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SHORT REPORT

Genetic homogeneity of lysinuric protein intolerance

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Lysinuric protein intolerance (LPI) is an autosomal recessive disorder in which transport of the cationic amino acids lysine, arginine and ornithine is defective at the basolateral membrane of the epithelial cells in the intestine and renal tubules. LPI is unusually common in Finland, but patients have been described on all continents. Linkage analysis in Finnish LPI families recently assigned the *LPI* gene locus to a 10 cM interval between markers D14S72 and MYH7 on the long arm of chromosome 14. In the present study linkage analysis of LPI families from six different non-Finnish populations strongly suggests genetic homogeneity in LPI. Peak lod scores were obtained at the chromosomal area between D14S72 and MYH7 with the same markers as in the Finnish families. The non-Finnish families showed no linkage disequilibrium except in an Italian family cluster, whereas strong allelic association in the Finnish families implies that LPI in Finland is caused by a founder mutation.

Keywords: genetic homogeneity; lysinuric protein intolerance; linkage analysis; haplotype analysis

Introduction

Lysinuric protein intolerance (LPI; MIM 222700) is an autosomal recessive disease caused by defective transport of the cationic amino acids lysine, arginine and ornithine at the cell membrane. The main symptoms of LPI are probably secondary to diminished dietary protein tolerance and protein-induced postprandial hyperammonaemia. As a consequence, most patients develop protective aversion to high-protein foods at an early age.[1] The disease is exceptionally prevalent in the Finnish population. Clusters of LPI families are also known in southern Italy,[2] where most patients come from the Naples region. Sporadic cases have been described in several other countries.

In Finnish families, we previously assigned the LPI locus to a 10 cM region on chromosome 14q11 between the markers D14S72 and MYH7 by linkage analysis.[3] The wide spectrum of clinical findings in non-Finnish LPI patients, including eg joint hyperextensibility, bone marrow abnormalities, and homocitrullinuria and homoargininuria[4,5] suggested that genetic heterogeneity in LPI is possible. We have now evaluated this hypothesis using linkage and haplotype analysis in LPI families

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representing six different non-related, non-Finnish patient populations.

Materials and Methods

Families

Linkage was studied in 19 non-Finnish families, of which 13 were Italian, one Swedish, one Latvian, two Moroccan, one Saudi-Arabian and one Turkish. Haplotype analysis included three additional Italian patients, and one Turkish and one Estonian patient. The diagnosis of LPI in all index patients was confirmed by clinical evaluation and by documentation of increased urinary excretion, low plasma concentrations of lysine, arginine and ornithine, and hyperammonemia and/or oroticaciduria after dietary or intravenous nitrogen load.

DNA-marker Analysis

Genomic DNA was extracted from peripheral blood, from Ebstein-Barr virus-transformed lymphoblast cell lines or from fibroblast cell lines using standard protocols. The polymorphic microsatellite markers D14S72, D14S50, D14S283, TCRA, MYH7 and D14S80 were studied in all families. To demonstrate recombination points in Italian families additional microsatellite markers D14S64, D14S275, D14S1042 and TCRD were analysed. PCR for microsatellite markers was performed as described in Lauteala *et al*.[3] The amplified fragments were separated by use of 6% polyacrylamide-7M urea sequencing gels and run and analysed with ABI Prism 377 automatic sequencer.

Statistical Analysis

Linkage analyses were performed with the LINKAGE program package[6] using MLINK for pairwise lod scores and GENEHUNTER program package[7] for multipoint analysis. The marker order and distances were based on combined CEPH-LPI data[3] and Généthon microsatellite marker map.[8] The heterogeneity test was done with the MTEST program from the HOMOG program package.[9]

Results

In pairwise linkage analysis performed with microsatellite markers in 19 non-Finnish LPI families with 24 affected individuals and 51 family members (13 families came from Italy), highest lod scores were obtained with the markers D14S50, TCRA and D14S283, with maximum lod scores of 4.37, 4.04 and 3.57, respectively, at $\theta = 0$ (Table 1). No recombinations were found between these three markers and the LPI locus, whereas marker D14S72 showed recombinations in one Italian family and marker MYH7 in one Italian, one Swedish and one Saudi-Arabian family. Seven-point linkage analysis between LPI and the markers D14S72, D14S50, D14S283, TCRA, MYH7 and D14S80 gave the highest lod score of 7.36 and the corresponding map location was 3.92 cM telomeric from the marker D14S72 (Figure 1).

Table 1 Pairwise lod scores between LPI and six marker loci in non-Finnish LPI families

Marker Locus	*Lod score at θ =*					
	0.00	*0.001*	*0.01*	*0.05*	*0.10*	Z_{max}
D14S72	–	2.83	3.69	3.75	3.23	3.85
D14S50	4.37	4.36	4.25	3.73	3.04	4.37
D14S283	3.57	3.56	3.47	3.03	2.46	3.57
TCRA	4.04	4.04	4.02	3.70	3.14	4.04
MYH7	–	–3.91	–0.72	1.36	1.78	1.79
D14S80	–	0.24	2.10	2.84	2.64	2.84

When the Italian families were analysed separately from the other non-Finnish families, slightly different results were obtained. The highest lod score of 3.08 was again obtained with the marker D14S50, whereas D14S283 and TCRA gave lod scores of 2.41 and 2.51, respectively, but in addition the marker D14S80 (located 10 cM telomeric from the framework marker MYH7),[8] also had a high lod score of 2.87. The multipoint linkage curve, however, displayed a peak at the cluster of D14S50, D14S283 and TCRA. We also generated haplotypes in Italian families with additional markers flanking D14S80 and demonstrated a recombination in one family between MYH7 and the marker

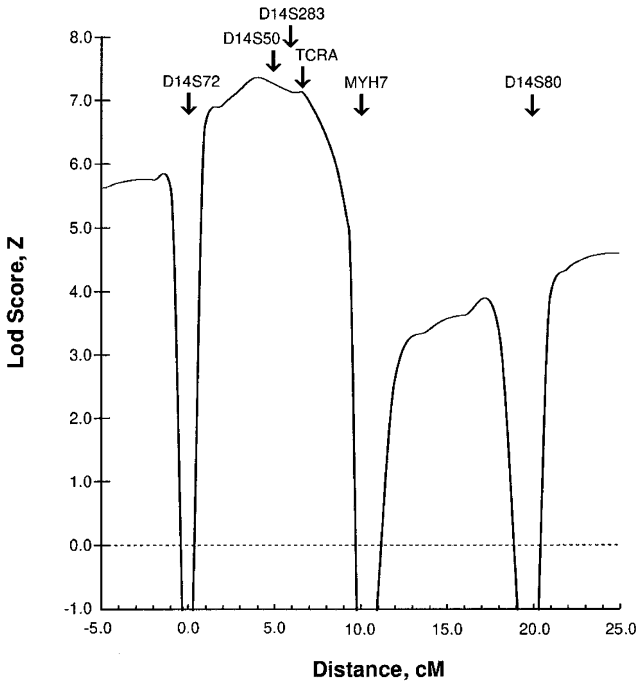

Figure 1 *Seven point linkage analysis between LPI locus and six marker loci. The family data include 19 non-Finnish LPI families.*

D14S64, which is located between MYH7 and D14S80. This restricts the critical region of the *LPI* gene also in the Italian families to the area between D14S72 and MYH7. The marker D14S80 revealed in our 20 Finnish LPI-families four recombination events, and two additional recombination events were even found in the non-Finnish, non-Italian families. Our results thus give no evidence of locus heterogeneity in LPI ($\chi^2 = 3.73$; $P > 0.05$).

We previously found strong linkage disequilibrium in Finnish families between LPI locus and several markers on the 5.1 cM region between D14S742 and MYH7. In the non-Finnish families shared haplotypes were seen only in the four Italian families originating from a small village near Naples. The 6–1 haplotype (D14S283, TCRA) was present in seven out of ten chromosomes from this subgroup of patients (Figure 2, lines 1 to 7 from the top). The Italian families, considering together, showed no common haplotypes and P_{excess} value of the best marker D14S283 was only 0.39 (P_{excess} was calculated as described in Lauteala *et al.*[3]) In addition two Italian patients shared the haplotype 6–4–13 (D14S283, TCRA and MYH7) that showed strong linkage disequilibrium in the Finnish LPI chromosomes. Swedish, Estonian and Lithuanian patients shared the TCRA allele 4.

Discussion

Two point linkage analysis in 19 non-Finnish LPI families gave positive lod scores on the proximal long arm of chromosome 14 with the same markers as were previously observed in the Finnish families. Such data strongly suggests genetic homogeneity in LPI. The peak lod scores in the non-Finnish families were obtained with the markers D14S283 and TCRA which also display the strongest linkage disequilibrium in the Finnish LPI chromosomes. The critical gene region, spanning a 10 cM interval at 14q11, was defined in non-Finnish families by recombinations with the markers D14S72 and MYH7, similarly to what was found in the Finnish families. The Italian families had relatively high lod scores also with the marker D14S80, which is located 10 cM distally from TCRA. However, extended haplotypes of the Italian families revealed that the critical region is between D14S72 and MYH7. Genetic homogeneity between the Finnish and Italian LPI patients was also confirmed by the MTEST computer program.

Allelic association was found in four of five Italian families that originated from a small village near Naples, but not in the rest of Italian LPI chromosomes nor in the whole group of the non-Finnish families. The subgroup of LPI families which showed allelic association probably shares a common founder mutation.

Nationality	D14S72		D14S50		D14S283		TCRA		MYH7	
Italian	3	—	6	—	6	—	1	—	12	*
	3	—	6	—	6	—	1	—	12	*
Italian	3	—	6	—	6	—	1	—	12	*
	8	—	6	—	6	—	1	—	12	*
Italian	3	—	6	—	6	—	1	—	12	*
	2	—	6	—	6	—	1	—	12	*
Italian	3	—	8	—	6	—	1	—	13	*
	3	—	3	—	1	—	3	—	12	*
Italian	7	—	4	—	2	—	9	—	11	*
	7	—	4	—	2	—	9	—	11	*
Italian	8	—	6	—	2	—	2	—	6	
	8	—	4	—	8	—	2	—	6	
Italian	3	—	4	—	2	—	1	—	13	
	3	—	4	—	2	—	1	—	5	
Italian	3	—	4	—	2	—	1	—	13	
	3	—	4	—	2	—	1	—	13	
Italian	3	—	6	—	2	—	1	—	14	
	3	—	6	—	2	—	1	—	14	
Italian	2	—	4	—	2	—	2	—	11	
	2	—	4	—	2	—	2	—	11	
Italian	1	—	2	—	6	—	4	—	13	
	1	—	2	—	6	—	4	—	13	
Italian	8	—	7	—	1	—	1	—	13	
	4	—	7	—	2	—	1	—	13	
Italian	8	—	6	—	6	—	9	—	6	
	6	—	3	—	2	—	2	—	6	
Italian	6	—	4	—	3	—	4	—	13	
	3	—	6	—	2	—	9	—	13	
Italian	3	—	6	—	6	—	4	—	13	
	2	—	4	—	6	—	4	—	13	
Italian	2	—	5	—	6	—	9	—	13	
	2	—	5	—	6	—	9	—	12	
Estonian	2	—	4	—	2	—	1	—	15	
	9	—	4	—	3	—	4	—	13	
Lithuanian	2	—	5	—	3	—	4	—	12	
	6	—	5	—	3	—	4	—	12	
Swedish	3	—	6	—	5	—	4	—	13	
	3	—	6	—	5	—	4	—	13	
Saudi-Arabian	7	—	6	—	6	—	1	—	12	
	9	—	5	—	4	—	1	—	11	
Moroccan	7	—	6	—	2	—	1	—	8	
	9	—	5	—	2	—	1	—	10	
Moroccan	2	—	4	—	2	—	1	—	10	
	2	—	4	—	2	—	1	—	8	
Turkish	3	—	4	—	6	—	1	—	10	
	3	—	4	—	6	—	1	—	10	
Turkish	3	—	4	—	2	—	1	—	11	
	9	—	4	—	2	—	1	—	11	

Figure 2 *Extended haplotypes in the non-Finnish LPI families. Haplotypes in lines 1 to 10 from the top are from the five families originating from a small village near Naples (*). Three of the patients are homozygous to an extended haplotype 6–6–1–12 and one patient has 6–1 haplotype in one of the chromosomes (grey box). Two Italian patients have the haplotype 6–4–13 (D14S283, TCRA and MYH7) which is in linkage disequilibrium in the Finnish LPI chromosomes (open box).*

Swedish, Estonian and Lithuanian patients had the same TCRA allele 4 that showed strong linkage disequilibrium in the Finnish LPI chromosomes. Swedish LPI chromosomes also shared D14S283 allele 6 in linkage disequilibrium with the Finnish LPI chromosomes, possibly reflecting a common origin of the mutation in the Finnish and Swedish patients.

The recognition of locus homogeneity in patients with LPI coming from ethnically different populations now allows linkage-based diagnosis and carrier detection for LPI families with at least one previous affected child. Mutation specific DNA diagnosis of LPI still waits for the cloning of the gene and identification of the disease-causing mutation. Strong linkage disequilibrium found in the Finnish LPI chromosomes with the most tightly linked markers provides a good basis for identification of the *LPI* gene.

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