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Association between Heat Shock Protein 72 Gene Polymorphism and Acute Renal Failure in Premature Neonates

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ABSTRACT

Heat shock protein (HSP)70 plays an important role in the ischemic tolerance of fetal and neonatal kidney. We have investigated the association of genetic polymorphisms of the constitutive HSP70 (HSP73) and the inducible HSP70 (HSP72) encoding genes with the risk of acute renal failure (ARF) in very low birth weight (VLBW) neonates. Thirty-seven VLBW neonates with ARF and 93 VLBW neonates without ARF were enrolled in the study. The presence of HSP72 (1267)AG and HSP73 (190)GC polymorphism was analyzed from dried blood samples by PCR and restriction length fragment polymorphism. Allelic prevalence was related to reference values obtained in 131 healthy adults. Stepwise binary logistic regression was applied to determine the independent effect of the established risk factors to the development of ARF. Sixteen of 37 VLBW neonates with ARF and 18 of 93 VLBW neonates without ARF were homozygous for HSP72 (1267)G allele ($p \leq 0.01$). The association between HSP72 (1267)GG genotype and ARF remained at the level of significance ($p = 0.05$) when it was adjusted for established risk factors of neonatal ARF. Prevalence of HSP72 (1267)GG was also higher in VLBW neonates than in the reference population ($p < 0.05$) and in VLBW neonates with infant respiratory distress syndrome than in those without ($p < 0.001$). We found that in VLBW neonates carrying HSP72 (1267)GG genetic variation, which is associated with low inducibility of HSP72, the risk of ARF was increased. Therefore, VLBW neonates with (1267)GG might express less HSP72 and might be less protected against ARF. **(*Pediatr Res* 54: 452–455, 2003)**

Abbreviations

HSP, heat shock protein
ARF, acute renal failure
VLBW, very low birth weight
NEC, necrotizing enterocolitis
IRDS, infant respiratory distress syndrome
PDA, patent ductus arteriosus

ARF is a severe complication of immaturity, affecting up to 40% of VLBW neonates born with a birth weight <1500 g (1). Despite evident progression in clinical therapy, ARF still remains a leading risk factor of perinatal morbidity (2). Pathologic conditions that are associated with disturbed renal hemodynamics (*e.g.* fetal distress, sepsis, cardiac failure, NEC, and IRDS) all predispose to ARF in preterm neonates (3).

HSPs, particularly the HSP70 family, are ideal candidates for cytoprotective substances against ARF. They act by refolding of disrupted proteins, thereby limiting tubular injury, restoring renal function and accelerating renal recovery (4). HSP70 family includes HSP73, which is expressed constitutively, whereas HSP72 is inducible by various cell stressors, such as hyperthermia, hypoxia, inflammation, and toxic agents (5). The significance of adequate HSP72 production has been extensively investigated in renal pathology of the fetus and neonate in animal models (6). Immature rat kidneys were previously demonstrated to exhibit a rapid HSP72 expression after heat stress and anoxia (7). Recent studies described that tolerance to renal ischemia of the neonatal rat kidney was associated with increased expression of HSP72 (8).

Besides environmental factors, genetic polymorphisms were also suggested to influence the production of HSP70s. Decreased HSP72 expression was measured in homozygous carriers of G variants at the 1267 site of the coding region of

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HSP72 gene (9). For HSP73, a biallelic G (190)C polymorphism in the 5'UT region was reported, but no functional consequence was established until now (10).

Recent studies indicate that HSP72 A(1267)G polymorphism does associate with the risk and outcome of a variety of hypoxia-related diseases (11, 12), but data have not been available for distressed neonates until now. In the present study, we tested the hypothesis that HSP72 A(1267)G and HSP73 G (190)C genotypes are associated with the prevalence of ARF in VLBW neonates.

METHODS

Patients. The present study was conducted at the neonatal intensive care unit (NICU) of the 1st Department of Pediatrics, Semmelweis University, Budapest. One hundred and thirty singleton VLBW neonates [48 boys and 72 girls; median gestational age: 29 wk (range, 24–33 wk); birth weight: 1180 g (range, 640–1500 g)] born between January 1 and June 30, 1999) were eligible for the study. This population is partly identical to that of a previously published study (13). Medical records of the enrolled VLBW neonates were systematically reviewed. Data on sex, birth weight, gestational age at birth, and neonatal clinical histories up to the end of first postnatal month were collected and entered to a database.

The diagnostic criteria for ARF were identical to those of Modi (3): serum creatinine >120 μmol/L and/or serum urea >9 mmol/L, diuresis 1.0 mL urine/kg/h. The median duration of oliguria was 65 h (range: 32–120 h). Based on these criteria, between d 3 and 8 of life, ARF developed in 37 VLBW neonates [ARF group; gestational age: 29 wk (range: 24–32 wk); birth weight: 1190 g (range: 640–1490 g)], whereas in 93 patients kidney function was not affected [gestational age: 29 wk (25–33 wk); birth weight: 1170 g (680–1500 g)]. The occurrence of established risk factors of ARF, such as severe hypotension (dobutamine requirement >9 μg/birth weight kg/min), sepsis, PDA, NEC, and IRDS were recorded. Neonates with ARF were treated by furosemide (1 mg/kg/dose) and dopamine (0.5–2.0 μg/kg/min) until oliguria resolved. One infant received peritoneal dialysis. ARF ceased after 120 h in each infant. All of the enrolled neonates survived the 10th postnatal day; five from the ARF group and three from non-ARF group died during the first postnatal month because of sepsis ($n = 6$) or intracerebral bleeding ($n = 2$). ARF did not lead to neonatal death in any of the patients.

Healthy subjects. The prevalence of HSP72 A(1267)G and HSP73 G (190)C genotypes was obtained by studying a random, unrelated population sample of healthy adults. Control dried blood samples were acquired from the National Institute for Hematology and Immunology. Each of control persons filled out a detailed questionnaire, in which none of them reported to be born as low birth weight infant. All cases and controls were of Hungarian ethnic origin and resided in Hungary.

Samples and genotyping. Remnant dried blood samples taken for phenylketonuria analysis after postnatal d 5, at the introduction of oral feeding, were used for genotyping. DNA was extracted by using a standard phenol-chloroform extraction (14). HSP72 gene A(1267)C polymorphism was detected with a restriction fragment length polymorphism method by *Pst*I restriction enzyme digestion from the fragment of 189 bp DNA, previously amplified with the following primers: forward: 5'-ACCCTGGAGCCCGTGGAGAA-3' and reverse: 5'-CACCCGCCCGCCCCGTAGG -3'. For G (190)C polymorphism of HSP73 gene *BsrBI*, restriction enzyme digestion was used from the fragment of 196 bp DNA, which was previously amplified by using the following primers: forward: 5'-CGACCTGGGCACCACCTACTCC-3' and reverse: 5'-AATCAGGCGCTTCGCGTCAAAC-3'. Annealing temperature was 61°C in each case. The cleaved PCR products were electrophoresed on 3% agarose gel stained with ethidium bromide (Fig. 1).

Statistical analysis. All samples received a unique three-digit number at the beginning of data processing, and during further analyses this number was used without referring to the patient name. Hardy-Weinberg equilibrium was calculated to evaluate the relationship between gene and genotype frequencies. Clinical characteristics (birth weight, gestational age) between premature neonates with and without ARF were compared by Mann-Whitney *U* test. Categorical data were analyzed by χ^2 test. A stepwise binary logistic regression approach was applied to determine the independent effect of the established risk factors to the development of ARF. For linkage and distribution calculations Arlequin software (available at: http://anthropologie.unige.ch/arlequin/) was used. The level of significance was set at $p < 0.05$.

Ethics. Ethical committees of the Institutional Review Board of Semmelweis University (TUKEB 13/2003) and the National Institute for Hematology and Immunology approved the present study. Informed consent of healthy controls and informed parental consent at the beginning of the therapy at the NICU was obtained to collect dried blood samples for diagnostic and scientific purposes.

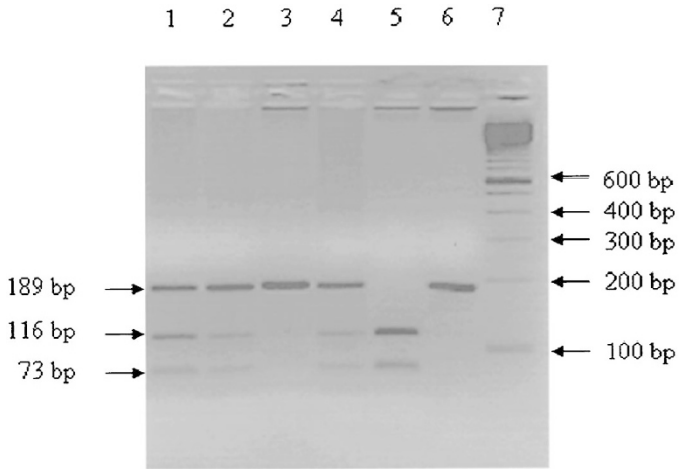

Figure 1. Restriction length fragment polymorphism analysis: *Pst* I digestion of the PCR product of HSP72 gene. The DNA molecular weight marker is shown in *lane 7*. *Lanes 3* and *6* represent samples from individuals of HSP72 (1267)AA wild-type allele; *lanes 1, 2,* and *4* samples from heterozygous (1267)AG allele; and *lane 5* a sample of homozygous allele for (1267)GG mutation. Sizes of DNA marker fragments are indicated on the right, sizes of the fragments from digestion are shown on the left.

RESULTS

Genotype distributions are presented in Table 1. There was no linkage between the prevalence of HSP73 G(190)C and HSP72 A(1267)G allele frequencies. Distribution of HSP73 G (190)C alleles was equal in VLBW neonates with and without ARF. This genetic variant was not associated with any of the perinatal complications.

HSP72 (1267)G allele occurred more frequently in VLBW neonates than in healthy subjects ($p = 0.01$). HSP72 (1267)GG genotype was associated with a higher risk of ARF (odds ratio, 3.17; 95% confidence interval, 1.34–7.45; $p < 0.01$). The length of ARF, however, did not associate with the carrier state of HSP72 allelic variant. The association between HSP72 (1267)GG genotype and ARF remained at the level of significance ($p = 0.05$) when it was adjusted for sepsis, PDA, NEC, severe hypotension, and IRDS (Table 2). Stepwise logistic regression analysis revealed HSP72 (1267)GG genotype, PDA, and early onset sepsis as independent risk factors of ARF ($p = 0.02$, $p = 0.05$, and $p = 0.001$, respectively). Besides ARF, IRDS was also more prevalent in VLBW neonates carrying (1267)GG genotype ($p < 0.05$). However, for HSP72 A(1267)G gene polymorphism, Hardy-Weinberg criteria were fulfilled neither in VLBW nor in the reference population ($p < 0.001$).

DISCUSSION

In the present study, we analyzed the prevalence of HSP73 G(190)C and HSP72 A(1267)G genetic polymorphisms in VLBW neonates with and without ARF during the first postnatal week. Our data indicate an association between HSP72 (1267)GG homozygosity and the risk of ARF in VLBW neonates.

Developmental studies suggest that HSP72 plays a fundamental role in the ischemic tolerance of the immature kidney (7). Vicencio *et al.* (8) have demonstrated a temporal correlation between a peak of HSP72 protein expression and a consequent protection of immature animals against hypoxic renal injury. In experimental ARF, renal ischemia has been shown to induce HSP72 synthesis during recovery of the proximal tubular cells (15), and low levels of HSP72 have been considered as detrimental for renal recovery (16, 17).

Although previous studies investigated the relevance of HSP72 and HSP73 genetic polymorphisms in heart and brain, there has been no data available in the kidney yet (11, 12). Our data are the first to demonstrate an association between ARF and HSP72 (1267)GG genetic variation in VLBW neonates. This genotype has been previously associated with impaired inducibility of HSP72 (8). Therefore, it is tempting to speculate that low HSP72 levels, which are putatively present in VLBW neonates carrying HSP72 (1267)GG, would increase the risk of ARF.

Literature data do support that HSP70s are significant determinants of the fetal development, inasmuch as they are expressed throughout the whole fetal maturation period and they have a variety of housekeeping and embryo-protective roles. In our study, we found that the HSP72 (1267)GG genetic variant, which has been associated with low HSP72 expression, is more prevalent in VLBW neonates than in healthy control population. This raises the possibility that the HSP72 (1267)GG genotype might be associated with premature birth as well.

As found in other studies, a genetic imbalance exists even in our reference population with the absence of AA genotype. This observation corresponds to those obtained in previous studies (18–20). These results suggest that HSP72 (1267)AG genetic variants might have biologic relevance associated with a selection bias (20). However, the relevance of these genetic polymorphisms should be treated cautiously in case of complex multifactorial diseases such as prematurity and acute renal failure.

Table 1. *Genotypic frequencies at the HSP72 A(1267)G and HSP73 G(190)C loci in VLBW neonates with and without ARF compared with healthy reference population*

		HSP 72 A(1267)G			HSP73 G(190)C		
Group	N	AA	AG	GG	GG	GC	CC
Healthy controls	131	25	93	13	3	35	93
VLBW neonates total	130	9*	87	34*	3	37	90
VLBW neonates with ARF	37	2	19	16**	0	12	25
VLBW neonates without ARF	93	7	68	18	3	22	68

* $p < 0.05$ *vs* healthy reference value; ** $p < 0.05$ *vs* VLBW neonates without ARF.

Table 2. *Perinatal complications and HSP 72 (1267)AG allele frequencies in VLBW neonates*

Group	N	HSP72 (1267)AA	HSP72 (1267)AG	HSP72 (1267)GG
Total VLBW neonates	130	9	87	34
Neonates with ARF	37	2	19	16
Established risk factors of ARF				
Severe hypotension	39	4	27	8
NEC	43	1	35	7
Early onset sepsis	32	2	21	9
IRDS	58	4	33	21*
PDA	46	3	30	13

* $p < 0.05$ *vs* VLBW neonates without (1267)AA.

In VLBW neonates, IRDS was also more prevalent in homozygous carriers of HSP72 (1267)G allele. This supports the assumption that altered expression of HSP70 proteins may influence fetal development of the lungs (21). The inherited partial deficiency of HSP72 inducibility might be associated with delayed lung development in VLBW neonates and might be an explanation for the increased prevalence of IRDS in the presence of HSP72 (1267)GG genotype.

In summary, our results indicate that the risk of ARF is increased in VLBW neonates carrying HSP72 (1267)GG genetic variation, independently of the presence of other risk factors. HSP72 (1267)GG genotype, which is associated with low HSP72 inducibility, is also more prevalent in a VLBW population than in healthy controls. These results could raise further questions about the clinical relevance of HSP polymorphisms.

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REFERENCES

1. Tóth-Heyn P, Drukker A, Guignard JP 2000 The stressed neonatal kidney: from pathophysiology to clinical management of neonatal vasomotor nephropathy. Pediatr Nephrol 14:227–239
2. Kelly KJ, Molitoris BA 2000 Acute renal failure in the new millennium: time to consider combination therapy. Semin Nephrol 20:4–19
3. Modi N 1999 Disorders of the kidney and urinary tract. In: Rennie JM, Roberton NR (eds) Textbook of Neonatology. Churchill-Livingston, Edinburgh, pp 1009–1037
4. Molitoris BA 1991 New insights into the cell biology of ischemic acute renal failure. J Am Soc Nephrol 1:1263–1270
5. Beck FX, Neuhofer W, Müller E 2000 Molecular chaperones in the kidney: distribution, putative roles and regulation. Am J Physiol 279:F203–F215
6. Ozer EA, Yilmaz O, Akhisaroglu M, Tuna B, Bakiler AR, Ozer E 2002 Heat shock protein 70 expression in neonatal rats after hypoxic stress. J Matern Fetal Neonatal Med 12:112–117
7. Gaudio KM, Thulin G, Mann A, Kashgarian M, Siegel NJ 1998 Role of heat stress response in the tolerance of immature renal tubules to anoxia. Am J Physiol 274:F1029–F1036
8. Vicencio A, Bidmon B, Ryu J, Reidy K, Thulin G, Mann A, Gaudio KM, Kashgarian M, Siegel NJ 2003 Developmental expression of HSP-72 and ischemic tolerance of the immature kidney. Pediatr Nephrol 18:85–91
9. Pociot F, Ronningen KS, Nerup J 1993 Polymorphic analysis of the human MHC-linked heat shock protein 70 (HSP70–2) and HSP70-Hom genes in insulin-dependent diabetes mellitus (IDDM). Scand J Immunol 38:491–495
10. Favatier F, Bornman L, Hightower LE, Gunther E, Polla BS 1997 Variation in HSP gene expression and HSP polymorphism: do they contribute to differential disease susceptibility and stress tolerance? Cell Stress Chaperones 2:141–155
11. Bolla MK, Miller GJ, Yellon DM, Evans A, Luc G, Cambou JP, Arveiler D, Cambien F, Latchman DS, Humphries SE, Day IN 1998 Analysis of the association of a heat shock protein 70–1 gene promoter polymorphism with myocardial infarction and coronary risk traits. Dis Markers 13:227–235
12. Zee RY, Bates D, Ridker PM 2002 A prospective evaluation of the heat shock protein 70 gene polymorphisms and the risk of stroke. Thromb Haemost 87:622–625
13. Nobilis A, Kocsis I, Tóth-Heyn P, Treszl A, Schuler A, Tulassay T, Vásárhelyi B 2001 Variance of ACE, and AT1 receptor gene do not influence the risk of neonatal acute renal failure. Pediatr Nephrol 16:1063–1066
14. Miller SA, Dykes DD, Polesky HE 1988 A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 44:628–632
15. Van Why SK, Hildebrandt F, Ardito T, Mann AS, Siegel NJ, Kashgarian M 1992 Induction and intracellular localization of HSP-72 after renal ischemia. Am J Physiol 263:F769–F775
16. Kelly KJ, Baird NR, Greene AL 2001 Induction of stress response proteins and experimental renal ischemia/reperfusion. Kidney Int 59:1798–1802
17. Luft JC, Dix DJ 1999 HsP 70 expression and function during embryogenesis. Cell Stress Chaperones 4:162–170
18. Schulte CM, Dignass AU, Goebell H, Roher HD, Schulte KM 2000 Genetic factors determine extent of bone loss in inflammatory bowel disease. Gastroenterology 119:909–920
19. Mestiri S, Bouaouina N, Ahmed SB, Khedhaier A, Jrad BB, Remadi S, Chouchane L 2001 Genetic variation in the tumor necrosis factor-alpha promoter region and in the stress protein hsp70–2: susceptibility and prognostic implications in breast carcinoma. Cancer 91:672–678
20. Schroder O, Schulte KM, Ostermann P, Roher HD, Ekkernkamp A, Laun RA 2003 Heat shock protein 70 genotypes HSPA1B and HSPA1L influence cytokine concentrations and interfere with outcome after major injury. Crit Care Med 31:73–79
21. David JC, Tanguay RM, Grongnet JF 2001 Perinatal expression of heat shock proteins HSC 70 and HSP 70 in neural and non-neural tissues of the piglet. Brain Res Dev Brain Res 128:91–99