Am J Hum Genet 37:1106–1111, 1985

Evidence for the Close Linkage between Lymphocyte Cytosol Polypeptide with Molecular Weight of 64,000 (LCP1) and Esterase D

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SUMMARY

The genetic linkage of the loci for lymphocyte cytosol polypeptide with molecular weight of 64,000 (LCP1) and esterase (ESD) were examined by two-dimensional gel electrophoresis using blood from four informative families. No recombinants were observed in the four families totaling 17 children, giving a summed lod score of 4.221 at recombination fraction 0. This result indicates that the gene for LCP1 is closely linked to the ESD locus, which is assigned to the chromosomal region 13q14.11. This finding also suggests that the LCP1 locus is linked to the retinoblastoma gene, which is assigned to the same chromosomal region and closely linked to the ESD locus.

INTRODUCTION

Lymphocyte cytosol polypeptide with molecular weight (mol. wt.) of 64,000 (LCP1: McKusick catalogue no. 15343) [1] is a human polymorphic polypeptide that can be detected in peripheral blood lymphocytes by two-dimensional gel electrophoresis (two-DE) [2]. The phenotype of LCP1 is determined by two common codominant alleles at an autosomal locus, and the gene frequencies of two common alleles, $LCP1^1$ and $LCP1^2$, are .94 and .06, respectively, in a Japanese population [2]. We recently demonstrated by deletion mapping that the gene for LCP1 is on human chromosomal region 13 q14.1-q32.1 [3]. This assignment prompted us to study the genetic linkage of the locus for LCP1 with the locus for esterase D (ESD), since both the ESD locus and retinoblastoma

Received December 27, 1984; revised May 21, 1985.

This study was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, nos. 5848413 and 59570972, and by University of Tsukuba Project Research, Japan.

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 0002-9297/85/3706-0006$02.00

gene (*RB1*) have been assigned to the same chromosomal region: 13q14.11 [4–8].

Here, we present the evidence for a close linkage between the loci encoding LCP1 and ESD. This finding strongly suggests that LCP1 may be the second polymorphic protein whose gene is closely linked to the retinoblastoma gene.

MATERIALS AND METHODS

Sample Preparation

Peripheral blood lymphocyte proteins were prepared as described [2, 9–11]. Briefly, mononuclear-rich fraction from 2 ml of heparinized venous blood was cultured in a 25-cm^2 culture flask containing 8 ml of culture medium consisting of about 90% RPMI 1640 medium (Gibco, Grand Island, N.Y.), 10% fetal calf serum (Gibco), and phytohemagglutinin-P (PHA-P)(Difco, Detroit, Mich.) at a final concentration of 1/200. Cells were cultured for 5 days at 37°C in a humidified atmosphere containing 5% CO_2. Subsequently, cells were washed three times with phosphate-buffered saline (PBS), and the cell pellet was lysed in 200 μl of cell lysis buffer consisting of 0.5% Nonidet P-40 (BDH Chemical, Pool, England), 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (Sigma, St Louis, Mo.), and 50 mM Tris-HCl at pH 7.4, with vigorous mixing on a Vortex and centrifuged at 105,000 *g* for 1 hour at 4°C. The supernatant was used as lymphocyte protein sample for electrophoresis; it was stored at −70°C until used. The protein concentration was measured by Lowry et al.'s method [12] using bovine serum albumin as the standard.

Erythrocytes for electrophoresis of ESD were prepared from venous blood: after removal of leukocyte-rich fraction, erythrocytes were washed three times with PBS and lysed by freezing and thawing three times.

Electrophoresis and Staining

The phenotypes of LCP1 and ESD were analyzed by two-DE of PHA-stimulated peripheral blood lymphocyte proteins [2, 11]. Two-DE was performed essentially according to the method of O'Farrell [13] with a minor modification [10]. Ampholine mixtures of 1.4%, pH range 5–8, and 0.6%, pH range 3.5–10, were used in the first-dimensional rod gel, and 30 μg of protein was applied. After two-DE, polypeptides were visualized by silver staining [14]. The pH gradient in the first dimension was determined in the presence of 9.2 M urea. Mol. wt. in the second dimension was determined by reference to mol. wt. markers, phosphorylase a, bovine serum albumin, ovalbumin, and ribonuclease (Pharmacia, Upsala, Sweden).

The phenotype of ESD was also analyzed according to the method A described by Harris and Hopkinson [15] to confirm the results obtained by two-DE.

RESULTS

Figure 1 shows a typical result of the analysis of the phenotypes of LCP1 and ESD using two-DE and peripheral blood lymphocyte proteins. Among 92 families we have examined so far, four were informative for linkage analysis of the loci for LCP1 and ESD. In each of the four families, one parent was heterozygous for the two loci and the other homozygous for each of them (fig. 2). In addition, the parental phases were determined by examination of the grandparents in one of the families including three parents-children combinations (fig. 2*D*). There were no proven recombinants in 17 children of informative parents. The linkage data were analyzed by the lod score method of Morton [16] as described by Maynard-Smith et al. [17]. Table 1 shows the lod

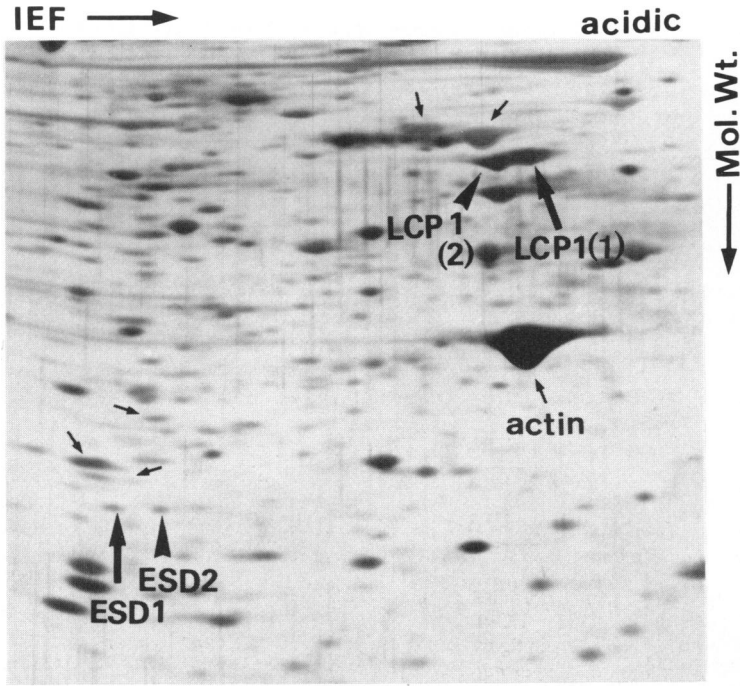

FIG. 1.—Two-DE pattern of lymphocyte proteins from an individual heterozygous for both loci encoding LCP1 and ESD. LCP1(1) and LCP1(2) indicate the products of two common alleles, LCP^1 and $LCP1^2$, respectively, at the LCP1 locus. The LCP1 alleles, $LCP1^1$ and $LCP1^2$, were originally designated $LC64P^1$ and $LC64P^2$, respectively [2]. ESD1 and ESD2 show the products of two common alleles, ESD^1 and ESD^2, respectively, at the ESD locus [11]. *Thin arrows* indicate the polypeptide spots that serve as reference markers for the identification of the LCP1 and ESD polypeptides [2, 11].

scores calculated for the four families. For both sexes combined, the summed lod score is 4.221 at recombination fraction 0, that is, 16,645:1 in favor of linkage. This result indicates that the locus for LCP1 is closely linked to the ESD locus and the subband 13q14.11.

As to the linkage relationship between the loci for LCP1 and other polymorphic cellular polypeptides reported previously [9, 10, 18], only a few families were informative. No evidence for close linkage has so far been obtained between the locus for LCP1 and the locus for cytosol 100 k polypeptide, cytosol 40 k polypeptide, or cytosol 31 k polypeptide (table 2).

DISCUSSION

Deletion [4–6, 8] and family [7] studies have shown that the locus for ESD is tightly linked to *RB1*. Therefore, the ESD polymorphism has been successfully utilized in studying somatic events that occur during tumorigenesis in retinoblastoma [19, 20]. Furthermore, it has been suggested that the ESD polymorphism may be useful for prenatal and preclinical detection of the autosomal dominant type of retinoblastoma, thereby allowing early institution of therapy [4, 7]. In order to perform the genetic analysis of retinoblastoma, especially in

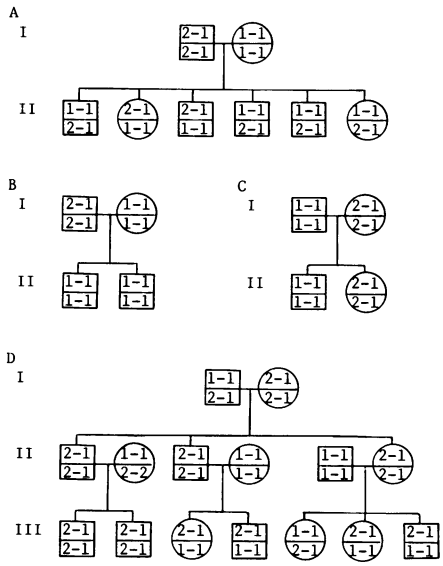

FIG. 2.—Pedigrees of four informative families for the linkage analysis of the loci encoding LCP1 and ESD. The phenotypes for LCP1 and ESD were analyzed by two-DE of peripheral blood lymphocyte proteins as shown in figure 1. For each individual, the genotype for LCP1 (1 = *LCP1*^{1}; 2 = *LCP1*^{2}) is shown in *the upper half of the symbol*, and for ESD (1 = *ESD*^{1}; 2 = *ESD*^{2}), *in the lower half.*

clinical medicine, more genetic markers closely linked to *RB1* are essential. Only the ESD locus has so far been demonstrated to be closely linked to *RB1*, although several DNA segments revealing polymorphic loci have been isolated from human chromosome 13 [21, 22]. We have now shown that the locus for LCP1 is closely linked to the locus for ESD at the narrow chromosomal subband 13q14.11. The data presented in this paper indicate the possibility of a close linkage between LCP1 and RB1 loci.

LCP1 seems to be identical with the polymorphic lymphocyte protein,

TABLE 1

LOD SCORES FOR LINKAGE OF *LCP1* AND *ESD*

FAMILY	No. CHILDREN	RECOMBINATION FRACTION, θ					
		0	.05	.10	.20	.30	.40
A	6	1.505	1.371	1.231	0.924	0.578	0.211
B	2	0.301	0.258	0.215	0.134	0.064	0.017
C	2	0.301	0.258	0.215	0.134	0.064	0.017
D	7	2.114	1.951	1.787	1.429	1.023	0.554
Sum of lod scores		4.221	3.838	3.448	2.621	1.729	0.799
Antilog (relative possibility of θ)		16,645	6,887	2,805	418	54	6

TABLE 2

LOD SCORES FOR LINKAGES OF LOCI FOR LCP1 AND OTHER POLYMORPHIC CELLULAR POLYPEPTIDES DETECTED BY TWO-DE

POLYPEPTIDES	No. FAMILIES	No. CHILDREN	RECOMBINATION FRACTION, θ		
			.05	.10	.20
Cytosol 100 k polypeptide	2	9	−1.906	−1.116	−0.448
Cytosol 40 k polypeptide	2	8	−0.933	−0.463	−0.124
Cytosol 31 k polypeptide	2	5	−0.464	−0.229	−0.060

NIMH 4, reported by Goldman and Merril [23], suggesting that LCP1 is also polymorphic in populations in the United States. The frequencies of the two alleles of NIMH4, p and q, are .84 and .16, respectively, in a population in the United States [23]. The usefulness of LCP1 for the genetic analysis of retinoblastoma depends on the degree of linkage between LCP1 and RB1 loci. Family and deletion studies pertaining to this question are currently being undertaken in our laboratory.

ACKNOWLEDGMENT

We thank Dr. V. M. Darly-Usmar for his comments on the manuscript.

REFERENCES

1. MCKUSICK V: 15343. Lymphocyte cytosol polypeptide, molecular weight 64,000 (LCP1), in *Mendelian Inheritance in Man. Catalogue of Autosomal Dominant, Autosomal Recessive, and X-linked Phenotypes,* 6th ed. Baltimore and London, Johns Hopkins Univ. Press, 1983, p 338
2. HAMAGUCHI H, YAMADA M, NOGUCHI A, ET AL.: Genetic analysis of human lymphocyte proteins by two-dimensional gel electrophoresis: 2. Genetic polymorphism of lymphocyte cytosol 64K polypeptide. *Hum Genet* 60:176–180, 1982
3. KONDO I, SHIN K, HONMURA S, ET AL.: A case report of the patient with retinoblastoma and chromosome 13 deletion: assignment of a new gene (gene for LCP1) on human chromosome 13. *Hum Genet.* In press, 1986
4. SPARKES RS, SPARKES MC, WILSON MG, ET AL.: Regional assignment of genes for human esterase D and retinoblastoma to chromosome band 13q14. *Science* 208:1042–1044, 1980
5. RIVERA H, TURLEAU C, DE GROUCHY J, JUNIEN C, DESPOISSE S, ZUCKER J-M: Retinoblastoma-del(13q14): report of two patients, one with a trisomic sib due to maternal insertion. Gene-dosage effect for esterase D. *Hum Genet* 59:211–214, 1981
6. MOTEGI T, KOMATSU M, NAKAZATO Y, OHUCHI M, MINODA K: Retinoblastoma in a boy with a de novo mutation of a 13/18 translocation. The assumption that the retinoblastoma locus is at 13q14.1, particularly at the distal portion of it. *Hum Genet* 60:193–195, 1982
7. SPARKES RS, MURPHREE AL, LINGUA RW, ET AL.: Gene for hereditary retinoblastoma assigned to human chromosome 13 by linkage to esterase D. *Science* 219:971–973, 1983
8. WARD P, PACKMAN S, LOUGHMAN W, ET AL.: Location of the retinoblastoma susceptibility gene(s) and the human esterase D locus. *J Med Genet* 21:92–95, 1984
9. HAMAGUCHI H, YAMADA M, SHIBASAKI M, MUKAI R, YABE T, KONDO I: Genetic analysis of human lymphocyte proteins by two-dimensional gel electrophoresis: 3.

Frequent occurrence of genetic variants in some abundant polypeptides of PHA-stimulated peripheral blood lymphocytes. *Hum Genet* 62:142–147, 1982

10. KONDO I, YAMAKAWA K, SHIBASAKI M, YAMAMOTO T, HAMAGUCHI H: Genetic analysis of human lymphocyte proteins by two-dimensional gel electrophoresis: V. Genetic polymorphism of cytosol 31k polypeptide. *Hum Genet* 66:244–247, 1984
11. KONDO I, YAMAMOTO T, YAMAKAWA K, ET AL.: Genetic analysis of human lymphocyte proteins by two-dimensional gel electrophoresis: VI. Identification of esterase D in the two-dimensional gel electrophoresis pattern of cellular proteins. *Hum Genet* 66:248–251, 1984
12. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275, 1951
13. O'FARRELL PH: High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 250:4007–4021, 1975
14. MERRIL CR, GOLDMAN D, SEDMAN SA, EBERT MH: Ultrasensitive stain for proteins in polyacrylamide gels shows regional variants in cerebrospinal fluid proteins. *Science* 211:1437–1438, 1981
15. HARRIS H, HOPKINSON DA: 3.1.1.1 esterase (ES), in *Handbook of Enzyme Electrophoresis in Human Genetics,* Amsterdam, New York, Oxford, North-Holland, 1977, pp 1–6
16. MORTON NE: Sequential tests for the detection of linkage. *Am J Hum Genet* 7:277–318, 1955
17. MAYNARD-SMITH S, PENROSE LS, SMITH CAB: in *Mathematical Tables for Research Workers in Human Genetics*. London, J. & A. Churchill, 1961, pp 47–74
18. HAMAGUCHI H, YAMADA M, SHIBASAKI M, KONDO I: Genetic analysis of human lymphocyte proteins by two-dimensional gel electrophoresis: 4. Genetic polymorphism of cytosol 100k polypeptide. *Hum Genet* 62:148–151, 1982
19. GODBOUT R, DRYJA TP, SQUIRE J, GALLIE BL, PHILLIPS RA: Somatic inactivation of genes on chromosome 13 is a common event in retinoblastoma. *Nature* 304:451–453, 1983
20. CAVENEE WK, DRYJA TP, PHILLIPS RA, ET AL.: Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. *Nature* 305:779–784, 1983
21. CEVENEE W, LEACH R, MOHANDAS T, PEARSON P, WHITE R: Isolation and regional localization of DNA segments revealing polymorphic loci from human chromosome 13. *Am J Hum Genet* 36:10–24, 1984
22. DRYJA TP, RAPAPORT JM, WEICHSELBAUM R, BRUNS GAP: Chromosome 13 restriction fragment length polymorphisms. *Hum Genet* 65:320–324, 1984
23. GOLDMAN D, MERRIL CR: Human lymphocyte polymorphisms detected by quantitative two-dimensional electrophoresis. *Am J Hum Genet* 35:827–837, 1983