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Possible role of intronic polymorphisms in the *PHACTR1* gene on the development of cardiovascular disease

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ABSTRACT

Cardiovascular disease (CVD) is a complex multifactorial and polygenetic disease in which the interaction of numerous genes, genetic variants, and environmental factors plays a major role in its development. In an attempt to demonstrate the association between certain genetic variants and CVD, researchers have run large genomic wild association studies (GWAS) in recent decades. These studies have correlated several genomic variants with the presence of CVD. Recently, certain polymorphisms in the phosphatase and actin regulator 1 *(PHACTR1)* gene have been shown to be associated with CVD (i.e., coronary artery disease, coronary artery calcification, early onset myocardial infarction, cervical artery dissection and hypertension) in different ethnic groups. It is important to state that all of the described *PHACTR1* genetic variants associated with CVD are located in non-translating gene regions known as introns. Thus, the purpose of this article is to hypothesize the effect of certain intronic polymorphisms in the *PHACTR1* gene on pathological processes in the cardiovascular system. In addition, we present compelling evidence that supports this hypothesis as well as a methodology that could be used to assess the allelic effect using *in vitro* and *in vivo* models, which will ultimately demonstrate the pathophysiological contribution of *PHACTR1* intronic polymorphisms to the development of CVD.

Introduction

Cardiovascular disease (CVD) is a group of pathologies that involve the heart and blood vessels. Furthermore, CVD remains the leading cause of death worldwide [1]. In 2010, the Global Burden of Disease study estimated that CVD is responsible for 15,616 million deaths annually [2]. Morbidity associated with CVD represents a mayor socio-economic burden. It is estimated that CVD is responsible for 10% and 18% of the disability-adjusted life years lost in low and middle-income countries and in high-income countries, respectively [1,3].

Several risk factors have been associated with the development and progression of CVD, such as insulin resistance, diabetes mellitus, hypertension, chronic kidney disease, obesity, dyslipidemia, a sedentary lifestyle, cigarette smoking, and excessive sodium and alcohol consumption [1,4]. Nevertheless, in the last century, some authors have begun to elucidate that a genetic component may be partially responsible for the development of CVD. Initially, in 1964, Rose reported a link between heredity and coronary artery disease (CAD) [5]. Then, other studies validated the role of family history as an independent risk factor for CAD [6,7]. Moreover, the American Heart Association considers a first degree relative with premature CVD (less than 55 years in males and less than 65 years in females) to be an independent risk factor for CVD [8].

CVD is a complex multifactorial and polygenetic disease that results from the interaction of numerous genetic and epigenetic variations in association with environmental factors [4,9,10]. For example, in CAD, these complex interactions make an important contribution to the mechanisms that lead to the deposition of atheroma plaques, endothelial dysfunction, vascular calcification, and a proinflammatory and prothrombotic state that is found in atheroma plaque growth and rupture [4,9,11].

Although investigators have made substantial progress in understanding the numerous genetic factors involved in CVD, the amount of knowledge produced by these studies has been insufficient. Thus, physicians have been unable to translate this

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information into the clinical setting. It is plausible that the genetic variants that are associated with CVD may become biomarkers that could be applied to a specific population to assess the risk of individual patients in the future [10].

In recent decades, numerous studies have attempted to describe the association between the presence of a genetic polymorphism and CVD. One of the tools that researchers have used to determine the aforementioned association is the genome wide association study (GWAS), which is an analysis of various common genetic variants in different populations to assess associations between a variant and a trait [12]. Although, GWAS has been helpful in detecting the association that exists between certain genetic variants and CVD, this technique has certain shortcomings [4]. For example, this method requires a large sample size and should be applied to specific populations [13].

One of the genes that is associated with an increase in the risk of developing CVD is the protein phosphatase and actin regulator 1 (*PHACTR1*) [14]; this association is based on certain polymorphisms at the intronic level. Of note, associations between non-intronic genomic variants located in other gene regions (i.e., exons or promoter) with CVD have not been reported.

The aim of this article is to discuss the association between the known polymorphisms in the *PHACTR1* gene and CVD. Because the scientific community does not possess complete knowledge of the mechanisms that are involved in the appearance of CVD in the presence of certain polymorphisms in *PHACTR1*, in this article, we will hypothesize the genetic effect of this genomic variants that leads to CVD. Furthermore, we will also state how we think that the search for such interactions should be approached.

Materials and methods

We conducted an exhaustive search in Scopus, EBSCO and PubMed to present the most accurate information regarding the interactions between the *PHACTR1* gene and CVD.

Theory

PHACTR1 gene and protein

Phosphatases are a large group of transmembrane proteins that have enzymatic properties that are encoded by several genes in the human genome. Phosphatases are fundamental in the physiological regulation of numerous aspects of eukaryotic cells. Some studies have suggested that phosphorylation regulates more than 70% of all eukaryotic cellular proteins. Furthermore, this regulatory capacity exerts its action through several molecular pathways that cause genomic and non-genomic responses [15,16]. PHACTRs are a family of four phosphatase (PHACTR1-4) proteins with different metabolic actions that mainly interact as mediators of protein phosphatase 1 (PP1) and bind to actin through the C-terminal RPEL domain. In addition, PP1 influences actin remodeling [17–19]. In addition, PHACTR1 is expressed in the testis, kidneys, lungs, brain (cortex, hippocampus and striatum), endothelium and heart [18–20].

The *PHACTR1* gene encodes the PHACTR1 protein. This gene is located on the 6p24.1 chromosome and is formed of 20 exons and 19 introns. *PHACTR1* homologous genes are conserved in chimpanzees, Rhesus monkeys, dogs, cows, mice, rats, chickens, zebrafish, and frogs [21]. Several single nucleotide polymorphisms (SNPs) located on *PHACTR1* are associated with CVD in humans (Table 1).

Cardiovascular effect of the PHACTR1 gene

The effect of PHACTR1 during the development of the cardiovascular system has been studied by Jarray et al. in human umbilical vascular endothelial cell models. These authors identified that PHACTR1 behaves as a vascular endothelial growth factor (VEGF)-dependent gene (VEGFs are a family of proteins that are required for angiogenesis). VEGF-A_{165}, a pro-angiogenic isoform, participates in the regulation of endothelial cell survival, proliferation and permeability. Consequently, knockdown of *PHACTR1* in human endothelial cells causes a major impairment in the vascular tubulogenesis process, which is an essential step in vascular development. In addition, depletion of PHACTR1 in human endothelial cells triggers apoptosis through the following receptors: DR4, DR5 and FAS [20]. The same group of researchers in another study also supported the premise that PHACTR1 is a key element in angiogenesis [22].

Table 1
PHACTR1 polymorphisms associated with CVD, in addition to its location, variant and the global MAF.

Polymorphism	Variant	Region	Global MAF
rs12526453	[C/G]	Intron	0.1727
rs1332844	[C/T]	Intron	0.3237
rs2026458	[C/T]	Intron	0.3480
rs2327620	[A/G]	Intron	0.4227
rs9349379	[A/G]	Intron	0.3774
rs9369640	[A/C]	Intron	0.3574

MAF: Minor allele frequency. Data obtain form the NCBI SNP database (http://www.ncbi.nlm.nih.gov/snp).

Additionally, PHACTRs interacts as an inhibitory mediator of PP1 by directly acting on a generic PP1 substrate (phosphorylase *a*) [18,19]. PP1 is an enzyme that dephosphorylates serine and threonine residues of several substrates and influences various cellular processes, such as the progression of the cell cycle, protein synthesis, muscle contraction, carbohydrate metabolism, transcription, and neuronal signaling [16,18]. Moreover, PP1 regulates endothelial nitric oxide (NO), which is synthesized in endothelial cells by a highly regulated enzyme known as NO synthase [23,24]. Therefore, this information suggests that PHACTR1 is involved in a pathway that modulates the vascular concentration of NO, a molecule that has a wide range of biological properties that are essential for vascular homeostasis and normal endothelial function, including regulation of vascular tone, modulation of local cell growth and vascular protection. Therefore, NO dysregulation is an essential component in the pathophysiology of endothelial dysfunction and atherosclerosis [25,26].

Recently, Jarray et al. [27] proved that down-expression of *PHACTR1* enhances matrix metalloproteinases (MMPs) regulators, which are known as tissue inhibitor of metalloproteinases (TIMPs), in a cellular model. MMPs are a family of enzymes that regulate the extracellular matrix by degrading the group of proteins (i.e., collagen) that are fundamental to architecture of this structural component. Hence, down-regulation of MMPs in the vascular connective tissue contributes to the stability of the plaque by stabilizing the connective tissue surrounding the plaque. Thus, increased MMP activity leads to a microenvironment that is prone to plaque instability and, consequently, to rupture of the plaque, which can culminate in a thrombotic event [28].

With the intent of investigating the expression of *PHACTR1* after a myocardial infarction (MI) in rats, Kelloniemi et al. demonstrated a 60% and 90% reduction in the mRNA and protein levels, respectively, one day after the MI. Furthermore, the authors observed that the mRNA and protein levels returned to control levels after 2 weeks. According to the authors, these results suggest that PHACTR1 has an important role in cardiac function. PHACTR1 is associated in early onset MI in GWAS irrespective of ethnic background. In addition, the function of PHACTR1 in the heart remains unclear; however, the authors demonstrated that PHACTR1 regulates the switch of skeletal and cardiac α-actin contractile isoforms [17].