Am. J. Hum. Genet. 46:286–292, 1990

Infantile Hypophosphatasia: Localization within Chromosome Region 1p36.1-34 and Prenatal Diagnosis Using Linked DNA Markers

Cheryl R. Greenberg,*,† Jane A. Evans,*,† Stephen McKendry-Smith,* Shawn Redekopp,* James C. Haworth,*,† Richard Mulivor,‡ and Bernard N. Chodirker*,†

Departments of *Pediatrics and Child Health and †Human Genetics, University of Manitoba, Winnipeg; and ‡Coriell Institute for Medical Research, Camden, NJ

Summary

Linkage analysis was performed on data from Manitoba Mennonite families identified by a proband with infantile hypophosphatasia (HOPS), an autosomal recessive disorder characterized by defective skeletal mineralization. Southern blot analysis of *Msp*-I-digested DNA from HOPS nuclear families probed with a 2.55-kb liver/bone/kidney alkaline phosphatase (ALPL) cDNA revealed two previously undescribed RFLPs at 2.4/2.3 kb and 2.0/1.9 kb. Maximum combined lod score equals 13.25 at $\theta = 0$. This establishes very close linkage between *ALPL* and *HOPS* and allows for the regional assignment of the HOPS gene to chromosome 1p36.1–34. Prenatal RFLP studies in an informative Mennonite family correctly predicted an unaffected fetus following chorionic villus sampling at 12 wk gestation. In addition in our Mennonite population, a nonrandom association exists between the polymorphic ALPL alleles and *HOPS*. These results suggest that strong linkage disequilibrium exists between *HOPS* and the ALPL markers. This will allow for improved carrier assignment in this high-risk population. Preliminary analysis suggests approximately 1/25 Manitoba Mennonites are HOPS carriers.

Introduction

Hypophosphatasia is a metabolic bone disease characterized by a disturbance in normal bone mineralization and by deficient serum and tissue alkaline phosphatase (ALP) activities (Rasmussen and Bartter 1978). Depending on the age of diagnosis, three clinical forms are currently recognized: (1) infantile, (2) childhood, and (3) adult.

Infantile hypophosphatasia (HOPS) is the most severe form and is usually lethal in the neonatal period. This form is generally accepted to be inherited as an autosomal recessive disorder (Rathbun 1948; Fraser 1957). The age of clinical presentation of HOPS is either in the neonatal period or before 6 mo and is characterized by a severe rachitic-like illness, bony ossification abnormalities, hypercalcemia, and failure to thrive. The exact incidence is unknown but, on the basis of Fraser's Toronto data (Fraser 1957), has been estimated to be approximately 1 in 100,000 live births. Juvenile hypophosphatasia presents after 6 mo of age and is associated with a more benign course characterized by premature loss of deciduous teeth, rachitic-like findings, and craniosynostosis. The adult form manifests generally later in life, with premature loss of adult teeth, recurrent fractures, and pseudofractures.

An increased frequency of HOPS has been documented in Mennonite communities of Manitoba and Saskatchewan (Macpherson et al. 1972). Following the birth of six severely affected neonates in southern Manitoba during the 10-year period 1977–86, we em-

Received September 12, 1988; final revision received October 16, 1989.

Address for correspondence and reprints: Cheryl R. Greenberg, M.D., Department of Human Genetics, University of Manitoba, 250–770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E OW3.

© 1990 by The American Society of Human Genetics. All rights reserved.
0002-9297/90/4602-0009$02.00

barked on a multidisciplinary study to elucidate the genetics of hypophosphatasia. With the recent cloning of the full-length cDNA encoding human liver/bone/kidney ALP (ALPL) (Weiss et al. 1986), the ALPL locus has been regionally assigned to 1p36.1-p34 (Smith et al. 1988) and genetically linked to *RH* (maximum lod score [$\hat{Z}$] = 15.66 at recombination value [θ = .10) (Smith et al. 1988). We have recently shown close linkage between *RH* and *HOPS* and provisionally assigned the HOPS gene to chromosome 1p (Chodirker et al. 1987; Coghlan et al. 1988). In the present paper we report on (1) the regional localization of *HOPS* to chromosome 1p36.1–34 by using linkage analysis between *HOPS* and *ALPL* and (2) as the first use of RFLP markers in the prenatal evaluation of a fetus at risk for HOPS.

Material and Methods

A. Family Studies

After informed consent was obtained, detailed clinical, dental, and radiologic information was collected on 269 individuals from eight infantile, three juvenile, and two adult hypophosphatasia Mennonite kindreds. All individuals were personally examined by one of four authors (C.R.G., B.N.C., J.A.E., or S.R.). Fasting blood specimens were collected for calcium, phosphate (Pi), and ALP determinations, vitamin B6 vitamers, blood group serology, and DNA extraction. Early-morning urine samples were obtained for phosphoethanolamine (PEA) determination. Results of radiologic studies, vitamin B6 analyses and detailed segregation analyses will be reported elsewhere. For the purpose of linkage analysis, the first-degree relatives of 20 obligate HOPS carriers from the eight infantile kindreds were classified as either HOPS carriers or HOPS noncarriers on the basis of the prediction from the diagnostic logistic regression analysis models based on (ALP+Pi) or (ALP+Pi+PEA), as described elsewhere (Chodirker et al. 1989). The accuracy of these diagnostic models for carrier assignment in our population has been established elsewhere (Chodirker et al. 1989). Thirty-six of the 269 individuals formed a predominantly Mennonite control group. This group consisted of the apparently unrelated spouses of the obligate carriers and the first-degree relatives. Only individuals older than 18 years were included in the linkage study.

B. Southern Blot Analysis

High-molecular-weight DNA was extracted from whole blood or from cultured skin fibroblasts, and Southern blot analysis was performed according to a method described elsewhere (Greenberg et al. 1987). Initially, a search for RFLPs was performed on the DNA from two obligate carriers and from their HOPS offspring digested with a battery of 10 restriction endonucleases (*Bcl*I, *Sst*I, *Pst*I, *Taq*I, *Eco*RV, *Xmn*I, *Hin*dIII, *Bst*XI, *Sac*I, and *Msp*I), size separated on 1% agarose gels for 1,375 V h, blotted to HY-BOND (Amersham), and probed with the ^{32}P-labeled 2.5-kb ALPL cDNA insert from the plasmid pS3–1 (courtesy of Dr. Harry Harris, Philadelphia). *Bcl*I digests were also probed with the ^{32}P-labeled ALPL genomic insert *8B/E5′* (American Type Culture Collection, Rockville, MD) that was isolated and characterized by Ray et al. (1988). The DNA probes were labeled by the random primer method (Feinberg and Vogelstein 1983) to specific activities of 1–4 $\times$ 10^8 cpm/μg DNA. Prehybridization and hybridization of the filters were performed at 42°C in the presence of 50% formamide, 5 $\times$ SSPE, 0.1% SDS, 0.05% Blotto with 600 μg denatured salmon sperm DNA/ml. In addition, the hybridization solution contained 5% dextran sulfate. Membranes were washed to 65°C in 0.015 M sodium chloride/0.015 M sodium citrate (0.1 $\times$ SSC) with 0.1% SDS, followed by autoradiographic exposure, with an intensifying screen, to Kodak XAR-5 film at −70°C for 1–7 d.

Following identification of two previously undescribed *Msp*I polymorphisms at the ALPL locus by using probe pS3–1, the allele frequencies in 69 apparently unrelated Mennonite adults (138 genomes) from the study population were calculated. The DNA from 25 unrelated non-Mennonite adults participating in investigations unrelated to this study was also screened for the *Msp*I polymorphisms to provide a comparison group.

C. Linkage Analysis

Linkage between *HOPS* and *ALPL* was studied by analysis of the *Msp*I RFLPs within the ALPL locus by using Southern blotting as described above and the two logistic regression analysis models of carrier assignment (Chodirker et al. 1989). Eighteen nuclear families with an average sibship size of five were included in the RFLP studies. Lod scores were calculated by the Mark III version (Côté 1975) of the linkage program based on Edwards's marker algebra (Edwards 1972), peak lods ($\hat{Z}$) were calculated by a computer-determined curve, and 95% likelihood limits were calculated by taking the θ values within the bounds of $\hat{Z} - 1$.

D. Prenatal Diagnostic Study

Transcervical chorionic villus sampling (CVS) was performed at 12 wk gestation on a 28-year-old G3P2 woman whose first child died in the neonatal period of well-documented HOPS. Having participated in our initial linkage studies, this Mennonite family was known to be informative for one of the two *Msp*I RFLP markers. Southern blot analysis of *Msp*I-digested DNA extracted from the fetal CVS tissue was performed as described above. Chromosome analysis was performed, and ALP activity in cultured CVS cells was studied according to a method described elsewhere (Mulivor et al. 1978). Serial fetal sonographic examinations were carried out at 17 and 21 wk gestation.

Results

Two *Msp*I polymorphisms at the ALPL locus were detected, characterized, and analyzed for linkage (fig. 1). Three constant bands are present at 5.5 kb (faint), 1.5 kb, and 0.5 kb. The two RFLPs observed are at 2.4 kb (A1)/2.3 kb (A2) and 1.98 kb (A3)/1.9 kb (A4). Twenty-four of the 25 unrelated non-Mennonite adults who were screened demonstrated only the A2A4 haplotype. The one apparently non-Mennonite adult who was heterozygous *A1A3/A2A4* was subsequently identified as having been adopted in southern Manitoba, which has a predominantly Mennonite population, but the ethnic background of this adult is not known. The allele frequencies in 69 apparently unrelated healthy Mennonite adults (138 genomes) are A1 = 0.27/A2 = 0.73 and A3 = 0.22/A4 = 0.78. Segregation of the (A1/A2) and (A3/A4) alleles was consistent with Mendelian codominant genetic markers ($P = 0.35$ and .86, χ^2 analysis with Yates' correction). The *BclI ALPL* RFLPs reported by Weiss et al. (1987) and Ray et al. (1988) were not informative in our families.

Lod score analysis of 14 informative nuclear families identified by an obligate HOPS carrier from eight large extended Mennonite kindreds was performed to assess linkage between *HOPS* and *ALPL* by using the A3/A4 polymorphism. The maternal lod score for 41 informative meioses was $\hat{Z} = 8.14$ at $\theta = 0$ (95% likelihood limits $\theta = 0.00$–.07). The paternal lod score for 19 informative meioses was $\hat{Z} = 5.13$ at $\theta = 0.00$ (95% likelihood limits $\theta = 0.00$-.11). The combined lod score for 60 informative meioses in 14 informative nuclear families was $\hat{Z} = 13.25$ at $\theta = 0$ (95% likelihood limits $\theta = 0.00$-.05), as shown in figure 2. A representative family study is shown in figure 1. In this family, family T, individuals 7 and 8 are obligate carriers, having had

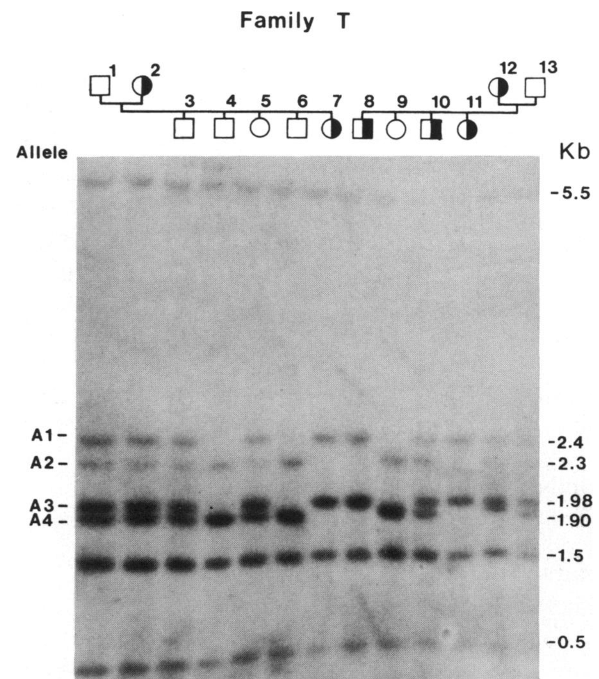

Figure 1 Southern blot pattern of the *Msp*I-digested DNA of family T with probe pS3–1. Constant bands are seen at 5.5 kb, 1.5 kb, and 0.5 kb. Polymorphic bands are at 2.4(A1)/2.3(A2) kb and 1.98(A3)/1.9(A4) kb. Open and half-shaded symbols denote individuals designated noncarriers and carriers, respectively, of the HOPS mutation, on the basis of logistic regression analysis using ALP, Pi, and PEA determinations. Individuals 4, 6, and 9, who are homozygous *A2A4/A2A4*, and individuals 7, 8, and 11, who are homozygous *A1A3/A1A3*, were scored for linkage analysis.

an affected infant who died in the neonatal period of classical HOPS but from whom no DNA was available. These parents are homozygous for the upper and lower RFLPs, and their genotypes are designated *A1A3h/A1A3H*. The maternal (individuals 1 and 2) and paternal (individuals 12 and 13) grandparents of the proband are heterozygotes *A1A3/A2A4*. We assigned to individuals 2 and 12 HOPS carrier status (Hh) biochemically, and their genotypes are designated *A1A3h/A2A4H*. Individuals 1 and 13 were assigned to noncarrier status biochemically, and their genotype designations are *A1A3H/A2A4H*. Individuals 4, 6, and 9, siblings of the obligate carriers and with genotypes *A2A4/A2A4*, would be predicted to be noncarriers. Individual 11, with genotype *A1A3/A1A3*, would be predicted to be a carrier. In all cases our biochemical carrier assignment was in agreement with the DNA prediction. Individuals 3, 5, and 10, who are heterozygous for the DNA markers, could not be scored. The contribution to the maternal lod score by this family was +.602 (no recom-

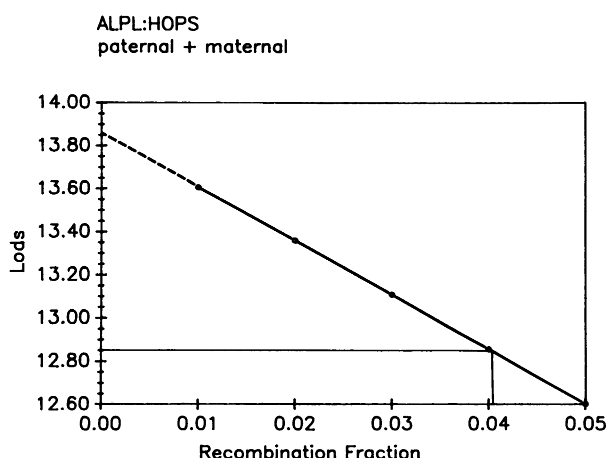

Figure 2 *ALPL:HOPS* combined peak lod ($\hat{Z}$) equals 13.85 at θ = 0 (95% likelihood limits θ = .00–.05).

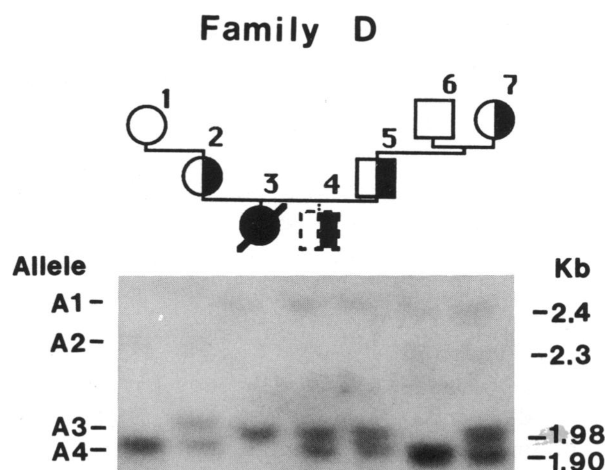

Figure 3 Southern blot pattern of prenatal diagnostic study of *Msp*I-digested DNA probed with pS3–1 in family D. The genotypes of the deceased affected female infant (lane 3) are designated *A3h/A3h*. The fetus at risk (lane 4) is heterozygous *A3/A4*, similar to the obligate carrier parents in lanes 2 and 5, predicting a carrier phenotype. Symbol notation of individuals 1, 6, and 7 is as in fig. 1. Alleles A1 and A2, faintly seen on this blot, were not informative in this family.

bination seen), and that to the paternal lod score was +.602 (no recombination seen). In the other 12 informative nuclear families scored, six first-degree relatives of obligate carriers were excluded from analysis on the basis of intermediate probabilities of carrier assignment by using logistic regression analysis based on (ALP+Pi) or (ALP+Pi+PEA). Four other nuclear families were noninformative for linkage analysis. Grandparental genotypes could not be inferred in three of these families, and in the fourth family the obligate-carrier parents were homozygous for both RFLP markers and their heterozygous children could not be scored.

Once close linkage between *ALPL* and *HOPS* had been established in our Mennonite kindreds, prenatal diagnosis using the linked DNA markers was possible. In family D the obligate-carrier parents were known to be completely informative for the lower *Msp*I RFLP only, and DNA was available from a previously affected deceased sibling. Southern blot analysis of *Msp*I-digested DNA extracted from fetal tissue obtained at 12 wk gestation by transcervical CVS was performed, and results are shown in figure 3. The fetus had alleles A3 and A4, similar to the parents and in contrast to the affected baby, who was homozygous *A3/A3*. This would predict a carrier phenotype. Chromosome analysis of 25 cells revealed a normal male 46, XY karyotype. Our DNA prediction of an unaffected but carrier male was in agreement with the results of two separate assays of ALP activity in cultured CVS cells showing specific activities with fluorescent substrate 4-methylumbelliferyl phosphate (4MUB) of 0.21 nmol and 0.14 nmol 4MUB/min/mg protein (normal >0.1 nmol 4MUB/min/mg protein) (Mulivor et al. 1978). Serial fetal sonographic examinations at 17 and 21 wk gestation revealed normal linear growth and no skeletal abnormalities. A normal male was born at term and is currently healthy and thriving.

Discussion

In this linkage study on HOPS, using RFLPs identified by the ALPL gene probe pS3–1, we show very tight linkage between *HOPS* and *ALPL*, with no recombination observed in 60 informative meioses studied to date. Genetic linkage analysis has previously shown *RH* and *HOPS* to be closely linked with a combined maternal and paternal *RH-HOPS* $\hat{Z}$ of 6.462 at θ = 0.11 (95% likelihood limits .02-.25). This provides the first evidence for regional assignment of the HOPS gene to chromosome 1p36.1–34 and supports the hypothesis that a mutation in the structural ALPL locus directly causes HOPS. No gene deletions or rearrangements have been seen when either the ALPL cDNA or the genomic DNA probe has been used. Northern analysis and studies to directly identify the suspected mutation in the HOPS gene are in progress.

Although our study has addressed the question of whether HOPS is genetically linked to the ALPL gene locus, we cannot answer the question of whether genetic heterogeneity exists. Recently Weiss et al. (1988)

Table 1

Number of Observed ALPL *MspI* Haplotypes For HOPS (h) AND NON HOPS (H) Chromosomes

Haplotypes	Total No.	h	H
A1A3	24	14	10
A2A3	0	0	0
A2A4	97	0	97
A1A4	7	0	7
Total	128	14	114

reported a missense mutation in *ALPL* that abolished ALPL activity and resulted in HOPS. The DNA of other unrelated HOPS patients who were screened, including our own Mennonite HOPS patients, did not demonstrate this point mutation (Weiss et al. 1989). Whyte et al. (1986) also reported the sustained biochemical, radiological, and clinical response of an infant with HOPS to intravenous infusion of pooled normal plasma, suggesting that *ALPL* may be intact and that a regulatory defect is more likely. Thus the mutations causing HOPS are likely to be very heterogeneous.

In our Mennonite population, an increased incidence of HOPS has been observed, and the disease in Manitoba has been essentially restricted to Mennonite kindreds. Preliminary population analysis suggests that the incidence of HOPS among Manitoba Mennonites is 1 in 2,500 births and that approximately 1 in 25 Mennonites is a carrier of the HOPS gene (Chodirker et al. 1988). In our population, first-trimester prenatal diagnosis using the linked DNA markers as demonstrated by the present report is now possible and will be an important adjunct to the more traditional biochemical assays and ultrasonographic scanning available (Mulivor et al. 1978; Warren et al. 1985).

In our Mennonite population, the *ALPL*-linked haplotypes of 128 chromosomes could be unequivocally assigned either by appropriate family studies or because the subjects were either homozygous at both *MspI* RFLP sites or heterozygous at only one site. Only four of eight possible haplotypes were observed. The distribution of these haplotypes is shown in table 1. Analysis of our data revealed that the mutant HOPS allele (h) always segregates with A1 and A3, defining an *A1A3h* haplotype. Three affected HOPS probands were homozygous *A1A3h/A1A3h,* two on the basis of DNA analysis directly and one in family T (fig. 1) who was inferred from the genotypes of the parents. However, not all *A1A3* haplotypes segregated with the h allele. The normal HOPS allele (H) was primarily inherited with *A2* and *A4* defining the *A2A4H* haplotype, but *A1A3H* and *A1A4H* were also observed. The *A2A4h, A1A4h, A2A3h,* and *A2A3H* haplotypes were not observed in our study of this Mennonite population. Only the *A2A4H* haplotype has been seen in the non-Mennonite comparison group studied. The χ^2 test for independence of variables by using 2 × 2 contingency tables was used to examine the data for nonrandom association between a specific *MspI* allele and a

Table 2

***MspI* ALPL Haplotypes: Association with Specific HOPS Alleles**

Haplotype				No. Observed	No. Expected	χ^2	*p*
h/H	*A1/A2*	*A3/A4*					
h	A1		....	14	3.39	44.6	<.00001
h	A2		....	0	10.6		
H	A1		....	17	27.6		
H	A2		....	97	86.4		
h		A3	....	14	2.6	62.3	<.00001
h		A4	....	0	11.4		
H		A3	....	10	21.4		
H		A4	....	104	92.6		
	A1	A3	....	23	5.75	79.3	<.00001
	A2	A3	....	0	17.25		
	A1	A4	....	9	26.2		
	A2	A4	....	96	78.75		

HOPS allele. Specifically, the number of each haplotype observed for alleles *A1* or *A2* and HOPS alleles and for alleles *A3* and *A4* and HOPS and for the two *Msp*I sites was compared with the number expected, given the allele frequency and the assumption of total linkage equilibrium. This analysis, shown in table 2, strongly favors linkage disequilibrium between alleles *A1* + A2 and *HOPS* alleles, between alleles *A3* + *A4* and *HOPS,* and between alleles *A1* + *A2* and *A3* + *A4* ($P < .00001$). These data suggest that a single mutational event likely accounts for the HOPS mutant gene in our particular Mennonite population. Presumably, founder effect and inbreeding have contributed to the high incidence of both the HOPS mutation and the A1A3 polymorphism present in our particular Mennonite group.

In addition, strategies for the development of a screening program designed to detect carriers of HOPS in our high-risk population are being formulated. We propose that, in our population, our logistic regression analysis model based on (ALP+Pi) will be suitable as a population screening test for HOPS carriers. This screening model has been shown to have a potential sensitivity of 100% and a specificity of 96% (Chodirker et al. 1989). In this screening test, any individual with a value above the cutoff of .2 would be considered a potential carrier. A carrier so detected on a screening test would be offered more definitive diagnostic carrier tests that include urinary PEA, family studies, and DNA typing. Two carriers ascertained in a screening program with informative *Msp*I RFLPs would be offered first-trimester prenatal diagnosis using DNA analysis and serial ultrasonography to monitor any pregnancy. An individual with an intermediate probability would be offered family studies including DNA typing to clarify the genotypes. The detection of *A2A4/A2A4* genotypes in such an individual would greatly reduce the likelihood that he or she is a HOPS carrier. The validity of our approach is supported by the three of the 36 Mennonite spouses who served as our control group but were classified as having a high likelihood of being HOPS carriers biochemically (Chodirker et al. 1989). Their genotypes were subsequently determined to be *A1A3h/A2A4H*. Such an approach has certainly been successful in screening for the sickle cell trait and, more recently, for screening for cystic fibrosis carriers (Estivill et al. 1987). Such a population approach, in combination with further analysis of the specific HOPS mutation in our families and, ultimately, with direct mutation detection, provides a comprehensive strategy to reduce the morbidity or mortality from this disorder.

Acknowledgments

We thank Dr. Harry Harris and Mitchell Weiss for receipt of probe pS3-1 and Josie Dumaran for expert secretarial assistance. We are indebted to Professor Marion Lewis and the Rh laboratory for their assistance, to Dr. Joanne Johnson and the Fetal Assessment Unit, to the Cytogenetics Laboratory, and especially to the families and their local health centers for their ongoing cooperation. This study was supported by the Manitoba Health Research Council and the Children's Hospital of Winnipeg Research Foundation.

References

Chodirker BN (1988) Infantile hypophosphatasia: carrier manifestations. M.Sc. thesis, University of Manitoba, Winnipeg

Chodirker BN, Evans JA, Lewis M, Coghlan G, Belcher E, Philipps S, Seargeant LE, et al (1987) Infantile hypophosphatasia-linkage with the *RH* locus. Genomics 1:280–282

Chodirker BN, Evans JA, Seargeant LE, Cheang MS, Greenberg CR (1990) Hyperphosphatemia in infantile hypophosphatasia: implications for carrier diagnosis and screening. Am J Hum Genet 46:000–000

Coghlan GE, Chodirker BN, Greenberg CR, Lewis M (1988) Genetic linkage between *RH* and the gene for infantile hypophosphatasia. In: Proceedings of the XX Congress of the International Society of Blood Transfusion and British Blood Transfusion Society. British Blood Transfusion Society, Manchester, p 301

Côté GB (1975) Centromeric linkage in man. Ph.D. thesis, University of Birmingham, England

Edwards JH (1972) A marker algebra. Clin Genet 3:371–380

Estivill X, Scrambler PJ, Wainwright BJ, Hawley K, Frederick P, Schwartz M, Baiget M, et al (1987) Pattern of polymorphism and linkage disequilibrium for cystic fibrosis. Genomics 1:257–263

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Fraser D (1957) Hypophosphatasia. Am J Med 22:730–746

Greenberg CR, Hamerton JL, Nigli M, Wrogemann K (1987) DNA studies in a family with Duchenne muscular dystrophy and a deletion at Xp21. Am J Hum Genet 41: 128–137

Macpherson RI, Kroeker M, Houston CS (1972) Hypophosphatasia. J Can Assoc Radiol 23:16–26

Mulivor RA, Mennuti M, Zackai EH, Harris H (1978) Prenatal diagnosis of hypophosphatasia: genetic biochemical and clinical studies. Am J Hum Genet 30:271–282

Rasmussen H, Bartter FC (1978) Hypophosphatasia. In: Stanbury JB, Wyngaarden JB, Frederickson DS (eds) The metabolic basis of inherited disease, 4th ed. McGraw-Hill, New York, pp 1340–1350

Rathbun JC (1948) Hypophosphatasia, a new developmental anomaly. Am J Dis Child 75:822–831

Ray K, Weiss MJ, Dracopoli NC, Harris H (1988) Probe 8B/E5′ detects a second RFLP at the human liver/bone/kidney alkaline phosphatase (ALPL) locus. Nucleic Acids Res 16:2361

Smith M, Weiss MJ, Griffin CA, Murray JC, Kuetow KH, Emanuel BS, Henthorn PS, et al (1988) Regional assignment of the gene for human liver/bone/kidney alkaline phosphatase to chromosome 1p36.1–34. Genomics 2: 139–143

Warren RC, McKenzie CF, Rodeck CH, Moscoso G, Brock DJH, Barron L (1985) First trimester prenatal diagnosis of hypophosphatasia with a monoclonal antibody to the liver/bone/kidney isozyme of alkaline phosphatase. Lancet 2:856–858

Weiss MJ, Cole DE, Ray K, Whyte M, Lafferty MA, Mulivor R, Harris H (1988) A missense mutation in the human liver/bone/kidney alkaline phosphatase gene causing a lethal form of hypophosphatasia. Proc Natl Acad Sci USA 85:7666–7669

Weiss MJ, Henthorn PS, Lafferty MA, Slaughter C, Raducha M, Harris H (1986) Isolation and characterization of a cDNA encoding a human liver/bone/kidney-type alkaline phosphatase. Proc Natl Acad Sci USA 83:7182–7186

Weiss MJ, Ray K, Fallon MD, Whyte MP, Fedde KN, Lafferty MA, Mulivor RA, et al (1989) Analysis of liver/bone/kidney alkaline phosphatase mRNA, DNA and enzymatic activity in cultured skin fibroblasts from 14 unrelated patients with severe hypophosphatasia. Am J Hum Genet 44: 686–694

Weiss MJ, Speilman RS, Harris H (1987) A high-frequency RFLP at the human liver/bone/kidney alkaline phosphatase locus. Nucleic Acids Res 15:860

Whyte MP, Magill HL, Fallon MD, Herrod HG (1986) Infantile hypophosphatasia: normalization of circulating bone alkaline phosphatase activity followed by skeletal remineralization. J Pediatr 108:82–88