Human heat shock protein gene polymorphisms and sudden infant death syndrome

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Abstract

Comparison of the frequency of occurrence of restriction fragment length polymorphisms in control human DNAs and DNAs from infants dying from sudden infant death syndrome has indicated no significant difference in the case of restriction fragment length polymorphisms associated with the heat shock protein genes hsp70 and hsp90. A highly significant difference was detected, however, in the case of the specific restriction fragment length polymorphisms detected by an hsp60 gene probe in MspI digests.

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Heat shock proteins play a central part in the normal physiology of cells.[1] Although they appear to be involved in the acquisition and maintenance of thermotolerance, other major roles include the repair of denatured cell proteins and functions as molecular 'chaperones' in the intracellular transmembrane transport of cellular proteins and in the construction of complex quaternary cellular protein structures. At present the cause of sudden infant death syndrome (SIDS) is unclear. Some deaths have been attributed to fever-like disorders,[2] possibly brought on by overwrapping or by a virus infection. A possibility is that infants are susceptible to SIDS because some of the heat shock proteins are not produced or are defective, thus providing inadequate thermal protection. This could arise from defects in the DNA associated with genes encoding critical heat shock proteins.

Materials and methods

Genomic DNA was isolated from the placenta of 28 different healthy subjects and from 1–1.5 g of frozen liver samples from infants diagnosed as having died from SIDS. These DNAs were digested with specific restriction endonucleases. After electrophoresis of the digests on agarose gels the DNA fragments were blotted onto nitrocellulose membranes and were hybridised with the appropriate radioactively labelled heat denatured DNA gene sequence probes for 16 hours at 69°C.[3] The hsp60 gene probe used was a 1.5 kilobase (kb) PstI fragment isolated from the plasmid pUCHS601. The hsp70 probe was a fragment of the 749 base pair of the coding sequence isolated, using EcoRI and HindIII, from the plasmid p17 containing 4 kb of hsp70. A 1.33 kb PstI segment of the hsp90 DNA insert from plasmid pUCHS901 was used as an hsp90 probe. The fragments were separated using low melting point agarose and labelled directly using random primer extension and [α-^{32}P] ATP. The heat shock protein cDNA plasmids were from StressGen Biotechnologies Corp, Victoria, Canada.

Results and discussion

An initial screen of healthy human subjects was undertaken to determine whether sequence probes to the heat shock protein genes, hsp60, hsp70, and hsp90 could reveal any restriction fragment length polymorphisms (RFLPs). A panel of 26 genomic DNA samples was digested with 19 different restriction enzymes to detect RFLPs. Use of the hsp70 probe detected variations in these control samples digested with BgII, PstI and PvuII, as previously reported by Goate *et al.*[4] The DNAs digested with MspI or ScaI were also polymorphic for hsp60, and the hsp90 probe detected variations in samples digested with Sau961. The frequency of the variable fragments was determined for all polymorphisms (table 1). The RFLPs detected using the hsp60 probe were more complex than those found using the hsp70 probe. With MspI, 16 constant fragments and six variant fragments were detected in 26 individuals. There was one hsp90 associated polymorphism, detected with Sau961 digested DNA. Nine of the fragments were invariant, whereas two others displayed a polymorphism characteristic of a two allele system.

To determine if there was any difference in the nature of heat shock protein gene polymorphisms in infants dying from SIDS compared with the controls, 12 DNA samples from SIDS infants were examined with the same 19 restriction enzymes used in the initial screening for heat shock protein polymorphisms. Although the sample size of SIDS DNAs used was small it was sufficiently large to detect frequent polymorphisms.[5] Importantly, no novel RFLP was detected in the DNAs from infants dying from SIDS. Although no new fragments were revealed, the 6.2 kb MspI fragment detected using hsp60 was a 'constant' fragment in the SIDS samples, but variable in the controls. For hsp70 and hsp90 there was no significant difference in the frequencies of the variable fragments. For hsp60 in DNA digested with MspI, however, there was a highly significant difference (at $p < 0.01$) in the frequency of occurrence between control

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Table 1 Frequency of polymorphic restriction fragments detected with hsp70, hsp60, and hsp90 in control DNAs and DNAs from infants dying from SIDS

Probe (restriction enzyme)	Variable fragments (kb)	Control DNA occurrence (frequency, %)	SIDS DNA occurrence (frequency, %)	χ^2
Hsp70				
BglI	3.5	7/19 (37)	3/12 (25)	0.09
PstI	8.9	18/19 (95)	10/12 (83)	0.18
PvuII	12.3	18/28 (64)	8/12 (67)	0.05
	6.6	26/28 (93)	10/12 (83)	0.12
	2.1	8/28 (29)	2/12 (17)	0.16
	1.8	28/28 (100)	11/12 (92)	0.20
Hsp60				
MspI	19.5	17/26 (65)	10/12 (83)	0.56
	15.0	16/26 (61)	1/12 (8)	7.37*
	12.0	6/26 (23)	3/12 (25)	0.08
	11.5	21/26 (80)	10/12 (83)	0.07
	7.7	13/26 (50)	6/12 (50)	0.12
	6.2	20/26 (77)	12/12 (100)	0.78
ScaI	11.6	13/21 (62)	4/12 (33)	1.48
Hsp90				
Sau961	4.3	22/26 (85)	11/12 (92)	0.01
	2.4	16/26 (62)	10/12 (83)	0.94

The relative frequencies of the polymorphic fragments detected by the different sequence probes were compared statistically in control and SIDS (cot death) DNAs using a χ^2 analysis with a 2 × 2 contingency table with Yates's correction.
* Significant difference at $p < 0.01$.

DNAs and those from infants dying from SIDS of a 15.0 kb fragment. Other variable fragments with MspI and ScaI, however, arose at similar frequencies (table 1).

Polymorphisms associated with hsp70 have been reported previously.[4] With regard to SIDS, however, the apparent significant association with a polymorphism in the gene encoding hsp60 is novel and of potential importance. The loss of the 15.0 kb MspI fragment hybridising to hsp60 in DNA from infants dying from SIDS could be due to the alteration of a base in the recognition site for MspI. Future research will aim to determine whether this is in a coding or regulatory nucleotide sequence. In terms of a potential cause it may be relevant that other studies have shown that the loss of the hsp60 15.0 kb MspI fragment also occurs in human cells exposed in vitro to the endogenous oxidant hydrogen peroxide.[6] In terms of function hsp60 itself appears to have an important role in mitochondrial biogenesis,[1] and impaired mitochondrial function could be a contributory factor in SIDS. Indeed, possible mitochondrial dysfunction in infants dying from SIDS has been observed in the shape of inappropriate brown adipose tissue thermogenesis.[7] This alteration of frequency of a polymorphic fragment between SIDS and control DNAs may in the future provide the basis of a test to indicate a predisposition to SIDS, the occurrence of which could be limited by good paediatric care.

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