

A Prospective Evaluation of the Heat Shock Protein 70 Gene Polymorphisms and the Risk of Stroke

Robert Y. L. Zee, David Bates, Paul M. Ridker

Center for Cardiovascular Disease Prevention, the Division of Preventive Medicine, and the Leducq Center for the Molecular and Genetic Epidemiology of Cardiovascular Diseases, Brigham and Women´s Hospital, Harvard Medical School, Boston, Mass., USA

Keywords

Heat shock protein 70-kD, stroke, prospective study, risk factors

Summary

Genetic polymorphisms of heat shock protein 70-kD (HSP70) gene family have recently been hypothesized to be risk factors for cerebral ischemia. However, no prospective epidemiological data evaluating this gene family are available. The present investigation was conducted to examine the possible associations between the HSP70-1 nucleotide 190, HSP70-2 nucleotide 1267, and HSP70-hom nucleotide 2437 polymorphisms and the incidence of stroke in a large cohort of initially healthy men. 14916 apparently healthy men were followed over a 12-year period for incident stroke. Employing a nested case-control study design, 338 study participants who developed stroke (cases) and 338 age- and smoking-matched study participants who remained healthy during follow-up (controls) were evaluated. All observed genotype frequencies were in Hardy-Weinberg equilibrium. The allele and genotype distributions of the polymorphisms tested were similar among cases and controls, such that the relative risk of future stroke was 0.89 for HSP70-1 nucleotide 190 (95%CI = 0.70-1.12; p = 0.31), 1.13 for HSP70-2 nucleotide 1267 (95%CI = 0.90-1.42; p = 0.29); and 0.89 for HSP70-hom nucleotide 2437 (95%CI = 0.65-1.21; p = 0.45), assuming an additive model. No evidence of association was observed assuming dominant or recessive mode of inheritance. In this large, prospective study, genetic polymorphisms in the HSP70 genes were not associated with risks of future stroke. Screening for these polymorphisms is unlikely to be a useful tool for risk assessment.

Introduction

Heat shock proteins are a class of stress proteins which exhibit well-established functions in cytoprotection/cell survival (1, 2). They are induced, at least in part, by denatured proteins produced during heat shock, ischemia and other cellular stresses (3, 4). Heat shock protein 70-kD (HSP70) is the most abundant in eukaryotic cells. Recent in vitro and in vivo studies have documented that an increased expression of HSP70 is associated with a variety of neurological insults including cerebral ischemia (5). Furthermore, it has been hypothesized that heat shock proteins may play an important role in cell survival and recovery in cerebral ischemia (4, 5).

Correspondence to: Robert Y. L. Zee, Ph. D., Division of Preventive Medicine, Brigham & Women's Hospital, Harvard Medical School, 900 Commonwealth Avenue East, Boston, MA 02215, USA – Tel.: 1-617-732 8175; Fax: 1-617-783 9212; E-mail: rzee@rics.bwh.harvard.edu

In humans, there are 3 genes encoding members of the HSP70 class (chromosome 6p21.3): HSP70-1, HSP70-2, and HSP70-hom. HSP70-1 and HSP70-2 encode an identical protein product, the major HSP70 heat-inducible form, but differ in their regulatory domains; HSP70-hom encodes a non-heat inducible form (6). Intriguingly, a recent report has shown that gene therapy for treatment of cerebro-vascular ischemia using HSP70 expressing vectors enhances neuron survival against cerebral insults (7). Furthermore, genetic variants of the HSP70 genes, in particular the HSP70-1 nucleotide 190, HSP70-2 nucleotide 1267, and HSP70-hom nucleotide 2437 polymorphisms, have been implicated in several inflammatory/autoimmune disease conditions (8). In part on the basis of these data, it has been hypothesized that identification and characterization of these gene markers may provide novel strategies to improve prognostication, treatment and prevention of stroke.

To address this hypothesis, we investigated the role of gene variants of HSP70-1, HSP70-2, and HSP70-hom as determinants of stroke in the Physicians Health Study (PHS), a large-scale, prospective cohort of apparently healthy middle-aged men.

Methods

We employed a nested case-control design within the PHS, a randomized, double-blinded, placebo-controlled trial of aspirin and beta-carotene initiated in 1982 among 22071 male, predominantly white, U.S. physicians, 40 to 84 years of age at study entry (9). Before randomization, 14916 participants provided an EDTA-anticoagulated blood sample. All participants were free of prior MI, stroke, transient ischemic attacks, or cancer at study entry. Yearly follow-up questionnaires provide updated information on newly developed diseases. Stroke was defined by the presence of a new focal neurological deficit, with symptoms and signs persisting for >24 h, and was ascertained from blinded review of medical records and autopsy results. For the present investigation, 338 incident cases of stroke (18.3% were fatal stroke cases) were identified among those study participants who had stored blood samples. For each case, a control matched by age, smoking history and length of follow-up was chosen among those subjects who remained free of vascular diseases.

Genotype Determination

Details of genotype determination for HSP70-1, HSP70-2, and HSP70-hom polymorphisms have been described previously (10). The polymorphisms tested were (i) a BsrBI restriction site at nucleotide 190 of HSP70-1, (ii) a PstI site at nucleotide 1267 of HSP70-2, and (iii) an NcoI site at nucleotide 2437 of HSP70-hom, a M(493)T polymorphism (10).

Statistical Analysis

Allele and genotype frequencies among cases and controls were compared with values predicted by the Hardy-Weinberg equilibrium using the χ^2 test. Relative risks of stroke associated with each genotype, with 95% confidence intervals (CI), were calculated by means of logistic regression models that

controlled for randomized treatment assignment, and presence or absence of hypertension and diabetes. All p values are two-tailed.

Results

Baseline characteristics of stroke cases and controls are shown in Table 1. The data reflect the expected recognized risk factors, with higher prevalence of hypertension, and diabetes among cases as compared to controls. Age and smoking status were identical in the two groups due to matching.

Table 2 shows the allele and genotype frequencies for the study population, which are consistent with that predicted by the Hardy-Weinberg equilibrium. As also shown in Table 2, the allele and genotype frequencies were similar among cases and controls such that no

Table 1 Baseline characteristics of the study population

parameter	all subjects		
	cases	controls	*p*
number of subjects	338	338	
age (years±S.D.)	61.0±8.3	60.5±8.2	n.s.
diagnosis (%)			
ischemic stroke	82.5	n/a	
hemorrhagic stroke	14.8	n/a	
unknown	2.7	n/a	
BMI (kg/m^2±S.D.)	25.6±3.2	25.0±2.8	<0.01
systolic BP (mmHg±S.D.)	134.4±12.7	129.2±12.2	<0.001
diastolic BP (mmHg±S.D.)	82.4±7.1	80.0±7.4	<0.001
history of hypertension (%)	34.7	15.5	<0.001
history of diabetes (%)	10.4	3.0	<0.001
history of H-chol (%)	13.3	9.5	n.s.
smoking history (%)			
never smoked	42.1	42.1	n.s.
former smoker	41.3	41.1	n.s.
current smoker	16.6	16.9	n.s.

BMI, body mass index; BP, blood pressure; H-chol, hypercholesterolemia; S.D., standard deviation; n.s., non-significance

Table 2 Genotype and allele distribution

	All Subjects			
Genotype	Cases		Controls	
	n	%	n	%
HSP70-1 (nucleotide 190)				
BB	49	14.5	46	13.6
Bb	170	50.3	148	43.8
bb	119	35.2	144	42.6
All	338	100	338	100
Allele				
B	268	39.6	240	35.5
b	408	60.4	436	64.5
HSP70-2 (nucleotide 1267)				
AA	103	30.5	131	38.8
AG	176	52.1	153	45.3
GG	59	17.4	54	15.9
All	338	100	338	100
Allele				
A	382	56.5	415	61.4
G	294	43.5	261	38.6
HSP70-hom (nucleotide 2437)				
MM	11	3.3	13	3.9
MT	95	28.1	89	26.3
TT	232	68.6	236	69.8
All	338	100	338	100
Allele				
M	117	17.3	115	17.0
T	559	82.7	561	83.0

Allele *B*, absence of *Bsr*BI restriction site; allele *b*, presence of *Bsr*BI site; allele *A*, absence of *Pst*I site; allele *G*, presence of *Pst*I site. *M* = Methionine, *T* = Threonine.

Table 3 Relative risks for stroke

Genotypes compared	All Subjects Relative Risk	95% C.I.	*p*
HSP70-1 (nucleotide 190)			
BB and Bb vs bb	1.26	0.91-1.75	0.17
BB vs Bb vs bb	0.89	0.70-1.12	0.31
BB vs Bb and bb	1.00	0.62-1.63	0.99
HSP70-2 (nucleotide 1267)			
GG and AG vs AA	1.28	0.92-1.79	0.14
GG vs AG vs AA	1.13	0.90-1.42	0.29
GG vs AG and AA	1.01	0.66-1.58	0.93
HSP70-hom (nucleotide 2437)			
MM and MT vs TT	1.14	0.79-1.64	0.48
MM vs MT vs TT	0.89	0.65-1.21	0.45
MM vs MT and TT	1.20	0.50-2.86	0.69

C.I., confidence interval. All models matched on age, smoking status, and adjusted for randomized treatment assignment, and history of hypertension and diabetes

overall difference in genotype distribution was observed (χ^2_{2df} = 3.99, p = 0.14 for HSP70-1 nucleotide 190; χ^2_{2df} = 5.18, p = 0.08 for HSP70-2 nucleotide 1267; χ^2_{2df} = 0.40, p = 0.82 for HSP70-hom nucleotide 2437). Thus, the relative risk of future stroke was 0.89 for HSP70-1 nucleotide 190 (95%CI = 0.70-1.12; p = 0.31), 1.13 for HSP70-2 nucleotide 1267 (95%CI = 0.90-1.42; p = 0.29); and 0.89 for HSP70-hom nucleotide 2437 (95%CI = 0.65-1.21; p = 0.45), assuming an additive model (Table 3). Since HSPs appear to play a role in response to the severity of cerebral-ischemic injury, we examined the genotype frequencies among fatal and non-fatal stroke cases, and found similar frequencies (χ^2_{2df} = 0.72, p = 0.70 for HSP70-1 nucleotide 190; χ^2_{2df} = 1.69, p = 0.43 for HSP70-2 nucleotide 1267; χ^2_{2df} = 3.89, p = 0.14 for HSP70-hom nucleotide 2437).

Similar null results were observed in models assuming recessive or dominant mode of inheritance. Virtually identical study results were obtained in a verified analysis limited to stroke with thrombo-embolic origin (data not shown).

Discussion

The HSP70 gene family is mapped to the same chromosomal region (6p21.3) of the class III region of the major histocompatibility complex (MHC) (6). Recent studies have suggested HSP70 gene polymorphisms as risk determinants in certain immunological/inflammatory disorders (8, 11). Since chronic low grade inflammation has been shown to be a major determinant of athero-thrombosis including stroke (12, 13), and because HSP70 has been implicated as neuroprotective (3), studies of the possible involvement of HSP70 gene family and MHC locus in the pathogenesis of stroke are of both clinical and pathophysiologic interest. However, the present prospective study, representing the first report on HSP70 gene polymorphisms and risk of future stroke, found little evidence of association between the gene variants tested and risk of stroke.

Potential limitations of the current data require consideration. For example, our study was limited to a cohort of almost exclusively Caucasian men and thus should not be construed to rule out a possible association in other ethnic groups and other socio-economic groups with different prevalences of other genetic or environmental risk factors (due to gene-gene or gene-environment interaction). However, unlike previous studies, the fact that our cases and controls derive from a homogeneous, prospective population greatly reduces the potential for selection bias or confounding by ethnicity. The majority of strokes in our study were ischemic; thus extrapolations from this study to other stroke types are not warranted. In addition, as the overall rate of stroke in the PHS cohort is low, the ability to detect risk in this population may be limited. Prior studies, however, have documented our ability to detect increased genetic risk in these individuals (14-16).

While our data are null, it is important to recognize that association studies, even when derived from very large study samples, can only examine the possible association between phenotype and the tested polymorphism(s); such studies cannot exclude the possibility that examination of a different polymorphism might obtain different results. Further, as in any null study, power to detect a true effect must be addressed. In this regard, based on the size of our study, we had the ability to detect, with 80% probability and at an alpha error of 0.05, a risk ratio of >1.23 for an association of the B allelic variant of HSP70-1 with stroke, a risk ratio of >1.22 for the G allele of HSP70-2 with stroke, and a risk ratio of >1.34 for the M allele of HSP70-hom with stroke, assuming an additive model. As these risk estimates are well below thresholds typically associated with substantive population attributable risk, we believe it unlikely that our sample size was inadequate to detect.

In conclusion, this large, prospective, nested case-control study among middle-aged US men provides little evidence of association between the genetic polymorphisms of HSP70 tested and risk of stroke. We believe that screening for these polymorphisms is unlikely to be a useful tool for risk assessment.

Acknowledgements

This study was supported by the National Heart, Lung, and Blood Institute (HL58755, HL63293), the American Heart Association, and the Doris Duke Charitable Foundation (P.M.R.).

References

1. Jaattela M. Heat Shock proteins as cellular lifeguards. Ann Med 1999; 31: 261-71.

2. Sharp FR, Massa SM, Swanson RA. Heat-shock protein protection. Trends Neurosci 1999; 22: 97-9.
3. Yenari MA, Giffard RG, Sapolsky RM, Steinberg GK. The neuroprotective potential of heat shock protein 70 (HSP70). Mol Med Today 1999; 5: 525-31.
4. Benjamin IJ, McMillan DR. Stress (Heat Shock) proteins. Molecular chaperones in cardiovascular biology and disease. Circ Res 1998; 83: 117-32.
5. Lee JE, Yenari MA, Sun GH, Xu L, Emond MR, Cheng D, Steinberg GK, Giffard RG. Differential neuroprotection from human heat shock protein 70 overexpression in in vitro and in vivo models of ischemia and ischemia-like conditions. Exp Neurol 2001; 170: 129-39.
6. Milner CM, Campbell RD. Structure and expression of the three MHC-linked HSP70 genes. Immunogenetics 1990; 32: 242-51.
7. Yenari MA, Dumas TC, Sapolsky RM, Steinberg GK. Gene therapy for treatment of cerebral ishcemia using defective herpes simplex viral vectors. Ann N Y Acad Sci 2001; 939: 340-57.
8. Favatier F, Bornman L, Hightower LE, Gunther E, Polla BS. Variation in hsp gene expression and Hsp polymorphism: do they contribute to differential disease susceptibility and stress tolerance? Cell Stress Chaperones 1997; 2: 141-55.
9. Steering committee of the physicians' health study research group. Final report of the aspirin component of the ongoing physicians' health study. N Engl J Med 1989; 312: 129-35.
10. Vinasco J, Beraun Y, Nieto A, Fraile A, Pareja E, Mataran L, Martin J. Heat shock protein 70 gene polymorphisms in rheumatoid arthritis. Tissue Antigens 1997; 50: 71-3.
11. Hunt PJ, Marshall SE, Weetman AP, Bunce M, Bell JI, Wass JA, Welsh KI. Histocompatibility leucocyte antigens and closely linked immunomodulatory genes in autoimmune thyroid disease. Clin Endocrinol 2001; 55: 491-9.
12. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000; 342: 836-43.
13. Ridker PM,Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997; 336: 973-9.
14. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. N Engl J Med 1995; 332: 912-7.
15. Ridker PM, Hennekens CH, Miletich JP. G20210A mutation in prothrombin gene and risk of myocardial infarction, stroke, and venous thrombosis in a large cohort of US men. Circulation 1999; 99: 999-1004.
16. Rubattu S, Ridker P, Stampfer MJ, Volpe M, Hennekens CH, Lindpaintner K. The gene encoding atrial natriuretic peptide and the risk of human stroke. Circulation 1999; 100: 1722-6.

Received October 19, 2001 Accepted after revision December 19, 2001