ANALYSIS OF HEAT SHOCK PROTEIN POLYMORPHISMS IN A LARGE FAMILY WITH AUTOIMMUNE THYROID DISEASES

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RESUME

La maladie de Basedow et la thyroïdite de Hashimoto sont des maladies autoimmunes thyroïdiennes dans lesquelles la contribution génétique est complexe. L'objectif de ce travail était d'analyser l'influence des polymorphismes des gènes hsp sur la susceptibilité aux maladies autoimmunes thyroïdiennes (MAIT). Les polymorphismes des gènes hsp70-2 et hsp70-hom étaient analysés par PCR-RFLP utilisant les enzymes de restriction PstI, et NcoI, respectivement, sur 40 patients atteints de MAIT et 38 sujets apparentés sains appartenant à une large famille consanguine appelée Akr. Le test de déséquilibre de transmission (TDT) a été appliqué sur des familles nucléaires, déduites de la famille Akr, avec au moins un parent hétérozygote pour chaque polymorphisme étudié. Les valeurs de x^2 trouvées pour hsp70-2 et hsp70-hom étaient de $x^2_{1ddl} = 0.52, p > 0.05$ et $x^2_{1ddl} = 2.77, p > 0.05$, respectivement. Nos résultats ont montré l'absence d'association entre ces deux polymorphismes et les MAIT dans la famille Akr.

Mots clés: *Association génétique, maladies autoimmunes thyroïdiennes, hsp70, déséquilibre de transmission*

ABSTRACT

Graves disease and Hashimoto's thyroiditis are autoimmune thyroid diseases (AITD) in which the genetic contribution is complex. The purpose of this work was to analyze the influence of hsp70 gene polymorphisms on the susceptibility to AITD. The hsp70-2 and hsp70-hom polymorphism was analyzed, by PCR-RFLP using PstI and NcoI enzymes, respectively, in 40 patients affected with AITD and 38 related healthy individuals belonging to a large consanguineous family named Akr. The transmission disequilibrium test (TDT) was applied on nuclear families, deduced from the Akr pedigree, with at least one heterozygous parent for each studied polymorphism. The corresponding x^2 values for hsp 70-2 and hsp 70-hom were, respectively, of 0.52, $p > 0.05$ and 2.77, $p > 0.05$. Our data indicated lack of association between these hsp polymorphisms and AITD in this large family.

Keywords: *Genetic association, thyroid autoimmune disorders, hsp 70, transmission disequilibrium*

Three genes encoding members of the hsp 70 family are located in the class III region of the human MHC. They have been defined as hsp 70-1, hsp 70-2 and hsp 70-hom. Polymorphism within each of these genes has been previously described [1, 2]. Polymorphism at a PstI site within hsp 70-2 is silent, whereas hsp 70-hom polymorphism corresponds to a Met-Thr amino acid substitution at residue 493. Genes encoding the hsps family are candidate loci in AITD susceptibility based upon: i) presence of hsp 70 antibodies in patients affected with autoimmune and non-autoimmune thyroid diseases [3] expression of HLA-DR and hsp 70 in eye muscle tissue in thyroid-associated ophthalmopathy [4] genetic association, previously described, between hsp 70, C4 complement factor and Graves disease [5].

To determine if defined polymorphisms in hsp 70 genes are associated with AITD susceptibility, genotyping by PCR-RFLP for polymorphic differences in hsp 70-2 at nucleotide 1267 and in hsp 70-hom at nucleotide 2437 was conducted in 40 patients affected with AITD and 38 related healthy individuals belonging to a large multiplex family named Akr 6. To evaluate the possible involvement of the hsp70 genes in AITD pathogenesis, we have applied the Transmission Disequilibrium Test (TDT), which compares transmission of the allele from heterozygous parent to affected offspring 7. This test has the advantage to detect even genes of modest effect in complex diseases and, is not altered by population statification. To apply the TDT, we have subdivided the Akr pedigree into 7 nuclear informative families with at least one heterozygous parent, for the studied polymorphism (figure 1). We have examined transmission of the D and A alleles of hsp70-2 and hsp70-hom polymorphisms respectively. The corresponding x^2

values were x^2 1df=0.52, p> 0.05 and x^2 1df=2.77, p> 0.05 respectively. These values were in agreement with lack of association between these two polymorphisms and AITD in Akr family. These results did not support data reported in previous studies which have shown hsp70 gene polymorphism involvement in Graves disease pathogenesis [5] and that of other autoimmune disorders such as celiac disease [8] type I diabetes mellitus [9, 10] and systemic lupus erythematosus [11] suggesting the genetic heterogeneity of these complex diseases. A recent study, undertaken on unrelated patients and ethnically matched control individuals from the mexicano mestizo population, has shown lack of association between hsp70-2 gene polymorphism and systemic lupus erythematosus [12].

Although genotyping by PCR-RFLP for polymorphic differences in hsp 70-02 and hsp 70-hom did not support a role for hsp 70 gene polymorphisms in AITD susceptibility, it remained likely that these loci may still play a role in disease initiation which is dependent on the currently identified polymorphisms.

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