The lipoprotein lipase Gly¹⁸⁸→Glu mutation in South Africans of Indian descent: evidence suggesting common origins and an increased frequency

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Abstract

Lipoprotein lipase (LPL) plays a crucial role in the hydrolysis of the triglyceride core of circulating chylomicrons and very low density lipoproteins (VLDL) and also has a major effect on the levels and lipid composition of high density lipoproteins (HDL). LPL deficiency is inherited as an autosomal recessive trait and most commonly presents with chylomicronaemia, abdominal pain, and eruptive xanthomata. We have previously described a mutation in exon 5 of the LPL gene which results in a substitution of glutamic acid for glycine at amino acid 188. We have now assessed 16 South African LPL deficient patients from nine separate kindreds for this mutation. Nine of these probands were homozygous for the mutation and were from four families, all of Indian descent. The ancestors of these probands have their origins in villages close to Bombay, India, which suggests a common ancestral mutation for the four Indian kindreds, particularly as the mutant allele in each family carried the identical restriction fragment length polymorphism (RFLP) haplotype. The presence of at least nine affected subjects in this small community around Cape Town is evidence for a higher than expected gene frequency for LPL deficiency in this population.

Lipoprotein lipase (LPL) is a prominent enzyme in lipoprotein triglyceride metabolism and is located primarily on the luminal surface of the capillary endothelium where it is active against the triacylglyceride core of circulating chylomicrons and very low density lipoproteins. It plays a major role in the transport of lipids by releasing free fatty acids in the plasma compartment for uptake by tissues for catabolism or storage.

Genetic deficiencies of LPL are uncommon and generally occur at a carrier frequency of approximately 1:500.¹ LPL deficiency is inherited as an autosomal recessive trait and usually presents in childhood with abdominal pain, pancreatitis, hepatosplenomegaly, eruptive xanthoma, failure to thrive, and chylomicronaemia.

DNA cloning and sequencing studies have recently yielded data on the structural organisation and sequence of the coding regions of

the human LPL gene.^{2,3} This information has facilitated the characterisation of numerous mutations which result in LPL deficiency through the impairment of gene expression or the synthesis of an abnormal gene product.⁴ The majority of these mutations are single base alterations leading to amino acid substitutions, adversely affecting the catalytic function of the mature enzyme.

One of these mutations results in the substitution of the charged residue, glutamic acid, for the neutral amino acid, glycine, at position 188 in the mature enzyme.^{5,6} This particular substitution would appear to be a common cause of LPL deficiency as it occurs in persons of differing ancestries and accounted for 22% of the mutant alleles in a cohort of 56 affected subjects⁶ who were ascertained from different parts of the world.

One of the affected patients in this cohort was a South African of Indian descent who is one of 16 South African subjects with LPL deficiency. These subjects come from nine separate families, comprising four Indian, three Dutch, and one each of Malay and Cape Coloured extraction. In this manuscript we report the results of screening for the Gly¹⁸⁸→Glu mutation in all these subjects. Interestingly, all nine probands of Indian descent were found to carry this particular amino acid substitution while it was not detected in the seven other South African patients. These probands come from a community of less than 100 000 persons who live in and around Cape Town, which would suggest a higher than expected gene frequency for LPL deficiency in this population. We have been unable to confirm an ancestral relationship between the Indian patients, but it is noteworthy that the parents or grandparents in the four kindreds were born in closely adjacent villages on the outskirts of Bombay. It is thus likely that these kindreds share a common ancestral mutation.

Materials and methods

SUBJECTS

All the South African probands presented with findings characteristic of LPL deficiency, including fasting chylomicronaemia with plasma triglycerides in excess of 20 mmol/l. The absence of LPL activity in post-heparin plasma was shown by an in vitro assay incorporating triolein as substrate with an exogenous source of ApoCII.⁷ Clinical and laboratory findings on probands in three of the four

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Received 31 May 1991.
Revised version accepted
22 July 1991.

Figure 1 The nucleotide change causing the Gly→Glu substitution at residue 188 of the LPL gene results in the loss of an *AvaII* site in exon 5 and a larger fragment after digestion with *AvaII*.

Indian kindreds have been described previously.^{6,8} In addition, the clinical and biochemical findings of the probands of Malaysian⁹ and Dutch descent¹⁰ have been described separately.

DNA ISOLATION AND RFLP ANALYSIS

Genomic DNA was isolated from blood leucocytes as described previously.⁶ RFLPs were determined by digesting 5 µg of DNA with 20 units of three restriction endonucleases known to show useful polymorphisms at the LPL locus using *PvuII*, *BamHI*,¹¹ and *HindIII* restriction enzymes.¹² The presence or absence of the restriction endonuclease cutting sites was recorded as (+) or (−) respectively. DNA was also digested with *AvaII* which shows an RFLP specific for the Gly¹⁸⁸→Glu substitution. Alternatively, this substitution was detected using PCR amplified DNA and

AvaII digestion as described previously.⁶ Restriction fragments were size separated in 1 to 1.5% agarose gels, transferred to nitrocellulose membranes, and hybridised to a LPL cDNA probe according to standard procedures.^{6,9,10} The full length human cDNA probe was a kind gift of Dr R Lawn. RFLP haplotypes at the LPL locus were determined for each patient and for 27 unrelated normolipidaemic subjects from the Cape Town Indian community.

Results

The nucleotide alteration underlying the Gly¹⁸⁸→Glu mutation abolishes one of the two cutting sites for *AvaII* within exon 5⁶ and results in an easily distinguishable restriction fragment pattern on Southern blot hybridisation analysis (fig 1). We have previously reported the Gly¹⁸⁸→Glu mutation in a South African patient of Indian extraction⁶ from a kindred with four affected members. We have now used this methodology to show the presence of the Gly¹⁸⁸→Glu mutation in a further five South African patients with LPL deficiency. These patients are also of Indian descent and come from three additional kindreds.

We have subsequently attempted to determine the origins of these nine affected probands through a detailed family history and assessment of family records provided by the patients' families. In each of the four kindreds (fig 2), the origins can be traced back to villages near the town of Khed approximately 150 km east of Bombay (fig 3). The respective villages are: kindred 1, Furus and Sakhrol; kindred 2, Morba; kindred 3, Shiv; and kindred 4, Furus and Kurgua.

LPL gene haplotypes were determined for the mutant alleles in each kindred and all were found to carry haplotype 1 (table). To determine whether the mutation occurred on a common haplotype in the Indian community, we analysed DNA from 27 normolipidaemic unrelated subjects. Haplotype 1 was found to be associated with only 11 (20%) of the 54 alleles examined and therefore represents a less common haplotype in this community. The use of three RFLPs to construct haplotypes gives a theoretical possibility of eight (2³) different haplotypes, six of which were found in the normolipidaemic control population. Two

Figure 2 The four separate kindreds with LPL deficiency have nine affected persons and numerous heterozygotes for the 188 mutation causing LPL deficiency.

Figure 3 The origins of the four kindreds from Cape Town with the Gly¹⁸⁸→Glu mutation in the LPL gene can be traced to different villages close to Bombay.

of these haplotypes, H1 and H2 (table), accounted for 83% of the alleles examined with H2 being the most common, occurring on 34 of the 54 alleles.

To prove that the substitution of glutamic acid for glycine at residue 188 caused the defective LPL activity in these patients, in vitro site directed mutagenesis and COS-1 cell transfections were performed as previously described.¹⁰ The mutagenic primer 5'-CAC-CAGAGAGTCCCCTG-3' was used to introduce the GGG(Gly) to GAG(Glu) transition.

Measurement of LPL activity in the medium from the cell cultures transfected with the mutant cDNA showed almost zero (1.1 nmol FFA/min/dish) lipolytic activity while the medium from cells transfected with the normal LPL cDNA showed a substantial level of activity at 124.7 nmol free fatty acid/min/dish (total medium per dish = 6 ml). These data show that the mutant LPL cDNA is catalytically inactive and supports a similar finding from another group.⁵

Discussion

In this manuscript we describe the presence of the lipoprotein lipase Gly¹⁸⁸→Glu substitution

in nine South African patients with LPL deficiency and confirm that this substitution results in a catalytically defective protein. These subjects come from four families of Indian descent and are all resident in Cape Town. These kindreds can be independently traced to immigrants who arrived in South Africa during the first half of this century. Pedigree analysis showed that either the parents or the grandparents or both were born in adjacent villages south-east of Bombay. Although we have not been able to establish a genetic relationship between these families it is likely that they share a common ancestral gene for LPL deficiency. This postulate is supported by the finding that the mutant allele in each kindred carries the same haplotype at the LPL locus. Further, this haplotype is one of the less common haplotypes in the Indian community, occurring in 20% of control alleles.

This mutation has previously been described in affected persons of Dutch, English, and German descent.⁶ The finding of this mutation in four separate Indian families from different villages but on the same haplotype (which is less common in this population) is further evidence for a common progenitor and might indicate that this mutation has occurred once and predates the spread of the Caucasian population to the Indian subcontinent.

The frequency of affected persons with LPL deficiency has been estimated at 1 in one million.¹ There are about 1 million South Africans of Indian origin who are descendants of persons arriving since 1860. The majority have their origins in Calcutta, Madras, and Bombay and settled in Natal where they worked on the sugar cane plantations. A small proportion (up to 5%) settled in the Cape, with most living in and around Cape Town. The isolation and rapid expansion of these communities has resulted in the increased prevalence of several genetic disorders among the

LPL haplotype frequencies in the Indian community of Cape Town, South Africa.

Haplotype	HindIII	BamHI	PvuII	Frequency	%	% in other Caucasian families
1	+	-	-	11	20	10
2	+	-	+	34	63	39
3	-	+	+	0	0	5
4	-	-	+	4	7	12
5	+	+	-	1	2	12
6	-	+	-	3	6	12
7	+	+	+	0	0	5
8	-	-	-	1	2	5

+ = presence of restriction site. - = absence of restriction site. Nomenclature according to Monsalve et al.⁵

Indian peoples. This has been well documented for thalassaemia, sickle cell anaemia, G6PD deficiency, and diabetes mellitus type 2¹³ and might reflect a rapid expansion of the community from a small number of founders, some of whom carried mutations for these genetic diseases.

A significant proportion of the Indian community is, however, descended from persons who arrived relatively recently in South Africa, during the early part of this century. This period has not been sufficient time for a significant founder effect and it is possible that the increased frequency of some genetic diseases in this population reflects multiple sources of introduction of mutant genes.

The identification of nine LPL deficient probands in the Cape Town Indian community, with a total population of approximately 100 000, reflects a high frequency for LPL deficiency. Interestingly, most of the Indian kindreds settled around Cape Town have similar origins to those of the affected kindreds. This may indicate a higher than expected carrier frequency of LPL deficiency in members of the Indian community as a whole.

The clinical consequences of being a carrier for lipoprotein lipase deficiency are yet to be determined. However, recent evidence suggests that these subjects might have a variable hyperlipidaemia with an increased predisposition to atherosclerosis.¹⁴ The ability to diagnose carrier status for LPL deficiency at the genomic level unequivocally will allow us to screen all members of these families and examine the phenotypic expression of LPL deficiency. Furthermore, screening for this mutation in persons of a similar origin who present with premature atherosclerosis and comparison with a control group will help to

determine whether this mutation is associated with predisposition to increased atherogenesis in this community.

This work was supported by the MRC-South Africa, the MRC-Canada, and the British Columbia Heart Foundation. Dr M R Hayden is an established investigator of the British Columbia Children's Hospital. We thank Dr Y Ha for constructive comments.

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