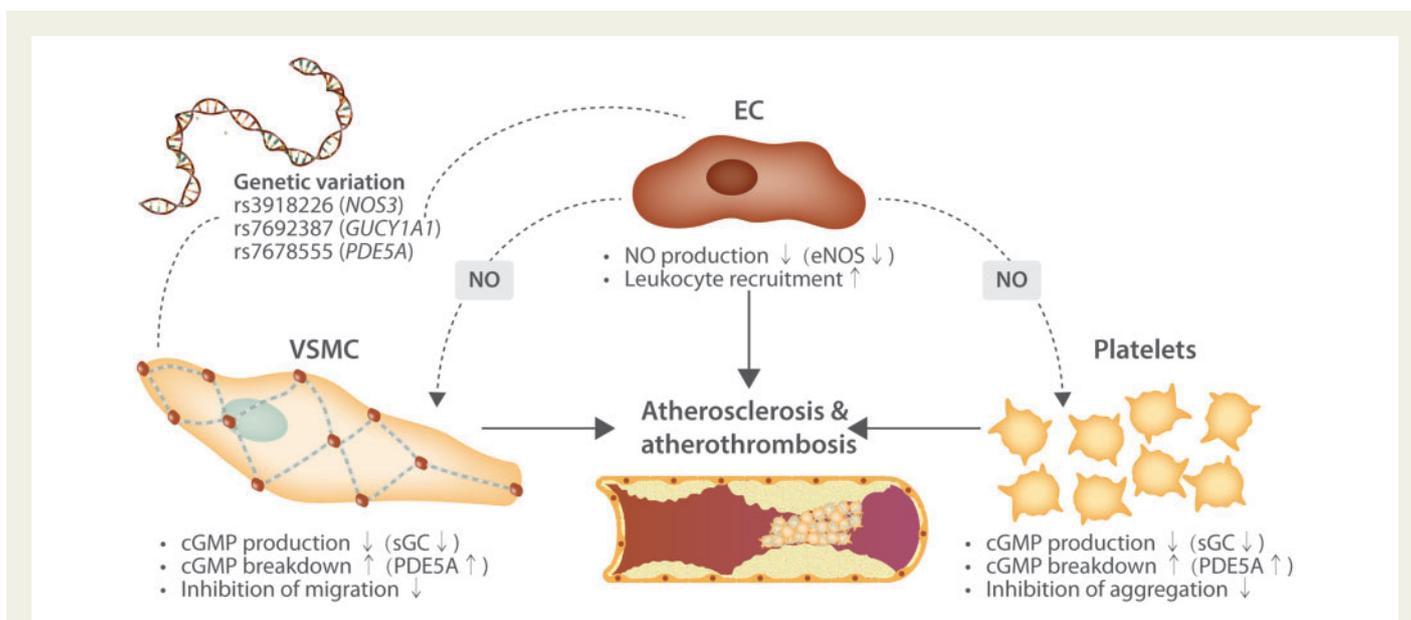
### 3.2 Network analysis

There are several tools available that can either prioritize variants found during an association analysis and/or perform tissue enrichment analysis. An interesting study published recently<sup>77</sup> used various tissue-specific regulatory networks and protein–protein interaction networks that do not rely solely on a priori knowledge to detect genes strongly implicated in the prevalence of CAD and also identify the novel key regulators of

CAD, *LUM*, *HGD*, *F2*, *ANXA3*, and *STAT3*. Recently, Vilne *et al.* demonstrated how hypercholesterolaemia can hinder mitochondrial activity during atherosclerosis progression and identified oestrogen-related receptor- $\alpha$  and its co-factors PGC1- $\alpha$  and - $\beta$  as potential therapeutic targets to counteract these processes, using a network approach.<sup>78</sup>

### 3.3 Systems genetics approaches in the cardiovascular field

Reduced costs of high-throughput analyses, as well as publicly available data sets, have allowed simultaneous analysis of data generated using multiple ‘-omics’ platforms (namely, genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiomics); cloud<sup>79</sup> and/or web<sup>80</sup> computing are vital to this type of approach. Awareness is increasing that (i) identified CAD loci only explain part of heritability; (ii) common diseases, such as CAD, tend to occur because of gene regulation changes; and (iii) similar genetic variants contribute to different final outcomes; hence, it is logical that systems genetics has evolved to integrate data from various ‘-omics’ studies and generate a broader view of the molecular mechanisms involved. Indeed, this approach has helped to explain the complexity of the molecular patterns associated with CAD.



**Figure 4** Role of NO-signalling in coronary artery disease.

Systems genetics, as defined by Björkegren *et al.*,<sup>81</sup> uses molecular mechanisms to define disease-driving molecular processes that underlie GWAS, whole-exome sequencing or whole-genome sequencing hits, and to integrate such processes with functional genomic data. One example of associated SNPs exerting tissue-dependent effects on gene expression was reported by Musunuru *et al.*<sup>82</sup> They integrated eQTL and protein QTL information to determine that an MI risk variant alters the expression of the *SORT1* gene in the liver (via a lipoprotein metabolism-regulated pathway). This observation supported the findings of a prior GWAS that identified a strong association between the 1p13 locus and plasma LDL-C levels in MI patients and, moreover, concluded that this locus influenced the risk of MI by conferring changes in plasma lipids. Another recent study<sup>83</sup> used a systems genetics approach to integrate DNA genotypes and gene expression profiles from seven CAD-relevant tissues with CAD CARDIoGRAM GWAS information. Using this method, the authors showed that RNA-processing genes are pivotal in causing CAD and, furthermore, identified several strongly inherited, evolutionarily conserved, risk-enriched CAD genes that cause regulatory gene network alterations across vascular and metabolic tissues.

Compared with individual -omics studies, the multi-omics approach provides a more comprehensive understanding of the flow of information from the disease driver to its functional consequence or interaction; however, multifactorial diseases, such as CAD, are extremely complex, which may partly account for the lack of multi-omics studies of CAD to date.

### 3.4 Mendelian randomization studies

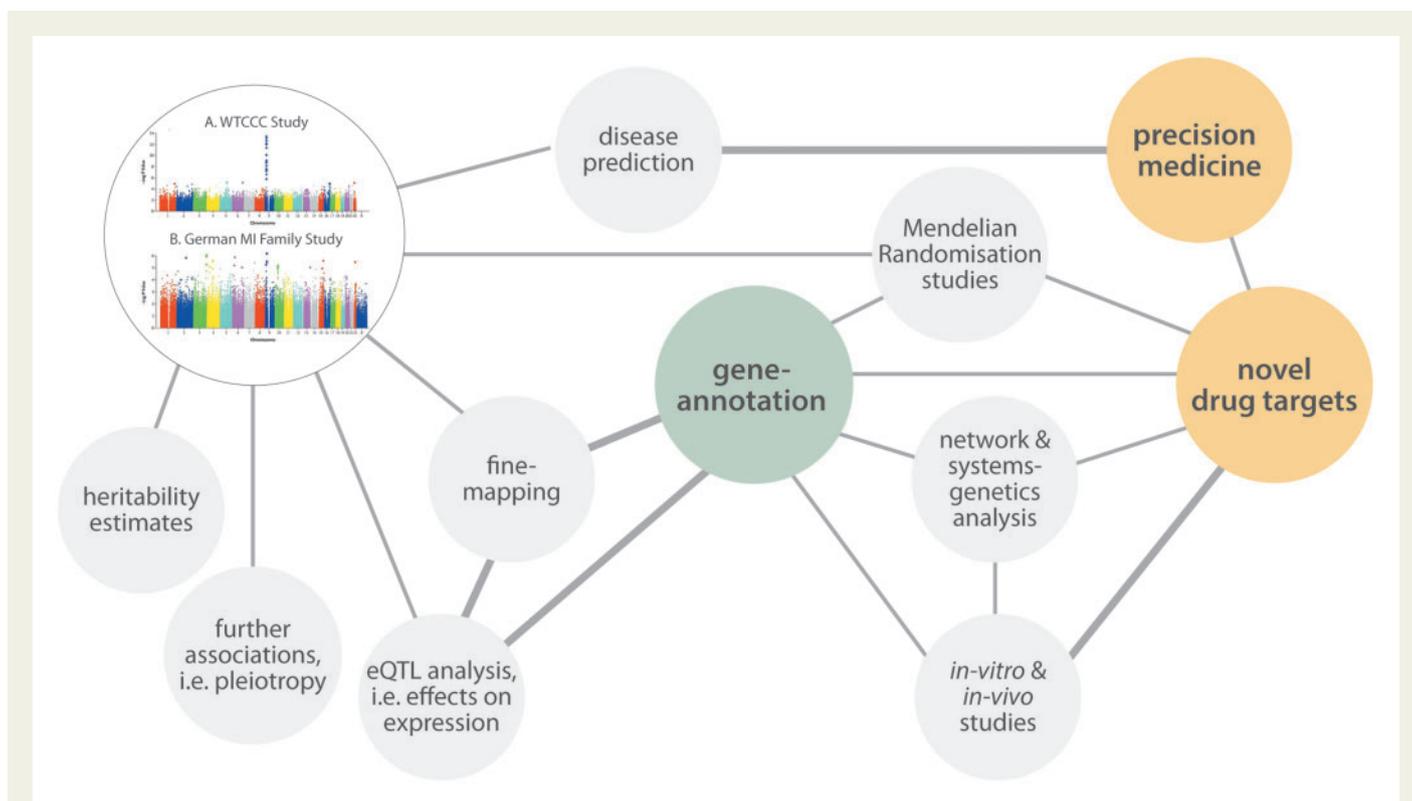
Although randomized controlled trial studies are the optimal way to establish causal relationships between risk factors, exposures, and a disease of interest (in our case, CAD), sometimes they are not possible. An alternative is to use Mendelian randomization (MR) analysis of GWAS data.<sup>84</sup> The rationale is to use genetic variants as proxies for potentially modifiable exposures to facilitate the identification of causal effects for risk of CAD. MR analysis is resistant to confounding factors, which is an

advantage over randomized controlled trials. An in-depth discussion of MR studies in the cardiovascular field can be found in this issue.<sup>85</sup>

### 3.5 Potential of precision medicine in CAD

One promise of GWAS, which has already partly been fulfilled, was that the results would facilitate the (re)identification of risk genes and disease-associated pathways useful for drug development or drug selection, as recently showcased for *GUCY1A1*,<sup>86</sup> *PCSK9*,<sup>87</sup> *ANGPTL4*,<sup>33</sup> and *ANGPTL3*<sup>88</sup> (more examples are compiled in Table 2). With more functional studies linking risk variants to underlying causal genes, it is likely that additional novel therapeutic targets will be identified.<sup>7,89</sup>

The examples depicted in Table 2 show how the discovery of (rare) genetic variants highlighted pathways influencing both an intermediate phenotype, i.e. lipid metabolism, and coronary atherosclerosis. An important fact, however, is that the therapies which are based on genetic findings are not limited to individuals carrying such variants. The importance of *NPC1L1* in lipid metabolism and CAD risk has been clearly shown from genetic point of view.<sup>90</sup> The potential of treating individuals at risk with the *NPC1L1* inhibitor ezetimibe, however, was successful in a large clinical trial that was not focusing on individuals with altered *NPC1L1* function.<sup>91</sup> The same is actually true for *PCSK9* inhibitors where treatment of hypercholesterolaemia and reduction of cardiovascular events was successful irrespectively of the presence or absence of *PCSK9* variants.<sup>92</sup> Hence, in the context of precision medicine, results from GWAS might lead to two developments that have to be analysed separately: The detection of rare variants/mutations or a genetic profile associated with a distinct pathway might lead to the development and/or use of a specific treatment. An example is the *GUCY1A1* locus, which has already been discussed above. Heterozygous carriers of rare mutations in this gene might benefit from the use of a pharmacological modulator of sGC function. Indeed, *in vitro* data suggest that coding variants which are present in individuals suffering from premature CAD lead to reduced formation of the second messenger cGMP but can be compensated by the use of an sGC stimulator.<sup>86</sup> This is an example of precision medicine in the sense of developing individualized treatment strategies. A second



**Figure 5** Complementarity of methods and analysis surveyed in yielding novel insights into disease biology. Thickness of lines reflect importance of methods/analysis to yield insights.

**Table 2** Examples of genes affecting CAD and MI risk identified by large-scale array-based or deep-sequencing projects with relevance for therapeutic development

Gene	<i>PCSK9</i>	<i>NPC1L1</i>	<i>LPA</i>	<i>LPL</i>	<i>APOC3</i>	<i>ANGPTL4</i>	<i>ANGPTL3</i>
Frequency	1 in 50 blacks	1 in 150	1 in 13	1 in 10	1 in 150	1 in 500	1 in 300
Phenotype	LDL	LDL	Lp(a)	TG	TG	TG	TG, LDL
Risk	80%	53%	14%	17%	40%	57%	34%
	lower risk	lower risk	higher risk	lower risk	lower risk	lower risk	lower risk
Therapy	Evolocumab, Bococizumab, Alirocumab	Ezetimibe	Antisense in development	?	Antisense in development	Monoclonal antibodies in development	Monoclonal antibodies in development
References	115	116	117	118	119	118, 120	121–123

possible use of genetic data in precision medicine is rather linked to the development of novel therapies sharply focusing on specific molecular processes or pathways which might be useful for all patients. Again NO/cGMP signalling represents an excellent example as common non-coding variants in *GUCY1A1* gene<sup>6,25</sup> and four other genes<sup>26,28,30,55</sup> encoding important proteins in this pathway have been identified to be associated with CAD. This raises the question whether modulating NO/cGMP signalling might be a promising therapeutic strategy to prevent CAD. In fact, stimulators of the sGC and inhibitors of PDE5A are already in clinical use, e.g. pulmonary hypertension. Data regarding prevention of cardiovascular endpoints are thus far lacking. Nevertheless, enhancing NO/cGMP signalling could emerge as a strategy comparable to reducing LDL cholesterol. Taken together, GWAS laid to ground to discover

novel therapies for particular individuals and for larger numbers of individuals at risk.

However, currently GWAS do not provide sufficient information necessary to stratify individuals according to severity, prognosis, and responsiveness, which is required for drug development and/or selection. To overcome these hurdles, Morita and Komuro<sup>93</sup> suggested stratifying large-scale prospective studies according to clinically affected sub-phenotypes in patients with similar clinical presentations, and then adding a second layer independent of the variant associated with disease onset, to search for associated variants (or driver pathways) with the disease sub-phenotype. One example of such stratification would be to discriminate between dyslipidaemia patients with and without CAD; here, a subpopulation analysis to identify genetic variants associated with

CAD susceptibility could facilitate the selection of individuals susceptible to CAD who should receive proactive, perhaps intensive, cardio-metabolic abnormality management to prevent the disease.<sup>93</sup> On a macro level, current pharmacogenomics research is proceeding along these lines. Contrary to the sub-phenotype method, some argue that for CAD patients it would be more useful to highlight the blend of genetic and environmental causal factors (or pathways) that underlie CAD development in large population-scale cohorts.<sup>94</sup> Such discussion can only promote this type of research approach and be used to facilitate progression towards a future of individualized precision medicine.

### 3.5.1 Polygenic risk scores for CAD

One potential application of genomic research outcomes is the prediction of the risk of an individual for a complex disorder, such as CAD, and use of this information to encourage the adoption of preventive measures. Familial hypercholesterolaemia is a condition characterized by monogenic mutations in the genes encoding *LDLR*, *PCSK9*, and *APOB*. Loss-of-function variants in these genes lead to increased cholesterol, and carriers have an up to four-fold elevated risk for CAD compared with non-carriers.<sup>95</sup> Early diagnosis, either on the basis of clinical criteria (including LDL cholesterol and family history) or DNA sequencing, can lead to timely treatment with lipid lowering medication and consequently lower the risk of cardiovascular disease to levels equivalent to those of the general population.<sup>96</sup> In contrast, the value of individual common variants is very limited for risk prediction. Polygenic risk scores (PRSs), derived by summing the number of risk variant alleles in each individual weighted by the impact of each allele on disease risk, perform better than individual variants; however, the predictive power remains limited.<sup>97,98</sup> Nevertheless, individuals with a high GRS, i.e. those in the top quintile of the risk score distribution, appear to have a larger benefit from statin treatment than those with low scores.<sup>99</sup> Moreover, among participants at high genetic risk, adherence to a healthy lifestyle is associated with an almost 50% lower relative risk of CAD.<sup>100</sup> A novel approach, developed by Khera et al.,<sup>101</sup> which aggregated information from 6.6 million common variants to build a PRS, demonstrated convincingly that such a PRS can identify a four-fold increased risk for CAD in 2.5% of the population, comparable to familial hypercholesterolaemia mutation carriers.<sup>101</sup> On a cautionary note, the potential benefits of disclosing the genetic risk to patients must be weighed against possible unfavourable consequences, such as increased treatment costs, psychological distress or discrimination, and a sense of fatalism in high-risk individuals. Clearly, more work is needed to optimize the disclosure of genetic risk to patients and their healthcare providers, and to assess whether such disclosure can improve clinical outcomes.

## 4. Conclusion

The last decade of genomic research has led to the identification of 163 common genetic loci conferring modest risk for CAD and MI. It is foreseeable that more variants will be identified by increasing GWAS sample sizes. In addition, whole-exome and whole-genome sequencing studies have identified rare risk variants in families and large patient cohorts with stronger effects. Nevertheless, although only part of the heritable risk for CAD is yet explained, we have developed a much more comprehensive picture of the biology underlying the disease, and with increasing numbers of functional studies aiming to decipher the link between genetic variation and CAD, we will finally identify novel treatment targets, as exemplified by *PCSK9*, *ANGPTL4*, *ANGPTL3*, and *GUCY1A1*. Hence, the

findings generated by CAD GWAS represent an excellent starting point for the development of individualized treatment strategies in the future.

## Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

## Disclosure statement

The authors have nothing to disclose.

**Conflict of interest:** none declared.

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