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# 1267 HSP70-2 polymorphism as a risk factor for carotid plaque rupture and cerebral ischaemia in old type 2 diabetes-atherosclerotic patients

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**Abstract**

Patients with type 2 diabetes mellitus (NIDDM) are at risk for macrovascular disease complications, such as myocardial infarction (MI) or stroke from plaque rupture.

Cytokines play a key role in plaque vulnerability. IFN-γ inhibits collagen synthesis thereby affecting plaque stability. High IL-6, TNF-α, and dyslipidemia are risk factors for thrombosis. Abnormal increments of HSP70 in atherosclerotic plaques might lead to plaque instability and rupture caused by chronic inflammation, which up-regulates the expression of pro-inflammatory cytokines (IL-6 and TNF-α) in human monocytes. Studies of a polymorphic *Pst*I site lying in the coding region at position 1267 of the HSP70-2 gene have shown that the BB genotype is associated with NIDDM. We screened 60 old NIDDM patients with carotid stenosis and 107 old healthy controls for 1267 HSP70-2 polymorphism in order to establish if an association with plaque frailty exists. Different genotypic distributions were observed between patients and healthy controls. An increased relative risk was associated with the B allele ($p = 0.0107$; odds ratio = 1.861). HSP70-2, IL-6, IFN-γ, TNF-α gene expressions within the plaques and serum levels of triglyceride, total cholesterol and LDL cholesterol were tested from patients stratified according to their B+ (AB and BB) and B− (AA) genotypes. Plaque morphology (soft or fibrous-calcified) and the incidence of cerebral ischaemia were also assessed.

B+ patients showed increased HSP70-2, IL-6, IFN-γ, TNF-α and dyslipidemia as compared to B− carriers. The frequency of soft plaques increased in B+ in comparison to B− patients (67% versus 13%; odds ratio 13.0, $p = 0.0006$). A higher frequency of cerebral ischaemia (ictus or transient ischaemic attack (TIA)) was present in B+ than in B− genotype (53% versus 20%; odds ratio 4.57, $p < 0.05$) Hence, 1267 HSP70-2 polymorphism may be of use in identifying B+ NIDDM patients at risk for carotid plaque rupture and cerebral ischaemia.


*Keywords:* HSP70-2; Polymorphism; Cytokines; Inflammation; Atherosclerosis; Type 2 diabetes mellitus

## 1. Introduction

Stroke represents a major cause of disability and death in the elderly. Carotid artery stenosis is an important risk factor for stroke especially in elderly men with diabetes mellitus or who present other risk factors (hypertension, smoking, dyslipidemia) (Thompson et al., 2003).

Type 2 diabetes mellitus patients (NIDDM), frequently, have coexistent dyslipidemia, hypertension and obesity. Moreover, NIDDM patients are at risk for microvascular and macrovascular disease complications, such as myocardial infarction (MI), stroke (Stolar and Chilton, 2003), which are the major causes of mortality in these patients (Vinik and Flemmer, 2002).

Macrovascular events, (MI and stroke), occur earlier in NIDDM patients than in non-diabetics and the underlying pathologies are often more common and severe. Indeed,

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more fibrosis and more thrombotic complications are observed in carotid atherectomies from NIDDM patients (Sommeijer et al., 2004). Endothelial dysfunction, hypercoagulability, changes in blood flow, and platelet abnormalities, contribute to the early evolution and progression of atherosclerosis in NIDDM (Vinik and Flemmer, 2002). Systemic inflammation plays a key role both in type 2 diabetes and atherosclerosis (Watson et al., 2003; Ito and Ikeda, 2003).

Recruitment of macrophages, involved in vascular intima lesions (Libby et al., 2002), is mediated by pro-inflammatory cytokines (IL-6 and TNF-α). Once resident in the arterial wall, the macrophages contribute and perpetuate a local inflammatory response through increased production of inflammatory cytokines, which in turn stimulate macrophages as well as vascular endothelial and smooth muscular cells (SMCs) (Ross, 1999). Activated macrophages release proteolytic enzymes able to degrade collagen fibres, thus thinning and weakening the fibrous cap of the plaque. As a consequence, plaques become unstable and susceptible to rupture (Libby et al., 1996). IFN-γ is involved in this process halting collagen synthesis by SMCs, limiting its capacity to renew the collagen fibres of the plaque (Libby and Simon, 2001; Libby et al., 1996). Plaque instability and eventual thrombosis are also related to IL-6, TNF-α production (Koukkunen et al., 2001; Libby and Simon, 2001). Increased IL-6 and TNF-α are observed in NIDDM (Kado et al., 1999; Spranger et al., 2003). It has been suggested that IL-6 participates in insulin resistance, decreasing glucose uptake (Bastard et al., 2002).

It has also been suggested that the heat shock protein HSP70 plays a relevant role in the development of both diabetic metabolic disturbances and their complications (Koranyi et al., 2004). In particular, a higher HSP70 production may improve insulin resistance (Koranyi et al., 2004), which is a key factor in the pathogenesis of NIDDM (Watson et al., 2003).

In general, high HSP70 levels protect cardiac cells from damage by stressful stimuli by binding to denatured or inappropriately folded proteins (Snoeckx et al., 2001; Xu, 2002). Highly expressed HSP70 was also found in cells localised within atherosclerotic plaques, including macrophages (Berberian et al., 1990). The specific effect of HSP70 expression within the plaques has yet to be fully elucidated. Insufficient HSP70 accumulation in SMCs leads to SMC death allowing resident macrophages to degrade and destabilise the matrix with subsequent plaque instability and rupture (Johnson et al., 1995). On the other hand, the intensity of the HSP70 expression correlates positively with the severity of atherosclerosis, as shown in normocholesterolemic rabbits immunised with HSPs (Xu et al., 1992). Moreover, the increased presence of protein binding molecules, such as HSPs, during chronic stress adversely influences protein homeostasis and a variety of intracellular functions (Shi et al., 1998). Consequently, abnormal increases in HSP70 in atherosclerotic plaques may not be protective and even be involved in plaque instability and rupture mechanisms. Genetic mechanisms may be involved in this possible harmful role of HSP70 in atherosclerotic plaques, especially in the advanced phase. A coding polymorphism, an A (A allele) to G (B Allele) transition, identified at nucleotide 1267 of HSP70-2 resulting in a silent change in the coding region, has been associated with NIDDM (Zouari Bouassida et al., 2004).

To investigate the role played by HSP70, we evaluated the genotypic frequency of 1267 HSP70-2 polymorphism in patients with carotid atherosclerotic stenosis and type 2 diabetes mellitus. The relationship among plaque morphology (soft or fibrous-calcified) clinical outcome (transient ischaemic attack (TIA) or stroke), as well as metabolic risk factors (serum triglycerides, total cholesterol and LDL cholesterol) (Stemerman, 2000; Laloux et al., 2004) and HSP70-2 polymorphisms was assessed.

We also analysed the effect of 1267 HSP70-2 genotypes on pro-inflammatory cytokine mRNA expression in atherosclerotic plaques.

## 2. Materials and methods

### 2.1. Patients and controls

Patients and controls were born in Central Italy and still living in the same area at the time of enrolment.

Sixty NIDDM patients (42 males and 18 females, mean age 70.4 ± 7.7 years) with bilateral or unilateral carotid stenosis who had been admitted for an endarterectomy were enrolled for the study. In accordance with the World Health Organization (WHO) 1980 guidelines, patients were classified as diabetic if they were taking insulin, oral antidiabetic medication or if they had a fasting glucose concentration >140 mg/dl (>7.8 mmol/l) or a 2-h glucose concentration >200 mg/dl (>11.1 mmol/l) after a 75-g oral glucose tolerance test. In this study, our patients and the control group had fasting glucose concentrations ≥140 mg/dl, and ≤110 mg/dl (American Diabetes Association), respectively.

The number of NIDDM patients with a characteristic atherosclerosis symptomatology at enrolment is summarised as follows and reported in Table 1: 48/60 patients had hypertension (systolic pressure >140 mm Hg; diastolic pressure >90 mm Hg or were put on hypertension medication). Thirty-three subjects had cardiovascular diseases (stable angina). Only 12/60 patients were current smokers.

27/60 patients had suffered a TIA or stroke. Twenty-three patients presented with unilateral carotid stenosis while 37 had bilateral carotid stenosis, considered severe if >80% and necessitating surgical removal of the plaques (Steiger, 1995).

The control group for genotype testing consisted of 107 individuals living at home matched by age and gender (75