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Authors
Smith, Moyra
Weiss, Mitchell J
Griffin, Constance A
et al.

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Regional Assignment of the Gene for Human Liver/Bone/Kidney Alkaline Phosphatase to Chromosome 1p36.1–p34

MOYRA SMITH,*,[1] MITCHELL J. WEISS,† CONSTANCE A. GRIFFIN,‡,[2] JEFFREY C. MURRAY,§ KENNETH H. BUETOW,‖ BEVERLY S. EMANUEL,‡ PAULA S. HENTHORN,† AND HARRY HARRIS†

*Department of Pediatrics, University of California, Irvine, California 92715; †Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104; ‡Children's Hospital of Philadelphia, Departments of Pediatrics and Genetics, Philadelphia, Pennsylvania 19104; §Department of Pediatrics, The University of Iowa, Iowa City, Iowa 52242; and ‖Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

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We have used three different methods to map the human liver/bone/kidney alkaline phosphatase (ALPL) locus: (1) Southern blot analysis of DNA derived from a panel of human–rodent somatic cell hybrids; (2) *in situ* hybridization to human chromosomes; and (3) genetic linkage analysis. Our results indicate that the ALPL locus maps to human chromosome bands 1p36.1–p34 and is genetically linked to the Rh (maximum lod score of 15.66 at a recombination value of 0.10) and fucosidase A (maximum lod score of 8.24 at a recombination value of 0.02) loci. These results, combined with restriction fragment length polymorphisms identified by ALPL DNA probes, provide a useful marker for gene mapping studies involving the short arm of chromosome 1. In addition, our results help to elucidate further the structure and evolution of the human alkaline phosphatase multigene enzyme family.

INTRODUCTION

Human alkaline phosphatases (ALPs)[3] are encoded by at least three gene loci that are named for the tissues in which they are characteristically expressed: placental (PALP), intestinal (IALP), and liver/bone/kidney ALP (ALPL, also commonly referred to as L/B/K ALP). The products of these loci differ in their thermostabilities, sensitivity to specific inhibitors, and antigenic properties (Harris, 1982). The intestinal and placental ALPs are expressed in a relatively tissue-specific manner. In contrast, ALPL has a widespread tissue distribution (McComb *et al.*, 1974; Goldstein *et al.*, 1982). It is the ALPL enzyme that is deficient in hereditary hypophosphatasia (Rasmussen and Bartter, 1983).

The ALPL locus has been provisionally assigned to human chromosome 1 by Swallow *et al.* (1986). It was shown that prior incubation of human fibroblast cell extracts with a monoclonal antibody specific for human ALPL led to retarded electrophoretic mobility of ALPL enzyme activity. In similar experiments with a panel of human–rodent somatic cell hybrids, the monoclonal antibody retarded ALPL mobility only in extracts which were derived from hybrids containing human chromosome 1.

Recently Weiss *et al.* isolated cDNA (1986) and genomic (M.W., unpublished data) clones representing the human ALPL locus. Two high-frequency restriction fragment length polymorphisms (RFLPs) within the human ALPL locus have been identified using the enzymes *Bcl*I and *Sst*I (Weiss *et al.*, 1987; Ray *et al.*, 1988). These tools have enabled us to use three different methods to confirm the provisional assignment of ALPL to human chromosome 1 and, furthermore, to regionally map ALPL to the distal short arm. The approaches include Southern blot analysis of somatic cell hybrid DNA, *in situ* hybridization to human chromosomes, and genetic linkage analysis.

MATERIALS AND METHODS

ALPL DNA Probes

The plasmid pS3-1 (Weiss *et al.*, 1986) contains a 2.5-kb ALPL cDNA cloned into the *Eco*RI site of the vector pAT153. The plasmid pLBK4 contains a 1.2-kb single-copy segment of ALPL genomic DNA cloned into the *Hin*dIII site of pAT153 (M.W., unpublished data). This fragment contains exon 6 (176 bp) of the ALPL gene which corresponds to bases 649–824 of the published cDNA sequence.

[1] To whom reprint requests should be addressed.

[2] Present address: The Johns Hopkins Oncology Center, Baltimore, MD 21205.

[3] Abbreviations used: ALP, alkaline phosphatase; L/B/K ALP, liver/bone/kidney alkaline phosphatase; PALP, placental alkaline phosphatase; IALP, intestinal alkaline phosphatase; lod, logarithm of the odds; RFLP, restriction fragment length polymorphism; CEPH, Centre d'Etude du Polymorphisme Humain.

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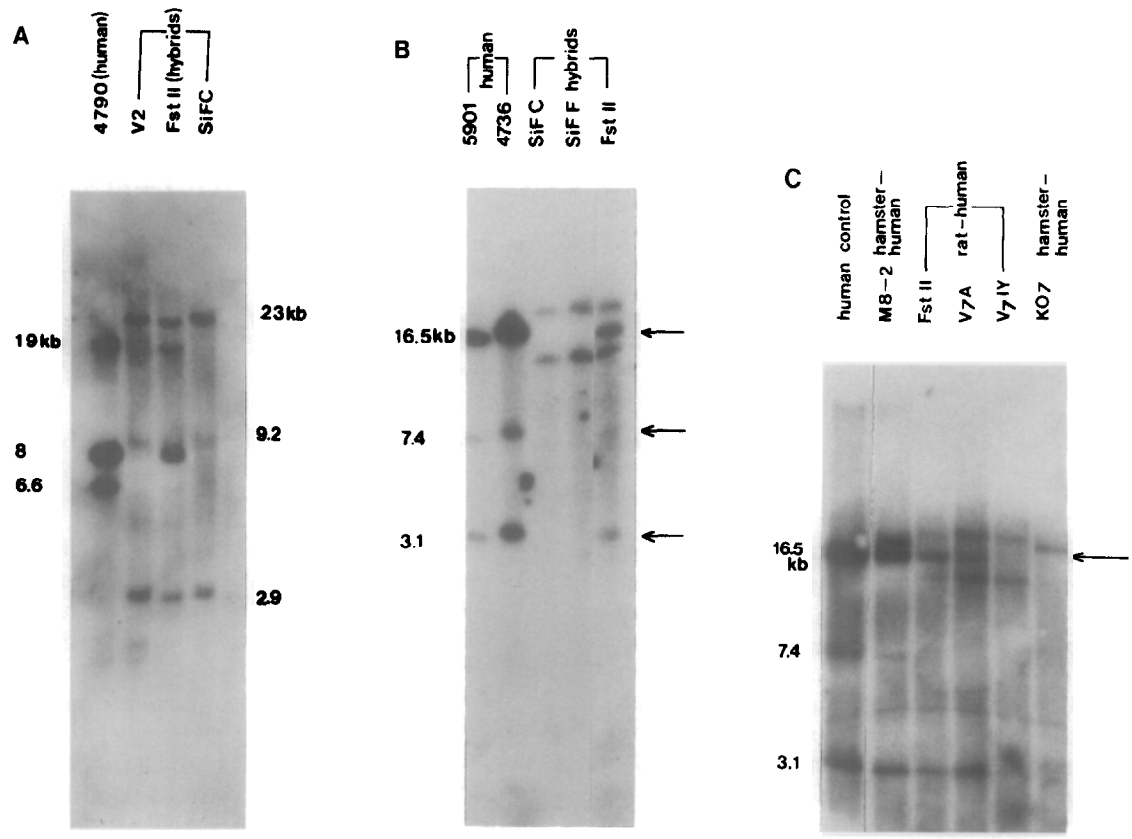

FIG. 1. Southern blots of DNA from human rodent somatic cell hybrids probed with the ALPL cDNA from the plasmid pS3-1. (**A**) *Bam*HI-digested DNA. (**B, C**) *Hind*III-digested DNA. Note that only hybrids Fst11, MR 8-2, and V7A contain ALPL hybridizing human fragments.

Somatic Cell Hybrids

Fourteen different human–rodent somatic cell hybrids were used to derive DNA for Southern blot analysis. Six of the hybrids were derived from the fusion of rat hepatoma cells and primary cultures of human cells. The hybrid clones Sif4D2A, Sif4D2C, and Sif4D2F were derived from the fusion of the hepatoma line Faza with human fibroblasts from one individual, while the clone Fst11 was derived from the fusion of the Faza line with cells from another individual (Kielty *et al.*, 1982). We derived hybrid clones V7A and V2 by fusing the rat hepatoma line Fu5 with human liver fibroblasts. The hybrid clones HHW 509, HHW 416, HHW 661, and B7-10 were derived from the fusion of human lymphocytes with a tRNA synthetase-deficient Chinese hamster ovary cell mutant (Carlock and Wasmuth, 1985). The hybrid line Cf 84/11 was derived from the fusion of the thymidine kinase-deficient hamster cell line GM34 with human fibroblasts (T. Mohandas, personal communication). The hybrid clones MR 8-1 and MR 8-2 were derived from the fusion of an hypoxanthine–guanine phosphoribosyltransferase-deficient hamster cell line and human fibroblasts (Ledbetter *et al.*, 1986). The human chromosomal content of hybrid cell lines (listed in Table 1) was determined by cytogenetic analysis and/or with marker enzyme and molecular probes with known assignments to specific human chromosomes.

Southern Blot Analysis of Somatic Cell Hybrid DNA

Unless otherwise mentioned, isolation and manipulation of DNA were performed according to standard methods (Maniatis *et al.*, 1982). The cDNA probe from the plasmid pS3-1 was used for analysis of hybrid cell DNA. The ALPL insert was isolated by digestion with *Eco*RI followed by agarose gel electrophoresis and electroelution. DNA was labeled with ^{32}P by the random primer method (Feinberg and Vogelstein, 1985).

Parental DNA (10 μg) or hybrid DNA (20 μg) was digested with *Bam*HI or *Hind*III and electrophoresed in 0.8% agarose gels prepared with 0.04 *M* Tris–acetate, pH 8.0/0.002 *M* EDTA. The DNA was then blotted onto nitrocellulose and hybridized with the radiolabeled ALPL cDNA probe. Prehybridization and hybridization were carried out in the presence of 50% formamide, 5× Denhardt's solution, 5% dextran

sulfate, 0.9 *M* NaCl/0.09 *M* sodium citrate (6× SSC), 0.1% SDS, 10 μg/ml polyadenylic acid, and 200 μg/ml herring sperm DNA. After hybridization, filters were washed at 60°C in 0.15 *M* NaCl/0.015 *M* sodium citrate (1× SSC) with 0.1% SDS followed by autoradiography.

Genetic Linkage Studies

Linkage analysis of ALPL was carried out using RFLP and Rh blood group typings on the CEPH reference panel pedigrees (Centre d'Etude du Polymorphisme Humain, Paris, France). The CEPH panel consists of 29 nuclear pedigrees and 11 nuclear families with an average sibship size of 8.2.

Two marker loci, Rh and fucosidase A (FUCA1), were chosen for linkage analysis because of their previous assignment to chromosome 1p34 (Cook and Hamerton, 1979; Carritt, 1985). Typing for the red cell antigen Rh was provided by CEPH. A fucosidase A cDNA probe, which identifies a *Pvu*II RFLP in human genomic DNA (Darby *et al.*, 1986), was provided by Dr. Patrick Willems. For analysis of RFLPs within the ALPL locus, genomic DNA was digested with *Bcl*I. Southern blots, performed as described above, were hybridized to the cDNA probe from pS3-1 which detects a two-allele *Bcl*I RFLP: either a 7.4-kb band (frequency = 0.67) or 3.1- and 4.3-kb bands (frequency = 0.33) (Weiss *et al.*, 1987).

Pairwise linkage analysis was performed by the lod (logarithm of the odds) score method of Morton (1955) using the LINKAGE program MLINK (Lathrop *et al.*, 1984) and CEPH database management software. A univariate ($\theta = \theta_{male} = \theta_{female}$) lod table was obtained and maximum likelihood estimates of the univariate recombination values were obtained by interpolation.

In Situ Hybridization of ALPL to Human Metaphase Chromosome Spreads

The plasmid pLBK4 was ^{3}H-labeled by nick translation to a specific activity of 2×10^7 cpm/μg. The probe was hybridized to human metaphase chromosome preparations made from short-term culture of peripheral lymphocytes of normal males. Hybridization was performed essentially by the method of Harper *et al.* (1981) at 37°C for 15–18 h in 50% formamide/0.3 *M* NaCl, 0.03 *M* sodium citrate (2× SSC)/10% dextran sulfate with 100 μg/ml of sonicated salmon sperm DNA. Washes were performed at 39°C in 50% formamide/2× SSC for 9 min and then in 2× SSC for 12 min, followed by dehydration in ethanol. Autoradiography was performed for 15–21 days using NTB-2 Kodak emulsion. Chromosome banding was achieved using borate buffer and Wrights-Giemsa stain (Cannizaro and Emanuel, 1984). Only well-spread, banded chromosomes were used for localization of grains.

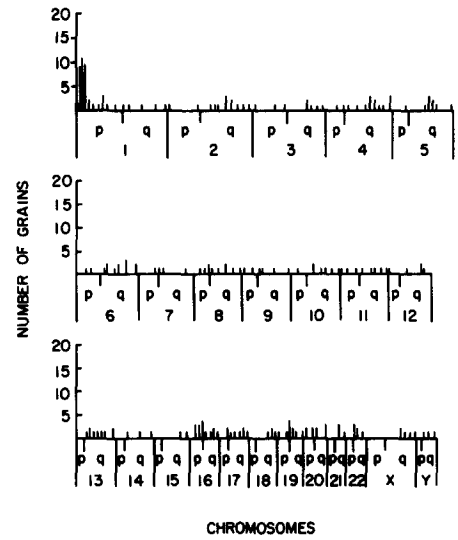

FIG. 2. Grain distributions from *in situ* hybridization of the ALPL genomic probe from the plasmid pLBK4 to human metaphase chromosomes. The abscissa represents the chromosomes in their relative size proportions and the ordinate shows the number of silver grains.

RESULTS

Somatic Cell Hybrid Studies

Fourteen different somatic cell hybrids were examined by Southern blot analysis for the presence of human-specific ALPL DNA. Digestion of DNA with *Bam*HI or *Hin*dIII enabled us to distinguish between human and rodent ALPL gene fragments (*Bam*HI: 19, 8.0, and 6.6 kb for human-specific ALPL sequences; 23, 9.2, and 2.9 kb for rat-specific ALPL sequences; *Hin*dIII: 16.5, 7.4, and 3.1 kb for human ALPL sequences; 18 and 15 kb for rat ALPL sequences, see Fig. 1). Concordant segregation of human-specific ALPL fragments in the hybrid DNA was observed only for human chromosome 1. Every other human chromosome could be excluded by at least two discordant hybrid clones (Table 1).

In Situ Hybridization of an ALPL-Specific Probe to Human Metaphase Chromosomes

One hundred metaphase chromosome spreads containing a total of 219 chromosomally localized grains were scored. The results are summarized in Fig. 2. The predominant site of hybridization was 1p36.1–p34. The 29 grains located in this region represent 13% of the total chromosomally localized grains and are at least three times the number on any other chromosomal segment of comparable length. Thus, there is a single locus for L/B/K ALP detected by *in situ* hybridization which maps to the distal end of the short end of chromosome 1.

Genetic Linkage Analysis

ALPL was observed to be genetically linked to Rh with a maximum lod score of 15.66 at 10% recombination, and to FUCA1 with a maximum lod score of 8.24 at 2% recombination.

DISCUSSION

This study confirms the assignment of the gene locus ALPL, which encodes the liver/bone/kidney form of ALP, to human chromosome 1. *In situ* hybridization experiments indicate that the ALPL locus is in the region 1p36.1–p34. This regional localization is further supported by linkage analyses which demonstrate that ALPL is closely linked to Rh and FUCA1, two markers known to map to the p34 region of human chromosome 1.

Two high-frequency RFLPs within the ALPL gene locus have been characterized (Weiss *et al.*, 1987; Ray *et al.*, 1988). A genomic DNA probe that identifies both of these RFLPs, 8B/E5′, will be useful for gene mapping studies involving the short arm of human chromosome 1. We have submitted this probe to the American Type Culture Collection (ATCC, Rockville, MD). A multipoint linkage map of chromosome 1p, which includes ALPL and six other markers, will be presented elsewhere.

The regional chromosomal assignment, and the estimation of close linkage between ALPL and Rh and ALPL and FUCA1, may prove to be clinically significant. The ALPL enzyme is deficient in hereditary hypophosphatasia, a disorder characterized by defective osteogenesis and low levels of ALPL in serum and all tissues (PALP and IALP levels are unaffected) (Rasmussen and Bartter, 1983). A wide variation in severity occurs in hypophosphatasia, suggesting that a number of different mutations may give rise to distinct clinical phenotypes. It is likely that some of these mutations involve the structural ALPL locus. However, it has also been suggested that some mutations that give rise to hypophosphatasia may affect the regulation of a structurally intact ALPL gene (Whyte *et al.*, 1986). Such mutations would not necessarily be linked to the ALPL locus. This issue of whether hypophosphatasia is genetically linked to the ALPL gene locus can now be addressed by linkage studies in affected pedigrees using RFLPs identified by ALPL gene probes. However, in pedigrees where ALPL probes are uninformative, the inheritance of hypophosphatasia may be tracked by following the segregation of Rh or FUCA1 polymorphisms.

Chromosome mapping of the loci that encode human ALPs further elucidates the evolutionary history of these genes. It is thought that the human ALP multigene enzyme family arose by a series of duplications from a single ancestral gene (Harris, 1982). Im-

TABLE 1

Correlation of the Human ALPL Gene and Human Chromosomal Content of Somatic Cell Hybrids

Hybrid clone	ALPL	Chromosome																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Sif4D2A	−	−	−	−	+	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	+
Sif4D2C	−	−	−	p+	+	+	+	+	−	+	−	+	+	−	+	+	+	+	−	−	−	+	+	+
Sif4D2F	−	−	+	−	+	−	−	−	−	−	+	+	+	−	−	+	−	−	+	+	+	+	−	+
FstII	+	+	−	−	+	−	+	−	+	−	+	+	−	−	−	+	−	−	+	+	−	−	−	+
Fu5 V2	−	−	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	−	+	+	+	−	+
Fu5 V7A	+	+	−	−	+	+	+	−	−	−	+	+	+	+	+	+	−	−	−	−	+	+	−	−
HHW 509	−	−	−	−	+	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HHW 455	−	−	−	−	p+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KO1	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KO8	−	−	−	−	+*	−	−	−	−	−	−	−	+*	−	−	−	−	−	−	−	−	−	−	−
B7-10	−	−	−	+	+	+	+	−	−	+	−	−	+	−	+	−	−	−	+	−	−	+	−	−
CF 84/11	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
MR 8-1	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
MR 8-2	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Discordancy rate		0/14	4/14	6/13	7/12	6/14	4/14	5/14	4/14	6/14	4/14	6/14	6/13	2/14	6/14	4/14	5/14	4/14	5/14	3/14	4/14	4/14	5/14	5/14

Note. *, Derivative chromosome: 4qter → 4q23–12p1-3 → pter. p+, Only the short arm of the indicated chromosome is present. Rearranged chromosomes were not included in the discordancy rates.

munologic and cDNA sequence data indicate that PALP and IALP are more similar to each other than either is to ALPL (Henthorn *et al.*, 1987; Harris, 1982). We believe that a duplication of the ancestral ALP gene gave rise to two ALP loci which subsequently became chromosomally separated in the course of evolution. An additional, more recent, duplication gave rise to the PALP and IALP genes which now reside on chromosome 2q34–q37 (Griffin *et al.*, 1987). There is evidence to suggest that yet another duplication followed, giving rise to a distinct ALP locus which is closely related to the PALP and IALP genes (Knoll *et al.*, 1987). This locus (which was isolated by hybridization to a PALP probe) must also map to chromosome 2q34–37, since *in situ* hybridization using IALP and PALP probes failed to localize any grains outside of this region (Griffin *et al.*, 1987). Thus, it is likely that there is a cluster of at least three ALP genes situated at the distal end of the long arm of chromosome 2. In contrast, the L/B/K ALP gene exists as a single copy (M.W., unpublished data) near the distal end of the short arm of chromosome 1.

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