

# Lysinuric Protein Intolerance (LPI) Gene Maps to the Long Arm of Chromosome 14

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## Summary

Lysinuric protein intolerance (LPI) is an autosomal recessive disease characterized by defective transport of cationic amino acids and by hyperammonemia. Linkage analysis in 20 Finnish LPI families assigned the LPI gene locus to the proximal long arm of chromosome 14. Recombinations placed the locus between framework markers D14S72 and MYH7, a 10-cM interval in which the markers D14S742, D14S50, D14S283, and TCRA showed no recombinations with the phenotype. The phenotype was in highly significant linkage disequilibrium with markers D14S50, D14S283, and TCRA. The strongest allelic association obtained with marker TCRA, resulting in a  $P_{\text{excess}}$  value of .98, suggests that the LPI gene locus lies in close proximity to this marker, probably within a distance of <100 kb.

## Introduction

Lysinuric protein intolerance (LPI; MIM 222700) is an autosomal recessive disease in which transport of the cationic amino acids lysine, ornithine, and arginine is defective at the basolateral (antiluminal) membranes of epithelial cells in the intestine (Desjeux et al. 1980; Rajantie et al. 1980) and in the renal tubules (Rajantie et al. 1981; Simell 1995). Through unexplained mechanisms, the transport defect also leads to hyperammonemia after high-protein meals. The clinical signs of LPI comprise failure to thrive, vomiting, growth retardation, and muscular hypotonia. Episodes of stupor may occur after high-protein meals, but strong protective aversion to dietary protein develops usually at an early age. The patients show hepatosplenomegaly and osteoporosis. Potentially fatal interstitial lung disease and progressive

renal failure may occur at any age (Parto et al. 1993, 1994).

LPI patients excrete large amounts of cationic amino acids into the urine, whereas the corresponding plasma concentrations are often subnormal. The molecular mechanisms leading to the clinical symptoms and signs of LPI have remained uncharacterized, and the gene locus and the gene itself are unknown.

The first patients with LPI were described in Finland in 1965 (Perheentupa and Visakorpi 1965). The disease shows worldwide distribution, but excess cases are known, in Finland (33 families), southern Italy (Incerti et al. 1993), and Japan (Kato et al. 1984).

Since three cationic amino acid-transporter proteins encoded by two genes have been described (Yoshimoto et al. 1991; Closs et al. 1993a, 1993b), we first applied the candidate-gene approach to map the LPI gene. Linkage studies, with flanking microsatellite markers, excluded both hCAT-1 and hCAT-2 (Lauteala et al., in press) as the mutated gene in LPI. The random-mapping approach, with the use of highly informative microsatellite markers in samples from 18 LPI patients and 48 of their healthy family members, localized the gene causing LPI to the proximal part of the long arm of chromosome 14. Linkage-disequilibrium data suggest that one founder mutation is responsible for the majority of the LPI cases in the Finnish population.

## Families, Material, and Methods

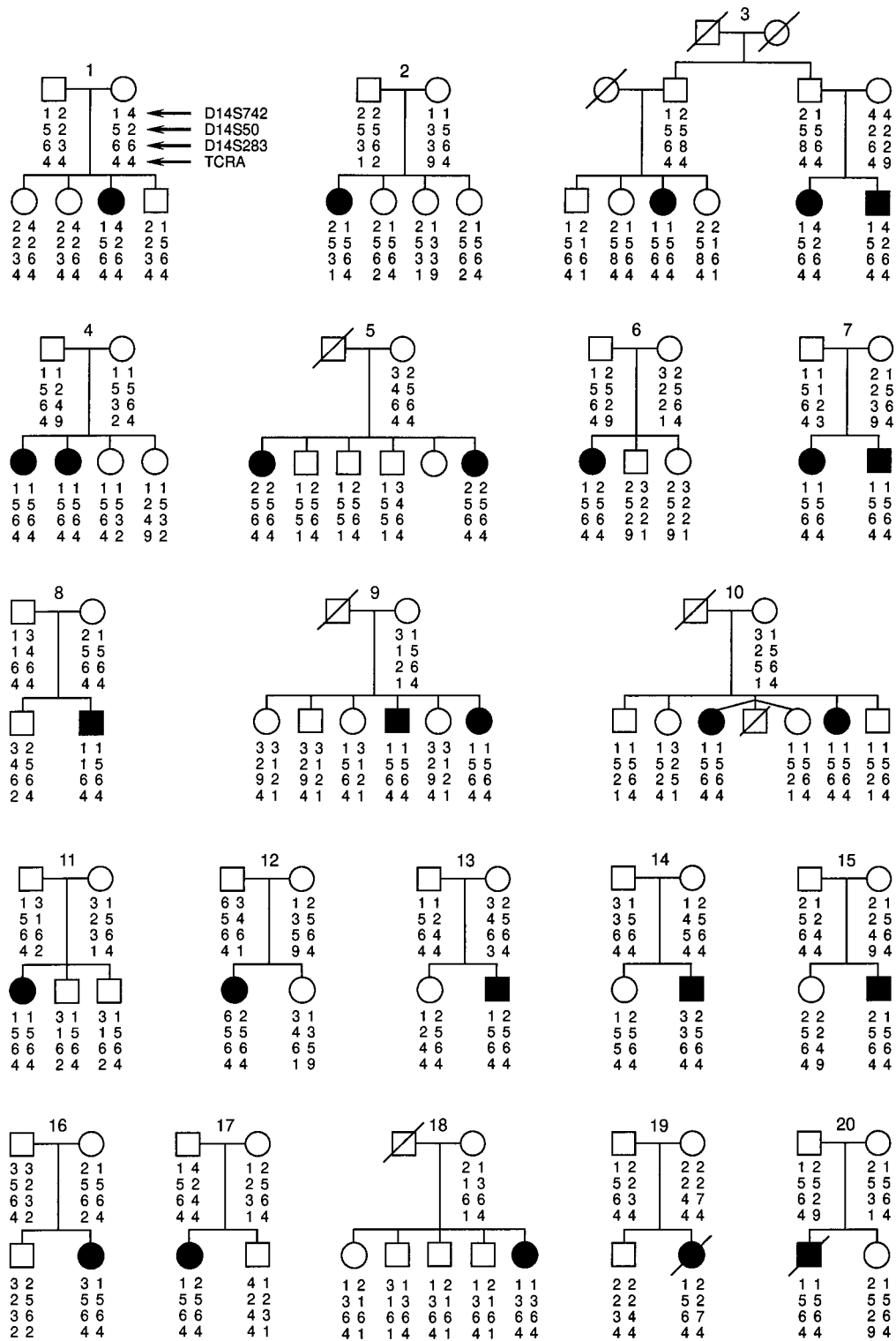
### Families

For the initial screening, two generations of 10 Finnish LPI families and three generations of 1 family were chosen. After linkage had been confirmed, two generations of 9 additional families were included. Altogether, the linkage study consisted of samples from 27 patients with LPI and from 77 healthy family members (fig. 1). Ten additional families with a single LPI child were included in the linkage-disequilibrium calculations. The birthplaces of the grandparents of the patients were distributed unevenly throughout Finland (fig. 2). Diagnosis of LPI in all index patients was confirmed by clinical evaluation; by documentation of increased urinary excretion and low plasma concentrations of lysine, arginine, and

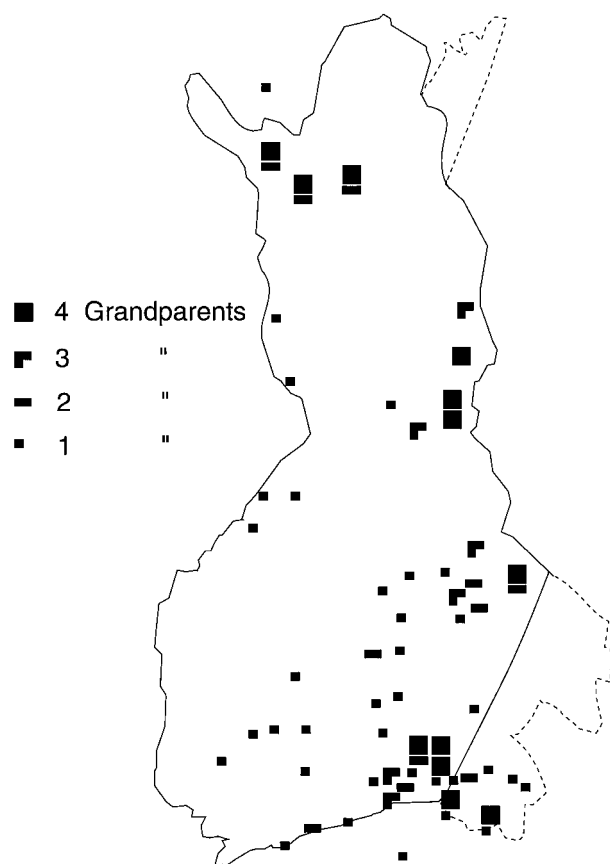
Received December 10, 1996; accepted for publication March 10, 1997.

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0002-9297/97/6006-0027\$02.00



**Figure 1** Pedigrees of the 20 Finnish LPI multiplex families used in the linkage study. The alleles in loci D14S742, D14S50, D14S283, and TCRA are given for each individual.



**Figure 2** Distribution of birthplaces of the grandparents of the Finnish LPI families

ornithine; by increased serum ferritin and lactate dehydrogenase values; and by postprandial hyperammonemia.

#### Samples

DNA was extracted from peripheral blood, from Epstein-Barr virus-transformed lymphoblast cell lines or from fibroblast cell lines, by use of standard protocols.

#### Markers

The microsatellite markers were provided by the Nordic Genome Resource Center (Uppsala) or were purchased from Isogen Bioscience. Some markers were synthesized according to published primer sequences (Gyapay et al. 1994; Dib et al. 1996). Polymorphic microsatellite markers were analyzed by use of PCR and polyacrylamide-gel electrophoresis. PCR was performed in a 25- $\mu$ l volume, by use of 100 ng of genomic DNA as a template, 3.6 pmol of each primer, 0.04  $\mu$ Ci of  $\alpha^{32}$ P-dCTP, the manufacturer's suggested volume of standard buffer and dNTP (Finnzymes or Promega), and 1.0 unit of DNA polymerase (Finnzymes or Promega). When fluorescent primers were used, PCR was performed in a

10- $\mu$ l volume, by use of 37.5 ng of genomic template, 2.0 pmol of each primer, 0.6–1.0 unit of DNA polymerase, 200  $\mu$ M of each nucleotide, and 1  $\mu$ l of standard buffer. Amplification was performed in an MJ Research PTC-100 thermal cycler for 27–35 cycles at 94°C for 30–60 s; annealing was performed at different temperatures, depending on the primers, for 30–60 s; and extension was performed at 72°C for 30–60 s. In the first cycle, denaturation was performed for 4–5 min, and extension was performed at 72°C for 6 min. The amplified fragments were separated by use of 6% polyacrylamide–7 M urea sequencing gels. Approximately one-third of the markers were run and were analyzed with an ABI Prism 377 automatic sequencer.

#### Linkage Analysis

Linkage analyses were performed with the LINKAGE program package (Lathrop et al. 1984), by use of MLINK for pairwise LOD scores and the VITESSE (O'Connell and Weeks 1995) program package for multipoint analysis. VITESSE allows fast multipoint analyses with highly polymorphic markers, through the use of the computationally efficient so-called fuzzy-inheritance algorithm. All pairwise and multipoint calculations were performed under the assumptions of no sex difference and complete penetrance and with the published allele frequencies in the CEPH pedigrees (Généthon 1996), or, if the allele frequencies were not available, they were calculated (by use of D14S742) from the families in this study. The order and distances and the sex-specific map differences of the loci on the fixed-marker map were computed by the CILINK program, by use of the available data in the CEPH v.8 database (Fondation Jean DAUSSET–CEPH 1996) for a total of 40 CEPH reference families, combined with the data from the 20 LPI families.

#### Linkage-Disequilibrium Analysis

The Luria-Delbrück formula (Hästbacka et al. 1992; de la Chapelle 1993; Lehesjoki et al. 1993) was used to estimate genetic distance between the closest markers and the major LPI locus, with the assumption that the number of generations ( $g$ ) since the introduction of the ancestral chromosome is 50; the mutation rate ( $\mu$ ) of the LPI locus is  $10^{-6}$ , and the LPI gene frequency ( $q$ ) is .008 (when the population in the eastern and northern parts of Finland, where the disease is most prevalent, is considered). Allelic excess was calculated by use of the equation  $P_{\text{excess}} = (P_{\text{affected}} - P_{\text{normal}}) / (1 - P_{\text{normal}})$ . Here,  $P_{\text{normal}}$  is the frequency of the selected allele at the marker locus, in normal chromosomes, and  $P_{\text{affected}}$  is the higher frequency of the same allele in chromosomes carrying the disease mutation. The  $\theta$  value was calculated by use of the formula  $P_{\text{excess}} = (1 - \mu g q^{-1})(1 - \theta)^g$ , where  $(1 - \mu g q^{-1})$  denotes the proportion of LPI chromosomes

**Table 1****Pairwise LOD Scores between the LPI Locus and Nine Marker Loci**

MARKER LOCUS	LOD SCORE AT $\theta =$							$Z_{\max}$	$\theta_{\max}$	90% CONFIDENCE LIMITS
	.00	.001	.01	.05	.10	.20	.30			
D14S261	$-\infty$	-3.25	.57	2.55	2.74	2.03	1.07	2.76	.09	...
D14S1023	$-\infty$	-.17	1.87	3.01	3.00	2.16	1.13	3.08	.07	.01 < $\theta$ < .21
D14S72	$-\infty$	1.50	3.36	4.08	3.79	2.61	1.36	4.08	.05	.007 < $\theta$ < .16
D14S742	5.82	5.81	5.65	4.94	4.09	2.53	1.24	5.82	.00	.00 < $\theta$ < .06
D14S50	4.02	4.01	3.91	3.45	2.88	1.82	.91	4.02	.00	.00 < $\theta$ < .09
D14S283	6.91	6.89	6.75	6.07	5.17	3.36	1.72	6.91	.00	.00 < $\theta$ < .06
TCRA	5.90	5.89	5.82	5.29	4.48	2.80	1.34	5.90	.00	.00 < $\theta$ < .07
MYH7	$-\infty$	-2.00	.04	1.38	1.69	1.43	.82	1.70	.11	...
D14S64	$-\infty$	2.98	3.85	3.96	3.52	2.36	1.23	4.04	.03	.001 < $\theta$ < .14

carrying the same ancestral mutation (Lehesjoki et al. 1993).

## Results

### Linkage Analysis

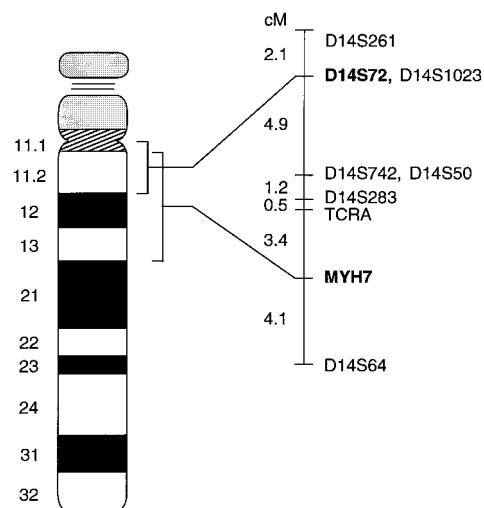
A total of 317 microsatellite markers randomly spread in all autosomes were analyzed in the primary families until highly promising LOD scores ( $>3$ ) eventually were obtained with the microsatellite marker GATA74E02, defining the locus D14S742 on the long arm of chromosome 14, near the centromere. No obligatory recombination events were found between the LPI locus and this marker. Pairwise linkage analysis revealed a maximum LOD score ( $Z_{\max}$ ) of 4.40 ( $\theta = .00$ ), for the initial material from 11 LPI families. Consequently, new markers from this region were analyzed, and linkage was confirmed with six other microsatellite markers (for LOD scores in pairwise linkage analyses between the LPI locus and the chromosome 14 markers, see table 1). No obligatory recombinants were observed between the LPI locus and the loci D14S742, D14S50, D14S283, and TCRA. Pairwise linkage analysis revealed  $Z_{\max}$  values of 5.82, 4.02, 6.91, and 5.90, respectively, at  $\theta = .00$  for each of the four markers.

Recombinations with the proximal marker D14S72 and the distal marker MYH7 preliminarily defined the genomic region harboring the LPI locus within a 10-cM chromosomal region on a sex-averaged genetic map. The 9-point fixed-marker map based on the combined CEPH-LPI data revealed no significant sex-specific differences (data not shown). The best-supported order and the estimated sex-averaged distances (in cM) between the analyzed markers are shown in figure 3. By the combination of information obtained by use of markers D14S72, D14S742, D14S50, D14S283, TCRA, and MYH7, in multipoint analyses, the highest LOD scores of 9.32 and 9.31 were measured at markers D14S283

(with a  $\pm 1$ -LOD-unit support interval of 2.7–8.6 cM) and TCRA, respectively (fig. 4).

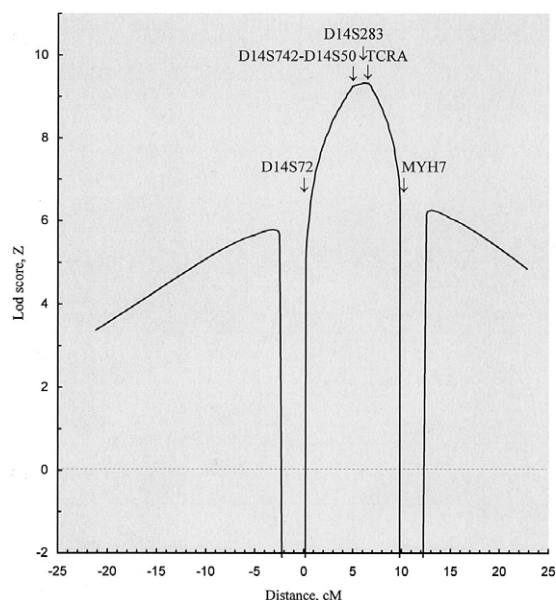
### Linkage Disequilibrium

Linkage-disequilibrium mapping is based on the observation that affected chromosomes descending from a common ancestral mutation show a distinctive haplotype in the immediate vicinity of the gene, reflecting the haplotype of the ancestral chromosome. We therefore tested for linkage disequilibrium in LPI families, with the completely linked markers D14S742, D14S50, D14S283, and TCRA. The alleles of these markers, as well as allele distribution in LPI and non-LPI chromo-

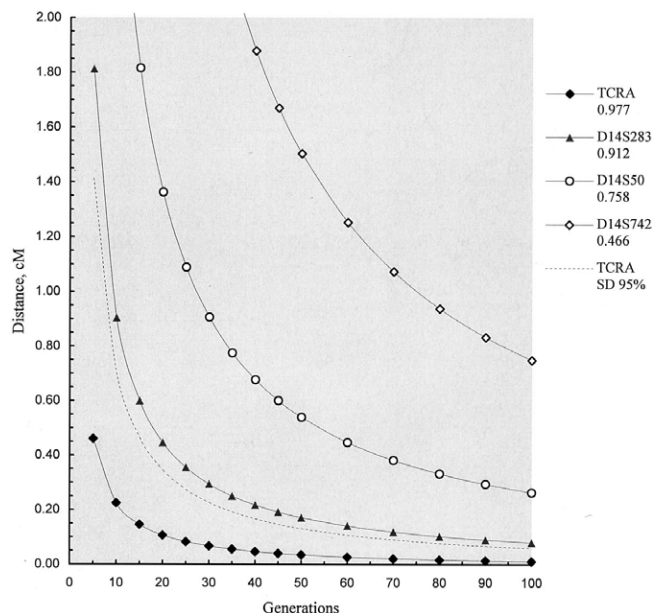


**Figure 3** The order and estimated sex-averaged distances (in cM) between the markers showing linkage to the LPI locus. The best-supported sex-averaged 9-point order is shown. The odds that this order is correct are at least  $2.6 \times 10^4$  better than those of the other 9-point orders (excluding pairs with no recombinants) produced by pairwise inversion of all loci. No sex differences, as estimated in the study by Hellsten et al. (1993), could be shown within this map region.

somes, are shown in table 2. Allele 4 of marker TCRA was present in 60 (98.4%) of the 61 LPI chromosomes, which is in contrast with its presence in 22 (35.5%) of the 62 non-LPI chromosomes. Similarly, allele 6 of marker D14S283 was present in 57 (93.6%) of the 61 LPI chromosomes and in 14 (22.6%) of the 62 non-LPI chromosomes. The marker alleles on the chromosomes carrying the LPI mutation also were distributed nonrandomly at loci D14S50 and D14S742. The  $P_{\text{excess}}$  values for the loci D14S742, D14S50, D14S283, and TCRA were .47, .76, .92, and .98, respectively (table 2). The estimated genetic region showing allelic association with the LPI locus spans 1.7 cM (fig. 3). To further estimate the distance between the disease and the marker loci, we applied a modified Luria-Delbrück method. When 50 generations of expansion were assumed, a mutation frequency of  $10^{-6}$  for the LPI locus and an LPI gene frequency of .008 resulted in genetic-distance estimates of 0.04 cM, 0.17 cM, and 0.54 cM, for the loci TCRA, D14S283, and D14S50, respectively (fig. 5). In the LPI chromosomes, ten different haplotypes were detected with four markers (D14S742, D14S50, D14S283, and TCRA), but only five haplotypes were detected when the alleles of the closest markers, D14S283 and TCRA, were used. The haplotype x-y-6-4 was present in 57 (93.4%) of the 61 LPI chromosomes but in only 5 (8.8%) of the 57 non-LPI chromosomes. The haplotype



**Figure 4** Seven-point linkage analysis between the LPI locus and six marker loci. The sex-averaged distances between markers on the horizontal axis are based on combined data from the LPI and CEPH families. Locus D14S72 was selected as a starting point for the map. The family data include 20 Finnish families with more than one child. The  $Z_{\text{max}}$  and the corresponding map location were 9.3 and 6.1 cM, respectively, with a  $\pm 1$ -LOD-unit support interval of 2.7–8.6 cM. The centromere is to the left.



**Figure 5** Luria-Delbrück-based analysis of the location of the LPI gene, with regard to marker loci showing no recombinations in linkage analysis. Genetic-distance estimates are shown as a function of the number of generations. The 95% confidence interval for TCRA (lower limit at 0) is based on the sampling error for chromosomes.

1-5-6-4 was carried by 31 (50.8%) of the 61 LPI chromosomes and by 2 (3.5%) of the 57 non-LPI chromosomes (table 3). Haplotype x-y-6-4 homozygosity was found in 34 (91.9%) of the 37 LPI patients.

## Discussion

The gene for LPI was assigned to the proximal part of chromosome 14 (14q11-13), by use of linkage analysis based on random screening of the autosomal genome. The closest flanking markers, D14S72 on the centromeric side and MYH7 on the telomeric side, defined an interval of  $\sim 10$  cM. Strong linkage disequilibrium, with markers TCRA, D14S283, and D14S50, was present in a genomic region of 1.7 cM, and, furthermore, the application of the Luria-Delbrück-based formula suggested that the distance between the closest marker (TCRA) and the LPI locus is only 0.04 cM. The results are completely consistent with autosomal recessive inheritance and full penetrance, in agreement with previously published conclusions based on segregation patterns and on careful clinical evaluation. We found no evidence of genetic heterogeneity, suggesting that, at least in Finland, the disease is caused by mutations at a single locus or at closely related loci. It remains to be determined if LPI families from other countries show evidence of mapping to the same region.

Our results are in line with previous linkage studies of recessive disorders in the isolated population of Fin-

**Table 2****Distribution of Alleles of TCRA, D14S283, D14S742, and D14S50, in LPI and Non-LPI Chromosomes**

ALLELE	TCRA		$P_{\text{excess}}$	D14S283		$P_{\text{excess}}$
	No. (%) of			No. (%) of		
	LPI	Non-LPI		LPI	Non-LPI	
1	1 (1.6)	15 (24.2)		0	1 (1.6)	
2	0	10 (16.1)		0	9 (14.5)	
3	0	3 (4.8)		1 (1.6)	14 (22.6)	
4	60* (98.4)	22 (35.5)	.98	1 (1.6)	9 (14.5)	
5	...	...		0	10 (16.1)	
6	...	...		57* (93.6)	14 (22.6)	.92
7	...	...		1 (1.6)	1 (1.6)	
8	...	...		0	2 (3.2)	
9	0	12 (19.4)		0	2 (3.2)	
10	...	...		1 (1.6)	0	
Total	61	62		61	62	
	D14S50			D14S742		
1	1 (1.6)	9 (14.3)		36* (58.1)	17 (27.0)	.47
2	4 (6.6)	26 (41.3)		17 (32.3)	24 (38.1)	
3	4 (6.6)	6 (9.5)		3 (4.8)	17 (27.0)	
4	2 (3.2)	6 (9.5)		2 (3.2)	5 (7.9)	
5	50* (82.0)	16 (25.4)	.76	...	...	
6	...	...		1 (1.6)	0	
Total	61	63		59	63	

\*  $P < .001$ .

land and again exemplify the power of linkage-disequilibrium mapping in the study of such populations. Approximately 30 recessive diseases (called “the Finnish disease heritage”; Norio et al. 1973) are exceptionally common in the Finnish population. The genes for at least 20 of these diseases have been mapped to a specific locus (Hellsten 1995). In six diseases (progressive myoclonus epilepsy [Pennacchio et al. 1996], diastrophic dysplasia [Hästbacka et al. 1994], choroideremia [Sanikila et al. 1992], infantile neuronal ceroid lipofuscinosis [Vesa et al. 1995], hypergonadotropic ovarian dysgenesis [Aittomäki et al. 1995], and congenital chloride diarrhea [Höglund et al. 1996]), the genes have been cloned by use of the positional cloning strategy. For the last four diseases listed above, the founder mutation has been described further. For four other diseases for which protein data was available (aspartylglucosaminuria [Ikonen et al. 1991], hereditary fructose intolerance [Cross et al. 1988], gyrate atrophy of the choroid and the retina [Mitchell et al. 1989], and nonketotic hyperglycinemia [Kure et al. 1992]), the gene was cloned by use of the functional cloning principle, followed by detection of the prevalent mutations.

Since its founding 100–200 generations ago, the population of Finland has grown in isolation. Therefore,

application of the Luria-Delbrück formula, originally developed to analyze mutations in rapidly growing bacterial populations, has been considered justified for the study of isolated human populations as well. Diastrophic dysplasia was a good example for the use of linkage-disequilibrium mapping in the Finnish population: Luria-Delbrück-based analysis predicted that the diastrophic dysplasia gene lies ~64 kb from the locus of the closest marker, CSF1R. When the diastrophic dysplasia gene subsequently was cloned, its location was 70 kb from CSF1R (Hästbacka et al. 1992, 1994).

The age of the ancestor mutation may be extrapolated from the distribution of the grandparents' birthplaces in Finland or from the size of the genetic region showing linkage disequilibrium. In the diastrophic dysplasia analysis, the number of generations was assumed to be 100. This estimate was based on the geographical distribution of the birthplaces of the grandparents, in the so-called earlier settlement areas in the southern and central parts of the country. In the LPI families, the distribution of the grandparents' birthplaces in the southeastern and northern parts of the country—that is, both the older and recent settlement areas—supports the estimate of ~50 generations as the age of the mutation. The genetic region of 1.7 cM that is in strong linkage disequilibrium

**Table 3****Distribution of Combined Haplotypes for the D14S742–D14S50–D14S283–TCRA Loci, in LPI and Non-LPI Chromosomes**

HAPLOTYPE	No. (%) OF	
	LPI [n = 61]	Non-LPI [n = 57]
1–5–6–4	31 (50.8)	2 (3.5)
x–5–6–4	48 (78.7)	3 (5.3)
x–y–6–4	57 (93.4)	5 (8.8)
Others	4 (6.6)	52 (91.2)

also supports evidence of a relatively high number of generations since expansion of the LPI mutation. The number of generations strongly influences the distance estimation (fig. 5). If the number of generations since the founding of the LPI mutation is set to be 20 instead of 50, the distance between the LPI and TCRA loci would increase from 0.04 cM to 0.12 cM.

The critical region harboring the LPI locus has three known gene assignments, TCRA, CTLA1, and TRP1 (Morton et al. 1992; Collins et al. 1996). None of these genes has any known amino acid-transport function. TCRA is a large 97.6-kb region containing the human T cell-receptor  $\alpha$  and  $\delta$  polypeptide-chain coding regions. T cell antigen receptor polypeptides ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) form two different heterodimers,  $\alpha\beta$  and  $\gamma\delta$ . The function of T cell receptor  $\alpha\beta$  is well established in antigen recognition, but less is known about the  $\gamma\delta$  receptor (Davis and Bjorkman 1988; Koop et al. 1994). Some LPI patients have associated immunological abnormalities, but a clear T cell defect is not a feature of the disease (Nagata et al. 1987). TRP1 is a tRNA proline 1 (Mitchell et al. 1991). The third gene in this region, CTLA1, codes for cytotoxic T-lymphocyte-associated serine esterase 1 (Heusel et al. 1991). Whether any of these genes is the disease-causing gene in LPI remains to be seen. If not, the assignment of the critical LPI region to a chromosomal area of <100 kb provides a good starting point for physical cloning and gene identification.

## Acknowledgments

We thank the lysinuric protein-intolerance families for their excellent cooperation. We also thank Mrs. A. Reinikainen and Mrs. Y. Bullen for their expert technical assistance. This study was supported by the Ulla Hjelt Fund, the Foundation for Pediatric Research, in Finland, the Sigrid Juselius Foundation, and the Signe and Arne Gyllenberg Foundation.

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