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REVIEW

Genetics and cardiovascular system: influence of human genetic variants on vascular function

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Abstract Candidate gene association studies in cardiovascular diseases have provided evidence on the molecular basis of phenotypic differences between individuals. The comprehension of how inherited genetic variants are able to affect protein functions has increased the knowledge of how genes interact with environment in order to modulate a particular phenotype. Although it is known that the human genome contains more than 10 million SNPs, only a minor part of them are supposed to be functional. A causative SNP in a particular gene may confer a small to moderate effect in complex phenotypes, such as functions important to cardiovascular homeostasis. This paper is a selective review of the literature on the evidence for interactions between vascular function and naturally occurring genetic variants in endothelial nitric oxide synthase (eNOS) and beta-2 adrenergic receptor (ADRB2), two genes among those influencing vascular phenotype and examples for which there is a strong evidence base. eNOS and ADRB2 will be characterized, as well as the mechanisms by which the enzyme and the receptor work to control vascular responses will be described. Understanding the molecular mechanisms underlying gene-mediated vascular function and their modification by genetic variants is expected to result in a better comprehension about individual's phenotypic differences.

Keywords Genetic · Genetic variant · Polymorphism · Vascular function · Gene-environment interaction · Phenotype

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Introduction

Advances in molecular biology have increased the knowledge of how genes control physiological functions involved in the modulation of specific phenotypes. The human genome consists of approximately 25,000 genes codified by approximately 3.1 billion base pairs, distributed in 23 pairs of chromosomes [21]. Considering the fact that the physiological systems of human beings have the same functioning pattern, one could imagine that the DNA sequence might be the same among individuals, at least for those genes codifying proteins involved in common cellular processes such as enzymes and receptors. This rationale seems not to be the case since it is common that nucleotide sequence variations can be identified in any particular gene of any two individuals. These genetic variants can take several forms, comprising a single base pair change, an insertion or deletion of a DNA segment and repetitive elements of DNA. Differences in single bases are by far the most common example of genetic variation, known as *single-nucleotide polymorphisms* (SNPs). The comparison of DNA sequences of two unrelated individuals has shown the existence of, on average, approximately 1 difference every 1,200 bases. The human genome contains about 10 million SNPs and a minor part of them is located in regulatory regions of genes and in protein coding sequences [4]. There are approximately 100,000 SNPs in

the protein coding regions of the genome. Many of these variants might be relevant for studies of human health and disease since they may alter an amino acid sequence or disrupt a motif that affects the function or structure of a protein involved in a biological process. While mutations are rare and maybe unique to an individual, polymorphisms are found in many individuals, at a specific frequency, usually 1% or greater in a particular population. In this case, a functional genetic variant affecting a specific protein may confer a small to moderate effect in a specific trait. This concept is particularly appealing in complex phenotypes, such as cardiovascular structure and function, which are controlled by a large number of genes. The knowledge about genes and SNPs function is crucial to establish the scientific basis for how genes interact with the environment in order to cause a particular phenotype. Nevertheless, while the assessment of a candidate variant on the basis of nucleotide sequence is relatively easy, the subsequent confirmation of its functional significance is usually more difficult. This is a major challenge affecting genetic association studies, which have the aim of assessing correlations between genetic variants and trait differences on a population scale. Exemplifying, a positive association between a variant in a candidate gene with a specific altered phenotype may not mean that the gene in consideration is responsible for the alteration. This gene might be in linkage disequilibrium with a truly causative variant or, perhaps, the association might represent a false-positive result. In case of disequilibrium, it means that the allele in a supposed causative gene under study is not inherited randomly, but together with a nearby and truly causative allele. This happens because close-linked genetic markers in a chromosome are not easily separated by recombination. In this situation, the allele located in that supposed causative gene works as a marker SNP for another causative SNP. The difficulty in identifying truly causative genes in association studies is also due to the synergistic effect of multiple genetic variants interacting with environmental factors in order to modulate a phenotype [36, 37]. Biologically relevant SNPs can alter cellular processes conferring an increased protection in some cases and a greater susceptibility to disease in other. Interestingly, environmental variables such as diet and exercise are able to interact with genes chronically activated during disease states, affecting disease progression. As the number of studies with diet- and exercise-gene interactions has increased, it is reasonable to expect, in the near future, the application of "personalized intervention" in health and disease, based on a set of SNPs in specific groups of genes.

This article will provide a brief overview of the complex interaction between genes and environmental factors in the control of vascular functions. Although a large number of genes influence vascular reactivity, to intentionally illustrate this complexity, this review will address specially the interactions involving two genes, endothelial nitric oxide synthase (eNOS) and beta-2 adrenergic receptor (ADRB2). There are an increasing number of published studies that have investigated their influence on vascular function, and they are examples for which there is strong evidence base. How eNOS and ADRB2 genotypes alter the harmful and beneficial impact of environment will be shown to illustrate gene–environment interaction in such scenarios. eNOS and ADRB2 genes will be molecularly described with the purpose of improving the comprehension of the mechanisms by which each protein interacts with others in their pathways to trigger cellular processes.

Nitric oxide, β_2-adrenoreceptors and vascular reactivity

While this review concentrates mainly on the role of nitric oxide and beta-2 adrenergic receptor in humans, it is important to note that other vasoactive substances control vascular tone as well. In this regard, endothelium plays a direct role in vasomotor function by integrating stimuli such as sympathetic discharge, mechanical forces, humoral and local factors. Among those endothelial-derived substances, nitric oxide, prostaglandins and endothelium-derived hyperpolarizing factor (EDHF) can be cited as vasorelaxing factors. On the other hand, endothelin, superoxide (O_2-) and thromboxane can be cited as vasoconstricting factors. For a better understanding of the role of these molecules and their function as vasoactive substances, we refer the readers to these papers [3, 22, 39, 41, 47].

Nitric oxide (NO) is an important signaling molecule which is synthesized from L-arginine by a family of enzymes called NO synthase (NOS). NO, an endothelium-derived relaxing factor (EDRF), is recognized as a vasoactive factor in the cardiovascular system mainly by its capacity to induce vasodilation and inhibit platelet aggregation [14, 15]. There is a basal NO release from the vascular endothelium and increases in NO discharge can be mechanically evoked by stimuli such as higher blood flow [31]. The proposed mechanism by which NO is synthesized in endothelial cells by eNOS leading to vascular smooth muscle relaxation is shown in Fig 1.

In humans, β_2-adrenoreceptors mediate physiologic responses such as vasodilatation, bronchial smooth muscle relaxation and lipolysis [8]. Stimulation of β_2-adrenoreceptors by catecholamines on vascular smooth muscle results in vasodilatation by an endothelium-independent mechanism. Additionally, β_2-adrenoreceptors-mediated vasodilatation has been linked to an increased activity of the L-arginine/NO pathway [13, 28]. The proposed mechanism by which β_2-adrenoreceptors activation on the

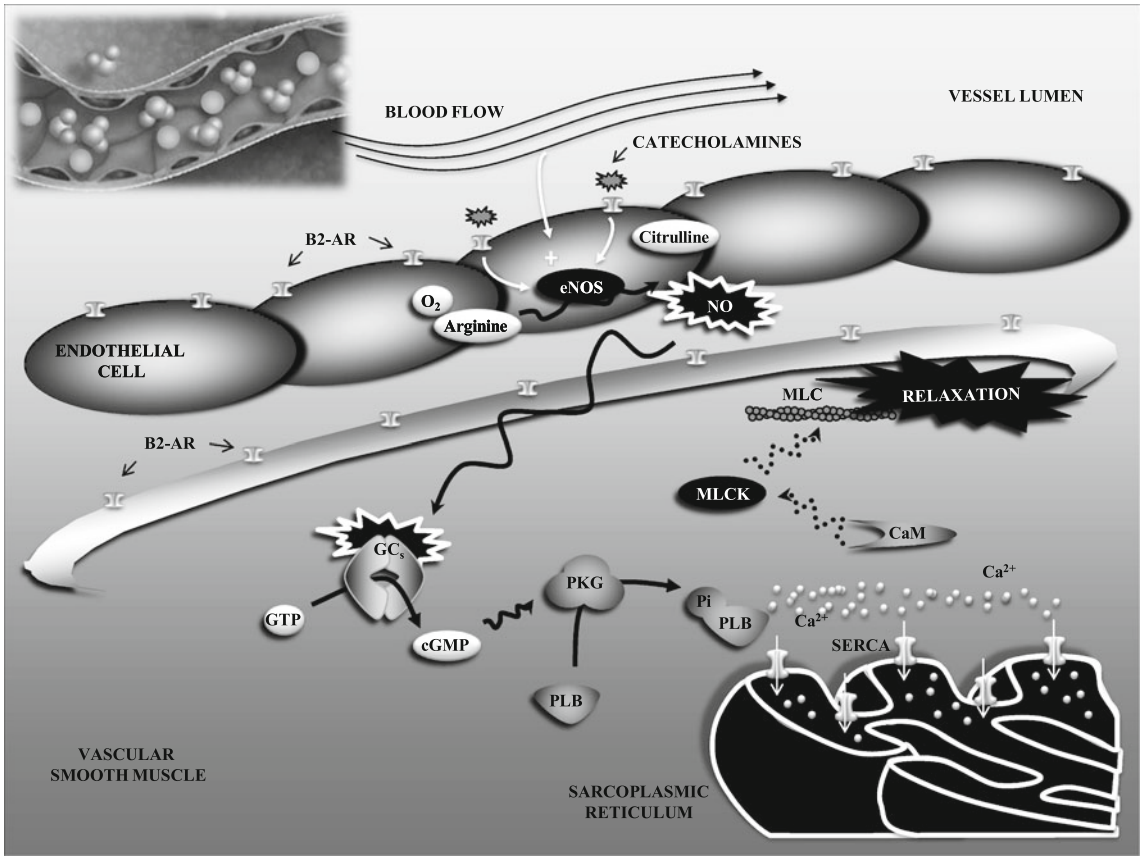

Fig. 1 Proposed mechanism by which nitric oxide (NO) synthesized by endothelial NO synthase (eNOS) induces relaxation in smooth muscle and increase vasodilatation. Shear stress, a frictional force exerted on the vessel surface by blood flow and stimulation of β_2-adrenoceptors, activates eNOS. NO, acting in a paracrine manner, stimulates soluble guanylyl cyclase (GCs) in vascular smooth muscle causing guanosine 3′, 5′-cyclic monophosphate (cGMP) rise. cGMP-dependent protein kinase (PKG) activates the uptake of Ca^{2+} into the superficial sarcoplasmic reticulum (SR) by the phosphorylation of phospholamban (PLB) and subsequently activation of SR Ca^{2+}-ATPase (SERCA). Myosin light chain (MLC) phosphorylation is necessary for actin activation of myosin ATPase, resulting in cross-bridge cycling. This phosphorylation is mediated by myosin light chain kinase (MLCK), a calcium/calmodulin-activated kinase. Since intracellular calcium is diminished by the increased influx into the superficial SR, the resultant effect is smooth muscle relaxation. *eNOS* endothelial nitric oxide synthase, *NO* nitric oxide, *B2-AR* beta2-adrenoreceptor, *GCs* soluble guanylyl cyclase, *cGMP* guanosine 3′, 5′-cyclic monophosphate, *PKG* cGMP-dependent protein kinase, *PLB* phospholamban, *MLC* myosin light chain, *MLCK* myosin light chain kinase, *SERCA* sarcoplasmic reticulum Ca^{2+}-ATPase

vascular endothelium contributes to vascular smooth muscle relaxation is shown in Fig 1.

Nitric oxide synthase gene

There are three isoforms of NOS codified by distinct genes: endothelial NOS (eNOS or NOS III; 7q35-36); neuronal NOS (nNOS or NOS I; 12q24.2); and inducible NOS (iNOS or NOS II; 17cen-q12). eNOS and nNOS are constitutively expressed, while iNOS is expressed in abnormal cell processes such as those occurring in heart failure [12] and after induction by cytokines and other inflammatory agents, which results in a high NO flow [1]. The eNOS gene is mainly expressed in endothelial cells and it is essential for baseline vascular tone maintenance.

The eNOS gene is located in chromosome 7q35-36 and comprises 26 exons that encode a 135-kD protein containing 1,203 amino acids. Variations in nucleotide sequence have been described in the promoter, exons and intronic regions [20]. These variations may result in differences in gene expression and may modulate interindividual differences in the activity of the encoded protein. These variations might predispose or increase an individual's susceptibility to the development of cardiovascular outcomes. As such, a polymorphic allele would be expected to be over-represented in populations of affected individuals, compared to healthy controls. Among the common genetic polymorphisms identified in the eNOS gene, the −786T>C (rs2070744) and 894G>T (rs1799983), located in the 5′-flanking region of the gene and in the exon 7 region, respectively, seem to affect cardiovascular phenotypes.

Differences in vascular reactivity to phenylephrine, an α_1-adrenoceptor agonist, are commonly observed in patients undergoing cardiopulmonary bypass. Philip et al. [38] identified that individuals with 894GT and 894TT genotypes of the eNOS 894G>T polymorphism showed similar increase in mean blood pressure but with lower phenylephrine doses, than 894GG genotype carriers. This result suggests that the eNOS 894G>T polymorphism may affect vascular responsiveness to α-adrenergic stimulation. In another study, Naber et al. [35] investigated coronary vasomotor tone in patients undergoing routine diagnostic cardiac catheterization for unclear chest pain. By means of intracoronary Doppler, they found no difference, at rest, in the cross-sectional area among eNOS 894G>T genotypes, but increased coronary vascular resistance and decreased average peak velocity in individuals harboring the 894TT genotype, more so than in other genotypes. Since epicardial vasomotor tone, which is represented by cross-sectional area, was not different among genotypes, the authors concluded that the supposed decreased eNOS enzyme activity in 894TT genotype carriers affects primarily the microvascular circulation, reflected by increased coronary vascular resistance and subsequent decreased average peak velocity. Suzuki et al. [43] investigated a possible association between the eNOS 894G>T polymorphism and coronary in-stent restenosis and found that the restenosis rate is higher among those carriers of the 894T allele. The positive results of these genetic association studies suggest that the protein encoded by the mutant allele may have impaired activity. Genetic variants in gene promoters have the potential to influence mRNA transcription, while those in gene exons, predicting amino acid substitution in the mature protein, have the potential to alter enzyme activity. The authors recognize that functional analysis would be necessary to confirm whether the observed vascular alterations are secondary to the eNOS 894T allele.

The eNOS 894G>T polymorphism results in the exchange of Glutamate (Glu) to Aspartate (Asp) at position 298 of the encoded protein. Interestingly, enzymatic studies for both, recombinant Asp298 and Glu298, showed no differences in enzyme activity, suggesting that the resultant amino acid substitution does not exert any functional effect via a mechanism dependent of NOS catalysis [20]. Nevertheless, it was shown by Tesauro et al. [44] that the Asp298 variant is more susceptible to proteolytic cleavage in endothelial cells and vascular tissues, which might result in reduced levels of functional eNOS. This result was criticized by the fact that the Asp298 increased susceptibility for proteolytic cleavage, observed in vitro, could be an artifact of nonspecific acid hydrolysis during sample preparation [11]. Although there is no conclusion about the underlying mechanism responsible for the reduced eNOS Asp298 functionality, some studies have observed that dysfunctions appear to be dependent of the Asp298 variant.

Endothelium-dependent mammary artery rings relaxation of patients undergoing coronary bypass surgery was measured in vitro. The carriers of −786C allele of eNOS −786T>C polymorphism or 894T allele of eNOS 894G>T polymorphism showed blunted acetylcholine-mediated relaxation, more so than in wild type for both polymorphisms. Additionally, among carriers of mutant alleles, those with more than three cardiovascular risk factors showed further aggravation of endothelial dysfunction compared with those with less than three risk factors [10]. These results exemplify the complex interaction between genes and environmental factors, as the eNOS variants represented an additional biological impact for those with more than three cardiovascular risk factors. Considering this idea, are eNOS gene polymorphisms functional in healthy conditions? To test this hypothesis, Dias et al. [7] investigated the potential influence of eNOS 894G>T polymorphism in exercise-induced reflex muscle vasodilatation in healthy volunteers. Forearm vasodilatation was blunted in 894TT genotype carriers, more so than in 894GG and 894TT genotypes carriers. Since this result would be mediated by increased sympathetic nerve activation and not by the eNOS 894G>T polymorphism, the authors studied exercise-induced reflex muscle vasodilatation under blockage of eNOS enzyme activity and direct measure of muscle sympathetic nerve activity. They found no difference in sympathetic discharge-mediated vasoconstriction among 894GG and 894TT genotypes carriers. On the other side, N^G –monomethyl-L-arginine (L-NMMA), a NOS enzyme active blocker, significantly reduced muscle vasodilatation in 894GG, but not in 894TT genotype carriers. This result showed association of eNOS 894T allele with impaired enzyme activity suggesting that, even in healthy conditions, this genetic variant may predict a higher susceptibility to certain cardiovascular diseases.

It is well known that exercise training improves endothelial function, either in healthy or disease states [27, 46]. In regard to eNOS gene polymorphisms, Erbs et al. [9] investigated the potential effect of exercise training-induced endothelial improvement in coronary artery disease patients. Interestingly, previous to exercise training only 894TT genotype carriers of eNOS 894G>T polymorphism showed a paradoxical vasoconstriction response to acetylcholine of the left internal mammary artery. After 4 weeks of training, the improvement in vasodilatory capacity was blunted in 894TT genotype, more so than in the other genotypes, including the genotypes of eNOS −786T>C polymorphism. In addition, in those −786TC genotype carriers of eNOS −786T>C polymorphism, the improvement in vasodilatory capacity was blunted, more so

than in carriers of the 894GT and 894TT genotypes of eNOS 894G>T polymorphism. Although changes in endothelial function varied depending on eNOS gene variants, the authors recognize the importance of exercise training as a nonpharmacological approach in cardiac rehabilitation programs.

There is a particular interest in the comprehension of how inherited genetic variants are able to affect the degree of influence of environmental factors in particular phenotypes. Leeson et al. [25] investigated, in young volunteers of both genders, the interaction between eNOS 894G>T polymorphism and two factors affecting vascular reactivity: the proatherogenic risk factor of cigarette smoking and the antiatherogenic influence of n-3 fatty acid intake. In male smokers, carriers of the 894GT and 894TT genotypes showed reduced flow-mediated dilatation compared with nonsmokers. Interestingly, smoking seems to not affect flow-mediated dilatation in males with 894GG genotype and in females with the three genotype groups for the eNOS 894G>T polymorphism. The authors suggest that, probably due to estrogen-mediated vasculoprotective action under the endothelium, premenopausal females follow a different pattern than men. In regard to n-3 fatty acid status, there was a positive association between plasma n-3 fatty acid levels and flow-mediated dilatation in 894T allele carriers (heterozygous plus homozygous). In this case, the results were similar between males and females. Although the mechanism for this interaction is not clear, raised levels of n-3 fatty acids have been shown to regulate gene expression and are associated with greater membrane fluidity. Considering the results of this study, the authors recognize that genetic effects on cardiovascular disease need to be investigated in conjunction with other modifying environmental factors to determine their impact on vascular disease development.

Since NO has an important role in normal vascular homeostasis, the reduction in its endothelial bioavailability is recognized as an important feature of an impending vascular disease state. As reviewed here, eNOS gene variants are associated with differences in vascular reactivity and this has been supported in some investigations by means of functional analysis. It provides evidence of how genes increase susceptibility to diseases and how genes interact with environment factors to modulate a specific phenotype. eNOS gene variants explain, at least in part, differences in susceptibility to cardiovascular complications and how they respond to interventions.

Beta-2 adrenergic receptor gene

The beta-2 adrenergic receptor gene (ADRB2) is expressed in vascular smooth muscle and in vascular endothelium. ADRB2 gene is located in the chromosome 5q31-q32. It is highly polymorphic and many naturally occurring SNPs were identified among different populations. Variations in gene nucleotide sequence can alter amino acid sequence of the protein and this may alter the receptor ligand binding and its down-regulation following agonist exposure. The substitution of glycine for arginine at position 16 (Arg16-Gly polymorphism; rs1042713) and the substitution of glutamic acid for glutamine at position 27 (Gln27Glu; rs1042714) are the most common SNP changes observed in the general population and seem to alter the receptor functional properties in different manners [5]. In addition, they might affect the function of the receptor in vitro and in vivo [24, 26]. The in vitro analysis showed that the Arg16Gly variant is associated with enhanced agonist-induced desensitization, and the Gln27Glu variant is associated with resistance to desensitization, relative to the responses associated with products of wild-type alleles, Arg16 and Gln27, respectively [17].

In vivo, Cockcroft et al. [5] demonstrated a relationship between the Gln27Glu β_2-adrenoreceptor variant and forearm vascular reactivity to isoproterenol. Compared with a group of individuals homozygous for the Gln27, the homozygous for the Glu27 β_2-adrenoreceptor allele have an increased vasodilator response to infused isoproterenol. An investigation has shown a marked disequilibrium between the polymorphisms at codons 16 and 27 of the β_2-adrenoreceptor [8]. Thus, almost all persons who are homozygous for Glu27 are also homozygous for Gly16, whereas persons who are homozygous for Gly16 can be homozygous for Gln27, homozygous for Glu27, or heterozygous at codon 27.

Trombetta et al. [45] reported an augmented reflex muscle vasodilatory response during physiological maneuvers in women who are homozygous for Gly16/Glu27 of the β_2-adrenoreceptor. In this study, the carriers of the allele Glu27 showed increased forearm vasodilatation during mental stress and handgrip exercise, regardless of the mutation encoding for the amino acids at position 16. Propranolol infusion into the brachial artery abolished the difference in forearm vasodilatory response between Gly16/Glu27 and Arg16/Gln27 women. These findings suggest a strong influence of Gly16/Glu27 β_2-adrenoreceptor variants in the receptor functionality.

Recently, one study from our group demonstrated that a high-fat meal reduces muscle vasodilatation during mental stress in healthy individuals. Moreover, the muscle vasodilatation reduction was markedly greater in individuals who were homozygous for the Glu27 allele of the ADRB2 gene than in those who were homozygous for the Gln27 allele [16]. As already proposed by previous studies, a high-fat meal increases plasma triglyceride levels, which may then lead to endothelial dysfunction by lowering

LDL-cholesterol particles and making them prone to oxidation [33]. The presence of oxidized products in the endothelium decreases nitric oxide synthase activity and impairs vasodilatation [18]. Based on the described link between high-fat meal ingestion, NO production and vasodilatation, the authors believe that the impaired vasodilatation response to mental stress seen after a high-fat ingestion by individuals who were homozygous for the Glu27 allele might be due to a greater dependency on NO synthesis in this particular subset of individuals.

The possibility that changes in the expression of the β_2-adrenoreceptors due to polymorphisms might have phenotypic consequences influencing their cardiovascular or metabolic function is an idea that has attracted much attention during the last years. Because individuals who are homozygous for Glu27 β_2-adrenoreceptor allele have greater vasodilatory response, some studies have speculated if those individuals could be somehow protected against cardiovascular disorders. Mansur et al. [29] were not able to demonstrate an influence of Gln27Glu and Arg16Gly β_2-adrenoreceptor variants on the prognosis of patients with heart failure. In addition, the allele Glu27 showed no association with preclinical atherosclerosis [19] but, on the other hand, it may represent a potential risk factor for fat accumulation and obesity in women [23, 24]. Another recent study suggested that individuals homozygous for Glu27 allele of β_2-adrenoreceptor had a decreased risk of myocardial infarction, compared to Gln27 homozygote subjects [48]. A reduction in cardiovascular events has been found in some [42, 48] but has not been replicated in other investigations [2, 40]. Moreover, a combination of the Arg16Gly and Glu298Asp (c.894G>T of eNOS) polymorphisms in β_2-adrenoreceptor and endothelial nitric oxide synthase genes, respectively, increased the risk for hypertension in elderly humans [34].

The importance of the ADRB2 gene goes beyond its role in vascular function, as described above, further adding to the complexity of gene-environment interactions and the resulting phenotype. It has been shown that the ADRB2 gene has also an important participation in energy-expenditure regulation as it encodes a major lipolytic receptor protein in human fat cells, what could partially explain the link between obesity and the Gln27Glu variant [30]. The presence of the Glu27 allele seems to influence the individual response to diet and physical activity [30]. A case–control study has found a significant interaction between obesity risk and levels of carbohydrate consumption above 49% total energy for carriers of the Glu27 allele of the β_2-adrenoreceptor [32]. Another investigation found that carriers of the Glu27 allele who were more active in their leisure time had a higher BMI compared to the noncarriers. Such results suggest that carriers of the Glu27 allele of the β_2-adrenoreceptor may represent a resistance to losing weight under nonpharmacological treatments, such as diet and physical activity [6]. This demonstrates that a single SNP in a particular gene may confer different functional properties of the respective coded-protein in different physiological systems in a same individual.

Conclusion

There are common genetic variants identified in nitric oxide synthase and beta-2 adrenergic receptor genes. Some of them are functionally important and influence vascular response. The interaction between these functional gene polymorphisms and environmental factors may play a substantial role in the risk of cardiovascular diseases. Despite the attractive scenario that has emerged with regards to the studies of diet- and exercise-gene interactions, to date, current results are not enough to start prescribe specific personalized interventions. As discussed above, vascular reactivity is controlled by a large number of genes and each one confers only a small to moderate effect in such phenotype. Nevertheless, the progression in functional genomics understanding promises a revolution in health care. As our understanding of the interplay between genetics and cardiovascular function increases, the optimistic expectation is that, in the near future, cardiovascular genetics could be integrated into clinical practice.

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