THE ROLE OF hsp70-2, hsp70-hom HEAT SHOCK PROTEINS ON MULTIPLE SCLEROSIS RISK AND SEVERITY

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Introduction

Oxidative stress is involved in MS pathogenesis and progression by direct and indirect mechanisms of action [1], which also encompass the heat shock proteins 70 (hsp70s) family [2].

Intracellular hsp70s act as chaperones and anti-apoptotic proteins[3]. Extracellular hsp70s process and present antigens, promoting the activation of immune system [4]. Polymorphisms leading to either quantitative or qualitative change in hsp70 expression likely affect both the hsp70 cyto-protective and/or immune-modulatory effects.

Among the several proteins included in hsp70s family, the two major stress-inducible members (i.e. the Hsp70-1 and Hsp70-2) are encoded by HSPA1A and HSPA1B gene respectively, and the constitutively expressed non-inducible protein (i.e. Hsp70-hom) is encoded by HSPA1L gene. These three genes are located on chromosome 6 (6p21.3)[5], within the human leukocyte antigen (HLA) class III region.

While polymorphisms within HSPA1A exons are silent, we recently demonstrated that +1267 A/G HSPA1B (rs1061581) polymorphism is associated with an increased MS risk and that MS patients with GG or GA genotype display a significant reduction of hsp70-2 expression compared to patients with AA genotype[6]. Polymorphisms in the HSPA1L gene are mainly located in the region coding for the substrate-binding domain.

Aims

To investigate the association of MS with HSP70-hom polymorphism and haplotypes including another HSP70 gene known as HSP70-2 and
To evaluate the relationship between Hsp70-hom protein expression level and MS severity, using peripheral blood mononuclear cells (PBMC) from MS patients and healthy donors.

Methods

- We included 195 MS Caucasian patients from the MS Centre of the National Neurological Institute "C. Mondino" (Pavia, Italy) and 439 Caucasian Healthy Controls.
- HSP70-hom polymorphism was studied with PCR-RFLP. Western blot analyses were performed to quantify the Hsp70-hom protein expression levels in PBMC.
- We performed additive and genotypic unconditional logistic regression analyses, sex and age adjusted, to assess the association between the HSP70 polymorphisms and MS risk.
- The quantitative expression of the protein was tested using linear regression models, sex and age adjusted.
- We also tested whether the overall HSP70-2 and HSP70-hom haplotype variation influences MS presence and severity (quantified by multiple sclerosis severity score, MSSS [7], performing omnibus and conditional LR-based tests (*haplotype-based association testing tool, plink 1.07*).

Results

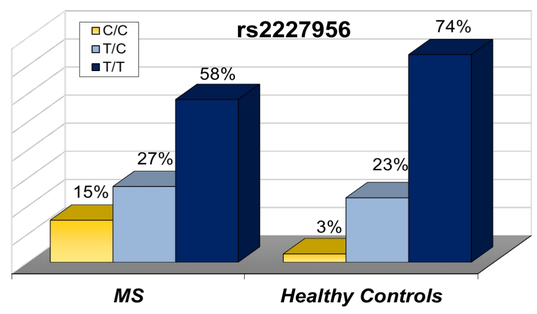
- The minor allele C of rs2227956 (HSP70-hom) conferred a risk against the MS (OR=2.13, P<0.0001) and having the CC genotype compared to TT genotype increased the risk of the MS almost 7 times (OR=6.71, P<0.0001)
- MSSS score was increased of 1.21 (P=0.017) among CC carriers (Figure 2) w.r.t. TT carriers
- The frequency of HSP70-2 and HSP70-hom GC haplotype (risk alleles combination) in MS patients respect to controls was significantly increased (OR: 3.489, p-value <0.0001) Intriguingly, HSP70-hom showed independent effect on MS risk after adjusting for the effect of the overall haplotype configuration (conditional LR test p-value<0.0001)
- The Hsp70-hom protein expression in PBMC of 47 MS patients and 37 controls were not significantly different by HSP70-hom genotype
- We explored the possible relationship between HSP70-hom protein low expression and mild MS : HSP70-hom protein total amount in low-severity patients (MSSS score <3, 1069±1035, median: 676 arbitrary units) was significantly lower vs. high-severity patients (MSSS score ≥3, 1730±1536, median: 1134 arbitrary units), Wilcoxon rank-sum test, P=0.038 (Figure 4).

Discussion and Conclusions

We observed an increased risk of MS in HSP70-hom rs2227956 C carriers and the reduced expression of hsp70-hom in MS patients with a mild form of the disease

HSP70-hom plays a more relevant role in promoting a pro-inflammatory immune system activation and an effective T cell response against the myelin antigens compared with its role in protecting CNS cells from inflammatory injury

However, the underlying mechanisms involved in this unfavourable outcome are not clear yet and further studies are required to clarify the exact roles of hsp70-hom and its possible applications as biomarker and/or as target therapy in MS



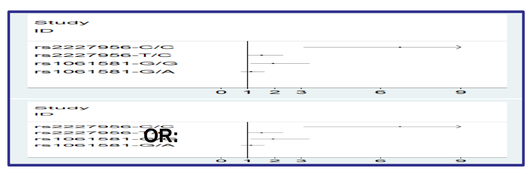
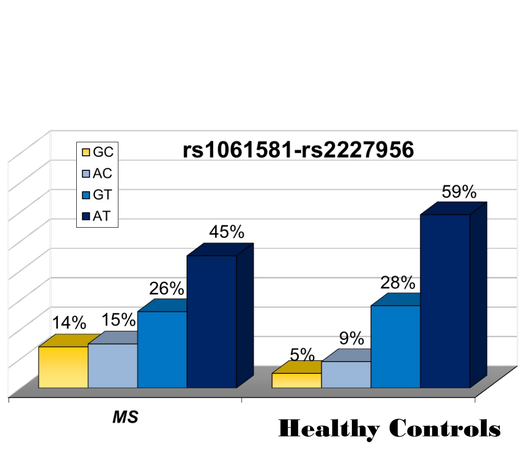


FIGURE 1. HSP70-hom polymorphism frequency distribution among MS cases and controls and MS estimates of risk among mutated genotypes



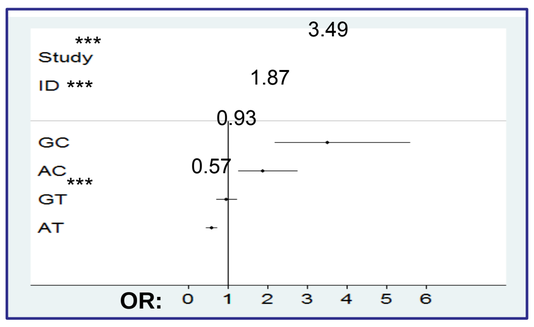


FIGURE 2. HSP70-2/HSP70-hom haplotypes frequency distribution among MS cases and controls and MS estimates of risk

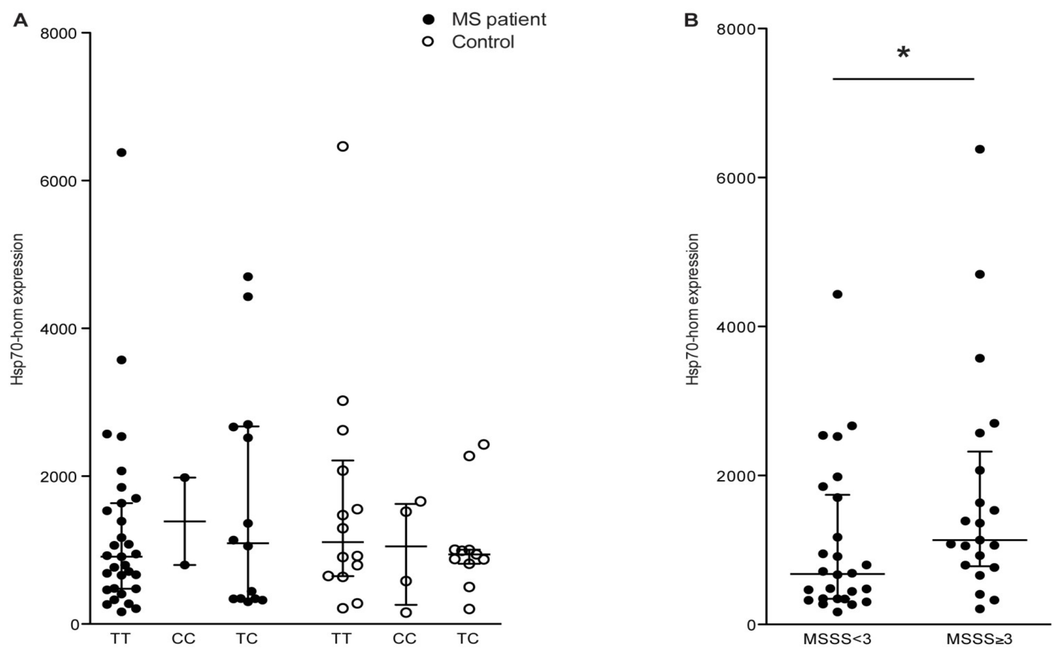


Figure 3

FIGURE 3. HSP70-hom expression by genotype and MS impact

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