Genomic Strategies Toward Identification of Novel Therapeutic Targets

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Abstract

Coronary artery disease, myocardial infarction, and secondary damages of the myocardium in the form of ischemic heart disease remain major causes of death in

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Western countries. Beyond traditional risk factors such as smoking, hypertension, dyslipidemia, or diabetes, a positive family history is known to increase risk. The genetic factors underlying this observation remained unknown for decades until genetic studies were able to identify multiple genomic loci contributing to the heritability of the trait. Knowledge of the affected genes and the resulting molecular and cellular mechanisms leads to improved understanding of the pathophysiology leading to coronary atherosclerosis. Major goals are also to improve prevention and therapy of coronary artery disease and its sequelae via improved risk prediction tools and pharmacological targets. In this chapter, we recapitulate recent major findings. We focus on established novel targets and discuss possible further targets which are currently explored in translational studies.

Keywords

Coronary artery disease · Exome sequencing · Genomics · Genome-wide association studies · Myocardial infarction

1 Introduction

Coronary artery disease (CAD) and myocardial infarction (MI) are the main causes of morbidity and mortality. The identification of risk factors is a prerequisite to improve prevention and therapy of the disease via gaining knowledge about the underlying pathophysiological processes as well as the identification of therapeutic targets. Hyperlipidemia, hypertension, and diabetes mellitus are examples for risk factors which can be treated via pharmacological intervention, whereas smoking and obesity can be addressed by lifestyle interventions. Age and male gender, which are also major risk factors, cannot be addressed therapeutically. In the past, a positive family history has also been regarded as a non-modifiable risk factor. However, the underlying risk factors were not known for decades. In this chapter, we summarize the developments in the past years which led to the identification of a plethora of genomic loci which are associated with CAD with high statistical certainty.

2 Methodological Aspects

Over the past decades, the methodological spectrum has been vastly expanded to identify novel genetic risk factors of CAD and MI. Details on these methods have been discussed elsewhere (Kessler et al. [2016](#)). In this section, we aim to briefly mention some important points.

2.1 Techniques

The development of arrays with hundred thousands of common single nucleotide polymorphisms (SNPs) distributed all over the genome of an individual enabled researchers to deeply genotype large numbers of individuals. An important prerequisite was knowledge of the human genome which has been mainly gained via projects as the human genome project (Lander et al. 2001; Sachidanandam et al. 2001). Subsequent projects as the *1000 Genomes Project* (1000 Genomes Project Consortium et al. 2012, 2015) raised further possibilities with the imputation of SNPs which had not been genotyped directly. As a consequence genome-wide association studies investigating millions of SNPs have been performed, and mostly common variants were found to be associated with the disease and lend support to the *common disease-common variant* hypothesis (Reich and Lander 2001). Thereby, since 2007, mostly genome-wide association studies and subsequent analyses led to the identification of genetic risk variants for various traits including CAD and traditional risk factors as blood pressure or lipids. Subsequently, large international consortia were formed that were responsible for the identification of most of the currently known CAD risk loci. Figure 1 illustrates the evolution of genome-wide association studies focusing on the CARDIoGRAMplusC4D consortium, a coalition of several individual studies to facilitate the identification of CAD risk factors (Schunkert et al. 2019) and further studies using data from UK Biobank (Littlejohns et al. 2019). The idea of exome-wide association studies is comparable to that of a genome-wide association study with the exception that the investigated SNPs are enriched for coding variants distributed over the genome (Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators et al. 2016). Most of the currently known genetic CAD risk factors have been identified using these methods (Kessler et al. 2016; Khera and Kathiresan 2017a; Erdmann et al. 2018).

Apart from common variants, the association of rare variants and private mutations has also been extensively studied. To identify such genetic variation, mostly exome sequencing has been used in the past (Erdmann et al. 2013). As the name says, this next-generation sequencing method is able to determine the genomic sequence of an individual enriched for coding regions and thus renders the identification of missense variants co-segregating with a certain disease in, e.g., a family with high prevalence possible. Whole-genome sequencing is not restricted to coding sequences. However, due to costs and computational challenges of the different methods, whole-genome sequencing has not yet contributed to the current knowledge of genetic variation in CAD.

2.2 Selection of Individuals

Most large-scale genomic studies use a case-control design, i.e., a cohort of patients suffering from the trait of interest is compared to a group of healthy individuals free of the disease. Obviously, clear definitions of phenotypes are important to gain reliable results. Most GWAS on CAD/MI focused on cases suffering from the

Fig. 1 *Discovery of genomic variants associated with CAD/MI using genome-/exome-wide association studies* (modified after Kessler et al. 2016). **(a)** Over the past years, ongoing research led to the identification of increasing numbers of SNPs which are associated with CAD/MI at the genome-wide level of significance. Results from the CARDIoGRAMplusC4D-Million Hearts Initiative are awaited for 2019/2020. **(b)** The number of investigated individuals correlates with the number of identified risk variants which highlights the importance of international collaborations

disease at an early age. This increased the possibility of identification of the genetic predisposition which, together with gender, still represents the strongest risk factor. For controls, population-based samples as well as individuals which have been

diagnosed to not to suffer from CAD/MI have been included. An important resource that has increasingly been used in recent projects is the UK Biobank (<https://www.ukbiobank.ac.uk/>). It includes health information as well as genetic data for 500,000 individuals which can be accessed by researchers after approval of specific projects (Littlejohns et al. 2019). In the USA, the Million Veteran Project has formed and stated to provide likewise genome-wide data (Klarin et al. 2018).

2.3 Statistical Analysis

To reduce the possibility of a false-positive finding, rigorous statistical thresholds have been determined to identify a certain variant to be associated with a trait as CAD/MI. Currently, a p -value below 5×10^{-8} is commonly accepted as a genome-wide significant finding. The strict p -value is a result of the large number of statistical tests which are performed in parallel on one dataset in a genome-wide association study (Pe'er et al. 2008). However, it cannot be excluded that true positive signals are discarded because they do not reach the stringent p -value. Recent studies also reported loci below certain false discovery rate thresholds to address this issue.

2.4 Mendelian Randomization Studies

Mendelian randomization studies (for an overview see Jansen et al. 2014) enable to investigate whether a biomarker or a trait is causal in the development of a disease. In epidemiological research, associations between biomarkers and diseases are frequently observed. However, the association can be influenced by unknown factors. On the other hand, the investigated disease itself can influence a given biomarker. In Mendelian randomization studies, causality between a biomarker and development of a disease can be assumed if a genetic variant influences this biomarker in the same direction as the biomarker is associated with the disease. Important examples in CAD are biomarkers such as LDL-cholesterol or C-reactive protein as well as behavioral traits such as educational attainment (Zeng et al. 2019a) which are discussed in more detail below.

3 Genetic Risk Factors Associated with Coronary Artery Disease

3.1 Genome-Wide Association Studies

The discovery of genetic CAD/MI risk factors was launched in 2007 by three independent studies which reported the chromosome 9p21 locus as the first and, until today, strongest genetic risk locus (Helgadottir et al. 2007; McPherson et al. 2007; Samani et al. 2007). Since then, with the inclusion of more individuals and the

possibility of analyzing a larger number of SNPs, more and more variants associated with CAD/MI have been reported (for an overview see Kessler et al. (2016), Khera and Kathiresan (2017b), and Erdmann et al. (2018)). The currently known loci are depicted in Table 1. Surprisingly, most of the SNPs tag genes which have not been studied in the pathophysiology of coronary atherosclerosis before. Another surprising finding was that only the minority of genes is also associated with traditional risk factors as hypertension or lipid metabolism rendering the involvement of other cellular mechanisms likely. Additionally, almost every lead SNP is located in a non-coding region of the genome. The most prominent example is again the chromosome 9p21 locus. Here, it is still unclear which gene might be responsible for the strong signal. Rather, there is much evidence that the effect might be mediated by the circular non-coding RNA *ANRIL* (Holdt et al. 2010, 2013, 2016). Overall, it has been estimated that the 163 so far known CAD risk loci explain between 30% and 40% of CAD heritability (Nelson et al. 2017). It has also been found that a large number of the reported loci also harbor multiple independent signals (van der Harst and Verweij 2018). Additionally, most of the loci show pleiotropy, i.e., a risk variant is associated with several phenotypes. About half of the currently known CAD risk loci have been reported to be associated with other traits (Webb et al. 2017).

The studies which led to the discovery of the currently known CAD/MI risk variants as well as studies which gave first insights into their involvement in CAD/MI are referenced in Table 1. Specific genes and pathways are further discussed below.

3.2 Exome-Wide Association Studies

As mentioned above, GWAS mainly led to the identification of non-coding variation associated with the disease. To specifically investigate the role of coding variants, dedicated arrays enriched for exonic variants were used. In an international effort, the Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators performed the largest exome-wide association study so far (Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators et al. 2016). The results are depicted in Table 2. In summary, only four variants were identified. First, it could be replicated that coding variation in the *LPA* and *PCSK9* genes is associated with CAD/MI. Second, two novel coding variants in *ANGPTL4* and *SVEP1* were found. Whereas *LPA*, *PCSK9*, and *ANGPTL4* are associated with lipid metabolism (see below), *SVEP1* only displayed association with blood pressure (Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators et al. 2016). However, this effect cannot fully explain the association signal for CAD/MI.

Table 1 Loci associated with CAD/MI identified by GWAS (modified after Erdmann et al. (2018))

Chr.	Lead SNP	EA (EAF)	OR	Gene(s)	Association with traditional risk factors		Ref.
					HTN	Lipids	
1	rs36096196	T (0.15)	1.05	MORNI, SKI			Van der Harst and Verweij (2018)
	rs2493298	A (0.14)	1.06	PRDM16, PEX10, PLCH2, RERI			Van der Harst and Verweij (2018)
	rs61776719	A (0.53)	1.04	FHL3, UTP11, SF3A3, MANEAL, INPP5B			Van der Harst and Verweij (2018)
	rs11206510	T (0.82)	1.08	PCSK9		+	Abifadel et al. (2003), Cohen et al. (2006), Myocardial Infarction Genetics Consortium et al. (2009), and Teslovich et al. (2010)
	rs17114036	A (0.91)	1.17	PPAP2B			Teslovich et al. (2010), Schunkert et al. (2011)
	rs599839	A (0.78)	1.11	SORT1, PCSRI, CELSR2		+	Samani et al. (2007), Teslovich et al. (2010), and Schunkert et al. (2011)
	rs11806316	G (0.66)	1.04	NGF, CASQ2			Van der Harst and Verweij (2018)
	rs11810571	G (0.79)	1.07	TDRKH, RP11-98D18.9			Verweij et al. (2017), Nelson et al. (2017)
	rs4845625	T (0.47)	1.06	IL6R, AQP10, ATP8B2, CHTOP, UBAP2L			CARDIoGRAMplusC4D Consortium et al. (2013)
	rs1892094	C (0.50)	1.04	ATPIB1, BLZF1, CCDC181, F5, NME7, SELP, SLC19A2			Howson et al. (2017)
	rs6700559	C (0.53)	1.04	DDX59, CAMSAP2, KIF14			
	rs2820315	T (0.30)	1.05	LMOD1, IPO9, NAV1, SHISA4, TIMM17A			
	rs60154123	T (0.15)	1.05	HHAT, SERTAD4, DIEXF			Van der Harst and Verweij (2018)
	rs17465637	C (0.74)	1.14	MIA3, AIDA, C1orf58			Samani et al. (2007), Schunkert et al. (2011)
	rs699	G (0.42)	1.04	AGT, CAPN9, GNPAT			Van der Harst and Verweij (2018)

(continued)

Table 1 (continued)

Chr.	Lead SNP	EA (EAF)	OR	Gene(s)	Association with traditional risk factors	Ref.
2	rs515135	G (0.83)	1.07	APOB	+	Teslovich et al. (2010), CARDIoGRAMplusC4D Consortium et al. (2013)
	rs6544713	T (0.30)	1.06	ABCG5, ABCG8	+	Teslovich et al. (2010), Schunkert et al. (2011), and IBC 50K CAD Consortium (2011)
	rs582384	A (0.53)	1.03	PRKCE, TMEM247		Van der Harst and Verweij (2018)
	rs1561198	A (0.45)	1.06	VAMP5, VAMP8, GGCX		CARDIoGRAMplusC4D Consortium et al. (2013)
	rs2252641	G (0.46)	1.06	ZEB2, TEX41		CARDIoGRAMplusC4D Consortium et al. (2013)
	rs12999907	A (0.82)	1.06	FIGN		Van der Harst and Verweij (2018)
	rs840616	C (0.65)	1.04	CALCRL, TFPI		Van der Harst and Verweij (2018)
	rs6725887	C (0.15)	1.14	WDR12, CARF, FAM117B, ICA1L, NBEAL1		Myocardial Infarction Genetics Consortium et al. (2009), Schunkert et al. (2011)
	rs1250229	T (0.26)	1.07	FN1, ATIC, LOC102724849, ABCA12, LINC00607	+	Klarin et al. (2017), Nelson et al. (2017)
	rs2571445	A (0.39)	1.04	TNSI, CXCR2, RUFY4		Howson et al. (2017)
rs2972146	T (0.65)	1.07	LOC646736, IRS1, MIR5702	+	Klarin et al. (2017)	
rs1801251	A (0.35)	1.05	KCNJ13, GIGYF2		Webb et al. (2017)	
rs11677932	G (0.68)	1.03	COL6A3		Van der Harst and Verweij (2018)	

3	rs748431	G (0.36)	1.04	FGD5			Klarin et al. (2017)
	rs7633770	A (0.41)	1.03	ALS2CL, RTP3			Van der Harst and Verweij (2018)
	rs7617773	T (0.67)	1.04	CDC25A, SPINK8, MAP4, ZNF589	+		
	rs7623687	A (0.86)	1.07	RHOA, AMT, TCTA, CDHRA, KLHDC8B, etc.			Verweij et al. (2017), Klarin et al. (2017), Nelson et al. (2017), and Howson et al. (2017)
	rs142695226	G (0.14)	1.08	UMPS, ITGB5			Verweij et al. (2017), Klarin et al. (2017), and Nelson et al. (2017)
	rs10512861	G (0.86)	1.04	DNAJC13, NPHP3, ACAD11, UBA5	+		Van der Harst and Verweij (2018)
	rs667920	T (0.78)	1.05	STAG1, MSL2, NCK1, PPP2R3A	+		
	rs2306374	C (0.18)	1.12	MRAS, CEP70			Erdmann et al. (2009), Schunkert et al. (2011)
4	rs12493885	C (0.85)	1.07	ARHGEF26			Verweij et al. (2017), Klarin et al. (2017), and Nelson et al. (2017)
	rs4266144	G (0.32)	1.03	CCNLI, TIPARP			Van der Harst and Verweij (2018)
	rs12897	G (0.41)	1.04	FNDC3B			
	rs16844401	A (0.07)	1.07	HGFAC, RGS12, MSANTD1	+		
	rs17087335	T (0.21)	1.06	REST, NOA1			Nikpay et al. (2015)
	rs12500824	A (0.36)	1.04	SHROOM3, SEPT11, FAM47E, STBD1			Van der Harst and Verweij (2018)
	rs10857147	T (0.27)	1.06	PRDM8, FGF5	+		Verweij et al. (2017), Klarin et al. (2017), and Nelson et al. (2017)
	rs11099493	A (0.69)	1.04	HNRNPD, RASGEF1B			Van der Harst and Verweij (2018)
	rs3775058	A (0.23)	1.04	UNC5C			
	rs11723436	G (0.31)	1.05	MAD2L1, PDE5A			Verweij et al. (2017), Klarin et al. (2017), and Nelson et al. (2017)
	rs35879803	C (0.70)	1.05	ZNF827			Verweij et al. (2017)
	rs1878406	T (0.15)	1.10	EDNRA			CARDIoGRAMplusC4D Consortium et al. (2013)
	rs7692387	G (0.81)	1.08	GUCY1A1	+		International Consortium for Blood Pressure Genome-Wide Association Studies et al. (2011), CARDIoGRAMplusC4D Consortium et al. (2013), and Erdmann et al. (2013)
	rs7696431	T (0.51)	1.04	PALLD, DDX60L			Van der Harst and Verweij (2018)

(continued)

Table 1 (continued)

Chr.	Lead SNP	EA (EAF)	OR	Gene(s)	Association with traditional risk factors		Ref.
					HTN	Lipids	
5	rs1508798	T (0.81)		SEMA5A , <i>TAS2R1</i>		+	
	rs3936511	G (0.18)	1.04	MAP3K1 , <i>MIER3</i>		+	
	rs1800449	T (0.17)	1.09	LOX			Klarin et al. (2017)
	rs273909	C (0.14)	1.07	SLC22A4			CARDIoGRAMplusC4D Consortium et al. (2013)
	rs2706399	G (0.51)	1.07	IL5 , RAD50			IBC 50K CAD Consortium (2011)
6	rs246600	T (0.48)	1.05	ARHGAP26			Howson et al. (2017)
	rs9501744	C (0.87)	1.05	FOXC1			Van der Harst and Verweij (2018)
	rs12526453	C (0.67)	1.10	PHACTR1 , EDN1			Myocardial Infarction Genetics Consortium et al. (2009), Schunkert et al. (2011)
	rs35541991	C (0.31)	1.05	HDGFL1			Verweij et al. (2017), Nelson et al. (2017)
	rs3130683	T (0.86)	1.09	C2 , <i>C4A</i> , etc.			Webb et al. (2017)
	rs17609940	G (0.75)	1.07	ANKS1A , <i>UHRF1BP1</i>			Schunkert et al. (2011)
	rs1321309	A (0.49)	1.03	<i>CDKN1A</i> , PH16		+	Van der Harst and Verweij (2018)
	rs10947789	T (0.76)	1.07	KCNK5			CARDIoGRAMplusC4D Consortium et al. (2013)
	rs6905288	A (0.57)	1.05	VEGFA , <i>MRPL14</i> , <i>TMEM63B</i>		+	Van der Harst and Verweij (2018)
	rs9367716	G (0.68)	1.04	<i>PRIM2</i> , RAB23 , <i>DST</i> , <i>BEND6</i>			
	rs4613862	A (0.53)	1.03	FAM46A			
	rs1591805	A (0.49)	1.04	CENPW			
	rs12190287	C (0.62)	1.08	TCF21 , <i>TARID</i> (<i>EYA4-AS1</i>)			Schunkert et al. (2011)
	rs17080091	C (0.92)	1.05	PLEKHG1 , <i>IYD</i>		+	Van der Harst and Verweij (2018)
	rs3798220	C (0.02)	1.51	LPA , <i>SLC22A3</i> , <i>LPAL2</i>		+	Tregouet et al. (2009), Teslovich et al. (2010), and Schunkert et al. (2011)
	rs4252120	T (0.73)	1.07	PLG , <i>LPAL2</i>		+	CARDIoGRAMplusC4D Consortium et al. (2013)

7	rs10267593	G (0.8)	1.04	MAD1L1				Van der Harst and Verweij (2018)
	rs7797644	C (0.77)	1.04	DAGLB, RAC1, FAM220A, KDELR2				
	rs11509880	A (0.36)	1.04	TMEM106B, THSD7A				
	rs2023938	G (0.10)	1.08	HDAC9				CARDIoGRAMplusC4D Consortium et al. (2013)
	rs2107732	G (0.91)	1.06	CCM2, MYO1G		+		Van der Harst and Verweij (2018)
	rs10953541	C (0.80)	1.08	BCAP29, GPR22				Coronary Artery Disease C4D Genetics Consortium (2011a)
	rs975722	G (0.4)	1.03	CTTNBP2, CFTR, ASZ1				Van der Harst and Verweij (2018)
8	rs11556924	C (0.62)	1.09	ZC3HC1, KLHDC10				Schunkert et al. (2011)
	rs10237377	G (0.65)	1.05	PARP12, TBXAS1				Howson et al. (2017)
	rs3918226	T (0.06)	1.14	NOS3		+		Nikpay et al. (2015)
	rs6997340	T (0.31)	1.04	NAT2			+	Van der Harst and Verweij (2018)
	rs264	G (0.86)	1.11	LPL			+	Testovich et al. (2010), CARDIoGRAMplusC4D Consortium et al. (2013), and Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators et al. (2016)
	rs6984210	G (0.06)	1.08	BMP1, SFTPC, DMTN, PHYHIP, DOK2, XPO7				Van der Harst and Verweij (2018)
	rs10093110	G (0.58)	1.03	ZFPM2				
	rs2954029	A (0.55)	1.06	TRIB1			+	Testovich et al. (2010), IBC 50K CAD Consortium (2011), and CARDIoGRAMplusC4D Consortium et al. (2013)
	rs1333049	G (0.46)	1.29	ANRIL, CDKN2B-AS				Helgadottir et al. (2007), McPherson et al. (2007), Samani et al. (2007), Holdt et al. (2010), and Schunkert et al. (2011)
	rs944172	C (0.28)	1.04	KLF4				Van der Harst and Verweij (2018)
9	rs885150	C (0.27)	1.03	DAB2IP				
	rs579459	C (0.21)	1.10	ABO, SURF6, GBGT1			+	Testovich et al. (2010), Reilly et al. (2011), and Schunkert et al. (2011)

(continued)

Table 1 (continued)

Chr.	Lead SNP	EA (EAF)	OR	Gene(s)	Association with traditional risk factors	Ref.
					HTN	Lipids
10	rs61848342	C (0.36)	1.04	CDC123, NUDT5, OPTN		Van der Harst and Verweij (2018)
	rs2505083	C (0.38)	1.07	KIAA1462		Coronary Artery Disease C4D Genetics Consortium (2011a), Erdmann et al. (2011)
	rs1746048	C (0.87)	1.09	CXCL12		Samani et al. (2007), Schunkert et al. (2011)
	rs17680741	T (0.72)	1.05	TSPAN14, MAT1A, FAM213A		Van der Harst and Verweij (2018)
	rs1412444	T (0.42)	1.09	LIPA		Coronary Artery Disease C4D Genetics Consortium (2011a)
	rs12413409	G (0.89)	1.12	CYP17A1, CNNM2, NT5C2	+	Levy et al. (2009), Newton-Cheh et al. (2009), and Schunkert et al. (2011)
11	rs4918072	A (0.27)	1.04	STN1, SH3PXD2A		Van der Harst and Verweij (2018)
	rs4752700	G (0.45)	1.03	HTRA1, PLEKHA1		
	rs11601507	A (0.07)	1.09	TRIM5, TRIM22, TRIM6, OR52N1, OR52B6		
	rs10840293	A (0.55)	1.06	SWAP70		Nikpay et al. (2015)
	rs11042937	T (0.49)	1.03	MRV11, CTR9		Webb et al. (2017)
	rs1351525	T (0.67)	1.05	ARNTL		Verweij et al. (2017), Nelson et al. (2017)
	rs7116641	G (0.31)	1.03	HSD17B12		Van der Harst and Verweij (2018)
	rs12801636	G (0.77)	1.05	PCNX3, POLA2, RELA, SIPA1, etc.	+	Howson et al. (2017)
	rs590121	T (0.30)	1.05	SERPINH1		Howson et al. (2017)
	rs7947761	G (0.28)	1.04	ARHGAP42		Van der Harst and Verweij (2018)
	rs974819	T (0.32)	1.07	PDGFD		Coronary Artery Disease C4D Genetics Consortium (2011a)
	rs964184	G (0.13)	1.13	APOA1-C3-A4-A5		Schunkert et al. (2011), Do et al. (2015)
	rs11838267	T (0.87)	1.05	C1S	+	Van der Harst and Verweij (2018)

12	rs10841443	G (0.67)	1.06	RP11-664H17.1, PDE3A				Klarin et al. (2017)
	rs11170820	G (0.08)	1.10	HOXC4				Verweij et al. (2017)
	rs11172113	C (0.41)	1.06	LRP1, STAT6				Webb et al. (2017)
	rs7306455	G (0.9)	1.05	NDUFA12, FGD6				Van der Harst and Verweij (2018)
	rs3184504	T (0.44)	1.07	SH2B3, FLJ21127, ATXN2, etc.	+			Gudbjartsson et al. (2009), Levy et al. (2009), Newton-Cheh et al. (2009), Teslovich et al. (2010), and Schunkert et al. (2011)
	rs11830157	G (0.36)	1.12	KSR2				Nikpay et al. (2015)
	rs2244608	G (0.35)	1.06	HNF1A, OASL, C12orf43, and others	+			Verweij et al. (2017), Klarin et al. (2017), Nelson et al. (2017), and Howson et al. (2017)
13	rs11057401	T (0.69)	1.08	CCDC92			+	Klarin et al. (2017)
	rs11057830	A (0.15)	1.07	SCARB1			+	Webb et al. (2017), Howson et al. (2017)
	rs9319428	A (0.32)	1.06	FLT1				CARDIoGRAMplusC4D Consortium et al. (2013)
	rs9591012	G (0.66)	1.04	N4BP2L2, PDS5B				Van der Harst and Verweij (2018)
	rs4773144	G (0.44)	1.07	COL4A1, COL4A2				Schunkert et al. (2011)
14	rs1317507	A (0.26)	1.04	MCF2L, PCID2, CUL4A				Van der Harst and Verweij (2018)
	rs2145598	G (0.42)	1.03	ARID4A, PSMA3				
	rs3832966	I (0.46)	1.05	TMED10, ZC2HC1C, RPS6KL1, NEK9, EIF2B2e, ACYP1				Verweij et al. (2017)
	rs112635299	G (0.92)	1.13	SERPINA2, SERPINA1				Van der Harst and Verweij (2018)
	rs2895811	C (0.43)	1.07	HHIPL1, YY1				Schunkert et al. (2011)
15	rs6494488	A (0.82)	1.05	OAZ2, RBPMS2, TRIP4, etc.				Howson et al. (2017)
	rs56062135	C (0.79)	1.07	SMAD3				Nikpay et al. (2015)
	rs3825807	A (0.57)	1.08	ADAMTS7				Reilly et al. (2011), Schunkert et al. (2011), and Coronary Artery Disease C4D Genetics Consortium (2011a)
	rs8042271	G (0.9)	1.10	MFG8, RPI1-326A19.4, ABHD2				Nikpay et al. (2015)
	rs17514846	A (0.44)	1.07	FURIN, FES	+			International Consortium for Blood Pressure Genome-Wide Association Studies et al. (2011), CARDIoGRAMplusC4D Consortium et al. (2013)
	rs17581137	A (0.75)	1.04	Gene desert				Van der Harst and Verweij (2018)

(continued)

Table 1 (continued)

Chr.	Lead SNP	EA (EAF)	OR	Gene(s)	Association with traditional risk factors		Ref.
					HTN	Lipids	
16	rs1800775	C (0.51)	1.03	CETP		+	Webb et al. (2017)
	rs1050362	A (0.38)	1.04	<i>DHX38</i> , HP , <i>DHODH</i>		+	Howson et al. (2017)
	rs3851738	C (0.60)	1.07	CFDP1 , BCAR1			Verweij et al. (2017), Klarin et al. (2017)
	rs7199941	A (0.4)	1.04	PLCG2 , <i>CENPN</i>			Van der Harst and Verweij (2018)
	rs7500448	A (0.77)	1.07	CDH13			Verweij et al. (2017), Klarin et al. (2017), and Nelson et al. (2017))
17	rs216172	C (0.37)	1.07	SMG6 , <i>SRR</i>			Schunkert et al. (2011)
	rs12936587	G (0.56)	1.07	Rai1 , PEMT , <i>RASD1</i> , <i>SMCR3</i> , <i>TOM1L2</i>			Schunkert et al. (2011)
	rs13723	G (0.49)	1.04	CORO6 , BLMH , ANKRD13B , GIT1 , SSH2 , EFCAB5			Van der Harst and Verweij (2018)
	rs76954792	T (0.22)	1.04	COPRS , RAB11FIP4			
	rs2074158	C (0.18)	1.05	DHX58 , KAT2A , RAB5 , NKIRAS2 , DNAJC7 , KCNH4 , HCRT , GHDC		+	
18	rs17608766	C (0.14)	1.07	GOSR2 , <i>MYL4</i> , <i>ARL17A</i> , etc.	+		Howson et al. (2017)
	rs46522	T (0.53)	1.06	UBE2Z , GIP , <i>ATP5G1</i>			Schunkert et al. (2011)
	rs7212798	C (0.15)	1.08	BCAS3			Nikpay et al. (2015)
	rs1867624	T (0.61)	1.04	PECAM1 , DDX5 , TEX2 , etc.			Howson et al. (2017)
	rs9964304	C (0.38)	1.04	ACAA2 , RPL17			Van der Harst and Verweij (2018)
	rs663129	A (0.26)	1.06	PMAIP1 , MC4R		+	Nikpay et al. (2015)

19	rs1122608	G (0.77)	1.14	LDLR, SMARCA4		+	Myocardial Infarction Genetics Consortium et al. (2009), Teslovich et al. (2010), Schunkert et al. (2011), and Do et al. (2015)
	rs73015714	G (0.2)	1.06	FCHO1, COLGALT1			Van der Harst and Verweij (2018)
	rs12976411	A (0.91)	1.33	ZNF507, LOC400684			Nikpay et al. (2015)
	rs8108632*	T (0.48)	1.05	HNRNPUL1, CCDC97, TGFB1, B9D2			Verweij et al. (2017), Klarin et al. (2017), and Nelson et al. (2017)
	rs2075650	G (0.14)	1.14	APOE, APOC1, TOMM40, PVRL2, COTL1		+	Teslovich et al. (2010), IBC 50K CAD Consortium (2011)
20	rs1964272	G (0.51)	1.04	SNRPD2, GIPR			Nelson et al. (2017)
	rs867186	A (0.89)	1.07	PROCR, ASIP, NCOA6, ITGB4BP, EIF6, etc.			Howson et al. (2017)
	rs6102343	A (0.25)	1.04	ZHX3, PLCG1, TOP1			Van der Harst and Verweij (2018)
	rs7270354	A (0.15)	1.06	PCIF1, ZNF335, NEURL2, PLTP, MMP9		+	Braenne et al. (2017)
	rs260020	T (0.13)	1.04	ZNF831			Van der Harst and Verweij (2018)
21	rs2832227	G (0.18)	1.04	MAP3K7CL, BACH1			
	rs9982601	T (0.15)	1.18	MRPS6, SLC5A3, KCNE2			Myocardial Infarction Genetics Consortium et al. (2009)
	rs180803	G (0.97)	1.20	ADORA2A			Nikpay et al. (2015)

The most likely candidate gene at the locus is marked in bold (after McPherson and Tybjaerg-Hansen (2016)). *Chr* chromosome, *EA* effect allele, *EAF* effect allele frequency, *HTN* hypertension, *I/D* Indel/Deletion, *OR* odds ratio, *Ref* reference, *SNP* single nucleotide polymorphism

Table 2 Coding variants associated with CAD/MI risk identified by exome-wide association studies

Chr.	SNP (AA change)	EA (EAF)	OR	Gene(s)	Association with traditional risk factors		Ref.
					HTN	Lipids	
1	rs11591147 (p. R46L)	T (0.0152)	0.78	PCSK9		+	Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators et al. (2016)
6	rs3798220 (p. I4399M)	C (0.019)	1.54	LPA		+	
9	rs111245230 (p. D2702G)	C (0.036)	1.14	SVEP1	+		
19	rs116843064 (p. E40K)	A (0.02)	0.86	ANGPTL4		+	

AA amino acid, Chr chromosome, EA effect allele, EAF effect allele frequency, HTN hypertension, OR odds ratio, Ref reference, SNP single nucleotide polymorphism

3.3 Exome and Whole-Genome Sequencing

As discussed above, due to still comparatively high costs, whole-genome sequencing has not yet significantly contributed to the knowledge of genetic CAD risk factors. In contrast, several studies made use of exome sequencing to identify variation in the coding sequence which is associated with CAD/MI. One scope of application the investigation of members of families with a high disease prevalence. Using this approach and subsequent co-segregation analyses genetic factors underlying the development of CAD/MI or its risk factors was possible. An important example is a mutation in the *GUCY1A3* gene, which has been – together with a coding mutation in the *CCT7* gene – shown to be responsible for the phenotype of premature CAD/MI in a family (Erdmann et al. 2013). As shown in Table 1, the *GUCY1A3* locus also harbors common, non-coding variants associated with CAD/MI. Thus, an allelic series has been shown at the locus with a mutation and common variants which lead to a strong or only moderate risk increase, respectively. Further loci have been identified by analyzing large cohorts of cases and controls comparable to the GWAS approach. Most of the genes that were thereby identified play a role in lipid metabolism. Some of the genes, e.g., *LDLR* or *PCSK9*, also demonstrate allelic series. A selection of genes which have been identified using exome sequencing is depicted in Table 3 and discussed below in more detail.

4 Genetic Overlap and Demarcation with Other Atherosclerotic Diseases

An increased prevalence of CAD risk alleles can be traced in a large number of cardiovascular conditions including heart failure, peripheral arterial disease, or atrial fibrillation (Ntalla et al. 2019). Large-scale genomic studies have also been published in particular for stroke (for a review see Dichgans et al. (2019)). Despite there is genetic overlap between CAD and atherosclerotic stroke (Dichgans et al. 2014; Kessler et al. 2015a), the genetic risk factors of CAD and stroke are not similar. The formation of large, international consortia has advanced the identification of risk genes in the fields of CAD and stroke genetics. In other atherosclerotic diseases as peripheral artery disease, genetic research will be facilitated by publicly available data from large-scale biobanks. Nevertheless, the identification of common genetic risk factors will be important to evaluate novel therapeutic strategies. Other genetic risk factors for CAD might also not play an important role in related diseases: as such, the strongest genetic risk factor reported so far, chromosome 9p21, is not associated with calcified aortic stenosis; in contrast, the *LPA* gene is associated with both diseases (Trenkwalder et al. 2018).

Table 3 Genes associated with CAD/MI which have been identified using exome sequencing

Chr.	Gene(s)	Mechanism	Ref.
1	<i>ANGPTL3</i>	<ul style="list-style-type: none"> • Angiopoietin-like 3 inhibits lipoprotein lipase • <i>ANGPTL3</i> loss-of-function mutations are associated with reduced LDL-cholesterol and triglycerides as well as reduced CAD risk 	Stitzel et al. (2017)
2	<i>APOB</i>	<ul style="list-style-type: none"> • Apolipoprotein B is a main component of LDL-cholesterol and triglyceride-rich lipoproteins • Truncating APOB mutations are associated with reduced LDL-cholesterol and reduced CAD risk 	Peloso et al. (2019)
4/2	<i>GUCY1A3/CCT7</i>	<ul style="list-style-type: none"> • Premature stop codon in <i>GUCY1A3</i> leads to loss of α_1-subunit of the soluble guanylyl cyclase (sGC); missense mutation in <i>CCT7</i> which encodes a chaperone protein stabilizing the sGC • Carriers of the <i>GUCY1A3</i>+<i>CCT7</i> mutation show reduced sGC-dependent cGMP formation in platelets • Coding variants in <i>GUCY1A3</i> are overrepresented in young MI patients 	CARDIoGRAMplusC4D Consortium et al. (2013), Erdmann et al. (2013), and Wobst et al. (2016)
7	<i>NPC1L1</i>	<ul style="list-style-type: none"> • Niemann-Pick C1-like protein 1 is responsible for the uptake of cholesterol from the intestine • <i>NPC1L1</i> loss-of-function mutations are associated with reduced LDL-cholesterol and reduced risk of CAD/MI 	Myocardial Infarction Genetics Consortium Investigators et al. (2014)
8	<i>LPL</i>	<ul style="list-style-type: none"> • Lipoprotein lipase reduced triglyceride levels • Loss-of-function variants are associated with increased triglyceride levels and CAD/MI 	Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators et al. (2016) and Khera et al. (2017)
11	<i>APOA5</i>	<ul style="list-style-type: none"> • Apolipoprotein A-V increases lipoprotein lipase activity • <i>APOA5</i> loss-of-function mutations are associated with high triglyceride levels and CAD/MI 	Do et al. (2015)
	<i>APOC3</i>	<ul style="list-style-type: none"> • Apolipoprotein C-III reduced lipoprotein lipase activity • <i>APOC3</i> loss-of-function mutations are associated with 	The TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute (2014)

(continued)

Table 3 (continued)

Chr.	Gene(s)	Mechanism	Ref.
		reduced triglyceride levels and CAD/MI risk	
16	<i>CETP</i>	<ul style="list-style-type: none">• Cholesteryl ester transfer protein reduces HDL-cholesterol levels• Carriers of truncating CETP mutation displayed higher levels of HDL-cholesterol, lower levels of LDL-cholesterol, and a lower CAD risk	Nomura et al. (2017)
19	<i>LDLR</i>	<ul style="list-style-type: none">• Loss of LDL-receptor function leads to reduced uptake of LDL-cholesterol and increased plasma LDL-cholesterol• High levels of LDL-cholesterol increase risk of CAD/MI	Do et al. (2015)

Fig. 2 Examples of CAD risk genes involved in the sequence of atherosclerotic plaque formation and rupture. Details see text. Contains modified image material available at Servier Medical Art under a Creative Commons Attribution 3.0 Unported License

5 Pharmacological Targets Identified by Genomic Studies

Genomic studies identified putative pharmacological targets at every stage of plaque formation, progression, and rupture (Fig. 2) which are discussed in this section.

5.1 Lipid Metabolism

While less than half of the identified variants tag genes which are associated with traditional risk factors, lipid metabolism represents a cluster of such variants.

LDL-Cholesterol Metabolism High levels of LDL-cholesterol are an established risk factors for CAD. Not surprisingly, the most prominent genes which are directly

involved in LDL-cholesterol metabolism and have been tagged by GWAS are *LDLR*, *PCSK9*, and *SORT1*. In line, mutations in the *LDLR* gene leading to reduced hepatic uptake of LDL-cholesterol have been shown to underlie familial hypercholesterolemia which itself increases CAD risk (Tolleshaug et al. 1983; Brown and Goldstein 1986), and common variants at the *LDLR* locus have been associated with both phenotypes (Myocardial Infarction Genetics Consortium et al. 2009; Teslovich et al. 2010; Schunkert et al. 2011; Do et al. 2015). *PCSK9* has been identified as an interaction partner of the LDL-receptor. If *PCSK9* binds to the LDL-receptor, it is internalized and degraded resulting in reduced hepatic uptake and high plasma levels of LDL-cholesterol (Cameron et al. 2006). Gain-of-function mutations in *PCSK9* have been shown to increase LDL-cholesterol (Abifadel et al. 2003), whereas loss-of-function variants have been shown to reduce LDL-cholesterol and CAD risk (Cohen et al. 2006). *PCSK9* has been targeted pharmacologically via different approaches. The inhibition of *PCSK9* using, e.g., neutralizing antibodies resulted in a marked reduction of LDL-cholesterol and reduced risk of cardiovascular events in CAD patients (Sabatine et al. 2015, 2017). Sortilin 1 encoded by *SORT1* has also been shown to affect LDL-cholesterol (Samani et al. 2007; Musunuru et al. 2010; Teslovich et al. 2010; Schunkert et al. 2011). However, as it seems to also play a role in other processes and as the mechanism involving sortilin 1 in LDL-cholesterol metabolism has still not been fully understood, it might not represent an ideal drug target. *NPC1L1*, a membrane transporter leading to the uptake of cholesterol from the intestine encoded by the *NPC1L1* gene, also revealed a signal. Using exome sequencing, loss-of-function mutations were identified to be associated with reduced LDL-cholesterol and protection from CAD (Myocardial Infarction Genetics Consortium Investigators et al. 2014). In parallel, it has been shown that pharmacological targeting of *NPC1L1* with ezetimibe also reduces the incidence of cardiovascular events in addition to a statin (Cannon et al. 2015). Figure 3 summarizes the multiple lines of evidence for an involvement of LDL-cholesterol metabolism in CAD/MI from a genetic point of view.

Triglyceride Metabolism Lipoprotein lipase has been identified as a central enzyme regulating triglyceride levels. In line, genetic variants in *LPL* have been associated with both triglyceride levels and CAD risk (Khera et al. 2017). Additionally, the genes encoding several modulators of lipoprotein lipase activity were found to be associated with CAD/MI risk: (1) apolipoprotein C-III reduces lipoprotein lipase activity, and mutations in *APOC3* are associated with both increased triglyceride levels and CAD risk (The TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute 2014; Klarin et al. 2018); (2) apolipoprotein A-V increases lipoprotein lipase activity, and mutations in *APOA5* increase triglyceride levels and CAD risk (Do et al. 2015); and (3) angiopoietin-like 4 inhibits lipoprotein lipase activity, and mutations in *ANGPTL4* reduce triglyceride levels and CAD risk (Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators et al. 2016). Angiopoietin-like 3 seems to have comparable effects as deficiency was also associated with reduced triglycerides and CAD risk (Stitzel et al. 2017). Mainly apolipoprotein C-III but also

Fig. 3 Multiple lines of genetic evidence for an involvement of LDL-cholesterol metabolism in CAD/MI. 1. Pharmacological treatments targeting genes which have been associated with LDL-cholesterol reduce risk of cardiovascular events. 2. Mendelian randomization studies have proven the causal influence of LDL-cholesterol metabolism in CAD/MI. 3. CAD/MI and LDL-cholesterol show shared genetic risk variants which are associated with both traits at the genome-wide level of statistical significance. 4. Genetic risk scores for LDL-cholesterol including genome-wide significant variants are also associated with CAD/MI risk, i.e., individuals with a high genetic LDL-cholesterol risk score are also at higher risk for CAD/MI and vice versa. Contains modified image material available at Servier Medical Art under a Creative Commons Attribution 3.0 Unported License

angiopoietin-like 3 are subjects of research for novel strategies targeting lipoprotein lipase metabolism (Graham et al. 2013; Gaudet et al. 2015; Ahmad et al. 2019).

5.2 Inflammation

Vascular inflammation and the recruitment of leukocytes are the hallmarks of atherosclerosis (for reviews see Lusis (2000), Libby et al. (2011), and Swirski and Nahrendorf (2013)). Several clinical studies already in the past decades thus targeted inflammatory parameters. A prominent example is C-reactive protein (CRP), which has been regarded for a long time as a pathophysiological player. This was mainly due to the observation of elevated CRP levels in individuals suffering from CAD in epidemiological studies (Koenig et al. 1999; Danesh et al. 2004) making CRP a promising therapeutic target (Pepys et al. 2006). Indeed, clinical trials, e.g., the JUPITER trial which investigated the effect of rosuvastatin in individuals with elevated CRP levels, showed a benefit (Ridker et al. 2008). Genetic studies were, however, able to dissect this association and trial outcomes. As such, LDL-cholesterol could be clearly proven as a causal risk factor for CAD as a genetic risk score for LDL-cholesterol elevating variants was also associated with CAD risk (Kathiresan et al. 2008). In contrast, several variants found to increase CRP levels did not show an association with CAD risk (Lange et al. 2006; Zacho et al. 2008; Linsel-Nitschke et al. 2008; Schunkert and Samani 2008; C Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC) et al. 2011). While these studies on CRP remained disappointing, targeting inflammation has indeed lately been proven to be effective in CAD. In the CANTOS trial, the administration of an interleukin-1 β neutralizing antibody was able to reduce the incidence of cardiovascular events ultimately proving the inflammation hypothesis (Ridker et al. 2017). This is in line with Mendelian randomization studies that revealed evidence for a causal role of interleukin-6/interleukin-6 receptor signaling, which is downstream of interleukin-1 β , in CAD (Interleukin-6 Receptor Mendelian Randomisation Analysis Consortium et al. 2012).

GWAS led to the identification of a number of variants tagging genes which play a role in inflammatory processes to be associated with CAD/MI.

Autoimmune Processes CAD/MI and autoimmune disease share genetic risk factors. As such, the *SH2B3* locus has been identified to be associated with CAD/MI (Coronary Artery Disease C4D Genetics Consortium 2011b; Schunkert et al. 2011) but also, e.g., type 1 diabetes mellitus (Barrett et al. 2009) and celiac disease (Hunt et al. 2008). A role for the encoded protein, SH2B adaptor protein 3 (SH2B3), has been shown in post MI remodeling: lack of Sh2b3 in rats was associated with increased fibrosis, increased leukocyte infiltration, and decreased cardiac function (Flister et al. 2015). Additionally, it is involved in dendritic cell function leading to T-cell interferon signaling (Mori et al. 2014). Nevertheless, uncertainties remain as the locus was also found to be associated with blood pressure (Levy et al. 2009; Newton-Cheh et al. 2009) and hematologic parameters (Soranzo

et al. 2009). The latter observation might be important as an involvement in megakaryopoiesis and stabilization of thrombi has also been shown experimentally (Tong and Lodish 2004; Takizawa et al. 2010). In a recent study, the *SH2B3* risk allele was found to be associated with decreased SH2B3 expression which, at least for the murine counterpart *Sh2b3/Lnk*, together with cholesterol loading promoted platelet production and activation (Wang et al. 2016).

Innate Immunity CXCL12 is a chemokine which is encoded by the CAD risk gene *CXCL12* (Myocardial Infarction Genetics Consortium et al. 2009; Coronary Artery Disease C4D Genetics Consortium 2011b) and has a function in various cellular processes. In atherosclerosis the CXCL12/CXCR4 axis has initially been described to have a rather protective role (Döring et al. 2014). Disruption of this axis led to increased plaque formation and proinflammatory plaque phenotypes in vivo (Zernecke et al. 2008). In line, CXCL12/CXCR4 signaling was shown to be important in endothelial cells and smooth muscle cells via maintaining barrier function and contractile responses, respectively (Döring et al. 2017). However, the interaction of CXCL12 and CXCR4 in atherosclerosis seems to be more complex. Whereas the atheroprotective findings were mainly derived from studies in which the interaction was inhibited by small molecules or genetic deletion of CXCR4, endothelial cell-derived CXCL12 was found to promote atherosclerosis (Döring et al. 2019). CXCR4 in contrast seems to be atheroprotective: in addition to the mentioned in vivo studies, the rs2322864 C-allele, which was found to be associated with CAD risk in a candidate gene study, was also associated with CXCR4 expression in plaques (Döring et al. 2017). The double-edged effects of CXCL12/CXCR4 signaling need to be considered to successfully target this pathway.

5.3 Platelet Function and Nitric Oxide Signaling

Genetic studies led to the identification of several genes which play a role in nitric oxide (NO) signaling leading to the formation or degradation of the second messenger cyclic guanosine monophosphate (cGMP), an endogenous inhibitor of platelet aggregation (Moro et al. 1996). Whereas several genes (e.g., *NOS3*, *PDE5A*, *MRVII*, *PDE3A*) fulfill important functions in this pathway, most is known about *GUCY1A3* which encodes the α_1 -subunit of the soluble guanylyl cyclase (α_1 -sGC). As discussed above, a digenic mutation in *GUCY1A3* and the *CCT7* gene, which encodes the chaperone protein, was identified in a family by exome sequencing (Erdmann et al. 2013). At the same time, also a common non-coding variant (rs7678555) was identified in Europeans to be associated with CAD by GWAS (CARDIoGRAMplusC4D Consortium et al. 2013). Whereas the digenic mutation led to loss of α_1 -sGC due to premature stop of translation (Erdmann et al. 2013), the common variant has been shown to influence *GUCY1A3* expression, i.e., the risk allele G was linked to reduced expression (Kessler et al. 2017). Both the digenic mutation and the common risk variant led to reduced cGMP formation in platelets (Erdmann et al. 2013; Kessler et al. 2017). As a consequence, platelets of carriers of

the common risk variants showed impaired inhibition of platelet aggregation secondary to NO stimulation (Kessler et al. 2017). The sGC is a known pharmacological target. Specific stimulators and activators of the sGC are available (for an overview see Stasch et al. (2011)) and approved for other traits, e.g., pulmonary hypertension (Ghofrani et al. 2013a, b). First preclinical data also render a positive influence of sGC stimulators on atherosclerotic phenotypes possible (Tsou et al. 2014). Additionally, the impaired response to nitric oxide in carriers of the common risk allele G in platelets might also be targeted via unspecific inhibitors of platelet aggregation. Recently, we showed that homozygous risk allele carriers might benefit from aspirin treatment in the primary prevention of cardiovascular diseases, whereas homozygous or heterozygous carriers of the non-risk allele A seem to even display increased risk (Hall et al. 2019). Additionally, homozygous carriers of the risk allele G are at an increased risk of ischemic events after the implantation of coronary stents, at least in part via higher on-aspirin platelet reactivity (Kessler et al. 2019). While platelets, in addition to their well-known role in atherothrombosis, are also involved in atherosclerotic plaque formation (Gawaz et al. 2005) and despite the known influence of sGC function on inflammatory phenotypes (Ahluwalia et al. 2004), the exact mechanisms are still unknown. This is complicated by the fact that a complete knockout of the murine counterpart *Gucyl1a3* led to the unexpected finding of reduced atherosclerotic plaque formation (Segura-Puimedon et al. 2016), whereas genetically determined reduced but not lacking expression of *Gucyl1a3* was also associated with increased plaque formation (Kessler et al. 2017). The genetic findings at the further mentioned NO-cGMP-signaling loci associated with CAD/MI have been extensively discussed elsewhere (Wobst et al. 2018). A promising target in addition to sGC is phosphodiesterase 5A encoded by the *PDE5A* gene (Nelson et al. 2017) which leads to degradation of cGMP. However, there is currently no evidence for a beneficial effect of PDE5A inhibition in atherosclerosis.

5.4 Vascular Phenotypes

Several genes identified by GWAS have been linked to vascular phenotypes including the regulation of vascular tone and vascular remodeling.

Vascular Tone and Blood Pressure Hypertension is a known risk factor for CAD (Yusuf et al. 2004). Some of the CAD risk genes also display genome-wide association with blood pressure. In particular, *NOS3*, encoding endothelial NO synthase (eNOS), and *GUCY1A3* (see above) have an established role also in smooth muscle cells leading to vasodilatation after production of cGMP (Moro et al. 1996). Both have been identified as blood pressure genes (International Consortium for Blood Pressure Genome-Wide Association Studies et al. 2011; Salvi et al. 2012). In line, a genetic risk score for *NOS3* and *GUCY1A3* was associated with increased CAD risk. However, the effect could only in part be explained by effects on blood pressure (Emdin et al. 2018). Further genes associated with both CAD and blood pressure

include *SH2B3*, *CYP17A1*, *FURIN*, *AGT*, and *ARHGAP42*. Profound knowledge about the underlying mechanisms is still lacking. However, a genetic risk score for hypertension was strongly associated with CAD/MI and vice versa highlighting the importance of this risk factor which also has a strong heritability (Ntalla et al. 2019).

Vascular Remodeling A large number of genes have been linked to vascular remodeling (for an overview see Erdmann et al. (2018)). One example is the *REST* gene which has been identified as a CAD risk gene in 2017 (Nelson et al. 2017). *REST* encodes the RE-1 silencing transcription factor (REST) which has been mainly studied in neuronal diseases as Huntington disease (Zuccato et al. 2003) or seizures (McClelland et al. 2014). However, there is also evidence for a role in cardiac development where REST led to increased proliferation (Zhang et al. 2017). Additionally, REST has been described to inhibit microRNA-21 and to inhibit proliferation in vascular smooth muscle cells. As a consequence, REST might influence the formation of vulnerable atherosclerotic plaques (Jin et al. 2018). As it acts as a transcriptional regulator, several downstream transcripts might play important pathophysiological roles and represent novel targets. Another novel target is a disintegrin and metalloproteinase with thrombospondin motifs 7 (*ADAMTS-7*) which has been identified as a CAD risk gene (*ADAMTS7*) in 2011 (Reilly et al. 2011; Schunkert et al. 2011; Coronary Artery Disease C4D Genetics Consortium 2011a). It has been shown that *Adamts7* deficiency leads to reduced neointima formation after vascular injury (Bauer et al. 2015; Kessler et al. 2015b) as well as reduced atherosclerotic plaque formation under proatherogenic conditions (Bauer et al. 2015). Whereas the influence on vascular remodeling is influenced by *ADAMTS-7*-dependent degradation of cartilage oligomeric matrix protein (COMP) (Wang et al. 2009) and thrombospondin-1 (TSP-1) (Kessler et al. 2015b) with effects on vascular smooth muscle cells and endothelial cells, respectively, the mechanism underlying reduced atherosclerotic plaque formation in mice lacking *Adamts7* remains unknown so far. Of note, the *ADAMTS7* locus is the only locus that has been identified to exert a gene-environment interaction with smoking. In smokers, the protective effect of the *ADAMTS7* non-risk allele was outweighed in smokers, presumably via upregulation of *ADAMTS7* expression secondary to exposure to tobacco ingredients (Saleheen et al. 2017).

5.5 Further Directions

Other CAD risk genes have been clustered into similarly interesting pathways which could in principle be targets of therapeutic interventions. Genes have, for example, also been annotated to *transcriptional gene regulation*, *mitosis and proliferation*, or *neovascularization and angiogenesis* (Lempiäinen et al. 2018; Zeng et al. 2019b). The majority of variants and genes has nevertheless not yet been classified to such pathways (Erdmann et al. 2018). Also, the role of the first and strongest risk locus chromosome 9p21 is still not fully understood. Whereas studies initially focused on the role of the cyclin-dependent kinase inhibitors 2A/2B (*CDKN2A/B*) (Harismendy

et al. 2011), the circular form of the non-coding RNA *ANRIL*, which is also located at the locus (Pasmant et al. 2011), was found to have an atheroprotective role through balanced regulation of vascular cells. In contrast, the linear form which is increased in carriers of the risk allele increases atherosclerosis risk (Holdt et al. 2016).

6 Risk Scores and Risk Prediction

Apart from identifying novel pharmacological targets, GWAS raised hope to identify patients at risk early on to improve prevention of CAD. Whereas initial studies including some of the identified variants only led to modest success in this regard (Hughes et al. 2012; for an overview see Kessler and Schunkert (2012)), recent studies using polygenic risk scores changed the scenario. A polygenic risk score for CSD including more than six millions of variants was able to identify individuals with a substantially elevated CAD risk. Strikingly, the score identified 20 times more individuals than familial hypercholesterolemia patients, while the carriers of a high genetic risk score were at even higher CAD risk (Khera et al. 2018). Even in a scenario in which high polygenic risk score and the presence of familial hypercholesterolemia lead to a same increase in CAD risk, the prevalence of a high polygenic risk score was ten times higher than that of familial hypercholesterolemia (Khera et al. 2019) illustrating the potential of including such scores in prevention programs.

Another field in which polygenic risk scores can be used is to investigate associations with other phenotypes. In particular, as discussed above it has been shown that CAD/MI and other cardiovascular phenotypes, e.g., peripheral arterial disease, stroke, or abdominal aneurysm, share genetic predisposition. In contrast, risk of migraine was reduced with increased genetic CAD/MI risk (Ntalla et al. 2019). Risk scores were also able to dissect an interrelationship between educational attainment, which is regarded as a determinant of lifestyle factors, and CAD/MI risk. Here, a genetic risk score including variants which are known to be associated with educational attainment was also associated with CAD risk. As the signal was lost after adjusting for body mass index and smoking, it can be hypothesized that a genetic predisposition to educational attainment might influence a healthier lifestyle and, subsequently, reduce CAD/MI risk (Zeng et al. 2019a).

Furthermore, there is hope that knowledge of particular genetic risk factors can be used to design individualized treatment strategies. *GUCY1A3* as an example has been discussed above. Here, knowledge of genotype could inform aspirin therapy in the setting of primary prevention (Hall et al. 2019) and ischemic risk in CAD patients after PCI (Kessler et al. 2019). Genetic risk scores have also been able to identify individuals who could have a larger benefit from statin treatment (Mega et al. 2015; Natarajan et al. 2017). In the future, polygenic risk scores might therefore also be used in the design of clinical trials. A recent post hoc study of the ODYSSEY trial which investigated the benefit from the PCSK9 inhibitor alirocumab in CAD (Schwartz et al. 2018) revealed that individuals with a higher polygenic risk score particularly benefited from treatment (Damask et al. 2020). Whereas this clearly

indicated that polygenic risk scores might provide an excellent tool for risk stratification, a prospective benefit in the design of clinical trials needs to be demonstrated.

7 Summary

Genomic studies led to the identification of a large and still growing number of genes which play a role in the pathophysiology of CAD/MI. While only a few have been functionally investigated so far, novel therapeutic strategies have been developed in, e.g., LDL-cholesterol metabolism, and further promising targets might be identified. In addition, knowledge of genetic risk factors might facilitate prevention of the disease through early identification of individuals at risk and therapy via individualized treatment strategies.

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