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Hunting the Risk NPY and ACE Polymorphisms as Predictors of Cardiovascular Diseases: Case Report and Review of the Literature

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Abstract

Many research efforts were addressed to identify individuals at high risk for multifactorial diseases, such as cardiovascular alterations and related diseases. Great interest was paid to investigate the genetic liability in multifactorial illnesses. The prognosis of high-risk patients might be greatly ameliorated using genetic predisposition risk factors, such as the polymorphisms of neuropeptide Y (NPY) and Angiotensin converting enzyme (ACE) genes. Epidemiologic results suggest that selected polymorphisms of both NPY and ACE might be helpful to improve the evaluation of patients, offering a powerful prognostic tool and paving the way to novel molecular therapeutic strategies.

We present a case report of a male, sudden-death from myocardial infarction, presenting with left ventricular hypertrophy. The patient carried polymorphisms of ACE and NPY genes, respectively ACE genotype ID and NPY genotype T-399C, actually considered as risk factors.

Keywords: ACE; NPY; Polymorphisms; Risk factor; Atherosclerosis; Cardiovascular disease; Left ventricular hypertrophy

Introduction

A number of factors contribute to the etio-pathogenesis of multifactorial diseases [1]. The identification of the sequence of events and of the acting molecules might help to define the natural history of multifactorial diseases, to ameliorate the diagnostic approach and to refine the prognosis, paving the way to novel molecular therapeutic strategies.

Environmental and genetic factors were analyzed in order to elucidate the nature and the role of the elements involved in the individual liability to multifactorial diseases. A number of studies reported associations of polymorphism with different pathological conditions [1-3]. Many studies found significant associations, whereas others did not confirm those findings [3,4]. Numerous factors, such as the matching factors or other linked genetic markers, could play confounding role upon results. Despite the controversial findings, great interest raised around selected polymorphisms associated to a number of multifactorial diseases, with special regard to atherosclerosis, cardiovascular and metabolic diseases [2-4].

We resumed literature data about polymorphisms of two genes, which codify respectively for neuropeptide Y (NPY) and Angiotensin converting enzyme (ACE), both molecules involved in the pathogenesis of cardiovascular disease (CD) and atherosclerosis [5]. We also present a case report of a male, suddenly deceased from myocardial infarction, presenting with left ventricular hypertrophy (LVH) at post-mortem examination, otherwise clinically silent. In the presented case, we analysed polymorphisms of NPY and ACE genes.

Materials and Methods

A 40 year old man suddenly had a collapse in a public store. Resuscitation attempts were unsuccessful and he died in a few minutes. Familial anamnestic data did not record cardiovascular or metabolic diseases. The patient was referred in good health and no information was available regarding his blood pressure.

Autopsy findings

The body length was 185 cm, weight 100 kg, with a body mass index (BMI) of 29.21. The patient was in good general hygienic conditions, with normal muscle trophism and abundant android fatty tissue distribution. Toxicological examination was negative for drug and alcohol abuse. The heart weighed 970 g, with longitudinal diameter of 16cm, transverse diameter of 15 cm and antero-posterior diameter of 8.5 cm. Heart's consistency was significantly increased. The section of the heart apex showed important LVH: the thickness was was 4.0 cm, 4.1 cm in the septum and 3.3 cm at the apex level. The myocardium was pale (boiled flesh-colour) with no macroscopic signs of necrosis. The histology examination confirmed concentric hypertrophy. The papillary muscles were significantly hypertrophic and increased in consistency, and cordae tendineae were slightly thickened. The valvular systems showed no alterations. The shape of the lungs was increased, as well as the volume and consistency. The right lung weighed 820g and the left 780g. The pulmonary tissue appeared smooth, moist, dark red, presenting petechiae in the interlobar fissures. Large, medium and small bronchi showed patent lumen and slightly hyperaemic mucosa. Histology findings identified congestive aspect of the respiratory mucosa. The histology of elastic arteries wall identified mild to moderate atherosclerosis evidences, especially in the abdominal aorta wall.

Molecular biology analyses

The polymorphism analyses were conducted by Geneticlab srl

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(Noventa Vicentina, Italy). Briefly, RNA was extracted from frozen heart tissue biopsy using TRIzol reagent (Invitrogen Corporation, Carlsbad, CA, USA), spectrophotometrically quantified at 260 nm, reverse-transcribed to cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Byosystems, Carlsbad, CA) and amplified using GoTaq® qPCR Master Mix (Promega, WI, USA). The following primer pairs (Bio Basic Inc., Amherst- NY, USA) were used for real-time amplification of ACE gene insertion/deletion allele: ACE1 primer 5′-CATCCTTTCTCCCATTTCTC-3′, ACE2 primer 5′-TGGGATTACAGGCGTGATACAG-3', and ACE3 primer 5′-ATTTCAGAGCTGGAATAAAATT-3′. Amplification and detection were performed using the CFX96™ Detection System (Biorad Laboratories, Hercules, CA). For RT-PCR amplification of the NPY 399T>C polymorphism, the following primers were used: forward 5'- TTGCCTCACTCCAACAGCG-3' and reverse 5'-ACAACACCAAAGCCCAAGTATCT-3'. The amplification reaction was conducted in Techne T-512 thermocycler (Techne, Staffordshire, UK) and the sequencing of the amplification product was obtained using the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Carlsbad, CA). The PCR products were digested by BsiEI (New England Biolabs, Beverly, Massachusetts) and digestions were analysed by electrophoresis on 2% agarose gel. For quality control, we used samples at random for repeat analysis using our standard genotyping protocol, which were in 100% concordance with the original.

Results

ACE: genotype ID. Analysis of PCR products on 2% agarose gel electrophoresis under UV light by staining etidium bromide detected a band of 190 bp shows deletion (D) and a band of 490 bp shows insertion (I).

NPY: genotype -399 T>C. We determined a T to C polymorphism which results in a substitution of leucine to proline substitution at codon 7 in the NPY gene. The percentage of efficiency was 100%.

Discussion

Selected polymorphisms of both ACE and NPY genes are considered to be risk factors for CD [2,3], as well as for metabolic diseases, including atherosclerosis [4-6]. However, the relationship and the specific role of those polymorphisms are not fully clear.

The post-mortem evaluation of our patient identified LVH, a major risk factor underlying coronary heart disease, and molecular biology analysis revealed the carrier status for selected ACE and NPY polymorphisms, respectively ACE genotype I/D and NPY genotype T-399C.

NPY and CD

The neuroendocrine regulation of the stress response is a complex event, modulated and influenced by polymorphisms of stress-related genes [6-11], including the NPY gene, which codifies for the most abundant peptide in heart and brain [9-11]. NPY is produced by sympathetic neurons, endothelial cells [12], and platelets [13]. NPY is involved in a number of functions including sympathetic nerve stimulation, immune regulation [14], food intake control [10], coronary blood flow, ventricular function [15], vascular smooth muscle cell proliferation and modulation of the contractile function during the development of cardiomyocite hypertrophy [9-11]. NPY is associated with pathological conditions, including schizophrenia [16], and eating disorders [17]. Measurement of NPY plasma levels contributes to predict cardiovascular complications in end-stage renal disease affected patients [18,19]. Moreover, NPY is claimed to be involved in CD [18] and congestive heart failure [20-22].

The regulatory roles of NPY are largely determined by the enzymatic processing of NPY (NPY1-36) and by its receptor subtypes (NPY-Y1 to Y5). The regulation of the cardiovascular system activities and of blood pressure by vasoconstriction and vascular smooth muscle cell proliferation is mediated by NPY-Y1 receptor and its selective agonist NPY (NPY1-36) [12-14, 23-26]. Ischemic angiogenesis is under the control of one or both NPY-Y2 or NPY-Y5 receptor subtypes and the N-terminally truncated form of NPY (NPY3-36) [12,27,28].

The NPY gene (NPY; OMIM *162640) is located on the short arm of chromosome 7 (7p15.1). Selected NPY polymorphisms are associated to cortisol and ACTH response to acute psychosocial stress. In Scandinavian populations, one NPY variant, otherwise rare in other populations, was associated with hyperlipidaemia and carotid atherosclerosis [20-22], coronary alterations in type 1 diabetes [23], and myocardial infarction following hypertensive status [24].

Many studies investigating the relationship between NPY polymorphisms and disease risk focused on the non-synonymous L7P (rs16139) polymorphism in preproneuropeptide Y (preproNPY), that might induce abnormal local NPY signalling [29]. The L7P polymorphism otherwise increases the risk for type 2 diabetes and CD concomitantly with further risk factors, such as obesity and hypertension [30,31]. Furthermore, Pro7 substitution for Leu7 is related to increased serum total cholesterol and low density lipoprotein levels in obese Caucasian individuals [21]. The L7P polymorphism is rare in Asians [29,32,33]. The association of ischemic stroke with polymorphisms TA and CC, constructed by two further polymorphisms, rs16135 and rs16476 was reported.

The intrinsic polymorphism rs16141 of NPY was related to cerebrospinal fluid cholesterol levels in Alzheimer disease (AD) patients [25-38], was reported in schizophrenia [29], and non-Hodgkin lymphoma [35]. It also affects stress response and emotion [28,35,36].

Linkage scans suggested that chromosome 7p might be involved in thermogenesis [37-41], as well as in pulse pressure, a measure of central arterial stiffness and a predictor of CD-related mortality [42]. In vascular homeostasis NPY plays two opposite roles, vaso-dilation or constriction, depending on control of the nervous systems [43]. Probably NPY polymorphisms, especially those involving the gene expression regulatory regions, may affect the vascular system. Special attention was paid to another NPY promoter polymorphism, C-399T, also known as rs16147 and C-485T [32-36]. A putative SP1 transcription factor binding site within the rs16147 stretch of NPY sequence is lost with the rs16147 an allele [32]. The rs16147 G allele (reverse strand C allele) overall increases the expression of the NPY gene [38-41] and subsequently the NPY plasma levels, thus promoting arterial smooth muscle cell proliferation [37] and thermogenesis [21,32]. C-399T was related to ischemic stroke with higher prevalence of hypertension affected individuals. Allele-specific effects of the C-399T polymorphism were reported [18,32,38,39]. Controversial observations were published [36] and no conclusive observations were reported. However, the effect of NPY on stroke susceptibility seems to act independently from hypertension [44]. The hypothesis that NPY contributes to atherosclerosis is supported by both the correlation of plasma NPY levels with selected NPY polymorphisms, and by the reported inhibition of murine atherosclerosis with an antagonist of the NPY1 receptor, which mediates most cardiovascular effects of NPY [9]. In the present case report, the patient carried the rs16147 or C-399T

genotype. Therefore, the identified polymorphism might indicate the liability to arterial smooth muscle proliferation and thermogenesis. Histology findings confirmed a moderate arterial atherosclerosis. Unfortunately, an attempt to measure the patient's plasma levels of NPY failed, probably due to the post-mortem interval.

A deeper understanding of the relationship among NPY, cardiovascular risk factors and clinically diagnosed CD will deserve further studies. The analysis of NPY polymorphisms might help to refine the risk estimates for selected individuals displaying a familial high risk predisposition to CD and/or atherosclerosis. In fact, individuals which share a genetic liability to their NPY risk profile might represent a prime target for prevention.

ACE polymorphisms, hypertension and atherosclerosis

ACE contributes to the finely tuned long-term regulation of blood pressure and volume [45]. ACE enzyme, a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells, plays a pivotal role in converting the inactive decapeptide, angiotensin I (Ang I or Ang 1-10), to the active octapeptide angiotensin II (Ang II or Ang 1-8), a powerful vasoconstrictor main active product of the renin-angiotensin system [46-49]. Ang II induces the adrenal cortex to release aldosterone [50], mediates cell growth and proliferation, [51], and contributes to endothelial dysfunction by reducing nitric oxide bioavailability [52]. ACE also plays a critical role in the kinin-kallikrein cascade, which also contributes to regulate the blood pressure [53,54].

The human ACE gene (OMIM +106180), located on the long arm of chromosome 17 (17q23), is 21 kilo bases (kb) long and comprises 26 exons and 25 introns. More than 160 ACE gene polymorphisms were described, most of which are single nucleotide polymorphisms (SNPs) [55,56]. The gene codifies for two isoforms: the somatic form (sACE), with a molecular mass of 170 kDa, expressed in somatic tissue, and the testicular form (tACE, germinal ACE -gACE), with a molecular mass of 100 kDa, exclusively expressed in male germinal cells [55]. A homologue of ACE, ACE2, was cloned from human heart failure and lymphoma cDNA libraries [57,58]. ACE2 gene contains 18 exons and maps to Xp22 [57]. *In vitro* studies indicate that the catalytic efficiency of ACE2 for Ang II is 400-fold greater than for Ang I [59].

The potential role of ACE and ACE2 as cardioprotective peptides with vasodilator, antigrowth, and antiproliferative actions was recently suggested [60].

Inter-individual differences in plasmatic ACE levels were described, suggesting efficient long-term tuning of plasma levels, possibly due to genetic regulation [61]. A polymorphism involving the presence (insertion I) or absence (deletion, D) of a 287-bp sequence of DNA in intron 16 of the gene (NCBI ref. SNP ID: rs1799752) was described [62]. Mean ACE activity levels in DD carriers were approximately twice those found in II genotype individuals [63]. Subjects with the ID genotype had intermediate levels, indicating allele codominancy. Involvement of the I/D polymorphism was detected in plasma, as well as in tissue ACE levels [64,65].

Beside the involvement in neurodegenerative diseases, such as AD [66-69], or metabolic illnesses, such as diabetes [70,71], ACE polymorphisms were investigated in a number of pathologies of the cardiovascular system in different populations. However, the complexity of association and linkage studies, as well as misclassifications of phenotypes may have lead to false-negative or false-positive results, and controversial observations were reported.

Although the nature, the specific role and location of ACE polymorphisms remain to elucidate, the I/D polymorphism is actually considered a valid marker to investigate the relationship between genetic predisposition and diseases. In normotensive men, after infusion of Ang I, venous levels of Ang II and increases in blood pressure were higher in DD carriers compared with II carriers [72]. However, controversial observations were reported [73]. Anomalies in further ACE activities, such as degradation of bradykinin, were also investigated [74]. Studies reported a positive association between the D allele and high blood pressure [75-85]. Significant relationship between the D allele and hypertension in women and in Asians was reported [55]. Controversial results were also reported in experimental models [86]. The possible association between the ACE I/D polymorphism and atherosclerosis was actively investigated using arterial intima-media thickness (IMT) measurement, a promising marker for very early metabolic disease liability, even in foetuses [86-89]. Positive association between the D allele and common carotid IMT in adults was reported demonstrating results concordance between Caucasians and Asians [89]. The association was stronger among high-risk individuals, including subjects with underlying diseases such as cerebrovascular disease, diabetes, or hypertension [89]. The association between ACE genotype and atherosclerosis was also assessed using coronary calcification as a measure of coronary atherosclerosis [90,91], and post-mortem measurements of aortic atherosclerosis [92,93]. However, also in this case, the results were controversial and inconclusive.

The D allele, considered as relevant exclusively in selected groups of patients, is associated to changes in the left ventricle [89-94]. The DD genotype was identified more frequently in male patients with myocardial infarction than in normal controls, particularly among low-risk controls, intended as individuals bearing low body mass index and low plasma levels of apolipoprotein B (ApoB) [94]. However, this observation was not confirmed in a large association study [95,96]. Association between the ACE polymorphism and physical performance was also described [97-103]. The association of the I allele with improved endurance is in line with the association of the D allele with LVH. Carriers of the D allele may develop hypertrophy following lower metabolic efficiency [103]. Moreover, a recently published report argued that the frequent association between short telomeres and cardiovascular risk factors and/or age related diseases is affected by the ACE genotype in the elderly hypertension subjects with LVH [104]. Genetic and epidemiological data documented that elevated plasma ACE levels increase LVH risk [105]. Moreover, animal studies suggested the contribution of cardiac ACE levels to atrial enlargement and cardiac arrhythmia [106]. Probably, genetic anomalies leading to ACE overexpression play an essential role in the pathogenesis of LVH. A significant association of ACE gene I/D polymorphisms with LVH, especially in East Asians, was described, with higher risk in males [107]. In the present case report, the molecular biology analyses identified the ID carrier status. However, histology findings did not identify strong evidence of IMT, although evidences of moderate aortic atherosclerosis were detected. Unfortunately, we do not have previous biochemical data regarding the plasma levels of ApoB in the patient.

Further studies are required in order to evaluate the role and the meaning of the D allele presence in the regulation of blood pressure and/or the increase in the left ventricle wall thickness. In fact, ACE polymorphisms might offer a powerful tool to identify high risk individuals in the population.

Conclusion

Epidemiologic results report the association ACE or NPY polymorphisms with atherosclerosis, metabolic, cardiovascular and/or

related diseases. The identification and prognosis of patients at high risk to develop CD and/or metabolic diseases might be greatly ameliorated using genetic liability risk factors, such as the polymorphisms of ACE and NPY genes. As an example, our patient was referred as a good health young man. However, he presented clinically silent LVH and presented ACE and NPY polymorphisms suggestive for CD/metabolic diseases high risk. One might speculate that knowledge of carrying risk factors, such as LVH and polymorphisms, might address to cardiological or internal medicine evaluation and to modify the life style.

Further investigations are required in order to investigate the functional effects of polymorphisms with respect to the molecular activities of ACE or NPY. To understand the meaning of polymorphisms might clarify the etiopathogenesis of a number of diseases, and also might help to improve the evaluation of patients, offering a further prognostic tool, thus paving the way to personalize molecular therapeutic strategies.

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