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CROSS-SECTIONAL STUDY OF A MICROSATELLITE MARKER IN THE LOW DENSITY LIPOPROTEIN RECEPTOR GENE IN OBESE NORMOTENSIVES

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SUMMARY

1. The low density lipoprotein receptor is an important regulator of serum cholesterol which may have implications for the development of both hypertension and obesity. In this study, genotypes for a low density lipoprotein receptor gene (*LDLR*) dinucleotide polymorphism were determined in both lean and obese normotensive populations.

2. In previous cross-sectional association studies an *ApaLI* and a *HincII* polymorphism for *LDLR* were shown to be associated with obesity in essential hypertensives. However, these polymorphisms did not show an association with obesity in normotensives.

3. In contrast, this study reports that preliminary results for an *LDLR* microsatellite marker, located more towards the 3' end of the gene, show a significant association with obesity in the normotensive population studied. These results indicate that *LDLR* could play an important role in the development of obesity, which might be independent of hypertension.

Key words: association studies, molecular genetics, obesity, PCR, polymorphism.

INTRODUCTION

The low density lipoprotein (LDL) receptor (*LDLR*) is involved in serum cholesterol regulation and its gene has been implicated in familial hypercholesterolaemia, a syndrome affecting 0.2% of Caucasians (Hobbs *et al.* 1990). *LDLR* has been reported to show linkage to the atherogenic lipoprotein phenotype, a common inherited trait that increases the risk of myocardial infarction (Nishina *et al.* 1992). More recently, restriction fragment length polymorphisms (RFLP) for *LDLR* have been

shown to have a marked association with obesity in essential hypertensives (Zee *et al.* 1992, 1995).

Obesity is generally regarded as being a multifactorial condition in which both environmental and genetic factors are important determinants in susceptibility to body fat accumulation (Despres *et al.* 1992). Adoption studies have recently implicated genetic control, rather than childhood environment, as the main influence on the development of adult obesity (Sorensen & Stunkard 1993). Obesity, essential hypertension, impaired glucose tolerance, non-insulin-dependent diabetes mellitus and dyslipidaemia tend to cluster in families. Collectively, these abnormalities constitute the multiple metabolic syndrome or Syndrome X, which is associated with cardiovascular disease (Kesaniemi *et al.* 1992). Hypertension, similar to obesity, is a multifactorial condition that involves environmental triggers and genetic determinants (Herrera & Ruiz-Opazo 1991).

In a study aimed at identifying genotypes that characterize hypertensives, *LDLR* was used as a candidate gene in cross-sectional association studies. Although an *ApaLI* polymorphism for the marker did not show an association with hypertension, we found that after subdividing the hypertensives into lean and obese individuals, the *LDLR* polymorphism showed a strongly significant association with obesity (Zee *et al.* 1992). To determine whether this association occurs only in hypertensive populations, *LDLR* genotypes were determined in obese and non-obese normotensives. In a population of 70 normotensives, the *ApaLI* polymorphism did not show a significant association with obesity (Morris *et al.* 1994). Similarly a *HincII* *LDLR* polymorphism showed a significant association with obesity in essential hypertensives but not in normotensives (Zee *et al.* 1995). In the present study we determined the genotypes for an *LDLR* dinucleotide repeat polymorphism in both lean and obese normotensives. The results for this microsatellite marker, which is located more towards the 3' end of the *LDLR* gene, are presented and discussed.

METHODS

Subjects

Twenty millilitre blood samples were collected from normotensives for the present cross-sectional association study.

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Individuals were classified as normotensive if their blood pressure was less than 140/90 mmHg, they were not on hypertensive medication and they had no family history of hypertension, diabetes or heart disease. The population was divided into lean and obese categories on the basis of body mass index (BMI); obese individuals had a BMI of ≥ 26 kg/m² and individuals were classified as lean if they had a BMI of < 26 kg/m².

Polymerase chain reaction analysis

DNA was extracted from white blood cells as previously described (Zee *et al.* 1992) and alleles for an *LDLR* dinucleotide repeat polymorphism were determined using fluorescently labelled primers and polymerase chain reaction (PCR) amplification. Polymerase chain reactions were performed as described previously (Zuliani & Hobbs 1990), but used a 94°C initial denaturing step for 3 min followed by 35 cycles of denaturation for 40 s at 94°C, annealing and extension at 60°C for 1 min and a final extension for 2 min at 72°C. Polymerase chain reaction products were fractionated on 6% polyacrylamide denaturing gels and alleles were determined by comparison to size standards using an Applied Biosystems DNA sequencer with Genescan software (Perkin-Elmer), as described by Ziegler *et al.* (1992).

Analysis of data

Electrophoretograms and spreadsheets were used to determine the genotypes for each subject tested. The total for each genotype was tabulated and allele frequencies calculated. Differences in frequencies were tested by Chi-squared analysis with two degrees of freedom.

RESULTS

Genotypes for the *LDLR* dinucleotide tandem repeat were determined for 83 normotensives, 33 of whom were obese and 50 lean. The polymorphic repeat marker has been localized to exon 18 of *LDLR*. Polymerase chain reaction amplification using the GZ-7 and GZ-8 *LDLR* oligonucleotide primers detects three DNA fragments, 106, 108, and 112 bp, containing 7, 8, and 10 (TA) repeats, respectively (Zuliani & Hobbs 1990). Genotypes were determined after fractionation of amplified, fluorescently labelled PCR products on polyacrylamide denaturing gels and comparison of fragment sizes to labelled standards using Genescan software. The total number of alleles was determined from genotype results and statistical analysis performed (Table 1). Statistical analysis of these results indicated that there was a significant difference between the lean and obese groups of normotensives ($\chi^2 = 9.8$; $P = 0.008$).

Table 1. Association analysis of a low density lipoprotein receptor dinucleotide repeat polymorphism in obese and lean normotensives

NT	No.	Allele frequencies (bp)			Total alleles (bp)*		
		106	108	112	106	108	112
Obese	33	0.60	0.20	0.20	40	13	13
Lean	50	0.78	0.17	0.05	78	17	5

* $\chi^2 = 9.8$; $P = 0.008$.

DISCUSSION

The low density lipoprotein receptor is an important regulator of cholesterol levels in serum. As such, it is possible that a defect in this gene could lead to an elevation in plasma lipids and increased triglyceride storage in adipose tissue.

LDLR is approximately 45 kb in length and contains 18 exons (Südhof *et al.* 1985). The *ApaLI* *LDLR* polymorphism detects a nucleotide substitution in intron 15 of this gene. Results from a previous study indicate that this polymorphism is associated with obesity in essential hypertensives (Zee *et al.* 1992) but is not associated with obesity in normotensives (Morris *et al.* 1994). Together these results imply that the association of this polymorphism with obesity is related to blood pressure. We have found similar recent results with another closely located *LDLR* polymorphism namely a *HincII* *LDLR* polymorphism which detects a substitution in exon 12 and, similar to the *ApaLI* RFLP, shows an association with obesity in a hypertensive population, but does not show an independent association with obesity in normotensives (Zee *et al.* 1995).

In contrast, the *LDLR* dinucleotide repeat polymorphism, which has been localized to exon 18, towards the 3' end of the gene, does show a significant association with obesity in a normotensive population. At present these results are preliminary as we are still continuing *LDLR* genotyping in our normotensives. However, this does appear to be the first time that a genetic association of *LDLR* with obesity has been detected in a normal population. We are currently completing these polymorphism studies in normotensives and commencing genotyping of this polymorphism in hypertensives. It will be interesting to see if this *LDLR* association also applies to a hypertensive population.

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