The Protein Polymorphism of Haptoglobin in Korean Elite Athletes

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Key Words

Endurance · Genotype and haptoglobin · Korean male athletes

Abstract

Objective: To investigate protein polymorphism of the haptoglobin (Hp) and the relationship between Hp phenotypes and anthropometric or biochemical parameters in elite Korean male athletes. **Materials and Methods:** Serum samples were collected from 120 Korean male elite athletes. The Hp phenotypes were determined by polyacrylamide gel electrophoresis, followed by peroxidase staining. Then anthropometric or biochemical measurements were made: body composition, blood pressures, ventilatory responses, cholesterol (total, LDL cholesterol and HDL cholesterol), triglyceride, apolipoprotein A1, lipoprotein (a), creatine phosphokinase and lactate dehydrogenase. **Results:** The gene frequencies of the Hp1-1, Hp2-1 and Hp2-2 phenotypes in Korean male athletes were 12, 37 and 51%, respectively; this polymorphism was significantly associated with the VO_{2max} index in the athletes. An excess of the Hp1 allele was also observed in marathon runners compared with the other sporting activities, although it did not have any statistical significance. **Conclusion:** Hp polymorphism exists in

elite Korean male athletes and Hp phenotype may be a useful marker for endurance performance in these male athletes.

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Introduction

Cardiovascular function is an important element of athletic performance. A number of studies have suggested that many genes contribute to this function [1, 2] and that several causative genes contribute to the genetic make-up of athletic performance [3–5]. One such candidate gene is the haptoglobin (Hp) [2]. Hp is one of the serum proteins that shows genetic polymorphism [6], and it functions as a hemoglobin-binding protein [7] or acute-phase protein [8]. In addition, it has many other properties [9]. Hp protein polymorphism manifests in three phenotypes: Hp1-1, Hp2-1 and Hp2-2, as shown by polyacrylamide gel electrophoresis. The synthesis of this protein is controlled by a single gene located on chromosome 16 [10]. Hp polymorphism is known to be associated with various cardiovascular diseases [11–15] or immune diseases [16–19]. However, to our knowledge, there is no report on the relationship between Hp polymorphism and athletic performance.

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In this study, the distribution of the Hp phenotypes was investigated among elite Korean male athletes. In addition, the relationship between Hp phenotypes and anthropometric or biochemical parameters of athletic groups was explored.

Subjects and Methods

Subjects

A total of 120 male elite athletes [15 basketball, 25 football, 32 baseball and 18 volleyball players, 12 gymnasts, 9 judo practitioners, 5 marathon and 4 middle-distant ($\geq 5,000$ m) runners] were randomly selected from the students of the Department of Physical Education, Hanyang University, Seoul, Korea.

Determination of Anthropometric and Biochemical Parameters

Blood samples were obtained in EDTA tubes from the subjects who had been fasting for 12–16 h. A sphygmomanometer was used to measure systolic and diastolic blood pressures (SBP and DBP, respectively). The mean arterial pressure was calculated by $DBP - 1/3(SBP - DBP)$ (mm Hg). The VO_{2max} index was measured by using motor-driven treadmills [20]. The body mass index value was calculated dividing the body weight (kg) by the square of the height (m^2). Concentration of total cholesterol and triglyceride was measured by enzymatic colorimetric methods with a commercial kit (Boehringer Mannheim, Germany) and chemistry analyzer. High-density lipoprotein cholesterol was determined by measuring cholesterol in the supernatant after precipitation of the serum with $MgCl_2$ and dextran sulfate, with a Gilford Impact 400E automated analyzer with reagents and calibrators from Boehringer Mannheim. Lipoprotein(a) level was measured by the immunoprecipitation method (SPQ Test System, Incstar Corporation, Stillwater, Minn., USA) and apolipoprotein A1 concentration was determined by the immunoturbidimetric method (Cobas Integra, Roche Diagnostics, USA). Low-density lipoprotein cholesterol level was calculated using the formula by Friedewald et al. [21]. Serum lactate dehydrogenase and creatine phosphokinase activity were measured by ultraviolet assay.

Hp Phenotyping

Hp phenotypes were determined by polyacrylamide gel electrophoresis and peroxidase staining with benzidine as previously described [22]. Briefly polyacrylamide gel was prepared with Peacock's gel buffer (pH 8.29). The composition of electrode buffer was 0.089 M of tris(hydroxymethyl)aminomethane, 0.0025 M of EDTA, and 0.091 M of boric acid (pH 8.29). Electrophoresis was carried out at 200 V for 30 min. The sampling mixture contained 50 μ l serum, 5 μ l blood hemolysate, and a little crystal sucrose. Staining of the gel was performed by adding the following mixture: 60 mg of benzidine, 2 ml of glacial acetic acid, 2 ml of dimethyl sulfoxide, 2 ml of H_2O_2 and 600 ml of distilled water.

Statistical Analysis

Allele frequencies were estimated by the gene counting method. Deviation in genotype distribution from that expected for the Hardy-Weinberg equilibrium was estimated by the χ^2 fitness test. The significance of differences in allele frequencies between populations was also estimated by the χ^2 independence test. The comparisons of the

Fig. 1. Polymorphic patterns of the Hp. Lanes 1 and 10: Hp1-1 genotypes; lanes 2, 5–7 and 9: Hp2-1 genotypes; lanes 3, 4 and 8: Hp2-2 genotypes.

variables across the phenotypes were performed by using a parametric one-way ANOVA test with Scheffe's multiple comparison test. Statistical significance was set at $p = 0.01$. All statistical analyses were performed by the computer program of Statistica (version 6.0).

Results

Phenotype Distribution

The polymorphic patterns of Hp in the Korean male athletes are shown in figure 1 and the gene frequencies in table 1. The frequencies of the Hp1-1, Hp2-1 and Hp2-2 phenotypes were 12, 37 and 51%, respectively. The observed phenotype distribution did not significantly deviate from the Hardy-Weinberg equilibrium. Derived allele frequencies for Hp1 and Hp2 were 0.31 and 0.69%, respectively. Among the 8 different athletic groups, the highest excess of the Hp1 allele was observed in marathon runners, although this was not statistically significant.

Association with Biochemical Parameters

Table 2 presents the comparison of anthropometric data and intermediate phenotypes across Hp polymorphism in male elite athletes. There were significant differences in the VO_{2max} index across the phenotypes (one-way ANOVA test, $p = 0.008$). Hp1-1 homozygotes (57.0 ± 1.3 ml/kg/min) had significantly higher values for the VO_{2max} index than Hp2-2 homozygotes (55.4 ± 1.7 ml/kg/min; Scheffe's multiple comparison, $p = 0.008$), but marginally higher than Hp2-1 heterozygotes (55.7 ± 1.3 ml/kg/min; Scheffe's multiple comparison, $p = 0.052$). However,

Table 1. Distribution of Hp protein polymorphism in the Korean male elite athletic groups

Subjects	Haptoglobin				
	phenotypes			alleles	
	Hp1-1	Hp2-1	Hp2-2	Hp1	Hp2
Basketball (n = 15)	1 (7)	3 (20)	11 (73)	5 (17)	25 (83)
Soccer (n = 25)	3 (12)	9 (36)	13 (52)	15 (30)	35 (70)
Baseball (n = 32)	3 (9)	14 (44)	15 (47)	20 (31)	44 (69)
Gymnastics (n = 12)	3 (25)	3 (25)	6 (50)	9 (38)	15 (62)
Volleyball (n = 18)	4 (22)	6 (33)	8 (45)	14 (39)	22 (61)
Runner ¹ (n = 4)	0 (0)	2 (50)	2 (50)	2 (25)	6 (75)
Judo (n = 9)	0 (0)	4 (44)	5 (56)	4 (22)	14 (78)
Marathon (n = 5)	1 (20)	3 (60)	1 (20)	5 (50)	5 (50)
Total (n = 120)	15 (12)	44 (37)	61 (51)	74 (31)	166 (69)

¹ Figures in parentheses are percentages.
Middle-distant runner ($\geq 5,000$ m).

Table 2. The comparison of the anthropometric data and biochemical parameters according to Hp phenotypes in male elite athletic groups among Koreans

Variables	Phenotypes					
	Hp1-1		Hp2-1		Hp2-2	
Age, year	19.9 $\pm$	1.1 (11)	20.3 $\pm$	1.2 (35)	20.4 $\pm$	1.2 (49)
BMI (kg/m ²)	21.6 $\pm$	1.7 (12)	23.0 $\pm$	1.6 (35)	23.3 $\pm$	2.1 (49)
VO _{2max} ^a , ml/kg/min	57.0 $\pm$	1.3 (12)	55.7 $\pm$	1.3 (35)	55.4 $\pm$	1.7 (49)
SBP, mm Hg	117.6 $\pm$	8.1 (12)	119.8 $\pm$	8.1 (36)	118.7 $\pm$	7.8 (51)
DBP, mm Hg	69.3 $\pm$	8.4 (12)	72.5 $\pm$	7.5 (36)	72.6 $\pm$	6.4 (51)
MAP, mm Hg	85.3 $\pm$	7.1 (12)	88.3 $\pm$	6.8 (36)	88.0 $\pm$	5.9 (51)
Tg, mg/dl	92.9 $\pm$	53.3 (15)	108.0 $\pm$	83.2 (44)	102.9 $\pm$	62.7 (61)
TC, mg/dl	161.4 $\pm$	23.4 (15)	169.6 $\pm$	27.4 (44)	179.8 $\pm$	46.8 (61)
LDL-chol, mg/dl	78.2 $\pm$	33.1 (15)	87.8 $\pm$	28.7 (44)	100.2 $\pm$	47.6 (61)
HDL-chol, mg/dl	57.7 $\pm$	12.6 (15)	58.6 $\pm$	12.6 (44)	57.5 $\pm$	12.2 (61)
Lp(a), mg/dl	13.0 $\pm$	18.0 (6)	7.2 $\pm$	4.3 (22)	8.2 $\pm$	7.7 (32)
Apo, mg/dl	88.4 $\pm$	20.3 (15)	111.0 $\pm$	33.2 (44)	102.4 $\pm$	33.8 (61)
CPK, IU/l	794.9 $\pm$	1,332.9 (15)	625.0 $\pm$	873.0 (43)	412.0 $\pm$	639.3 (61)
LDH, IU/l	486.7 $\pm$	139.7 (15)	457.1 $\pm$	99.0 (44)	449.8 $\pm$	89.4 (61)

BMI = Body mass index; MAP = mean arterial pressure; Tg = triglyceride; TC = total cholesterol; LDL-chol = LDL cholesterol; HDL-chol = HDL cholesterol; Lp(a) = lipoprotein(a); ApoA1 = apolipoprotein A1; CPK = creatine phosphokinase; LDH = lactate dehydrogenase. Values are mean $\pm$ SD. Figures in parentheses indicate number of subjects.

^a Statistically significant difference (one-way ANOVA test, $p = 0.008$).

there was no statistically significant difference between the Hp2-1 and Hp2-2 phenotypes (Scheffe's multiple comparison, $p = 0.645$). A recessive model on the Hp1 allele of the VO_{2max} index provides the best fit for our data, so the Hp1-1 phenotype might be considered a factor for endurance performance in our male athletic group. No association was found between Hp phenotypes and the other anthropometric or biochemical parameters.

Discussion

Many genetic and environmental factors contribute to athletic performance. Specific candidate genes have been tested for association with athletic performance [22–35], and recently, several genetic factors for this phenotype have been gradually elucidated. These include the I/D polymorphism of the angiotensin-I-converting enzyme

gene [22, 25, 26] and the *Dra* I RFLP of the α_{2A} -adrenergic receptor gene [32]. Nevertheless, the genetic variations responsible for athletic performance remain largely unknown [36], and the success to date in identifying causative genes has been very limited.

Delanghe et al. [37] reported that Hp polymorphism was associated with maximal walking distance in peripheral arterial occlusive disease patients with severe atherosclerotic lesions, but the role of Hp polymorphism in elite athletic groups has not been studied. The present study revealed that the VO_{2max} index is significantly associated with the Hp phenotype in elite Korean male athletes. However, if the Bonferroni correction is applied in 16 tests, our result is no longer significant with a p value of 0.003 (0.05/16). Nevertheless, it could be argued that the Bonferroni correction may give a low probability of detecting a small effect of the Hp phenotype, and eventually increase the possibility of type II error. This is why we cannot exclude the possibility that the Hp phenotype is somehow involved in the VO_{2max} index, although multiple comparisons such as these have the potential to generate spurious significances at the $p = 0.01$ level for 1 in every 16 tests. Thus, our result awaits precise confirmation from a large-scale study.

When we examined phenotype and allele frequency of Hp according to each sporting event, there was an excess of the Hp1 allele in marathon runners compared with the other sporting events. This result may imply that the Hp phenotype accounts for a portion of the interindividual differences in athletic endurance performance. It is difficult to explain the mechanism for this association of the Hp phenotypes with endurance performance. However, a plausible explanation is the functional differences between Hp phenotypes. At present, multiple functional differences among these Hp phenotypes [9, 11–15] have been

reported. The Hp1-1 phenotype may influence the athletic performance through mechanisms such as stronger hemoglobin-binding ability and antioxidative capacity compared with other phenotypes [9]. Also, Hp2-2 is associated with various cardiovascular diseases when compared with Hp1-1 phenotypes, suggesting a possible advantage for the Hp1-1 phenotype in cardiovascular function [11–15]. Another possibility is that the effect of the Hp phenotype on the interindividual difference in the VO_{2max} index results from linkage disequilibrium between the Hp phenotype and another causative allele. Some studies have suggested linkage disequilibrium as a mechanism for the association between the Hp phenotype and serum lipid levels [38–40].

This study is the first report of an association between endurance performance and Hp polymorphism in an elite athletic group. Thus, our study may help clarify the genetic basis for endurance performance, and provide a basis for further investigation. It is, however, premature to draw any firm conclusions about the genetic basis of endurance performance in elite Korean athletes because of the relatively small number of subjects ($n = 120$), especially for middle-distance ($n = 4$) and marathon runners ($n = 5$). These studies also need to be confirmed in elite athletes from other ethnic groups.

Conclusion

The results suggest that the distribution of Hp phenotypes in Korean male elite athletes is significantly associated with the VO_{2max} index and may be a useful marker for endurance performance in these elite Korean athletes.

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