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Genetic Polymorphisms and Ischemic Heart Disease

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Abstract

Although the progression in diagnostic tools, prevention strategies, and therapies, ischemic heart disease still represents the major cause of mortality and morbidity worldwide that globally represents an important problem for individuals and healthcare resources. By convention, ischemic heart disease is associated with the presence of an atherosclerotic plaque that is able to limit the flow in large-medium-sized coronary arteries. Nevertheless, several findings suggest a more complex pathophysiology of ischemic heart disease. At this time, there is no well-defined assessment of myocardial ischemia pathophysiology. Moreover, several data have identified genetic variations at different loci that are linked with ischemic heart disease susceptibility. This chapter aims to examine this complicated disease and to review the evidence on the genetic heritability acting with other factors in determining the presence of ischemic heart disease, due to either an obstruction in epicardial vessels or a dysfunction of coronary microcirculation.

Keywords: atherosclerosis, coronary artery disease, coronary microvascular disease, endothelial dysfunction, ion channel, ischemic heart disease, myocardial infarction, myocardial ischemia, risk factor, single-nucleotide polymorphism

1. Introduction

Nowadays, ischemic heart disease remains a major cause of death and disability worldwide for both men and women, although the evolution in prevention and therapy strategies [1]. By convention, ischemic heart disease is equated with atherosclerotic plaque due to flow-limiting obstruction in epicardial coronary arteries. Nevertheless, several findings suggest a more complex pathophysiology of this complex disease [2–8]. In fact, it has been showed that beyond the presence of epicardial atherosclerotic plaques, coronary microcirculation is crucial in the genesis of ischemic heart disease [9, 10]. In addition, in the last few years, a growing

body of data is underlying the importance of heritability acting with other factors in order to determine the presence of ischemic heart disease, due to either an obstruction in epicardial vessels [11–13] or a dysfunction of coronary microcirculation [14, 15]. In fact, over the past decade, genome-wide association studies have identified several loci, explaining a part of the ischemic heart disease's heritability. Existing knowledge of genetic variants affecting risk of ischemic heart disease is largely based on genome-wide association studies analysis of common single-nucleotide polymorphisms.

Therefore, this chapter proposes an overview of the loci that have been identified as connected with ischemic heart disease genesis, from epicardial plaque to microvascular dysfunction.

2. Coronary artery disease

Coronary artery disease is defined by the presence of a plaque in the epicardial coronary arteries. Usually, the atherosclerotic plaque narrow coronary arteries, decreasing blood flow. In subjects with genetic susceptibility factors, coronary artery disease is determined by exposure to some risk factors. In the last years, several researches have enhanced our understanding of the risk factors as well as the genetic basis of coronary artery disease. Some of these factors relate to lifestyles, such as tobacco smoking, lack of physical activity, and dietary habits, and are thus modifiable. Other risk factors are also modifiable, such as elevated blood pressure, type 2 diabetes, and dyslipidemias, or non-modifiable, such as age, male gender, and genetic susceptibility.

To date, more than 50 loci have been identified for susceptibility of coronary artery disease, as described by the meta-analysis carried out by the CARDIoGRAMplusC4D Consortium, on almost 185,000 cases and controls [11]. Moreover, data from a meta-analysis of genome-wide association studies have also been carried out for conventional risk factors and biomarker for coronary artery disease, like dyslipidemia and blood pressure, diabetes mellitus, atherosclerosis, inflammation, coagulation, oxidation, and amino acid metabolism, identifying several genes for each respective factor [12].

2.1. Genetic polymorphisms and risk factors for coronary artery disease

2.1.1. *Dyslipidemia*

Lipid metabolism can be altered in different ways, leading to variations in plasma lipoprotein function and concentration. Through interaction with other cardiovascular risk factors, dyslipidemias may affect the development of coronary artery disease. Dyslipidemias may be related to the interaction between genetic predisposition and environmental factors. Up to the present time, 12 loci were significantly associated by genome-wide association studies with the concentrations of blood lipids and coronary artery disease. Among these, eight loci were associated with LDL concentration (ABCG5, ABCG8, ABO, APOB, APOE, LDLR, LPA PCSK9, and SORT1), two loci with triglyceride concentration (APOA5 and TRIB1), and one locus with HDL concentration

(ANKS1A). At another locus, there was a near-equivalent association for triglyceride and HDL (LPL). In severely obese patients, a single-nucleotide polymorphism within the ARPC3 Gene Promoter was associated with hypertriglyceridemia [16]. Moreover, among different ethnicities Lysosomal Acid Lipase A (LIPA), polymorphisms have been described as associated with susceptibility to premature coronary artery disease [17]. APOA5 polymorphisms, air pollution, and the development of coronary artery disease have been associated, although methylation studies are needed to examine epigenetic factors associated with those single-nucleotide polymorphisms. Another interesting correlation is between TNNT1 variations, HDL levels, and coronary artery disease [15]. Finally, it has been suggested that Numb gene haplotypes, the regulating factor for intestinal cholesterol absorption and plasma cholesterol level, are related to coronary artery disease in Han Chinese [18].

2.1.2. Arterial hypertension

The relationship between blood pressure values and cardiovascular fatal events has been determined in several studies [19]. Numerous rare, monogenic forms of hypertension have been described, where a single gene mutation explains the pathogenesis of hypertension [20]. On the other side, essential hypertension is a heterogeneous disease with a multifactorial etiology. Genetic approaches raised the understanding of pathways underlying individual variations in blood pressure. Primary analyses evaluated associations between 2.5 million genotyped or imputed single-nucleotide polymorphisms (single-nucleotide polymorphisms) and SBP and DBP. Several genome-wide association studies and their meta-analyses point to a total of 29 single-nucleotide polymorphisms, which are associated with systolic and/or diastolic blood pressure [21]. In particular, latest genome-wide association studies data described four coronary artery disease risk loci (CYP17A1- NT5C2, SH2B3, ZC3HC1, GUCY1A3, and FES) associated with systolic and diastolic blood pressure [11, 21]. Moreover, endothelial nitric oxide synthase (eNOS) single-nucleotide polymorphism G894T significantly increases hypertension risk and coronary artery disease [22]. Selective expression of the Rho GTPase-activating protein ARHGAP42 in vascular smooth muscle cells regulates arterial blood pressure, as it inhibits RhoA-dependent contractility [23]. Furthermore, PDE3A, PRDM6, IGFBP3, and KCNK3 genes regulate vascular smooth muscle cells [24]. Particularly, PDE3A is phosphodiesterase acting in cyclic GMP metabolism [25], whereas KCNK3 has been related to pulmonary hypertension [26]. Other genes related to renal function have been described as acting in blood pressure regulation: ARHGAP24 influences podocyte formation [27], OSR1 influences renal mass and function [28], and SLC22A7 encodes for a renal solute transporter [29]; DNA methylation is probably involved in the regulatory pathway linking common single-nucleotide polymorphisms with blood pressure, according to data from experimental models of hypertension [30].

The single-nucleotide polymorphism Gly460Trp has been associated with hypertension and salt sensitivity [31] and an increased risk for coronary heart disease or peripheral vascular disease [32] and stroke [33]. An individual with this single-nucleotide polymorphism is a responder to diuretics better than wild-type homozygotes [31] in terms mostly of reduction of cardiovascular risk [34].

2.1.3. *Diabetes mellitus*

Diabetes mellitus has an increasing prevalence worldwide: 360 million people had diabetes in 2011, of which more than 95% type 2 diabetes. Diabetes mellitus is a complex, chronic disease requiring multifactorial risk-reduction strategies beyond glycemic control. Diabetes mellitus and cardiovascular disease develop with metabolic abnormalities causing dysfunction in the vasculature. Mortality and morbidity in people with diabetes are related to cardiovascular disease. Diabetes is a condition defined by an elevated level of blood glucose, and it can be classified into general categories: type 1 diabetes (due to b-cell destruction) and type 2 diabetes (due to a progressive loss of insulin secretion on the background of insulin resistance).

Usually, type 2 diabetes mellitus is more frequent with obesity, lack of physical activity, in women with prior gestational diabetes, in association with hypertension or dyslipidemia, and in certain ethnic groups. Insulin resistance plays an important role in the pathophysiology of type 2 diabetes mellitus and coronary artery disease: both genetic and environmental factors collaborate to its development. In fact, more than 90% of people with type 2 diabetes mellitus are obese [35]. Nevertheless, type 2 diabetes mellitus is often associated with a strong genetic predisposition, more so than type 1 diabetes. However, the genetics of type 2 diabetes is poorly understood. Classically, both the single-nucleotide polymorphism Pro12Ala in the peroxisome proliferator-activated receptor gamma (PPARG) gene [36] and the single-nucleotide polymorphism Glu23Lys in KCNJ11 gene [37] are common polymorphisms connected with influence risk of diabetes mellitus [38]. In particular, single-nucleotide polymorphisms for KCNJ11 have been described as involved in the susceptibility of ischemic heart disease, including coronary artery disease and CM (see below). Moreover, transcription factor 7-like 2 (TCF7L2) gene is involved in diabetes mellitus susceptibility [39]. Some single-nucleotide polymorphisms of the gene associated with fat mass and obesity (FTO) [40] have an impact on body mass index (BMI). Other single-nucleotide polymorphisms of the gene within or adjacent to hematopoietically expressed homeobox (HHEX)/insulin degrading enzyme (IDE), CDK5 regulatory subunit associated protein 1-like 1 (CDKAL1), insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2), cyclin-dependent kinase inhibitors 2a, b (CDKN2A and CDKN2B), and solute carrier family 30, member 8 (SLC30A8) have an effect on insulin secretion [41–43].

Several other loci are implicated in diabetes mellitus as PPARG [44] and KCNJ11 [45] and for HNF1B (TCF2) [46] and WFS1 [47]. However, recent data suggest a possible role of other genes [48]: Notch homologue 2, *Drosophila* (NOTCH2) that is known to be involved in pancreatic development. Mapping within or adjacent to ADAM metalloproteinase with thrombospondin type 1 motif 9 (ADAMTS9), calcium/calmodulin-dependent protein kinase 1D (CAMK1D), compared with another zinc finger gene 1 (JAZF1), tetraspanin 8 (TSPAN8)/leucine-rich repeat containing G-protein coupled (LGR5), and thyroid adenoma associated (THADA), the mechanisms involved remain unclear [38].

2.1.4. *Atherosclerosis*

Cardiovascular disease due to atherosclerosis of the arterial vessel wall and to thrombosis is the foremost cause of premature mortality and of disability-adjusted life years (DALYs) in Europe and is also increasingly common in developing countries. Atherosclerotic lesions (i.e., atheroma)

are asymmetric focal thickenings of the innermost layer of the artery, the intima. The pathophysiology of atherosclerosis suggests an inflammatory disease characterized by arterial plaque rich in cholesterol, inflammatory cell infiltrates, and connective tissue [49]. Data from clinical researches, studies in animal models, and cell culture experiments found important evidences to the pathogenesis of atherosclerosis. Several types of research demonstrated an association of IL6R-related gene pathways with atherosclerosis and coronary artery disease [50].

In patients with established coronary artery disease, it has been showed that the -174 C allele of the IL-6 gene increases the risk for progression of coronary plaques [14]. Moreover, patients with the Cox-2 GG single-nucleotide polymorphism have a higher risk of coronary artery disease while the Cox-2 (-765G>C) polymorphism is associated with lower interleukin-6 levels [51]. Moreover, multiple single-nucleotide polymorphisms as FGB-FGA-FGG, NLRP3, IL1RN, and IRF1-SCL22A5 show a strong association with fibrinogen expression and function [52]. Analogously the plaque composition has been associated with specific genes: matrix metalloproteinase genes (MMP1, 9, 12, 14) and the co-stimulatory ligands CD80 and CD86 [53] are associated with vulnerable plaques. Moreover, indoleamine 2, 3-dioxygenase 1 (IDO1) and integrin alpha V expression levels seem to be higher in vulnerable than in stable plaques [53].

In atherosclerotic plaques, there is an overexpression of CB2 gene (CNR2), that is an inflammatory marker, compared with normal arteries, whereas stable and vulnerable plaques displayed similar CNR2 levels [53]. Lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1) is the main scavenger receptor for oxidized low-density lipoprotein (ox-LDL) in endothelial cells. The single-nucleotide polymorphism c.501G>C determines a single amino acid change (K>N at codon 167) reduces ox-LDL binding and uptake. Ox-LDL activated extracellular signal-regulated kinases 1 and 2 (ERK 1/2) is inhibited [54–56].

3. Coronary microvascular disease

The coronary microcirculation is often considered an underwater world beyond the epicardial arteries, often inaccessible for routine investigation, unachievable for targeted treatment. However, microcirculation is crucial in the cross talk between perfusion to myocardial demand. Pathway for coronary metabolic dilation is determining genetic associations in genes encoding for coronary blood flow regulators (i.e., ion channels, nitric oxide synthase, SERCA pump, and so on) with the susceptibility for microcirculation dysfunction and ischemic heart disease [57].

Recently, a study comparing the prevalence of single-nucleotide polymorphisms in genes encoding coronary ion channels between patients with coronary artery disease or microvascular dysfunction and those with both anatomically and functionally normal coronary arteries suggested the possibility of associations between single-nucleotide polymorphisms and ischemic heart disease in term of coronary artery disease and microvascular dysfunction [57]. In fact, data show that specific single-nucleotide polymorphisms detected in NOS3 gene encoding for endothelial nitric oxide synthase (eNOS), as well as in KCNJ8 encoding for the inward rectifying subunit of ATP-sensitive potassium channel (Kir6.2) and SCN5A encoding for voltage-dependent Na⁺ channel (Nav1.5) were found to be correlated with ischemic heart

disease and microvascular dysfunction [58]. Specifically, the single-nucleotide polymorphisms rs5215_GG, rs5218_CT, and rs5219_AA for Kir6.2/KCJ11 could reduce susceptibility to ischemic heart disease; the single-nucleotide polymorphism rs5219_AA of Kir6.2/KCNJ11 may suggest a protecting role against coronary microvascular dysfunction; the rs1805124_GG genotype of Nav1.5/SCN5A seems to play a role in coronary artery disease. In the same study, also eNOS/NOS3 gene was investigated, demonstrating that rs1799983 polymorphism for this gene seems to be an independent risk factor for microvascular dysfunction [57]. The Transient Receptor Potential Ankyrin 1 (TRPA1) has been evaluated in vasodilation using KO mice [59]. On the other side, smooth muscle Kv7 channels have been associated with the control of vascular reactivity and vasorelaxant responses in coronary circulation [60]. To date, five subtypes of Kv7 channels encoded by KCNQ genes have been identified [61]. Kv7.1 and Kv7.4 were expressed at higher levels compared to Kv7.2, Kv7.3, and Kv7.5 in coronary arteries [62].

Regarding nitric oxide synthase, the allele “a” of intron 4a/b (eNOS4) has been described as a risk factor for patients with microvascular endothelial dysfunction and slow coronary flow [63].

Microvascular angina has been also associated with CYP2C19 variants that may affect coronary microvascular dysfunction [64]. Moreover, recent data in the female population showed that the specific CYP2C19 poor metabolizer genotype can lead to coronary microvascular disorders via inflammation [65].

Nuclear factor (erythroid-derived 2)-like-2 (NRF2) is an antioxidant and cell protective transcription factor that controls antioxidant defenses. NRF2 suppression plays an essential role in the development of oxidant stress, endothelial dysfunction, and microvessel rarefaction [66]. Moreover, Nox4 has been described as a positive transcriptional regulator of cystathionine- γ -lyase (CSE) in endothelial cells. It regulates vascular tone via the modulation of gasotransmitter, hydrogen sulfide (H₂S) production [67].

Hypoxia-inducible factor-1 (HIF-1) is a peptide regulator of genes such as heme oxygenase (HO)-1 expressed during hypoxia. HIF-1 activation induces HO-1 expression attenuating proinflammatory chemokine production by microvascular endothelium in vitro and in vivo [68]. The single-nucleotide polymorphism C242T causes p22 (phox) structural changes that inhibit endothelial Nox2 activation and oxidative response to tumor necrosis factor- α or high-glucose stimulation. The single-nucleotide polymorphism C242T has been proposed a protective factor against cardiovascular diseases [69]. Novel regions of genetic variations within vascular endothelial growth factor A (VEGFA) and CDKN2B antisense RNA1 (CDKN2B-AS1) genes have been associated with coronary microvascular dysfunction. Furthermore, there were sex-specific differences in single-nucleotide polymorphisms which are associated with microvascular dysfunction, in particular, myosin heavy chain 15 (MYH15), VEGFA, and NT5E. In the male, single-nucleotide polymorphisms for NT5E are associated with abnormal coronary flow reserve; however, mutations in NT5E are associated with arterial calcification [70]. NT5E gene encodes for CD73 that transforms adenosine monophosphate (AMP) to adenosine, supporting a role for this metabolic pathway in inhibiting vascular calcification [70, 71]. In fact, lack of CD73 leads to a reduction in extracellular adenosine levels, causing vascular calcification [72].

In a meta-analysis of genome-wide association studies, data identified four novel loci on chromosomes 19q13, 6q24, 12q24, and 5q14 that were associated with retinal venular caliber. The retinal vasculature is comparable with human microcirculation. Retinal venular caliber has been shown to predict a range of subclinical [73] and clinical cardiovascular disease. RASIP1 gene (rs2287921, $p = 1.61610225$) on chromosome 19q13 is the most significant single-nucleotide polymorphism associated with retinal venular caliber, and the single-nucleotide polymorphisms were located in or adjacent to VTA1 and NMBR genes on chromosome 6q24. VTA1 gene encodes for a protein involved in trafficking of the multivesicular body. The signals for the association on chromosome 12q24 were spread across a large one Mb LD block, including genes such as SH2B3, ATXN2, and PTPN11. The most significant single-nucleotide polymorphisms at the 5q14 locus were located closest to MEF2C that plays an important role in cardiogenesis, epithelial cell survival, and maintenance of blood vessel integrity [74].

Finally, sarcomere gene mutations are associated with adverse remodeling of the microcirculation in hypertrophic cardiomyopathy [75, 76]. In fact, patients with hypertrophic cardiomyopathy with sarcomere myofilament mutations are characterized by more severe impairment of microvascular function and increased prevalence of myocardial fibrosis, compared with genotype-negative individuals [75].

4. Sex differences

Gender difference deserves a separate section. Scientific interest in ischemic heart disease in women has grown significantly over the past decades, mostly on clinical aspects. In fact, ischemic heart disease differs in term of pathogenesis, symptoms, and prognosis between males and females. Several studies show that different single-nucleotide polymorphisms of different genes can be involved. Single-nucleotide polymorphisms within genes of MYH15, linked to the maintenance of tonic force in vascular smooth muscle cells, VEGFA, involved in cell proliferation, migration, and angiogenic potential and NT5E which contributes to overall microvessel stiffness were associated with microvascular dysfunction in men [63, 77]. Studies on polymorphisms at the cholesteryl ester transfer protein (CETP) locus showed that women displayed a higher HDL-C than men and an equally high incidence of coronary heart disease in B2 homozygotes as in other genotypes [78]. Thus, in type 2 diabetic patients, the B polymorphism seems to exert a modulating role in males only. This may contribute to the loss of macrovascular protection in type 2 diabetic females [78].

Moreover, the specific CYP2C19 poor metabolizer has been described as a risk factor for coronary microvascular disorders via inflammation exclusively in the female population [65]. Usually, in fertile female exhibits a protection in ischemic injury compared to age-related men, a phenomenon designated as sex-specific cardioprotection. PKC-mediated regulation of sarcolemmal ATP-dependent K [sarK(ATP)] channels may account for the gender difference in cardioprotection upon both PKC and sarK(ATP). It involves PKC-dependent sarcolemma increase with a major expression of sarK(ATP) in female [77]. Microcirculation dysfunction can also be associated with cardiac hypertrophy, which can be related to the expression of Kv β 1.1, particularly in females. In an animal model, Kv β 1 KO female mice have a growing

myosin heavy chain α expression in myocytes. Changes in molecular and cell signaling pathways clearly point toward a distinct electrical and structural remodeling consistent with cardiac hypertrophy in the Kv β 1.1 KO female mice [79].

5. Conclusions

Ischemic heart disease is a common disease that globally represents an important problem for individuals and healthcare resources. An enhanced understanding of its pathophysiology is needed. By convention, ischemic heart disease is associated with the presence of an atherosclerotic plaque that is able to limit the flow in large-medium sized coronary arteries. Multiple different mechanisms are responsible for symptoms suggesting ischemic heart disease without apparent flow-limiting obstruction on angiography. In fact, both coronary artery disease and coronary microvascular disease can be responsible for an impaired cross talk between myocardial demand and oxygen supply. Interestingly, discordance between epicardial coronary function and microvascular function has generated recent interest. Finding no obstructive epicardial stenosis, but reduced microvascular function, indicating coronary microvascular disease, is associated with a negative prognosis [80].

In contrast, preserved microvascular function in the presence of flow-limiting epicardial stenosis has been associated with a long-term clinical outcome. In fact, several findings suggest a complex pathophysiology of ischemic heart disease. Both genetic and lifestyle factors contribute to the individual-level risk of ischemic heart disease, both coronary artery disease and coronary microvascular disease. Genetic susceptibility is determined by several single-nucleotide polymorphisms of genes encoding for both elements involved in the coronary homeostasis as well as for major risk factors for cardiovascular events (i.e., hypertension, diabetes, dyslipidemia). Genetic susceptibility is independent of healthy lifestyle behaviors and can be associated with an increased risk of coronary events, although a healthy lifestyle is associated with event risk reductions in rates.

Nowadays, associative hypothesis between single-nucleotide polymorphisms and ischemic heart disease has been numerous in literature, and, in some case, researches show speculative data with no provided plausible causal mechanism. Large-scale studies in human populations, genome-wide association studies, and meta-analysis, together with genetic technologies improvements, are helping the comprehension of genetic susceptibility of ischemic heart disease over the last decade. Moreover, a functional understanding of the discovered genetic associations with ischemic heart disease could help in the development of novel therapeutic strategies. However, we are still distant to the fully knowledge of the pathophysiology of this complex disease and the real role of heritability.

Conflict of interest

No conflict of interest to declare, no relationship with industry.

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