Genes associated with Atherosclerosis in type 2 diabetes mellitus


ABSTRACT
Available statistics and research reveal that most patients with diabetes die from cardiovascular diseases, mainly due to markedly advanced and untreated atherosclerosis. Although the risk of cardiovascular disease increases additively with the number of conventional risk factors, including diabetes, hypertension and dyslipidemia, these conventional risk factors cannot fully account for the risk of cardiovascular diseases. Atherosclerosis is a complex multifactorial and polygenic disorder resulting from interactions among individual genetic and various environmental risk factors. Of late, research field has been focusing on arterial calcifications in setting of atherosclerosis. Many features of atherosclerosis-related calcification provide useful clinical information. Several mechanisms leading to calcification associated with atherosclerosis have been proposed; however, no direct testing of proposed mechanisms has yet been reported. Studies in genetically altered animals and in humans have shed light on potential genetic determinants, which in turn could form the basis for more comprehensive understanding of the factors affecting calcification within plaque and the associated pathobiologic implications. Understanding the molecular and genetic determinants of specific structural plaque components such as calcification can provide a solid foundation for the development of novel therapeutic approaches. Although it is possible that some of these atherosclerosis-susceptibility gene polymorphisms are synergistically responsible for advanced atherosclerosis, few studies have reported on the involvement of a combination of potentially susceptible genes in cardiovascular diseases. The objective of the present review is to identify Single Nucleotide Polymorphisms associated with carotid atherosclerosis of type 2 diabetic patients, which can be considered as a surrogate end point of cardiovascular events.

Keywords: Type 2 diabetes mellitus, cardiovascular disease, atherosclerosis, SNPs, genes, monogenetic heredity, polygenic heredity.

1. Introduction
Type 2 diabetes mellitus (T2DM) is characterized by impaired insulin secretion, by definition is a heterogeneous, multifactorial, polygenic syndrome which results from insulin receptor dysfunction. Some study conclude that β-cell mass is decreased in T2DM and that the mechanism underlying is due to increased β-cell apoptosis. The major defect leading to a decrease in β-cell mass in T2DM is increased apoptosis, while new islet formation and β-cell replication are normal, therapeutic approaches designed to arrest apoptosis could be a significant new development in the management of T2DM, since this approach might actually reverse the disease to a degree rather than just palliate glycemia [1]. The dyslipidemia in prolonged diabetes mellitus (DM) results in atherosclerosis. Atherosclerosis is characterized by endothelial dysfunction, vascular inflammation and the buildup of lipids, cholesterol, calcium and cellular debris within the intima of the vessel wall [2]. This constriction results in plaque formation accumulating on the inner walls of arteries, and as the artery walls thicken, the pathway for blood narrows and this can decrease or block blood flow diminishing oxygen supply to target organs. Atherosclerosis represents a leading global cause of death and disability [3]. Based on greater understanding of the relative roles of insulin resistance and β-cell dysfunction in T2DM, it can be anticipated advances in the identification of genes contributing to the development of the disease as well as approaches to the treatment and prevention of T2DM. Although environmental factors such as diet or smoking play an important role in atherosclerosis development, genetic factors represent consequential determinant of atherosclerotic cardiovascular disease risk. Advances in molecular genetics have revealed that genetic disorders significantly influence susceptibility to atherosclerotic vascular diseases [4].
2. Atherosclerosis and Type 2 Diabetes Mellitus

Atherosclerosis, formerly considered a bland lipid storage disease, actually involves an ongoing inflammatory response. Recent advances in basic medical science have established a fundamental role for inflammation in mediating all stages of this disease from initiation through progression and ultimately, the thrombotic complications of atherosclerosis. These new findings provide important links between risk factors and the mechanisms of atherogenesis. Recent studies have shown that this emerging biology of inflammation in atherosclerosis applies directly to patients. Elevation in markers of inflammation predicts outcomes of patients with acute coronary syndromes, independently of myocardial damage. In addition, low-grade chronic inflammation, as indicated by levels of the inflammatory marker, C-reactive protein (CRP), prospectively defines risk of atherosclerotic complications, thus adding to prognostic information provided by traditional risk factors. In the case of lipid lowering with statins, this anti-inflammatory effect does not appear to correlate with reduction in low-density lipoprotein (LDL) levels [3].

Since most patients with diabetes die from complications of atherosclerosis, they should receive intensive preventive interventions proven to reduce their cardiovascular risk. Diabetes Mellitus (DM) magnifies the risk of cardiovascular morbidity and mortality. Besides the well-recognized microvascular complications of diabetes, such as nephropathy and retinopathy, there is a growing epidemic of macrovascular complications, including diseases of coronary arteries, peripheral arteries and carotid vessels, particularly in the burgeoning T2DM population. Understanding atherosclerosis in DM and instituting therapy guided by emerging evidence should improve outcomes in patients. The evidence supports aggressive antiatherosclerotic management strategies upon diagnosis of T2DM to minimize the risk of cardiovascular morbidity and mortality [6].

T2DM should also be considered a vascular disease because diabetic patients have a strong predilection for atherosclerosis. The mechanisms of the high rate of atherosclerosis are multifactorial and give clinicians and researchers insights into potential preventive therapies [7]. T2DM and obesity are major risk factors for the development of coronary artery disease (CAD) and premature atherosclerosis. Both the conditions are associated with insulin resistance, oxidative stress and inflammation. Inflammatory mediators, including plasma interleukin 6, tumor necrosis factor α and tumor necrosis factor-R are elevated in these individuals. The elevations of inflammatory mediators may contribute to the pathogenesis of atherosclerosis, because atherosclerosis is an inflammation of the arterial wall. There is evidence that the thiazolidinedione (TZD) class of drugs may alleviate some of the adverse atherosclerotic effects common in patients with T2DM. The use of TZDs as potent anti-inflammatory agents in patients with T2DM is an approach that would normalize glucose levels, as well as potentially alleviate the long-term risk of atherosclerosis [8].

3. Genes Associated with Atherosclerosis

The genes that provide the predictive power include many previously suspected to play a role in atherosclerosis and additional genes without prior association with atherosclerosis. Studies report a novel method for generating a molecular phenotype of disease and then identifying genes whose discriminatory capability strongly implicates their potential roles in human atherosclerosis [9]. T2DM is considered a model of premature atherosclerosis with a strong genetic component. A deletion-polymorphism in the angiotensin-converting enzyme (ACE) gene was recently reported to be associated with myocardial infarction especially in people classified as low risk. A recent report confirms that the D allele of the ACE gene is a strong and independent risk factor for Coronary Heart Disease (CHD) in T2DM patients. The D allele is associated with early-onset of CHD in T2DM, independently of hypertension and lipid values. A progressively increasing relative risk in individuals heterozygous and homozygous for the D allele was observed, suggesting a codominant effect on the cardiovascular risk. Identification of T2DM patients carrying this putative CHD-susceptibility genotype would help early detection and treatment of CHD [10]. There is considerable evidence that the antioxidant activity of high density lipoprotein (HDL) is largely due to the paraoxonase-1 (PON1) located on it. Preliminary case-control evidence suggests that the quest for dietary and pharmacological means of modifying serum PON1 activity may allow the oxidant model of atherosclerosis to be tested in clinical trials [11].

PON1 was identified as a genetic risk factor for cardiovascular disease (CVD) in recent studies focusing on a polymorphism affecting position 191. A second polymorphism of the paraoxonase gene affects position 54 and involves a methionine (M allele) to leucine (L allele) change. It was investigated in diabetic patients with and without vascular disease. Susceptibility to CVD correlates with high activity paraoxonase alleles. The 54 polymorphism would appear to be of central importance to paraoxonase function by virtue of its association with modulated concentrations. The latter could explain the association between the 54 and 191 polymorphisms and CVD [12]. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily. The 3 PPAR isotypes, PPAR-α, PPAR-γ and PPAR-δ, play a key role in the regulation of lipid and glucose metabolism. Obesity and the interrelated disorders of the metabolic syndrome have become a major worldwide health problem [13].

Genetic association studies are viewed as problematic and plagued by irreproducibility. Many associations have been reported for T2DM, but none have been confirmed in multiple samples and with comprehensive controls. There is an association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor-α (PPAR-α) with T2DM. There is implications of inherited variation in PPAR in the pathogenesis of T2DM. Because the risk allele occurs at such high frequency, its modest effect translates into a large population attributable risk— influencing as much as 25% of T2DM in the general population [14].

4. Monogenetic Heredity

Single-gene ( mendelian) disorders represent the most remarkable examples of the genetic implication to atherosclerosis [15]. Several monogenic diseases elevate plasma levels of LDL by impairing the activity of hepatic
LDL receptors, which normally clear LDL from the plasma. Familial hypercholesterolemia was the first monogenic disorder shown to cause elevated plasma cholesterol levels. The primary defect in familial hypercholesterolemia is a deficit of LDL receptors and more than 600 mutations in the LDL-R gene have been identified in patients with this disorder [16]. The frequency of this genetic defect is 1 in 1,000,000. Deficiency of lipoprotein transport abolishes transporter activity, resulting in elevated cholesterol absorption and LDL synthesis. Mutations in the APOB-100 gene, which encodes apolipoprotein B-100, reduce the binding of apolipoprotein B-100 to LDL receptors and slow the clearance of plasma LDL, causing a disorder known as familial ligand-defective apolipoprotein B-100 [17]. Five different mutations located in this region of the APO-B gene are reported to cause a high-cholesterol phenotype. One in 1000 people is heterozygous for one of these mutations. These patients are diagnosed as familial defective APOB (FDB), which is clinically indistinguishable from familial hypercholesterolemia. Mutations in PCSK9 have recently been shown to result in Mendelian forms of increased LDL-C levels. PCSK9 encodes NARC-1 (neural apoptosis regulated convertase), a newly identified human subtilase that is highly expressed in liver and contributes to cholesterol homeostasis [18,19].

The mutations in the Adenosine Triphosphate (ATP) binding cassette transporter 1 (ABCA1) gene cause Tangier disease (TD), an autosomal recessive hereditary disorder characterized by severe HDL deficiency, sterol deposition in macrophages and premature atherosclerosis [19]. ABCA1 promotes cholesterol and phospholipid efflux from cells to lipid poor apolipoprotein (apoA1), the precursor of HDL and plays a major role in cholesterol homeostasis and reverse cholesterol transport [20]. Sitosterolemia, a rare autosomal disorder, results from loss-of-functional mutations in genes encoding two ABC transporters, ABCG5 and ABCG8, which act in concert to export cholesterol into the intestinal lumen, thereby diminishing cholesterol absorption [21]. Very rare hereditary hypercholesterolemia with the prevalence <1 case per 10 million persons is autosomal recessive hypercholesterolemia. The molecular cause is the presence of defects in a putative hepatic adaptor protein, which then fails to clear plasma LDL with LDL receptors. Mutations in the gene encoding that protein elevate plasma LDL to levels similar to those seen in homozygous familial hypercholesterolemia [22].

5. Polygenetic Heredity

Within the general population, polymorphisms within genes in lipid metabolism, inflammation and thrombogenesis are probably responsible for the wide range of atherosclerotic diseases. A good example of a candidate gene is apolipoprotein E (apoE). The apoE gene is located at chromosome 19q13.2. Among the variants of this gene, alleles E2, E3 and E4 constitute the common polymorphism found in most populations. Of these variants, apoE3 is the most frequent (>60%) in all populations studied [23]. The polymorphism has functional effects on lipoprotein metabolism mediated through the hepatic binding, uptake and catabolism of chylomicrons, chyomicron remnants, very low-density lipoprotein (VLDL) and HDL subspecies. Type III hyperlipidemia is an interesting example of a genetic interaction. Almost all individuals with this uncommon hyperlipidemia are homozygous for the E2 allele, but most individuals who are homozygous for the E2 allele do not have the disorder. CYBA gene polymorphisms, including C242T (rs4673) and −930A/G (rs9932581) are implicated in the process of atherosclerosis from a very early stage to the clinical phase of cardiovascular diseases. The CYBA 242T allele consistently shows a protective association against the chronic inflammatory process presenting as impairment of endothelial function and the development of coronary artery disease. In addition, the CYBA −930A/G gene polymorphisms might modify the effect of smoking and hypertension on early structural alterations on the arterial wall [24]. Atherosclerosis is a complex multifactorial and polygenic disorder that is thought to result from interactions among individual genetic risk factors and various environmental factors [25]. To identify disease susceptibility genes, genome-wide scanning of SNPs & candidate gene analysis have been performed. Genes potentially associated with atherosclerosis and diabetes mellitus are described below.

i. Adiponectin

Adiponectin is an adipose tissue-specific hormone which is inversely associated with metabolic alterations related to atherosclerosis. Polymorphisms in the adiponectin gene (AdipoQ) have been related to low adiponectin levels as well as several cardiovascular risk factors, but this association remains controversial. An association between the AdipoQ G276T polymorphism and lipid levels exist in overweight boys alone, thereby suggesting that the influence of the AdipoQ polymorphisms on cardiovascular risk factors may be dependent on BMI [26]. Serum adiponectin levels were significantly higher in normotensive subjects compared with hypertensive subjects. High circulating levels of adiponectin were found to be associated with hypertension only in female T2DM patients [27] and obese patients presented with higher values of atherogenic indicators than the non-obese patients. The indicators positively correlated with CRP and lipid concentrations, and adiponectin concentrations were decreased in children with polymorphism G276T in adiponectin gene [28].

ii. Apolipoprotein E

Several SNPs have been recognized as associated with ischemic heart disease (IHD) although the optimal set of risk genotypes has not been identified. Currently identified high-risk SNPs confer an additive biomarker for cardiovascular events [29]. The apo ε genotype yields poor predictive values when screening for clinically defined atherosclerosis despite positive, but modest associations with plaque and CHD outcomes [30].

iii. β-3 Adrenergic Receptor

The ADRβ3 T190C may be involved in the pathophysiology of gall bladder cancer by both gallstone-dependent pathway and by some other independent mechanisms [31]. Cells express the β3AR, and its activation contributes to the regulation of insulin secretion. These findings may help explain the low levels of insulin secretion in response to glucose tolerance test observed in humans carrying the Arg64 polymorphism [32]. Unlike β1- and β2- adrenergic receptors, it has been shown that β3-adrenergic receptors possess the cardio depressant effects in human ventricles, what did not fit to its stimulatory properties of adenylyl
cyclase in other tissues. In this regard, the role of β3-adrenergic receptors in the regulation of cardiac function may be of great importance in pathological conditions and remains undetermined [33].

iv. Cholesteryl Ester Transfer Protein
Controversy remains about whether CETP deficiency and the resultant rise in HDL-C are antiatherogenic or whether CETP has the opposite effect due to its role in reverse cholesterol transport and it indicates that HDL-C remains an important risk factor for CHD in the elderly [34]. Epidemiological studies have shown an increased incidence of coronary atherosclerosis in CETP-deficient patients. The plasma CETP is a key protein in reverse cholesterol transport [35]. The CETP is localized primarily on larger, Lp (AI)-containing HDL particles, and its principal role is to catalyze the exchange of triglycerides from apo B-containing particles (e.g., LDL, VLDL) to cholesteryl esters from HDL [36].

v. C- Reactive Protein
Carriers of the rarer G allele had significantly higher CRP plasma concentrations in CAD (+) subjects and higher homocysteine concentrations in CAD (+) group. Atherosclerosis is an inflammatory disease resulting from different genetic and environmental factors. Studies support the contribution of CRP genetic variations in the development of CAD [37]. CRP 1059G/C gene variation influences plasma CRP levels. Conversely, this polymorphism was not associated with the risk for Acute Myocardial Infarction (AMI) [38].

vi. Ghrelin
No association was found in the genetic variation in the ghrelin gene and T2DM; however, results support the speculation that weight regulation may be affected by the genetic variation observed in the ghrelin gene in patients with DM [39]. Previous studies suggested that polymorphisms in the coding region of the preproghrelin were involved in the etiology of obesity and might modulate glucose-induced insulin secretion [40].

Vii. Glucose Transporter 1 (GLUT 1)
The ketogenic diet (KD) effectively restores brain energy metabolism. Ketosis does not influence impaired GLUT1-mediated glucose transport into brain: hypoglycorrachia, the biochemical hallmark of the disease, can be identified in GLUT 1-deficient patients on a KD [41]. There were no obvious correlations between phenotype, genotype or biochemical measures. The KD produced good seizure control [42].

viii. Hepatic Lipase
Hepatic lipase (HL) may affect intracellular lipid content. The prime role of HL is to maintain, in concert with other factors (e.g., lipoprotein receptors), intracellular lipid homeostasis. This, and the uncertainties about its impact on human atherosclerosis, makes it difficult to predict whether HL is a suitable target for intervention to lower CAD risk [43]. The variation in lipid profile associated with the C-514T polymorphism is significant and the T allele is associated with the best response to Estrogen Replacement Therapy (ERT) [44].

ix. Insulin Receptor Substrate-1
Overall, carriers of the 972Arg variant of the IRS-1 gene are at a 25% increased risk of having T2DM compared with non-carriers. The odds ratios are generally higher in hospital-based studies, including relatively young, symptomatic cases [45].

x. Interleukin (IL)-6
A statistically significant difference of the frequency of the A allele of the Protein Z (PZ) intron F G79A polymorphism was found with a higher prevalence of the A allele among the controls compared with the patients, suggesting a lower risk of recurrent pregnancy loss among the studied patients, but the IL6 C634G polymorphism did not prove to have an equivalent effect [46]. A common G/C promoter polymorphism at nucleotide (~174) of the IL–6 gene has been shown to affect basal IL-6 levels. Consequently, the IL-6 genotype may be associated with risk and outcome of ischemic stroke (IS). (~174) GG-genotype of the IL-6 gene is associated with severe stroke in young patients with acute cerebrovascular events [47]. IL-6 -174 G/C polymorphism is not associated with the risk of premature CAD, and does not contribute to cardiovascular risk stratification [48].

xi. LDL Receptor-Related Protein
The low-density lipoprotein receptor-related protein (LRP) polymorphism influences the risk as well as the age at onset of Alzheimer’s Disease (AD). Results contrast with other studies which described the C-allele to be a risk-factor for AD, but are in line with a recent publication on the effect of LRP polymorphism on longevity and on the risk for CAD. Further research on LRP polymorphisms is needed to evaluate their effects on the risk of AD, on CAD and on longevity [49].

xii. Lipoprotein Lipase
Researches established a central role for Lipoprotein lipase (LPL) in the overall lipid metabolism and transport but have also identified additional, non-catalytic functions of the enzyme. Abnormalities in LPL function have been found to be associated with a number of pathophysiological conditions, including atherosclerosis, chylomicronaemia, obesity, Alzheimer’s disease and dyslipidemia associated with diabetes, insulin resistance and infection. Advances have also been made in relating the various domains in the protein to different functions, and in understanding the mechanisms that are responsible for the changes in LPL expression seen in response to nutritional and other physiological changes and during disease [50]. A previously uncharacterized pathway in which the key lipolytic enzyme LPL can act on circulating lipoproteins to generate PPARα ligands, providing a potentially important link between lipoprotein metabolism and distal PPARα transcriptional effects [51]. LPL is a fascinating enzyme that contributes in a pronounced way to normal lipoprotein metabolism, tissue-specific substrate delivery and utilization, and the many aspects of obesity and other metabolic disorders that relate to energy balance, insulin action and body weight regulation [52].

xiii. Microsomal Triglyceride Transfer Protein
There was no significant association between the G-493T polymorphism and variability of lipoprotein subclass...
distributions or lipoprotein particle size. The G-493T mutation in the Microsomal triglyceride transfer protein (MTP) promoter is unlikely to have significant implications for CVD in men and women [53]. MTP is required to produce triglyceride rich droplets in the smooth endoplasmic reticulum which may supply the core lipids for conversion of nascent, dense apolipoprotein B-48 particles to mature VLDL. In addition, assembly of dense apolipoprotein B-48 containing lipoproteins has been observed in mouse liver in the absence of MTP [54].

xiv. Myeloperoxidase
A meta-analysis showed that there was no association between the myeloperoxidase G-463/A polymorphism and the risk of developing ANCA-associated vasculitis [55]. Granulocyte macrophage colony-stimulating factor as an endogenous regulator of macrophage myeloperoxidase expression in human atherosclerosis and support a particular role for the myeloperoxidase-expressing macrophages in atheroma complication and the acute coronary syndromes [56]. MPO and its downstream inflammatory pathways represent attractive targets for both prognostication and therapeutic intervention in the prophylaxis of atherosclerotic cardiovascular disease [57].

xv. Plasminogen Activator Inhibitor-1
CRP induces Plasminogen activator inhibitor-1 (PAI-1) expression and activity in HAECs and thus has implications for both the metabolic syndrome and atherothrombosis [58]. PAI-1 still remained significantly related to incident T2DM. Chronic inflammation emerges as a new risk factor for the development of T2DM; PAI-1 predicts T2DM independent of insulin resistance and other known risk factors for diabetes [59]. Modifying PAI-1 expression by PAI-1 inhibitors provides a new challenge and may reveal the true role of PAI-1 in atherosclerotic and insulin resistance processes [60].

xvi. Prothrombin (PT)
The association between the inherited gene mutations of factor V, prothrombin and homocysteine metabolism and venous thromboembolic events is accepted widely; however, their influence on the arterial circulatory system remains controversial [61].

xvii. Serotonin
The TT genotype of the Serotonin (5-HT) 2A receptor gene may enhance susceptibility to AMI suggesting that the T102C polymorphism of the 5-HT2A receptor gene can serve as a new genetic marker for AMI [62].

xviii. Thrombomodulin
Findings support the hypothesis that both endothelial cell protein C receptor and thrombomodulin are down-regulated in coronary arteries with atherosclerosis. These changes would be expected to result in reduced inhibition of thrombogenic and anti-inflammatory activity on the endothelium overlying atherosclerotic regions and thus could contribute to coronary thrombosis [63].

xix. Vascular Endothelial Growth Factor
The C (~634) G polymorphism in the 5’UTR of the Vascular endothelial growth factor (VEGF) gene is a novel genetic risk factor for diabetic retinopathy [64]. The multivariate logistic regression analysis showed that the D allele of the VEGF polymorphism is an independent risk factor of diabetic retinopathy after controlling for other clinical variables [65]. VEGF is important in atherosclerotic development and two VEGF polymorphisms, including +405C/G (rs2010963) and −2578C/A (rs699947), to assess their relation to the extent of coronary atherosclerosis [66].

xx. Von Willebrand Factor
Enlightening findings have been obtained on the link between regulation of Von Willebrand factor multimer size and microvascular thrombosis. This progress in basic research has provided critical information to define with greater precision the role of Von Willebrand factor in vascular biology and pathology [67].

6. Conclusion
The genes that provide the predictive power include many previously suspected to play a role in atherosclerosis and additional genes without prior association with atherosclerosis. Single-gene disorders represent the most remarkable examples of the genetic implication to atherosclerosis. In general population, polymorphisms in genes of lipid metabolism, inflammation and thrombogenesis are probably responsible for the wide range of atherosclerotic diseases. In this paper we have reviewed various SNPs associated with carotid atherosclerosis of T2DM and understanding the molecular and genetic determinants of atherosclerosis can provide a solid foundation for the development of novel therapeutic approaches.

7. References


