Coronary artery disease and depression: Possible role of brain-derived neurotrophic factor and serotonin transporter gene polymorphisms

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Abstract. Cardiovascular disease (CVD) and depression are two of the most common human health problems. Patients with depression have an increased risk of developing cardiovascular disease and mortality after experiencing a cardiac event. Both diseases are complex disorders that are influenced by genetic and environmental factors. Brain-derived neurotrophic factor (BDNF) plays a critical role in regulating both vascular development and response to injury, and promotes survival, differentiation, and maintenance of neurons in the peripheral and nervous system. Evidence suggests that BDNF can enhance serotoninergic transmission. Serotonin modulates different brain functions and is known to regulate sleep, appetite, pain and inflammation. The aims of the present case-control study were to investigate the possible role of BDNF Val66Met, 5-HTTLPR and -1438 G/A polymorphisms in the development of coronary artery disease (CAD) in patients with and without depression. Regarding BDNF, our data suggest an involvement of the AA genotype in the pathogenesis of CAD in females and in the predisposition to CAD associated with depression. Furthermore, it could be argued that the GG genotype is protective against CAD in the female population and against CAD associated with depression. In our CAD population we also observed a significant increase in the L/L genotype and a decrease in the S/L genotype with respect to the controls. A higher frequency of the L allele, responsible for enhancing the efficiency of transcription, was found in CAD patients. These findings may be responsible for the increased capacity of platelet serotonin uptake previously observed in patients with CAD. Although no differences were found for genotype and allelic frequencies of the -1438 G/A polymorphism between the CAD patients and controls, we cannot exclude the possible role of this receptor in coronary artery disease.

Introduction

Cardiovascular disease (CVD) and depression are two of the most common human health problems. Patients with depression have an increased risk of developing cardiovascular disease and a 2- to 4-fold risk of mortality after experiencing a cardiac event (1). Despite the evidence that heart disease and depression are epidemiologically linked, this correlation is not well understood. Depression is associated with changes in an individual's health status that may influence the development and course of cardiovascular disease, including noncompliance with medical recommendations, as well as the presence of cardiovascular risk factors such as smoking and hypertension. In addition, depression is associated with physiologic and pathologic changes, including nervous system activations, cardiac rhythm disturbances, multidistrectual inflammation, and hypercoagulability that negatively influence the cardiovascular system. Furthermore, stress may be an underlying trigger that leads to the development of both depression and cardiovascular disease (2).

Depression and CVD are complex disorders that are influenced by genetic and environmental factors. Previous studies have demonstrated that brain-derived neurotrophic factor (BDNF) plays a critical role in regulating both vascular development and response to injury (3). BDNF is a member of the neurotrophin family of growth factors which promotes survival, differentiation, and maintenance of neurons in the peripheral and nervous system (4). BDNF is expressed in atherosclerotic coronary arteries, preferentially localized in the atheromatous intima and around the vasa vasorum in the adventitia. The slight detection of BDNF in non-atherosclerotic coronary arteries (5) suggests its possible role in the pathogenesis of CAD.

A single nucleotide polymorphism (SNP) in the BDNF gene leading to a G (Val) to A (Met) substitution at position

196 (codon 66) in the prodomain has been found to be associated with neuropsychiatric disorders including Alzheimer's disease, Parkinson's disease, depression and bipolar disorder (6-9). Humans carrying the Met allele have smaller hippocampal volumes and perform poorly on hippocampal-dependent memory tasks (10,11). It has previously been shown that the Met variant alters the intracellular trafficking and activity-dependent secretion of BDNF in neurosecretory cells and neurons (10,12).

Several studies have suggested the possible implication of the serotonin (5-hydroxytryptamine) 5-HT_{2A} receptor in depression (13-16). Serotonin modulates different brain functions through interactions with different 5-HT receptor subtypes and is known to regulate sleep, appetite, pain and inflammation. The G protein-coupled serotonin 5-HT_{2A} receptor is primarily recognized for its role in brain neurotransmission, where it mediates a wide variety of functions including certain aspects of cognition. However, there is significant expression of this receptor in peripheral tissues, where its importance is largely unknown. A recent study revealed that activation of 5-HT_{2A} receptors in primary aortic smooth muscle cells provides a previously unknown and extremely potent inhibition of tumor necrosis factor (TNF)-α-mediated inflammation (17). TNF-α-mediated inflammatory pathways have been strongly implicated in a number of diseases, including atherosclerosis, rheumatoid arthritis, psoriasis, type II diabetes, depression, schizophrenia and Alzheimer's disease.

The complex 5-HT neuronal system is under a bottleneck control by a single protein, 5-HT transporter (5-HTT). By controlling reuptake of 5-HT from the extracellular space, 5-HTT regulates the duration and strength of the interactions between 5-HT and its receptors. A polymorphism within the 5-htt 5' upstream region (5-HTTLPR) has been reported, the majority of which is composed of either 14 (S) or 16 (L) repetitive elements. In humans, although infrequent, 18 and 20 repetitive elements (XL) are also present (18-21). An *in vitro* transcriptional assay has indicated that the activity of the human 5-htt promoter is regulated by these polymorphic repetitive elements, resulting in differences in the efficacy of 5-HTT reuptake among the allelic variants (22).

The aims of the present case-control study were to investigate the possible role of BDNF Val66Met, 5-HTTLPR and -1438 G/A polymorphisms in the development of coronary artery disease (CAD) in patients with and without depression.

Patients and methods

Study subjects. Genotype frequencies were investigated in 99 Caucasian Italian patients with CAD (87 males, 12 females) who were consecutively recruited among subjects referred for coronary angiography at the Cardiology Division of the IRCCS San Matteo Hospital, Pavia, Italy. CAD was defined as angiographic evidence of stenosis in any epicardial coronary artery ≥50% of the diameter. A total of 143 healthy blood donors (49 males, 94 females), matched for age, gender and geographical origins, served as controls. The population of patients and controls were in Hardy-Weinberg equilibrium.

The study protocol was approved by our university ethics committee. After a complete explanation of the aims and details of the study, written informed consent was obtained from the subjects.

Measures of depression. All of the CAD patients completed the Beck Depression Inventory (BDI) (23). The BDI is a widely used, 21-item instrument for assessing a variety of symptoms of depression and has been shown to predict cardiovascular disease outcome among cardiovascular patient populations in several studies (24). Although completion of the BDI provides a continuous range of scores from 0 to 48, previous research suggests that scores ≥17 indicate the presence of moderate to severe depression.

We identified 29 patients who had high BDI results. We also clinically confirmed depression in these patients.

Genetic analysis. Genomic DNA was extracted from EDTA-treated blood using the DNA Blood Mini Kit (Qiagen). The SNPs considered in this study were analyzed using the PCR-RFLP method. PCR amplifications were carried out in a total volume of 25 μl containing 20 ng of genomic DNA, 0.1 μl of Taq polymerase (0.02 U/μl), 1 μl of each specific primer forward and reverse (4 ng/μl), 2.5 μl PCR buffer 10X, 0.75 μl $MgCl_2$ (1.5 mM), 2.5 μl dNTPs (2 mM each) and 16.15 μl of sterile H_2O.

The Val66Met and -1438 G/A polymorphisms were analyzed using the PCR-RFLP technique as previously described. The thermocycling program consisted of 95°C for 10 min, then 35 cycles at 95°C for 30 sec, 60°C for 1 min, 72°C for 30 sec, and a final extension step at 72°C for 10 min. The amplified segments were digested using *Pml*I (New England Biolabs) and *Hpa*II (Fermentas) repeatedly for 4 h at 37°C and the digestion was run on a 3% agarose gel and read using a UV transilluminator.

The 5-HTTLPR polymorphism was analyzed using the same technique as previously described. The thermocycling program consisted of 95°C at 10 min, 35 cycles at 95°C for 1 min, 60°C for 30 sec, 72°C for 30 sec, and a final extension step at 72°C for 10 min. The amplified segment was run on a 3% agarose gel and read using a UV transilluminator.

Statistical analyses. The χ^2 test for goodness of fit was used to verify whether the observed allelic frequencies agreed with those expected under Hardy-Weinberg equilibrium. Allelic and genotypic distributions were estimated using the χ^2 test, and differences were considered statistically significant at a p-value <0.05. Crude and adjusted odds ratios (ORs) are reported with their 95% confidence intervals (CIs) using univariate analyses.

Results

The distribution of the BDNF Val66Met polymorphism in the CAD population is shown in Table I.

Genotypes were in Hardy-Weinberg equilibrium both in the patients and controls.

In our study population, the allelic and genotypic frequencies of the Val66Met polymorphism in the BDNF gene were similar in the CAD patients and healthy controls.

Table I. Distribution of BDNF Val66Met alleles and genotypes in female CAD patients and controls.

	Female CAD (n=12)	Female controls (n=94)	OR (95% CI)	P-value
Genotype				
GG	1	51	0.0766 (0.0096-0.6178)	0.00270
AG	3	34		0.44450
AA	8	9	18.8890 (4.7379-75.3057)	<0.00001
Allele				
G	5	136	0.1006 (0.0357-0.2834)	<0.00001
A	19	52	9.9385 (3.5280-27.9969)	<0.00001

Table II. Distribution of BDNF Val66Met alleles and genotypes in CAD patients with and without depression.

	Depressed CAD (n=29)	Non-depressed CAD (n=70)	OR (95% CI)	P-value
Genotype				
GG	5	48	0.0955 (0.0322-0.2833)	<0.00001
AG	12	18		0.12300
AA	12	4	11.6471 (3.334-40.6883)	<0.00010
Allele				
G	22	114	0.1394 (0.0706-0.2752)	<0.00001
A	36	26	7.1748 (3.6339-14.1662)	<0.00001

For the same polymorphisms in the CAD patient population, divided according to gender, no statistically significant data were found for the CAD and control male population. In the female CAD population, the frequency of the homozygous AA (Met/Met) genotype was significantly higher in the patients (66.7%) as compared to the controls (9.6%) (p<0.00001). The crude odds ratio for the presence of CAD in subjects bearing the homozygous AA genotype was 18.89 (95% CI, 4.74-75.3). Furthermore, the frequency of the homozygous GG (Val/Val) genotype was significantly lower in the patients (8.3%) as compared to the controls (54.2%) (p=0.0027). The frequencies of heterozygotes were not statistically different. Allelic frequencies also appeared to differ significantly (p<0.00001); and in particular the A allele coded for methionine in the sequence of proBDNF was more prevalent in the patients (79.1%) as compared with the healthy controls (27.6%).

The distribution of the BDNF Val66Met polymorphism in depressed and non-depressed CAD patients is shown in Table II. The genotypic and allelic frequencies showed a statistically significant difference between the genotypes GG and AA, while no difference was observed between the frequencies of the heterozygous AG. In particular, the frequency of the AA genotype was higher in the depressed patients (41.3%) compared to the patients with no depression (5.71%) (p<0.00001), whereas the GG genotype was lower in the depressed patients (17.2%) as compared to the non-depressed patients (68.5%) (p<0.00001). The crude odds ratio for the presence of CAD associated with depression in subjects bearing the homozygous AA genotype was 11.65 (95% CI, 3.3-40.7). The distribution of alleles was significantly different (p<0.00001) between the two groups; and, in particular, the A allele (Met) had a higher frequency in patients exhibiting symptoms of depression (62.1%) than in the patients without clinically defined symptoms of depression (18.6%).

Regarding the 5-HTTLPR polymorphism, the distribution in CAD patients is shown in Table III. The frequency of the heterozygous S/L genotype was significantly lower in the patients (39.4%) as compared to the controls (53.1%), and the frequency of the homozygous L/L genotype was significantly higher in the patients (45.5%) as compared to the controls (28.7%); while we did not find any significant difference for the S/S genotype. Statistically significant difference between the frequencies of the S and L alleles in the CAD patients was found as compared with the healthy controls (p=0.0291); and, in particular, we observed an increase in the frequency of L in the patients with coronary artery disease (65.2%) as compared to the healthy controls (55.2%). The crude odds ratio for the presence of CAD in subjects bearing the L allele was 1.51 (95% CI, 1.04-2.2).

Dividing the population into males and females, no statistically significant difference was found for the female population while the L/L genotype in the male population was more frequent in the CAD population (46%) compared with the healthy controls (29%) (Table IV). Allelic and geno-

Table III. Distribution of BDNF Val66Met alleles and genotypes in CAD patients and controls.

	CAD (n=99)	Controls (n=143)	OR (95% CI)	P-value
Genotype				
S/S	15	26		0.5370
S/L	39	76	0.5730 (0.3407-0.9639)	0.0350
L/L	45	41	2.0732 (1.2123-3.5453)	0.0073
Allele				
S	69	128	0.6602 (0.4543-0.9596)	0.0291
L	129	158	1.5146 (1.0421-2.2012)	0.0291

Table IV. Distribution of BDNF Val66Met alleles and genotypes in male CAD patients and controls.

	Male CAD (n=87)	Male controls (n=49)	OR (95% CI)	P-value
Genotype				
S/S	14	10		0.5261
S/L	33	25		0.1383
L/L	40	14	2.1277 (1.0054-4.5026)	0.0464
Allele				
S	61	45	0.6358 (0.3838-1.0533)	0.0780
L	113	53	1.5728 (0.9494-2.6057)	0.0780

typic study did not reveal any statistically significant data comparing the CAD patients with and without depression as based on the results of the BDI.

Upon univariate analysis of the genotypic and allelic frequencies for the -1438 G/A polymorphism in 5HTR2A, we did not find statistically significant differences between the CAD patients and healthy controls. In addition, the study of this polymorphism did not reveal any association in the gender study and when comparing the frequencies of depressed and not depressed patients.

Discussion

CAD (coronary artery disease) is a leading cause of human death worldwide (25). A number of factors increase an individual's risk for cardiovascular disease, including smoking, hypertension, diabetes, hypercholesterolemia and obesity (26,27). In addition, other risk factors such as a high level of homocysteine have been identified (28), and several studies indicate that depression may have behavioral and direct pathophysiologic effects on CAD. Abnormal platelet function and endothelial dysfunction have been identified as possible links between depression and CAD.

Cardiovascular disease and depression are often comorbid conditions, and it has been widely demonstrated that both diseases are epidemiologically linked. The presence of depression increases the risk of cardiovascular events and mortality, predicts poor adherence to treatment and more frequent and earlier hospital readmissions (29-31).

Furthermore, even subclinical levels of depression severity are associated with changes in sympathetic and parasympathetic nervous system activity and alterations in platelet responsiveness (32-34), providing support for plausible biological pathways.

BDNF is the neurotrophin most widespread in the central nervous system, especially in the hippocampus, neocortex and hypothalamus (35-37). BDNF is also synthesized and released from non-neuronal cell populations; it is in particular present in the bloodstream and especially in platelets (38).

In patients with acute coronary syndromes (ACS), plasma levels of BDNF were found to be reduced, suggesting a possible involvement of this neurotrophic factor in these diseases (4). Some studies have shown that BDNF plays a crucial role in regulating response to injury (4). The lack of BDNF results in a reduction of endothelial cell contacts and their cell apoptosis, causing bleeding into the ventricular wall, depression of cardiac contractility and early postnatal death (39). Its receptor is shown to be expressed in atherosclerotic vessels, endothelial cells and smooth muscle cells, suggesting that BDNF may influence the regulation of vascular development (5).

The precise role of BDNF in the pathogenesis of CAD is not clear but appears to be associated with an increased inflammatory response by activated T cells and macrophages in atherosclerotic coronary arteries (40).

Women have traditionally received less attention in heart disease research relative to men, despite well-know gender differences indicating comparative less aggressive treatments,

less accurate diagnostic tests, and higher post-myocardial infarction mortality among women (41,42). Depression rates among women exceed those for men by a factor of more than 2 to 1 (43), and they may be associated with clinical symptoms that affect cardiac diagnosis and treatment (41).

We found a variation in allelic frequencies in female patients with CAD compared with healthy women: the G allele frequency, coding for valine, was lower in the patients compared to the controls; while the frequency of the A allele, coding for methionine, was higher in the CAD patients with respect to the controls. We also found differences in the frequencies of the GG and AA genotypes; the frequency of the genotype GG (Val/Val) was lower in the patients compared with the controls. In contrast, the frequency of the AA genotype (Met/Met) in the patients was higher. This association could be due to the positive trophic effect exerted by BDNF on vascular endothelial cells which, in combination with hormonal factors, may provide females with reduced BDNF (as a result of the AA genotype) at a greater risk of developing atherosclerotic lesions.

The Val66Met polymorphism has been found to be associated with neuropsychiatric disorders such as Alzheimer's disease, Parkinson's disease, bipolar disorder and depression (6-9). Our study population, depressed and non-depressed CAD patients, identified by BDI administration, showed a higher frequency of the AA genotype in the group with depression while the GG genotype was lower in the same group.

Our data suggest an involvement of the AA genotype, linked to a low secretion of BDNF, in the pathogenesis of CAD in women and in the predisposition to CAD associated with depression. Furthermore, it could be argued that the GG genotype is protective against CAD in the female population and against CAD associated with depression.

Evidence suggests that BDNF can enhance serotoninergic transmission (44). The direct infusion of BDNF in the brain influences the survival and function of serotoninergic neurons by enhancing the release of serotonin. The molecular mechanism by which BDNF modulates the serotoninergic system is still unknown. Even if assumed that a deficiency of serotonin is specifically linked to depression, efforts to connect values of serotonin to depression have been inconclusive. Serotonin may contribute to the onset of CAD through central and peripheral mechanisms. In the periphery, serotonin induces platelet aggregation, and in the presence of endothelial damage induces vasoconstriction and hyperplasia of smooth muscle cells of arteries, conditions that can lead to the formation of thrombi (45,46). The effects of serotonin on platelets are mediated by 5-HT_{2A} receptors (47), and the reuptake of circulating serotonin in platelets is mediated through its specific transporter (5-HTT). Many studies have shown that in depressed patients there is an increase in the platelet binding sites for 5-HT_{2A} and a reduction in the amount of carrier, indicating that depressed patients are particularly vulnerable to aggregating platelets and vasoconstriction associated with CAD (33).

In our CAD population we observed a significant increase in the L/L genotype and a decrease in the S/L genotype with respect to the controls. We also found a statistical difference in the allelic distribution in the CAD patients compared to the healthy controls: a greater frequency of the L allele, responsible for enhancing the efficiency of transcription, was found in the CAD patients, while the S allele was less frequent. These findings may be responsible for the increased capacity of platelet serotonin uptake previously observed in patients with CAD (48).

Although no differences were found for the genotypic and allelic frequencies of the -1438 G/A polymorphism between the CAD patients and the controls, we cannot exclude the possible role of this receptor in this disease. Further studies genotyping several polymorphisms in this gene are needed to exclude a potential involvement in the susceptibility to coronary atherosclerosis.

We confirmed the multifactorial genesis of the CAD-depression association. In interpreting our findings, important limitations must be considered. Firstly, this is a preliminary study with a group of Caucasian Italian subject. One future objective is to increase the number of subjects, in particular females, to confirm the importance of these polymorphisms in affecting the risk of CAD and depression. Furthermore, all polymorphisms considered change the amount of protein secreted. For this reason our intent is to measure the BDNF and serotonin protein levels in serum in order to identify a possible correlation. Further studies aimed at genotyping several polymorphisms in these and other genes will be required to confirm or rule out their potential involvement in the development of CAD.

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