

Heat-Shock Protein Gene Is Not Associated with Type-1 Diabetes in African Americans

Noureddine Berka, PhD; Gail N. Bland, MD; El Hajja Erabhaoui, BS; and Georgia M. Dunston, PhD

Financial support: This work was supported in part by National Institutes of Health grant #RR03048.

The polymorphism at the heat-shock protein gene was reported to be associated with type-1 diabetes in Caucasians but not in the Japanese. We report in this study the association between HSP70-1 alleles and type-1 diabetes in 30 unrelated African-American patients and 96 ethnically matched controls from the Washington, DC area. The polymorphic variation (A-C transversion) at position -110 in the HSP70-1 promoter region was amplified using the polymerase chain reaction. No significant differences between patients and controls were detected. These data suggest that in African Americans, HSP70-1 polymorphism is not associated with type-1 diabetes and is similar to findings in Japanese patients. The difference between results from this study and that of Caucasians may be due to population differences in genetic polymorphism or to linkage disequilibrium of HSP70-1 with human leukocyte antigen class-II alleles associated with type-1 diabetes susceptibility genes.

Key words: human leukocyte antigen ■ diabetes ■ African Americans

© 2007. From the Tissue Typing Laboratory, Calgary Laboratory Services, Alberta, Canada (Berka; Erabhaoui, research assistant); and the Department of Pediatrics (Berka; Bland, pediatric endocrinologist) and Clinical Immunogenetics Laboratory (Berka), Howard University Hospital, and National Human Genome Center, Howard University (Dunston, professor of microbiology), Washington, DC. Send correspondence and reprint requests for *J Natl Med Assoc.* 2007;99:715-717 to: Dr. Noureddine Berka, Tissue Typing Laboratory, Calgary Laboratory Services, 9, 3535 Research Road NW, Calgary, Alberta, Canada T2L 2K8; phone: (403) 770-3655; fax: (403) 770-3738; e-mail: noureddine.berka@cls.ab.ca

In the early 1970s, increased frequencies of certain human leukocyte antigens (HLAs) in unrelated type-1 diabetes individuals, compared with the general population, pointed to the HLA region on chromosome 6p as the location for type-1 diabetes susceptibility genes.¹ The increased sharing of HLA haplotypes in pairs of siblings affected with type-1 diabetes added to the evidence of the importance of the HLA region. Since then, other candidate genes were found to be associated with susceptibility to type-1 diabetes.²⁻¹⁴ The genes for the heat-shock pro-

teins (HSPs)—HSP70—that map within the HLA class-III region are also suspected to have a role in conferring genetic susceptibility or resistance to type-1 diabetes.¹⁵ The HSP70 genes are located between the genes encoding for the complement system and those encoding for the tumor necrosis factor.^{16,17} The product of these genes belongs to a major group of HSPs that are constitutively expressed primarily in the cytosol and mitochondria, and the members of their family are equipped with two major functional domains, including a C-terminal region that binds peptides and denatured proteins, and an N-terminal ATPase domain that controls the opening and closing of the peptide-binding domain.¹⁸

HSP70 was found to enhance antigen-specific proliferation of human CD4+ memory T cells and to increase the immunogenicity of presented peptides.¹⁹ At low doses of antigen, stimulation with HSP70-peptide complexes was found to be far superior to stimulation with peptide alone. The complex formation of the antigenic peptide with HSP70 was found to be absolutely required in order to elicit an antigen-specific amplification. In this situation, the induction of HSP70 decreases the threshold of activation of human CD4+ T cells by the antigenic peptide. The increased reactivity of T cells against a peptide chaperoned by HSP indicates a putative involvement of HSP in the pathogenesis of autoimmune diseases. Thus, the induction of HSP by certain stress factors, such as infection, would facilitate an immune response to a given peptide of the self that would not be immunogenic under conditions where HSP is not available.

On the other hand, HSPs have been shown to play a protective role in islets exposed to cytokines. Expression profiling studies have demonstrated that HSP70 is one of these protective molecules.²⁰ HSP70 has also been suggested to protect mitochondrial function against oxidative injury, as heat-shock-induced HSP70 prevented H₂O₂-induced mitochondrial damage.²¹ Another mechanism by which HSP70 can play a protective role is via cellular protection by interfering with apoptosis induction^{22,23} possibly by inhibition of JNK and p38.^{24,25}

The product of certain HSP genes are autoantigen, which have the potential to trigger immunoregulatory

pathways that can suppress immune responses that occur in human inflammatory diseases, such as type-1 diabetes. Multiple studies have presented data supporting the possibility that HSPs may serve as endogenous signals for antigen-presenting cells (APC) maturation and may have a role in creating a balance between immune response and tolerance.²⁶ Moreover, studies in the nonobese diabetic mouse model of type-1 diabetes have shown that immunization with mycobacterial HSP60 protects against disease.²⁷ In particular, HSP60-derived peptide p277 was shown to be effective in protecting nonobese diabetic mice against insulinitis, and initial results of clinical trials of therapeutic vaccination with this peptide in patients with type-1 diabetes have indicated the potential of HSPs to be a source of immunoregulatory peptides.

It has also been shown that the p277 could bind human Major Histocompatibility Complex class-II molecules and induces proliferative T-cell responses in patients with type-1 diabetes.²⁸ With this promising data of the role of HSPs in type-1 diabetes and autoimmunity, it is important to analyze the association of HSP70 genes with type-1 diabetes. Previous studies looking at this area of the genome have yielded conflicting results. Caplen and colleagues showed significant association between HSP70 and type-1 diabetes, which was less than the HLA DR/DQ association.¹⁵ Others have failed to show such association.²⁹ In this study, we investigated the association of HSP70-1 with type-1 diabetes among African-American patients and controls. Variation in the 5' flanking region of the HSP70-1 gene was analyzed, using polymerase chain reaction-based amplification and polyacrylamide gel electrophoresis of the DNA fragment generated. The bands of different mobility were interpreted as corresponding to different alleles and were named A (slow) and C (fast). The combinations found in this study were the following: AA, AC and CC (Table 1). The frequencies of the two alleles in the control group were

estimated from 192 chromosomes and were as follows: A=0.23, C=0.76. Similar frequencies were derived from 60 chromosomes of type-1 diabetes individuals (A=0.18 and C=0.82). All three genotype combinations were observed in controls, but only AC and CC were found in type-1 diabetes. The AA genotype was found in 8.3% of the controls and was absent in the type-1 diabetes group. The homozygous CC and the heterozygous AC genotypes were equally distributed in type-1 diabetes and controls. No significant differences were detected between these two groups.

When analyzed independently from the HSP gene HSP70-1, the HLA DR3, DR4 and DR3/DR4 genotypes were all significantly increased in type-1 diabetes (Table 2).

HSP70-1 allele frequencies in this study showed a predominance of the C allele over the A allele. Previous studies in Europeans have shown that the A allele was more frequent than the C allele with respective frequencies of 0.614 and 0.386.³⁰ Casino and colleagues showed no association between HSP70-1 and type-1 diabetes. Others have suggested that HSP70-1 alleles are associated with type-1 diabetes.¹⁵ In Caucasians, Casino's group has reported that DR3 was strongly associated with HSP70-1 polymorphism.³⁰ This strong association shows that the results obtained by Caplen et al. in 1990 are probably due to linkage disequilibrium between HSP70-1 and DR3 in Caucasians. Our data suggest that in African-American patients, HSP70-1 polymorphism is not associated with type-1 diabetes, which is similar to that reported among Japanese individuals.³¹ This difference between results from the African-American and Japanese populations in one hand and that of Caucasians may be due to population differences in genetic polymorphism or to linkage disequilibrium of HSP70-1 with HLA class-II alleles associated with type-1 diabetes susceptibility genes. It is noteworthy to mention that our study only investigated the association of HSP70-1 gene with type-1 diabetes but did not look at the role of

Table 1. HSP70-1 polymorphism at 5' flanking region tested in type-1 diabetes patients and controls

HSP70-1 Genotypes	Type-1 Diabetes N=30 (%)	Controls N=96 (%)	χ^2	P
AA	0 (0)	8 (8.33)	2.67	0.1023
CC	19 (63.33)	59 (61.46)	0.03	0.8535
AC	11 (36.67)	29 (30.21)	0.44	0.5071

Table 2. DR3 and DR4 alleles in type-1 diabetes and controls

HLA-DRB1	Type-1 Diabetes N=70 (%)	Controls N=214 (%)	χ^2	P	RR
DR3/-	7 (10.00)	8 (3.74)	4.13	0.0420	2.8611
DR4/-	2 (2.86)	6 (2.80)	0.00	0.9813	1.0196
DR3/X	23 (32.86)	46 (21.49)	3.70	0.0543	1.7872
DR4/X	12 (17.14)	17 (7.94)	4.87	0.0274	2.3976
DR3/DR4	12 (17.14)	2 (0.93)	29.5	0.00001	21.931
Non-DR3/non-DR4	14 (20.00)	135 (63.08)	39.26	0.00001	0.1463

X: non-DR3, non-DR4

HSP70-2 and HSP70-hom polymorphism also found within the HLA region, although these two genes are closely linked and are likely to have the same association with type-1 diabetes. In addition to the disease association mentioned above, several studies have demonstrated an association between HSP-specific T cells with a long-lasting auto-tolerance that prevent autoimmune diseases via T cells. In a recent review, Raska and Weigl proposed three possible mechanisms to explain this association:³² 1) bystander anti-inflammatory effects of lymphocytes educated in gut-associated lymphoid tissues through interleukin 10 (IL-10) and transforming growth factor beta (TGF- β), 2) low affinity TCR recognition of self HSP peptide leads to suppression of inflammatory T-cell response through B7.2–CTLA-4 interaction, and 3) nonprofessional APC presentation of HSP epitopes without costimulatory molecules drives T cell to anergy. The current study could not confirm the association of HSPs with either susceptibility or resistance to type-1 diabetes. It is important to study the polymorphism of HSP70-2 and HSP70-hom genes together with other HSP genes located in other regions of the genome for possible association with type-1 diabetes in this African-American population and other populations. To achieve good power statistics, a larger sample size is required to allow for stratification of the data to eliminate the strong effect of classical HLA genes. The type-1 diabetes genetics consortium has gathered a large repository of cells and DNA samples that were collected mainly from North America and Europe. There is a need for more diverse population sampling in particular, people of Asian and African descent.

ACKNOWLEDGEMENTS

We thank Aaliah Alim, Georgia Mcintosh, Prabha Chundur and Maher Belmamoun for their technical support.

REFERENCES

1. Singal DP, Blajchman MA. Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. *Diabetes*. 1973;22:429-433.
2. Todd JA, Bain SC. A practical approach to the identification of susceptibility genes for type 1 diabetes. *Diabetes*. 1992;41:1029-1034.
3. Bell GI, Horita S, Karam JH. A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. *Diabetes*. 1984;33:176-183.
4. Field LL, Tobias R, Magnus T. A locus on chromosome 15q26 (IDDM3) produces susceptibility to insulin-dependent diabetes mellitus. *Nat Genet*. 1994;8:189-194.
5. Field LL, Nagtomi J. Linkage analysis: inadequate for detecting susceptibility loci in complex disorders? *Am J Hum Genet*. 1994;55:A347.
6. Hashimoto L, Habita C, Beressi JP, et al. Genetic mapping of susceptibility locus for insulin-dependent diabetes mellitus on chromosome 11q. *Nature*. 1994;371:161-164.
7. Davies JL, Kawaguchi Y, Bennett ST, et al. A genome-wide search for human type 1 diabetes susceptibility genes. *Nature*. 1994;371:130-136.
8. Delepine M, Pociot F, Habita C, et al. Evidence of a non-MHC susceptibility locus in type 1 diabetes linked to HLA on chromosome 6. *Am J Hum Genet*. 1997;60:174-187.
9. Owerbach D, Gabbay KH. Localization of a type 1 diabetes susceptibility locus to the various tandem repeat regions flanking the insulin gene. *Diabetes*. 1993;42:1708-1714.

10. Luo D-F, Bui MM, Muir A, et al. Affected sibpair mapping of a novel susceptibility gene to insulin-dependent diabetes mellitus (IDDM8) on chromosome 6q25-q27. *Am J Hum Genet*. 1995;57:911-919.
11. Field LL, Tobias R, Thomson G, et al. Susceptibility to insulin-dependent diabetes mellitus maps to a locus (IDDM11) on chromosome 14q24.3-q31. *Genomics*. 1996;33:1-8.
12. Nistico L, Buzzetti R, Pritchard LE, et al. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. *Hum Mol Genet*. 1996;5:1075-1080.
13. Awata T, Kurihara S, Iitaka M, et al. Association of CTLA-4 gene A-G polymorphism (IDDM12 Locus) with acute-onset and insulin-dependent IDDM as well as autoimmune thyroid disease (Graves' disease and Hashimoto's thyroiditis) in the Japanese population. *Diabetes*. 1998;47:128-133.
14. Concannon P, Erlich HA, Julier C, et al. Type 1 diabetes genetics consortium. Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. *Diabetes*. 2005;54:2995-3001.
15. Caplen NJ, Patel A, Millward A, et al. Complement C4 and heat shock protein 70 (HSP70) genotypes and type 1 diabetes mellitus. *Immunogenetics*. 1990;32:427-430.
16. Sargent CA, Dunham I, Trowsdale J, et al. Human major histocompatibility complex contains genes for the major heat shock protein HSP70. *Proc Natl Acad Sci USA*. 1989;86(6):1968-1972.
17. Sargent CA, Dunham I, Campbell RD. Identification of multiple HLA class III region associated genes in the human major histocompatibility complex class III region. *EMBO J*. 1989b;8:2305-2312.
18. Bukau B, and Horwich AL. The HSP70 and HSP60 chaperone machines. *Cell*. 1998;92:351-366.
19. Haug M, Dannecker L, Schepp CP, et al. The heat shock protein HSP70 enhances antigen-specific proliferation of human CD4+ memory T cells. *Eur J Immunol*. 2005;35:3163-3172.
20. Larsen PM, Fey SJ, Larsen MR, et al. Proteome analysis of interleukin-1 β -induced changes in protein expression in rat islets of Langerhans. *Diabetes*. 2001;50:5:1056-63.
21. Polla BS, Kantengwa S, François D, et al. Mitochondria are selective targets for the protective effects of heat shock against oxidative injury. Proceedings of the National Academy of Sciences of the United States of America. 1996;93;13:6458-6463.
22. Buzzard K A, Giaccia AJ, Killender M, et al. Heat shock protein 72 modulates pathways of stress-induced apoptosis. *J Biol Chem*. 1998;273;27:17147-17153.
23. Jaattela M, Wissing D, Kokholm K, et al. Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases. *EMBO J*. 1998;17;21:6124-6134.
24. Gabai VL, Meriin AB, Mosser DD, et al. Hsp70 prevents activation of stress kinases. A novel pathway of cellular thermotolerance. *J Biol Chem*. 1997;272;29:18033-18037.
25. Mosser DD, Caron AW, Bourget L, et al. Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. *Mol Cell Biol*. 1997;17;9:5317-5327.
26. Kaufman SH. Heat shock proteins and the immune response. *Immunol Today*. 1990;11:129-136.
27. Brugman S, Klatter FA, Visser J, et al. Neonatal oral administration of DiaPep277, combined with hydrolysed casein diet, protects against type 1 diabetes in BB-DP rats. An experimental study. *Diabetologia*. 2004;47:1331-1333.
28. Raz I, Elias D, Avron A, et al. Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet*. 2001;358:1749-1753.
29. Pugliese A, Awdeh ZL, Galluzzo A, et al. No independent association between HSP70 gene polymorphism and IDDM. *Diabetes*. 1992;41:788-791.
30. Cascino I, Sorrentino R, and Tosi, R. Strong genetic association between HLA-DR3 and polymorphic variation in the regulatory region of the HSP70-1 gene. *Immunogenetics*. 1993;37:177-182.
31. Kawaguchi Y, Ikegami H, Fukuda M, et al. Polymorphism of HSP70 gene is not associated with type 1 (insulin-dependent) diabetes mellitus in Japanese. *Diabetes Res Clin Pract*. 1993;21:103-107.
32. Raska M, Weigl E. Heat shock proteins in autoimmune diseases. *Biomed Pap Med. Fac Univ Palacky Olomouc, Czech Repub*. 2005;149:243-249. ■