Hypophosphatasia: diagnostic application of linked DNA markers in the dominantly inherited adult form

S. J. IQBAL*, D. S. PLAHA†, G. H. LINFORTH† and R. DALGLEISH†
*Department of Chemical Pathology, Leicester Royal Infirmary, Leicester LE1 SWW, U.K., †Leicestershire Genetics Centre, Leicester Royal Infirmary, Leicester LE1 SWW, U.K. and ‡Department of Genetics, University of Leicester, Leicester LE1 7RH, U.K.

ABSTRACT

Hypophosphatasia is a rare disease characterized by low serum levels of tissue non-specific alkaline phosphatase (TNSALP) and a spectrum of skeletal disease varying from the severest form with death in utero to mild with no clinical abnormality in adults. Currently, the diagnosis of hypophosphatasia is made on the basis of clinical findings, radiography, low serum alkaline phosphatase levels and raised abnormal phosphorylated metabolites; there are elevations in serum pyridoxal 5′-phosphate, urinary phosphoethanolamine and inorganic pyrophosphate. In borderline cases the biochemical diagnosis remains uncertain. Prenatally, diagnosis is made using radiography and ultrasonography together with chorionic villus tissue biopsy, in which TNSALP levels are measured using an antibody-based assay. Since hypophosphatasia results from mutations in the TNSALP gene we have, for the first time in two U.K. families, undertaken restriction fragment length polymorphism (RFLP) analysis using three intragenic RFLPs for BclI and MspI at the ALPL locus. One family was informative, and a mutant-allele-specific haplotype with respect to three RFLPs was defined. In the other family the disease was shown to segregate with one allele of the BclI RFLP, but the MspI RFLPs were not informative. The disease segregated in the two families with different alleles of the BclI RFLP, suggesting that the mutations are likely to be different. We confirm that DNA analysis is likely to be the way ahead for diagnosing hypophosphatasia, and that standardized screening methods need to be developed for detecting mutations in these and other families.

INTRODUCTION

Hypophosphatasia is a rare metabolic bone disease characterized by defective bone mineralization and low serum levels of tissue non-specific alkaline phosphatase (TNSALP) [1]. TNSALP is one of the four isoenzymes of alkaline phosphatase; the others are intestinal, placental and placental-like (also known as germ cell type) alkaline phosphatases. The alkaline phosphatases are a group of membrane-bound glycoprotein enzymes that hydrolyse a range of monophosphate esters at an alkaline pH. They are widely distributed in animals and plants, and are present in virtually all mammalian tissues [2]. In humans, four separate genes encode the four different isoenzymes of alkaline phosphatase. Three of the genes, i.e. those encoding the placental, intestinal and germ cell alkaline phosphatase forms, are located on chromosome 2q34–2q37 [3]. TNSALP is encoded at the ALPL locus on chromosome 1p36.1–34 [4]. The ALPL gene is present as a single copy in the haploid genome and comprises 13 exons that are distributed over more than 50 kb of DNA [5,6]. The enzyme itself is widely distributed in virtually all mammalian tissues.
distributed and expressed in many tissues, and post-translational modification in these tissues produces secondary TNSALP isoforms with different physiochemical properties [2].

Hypophosphatasia has been classified into different clinical types: perinatal, infantile, childhood, adult, odontohypophosphatasia and pseudohypophosphatasia. In its severest form, death may occur in utero from skeletal malformation due to under-mineralization (perinatal form), while in its mildest form there may be very minor or no skeletal defects in adults. There is a disturbance of metabolism in a number of phosphorylated metabolites. Raised levels of serum pyridoxal 5’-phosphate, urinary phosphoethanolamine and inorganic pyrophosphate (PPi) characteristically occur [1].

Soon after hypophosphatasia was first described, it was recognized that this may be an inherited condition following the description of two affected siblings [7]. Although the mode of inheritance of the severe perinatal and infantile forms is more clear, and thought to be autosomal recessive, the mode of inheritance for the other and adult varieties of hypophosphatasia is less clear, and a dominant mode of inheritance has been suggested [1]. Hypophosphatasia is now known to result from mutations in the gene encoding TNSALP [8].

Since its original description, the diagnosis of hypophosphatasia has been made on the basis of a combination of low levels of serum alkaline phosphatase activity, abnormal levels of the phosphorylated metabolites, radiography and the clinical features of the disease. As hypophosphatasia results from a defect in the gene for TNSALP, diagnoses have recently been made using DNA analysis [8]. In the U.K., to date, no attempts have yet been made to diagnose hypophosphatasia using DNA analysis. In two families with hypophosphatasia, we have had the opportunity of analysing DNA using restriction fragment length polymorphism (RFLP) analysis.

**METHODS**

**Patients**

Details of patients are given in Figures 1 and 2 and Table 1. This research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and approval was obtained from the Ethical Committee of Leicestershire Health Authority to carry out diagnostic DNA tests for hypophosphatasia on these subjects. Informed consent was obtained from all subjects.

**Family 1**

The proband of this family (I-3) was diagnosed as having adult-onset hypophosphatasia with short stature (height...
143 cm; < third centile). She presented at the age of 47 with bowing of her legs and a waddling gait. She was noted to have a deformed pelvis on radiography and had lost all her teeth by the age of 20 years. She had two daughters that had been delivered by Caesarean section, and she had one male stillbirth. She had the characteristic biochemical features of hypophosphatasia. Total serum alkaline phosphatase activity was consistently low: mean biochemical features of hypophosphatasia. Total serum alkaline phosphatase activity was consistently low: mean biochemical features of hypophosphatasia. Total serum alkaline phosphatase activity was consistently low: mean biochemical features of hypophosphatasia. Total serum alkaline phosphatase activity was consistently low: mean biochemical features of hypophosphatasia. Total serum alkaline phosphatase activity was consistently low: mean biochemical features of hypophosphatasia. 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Only the proband had clinical features of hypophosphatasia. Her father (I-2) and brother (II-2), although not having clinical features of hypophosphatasia, had biochemical features of the disease (see Table 1 for results).
The inheritance in this family is also compatible with an autosomal dominant mode.

**Biochemical analysis**

Routine biochemical measurements, including serum alkaline phosphatase, were carried out using an SMAC I/II analyser (Technicon, Tarrytown, NY, U.S.A.). Alkaline phosphatase activity was measured using p-nitrophenyl phosphate as substrate with a 2-amino-2-methyl-1-propanol buffer, pH 10.3, at 30 °C. Urine phosphoethanolamine was measured using an amino acid analyser, with ion-exchange gradient HPLC on a 0.15 M NaCl intensifying screens at radiography was performed using Kodak X-AR film and serum pyridoxal 5'-phosphate was measured by HPLC at the Royal Gwent Hospital (Newport, Wales, U.K.).

**DNA analysis**

Genomic DNA was extracted from peripheral blood by standard phenol/chloroform techniques, and portions of 5 μg were digested with MspI or BclI (Life Technologies, Paisley, Scotland, U.K.). For one subject (Family 1, subject III-1), post-delivery placental tissue was used to obtain DNA. Following agarose-gel electrophoresis, the DNA was Southern-blotted on to a Hybond N nylon membrane (Amersham Pharmacia Biotech, Little Chalfont, Bucks., U.K.). The blots were hybridized with 10 ng of the 2.5 kb insert of the human TNSALP cDNA clone pS3-pS1 [10] labelled with [z-32P]dCTP using the random-primer method. Post-hybridization washes were carried out with 0.1 × SSC/0.1 % SDS at 65 °C (1 × SSC is 0.15 M NaCl/0.015 M sodium citrate), and autoradiography was performed using Kodak X-AR film and intensifying screens at −70 °C for 2 days.

**RESULTS**

**Biochemical analyses**

The results of the biochemical analyses are summarized in Table 1.

**DNA analyses**

The cDNA probe pS3-pS1 detects a BclI RFLP [11] and two MspI RFLPs [12] at the ALPL locus, which encodes TNSALP. The alleles of the BclI RFLP are detected as a band of 7.4 kb (allele B1) or a pair of bands of sizes 4.3 and 3.1 kb (allele B2) (Figure 1). One MspI RFLP has allele sizes of 2.4 kb and 2.3 kb (alleles A1 and A2 respectively); the other has allele sizes of 1.98 kb and 1.9 kb (alleles A3 and A4 respectively) (Figure 2). Consequently, four haplotypes with respect to MspI are possible (A1A3, A1A4, A2A3 and A2A4). Individuals from both families were typed with respect to the BclI and MspI RFLPs, and the data are presented in Table 1.

**DISCUSSION**

An accurate diagnosis of hypophosphatasia would be helpful in symptomatic individuals suspected of the disease, in possible healthy carriers and prenatally. Currently the diagnosis of hypophosphatasia is made on the basis of clinical features, subnormal alkaline phosphate levels and raised levels of phosphorylated metabolites. Although the diagnosis is clear-cut in some individuals, in milder forms of hypophosphatasia the biochemical abnormalities may not be marked, and values often overlap with those in normal subjects [13]. In view
of the plethora of methods available for measuring serum alkaline phosphatase, reference ranges can vary considerably, notably in children, especially at times of skeletal growth [14]. Although in children with very low levels of alkaline phosphatase the diagnosis of hypophosphatasia would be clear, in milder forms of the disease children may show a higher alkaline phosphatase level and, as Whyte [1] has pointed out, careful interpretation of results is needed. Alkaline phosphatase and phosphoethanolamine levels are known to vary with age, and this is also likely to be the case for PP levels. This adds further to diagnostic difficulties; therefore definitive diagnostic tests using DNA-based methods would clearly be advantageous in this context.

Among the currently available methods in the U.K., the prenatal diagnosis of hypophosphatasia is made by measuring TNSALP levels in chorionic villus biopsies obtained in the first trimester [15,16]. A combination of radiography and ultrasound inspection of the foetal skull and other skeletal morphometry is also used in the second trimester [17,18]. Culture of cells from the amniotic fluid has also been used [19]. However, direct measurement of alkaline phosphatase levels in the amniotic fluid has produced variable results. This method is likely to produce possible errors, as different alkaline phosphatase isoenzymes, expressed by different alkaline phosphatase genes, are present in the amniotic fluid at different stages of pregnancy; e.g. in the first trimester, intestinal alkaline phosphatase is present at its highest concentration [20]. It is not surprising that measurements of this type have produced inconsistent results [16]. Some caution has also been expressed with regard to the measurement of TNSALP levels in chorionic villus biopsies, as significant overlap can occur between normal and abnormal ranges [15]. The culture of amniotic fluid cells not only takes time, but may also produce results that overlap with normal values [16]. Since hypophosphatasia is the result of mutations at the \textit{ALPL} locus, methods that utilize DNA analysis are likely to be diagnostically the most specific.

In the present study, RFLP analysis has, in some instances, distinguished between affected and non-affected cases. In Family 1 it is clear that hypophosphatasia segregates with the Bcl \textit{I} allele of the \textit{Bcl} \textit{I} RFLP. However, the two affected individuals, I-3 and II-2, are both homozygous for \textit{B}2, which means that it is impossible to distinguish whether the normal or mutant allele has been inherited by individual III-1. Unfortunately, the family is not informative for the \textit{Msp} \textit{I} RFLPs, and it remains impossible to diagnose hypophosphatasia in II-2’s offspring using RFLP inheritance as a criterion. The proband’s unaffected nephew (II-3) is homozygous for the \textit{B}1 allele, consistent with the biochemical data. Hence the \textit{Bcl} \textit{I} RFLP will not be of help in prenatal diagnoses in future offspring of II-2 (who has already made enquiries to this effect), but may be of value in the extended family.

In Family 2, hypophosphatasia segregates with the \textit{Bcl}\textit{I} B1 allele and the \textit{Msp} \textit{I} A1A4 haplotype, consistent with the biochemical data. The two affected individuals in the second generation (II-2 and II-3) are homozygous for \textit{B}1, but are heterozygous for A1A4/A2A4. Hence there is the future possibility of prenatal diagnoses on the basis of \textit{Msp} \textit{I} haplotype inheritance, depending on the haplotypes carried by their potential partners. The success of this approach has been reported in a number of families [8,12], but clearly the ideal solution would be direct detection of the mutation.

The mode of inheritance in the different clinical types of hypophosphatasia is not uniform. In the perinatal and infantile types of the disorder, in which the subjects are often severely affected, the autosomal recessive mode of inheritance is well accepted [1,11]. In the adult type, inheritance is thought to be autosomal dominant [21–23], although there is clear evidence that some affected adult individuals are compound heterozygotes for recessive alleles [23,24]. Such cases, however, would be expected to be sporadic, and not to exhibit the dominant inheritance pattern seen in the families presented here.

\textbf{REFERENCES}


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