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Carotid Artery Atherosclerosis: A Review on Heritability and Genetics

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Carotid atherosclerosis (CAS) is associated with increased cardiovascular risk, and therefore, assessing the genetic versus environmental background of CAS traits is of key importance. Carotid intima-media-thickness and plaque characteristics seem to be moderately heritable, with remarkable differences in both heritability and presence or severity of these traits among ethnicities. Although the considerable role of additive genetic effects is obvious, based on the results so far, there is an important emphasis on non-shared environmental factors as well. We aimed to collect and summarize the papers that investigate twin and family studies assessing the phenotypic variance attributable to genetic associations with CAS. Genes in relation to CAS markers were overviewed with a focus on genetic association studies and genome-wide association studies. Although the role of certain genes is confirmed by studies conducted on large populations and meta-analyses, many of them show conflicting results. A great focus should be on future studies elucidating the exact pathomechanism of these genes in CAS in order to imply them as novel therapeutic targets.

■ **Keywords:** carotid atherosclerosis, heritability, genetics, genome-wide association study, cardiovascular risk

Atherosclerosis is a chronic disease of the arteries characterized by inflammation and plaque building in the arterial wall, eventually leading to stenosis of the vessel. Carotid atherosclerosis (CAS), which is the manifestation of atherosclerotic disease in the cervical arteries, is associated with increased risk for cardiovascular diseases (CVDs) (Chambless et al., 1997; Ebrahim et al., 1999; Lorenz et al., 2007). CVDs are responsible for 31% of deaths globally, being the leading cause of death worldwide, and of all CVD deaths, 80% are attributable to stroke and myocardial infarction (World Health Organization, 2017). Carotid intima-media-thickness (CIMT), plaque, and stenosis are measurable traits indicating CAS. Several studies have confirmed that increased CIMT is the distance between the lumen-intima and the media-adventitia interfaces as assessed by ultrasound and is a predictor of coronary heart disease and cerebrovascular events, such as stroke (Bots et al., 1997; Chambless et al., 1997, 2000; Howard et al., 1993; Lorenz et al., 2007; Polak et al., 2011; Salonen & Salonen, 1991; van der Meer et al., 2004). Furthermore, CIMT is a marker of subclinical atherosclerosis (Naqvi et al., 2010), and prospective studies have shown that the subclinical stage of atherosclerosis is associated with clinical coronary artery disease (Kuller et al., 1995).

The tools for non-invasive assessment of CAS include B-mode ultrasound and time-of-flight magnetic resonance angiography, or contrast-enhanced magnetic resonance angiography. Ultrasound is a well-established and widely used method to detect carotid artery pathologies since it is highly repeatable, reproducible, and sensitive (Heiss et al., 1991; Stein et al., 2008). According to a meta-analysis on CIMT reproducibility, intra- and interobserver variability varied between 62% and 97% and between 58% and 100%, respectively (Kanters et al., 1997). However, the measurement error increased if maximal instead of mean CIMT was registered and if internal CIMT instead of common CIMT was measured (Kanters et al., 1997). MRI may provide additional information on plaque characteristics (Kerwin et al., 2013). The invasive imaging methods of the CAS include contrast-enhanced computer tomography, magnetic

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resonance, and catheter (digital subtraction) angiography. Plaque size relative to the resolution of magnetic resonance imaging influences the reliability of magnetic resonance angiography, which ranges between 0.38 and 0.89 for wall and cap thickness and between 0.66 and 0.94 for vessel area (Wasserman et al., 2010). Reliability or measurement error is an important factor to consider since it is a part of unique environmental variance (E), an important driver of the magnitude of heritability estimates.

Twin and family studies along with candidate gene analyses and genome-wide association studies (GWAS) have a crucial role to explore detailed genetic and environmental effects on these important markers of cardiovascular disease. Investigating underlying factors affecting the condition of carotid arteries is essential for future individualized treatment and prevention aspects. The present review aims to overview the studies that investigate the background of phenotypic variances of carotid artery atherosclerosis markers and possible responsible genes, with the focus on recent results.

Methods

To find the most appropriate articles for our topic, searches in PUBMED and the Web of Science database were conducted. First, we searched using the following keywords in the title or abstract: carotid, atherosclerosis, family study, twin study, heritability, heredity, parent offspring, parent child, and atherosclerosis. Second, we performed a separate search in order to review genes associated with carotid artery atherosclerosis with the key words candidate gene, genome-wide association, and carotid. The first search resulted in 152 articles, of which 41 were considered as relevant to our topic, and the second search resulted in 125 articles, of which 22 were included. Articles exclusively in English were considered for inclusion, as well as articles that were the subject of clinical twin research or family studies.

Twin Studies on CAS

The features of carotid arteries have been the focus of many twin studies since the 1980s. The first twin studies aimed to investigate the effect of environmental factors (e.g., smoking) on atherosclerosis in monozygotic (MZ) twins discordant for smoking (Haapanen et al., 1989; Lassila et al., 1988). Recently, CIMT and plaques on several segments of the carotid artery, which are important predictors of future cardiovascular events, have been the focus of investigations in twins (Naqvi & Lee, 2014).

Heritability of CIMT and its genetic correlation with other non-vascular traits. CIMT is the distance between the luminal surface of the intima (inner layer of the carotid artery) and the media-adventitia interface measured by ultrasound. Substantial genetic effects on CIMT have been described using the ACE + age model (Medda et al., 2014). Variance in the common CIMT was genetically determined in 31% of participants (Medda et al., 2014). Similar results were observed in the Korean population (Lee et al., 2012a). Using the ACE model, heritability was estimated at 48%, 38%, and 45% regarding common CIMT, bifurcation intima-media thickness (IMT), and internal CIMT, respectively. When taking into account measured covariates (cardiovascular risk factors), the variance was explained by additive genetics in 21% in the common carotid artery (CCA) and 24% in the bifurcation and in 31% in the internal carotid artery (ICA) (Lee et al., 2012a). Cardiovascular risk factors determined the total phenotypic variance in 46%, 37%, and 26% in the CCA, bifurcation IMT, and ICA, respectively (Lee et al., 2012a). Zhao et al. (2008) reported higher heritability (69%) of CIMT, assuming equal common environmental factors, and the heritability remained high (59%) after adjusting for age, high-density lipoprotein (HDL), and systolic blood pressure (SBP). However, the study population consisted only of middle-aged male twins. Swan et al. (2003) found higher MZ than dizygotic correlations regarding far-wall common CIMT, but the significant heritability was not confirmed. Near-wall common CIMT on the right side did not show a higher MZ correlation (Swan et al., 2003); however, far-wall common CIMT is the preferred location to determine CIMT (Wikstrand, 2007). The differences observed among segments of the carotid arteries might be attributable to the carotid geometry (Bijari et al., 2014; Phan et al., 2012). Furthermore, Polak et al. (2010) suggested that there is a segmental difference regarding effects of cardiovascular risk factors on the carotid arteries. According to their results, fasting glucose and diastolic blood pressure showed a stronger association with common CIMT than with the other segments. Hypertension, diabetes, and current smoking were associated with carotid bulb IMT, and low-density lipoprotein (LDL) cholesterol with ICA IMT (Polak et al., 2010).

Interestingly, the association of anthropometric parameters showed differences in genetic basis between men and women. In men, the genetic correlation between body mass index (BMI) and IMT in the common and internal carotid arteries remained significant even after adjustment for covariates, whereas in women, these traits did not genetically correlate with CIMT after adjustment (Song et al., 2012). Similarly, Juo et al. (2004) found genetic correlation between common CIMT and BMI in a family study conducted on the Hispanic population. In a study conducted on women twin pairs, substantial additive genetic basis (92%) was found regarding the association between carotid-femoral pulse wave velocity and CIMT (Cecelja et al., 2011). However, no independent association was found between these two traits (Cecelja et al., 2011). Kulshreshtha et al. (2014) implied that cardiovascular health index (which considers blood pressure, fasting glucose, total cholesterol, BMI, physical activity, healthy diet, and smoking) and CIMT are independently associated and this relation has a unique environmental basis.

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TABLE 1

Heritability Values for CAS Markers in Twin Studies

	Twin studies		
Author	Variable	Heritability	Adjustment for covariates
Medda et al. (2014)		Model: ACE + age	
	CCA IMT	31%	Sex, SBPao
Lee et al. (2012a)		Model: ACE + covariates	
	CCA IMT	21%	Age, BMI, diabetes, hypertension
	Bifurcation IMT	24%	Age, sex, alcohol use, diabetes hypertension
	ICA IMT	31%	Age, sex, BMI
Lee et al. (2012b)	CCA IMT	47%	Sex, FRS, study site, alcohol consumption, exercise, BMI,
	ICA IMT	45%	triglycerides and high-sensitivity C-reactive protein
	Bifurcation IMT	36%	
Zhao et al. (2008)	CCA IMT	59%	Age, HDL, SBP
Swan et al. (2003)	Non-significant, raw heritability estimates		Age, sex, height, blood pressure
	Right CCA far wall mean IMT	31%	
	Right CCA far wall max IMT	19%	
	Left CCA far wall mean	2%	
	Left CCA far wall max	26%	
Lucatelli et al. (2017)	A-E model		Age and country
	Presence of carotid plaque	52%	
Tarnoki et al. (2012)	ACE-model		Age-, sex-, and country-adjusted
	Plaque area	69%	
	Plaque sidedness	74%	
	Plaque quantity	74%	
	Presence of carotid plaque in CCA	78%	
	Presence of carotid plaque in bulb	68%	
	Presence of carotid plaque in ICA	66%	
	Plaque composition	74%	

Note: ACE-model: A = additive genetic effects, C = shared environment effects, E = unshared environment effects, BMI = body mass index, CCA = common carotid artery, FRS = Framingham Risk Score, HDL = high-density lipoprotein, ICA = internal carotid artery, IMT = intimamedia thickness, MAP = mean arterial pressure, RSES = Rosenberg Self-Esteem Scale, SBPao = aortic systolic blood pressure, SBP = systolic blood pressure.

Heritability of carotid artery plaque features. On a relatively large sample (328 individuals) of Hungarian and Italian twins, additive genetics was responsible for the variance in the presence of carotid plaques in 52% of participants, and this trait was explained by unique environmental factors in 48% (Lucatelli et al., 2017). Moreover, substantial relation in the co-occurrence of femoral and carotid plaques was found using the Cholesky model (42%) and was highly heritable (77%) (Lucatelli et al., 2017). Besides, the contribution of unique environmental factors to co-occurrence of these plaques was lower but not negligible (23%) (Lucatelli et al., 2017). The same study group reported an even higher additive genetic basis of carotid plaque presence (78%), plaque composition (74%), plaque sidedness (74%), and plaque quantity (74%) (Tarnoki et al., 2012). Although heritability seems to have a strong influence on both CIMT and carotid plaques, there are twin studies emphasizing the role of mainly non-shared environmental factors. Investigating co-twins living in different countries is an ideal model to assess unique environmental effects. Jartti et al. (2009) described interesting results when comparing CIMT in Finnish twin pairs when one co-twin was resident in Sweden. Being a resident in a lower coronary heart disease risk country had a significant impact on CIMT, but the difference was only significant when moving at an age less than 21 years (Jartti et al., 2009). On the other hand, the genetic susceptibility to have a greater CIMT in certain populations is also confirmed by the results of the same study group, showing that men from Eastern Finland had significantly higher IMT compared to men from Western Finland, and this was independent from their current resident country (Jartti et al., 2002). Table 1 summarizes the twin studies and heritability values on carotid plaque features and CIMT.

Proust et al. (2015) conducted an exome-array analysis and reported a heritability of 10.6% attributable to variants on the exome-array regarding common CIMT using genome-wide complex trait analysis (GCTA). However, despite the significant heritability, the large standard errors may indicate inconclusive results. None of the investigated exomes reached the significance thresholds in this study (Proust et al., 2015). GCTA is a tool to investigate heritability of phenotypes based on the differences in genotype data between cases and controls (Yang et al., 2011). According to our knowledge, no heritability was estimated using LD score regression for CIMT phenotypes.

Family studies and CAS. Although twin studies are the focus of this article, family studies in regard to CAS also have to be mentioned in brief. In family studies, the heritability of CIMT varied between 16% and 66% (Fox et al., 2003; Juo et al., 2005; Kao et al., 2005; Kuipers et al., 2013; Mayosi et al., 2005; Moskau et al., 2005; Ryder et al., 2017; Sayed-Tabatabaei et al., 2005; Xiang et al., 2002). There were two findings for heritability of plaque presence of 23% (Hunt et al., 2002) and 15% (Dong et al., 2010). Whether

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heritability is calculated for offspring only or offspring and parents influences the results heavily. The population characteristics – for example, hypertensive or diabetic individuals – also may explain the wide range of heritability results. Although the family study is a good method to assess the difference between generations, it may not differentiate shared environmental and genetic effects, and this can be a cause of differences compared to twin studies (Susser & Susser, 1987). Table 2 summarizes the family and sib studies and heritability values on carotid plaque features and CIMT.

The methodology of CIMT measurement may be responsible for the observed differences in heritability values. Far-wall CIMT values are preferred over near-wall CIMT values as layers of the near-wall cannot be accurately visualized and clearly distinguished because the ultrasound beam crosses the arterial wall layers in a different order (from high to low echogenic structures) as compared to far-wall CIMT. Far-wall CIMT, on the other hand, is highly correlated with wall thickness measurements on histological specimens (Pignoli et al., 1986; Wong et al., 1993). Differences also arise in carotid artery segments, as visualization of the CCA is more accurate compared to visualization of the carotid bulb and ICA. This is explained by the CCA being perpendicular to the ultrasound beam. Mean CIMT values have the advantage of better reproducibility; however, they are less sensitive to changes in CIMT (Stein et al., 2008). Most protocols are made for the registration of CCA far-wall mean IMT. On the contrary, plaques should be screened and registered on the CCA, carotid bulb and ICA segments separately (Stein et al., 2008).

Other non-modifiable factors, such as gender and ethnic differences, have to be considered when interpreting heritability results of CIMT. Several studies have confirmed that male gender is associated with significantly higher CIMT values both in adults (Mazurek et al., 2014) and children (Whincup et al., 2012). Relevant ethnic differences have been observed, with Black African Caribbeans having significantly higher CIMT compared to White Europeans (Markus et al., 2001; Whincup et al., 2012). Significantly higher CIMT values were reported among African-American individuals as compared to Whites and Asians (Breton et al., 2011). Furthermore, the impact of significant covariates on a given phenotype should always be taken into account when interpreting heritability results. Similar heritability estimates with and without adjustment for covariates indicate a more powerful heritability result and the effect of the covariates on the variance. Different models may be created based on the significantly associated covariates. For example, features of carotid plaque were highly heritable when adjusting for age, sex, and country only; when taking into account significant covariates, such as smoking, hyperlipidemia, peripheral arterial disease, and diabetes, the heritability did not change significantly (Tarnoki et al., 2012).

Genes Associated with Carotid Artery Atherosclerosis

Heritability of carotid artery atherosclerosis traits has been confirmed by the twin and family studies described above in detail, even though the results show great variation in heritability values depending on the methods and the populations investigated. In order to obtain a more detailed understanding of the genes beyond phenotype-based heritability, various studies investigating the exact gene variations have been conducted via linkage analysis, candidate gene association studies, and GWAS. These studies aim to determine the risk or the eventual causative role of specific alleles regarding the CAS phenotype; however, differences between these study designs have to be emphasized. Candidate gene association studies depend heavily on the right choice of genes, which can bias the outcomes. This approach may be successful if there is a presumption or knowledge about the function of the gene of interest. GWAS, on the other hand, can be conducted in order to find an association between a phenotype or disease and genetic variants without any prior knowledge on the function or action of a given gene. GWASs allow the sequencing of the entire genome, and therefore, are less biased by the choice of candidate genes. Furthermore, GWAS is a more powerful method to identify low-penetrance variants. This is also the reason why GWAS studies are well-established methods for the investigation of the genetic background of common genetic variations and complex diseases and phenotypes, such as CIMT and plaque. On the other hand, GWASs are not suitable for the analysis of the genetic background of rare diseases (Wilkening et al., 2009). Compared to linkage analysis, GWAS has a better resolution. Previous reviews summarized the most relevant genes and single-nucleotide polymorphisms (SNP) (Humphries & Morgan, 2004; Juo, 2009). Therefore, the current review is restricted to the advances and most relevant studies in this field since the latest reviews (Humphries & Morgan, 2004; Juo, 2009).

Candidate Gene Analyses and GWAS on CAS

Since atherosclerosis is a complex procedure, prior candidate gene association studies aimed to investigate regulators of the process at several points, such as inflammation and the function of extracellular matrix components.

The matrix-metalloproteinase-3 (MMP-3) and other matrix-metalloproteinases (MMPs). Members of the MMP family, which are important regulators of the extracellular matrix degradation, were investigated as target genes influencing atherogenesis in both coronary and carotid arteries. The MMP-3 enzyme has broad substrate specificity in extracellular matrix degradation and can regulate other MMPs (Woessner, 1991). Extensive research has been conducted on the 5A/6A polymorphisms found in the promoter region of the MMP-3 gene with regard to both coronary and CAS, as the 5A allele is associated with higher and the 6A allele with lower MMP-3

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TABLE 2

Heritability Values for CAS and Stiffness Markers in Family- and Sib-Studies

	Family studies		
Author	Variable	Heritability	Adjustment for covariates
Chen et al. (2008)	CCA IMT	37%	Age, sex, BMI
Li et al. (2013)	CCA IMT	47%	Age, sex
Juo et al. (2004)	Total maximum IMT	40%	Age, sex
	Total mean IMT	36%	
	CCA maximum IMT	35%	
	CCA mean IMT	39%	
	Bifurcation maximum IMT	25%	
	Bifurcation mean IMT	26%	
	ICA maximum IMT	9%	
	ICA mean IMT	12%	
Xiang et al. (2002)	Offspring	64%	Offspring: age, fasting glucose, and insulin sensitivity, SBP
	Parents to offspring	34%	Parents to offspring: age, SBP, total cholesterol, sex
Kao et al. (2005)	CCA IMT	16%	Age, sex
Kuipers et al. (2013)	Mean CCA IMT	47%	Residual heritability
	Max CCA IMT	35%	
	Mean AD	64%	
	Max AD	62%	
	Mean LD	58%	
	Min LD	57%	
Dong et al. (2010)	Plaque presence	50%	Plaque presence: Age, diabetes, smoking, BMI, hypertension
	Plaque area	17%	Plaque area: Age, diabetes, smoking, BMI, and hypertension, age^2 WHR
Mayosi et al. (2005)	$\log_e$ maximal CCA IMT	24%	Age, sex, body mass index, physical exercise
North et al. (2002)	CCA lumen diameter	44%	CCA lumen diameter: sex, age, center, diabetes, hypertension, BSA
	CCA IMT	21%	CCA IMT: sex, age, smoking, diabetes
Fox et al. (2003)	CCA mean IMT	38%	Age, sex, systolic blood pressure, number of cigarettes/day, total cholesterol, HDL cholesterol, triglycerides, diabetes status, body mass index, anti-hypertensive treatment, menopausal status, and hormone replacement therapy
	CCA maximum IMT	39%	
	ICA mean IMT	35%	
	ICA maximum IMT	31%	
Hunt et al. (2002)	Carotid artery plaque	23%	Age, sex, BMI, BMI, waist circumference, diabetes, hypertension, and smoking status
Wang et al. (2005)	CCA IMT unadjusted	68%	Multivariate-adjusted: gender, age, age^2 SBP, fasting insulin, and smoking
	CCA IMT adjusted for sex and age	45%	
	CCA IMT multivariate-adjusted	40%	
Sayed-Tabatabaei et al. (2005)	CCA IMT adjusted for age and gender	61%	1. CCA IMT covariates: age, gender, mean arterial pressure, LDL cholesterol, fasting glucose, and heart rate
	CCA IMT adjusted for other covariates	66%	2. Plaque score covariates: age, gender, body mass index, systolic blood pressures, LDL and HDL cholesterol, and smoking.
	Plaque score adjusted for age and gender	40%	
	Plaque score adjusted for other covariates	44%	
Ryabikov et al. (2007)	CCA IMT	54%	Model 1: age, sex, race
Lange et al. (2002)	CCA IMT (model 1)	31%	Model 2: age, sex, race, total cholesterol
	CCA IMT (model 2)	36%	Model 3: age, sex, race, total cholesterol, hypertension status, current smoking status
	CCA IMT (model 3)	41%	
Chien et al. (2008)	CCA IMT	19%	
Moskau et al. (2005)	CCA IMT (model 1)	61%	Model 1: age, sex, arterial hypertension, diabetes mellitus, Lipoprotein a
	CCA IMT (model 2)	26%	Model 2: age, sex, arterial hypertension, diabetes mellitus
Bella et al. (2013)	Carotid artery lumen diameter	28–56% in various centers	Sex, age
Ryder et al. (2017)	Whole cohort		Whole cohort: age, sex, race, BMI, MAP, and smoking of both parents and offspring
	Lumen diameter	55%	
	CCA IMT	29%	Complete trios: Age, sex, race, BMI, MAP, and smoking of both parents and offspring
	Diameter distensibility	28%	
	Cross-sectional distensibility	27%	
	Diameter compliance	3%	
	Cross-sectional compliance	27%	
	Among complete trios		
	Lumen diameter	58%	

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TABLE 2
Continued

	Family studies		
Author	Variable	Heritability	Adjustment for covariates
Rampersaud et al. (2008)	CCA IMT	29%	Age, sex
Sacco et al. (2009)	Mean Total IMT	65%	Mean total and mean bifurcation IMT, max total IMT, and max ICA IMT: Age, age^2, sex, PackYears, WHR, BMI
	Mean Bifurcation IMT	58%	
	Mean ICA IMT	47%	Mean ICA IMT: Age, age^2, sex, hypercholesterolemia, WHR, BMI
	Mean CCA IMT	56%	
	Max Total IMT	62%	Mean CCA IMT: Age, sex, hypertension, diabetes, PackYears, WHR BMI
	Max Bifurcation IMT	51%	
	Max ICA IMT	41%	Max bifurcation IMT: Age, age^2, sex, age by sex, PackYears, WHR, BMI
	Max CCA IMT	48%	
		Sib-study	Max CCA IMT: Age, sex, hypertension, hypercholesterolemia, diabetes, PackYears, WHR, BMI
Duggirala et al. (1996)	CCA IMT	92%	Age, diabetes mellitus, SBP, TC, and HDL cholesterol
	ICA IMT	86%	

Note: AD = adventitial diameter, BMI = body mass index, BSA = body surface area, CCA = common carotid artery, HDL = high-density lipoprotein, ICA = internal carotid artery, IMT = intima-media thickness, LD = lumen diameter, LDL = low-density lipoprotein, MAP = mean arterial pressure, SBP = systolic blood pressure, TC = total cholesterol, WHR = waist-hip ratio.

transcription (Ye et al., 1996), the former leading to decreased plaque stability and the latter leading to (stable) plaque progression. The 5A variant seems to be related to coronary plaque rupture and consequent myocardial infarction and the 6A variant may be related to coronary artery disease (Abilleira et al., 2006). Increased levels of MMP-3 have been described in patients with vulnerable plaques in a recent study (Hu et al., 2018), which is in line with the aforementioned assumption regarding the effect of the 5A variant. The MMP-3 6A allele was significantly associated with greater IMT (Rauramaa et al., 2000), even after adjustment for covariates (Djuric et al., 2008; Rundek et al., 2002). This was confirmed by a meta-analysis including roughly 180 individuals (Humphries & Morgan, 2004). Recent results also indicate that the 5A/6A polymorphism is associated with CIMT progression in patients with type 2 diabetes mellitus (Pleskovic et al., 2017). A recent GWAS study identified four SNPs on the 11q22.3 region that were independently associated with plasma MMP-12 levels on a genome-wide significant level, but expression quantitative trait loci analysis did not reveal a direct, causative role of these SNPs (Mahdessian et al., 2017). MMP-8 promoter gene polymorphisms were associated with plaque presence in Caucasian females and elevated MMP-8 mRNA levels in carotid artery plaques were associated with this allele ex vivo; however, the power of this study was limited by the low number of cases (Djuric et al., 2011). Other MMPs and their gene polymorphisms, such as MMP-14 (Li et al., 2014) and MMP-7 (Hu et al., 2011; Wang et al., 2011), might have a role in plaque vulnerability, but these results have not been confirmed on larger populations.

CDKN2A/B. *CDKN2A* and *2B* are important modulators of cell proliferation. Zhang et al. (2015) identified the association between the SNP near the *CDKN2A/B* gene and carotid artery calcification in a population of nearly 900 individuals. The same SNP was significantly associated with plaque presence in a meta-analysis conducted on a much larger sample (den Hoed et al., 2015). The *CDKN2A/B* gene is located at the 9p21 locus, which is subject of intensive research and the relevance of which in atherosclerosis is undoubted (Holdt & Teupser, 2012; Holdt et al., 2010, 2011, 2013, 2016). Results of genome-wide significance of this polymorphism were replicated by large-scale meta-analyses investigating its relation to coronary heart disease (Nikpay et al., 2015) and carotid plaque score (Pott et al., 2017). The *CDKN2A/B* polymorphism was strongly associated with carotid plaque score (Pott et al., 2017), but not with CIMT in a large-scale, multi-ethnic candidate gene association study conducted on more than 8,000 individuals (Vargas et al., 2016), which raises the possibility of clinical and subclinical atherosclerosis having a partly different genetic background (Holdt & Teupser, 2012).

IL-6 (interleukin-6) and IL-10 (interleukin-10). Great attention has been dedicated to inflammatory molecules and variations of their genes in the atherogenic process. The role of IL-6 and IL-10 was reviewed previously and conflicting results were found (Humphries & Morgan, 2004). Although it is logical that the genetic variants coding these inflammatory molecules affect CAS, results are conflicting regarding IL-6 (Chapman et al., 2003; Mayosi et al., 2005) and IL-10 (Heiskanen et al., 2010; Yu et al., 2015), and epigenetic regulatory mechanisms are possible (Pessi et al., 2015). In a family study of roughly 800 individuals, the functional polymorphism of IL-6 explained 2.5% of the heritable component of CIMT (Mayosi et al., 2005). Hulkkonen et al. (2009) and Riikola et al. (2009) did not find any association between CIMT and IL-6 gene polymorphisms in a gene-association study of ~2,000 individuals. Cunnington et al. (2009) could not demonstrate the association between IL-6 gene polymorphisms (which had been associated with coronary artery disease) and CIMT.

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Since the IL-10 is a molecule of potent anti-inflammatory character, it may have therapeutic implications in the future through gene therapy and is a subject of animal model studies (Dronadula et al., 2017; Du et al., 2011).

APOE. Although earlier studies emphasize the role of the E4 genotype of the *APOE* gene in carotid artery atherosclerosis and the atheroprotective role of the E2 genotype (Humphries & Morgan, 2004; Paternoster et al., 2010), large-scale meta-analyses did not confirm the relevance of this gene regarding CIMT. Instead of the *APOE* gene, the *APOC1* gene was associated with CIMT (Bis et al., 2011; Geisel et al., 2016). The authors speculate whether the significance of *APOE* gene may be restricted to early atherosclerosis and familial dyslipidemia (Bis et al., 2011).

ACE. The *ACE* gene codes the angiotensin-converting enzyme which converts angiotensin I to angiotensin II. A well-known association exists between the enzyme and hypertension. The insertion/deletion (I/D) in the non-coding region of this gene affecting the enzyme activity has been associated with increased CIMT. A meta-analysis including more than 9,800 individuals described the significant positive association between the D-allele and CIMT (Sayed-Tabatabaei et al., 2003). The association was significant only among Whites in low-risk populations, whereas it was significant among both Asians and Whites in high-risk populations (including symptomatic cerebrovascular disease, type I and II diabetes, non-diabetic hemodialysis, and hypertensive patients), indicating relevant ethnic differences possibly in both genetic and environmental effects regarding this trait (Sayed-Tabatabaei et al., 2003). The D/D genotype had a low frequency amongst Asians (Sayed-Tabatabaei et al., 2003). Several other studies with smaller hypertensive populations from both Asian (Park et al., 2009) and European (Imbalzano et al., 2017) ancestries found association between the D/D phenotype and increased CIMT. Some other studies found no significant association with this trait (Hung et al., 1999), which may depend on smaller sample sizes or other methodological aspects as an association of opposite direction has not been described (Humphries & Morgan, 2004).

Paraoxonase-1 (PON-1). PON-1 is an enzyme that binds to plasma HDL and protects it from oxidation (Litvinov et al., 2012). PON-1 may affect CAS through its influence on HDL (Kim et al., 2016) and LDL particles (Mackness et al., 1998). The genetic variants and expression of the *PON-1* gene is highly dependent on environmental influences, such as smoking, and it has been a subject of research regarding epigenetic effects (Aviram & Vaya, 2013). Details regarding *PON-1* polymorphisms and CAS have recently been reviewed (Lioudaki et al., 2017), and therefore, they are not further discussed in this article. Briefly, two polymorphisms of the enzyme gene have been described and their effect on CIMT and carotid plaque formation is controversial (Humphries & Morgan, 2004; Lioudaki et al., 2017).

Cholestryl-esther transfer protein (CETP). CETP is a protein taking part in the cholesterol transport from HDL to very low-density lipoprotein. The inhibition of CETP increases serum HDL levels, and therefore, it is a potential pharmacological target. Millwood et al. (2018) investigated the association between the loss-of-function CETP variant and CIMT. Furthermore, a genetic risk score was created consisting of the loss-of-function variant and four other CETP variants and its relation to CIMT was studied. No significant associations were found in this study involving more than 20,000 Asian individuals (Millwood et al., 2018). Two polymorphisms of the enzyme gene, the TaqIB, and the I405V polymorphisms, have been studied but the results are inconclusive regarding their relation to CIMT (Humphries & Morgan, 2004). A meta-analysis conducted on circa 2,200 individuals did not find any association between these polymorphisms and CIMT either in the total population of the meta-analysis, nor in Asians and Europeans separately (Li et al., 2014). Neither TaqIB nor other rare variants showed significant associations with CIMT in a study including 855 patients from different ethnicities, despite the fact that serum HDL and CETP levels depended on CETP polymorphisms (Tsai et al., 2008).

MTHFR. *MTHFR* is involved in homocysteine metabolism and the C to T substitution at nucleotide 677 in the *MTHFR* gene has been associated with lower enzyme activity and higher homocysteine levels (Miyaki, 2010). Increased homocysteine levels are associated with higher cardiovascular risk (Graham et al., 1997). Previous reviews have summarized the findings regarding *MTHFR* gene polymorphisms (Humphries & Morgan, 2004; Juo, 2009). The association between the *MTHFR* gene and homocysteine levels is confirmed, but recent candidate gene studies including less than 1,000 individuals did not confirm the gene's direct effect on CIMT (Hernandez-Socorro et al., 2017; Pramukarso et al., 2015; Sun et al., 2017). Whether hyperhomocysteinemia is directly or indirectly related to atherosclerosis (the latter because of renal dysfunction) is to be elucidated (Durga et al., 2004).

Other genes. Further novel findings of large-scale meta-analyses include the role of certain SNPs of the *EDNRA* gene (coding the endothelin receptor type-1) in multiple carotid artery plaque phenotypes (Bis et al., 2011; Hemerich et al., 2015). Regarding the relation of *EDNRA* gene to CIMT, the results are controversial (Li et al., 2015; Lopez-Mejias et al., 2014); however, gene–environmental interactions regarding this gene have been studied (Li et al., 2015; 2015). Another gene, *PINX1*, the product of which is a telomerase inhibitor, was associated with common CIMT

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in the general population (Bis et al., 2011; Geisel et al., 2016; Li et al., 2015), although no similar association was found in a population with rheumatoid arthritis (Lopez-Mejias et al., 2014). The *SMG6* gene showed significant association with common CIMT with increased heterogeneity across ethnicities, whereas LPA and TRIB1 loci were significantly associated with internal CIMT (Vargas et al., 2016).

In the Asian population, the highly significant association between Early B-cell Factor 1 (*EBF1*) gene SNPs and CIMT progression was emphasized in a large-scale GWAS study (Xie et al., 2015), but other results point toward the epigenetic regulation of this gene and its effect on atherosclerosis (Singh et al., 2015). In the same study, another SNP near the procadherin 15 (*PCDH15*) gene was linked to CIMT progression on a genome-wide significant level (Xie et al., 2015), and although other similar SNPs in this region have been identified, the relevance of this SNP is not confirmed.

More recently, the ryanodine receptor 3 (*RYR3*) gene SNPs and their relation to CAS have been investigated. This gene codes a calcium channel regulating intracellular calcium and inflammatory processes. Two variants of this gene showed an association with subclinical and clinical CAS in a subpopulation of men (Shrestha et al., 2010), women (Shendre et al., 2014), and a population of both sexes (Zhi et al., 2015) suffering from human immunodeficiency virus (HIV) as well as clinical manifestation of CAS in a postmortem study of Japanese elderly (Zhao et al., 2014).

Bis et al. (2011) conducted a GWAS meta-analysis on ∼25,000, ∼11,000, and∼10,000 individuals regarding carotid artery plaque, internal CIMT, and common CIMT, respectively. Three SNPs reached genome-wide significance regarding common CIMT, including a SNP near the *ZHX2* gene on chromosome 8q24, a SNP near the *APOC1* gene on chromosome 19q13, and a SNP within the *PINX1* gene on 8q23.1. The first two SNPs were associated with decreased common CIMT. More specifically, the rs11781551 near the *ZHX2* gene lowered CIMT by 0.8% per copy of the allele, and the G allele of rs445925 near the *APOC1* gene lowered common CIMT by 1.6%. The G allele on the third SNP near the *PINX1* gene, rs6601530, was associated with increased common CIMT by 0.08% per allele copy. Two SNPs near the *PIK3CG* and *EDNRA* genes were associated with plaque presence on a genome-wide significant level. No SNP achieved genome-wide significant association with regard to internal CIMT (Bis et al., 2011).

Melton et al. (2013) investigated SNPs associated with CIMT in 772 Mexican American individuals. No genome-wide significant association was detected, but there were some nominally significant SNPs on chromosome 20p11 near the gene *PAX1* and on chromosome 2q21 in the gene *NCKAP5* (Nck-associated protein 5) in relation to internal CIMT. Another SNP upstream of *EXOC3L2* on chromosome 19q13 was nominally significantly associated with common CIMT. The number of cases was small in this study and therefore the study power was relatively low. There was a genetic correlation of 51% between common and internal CIMT, indicating that the genetic basis of these two segments CIMT is partly overlapping (Melton et al., 2013).

Shendre et al. (2017) performed genome-wide association and admixture analysis on CIMT-related genes in a population of ∼1,000 individuals including HIV-positive and negative African-American female individuals (Shendre et al., 2017). None of the SNPs reached genome-wide significance, although some SNPs almost reached this level, such as mediator complex subunit 30 and exostosin glycosyltransferase 1 (*MED30* and *EXT1*) genes on chromosome 8 in all women and in the HIV-positive group, catenin delta 2 (*CTNND2*) gene on chromosome 5, transmembrane and coiled-coil domain family 3 gene, and the NADH: Ubiquinone oxidoreductase subunit A12 (*TMCC3* and *NDUFA12*) in the HIV-positive group and family with sequence similarity 5, member C, and regulator of G-protein signaling 18 genes (*FAM5C* and *RGS18*) on chromosome 1 in the HIV-negative group. *CTNND2* and *TMCC3* | *NDUFA12* were significantly associated with local European ancestry. However, the study power was relatively low because of the number of cases (Shendre et al., 2017). The relevance of these novel findings remains to be elucidated.

Other GWAS in Relation to CAS (Genetic Risk Score)

Large-scale studies attempted to find associations between certain risk scores and SNPs previously identified by GWAS.

Den Hoed et al. (2015) investigated the association between genetic risk score consisting of 45 genes and carotid plaque and CIMT in a meta-analysis involving more than 7,000 individuals. The risk alleles of these 45 loci had previously been associated with coronary heart disease. Carotid bulb CIMT and plaque presence showed significant associations with the genetic risk score, but not CCA IMT (den Hoed et al., 2015). Each risk allele of the 45 loci increased the odds of having plaque by 2.8% and that of having increased CIMT by 0.24% (den Hoed et al., 2015). SNPs near *CDKN2B/A* were significantly associated with plaque presence, but this large-scale meta-analysis failed to demonstrate any such association between plaque presence and SNPs near *EDNRA*. Furthermore, additional risk-alleles on chromosome 9p21.3, where *CDKN2B/A* is located, increased the odds of having plaque with an additional 13.9%. With regard to CIMT, no significant association was observed between the individual loci and CIMT of the carotid bulb or CCA; however, the association between risk alleles near the apolipoprotein gene cluster and common CIMT was confirmed (den Hoed et al., 2015). No association was found between SNPs affecting coronary artery disease and common CIMT in a meta-analysis involving roughly 5,000 individuals (Conde et al., 2011). The different outcomes regarding CIMT of the CCA and bulb might relate to

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their associations with different conditions as previously described in the literature, as CCA IMT tends to be associated with stroke whereas carotid bulb IMT tends to reflect the risk for ischemic heart disease (Ebrahim et al., 1999).

Conclusion

Although heritability varies widely regarding carotid artery atherosclerosis traits, moderate additive genetic influence seems to determine the variance in these phenotypes. Future international collaborative twin studies (such as on discordant MZ twins) should elucidate how the different environmental interventions and effects influence this genetic susceptibility in various ethnicities. The co-occurrence of CAS with coronary and peripheral atherosclerosis and other diseases have been certified with twin studies. Based on these findings, new therapeutic targets and preventive individualized strategies may be established. Numerous SNPs have been described to increase the risk for development of subclinical or clinical CAS. However, the results are often conflicting, and only a minority of these genes seems to be potential future therapeutic targets. This warrants the need for future research aiming to obtain a deeper knowledge of the exact pathomechanism of these genetic variants and gene-environmental interactions, which would be essential for practical implications of the enormous amount of genes found in GWAS-studies in relation to CAS.

Conflict of Interest

None.

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