The relation between the PST1 restriction fragment length polymorphism at the APO-AI locus and plasma APO-AI concentrations in marathon runners

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A PST1 restriction fragment length polymorphism (RFLP), located close to the apolipoprotein Al (apo-Al) gene on chromosome 11, is associated with elevated levels of apo-Al in normal healthy individuals and with depressed levels in patients with coronary heart disease. In both cases the association is with the P2 allele (the allele not containing the PST1 cutting site). Prolonged exercise is known to increase steady-state plasma apo-Al concentrations. We investigated the effect of adaptation to endurance exercise on the association of the PST1 marker with plasma apo-Al levels. Eighty-two male subjects between the ages of 20 and 50 years were randomly selected from a group of local marathon runners. The frequency of the P2 allele was 14% in this group. This was similar to the frequency reported for this RFLP in other population groups of healthy men. Plasma levels of apo-Al were elevated in the marathon runners compared with randomly selected healthy South African men in the same age group. There was, however, no association between the PST1 RFLP and the plasma high-density lipoprotein cholesterol and/or apo-Al concentrations in this group. The elevated apo-Al levels in marathon runners therefore bear no relation to differences associated with the PST1 RFLP at the apo-Al gene locus.

Studies of genetic variation at the apo-Al gene have provided useful but inconclusive information on the effect of variation at this locus on the incidence of CAD. The apo-Al gene is part of a multigene complex also containing the genes for apo-CIII and apo-AIV and is located on chromosome 11 (Fig. 1). Population studies of several polymorphisms of the apo-Al-CIII-AIV gene cluster have shown that some restriction fragment length polymorphisms (RFLPs) of the apo-Al gene are associated with small but significant changes in the levels of plasma HDL cholesterol, apo-Al and triglycerides.

A particular RFLP of the apo-Al gene, detected by digestion with the PST1 enzyme (Fig. 1), has been used as a marker to study genetic variation at the apo-Al gene locus. Studies of this RFLP in patients with CAD from both America and London showed that the frequency of the P2 allele (absence of a PST1 cutting site) was higher in the patient group than in the controls. Furthermore, in the patient group, the P2 allele of this RFLP was associated with lower plasma HDL cholesterol values as well as with lower plasma apo-Al levels. In contrast to these results, studies have shown a negative correlation between total plasma high-density lipoprotein (HDL) cholesterol levels and the development of coronary heart disease (CAD). The level of apolipoprotein Al (apo-Al), the major protein component of HDL, has also been proposed as a negative risk factor for this form of heart disease. Studies of genetic variation at the apo-Al gene have provided useful but inconclusive information on the effect of variation at this locus on the incidence of CAD.

A number of genetic and environmental factors affect steady-state concentrations of plasma lipids and have been implicated as risk factors for atherosclerosis. Environmental factors, including smoking, alcohol, exogenous steroids, diet and exercise, are reported to be responsible for as much as 50% of the variance in plasma lipid levels, with the remainder of the variance being attributed to genetic differences between individuals. Epidemiological studies have shown a negative correlation between total plasma high-density lipoprotein (HDL) levels and the development of coronary heart disease (CAD). The level of apolipoprotein Al (apo-Al), the major protein component of HDL, has also been proposed as a negative risk factor for this form of heart disease. Studies of genetic variation at the apo-Al gene have provided useful but inconclusive information on the effect of variation at this locus on the incidence of CAD.

Fig. 1. Top: map of apo-Al/CIII/AIV gene cluster on chromosome 11: middle: sizes and positions of the different restriction fragments produced when genomic DNA is amplified by PCR using primers specific for the apo-Al gene followed by digestion with the restriction enzyme PST1; bottom: a typical gel shows subjects who are heterozygous (P1P2 genotype) and homozygous (P1P1 genotype) for the PST1 RFLP.
of the PST1 RFLP in healthy subjects revealed that the P2 allele was associated with higher apo-AI and HDL levels.\(^6\) The data from these studies suggest that the levels of apo-AI may be determined by the interaction between variations associated with the PST1 RFLP and a second genetic or environmental factor. An analogous interaction has previously been observed for the apo-E gene.\(^11\)\(^13\)

Training and exercise have been shown in a number of different studies to increase plasma HDL cholesterol and apo-AI levels.\(^14\)\(^16\) Using exercise as an environmental factor, we investigated the effect of adaptation to endurance exercise on the association of the PST1 marker of the apo-AI allele with plasma apo-AI levels. The effect of this adaptation on the levels of plasma lipids associated with the apo-AI gene was also assessed in a group of highly trained male Caucasian marathon runners in South Africa.

Materials and methods

Materials

Restriction endonucleases and DNA polymerase 1 (Klenow) fragment were obtained from Boehringer Mannheim and Amersham, respectively. Thermus aquaticus DNA polymerase 1 (Taq) was obtained from Promega. 5' GACTGGCTTCCACTGCTGTGC 3' [bp 2956 - 2976] and 5' GAGCGCTCTCGAGGAGTACAC 3' [bp 2238 - 2258] and Iipoproteins from the runners are shown in Table I. The mean value of HDL cholesterol for the runners (1,48 mmol/l) was higher than that previously reported for Europeans and South African Caucasians (the average value being 1,2 mmol/l).\(^12\)\(^15\) These data would confirm many previous reports showing that endurance training markedly increases HDL cholesterol levels.\(^6\)\(^5\)

Study population

Blood samples were drawn after 10 - 12 hours of fasting from 82 male Caucasian marathon runners between 20 and 50 years of age. Only those individuals who had taken part in a 56 km marathon and who regularly ran 75 km a week were chosen to take part in the study. Plasma cholesterol and triglyceride levels were determined using commercially available enzymatic procedures (Boehringer Mannheim). Lipoproteins were separated by density gradient ultracentrifugation,\(^6\) and their cholesterol and apoprotein contents were measured using standard methods.\(^6\)

DNA analysis using the polymerase chain reaction (PCR)

Genomic DNA was prepared from leucocytes using a Triton X-100 lysis method.\(^7\) DNA (1 - 3 µg) was amplified by PCR using 1 unit of Taq1 polymerase in 50 µl of reaction mixture containing polymerase buffer and 1 pmol of oligonucleotides. The primers used were: 5' GAGCGCTCTCGAGGAGTACAC 3' [bp 2238 - 2258] and 5' GACTGGCTTCCACTGCTGTGC 3' [bp 2956 - 2976].

The cycling reactions were carried out in a programmable heating block as follows: 93° for 5 minutes, 62° for 2.5 minutes and 72° for 3 minutes over 1 cycle, followed by 30 cycles at 93° for 0.5 minute, 62° for 1 minute and 72° for 2 minutes. The amplified DNA was digested with PST1 for 24 hours at 37°. Fragments were separated on a 2% agarose gel and ethidium bromide-stained gels were visualised and photographed under ultraviolet light (Fig. 1).

Statistical analysis

Statistical analyses were performed using the computer programme STATPAK.\(^8\) The level of statistical significance was taken as \(P < 0.05\). For the polymorphisms, gene frequencies were determined by gene counting. In this sample, the HDL cholesterol, apo-AI and triglyceride concentrations were normally distributed. One-way analysis of variance was used to test the null hypothesis that phenotypic variation was not associated with genotype.

Results

A gene-environment interaction, in this case between endurance exercise and variation at the apo-AI gene, could manifest itself in one of two ways in a population study: by alteration of the frequency of the polymorphism ('self-selection' as marathon runners), or by association of the genotype with the apolipoprotein phenotype. We attempted to verify this in marathon runners.

The means and standard deviations (SDs) of plasma lipids and lipoproteins from the runners are shown in Table I. The mean value of HDL cholesterol for the runners (1,48 mmol/l) was higher than that previously reported for Europeans and South African Caucasians (the average value being 1,2 mmol/l).\(^12\)\(^15\) These data would confirm many previous reports showing that endurance training markedly increases HDL cholesterol levels.\(^6\)\(^5\)

The PST1 polymorphism is present at bp 2636 from the apo-AI transcriptional start site, between the apo-AI and apo-CIII genes (Fig. 1). This PST1 polymorphism was detected using PCR amplification of DNA followed by PST1 digestion of the amplified DNA. In the absence of the cutting site (P2 allele) a 740 bp band was visualised, whereas in the presence of the cutting site (P1 allele) two bands were observed, one at 400 bp and the other at 340 bp (Fig. 1).

The genotypes and allele frequencies of the PST1 polymorphism are presented in Table II. In this population, the rare allele is the P2 allele with a frequency of 0,14 (Table II). This frequency does not differ from values reported for other population groups at this RFLP (values range from 0,06 to 0,11).\(^12\)\(^23\)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>Allele</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1P1</td>
<td>0,75</td>
<td>P1</td>
<td>0,86</td>
</tr>
<tr>
<td>P1P2</td>
<td>0,20</td>
<td>P2</td>
<td>0,14</td>
</tr>
<tr>
<td>P2P2</td>
<td>0,05</td>
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Distribution did not differ from expected Hardy Weinberg proportions.\(^6\) Allele frequencies were determined by gene counting.
To assess the effect of apo-AI genotype on phenotypic variation in lipid levels, individuals were grouped according to their genotype, and within each group the means and SDs of their lipid and lipoprotein levels were determined (Table III). One-way analysis of variance was used to compare lipid and lipoprotein levels between the groups containing a particular genotype.

Individuals homozygous for the rare P2 allele had 10% higher apo-AI concentrations than individuals with the P1P1 genotype (Table III). The increase did not, however, reach statistical significance. The increase in apo-AI levels was accompanied by a small but non-significant decrease in triglyceride levels in individuals homozygous for the P2 allele, relative to those of the P1P1 genotype (Table III).

**Discussion**

The candidate gene approach in the study of CAD risk factors has been plagued with conflicting results. These are highlighted in association studies between RFLPs at the apo-AI gene locus and changes in lipid and apolipoprotein levels.

In 1986, Ordovas et al. showed a strong association between the rare P2 allele and CAD. In a 1991 study, however, the PST1 RFLP data did not reveal any significant differences in lipid or apolipoprotein levels associated with the different genotypes, and although the P2 allele frequency was higher in the CAD patients, this increase was not significant. In contrast, epidemiological studies of the PST1 RFLP in healthy Caucasians have shown an increase in apo-AI and HDL levels associated with the P2 allele.

Association studies are complicated by gene-gene and gene-environment interactions. Pedersen and Berg have shown, in two separate studies, that the interaction between variation at the LDL receptor and apo-E loci enhances the association with changes in lipid levels.

In this study we attempted to address the gene-environmental relationship and its influence on the association between the PST1 RFLP and lipid changes, by investigating this association in a group of highly trained endurance athletes.

Exercise has been shown to increase HDL cholesterol values in a number of studies. There is also a significant correlation between levels of physical activity and apo-AI levels. In the group of runners studied here, the levels of HDL cholesterol were on average higher than those from a control population of South African Caucasians. Furthermore, the levels of HDL cholesterol and apo-AI in the marathon runners were on average higher than those previously reported for Caucasians in Europe. The higher levels of HDL cholesterol and apo-AI observed in this population were therefore presumably due to the effects of training on plasma HDL levels. These results provide us with an opportunity to investigate the influence of the environmental factor, exercise, on the association between the PST1 RFLP and lipid measurements.

The frequency of the P2 allele in this population, viz. South African male marathon runners, was similar to that reported for other populations, and this would imply that there is no association between either the P1 or the P2 allele and the ability to perform sustained strenuous exercise. It was important to establish this so as to be certain that we were not working with a selected population.

Analysis of the phenotypic data according to the genotype in these marathon runners showed that there was a slight association between the P2P2 genotype and higher apo-AI levels, but this was not statistically significant (Table III). This seems to be consistent with previous results where the P2 allele was associated with higher apo-AI levels in healthy individuals.

The small decrease observed in triglyceride levels associated with the P2 allele in marathon runners was also not statistically significant (Table III). No other differences in lipid levels between the various PST1 genotypes were observed. These data indicate that exercise does not induce an association of the genotype with the apo-AI phenotype.

Our original postulate was that the increase in apo-AI levels due to training was mediated via the variation associated with the P2 allele. The lack of fitness in CAD patients would have been responsible for the association of the P2 allele with low apo-AI in this group. The converse of this would have been true in healthy individuals (i.e. those with a higher level of fitness) where the P2 allele was associated with high apo-AI levels.

Our results indicate that exercise is not an environmental factor affecting variation in apo-AI levels associated with the PST1 RFLP. Furthermore, the data presented in this paper would imply that the mechanism responsible for the exercise-related increase in apo-AI levels is not related to the variation associated with the PST1 RFLP at the apo-AI gene locus. Exercise would also not be the factor responsible for the contrasting data obtained in various association studies between the PST1 RFLP and lipid levels. Further studies are required to elucidate those factors responsible for the association of the P2 allele with increased apo-AI levels in healthy individuals but lower apo-AI levels in CAD subjects.

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**REFERENCES**

Short-term relationships between winter temperatures and cardiac disease mortality in Cape Town

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The short-term relationship between winter temperatures and cardiac disease mortality in Cape Town was investigated for the period 1978 - 1985. The mortality data were stratified according to age (0 - 4 years, 5 - 59 years, 60 years and older), while the temperature data comprised daily maximum and minimum temperatures at Cape Town International Airport. The analyses identified a strong negative relationship between extreme (high and low) temperatures and daily mortality, and it was established that large daily temperature variations were associated with above-average mortality rates.


Cardiovascular disease is the leading cause of death among white, coloured and Asian South Africans. According to 1986 statistics, it accounts for approximately 40% of all natural deaths in these population groups (whites 46%; Asians 43%; coloureds 26%). Among blacks, deaths due to 'symptoms, signs and ill-defined conditions' are most prevalent, followed by those resulting from infections and parasitic diseases. Diseases of the circulatory system rank third (15%) as a cause of natural deaths, and accounted for 11 367 deaths in 1986.

In most countries, cardiovascular disease mortality (CDM) has a seasonal distribution, peaking during winter. A number of studies have, in fact, linked rises in CDM to cold weather. Although these associations are most marked when temperatures are abnormally low, moderate temperature falls are also at times accompanied by small increases in mortality. As a result of these factors, the estimation of the magnitude of the mortality increase also appears to be associated with the suddenness of onset of severe weather and with its duration. However, while the

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