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Genetics of human longevity with emphasis on the relevance of *HSP70* as candidate genes

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1. ABSTRACT

Human longevity is determined to a certain extent by genetic factors. Several candidate genes have been studied for their association with human longevity, but the data collected so far are inconclusive. One of the reasons is the choice of the candidate genes in addition to the choice of an appropriate study design and methodology. Since aging is characterized by a progressive accumulation of molecular damage and an attenuation of the cellular defense mechanisms, the focus of studies on human longevity association with genes has now shifted to the pathways of cellular maintenance and repair mechanisms. One such pathway includes the battery of stress response genes, especially the heat shock protein *HSP70* genes. Three such genes, *HSPA1A*, *HSPA1B* and *HSPA1L*, are present within the MHC-III region on the short arm of chromosome 6. We and others have found alleles, genotypes and haplotypes which have been significantly associated with human longevity and survival. We have also provided some functional evidence for these genetic associations by showing that isolated peripheral blood cells from those genotypes which are negatively associated with human longevity also have less ability to respond to heat shock. Stress response genes, particularly *HSP70*, are now the major candidates in the gene-longevity association studies.

2. GENES AND LONGEVITY

Longevity is a complex trait in which genes, milieu and chance play a deterministic role in its manifestation (1,2). It is widely accepted that there are no real gerontogenes that might have evolved with the specific function of causing aging, nor is there an evolutionarily conserved genetic program that would control the aging process across the animal kingdoms; such genes would have affected the reproductive fitness, and hence would have been negatively selected for (3,4). But significant differences in the maximum lifespan between and within species do exist, indicating a genetic basis for longevity. Search for genes affecting aging and longevity in experimental model systems have most commonly depended on the isolation of induced or natural mutants with extended lifespan. In invertebrates, it has been relatively easy to find the differences in lifespan that can be defined by genetic variability. The best measure of aging in such organisms after a genetic intervention has been an increased mean and maximum lifespan. So far, a large number of genes have been isolated and identified that influence aging and longevity in nematodes, fruitflies and rodents. The molecular pathways affected by these genes range from signal transduction and metabolic rate regulation to protein transport, chaperoning and stress tolerance (Table 1). For a complete list of such genes see

Table 1. Examples of Longevity genes in *C. elegans*, Drosophila and rodents

Model Organism	Gene	Function	Reference
C.elegans	*Age-1*	Codes for a protein with homology to enzyme phosphatidyl-inositol 3-kinase, increases catalase and superoxide dimutase activity in C.elegans and hence increases resistance to oxidative stress	77,78
	daf-2	Codes for a protein with homology to the gene family that includes insulin receptor in mammals	79
	daf-16	Codes for a transcription factor, daf-16	80
	old-1& old-2	Overexpression leads to increased resistance to environmental stress	81
	eat-2	Leads to defect in feeding and also to increase in lifespan. This process may mimic calorie restriction	82
	clk-1	Encodes an enzyme demethoxyubiquinone mono-oxygenase that is necessary for ubiquinone biosynthesis	83
	sir-2	Regulate nematode aging via the insulin/IGF pathway involving transcription factor daf-16	84
Drosophila	*mth*	Gene mutants have been shown to have increased resistance to stress as well as 35% increased lifespan as compared to the wild type	85
	Indy	Codes for a transporter protein	86
	InR	Insulin receptor homologue	87
	hsp70	Chaperone protein	65
Rodents	*Prop-1 & Pit-1*	Growth deficiency due to the absence of pituitary gland hormone and thyroid stimulating hormone	88
	Ghr	Dysfunctional growth hormone receptors	89
	p66^{shc}	Codes for signal transduction protein	90
	klotho	Homologue to a gene for putative membrane protein with β-glucosidase activity	91

review by Warner *et al.* (5). Interestingly, almost all such genes that are associated with the prolongation of lifespan in model organisms are mutated and become completely or partially non-functional. A common feature of all such 'genes for longevity' is that in their natural state their products have biochemical functions none of which is directly involved with causing aging. Hence such genes qualify as being termed 'virtual gerontogenes' by virtue of their indirect role in aging and longevity (3).

2.1. Longevity genes in humans

Although no gene mutations in humans have yet been identified that can lead to increased lifespan, several single gene mutations are known that lead to premature aging disorders such as the Hutchinson-Gilford's progeria syndrome (6) and Werner's syndrome (7-9). On the other hand, many genes modulating the same pathways as in lower organisms have been studied for their association with human longevity. Such genes have been called by different names viz. 'longevity assurance genes (LAG),' 'longevity enabling genes,' 'longevity predisposing genes,' or just 'longevity genes' (10).

The evidence for the presence of longevity genes in humans has mainly come from the studies on centenarians and their siblings (11), and also from the studies on twins. It was shown that the siblings of centenarians have a four-fold greater survival to more than 85 years of age than sibs of those who died by age 73 years (12). The actual value of the genetic determinant of longevity was calculated from studies on Danish twins, and it was shown that the heritability of longevity in males and females was 0.26 and 0.23, respectively (13). But the search for the molecular basis behind this heritability of longevity is far from over. As with other complex traits, the genetic determinants of human longevity are most likely defined by subtle variations in many genes involved in different functional pathways.

2.2. Choice of candidate genes

Once it is accepted that genes do exist in humans that can explain some variability in lifespan, the next step is to choose candidate genes and a suitable approach to study the genetics of longevity. One can either choose a candidate gene, or perform a genome wide scan, looking for the target areas associated with longevity. The candidate gene approach requires understanding of the related trait and *a priori* assumptions about the biological process(es) pertinent to the gene, and a hypothesis behind choosing the gene. Then the variations in the candidate genes are studied and it is analyzed if the gene variant co-segregates with the phenotype (long life), in a family-based linkage analysis; or if the frequency of a gene variant is more common in a group of old cases as compared to young controls, in a population-based analysis. If the gene variant is shown to be associated with longevity then the next step is to demonstrate how the gene variant alters function, and how the altered function manifests itself into exceptional longevity.

On the other hand, the genome-wide scan approach does not make any *a priori* assumptions about the pertinent biological process(es). Completion of the Human Genome Project has now given the opportunity to perform such an analysis for identifying genes involved in complex traits, such as longevity. In this approach, whole genome scan can be performed to map genes that are linked to longevity using both linkage and association analyses. Then the genes within that target area are studied to localize specific candidate genes. High-throughput genomic technologies have made it possible to perform such studies. For example, a genome-wide scan has linked marker D4S1546 on chromosome 4 with human longevity (14).

Most of the data currently available on the genetics of human longevity come from the candidate gene approach. Several genes involved in determining susceptibility of age-related diseases, DNA repair, antioxidants defense systems, and heat shock response (HSR), have been studied in humans [for review see (15)]. But many of these studies have rendered contradictory results in different populations, which may be due to the choice of study designs and methodologies.

2.3. Human longevity associated with genes

Dysregulation of the immune system with age leads to greater incidence of infections, cancer and autoimmune diseases (16). Studies on centenarians have shown that a well preserved immune function is associated with extended longevity (17), suggesting that genetic determinants of longevity also reside in the polymorphisms for the immune system such as human leukocyte antigen (HLA). Scores of markers within the HLA region have been studied for their association with human longevity. But it has been shown that HLA/longevity association is population-specific being heavily affected by population-specific genetic and environmental history. For example, in the Caucasian population studies have been performed on longevity with respect to haplotype within the major histocompatibility complex (MHC) region. In a study done on the French (18) and Northern Irish (19) Caucasian populations, haplotype A1B8Cw7DR3 has been found to have positive association with longevity in male nonagenarians. Haplotypes bearing DR3 were increased in male nonagenarians (20). In a Greek study DR7 frequency increased in aged men, whereas DR13 increased in centenarians among both the genders (21). Again, as with the other genes, most of the data from the genetics of HLA on human longevity are ambiguous.

Release of cytokines is important for the regulation of immune response in the elderly. There are different levels of cytokine production, and elevated interleukins (IL) levels are associated with disease, disability and mortality in the elderly. Hence various cytokine markers (IL-2, IL-6, IL-8, IL-10. etc.) have been studied as longevity genes in different populations with positive and negative associations (22-24).

The key to successfully identifying a longevity gene is the reproducibility of the association in different populations. Until now results from ApoE gene, whose variants are associated with susceptibility to Alzheimer's disease and cardiovascular disease, have been successfully reproduced independently in different populations (25-29) with the exception of a study of an Italian population (30). Consistently, results from most of these studies show that allele e2, relative to the e4, is present in higher frequency among the centenarians.

Recently, a lot of interest has shifted to the network of genes involved in cellular and molecular repair and defense mechanisms such as DNA repair genes, and HSR genes. Many features of mammalian aging could be considered to be the consequence of the long-term effects of chronic stress, and aging and longevity are related to the ability to cope with a variety of stressors. It has been shown in rodents, rhesus monkey (31-33), and humans (34-37), that this ability, HSR, decreases with age. The biological relevance and regulatory mechanisms involved in this decrease are not yet fully known. However, inter-individual differences in the ability to respond to stress can be understood by studying the genetic polymorphisms in the stress response genes. This approach will give us insights into whether the carriers of certain variants have increased ability to respond to stress as compared to non-carriers.

3. HEAT SHOCK PROTEINS: BRIEF BACKGROUND

At the cellular level, all organisms respond to stressors, such as heat, nutrient deprivation, oxygen radicals, metabolic disruptions, viral infections, heavy metals and others, by inducing the synthesis of a group of cytoprotective proteins called heat shock proteins (Hsps) (38-42). Following stress, Hsps act as chaperones, preventing misfolding of proteins, permitting them to cross biological membranes and allowing them to fold properly, hence helping in their restoration after cellular shock (42). Hsps also act as indirect proteases facilitating the transport of abnormal proteins to the proteasome for degradation and removal of damaged proteins from the cells (42). Hence they are a part of cellular safety and rescue mechanism. This process of preferential transcription and translation of Hsps, at the expense of the synthesis of other cellular proteins is called the HSR (43). The HSR protects cells from subsequent damage and aids them to counteract the effects of stress. Hsps are among the most highly conserved proteins in existence, both in terms of function and structure, reiterating their important role in cell survival throughout evolution. Most of the Hsps are inducible, that is they are expressed in response to heat shock or other kinds of stress. But a few, called cognate or constitutive, are expressed in the cells under normal physiological conditions and their synthesis is increased following heat stress but this increase is, as a rule, only moderate.

Hsps are classified into different families based on their molecular weights (42). In mammalian cells, there are several Hsp families: the Hsp90, Hsp70, Hsp60, Hsp40, low molecular weight, and high molecular weight families (42,44). Some Hsps like Hsp90, and 110kDa can be found in the nucleolus, are constitutively synthesised in mammalian cells and their levels increase in response to stress (44). The Hsp70 proteins are encoded in a multigene family, consisting of at least 17 distinct genes in humans (45,46). Among the members of the Hsp60 family is the mitochondrial Cpn60, while Hsp47 belongs to the Hsp40 family. Hsp32, Hsp28 and Hsp10 are low-molecular weight Hsps (42).

3.1. Heat shock protein 70

Of the various Hsps, Hsp70 is the most prominent and best characterised (47). It is a highly inducible and most actively synthesised protein in the cell upon heat shock. The Hsp70 protein family members have a highly conserved sequence from *E. coli* to man (41). In mammals, there are at least 17 *hsp70* genes located in various chromosomes (45,46). Three *HSP70* genes are mapped within major histocompatibility complex (MHC) class III region on the short arm of chromosome 6 (6p21.3) (48).

3.2. MHC-III linked *HSP70* genes

The three genes mapped within the MHC-III region are intronless *HSP70-1* (*HSPA1A*), *HSP70-2* (*HSPA1B*), and *HSP70-Hom* (*HSPA1L*) (49) (Figure 1). A continuous reading frame, with no introns, is significant for genes that are rapidly activated at the transcriptional level.

Table 2. Single Nucleotide Polymorphism in three *HSP70* genes in humans

SNP position	Nucleotide change
HSP70-1 (HSPA1A)	
-110 (5' flanking)	A to C transversion
120 (5'UTR)	T to C transition
190 (5'UTR)	G to C transversion
438 (coding)	C to T transition
1911 (coding)	C to G transversion
1926 (coding)	G to T transversion
HSP70-2 (HSPA1B)	
145 (5'UTR)	C to T transition
1267 (coding)	A to G transition
2074 (coding)	C to G transversion
2257	Penta-duplication
HSP70-Hom (HSPA1L)	
1097 (coding)	C to T transition
2180 (coding)	G to A transition
2437 (coding)	C to T transition (amino acid substitution)
2763 (coding)	G to A transition (amino acid substitution)

Contrary to this, the *HSC70* gene, which encodes a constitutively expressed protein, Hsc70, contains 8 introns.

All the three genes have similar sequences but differ in the regulation. *HSPA1A* and *HSPA1B* genes are both expressed at very high levels in mammalian cells heat shocked at 42°C (49). *HSPA1A* and *HSPA1L* are constitutively expressed at low levels but *HSPA1L* is not induced by heat shock. *HSPA1A* and *HSPA1B* genes have open reading frames (ORF) of 1923bp with similar sequences and code for identical heat-inducible proteins of 641 amino acids (70,053 Dalton). These two genes differ in the 3' untranslated region. *HSPA1L* has an ORF of 1923bp and encodes a basic protein of 641 amino acids that has 91% sequence similarity with HspA1A and HspA1B.

3.3. Polymorphisms in *HSP70* genes

In humans, data on the *HSP* gene polymorphism are restricted to members of *HSP70* family (Table 2). MHC is one of the highly polymorphic regions in human genome, and polymorphisms have also been described in all the three *HSP70* genes mapped to MHC-III region (50).

In *HSPA1A* four different polymorphic locations have been described, at positions -110 (A to C transversion), 120 (T to C transition), 190 (G to C transversion), and 438 (C to T transition). The first three are in the 5' flanking region and the fourth one is in the coding region of the gene. Three different alleles, namely A, B and C have been described on the basis of electrophoretic mobility on polyacrylamide gel (48).

There are four different polymorphic sites present in the *HSPA1B* gene. These are at positions 145 (5' flanking region), 1267 (A to G transition) and 2074 (both 1267 and 2074 lie in coding region); and at 2257 located in 3'untranslated region. The polymorphism at 1267 generates a *Pst I* site giving rise to two alleles, 'L' allele (8.5 kb) and 'U' allele (9.0 kb), identified according to their length. Polymorphism at position 2257 is a result of penta-duplication of the sequence AAGTT giving rise to two alleles A1 (183 bp) and A2 (188 bp) differing in 5bp.

Also in case of the *HSPA1L*, four polymorphic sites have been discovered at positions 1097, 2437 (C to T transition), 2180 (G to A transition) and 2763 (G to A transition). The polymorphism at position 2437 leads to an amino acid change at position 493 from a non-polar hydrophobic Methionine (Met) to a polar neutral Threonine (Thr), and may have biofunctional relevance, since amino acid 493 is present in the 18kDa peptide binding domain on the beta sheet which forms the floor of the peptide binding groove (51). An amino acid change at this position could be associated with altered peptide-binding specificity and efficiency of Hsp70. A change to a polar neutral Thr may thus affect the chaperone activity and hence the functional efficiency of *HSPA1L* by lowering the strength of the hydrophobic interactions between chaperones and the target protein (52). This change lies within the *Nco I* restriction site. The G to A transition at position 2763 is a novel coding mutation leading to the amino acid substitution Glutamic acid (Glu) to Lysine (Lys) at position 602. Also a high level of Linkage Disequilibrium (D'=1) is present between the SNP (single nucleotide polymorphism) at *HSPA1A* with the other two SNPs at *HSPA1B* and *HSPA1L* (53).

4. *HSP70*, AGING AND LONGEVITY

Since aging is characterized by a progressive accumulation of molecular damage, and an attenuation of the cellular defense mechanisms, longevity is related to the ability of the biological system to cope with variety of stressors (1,2). One of the indicators of such an ability to cope with stressors, measured by the induction of Hsp70 upon transient heat shock, decreases with age (54). HSR protects cells from subsequent damage and aids them to counteract the effects of the stress (43). The capacity to respond rapidly to stress determines the adaptive and, therefore, the survival capacity and longevity of the organism (55,56). The decrease in the HSR with age may be due to the presence of the less active oligomeric form of heat shock factor (HSF) which may in turn reduce the HSF binding activity leading to the decrease in Hsp70 transcription (57). The relationship between Hsp70 and longevity can either be studied at the protein level, measuring the basal and the induced levels of Hsp70 at different ages, or at the gene level studying the association of polymorphisms present in *HSP70* genes with longevity and seeing if a particular allele, genotype, or haplotype co-segregates with increasing age.

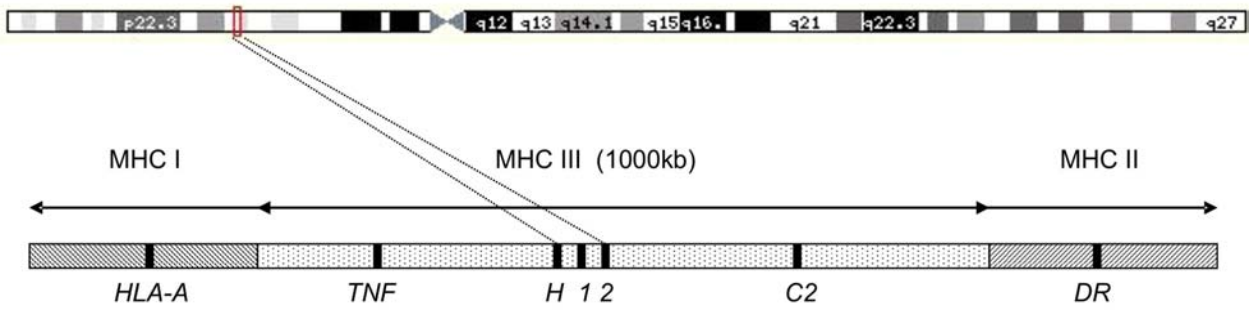

Figure 1. Localization of the three *HSP70* genes (denoted as 2, 1, and H) within the MHC-III region of the short arm of chromosome 6. MHC spans 3-4 Mbp on the short arm of Chromosome 6. The distance between different genetic markers is not shown in scale. *HSP70-1* and *HSP70-2* are 11 kb apart. *HSP70-Hom* gene is 4 kb telomeric to *HSP70-1*. MHC I contains 17 highly related genes including HLA-A, HLA-B and HLA-C. MHC II contains four sub regions DP, DO/DZ, DQ and DR, each containing at least one alpha and beta pair of genes.

4.1. Hsp70 level with age – cellular and extracellular

A general age-related variation in the ability to respond to stress, measured by the inducibility of Hsp70 following heat shock at a certain temperature (42°C), has been studied at the cellular level. Consistently in all the studies an age-related decrease in the induced levels of Hsp70 has been observed in lymphocytes (36) and other peripheral blood mononuclear cells (34,37,54,58). Similarly a decrease in the synthesis and accumulation of inducible Hsp70 has been reported for other human cells undergoing aging *in vitro* and *in vivo* [For review see (42,43)].

Though members of Hsp70 family are primarily intracellular proteins, soluble form of Hsp70 are also present in the peripheral circulation of normal individuals (59,60). As with the intracellular levels, Hsp70 levels in serum are also negatively correlated with human age (35,36,61). High levels of circulating Hsp70 predict the development of peripheral vascular disease, renal vascular disease (62), and atherosclerosis in subjects with established hypertension (63). Elevated levels of circulating cognate Hsp70 have also been related with predisposition to cardiovascular diseases (64). In the same study it was found that the offspring of centenarians had 10-fold lower levels of circulating Hsp70 as compared to spousal controls suggesting that among the same age group low levels of Hsp70 may be an indicator of healthy state and a marker of longevity.

4.2. *HSP70* gene polymorphisms with age

It is clear that the ability of human cells to respond to heat stress decreases with age, and so it is important to see if this age-related difference has any genetic predisposition to it. And also, if inter-individual differences in the ability to respond to stress can be understood by studying the gene variations in stress response genes. It is also important to find out if polymorphisms in the *HSP70* genes are directly associated with survival capacity of an individual and hence with longevity.

Resistance to stress with increased functionality of Hsp70 has been observed. Cells from individuals homozygous for 9.0kb allele of *HSPA1B* gene tend to display higher HspA1B mRNA levels, *in vitro*, after exposure to heat stress, than cells from individuals with 8.5kb allele (51). In transgenic *Drosophila melanogaster*, varying copy numbers of the gene *hsp70* encoding heat induced expression of Hsp70 increased lifespan at normal temperature (65). Adding an extra copy of Hsp70F, a homolog of mot-2 (mortalin)/Grp75 is shown to extend lifespan in *Caenorhabditis elegans* (66).

In humans, the first study relating variations in *HSP70* genes with longevity was performed on an Italian population where it was shown that allele A of a promoter region polymorphism, *HSPA1A*(A-110C), was unfavorable for female longevity (67). Later in a study done on Irish population, Ross *et al.* observed an increase in the frequency of T allele of coding polymorphism *HSPA1L*(T2437C) and decrease in the frequency of allele C with age (52). This difference was maintained when a gender-specific analysis was done. Increasing number of elderly people in the population, access to the unique set of biological samples from various well established age-related population databases in Denmark (53,68,69) and new methods to perform gene association studies (70), provided us with the unique opportunity to perform novel gene association studies on the polymorphisms in *HSP70* genes with aging (71), longevity (53), survival (manuscript submitted) and stress response (54). Apart from showing that female carriers of allele A of *HSPA1A* have decreased survival as compared with non-carriers (53), we also found a haplotype G-C-T that significantly influenced female longevity. Figure 2 summarizes the results from the study of *HSP70* polymorphisms with human longevity.

4.3. From *HSP70* genes to function

The strength and reliability of a genetic marker used for gene-phenotype association studies depend on whether its association with phenotype can be reproduced across different populations and by using different methodological approaches, and also by studying if the genetic variation manifests itself at the functional level. In this case, the main purpose would be to determine if the genetic polymorphisms are also associated with differential induction of Hsp70 following stress. The C allele of the

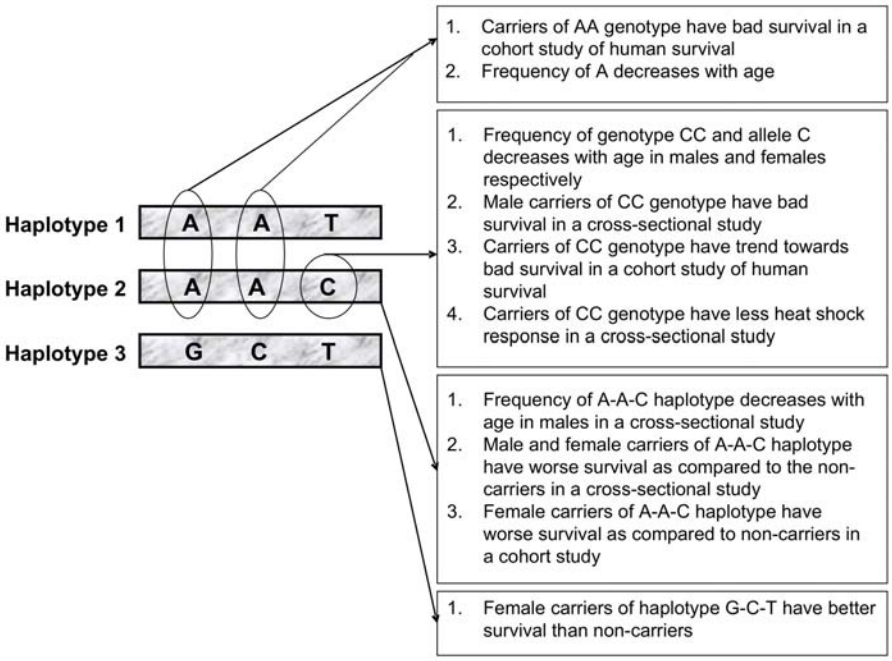

Figure 2. Summary of the results from the association of *HSP70* genes with human longevity. The figure shows three frequent haplotypes AAT, AAC and GCT. The first, second and third positions in the haplotype correspond to genes *HSPA1B*, *HSPA1A* and *HSPA1L* respectively.

functional polymorphism *HSPA1L*(T2437C) was found to be negatively associated with longevity both in Irish (52) and Danish (53) populations. As mentioned earlier, an amino acid change at a key position on Hsp70 could be associated with changes in the peptide-binding specificity of the chaperone (51). We undertook studies to find out if genetic polymorphism at this position was associated with differential induction of Hsp70 following heat treatment of peripheral blood mononuclear cells (PBMC). For this, blood samples was collected from young (mean age = 22.6±1.7 years) and middle-aged (mean age = 56.3±4.7 years) individuals. PBMC were isolated and then were subjected to one hour of heat shock at 42°C. Age-dependent changes in HSR were studied. Also genotype-specific HSR was measured by genotyping the subjects for three SNPs, *HSPA1A*(A-110C), *HSPA1B*(A1267G) and *HSPA1L*(T2437C), one each in the three *HSP70* genes. There was a significant age-related decrease in the induction of Hsp70 after heat shock, in both monocytes and lymphocytes (54). We also observed that there was a significant difference in the induction of Hsp70 between carriers of Allele C and T (54). The carriers of allele C had much lower induction than the carriers of allele T. And this difference was more significant in younger subjects. Also the fact that it is possible to give a plausible functional explanation to the observations from gene association studies emphasises that *HSP70* genes are ideal candidates for performing gene-longevity association studies.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Global variations within the human genome manifested as SNPs and the possibility of high throughput genotyping have led to scores of candidate genes being studied for their association with longevity, age-related diseases, and parameters of aging in different populations. However, the data thus derived are inconclusive (72). The success of finding a longevity gene depends on the choice of the samples (study design), methodology, reproducibility of results in different populations, and substantiating the results derived from genetic studies with functional tests (73). Once these factors are controlled the chances of finding a longevity-assuring gene are increased. The candidacy of the target gene is as much important (74,75).

Since aging is a result of a progressive accumulation of molecular damage, and longevity of an organism is directly related to its ability to counteract this damage (1,2), genetic pathways involved in such defense mechanisms are obvious candidates for gene-longevity association studies. As presented schematically in Figure 3, whenever a biological system experiences stress it initiates a stress response whose success determines the survival of the system. The extent and the efficiency of the stress response are dependent on the genetic background of the individual and the state of aging (Figure 3). HSR is one of the primordial and crucial pathways of stress response, and our studies have shown that differential induction of HSR is related with polymorphisms in the Hsp genes (54). An age-related decrease in the ability to respond to stress is further affected by the genetic makeup of the individual with respect to different stresses.

Also a three point categorization of a gene applicable for gene-longevity association study has been proposed: genes with homologs that influence longevity in

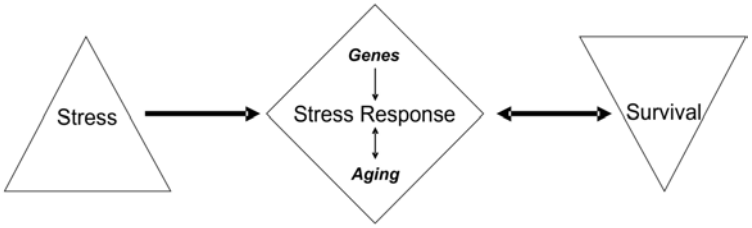

Figure 3. The survival of an organism depends on successful stress response, which itself depends on genes and aging. *HSP70* genes are one of the crucial mediators of stress response, and thereby of aging and survival.

other species, genes that mediate cellular maintenance and repair, and genes that are associated with susceptibility to major age-related diseases (76). Increase in life span with varying copy number of *HSP70* in *Drosophila* has been observed; *HSP70* gene products acting either as chaperones or helping in the proteolytic pathway, are a part of cellular safety and rescue mechanisms; and *HSP70* genes have also been shown to be associated with autoimmune diseases. If these criteria are adopted, *HSP70* genes become promising candidates for studying genetics of human longevity.

By using different study designs and methodologies, we and others have obtained significant and novel results on the association of three SNPs in three *HSP70* genes with respect to parameters of aging, longevity and human survival. Alleles, genotypes and haplotypes that influence human longevity have been found. From Figure 2 it is clear that altogether the epidemiological observations can be explained by haplotype A-A-C causing decreased survival in old age. The fact that the T to C substitution in the A-A-C haplotype causes diminished HSR in younger individuals may play a role in this decreased survival, possibly by causing an increased stress-related cell death of specific cell types in young, A-A-C carrying individuals. Such an increased cell death in the first half of life could then result in increased cellular turnover, possibly resulting in a tendency to premature replicative senescence of progenitor cells late in life.

Data accumulated on the association of *HSP70* genes and human longevity are promising in the populations studied so far. Still it would be interesting to see if these genetic associations can be reproduced across other populations. The *HSP70* genes studied lie in a highly polymorphic region of MHC-III on chromosome 6. Thus other functional polymorphisms within these genes are natural candidates for further pursuing gene-longevity association studies.

6. ACKNOWLEDGEMENTS

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Abbreviations: AD, autoimmune disease; HLA, human leukocyte antigen; HS, Heat shock; HSE, heat shock element; HSF, heat shock factor; Hsp, heat shock protein; *HSP*, heat shock protein gene; HSR, heat shock response; IL, interleukin; MHC, major histocompatibility complex; ORF, open reading frame; PBMC, peripheral blood mononuclear cells; SNP, single nucleotide polymorphism

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