Vascular Calcification and Cardiovascular Outcome in Dialysis Patients: The Role of Gene Polymorphisms

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Key Words
Hemodialysis · Gene polymorphism · Vascular calcification · Atherosclerosis

Abstract
Vascular calcification and accelerated atherosclerosis are major causes of death in hemodialysis (HD) patients. Epigenetic mechanisms of gene regulation may be crucial determinants of cellular behavior in uremic conditions, determining an increased risk of cardiovascular morbidity and mortality. The common polymorphisms on different gene promoters have been related to increased coronary artery calcification and associated with cardiovascular outcome in HD population. In this review, we reported the gene polymorphisms of different proteins as negative prognostic risk factors for all-cause mortality in HD patients, independent of traditional risk factors. These data may have important implications for better understanding the pathogenesis of the increased mortality in this population.

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Introduction
Cardiovascular (CV) disease (CVD) is the leading cause of mortality in hemodialysis (HD) patients [1]. The very high CV mortality and morbidity rates in this population are only partially explained by the high prevalence of traditional CV risk factors [2], which are classically related to atherosclerosis. The vascular changes observed in chronic kidney disease (CKD) patients consist not only in atherosclerosis, but also in arteriosclerosis associated with both medial and intimal vascular calcification [3]. The degree of arterial stiffening and the extent of calcification are closely related [4], and both of these variables are strong and independent prognostic markers of all-cause and CV mortality in patients on HD [5, 6].

In particular, over the last few years, different authors have focalized their own research on investigating the role of genetic polymorphism of different proteins as a negative prognostic risk factor for all-cause mortality in HD patients, independent of traditional risk factors [7, 8]. These data may have important implications for better understanding the pathogenesis of the increased mortality in this population.
Vascular Calcification in Dialysis: Which Is the ‘Guilty’ Protein?

A mounting number of vascular calcification risk factors are involved in the dialysis population: age, gender, dialysis vintage, inflammation, mineral metabolism abnormalities, and diabetes [9]. In addition to these well-known features of CKD patients, in the last decade new pathogenetic tools are emerging in the nephrology community to better understand vascular mineralization. In fact, although the calcification process has not been completely elucidated, it is now clear that it does not merely consist in passive deposition of calcium-phosphate crystals; on the contrary, it is a well-organized process, involving cell activity and specific protein synthesis [9].

Accordingly, a large amount of proteins are now certified for their capacity of inducing or inhibiting the process of extra-skeletal calcification, and their potential role as ‘protective’ or ‘noxious’ proteins associated with vascular calcification in CKD needs to be clarified. Analyzing those substances recognized as regulatory key factors in inducing vascular calcification in uremic conditions, matrix metalloproteinases (MMPs) have been increasingly implicated in connective tissue remodeling during atherogenesis, contributing to the enlargement and instability of atherosclerotic plaque [10]. Recently, the association of MMP1 and MMP3 polymorphisms with all-cause mortality risk in HD patients has been reported [11]. On the other hand, vascular calcification inhibitors, such as fetuin-A (α2-Heremans-Schmid glycoprotein, AHSG) and the matrix GLA protein (MGP), have been considered in their gene polymorphism modification in HD patients [12, 13]. In fact, while fetuin-A is considered an important circulating inhibitory protein involved in vascular calcification, MGP is a potent in situ regulator of this dramatic disease.

Role of Atherosclerosis: MMP Gene Polymorphisms

Recently, we demonstrated that HD patients have a different distribution of MMP1 and MMP3 gene polymorphisms when compared to the normal population [11]. Moreover, HD patients who have combined alterations of gene polymorphisms of the MMP1 and MMP3 appear to have a significantly worse prognosis in terms of mortality [11].

The MMPs are a family of enzymes involved in the biology of extracellular matrix and in atherogenesis. MMP1 and MMP3 participate in the enlargement and instability of atherosclerotic plaque, respectively [14, 15]. The common polymorphisms on MMP1 (2G/2G) and MMP3 (6A/6A) gene promoters have been related to increased coronary artery calcification and to carotid artery stenosis [16].

Accelerated atherosclerosis and CVD have been shown to be associated with the MMP3 gene 5A/6A polymorphism. MMP3 has proteolytic activity on different extracellular matrix proteins [17, 18] and can activate other MMPs [18]. Therefore, MMP3 has an important role in vascular and cardiac matrix remodeling [18]. Interestingly, Humphries et al. [19] have shown that subjects with the 6A/6A genotype have a higher rate of coronary atherosclerotic lesion growth compared with individuals with the 5A/5A or 5A/6A genotype.

Vascular calcification increases the risk of CV events [9]. MMP3 expression is colocalized with calcium deposition in atherosclerotic lesions [20]. In an autopsy study of men who died of cardiac disease or other causes, Pollanen et al. [21] have found that patients of the 5A/5A or 5A/6A genotype had more calcification in atherosclerotic lesions than subjects of the 6A/6A genotype. Furthermore, individuals of the 6A/6A genotype more likely have advanced carotid atherosclerosis resulting in significant carotid stenosis. In a study of patients with coronary atherosclerosis and controls with no evidence of the disease, Ghilardi et al. [16] showed that the frequency of the 6A/6A genotype was higher in the case group than in the control group, and that among the cases, carriers of the 6A/6A genotype had a higher degree of carotid stenosis. The major constituents of atherosclerotic lesions are matrix proteins (collagen, proteoglycans, elastin, etc.), smooth muscle cells, macrophages, and lipids [22]. Since MMP3 is considered to play an important role in the degradation of matrix proteins in atherosclerotic lesions, and since MMP3 expression in vascular tissues is higher in individuals carrying the 5A allele than in individuals of the 6A/6A genotype, a possible explanation for our findings that the 6A/6A genotype is associated with greater mortality risk is that HD patients with the low MMP3 expression 6A/6A genotype are prone to developing atherosclerotic plaques that are rich in matrix proteins and hence relatively large and stable, whereas individuals with the high MMP3 expression 5A/5A or 5A/6A genotype are predisposed to developing atherosclerotic plaques which have less matrix proteins and hence are smaller but prone to rupture.

Furthermore, in a recent study, Lehrke et al. [23] observed 260 patients with typical or atypical chest pain who underwent dual-source CT coronary angiography...
for exclusion of coronary artery stenosis. In a multivariable regression analysis, serum MMP1 levels were associated with calcified plaque burden, while no association was found between serum MMP9 levels and total plaque burden or plaque morphology.

**Role of Inflammation: Fetuin-A Gene Polymorphisms**

Fetuin-A, also known as AHSG, is an abundant serum protein of the cystatin superfamily of cysteine protease inhibitors synthesized by the liver and found throughout the body in the extracellular space. Even if AHSG physiological roles are still under investigation, numerous reports have supported evidence that fetuin-A is a multifunctional protein. It is a negative, acute-phase 62-kDa glycoprotein that is able to prevent ectopic calcification [24].

A considerable body of evidence indicates that deficiency of fetuin-A may contribute to vascular calcification in CKD: massive ectopic calcification occurs in fetuin-A knockout mice receiving a diet with a high content of calcium and vitamin D [24]; fetuin-A serum levels are significantly lower in CKD patients with calciphylaxis compared to other CKD subjects [25]; the inability of human uremic plasma to inhibit the precipitation of calcium and phosphorus is corrected by the addition of fetuin-A, which accounts for more than 50% of the precipitation inhibitory effect of serum [24].

Furthermore, HD patients are affected by a chronic inflammatory state, represented by lower levels of fetuin-A. Chronic inflammation is also associated with a higher risk of CVD in HD. Recently, Ketteler et al. [26] have shown that in HD patients fetuin-A is an independent predictor of all-cause and CV mortality. Moreover, we have recently shown the independent and significant association between reduced serum fetuin-A levels and multi-site vascular calcification in HD patients, investigated with an ultrasound technique [27].

Indeed, in a recent study, Stenvinkel et al. [28] demonstrated that CKD patients with elevated inflammatory markers and with AHSG T256S allele had lower serum fetuin-A levels and higher all-cause and CV mortality rates.

We performed a similar study in Italian HD patients to assess the relationship between serum fetuin-A levels and its gene (AHSG) polymorphisms [12]. This study suggests that this group of Italian HD patients have a similar distribution of AHSG gene polymorphism as compared to the normal population. In contrast to previous reports [28], this study suggests that CKD patients that receive HD treatment have a reduction in serum fetuin-A levels that is not associated with alteration on the distribution of AHSG T256S polymorphisms. Consequently, altered polymorphism of the fetuin-A gene does not appear to be a negative prognostic factor for the progression to CVD in this population.

Recently, different authors have investigated the distribution of AHSG gene polymorphism in dialysis patients [28], in subjects with subclinical atherosclerosis [29], and in Alzheimer's disease patients [30]. Stenvinkel et al. [28] recently reported significantly lower serum fetuin-A levels in Swedish dialysis patients with the AHSG T256S allele, in association with an increased CV and all-cause mortality rates. Indeed, other recent studies demonstrated that serum fetuin-A levels are linked to inflammation in early stages of renal insufficiency [31], in dialysis population [27], and in renal transplant patients [32] independently by AHSG gene polymorphism distribution.

Probably, the reason that some recent clinical results are different from our findings may be explained by the differences in races. It should be borne in mind that numerous factors contribute towards the marked arterial calcifi-
cation observed in CKD patients: all the ‘classic’ risk factors for atherosclerosis plus ‘uremia-associated’ risk factors, such as duration of dialysis, uremic toxins, inflammation, increased serum levels of phosphate, calcium-phosphate product and PTH. However, the importance of fetuin-A in arterial calcification has been demonstrated as an important protective factor against arterial calcification, even if its definitive role remains to be elucidated.

Role of in situ Vascular Calcification Inhibition: MGP Gene Polymorphisms

MGP is a vitamin K-dependent extracellular matrix protein with a molecular weight of 10 kDa. MGP is present in cartilage, bone matrix and the arterial wall, where it inhibits the deposition of calcium phosphate. MGP knockout mice are normal at birth, but rapidly develop severe and arterial calcifications and almost invariably die of rupture of the aorta within 6–8 weeks [33]. Moreover, MGP is highly expressed in human atheromatous plaques [34].

The most common MGP gene polymorphisms identified are MGP-7 and MGP-138 isoforms [35]. Both of these polymorphisms have an important impact on in vitro promoter activity when they are transfected into vascular smooth muscle cells. In particular, the MGP-138C variant is associated with higher MGP serum levels (+30%) [36]. In clinical studies, MGP-7 AA homozygotes have been shown to have a significantly higher risk of myocardial infarction in a low-risk male population (OR 3.82) and to exhibit femoral artery calcification in the presence of femoral atherosclerotic plaque more frequently than subjects with other genotypes (p < 0.025) [35]. In a recent clinical study in 204 patients with end-stage renal disease (ESRD), MGP-7 AA homozygosity was found to be associated with a higher level of left ventricular hypertrophy (p < 0.05) and accelerated progress of atherosclerosis in 1 year, based on carotid artery ultrasound assessments, than other genotypes [36]; MGP-138CC genotype was associated with an increase in CV and total mortality in ESRD patients [37].

Recently, we investigated the distribution of MGP-7 and MGP-138 genotypes in CKD and HD patients, followed up for 12 months, to evaluate the relationship between genotype and CV mortality [13]. We found that CKD and HD patients have a different distribution of MGP gene polymorphism when compared to the normal population. Consequently, altered polymorphism of the MGP gene appears to be a negative prognostic factor in terms of CV mortality in HD patients. Wang et al. [38] have reported significantly more severe left ventricular hypertrophy and an accelerated progress of atherosclerosis, defined as the change in calculated carotid intima media area, in 1 year in 58 ESRD patients. Thus, our findings contribute towards the body of evidence suggesting that –7A allele carriers with CKD have an increased risk of calcification and related CV events. The findings related to –138 polymorphisms are consistent with previous in vitro data suggesting the –138 T variant is 4-fold less active than the –138C variant [37].

Conclusions

‘Classical’ CV risk factors are common in dialysis patients, but they cannot explain alone the dramatic high prevalence of CV morbidity and mortality. The role of various ‘non-classical’ CV risk factors, such as vascular calcification, inflammation, and oxidative stress, is under investigation. In addition, genetic factors such as gene polymorphisms of specific proteins may significantly contribute to the prevalence of vascular calcification in this group of HD patients.

During the last decade, there has been a great interest in vascular calcification as a nontraditional risk factor for CVD in CKD and HD patients. To better elucidate the respective roles of gene polymorphisms of proteins involved in production (such as MMP1 and MMP3) or inhibition (such as fetuin-A and MGP) of vascular calcification, prospective studies are warranted. This will help the nephrology community to identify the role of gene polymorphism in determining CV calcification in HD patients in the attempt to improve our understanding of the pathogenesis of increased risk of ectopic calcification and CV events in patients with renal failure.

Acknowledgements

This research was supported in part by an investigator supported trial grant from Shire Pharmaceuticals (M.C., D.B.) and in part by Ingenious Hypercare LSHM-CT-2006-037093 (D.C.), by HYPERGENES grant HEALTH-F4-2007-201550 (D.C.).