Heat-Shock Protein 70 Genes and Human Longevity

A View from Denmark

RIPUDAMAN SINGH,[a] STEEN KØLVRAA,[b] PETER BROSS,[c] KAARE CHRISTENSEN,[d] NIELS GREGERSEN,[c] QIHUA TAN,[e] UFFE BIRK JENSEN,[a,h] HANS EIBERG,[f] AND SURESH I. S. RATTAN[g]

[a]*Department of Human Genetics, University of Aarhus, Aarhus, Denmark*

[b]*Department of Clinical Genetics, Vejle Hospital, Vejle, Denmark*

[c]*Research Unit for Molecular Medicine, Aarhus University Hospital and Faculty of Health Sciences, Skejby Hospital, Aarhus, Denmark*

[d]*Institute of Public Health, University of Southern Denmark, Odense, Denmark*

[e]*Odense University Hospital, KKA, Department of Clinical Biochemistry and Genetics, Odense, Denmark*

[f]*Panum Institut, Copenhagen, Denmark*

[g]*Department of Molecular Biology, University of Aarhus, Aarhus, Denmark*

[h]*Department of Clinical Genetics, University of Aarhus, Aarhus, Denmark*

ABSTRACT: We have studied the association of three single nucleotide polymorphisms (SNPs) present in the three *HSP70* (heat-shock protein) genes on 6p21 with human longevity. The availability of biological samples from various population cohorts in Denmark has given us the opportunity to try novel methods of gene association with human longevity. A significant association of one haplotype with male longevity was observed. Furthermore, a significant difference in the survival of the carriers of the different genotypes in females was observed. We also found an age-dependant decline in the ability of peripheral blood mononuclear cells to respond to heat stress in terms of Hsp70 induction.

KEYWORDS: human longevity; aging; heat-shock proteins; heat-shock response; gene association; linkage disequilibrium

Address for correspondence: Ripudaman Singh, Department of Human Genetics, Bartholin Building, University of Aarhus, Aarhus C. Denmark. Voice: +45-8942-1682; fax: +45-8612-3173.
e-mail: singh@humgen.au.dk

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INTRODUCTION

Human longevity is a multifactorial trait with both genetic and environmental factors playing an important role in its manifestation. Studies in twins on the genetic basis of human longevity indicated that approximately 25% of the observed differences in life expectancy are due to genetic variations.[1,2] The conventional approach for identifying longevity-determining genes has been by population-based association studies, where a group of long-lived individuals (LLIs) is compared to a group of young individuals for differences in the gene and genotype frequencies of candidate genes.[3] This approach, though common, has several limiting factors.[3]

HSP70 GENE AND HUMAN LONGEVITY

Heat-shock proteins (Hsps)[4] are ubiquitous, highly conserved proteins which are a part of the cellular safety and rescue mechanisms. At the cellular level all organisms respond to stress by synthesizing Hsps at the expense of other proteins. This phenomenon is called "heat-shock response" (HSR),[5] which protects cells from subsequent damage and aids them to counteract the effects of the stress. The capacity to respond rapidly to stress at the gene level determines the adaptive and, therefore, the survival capacity and longevity of the organism.[6] One way of indicating the relationship between human longevity and stress response is to demonstrate an association between single nucleotide polymorphisms (SNPs) in *Hsp* genes (*HSP*) and longevity[7,8] or parameters of human aging.[9]

Of all the various heat-shock proteins, Hsp70 is the most prominent and best characterized of the stress protein families. Hsp70 is a highly inducible and most actively synthesized protein in the cell upon heat shock. In humans there are 11 different isoforms of *HSP70* encoded by different genes located at dispersed loci. Three of the 11 isoforms of *HSP70* are localized within the major histocompatibility complex (MHC) class III region on chromosome 6p21.[10] These are intron-less *HSP70A1A (HSP70-1), HSP70A1B (HSP70-2),* and *HSP70A1L (HSP70-Hom)*[11] genes. They display a very high sequence similarity but differ in their regulation. We have studied the association of three SNPs, one in each MHC-linked *HSP70* gene, with human longevity, survival, and parameters of aging (TABLE 1).

CHOICE OF SUBJECTS AND METHODS

With the ever-increasing number of elderly persons in the population and in well-maintained health and population registries, [12,13] the availability of a unique set of biological samples for research from various well-established

TABLE 1. SNPs in *HSP70* genes studied for their association with human longevity

SNP (position)	Marker	Nucleotide change
HSPA1A A>C (−110; 5′ flanking)	rs1008438	A to C transversion
HSPA1B A>G (1267 coding)	rs1061581	A to G transition
HSPA1L T>C (2437 coding)	rs2227956	T to C transition (Met to Thr substitution)[a]

[a]Variation may affect the substrate specificity and chaperone activity of the Hsp70.

TABLE 2. Different population cohorts in Denmark used for studying the association of *HSP70* genes with human longevity

Population cohorts	Mean age at the time of sampling (in years)
Twins (LSADT)	75.6
Danish families (only parents)	40.8
1905 Cohort	92.8
Centenarians (DLCS)	—
Heat-shock response study	young = 22.6; middle-aged = 56.3

population databases, and from different age-related cohorts, in Denmark, provides a rare opportunity to perform novel population-based genetic association studies by mitigating many of the limiting factors [9,14–17] (TABLE 2).

For our research we have used DNA samples from 426 participants from the following groups:

(1) Longitudinal Study of Ageing Danish Twins (LSADT), which is the oldest twin registry in the world.[18] These twins, sampled in 1999, are between the ages of 70 and 91 years (mean age 75.6 years), and have been categorized according to the absence or presence of various age-related diseases and for various age-related parameters including scores of physical and cognitive tests.[19]

(2) One hundred fifty-seven DNA samples collected in 1998 from individuals born in 1905[13] have been used to perform survival analyses.

(3) Trio samples (mother, father, and child) from 42 Danish families[20,21] were used for molecular haplotyping.

(4) Sixty-two samples from the Danish Longitudinal Centenarian Study (DLCS) were also used. The DLCS is a clinical epidemiological survey of all persons living in Denmark who celebrated their 100th birthday during the period April 1, 1995 to May 31, 1996 (276 people).

The three SNPs in the three *HSP70* genes were genotyped using real-time polymerase chain reaction on the LightCycler system (Roche Applied Sciences), which allows monitoring of the amplification of the PCR product in real time, using fluorescent labeled oligonucleotide probes (TIB MOLBIOL, Germany) and primers (DNA Technology A/S, Denmark) specific for each SNP (sequences of primers and probes can be provided on request).

For different population cohorts haplotypes were generated by using the computer program PHASE (Version 2.0.2), which implements a Bayesian statistical method for reconstructing haplotypes from population data.[22,23]

Pair-wise linkage Disequilibrium (LD) among the three markers was calculated using Java LINkage Disequilibrium Plotter (JLIN) (http//www.genepi.com.au/projects/jlin).

Blood samples were also collected from a group of young (mean age 22.6 years) and middle-aged individuals (mean age 56.3 years). Mononuclear cells (monocytes and lymphocytes) were isolated and then studied for age-related HSR after giving 1 h of heat shock for 42°C followed by 5 h of recovery. The amount of induced protein was quantified by using a flow cytometer.

RESULTS AND DISCUSSION

The gene and genotype frequency of the three SNPs was found to be in a Hardy–Weinberg equilibrium. In LSADT "Self-rated health" ($P = 0.0046$) and "Relative self-rated health" ($P = 0.018$), which represent an individual's overall sense of physical well-being and which have been shown to be both predictors of survival at older ages and better indicators of future survival than objectively measured health status, were associated with heterozygosity for -110A>C polymorphism in the promoter region of *HSP70-1*.[9]

The molecular haplotype analyses on Danish family samples have revealed high LD among the three markers (TABLE 3). Pair-wise LD calculated among the three markers also showed a high LD ($D'=1$) between the SNP at *HSPA1A* with the other two SNPs at *HSP70A1B* and *HSP70A1L* (FIG. 1). This information is very important when it comes to discovering genes that influence complex traits such as longevity.[24] On analyzing the frequency distribution of

TABLE 3. Comparison of haplotype frequencies in the family samples (only parents) generated by PHASE and from allele frequencies

Haplotypes[a]	Observed frequency (generated by PHASE)	Expected frequency (generated from allele frequency)
A-A-T	0.41	0.3
A-A-C	0.18	0.09
G-A-T	0.03	0.2
G-A-C	0.02	0.06
A-C-T	0	0.16
G-C-T	0.34	0.1
G-C-C	0	0.03
A-C-C	0	0.05

Chi-square = 171.457; df = 7; P value < 0.0001.

[a]The first position in the haplotypes is the marker at gene *HSPA1B*, second at *HSPA1A*, and third at *HSPA1L*.

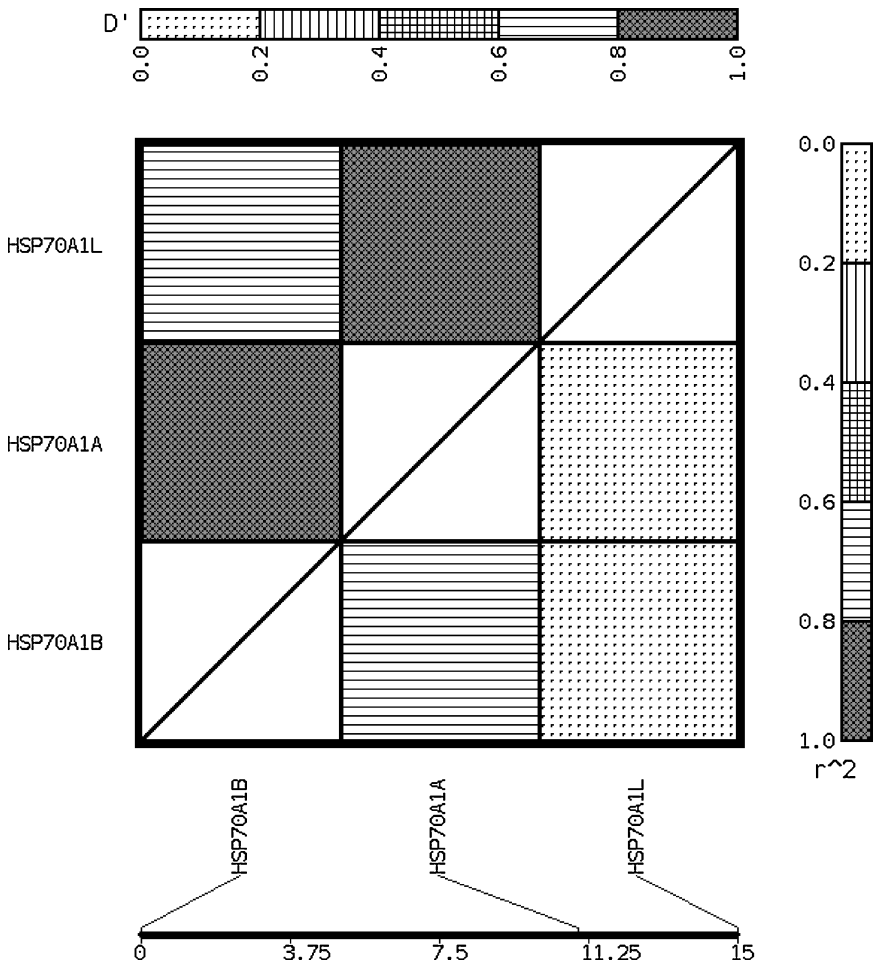

FIGURE 1. Pair-wise measurement of LD among the three *HSP70* gene markers. The figure shows two common ways of measuring LD, D′, and r^2. The distance shown among the markers is the relative distance, in kilobases, where position 0 corresponds to *HSP70A1B*.

haplotypes across different age groups from young individuals to centenarians we found a significant age-dependent increase in the frequency of one haplotype in males (Singh *et al.*, in preparation).

As mentioned above most of the genetic association studies on human longevity are performed in a case–control manner by comparing the gene/genotype frequencies between young and LLIs. This method, though widely used, has a shortcoming in that comparing the young with LLIs may give false-positive results, where the differences in the frequencies of the gene

in study might just be owing to secular changes in population demographic and genetic parameters of the two groups and not necessarily age. To mitigate this factor, cohort studies, which focus on tracking the survival of a group of individuals born in the same time period, are appropriate alternatives. Our work on the cohort persons of Danish form in the year 1905 has provided us with the opportunity to perform such studies. We have seen a significant difference in the survival of the carriers of different genotypes. Female carriers of CC genotype of *HSP70A1A* survive better than the noncarriers (AA and AC) ($P = 0.005$). It was also observed that female carriers of GG genotype in *HSP70A1B* survive better than noncarriers ($P = 0.04$). (Singh *et al.*, in preparation).

We have also seen that peripheral blood mononuclear cells of young and middle-aged individuals respond differently to heat stress, confirming that in humans there is significant age-dependent attenuation in the ability to respond to stress (Singh *et al.*, in preparation). Also, in the young subjects a positive association was found between the *HSPA1L* (T2Y37C) polymorphism and HSR. CC carriers had significantly lower induction than TT carriers in both monocytes ($P = 0.015$) and lymphocytes ($P = 0.004$). This polymorphism, which is present in the coding region of the *HSPA1L* gene, can affect the chaperoning function of Hsp70.

Our results from the association of SNPs in three *HSP70* genes with human longevity, survival, and parameters of aging have reiterated our belief that the genes involved in cellular maintenance and repair mechanisms are indeed important candidates for deciphering the genetics of human longevity. Additionally, the availability of samples from different population cohorts in Denmark has provided us with the opportunity to perform novel studies by mitigating many confounding factors.

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REFERENCES

1. Herskind, A.M., M. McGue, N.V. Holm, *et al*. 1996. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870–1900. Hum. Genet. **97:** 319–323.
2. Tan, Q.H., G. De Benedictis, A.I. Yashin, *et al*. 2001. Measuring the genetic influence in modulating the human life span: gene-environment interaction and the sex-specific genetic effect. Biogerontology **2:** 141–153.

3. De Benedictis, G., Q. Tan, B. Jeune, *et al*. 2001. Recent advances in human gene-longevity association studies. Mech. Ageing Dev. **122:** 909–920.
4. Verbeke, P., J. Fonager, B.F.C. Clark, *et al*. 2001. Heat shock response and ageing: mechanisms and applications. Cell Biol. Int. **25:** 845–857.
5. Verbeke, P., B.F.C. Clark & S.I.S. Rattan. 2001. Reduced levels of oxidized and glycoxidized proteins in human fibroblasts exposed to repeated mild heat shock during serial passaging in vitro. Free Radic. Biol. Med. **31:** 1593–1602.
6. Rattan, S.I.S. 2004. Aging intervention, prevention, and therapy through hormesis. J. Gerontol. Series A-Biol. Sci. Med. Sci. **59:** 705–709.
7. Altomare, K., V. Greco, D. Bellizzi, *et al*. 2003. The allele (A)(-110) in the promoter region of the HSP70-1 gene is unfavorable to longevity in women. Biogerontology **4:** 215–220.
8. Ross, O.A., M.D. Curran, K.A. Crum, *et al*. 2003. Increased frequency of the 2437T allele of the heat shock protein 70-Hom gene in an aged Irish population. Exp. Gerontol. **38:** 561–565.
9. Singh, R., S. Kolvraa, P. Bross, *et al*. 2004. Association between low self-rated health and heterozygosity for −110A > C polymorphism in the promoter region of HSP70-1 in aged Danish twins. Biogerontology **5:** 169–176.
10. Goate, A.M., D.N. Cooper, C. Hall, *et al*. 1987. Localization of a human heat-shock Hsp-70 gene sequence to chromosome-6 and detection of 2 other loci by somatic-cell hybrid and restriction-fragment-length-polymorphism analysis. Hum. Genet. **75:** 123–128.
11. Milner, C.M. & R.D. Campbell. 1990. Structure and expression of the three MHC-linked HSP70 genes. Immunogenetics **32:** 242–251.
12. Kyvik, K.O., K. Christensen, A. Skytthe, *et al*. 1996. The Danish twin register. Dan. Med. Bull. **43:** 467–470.
13. Nybo, H., D. Gaist, B. Jeune, *et al*. 2001. The Danish 1905 cohort: a genetic-epidemiological nationwide survey. J. Aging Health **13:** 32–46.
14. Skytthe, A., K. Kyvik, N.V. Holm, *et al*. 2002. The Danish twin registry: 127 birth cohorts of twins. Twin Res. **5:** 352–357.
15. McGue, M., J.W. Vaupel, N. Holm, *et al*. 1993. Longevity is moderately heritable in a sample of Danish twins born 1870–1880. J. Gerontol. **48:** B237–B244.
16. Frederiksen, H. & K. Christensen. 2003. The influence of genetic factors on physical functioning and exercise in second half of life. Scand. J. Med. Sci. Sports **13:** 9–18.
17. Christensen, K., D. Gaist, J.W. Vaupel, *et al*. 2002. Genetic contribution to rate of change in functional abilities among Danish twins aged 75 years or more. Am. J. Epidemiol. **155:** 132–139.
18. Christensen, K., N.V. Holm, M. McGue, *et al*. 1999. A Danish population-based twin study on general health in the elderly. J. Aging Health **11:** 49–64.
19. Frederiksen, H., D. Gaist, H.C. Petersen, *et al*. 2002. Hand grip strength: a phenotype suitable for identifying genetic variants affecting mid- and late-life physical functioning. Genet. Epidemiol. **23:** 110–122.
20. Eiberg, H. & J. Mohr. 1981. Genetics of paraoxonase. Ann. Hum. Genet. **45:** 323–330.
21. Mohr, J. & H. Eiberg. 1977. Colton blood groups: indication of linkage with the Kidd (Jk) system as support for assignment to chromosome 7. Clin. Genet. **11:** 372–374.

22. STEPHENS, M., N.J. SMITH & P. DONNELLY. 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. **68:** 978–989.
23. STEPHENS, M. & P. DONNELLY. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am. J. Hum. Genet. **73:** 1162–1169.
24. CARDON, L.R. & G.R. ABECASIS. 2003. Using haplotype blocks to map human complex trait loci. Trends Genet. **19:** 135–140.