SNP A79G in the second exon of the myoglobin gene in elite long distance runners

Myoglobin contains 153 amino acid residues in a compactly folded polypeptide chain and a haem. This small protein facilitates oxygen transport in skeletal and cardiac muscle. Myoglobin plays a crucial role in energy metabolism by carrying molecular oxygen between the capillaries and the mitochondria to satisfy the requirement for sustained work.

The human myoglobin gene has been mapped to chromosome 22q11.2-q13, with three exons and two introns in 10.5 kb pairs. Previous studies found a single nucleotide polymorphism (SNP A79G) in the second exon of the myoglobin gene, with a tendency towards a higher frequency of the 79A allele in high altitude Tibetans than in sea level residents. Whether the genotypes of this gene are associated with aerobic capacity remains an interesting question. We compared the SNP A79G in elite long distance runners and normal people, all members of the Han population of China.

Materials and methods

Subjects

The Han people constitute the majority (93.3%) of China’s 1.3 billion inhabitants, and generally reside in the northern plains. Elite long distance runners mainly come from northern China, particularly northeast China. All the subjects selected for this study came from the Han population of northern China.

DNA samples were obtained from two groups, controls and athletes. There were 5000 m, 10 000 m, and marathon; 53 men and 55.4 (7.6) kg. All subjects were informed about the protocols and aims of the study and gave their written consent.

Genotype analysis

Previous studies analysed the SNP A79G by using polymerase chain reaction (PCR)/single strand conformation polymorphism (SSCP) or DNA sequencing. In this study, we developed a method to detect SNP using PCR/restriction fragment length polymorphism (RFLP). DNA was extracted from lymphoblastoid cell lines using a kit (Promega, Madison, Wisconsin, USA). The forward primer for the PCR/RFLP of SNP A79G was specially designed using Primer 5.0 software. The reverse primer used was the oligonucleotide sequence reported by Fernandez et al. The concrete sequences of primers were as follows:

Forward: 5’-TGAATGCAGGAGGAGATGAAAGC-3’
Reverse: 5’-GCCAGCTCTGCTCTACCCAGTAC-3’

The amplification protocol was: (a) one cycle of denaturation at 95°C for five minutes; (b) 35 cycles of denaturation at 95°C for 45 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 45 seconds; (c) one final 10 minute elongation cycle at 72°C. The final amplification of the 189 bp sequence produced was digested with the restriction enzyme EcoT221 (Takara, Tokyo, Japan) for five hours at 37°C. EcoT221 detected nucleotide A in the SNP A79G. The allele without the EcoT221 restriction site was designated as 189 bp, and the allele with the EcoT221 site was designated as 163/26 bp. These different fragments were separated by electrophoresis at 3% agarose gel at 100 V for two hours, and photographed under UV transmitted lights. The PUC19 DNA marker (MBI, Cascade, Colorado, USA) was used to detect the differences in the alleles and genotypes between the two groups and for comparison with results reported in previous studies. p<0.05 was considered a significant difference between the groups. All statistical analyses were performed with SPSS software for Windows 11.5 package.

Results

The amplified fragment was 189 bp in the SNP A79G and digested by the EcoT221 restriction enzyme. Three genotypes AA, AG and GG were obtained (fig 1 (electrophoretic patterns) and fig 2 (sequence chromatogram)). As shown in table 1, the genotype frequencies were in Hardy-Weinberg equilibrium (p>0.05). There were no significant differences in the frequencies of alleles and genotypes between the athlete and control groups (p>0.05).

The frequency of the 79A allele in the Han population of northern China was significantly different from those reported in white, black, and Hispanic populations and residents of Dallas, Texas (p<0.05), but not significantly different from Japanese (p>0.05, table 2).

Statistical analysis

Allele and genotype frequencies were estimated by the gene counting method. A χ2 test was used to detect the differences in the alleles and genotypes between the two groups and for comparison with results reported in previous studies. p<0.05 was considered a significant difference between the groups. All statistical analyses were performed with SPSS software for Windows 11.5 package.

Figure 1 Electrophoretic patterns observed on poly-merase chain reaction/restriction fragment length polymorphism analysis of the second exon of the myoglobin gene. Lanes 1, 4, 5, homozygous AA; lanes 2, 6, 7, heterozygous AG; lane 3, homozygous GG. The marker is PUC19 DNA marker (MBI, Cascade, Colorado, USA).

Figure 2 Sequence chromatogram of single nucleotide polymorphism in exon 2 of the myoglobin gene from three subjects. (A) Homozygous AA; (B) heterozygous AG; (C) homozygous GG. N is used to denote the nucleotide at the heterozygous AG.
Discussion

Myoglobin is an important protein in oxygen dependent metabolism. Myoglobin gene expression increases under conditions of chronic hypoxia. The second exon of the myoglobin gene has been shown to encode the principal functional portion of the molecule. A tendency towards a higher frequency of the 79A allele has been reported in Tibetans than in sea level residents. It is reasonable to consider that the SNP A79G may be related to aerobic performance, as Tibetans are an ethnographically distinct population with remarkable tolerance to hypoxia.

Although genetic variation in myoglobin has long been recognised in humans, there is little information on the functional implications of such variation and no data from endurance athletes. This study is the first to investigate the SNP A79G in elite long distance runners. However, no significant differences were found in the allele frequencies between athlete and control groups. The reasons for this result are not known, but it might be related to the SNP, which did not affect the coding of amino acids in the myoglobin protein, and no significant changes were shown in the functions of the protein.

Previous studies analysed this SNP using PCR/SSCP or DNA sequencing. We developed a method to detect it using PCR/RFLP and found that the frequency of the 79A allele in the Han population of northern China is different from those reported in white, black, and Hispanic populations and the residents of Dallas, Texas (p<0.05), but not significantly different from a Japanese population (p>0.05). The findings suggest that the differences in races and regions is due to differences in the frequencies of the 79A allele between Asian and other ethnic groups. There is no evidence of an association between the SNP A79G and aerobic capacity.

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Competing interests: none declared

References


Table 1 Frequencies of the single nucleotide polymorphism A79G

<table>
<thead>
<tr>
<th>Genotype (frequency)</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>n AA AG GG A G</td>
<td>A G</td>
</tr>
<tr>
<td>Han population of northern China 312 162 (0.52) 128 (0.41) 22 (0.07) 0.72 0.28</td>
<td></td>
</tr>
<tr>
<td>Elite long distance runners from northern China 106 51 (0.48) 40 (0.38) 15 (0.14) 0.67 0.33</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different (p<0.05) from the Han population of northern China.

Table 2 Frequency of the myoglobin gene 79A allele in different ethnic groups

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>n</th>
<th>79A allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Han population of northern China</td>
<td>312</td>
<td>0.72</td>
</tr>
<tr>
<td>Japanese</td>
<td>100</td>
<td>0.755</td>
</tr>
<tr>
<td>White</td>
<td>238</td>
<td>0.46*</td>
</tr>
<tr>
<td>Black</td>
<td>189</td>
<td>0.41*</td>
</tr>
<tr>
<td>Hispanic</td>
<td>67</td>
<td>0.42</td>
</tr>
<tr>
<td>Dallas, Texas, USA</td>
<td>525</td>
<td>0.43*</td>
</tr>
</tbody>
</table>

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