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

MOYRA SMITH,*† Mitchell J. Weiss,‡ Constance A. Griffin,‡ Jeffrey C. Murray,§ Kenneth H. Buetow,¶ Beverly S. Emanuel,¶ Paula S. Henthorn,† and Harry HARRIS†

*Department of Pediatrics, University of California, Irvine, California 92712; †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

Received December 21, 1987

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) 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 BclI and SstI (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 EcoRI site of the vector pAT153. The plasmid pLBK4 contains a 1.2-kb single-copy segment of ALPL genomic DNA cloned into the HindIII 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.
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 EcoRI 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 BamHI or HindIII 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 (2× 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 (FUCAl), 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 PvuII 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 BclI. Southern blots, performed as described above, were hybridized to the cDNA probe from pSL-1 which detects a two-allele BclI 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 (θ = θmale = θfemale) 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 3H-labeled by nick translation to a specific activity of 2 × 10^6 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.

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 BamHI or HindIII enabled us to distinguish between human and rodent ALPL gene fragments (BamHI: 19, 8.0, and 6.6 kb for human-specific ALPL sequences; 23, 9.2, and 2.9 kb for rat-specific ALPL sequences; HindIII: 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-
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.

ACKNOWLEDGMENTS

We thank Robert Nussbaum for the gift of genomic DNA from somatic cell hybrids MR 8-1 and MR 8-2, Anita Hawkins and Catherine DeHaven for technical assistance, and Max Minne for somatic cell hybrids MR 8-1 and MR 8-2, Anita Hawkins and Catherine DeHaven for technical assistance, and Max Minne for the distal end of the short arm of chromosome 1. This work was supported by Grants NIH GM 27018 and March of Dimes 858 (to H.H.), NIH Medical Scientist Training Program T32-GM07170 (for M.W.), and the NIAAA Intramural Research Program (for M.S.). Catherine DeHaven for technical assistance, and Max Minne for the distal end of the short arm of chromosome 1. This work was supported by Grants NIH GM 27018 and March of Dimes 858 (to H.H.), NIH Medical Scientist Training Program T32-GM07170 (for M.W.), and the NIAAA Intramural Research Program (for M.S.).

REFERENCES

1. CANNIZARO, L., AND EMANUEL, B. (1984). An improved method for G banding of chromosomes after in situ hybridization. Cytogenet. Cell Genet. 38: 308–309.
2. CARLOCK, L., AND WASMUTH, J. (1985). Molecular approach to analyzing the human 5p deletion syndrome Cri du Chat. Somat. Cell Mol. Genet. 11: 267–276.
3. CARRITT, B. (1986). Genomic cloning of the short arm of chromosome 1. Cytogenet. Cell Genet. 40: 599.
4. COOK, P. J. L., AND HAMERTON, J. (1979). Report of the committee on human chromosome 1: Human Gene Mapping 5. Cytogenet. Cell Genet. 25: 9–20.
5. DARBY, J. K., JOHNSEN, J., NAKASHIMA, P., WILLEM, P. J., O'BRIEN, J. S. O., FOWLER, M. L., SHOWS, T. B., SHOOTER, E. M., AND CAVALLI-SFORZA, L. L. (1986). PouII RFLP at the human chromosome 1 alpha-L-fucosidase gene locus (FUCA1). Nucleic Acids Res. 14: 9543.
6. FEINBERG, A. P., AND VOGLERSTEIN, B. (1985). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132: 6–13.
7. GOLDSTEIN, D. J., ROGERS, C., AND HARRIS, H. (1982). A search for expression of placental-like alkaline phosphatase in non-malignant human tissues: Demonstration of its occurrence in lung, cervix, testis and thymus. Clin. Chim. Acta 125: 63–65.
8. GRIFFIN, C., SMITH, M., HENTHORN, P., HARRE, H., WEISS, M., RADUCHA, M. AND EMANUEL, B. (1987). Human placental and intestinal alkaline phosphatase genes map to 2q34–q37. Amer. J. Hum. Genet. 41: 1025–1034.
9. HARPER, M. E., ULLRICH, A., AND SAUNDERS, G. F. (1961). Localization of the human insulin gene to the distal end of the short arm of chromosome 11. Proc. Natl. Acad. Sci. USA 78: 4458–4460.
10. HARRIS, H. (1982). Human alkaline phosphatases. In “The Harvey Lectures Series 76” (D. M. Marcus, Ed.), pp. 95–124, Academic Press, New York.
11. HENTHORN, P. S., RADUCHA, M., EDWARDS, V. H., WEISS, M. J., SLAUGHTER, C., LAFERTY, M. A., AND HARRIS, H. (1987). Nucleotide and amino acid sequences of human intestinal alkaline phosphatase: Close homology to placental alkaline phosphatase. Proc. Natl. Acad. Sci. USA 84: 1234–1238.
12. KIELTY, C. M., POVEY, M. S., AND HAMPSON, D. A. (1989). Regulation of expression of liver specific enzymes. Ann. Hum. Genet. 53: 135–143.
13. KNOLL, B. J., ROTHJUL, N. K., AND LONGLEY, M. A. (1987). Two gene duplication events in the evolution of the heat stable alkaline phosphatases. Gene, in press.
14. LATHROP, G. M., LALOUEL, J. M., JULIERS, C., AND OTT, J. (1984). Strategies for multilocus linkage analysis in humans. Proc. Natl. Acad. Sci. USA 81: 3443–3446.
15. LEDGETTER, D. H., AIRHART, S. D., AND NUSSEBAUM, R. (1996). Somatic cell hybrid studies of fragile X expression in a carrier female and transmitting male. Amer. J. Hum. Genet. 23: 499–443.
16. MANIATIS, T., FRITSCH, E. F., AND SAMBROOK, J. (1982). “Molecular Cloning.” Cold Spring Harbor Laboratories, New York.
17. MCCOMB, R. B., BOWERS, G. N., AND POSEN, S. (1974). “Alkaline Phosphatase,” Plenum Press, New York.
18. MORTON, N. E. (1955). Sequential tests for detection of linkage. Amer. J. Hum. Genet. 7: 277–238.
19. RASMUSSEN, H., AND BARTTER, F. C. (1983). Hypophosphatasia. In “The Metabolic Basis of Inherited Diseases” (J. B. Stanbury, J. B. Wyngaarden, D. S. Frederickson, J. H. Goldstein, and M. S. Brown, Eds.), 6th ed., pp. 1497–1507, McGraw–Hill, New York.
20. RAY, K., WEISS, M. J., DRACOPOLI, N., AND HARRIS, H. (1988). A second RFLP at the human liver/bone/kidney alkaline phosphatase locus. Nucleic Acids Res., in press.
21. SWALLOW, D. M., POVEY, S., PARKAR, M., ANDREW, P. W., HARRIS, H., PYM, B., AND GOODFELLOW, P. (1986). Mapping of the gene coding for the human liver/bone/kidney isozyme of alkaline phosphatase to chromosome 1. Ann. Hum. Genet. 50: 229–235.
22. WEISS, M. J., HENTHORN, P. S., LAFERTY, M. A., SLAUGHTER, C., RADUCHA, M., AND HARRIS, N. (1986). Isolation and characterization of a cDNA encoding human liver/bone/kidney-type alkaline phosphatase. Proc. Natl. Acad. Sci. USA 83: 7182–7186.
23. WEISS, M. J., SPEILMAN, R. S., AND HARRIS, H. (1987). A high-frequency RFLP at the human liver/bone/kidney alkaline phosphatase locus. Nucleic Acids Res. 15: 889.
24. WHEAT, M. P., MAGILL, H. L., FALLON, M. D., AND HERROD, H. G. (1986). Infantile hypophosphatasia: Normalization of circulating bone alkaline phosphatase activity followed by skeletal remineralization (evidence for an intact structural gene for tissue nonspecific alkaline phosphatase). J. Pediatr. 108: 82–88.