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Computer assisted analysis of hand radiographs in infantile hypophosphatasia carriers

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Abstract. Hand radiographs of 49 carriers of infantile hypophosphatasia and 67 non-carriers were evaluated using two Apple IIe Computer Programs and an Apple Graphics Tablet. CAMPS (1) was used to determine the bone lengths and calculate the metacarpophalangeal profiles. A newly developed program (ADAM) was used to determine bone density based on percent cortical area of the second metacarpal. Carriers of infantile hypophosphatasia had significantly less dense bones.

Infantile hypophosphatasia (HOPS) is a severe autosomal recessive metabolic bone disorder. MacPherson et al. [1] described the radiologic features of this and other forms of hypophosphatasia and reported that there was an increased incidence of hypophosphatasia in the Mennonite communities of Manitoba and Saskatchewan.

As part of a larger study on the genetics of hypophosphatasia, we have developed very accurate methods of carrier assignment [2]. This allowed us to study a group of assigned carriers and non-carriers and to test the hypothesis that bones of carriers would differ from those of non-carriers in a manner that could be determined from a hand radiograph. We evaluated the hand radiographs using two Apple IIe Computer Programs and an Apple Graphics Tablet. CAMPS [3] was used for determination of the metacarpophalangeal profile (MCP) while bone density was analyzed using a newly developed computer program. The MCP, which is an objective method of describing a hand radiograph, has been shown to be a useful method in the study of various genetic syndromes [4].

Methods

Collection of radiographs and preparation for analysis

As part of a larger study on the genetics of hypophosphatasia, 20 obligate carriers for infantile hypophosphatasia, 104 of their first degree relatives and 36 controls (unrelated spouses of the first degree relatives) were studied [2, 3]. All obligate carriers and first degree relatives and most (32/36) controls were of Mennonite descent. All were over 18 years of age. We were able to assign carrier status in 140 of these 160 individuals using logistic regression analysis based on serum alkaline phosphatase activity (ALP), serum phosphate level (Pi) and if necessary urinary phosphoethanolamine excretion (PEA). These methods of carrier assignment are felt to be extremely accurate in this population [2].

We obtained informed consent to take hand radiographs of 116 of these individuals. The radiographs were taken under routine conditions (i.e. focal-film distance = 40 inches). All were postero-anterior views of the right hand.

The radiographs were analyzed by one observer (BNC) without prior knowledge of the carrier status of the individual. Each radiograph was placed on a viewing box in a room without any other light source. The portion of the viewing box not directly under the radiograph was then covered. A sheet of white paper was then placed over the radiograph. Under these conditions the details of the bony structures could be seen through the paper. The ends and outer margins of the phalanges and metacarpals as well as the medullary space of the second metacarpal were traced onto the paper with a sharp pencil.

Metacarpophalangeal profile analysis

Metacarpophalangeal (MCPs) profiles were determined from the tracings using the "CAMPS" computer program and an Apple Graphics Tablet. The analysis was done according to the method of Coupland et al. [3], except that the tracings from the radiographs were used instead of contact prints. The program calculated the lengths of the 19 metacarpal and phalangeal bones and converted them to Z-scores based on published age and sex matched norms [3, 5]. The pattern variability index (σ_z) was calculated according to the method of Garn et al. [6].

Determination of bone density

For determination of the bone density we developed an Applesoft Basic program to run on an Apple IIe microcomputer with an Apple Graphics Tablet. The program is called ADAM (Automated Density Analysis Machine). After calibration, the user is prompted to place the graphics tablet pen at one end of the second metacarpal. The user is then prompted to place the pen at the opposite end. The program then instructs the user via a series of directional arrows how

to locate the midway point of the 2nd metacarpal. Once this is found the user is prompted to place the pen at the outer margins of the bone at that point and then at the outer margins of the medullary cavity. This process is then repeated for other radiographs (tracings) to be analyzed at that session. Following this the program then calculates the percent cortical area according to the formula described by Poznanski [4]. This is then compared to the means and standard deviations (SD) for the appropriate sex and age group as given by Poznansi. The mean percent cortical area and its standard deviation (SD) for ages between those supplied by Poznansi were calculated by interpolation. The Z-score was calculated by ADAM as the percent cortical area – mean/SD. The Z-score was then used to provide a relative index of bone density.

Statistical analysis

Student *t*-tests or chi-squared analysis was used to check for statistical differences where appropriate. Logistic regression analysis was used to develop the best model for separating carriers from non-carriers based on the MCP [7].

Results

Table 1 shows the distribution of sex, age, MCP length, and mean Z as well as height and bone density in 49 carriers and 67 non-carriers. There were no significant differences in the age or sex distributions of the two groups. Bone density (as reflected by the percent cortical area Z-score) was significantly less in carriers than in non-carriers. Seventeen of the 19 hand bones measured were on average longer in carriers than in controls. For only six bones, however, were the differences statistically significant. There was no difference in the σ_z or mean Z.

Tables 2 and 3 show the results obtained when the data for female and males are analyzed separately. A significant difference is seen in the age distribution for males with carriers being younger than non-carriers. Male carriers still had significantly less dense bones than male non-carriers. The mean bone density for female carriers was also less than female non-carriers although the difference was no longer significant. Similar trends were seen in the MCP data.

Information regarding the heights of the study participants was available for 102 of the 116 individuals (57 non-carriers, 45 carriers). No significant difference in the heights was seen between the carriers and non-carriers.

Logistic regression analysis showed that a model based on three of the five proximal phalanges was the best for predicting carrier status. The sensitivities and specificities of a test based on either the bone density or the MCP were determined (calculations not shown). Neither test had a sensitivity of greater than 70% if the specificity was greater than 70%.

Table 1. Characteristics of carriers and non-carriers (total)

Test	Carrier mean (±SD)		Non-carrier mean (±SD)		*P*
Age (years)	36.2	(±13.8)	34.8	(±13.6)	NS
Sex	21 Male/28 Female		35 Male/32 Female		NS
BD	−0.38	(±1.0)	0.18	(±0.9)	0.002
Height (cm)	167.3	(±9.4)	170.1	(±8.8)	NS
D5	−0.06	(±1.00)	−0.37	(±0.94)	NS
D4	−0.16	(±1.33)	−0.23	(±0.98)	NS
D3	−0.29	(±1.22)	−0.25	(±0.99)	NS
D2	−0.09	(±1.00)	−0.26	(±0.87)	NS
D1	0.24	(±1.10)	0.07	(±1.12)	NS
M5	−0.07	(±1.20)	−0.53	(±1.38)	NS
M4	0.12	(±1.09)	−0.24	(±1.16)	NS
M3	0.16	(±1.04)	−0.22	(±1.01)	0.05
M2	−0.26	(±1.06)	−0.54	(±0.99)	NS
P5	0.30	(±1.06)	−0.23	(±1.11)	0.01
P4	0.29	(±0.86)	−0.09	(±1.16)	0.04
P3	0.37	(±0.89)	−0.04	(±1.02)	0.03
P2	0.16	(±0.93)	−0.21	(±1.22)	NS
P1	−0.04	(±1.00)	−0.58	(±1.30)	0.01
ME5	0.07	(±0.86)	−0.18	(±1.23)	NS
ME4	−0.11	(±0.84)	−0.09	(±1.03)	NS
ME3	0.10	(±0.83)	0.06	(±0.97)	NS
ME2	0.05	(±0.87)	0.04	(±1.03)	NS
ME1	0.32	(±0.84)	−0.08	(±0.98)	0.002
Mean Z	−0.08	(±0.68)	−0.16	(±0.61)	NS
σ_z	0.79	(±0.31)	0.81	(±0.36)	NS

NS = Not statistically significant; σ_z = Pattern variability index; BD = Bone density (Z score); D = Distal phalanx length (Z score); M = Middle phalanx (Z score); P = Proximal phalanx (Z score); ME = Metacarpal (Z score); Mean Z = Mean Z score for all 19 metacarpal phalangeal bones

Table 2. Characteristics of female carriers and non-carriers

Test	Carrier mean (±SD)		Non-carrier mean (±SD)		*P*
n	28		32		
Age	40.3	(±15.1)	36.3	(±12.3)	NS
BD	−0.36	(±1.13)	0.0	(±0.94)	NS
Height (cm)	160.7	(±5.2)	163.2	(±6.6)	NS
D5	0.21	(±0.91)	−0.17	(±0.90)	NS
D4	0.06	(±1.02)	0.02	(±1.01)	NS
D3	−0.10	(±0.99)	−0.07	(±1.08)	NS
D2	0.09	(±0.97)	−0.06	(±1.05)	NS
D1	0.48	(±1.05)	0.05	(±1.11)	NS
M5	0.30	(±1.17)	−0.18	(±1.19)	NS
M4	0.30	(±1.01)	−0.13	(±0.97)	NS
M3	0.38	(±0.92)	−0.08	(±0.91)	0.05
M2	0.00	(±0.93)	−0.31	(±0.96)	NS
P5	0.51	(±0.98)	−0.22	(±0.90)	0.004
P4	0.41	(±0.78)	−0.06	(±1.07)	NS
P3	0.56	(±0.81)	−0.01	(±0.97)	0.02
P2	0.35	(±0.84)	−0.15	(±1.22)	NS
P1	0.11	(±0.90)	−0.45	(±1.47)	NS
ME5	0.25	(±0.68)	−0.27	(±1.38)	NS
ME4	−0.01	(±0.78)	−0.03	(±1.10)	NS
ME3	0.18	(±0.73)	0.14	(±1.08)	NS
ME2	0.16	(±0.80)	0.15	(±1.00)	NS
ME1	0.42	(±0.82)	−0.02	(±1.07)	NS
Mean Z	−0.03	(±0.68)	−0.03	(±0.72)	NS
σ_z	0.70	(±0.20)	0.79	(±0.45)	NS

NS = Not statistically significant; σ_z = Pattern variability index; BD = Bone density (Z score); D = Distal phalanx length (Z score); *n* = Number of participants; M = Middle phalanx (Z score); P = Proximal phalanx (Z score); ME = Metacarpal (Z score)

Table 3. Characteristics of male carriers and non-carriers

Test	Carrier mean (± SD)		Non-carrier mean (± SD)		P
n	21	(± 9.2)	35		
Age	30.4	(± 0.8)	39.7	(± 14.7)	0.006
BD	−0.42		0.36	(± 0.84)	0.002
Height (cm)	176.4	(± 6.0)	175.9	(± 5.6)	NS
D5	−0.40	(± 1.02)	−0.56	(± 0.94)	NS
D4	−0.46	(± 1.64)	−0.46	(± 0.91)	NS
D3	−0.55	(± 1.50)	−0.43	(± 0.89)	NS
D2	−0.32	(± 0.94)	−0.44	(± 0.63)	NS
D1	−0.08	(± 1.09)	0.08	(± 1.15)	NS
M5	−0.55	(± 1.07)	−0.84	(± 1.49)	NS
M4	−0.11	(± 1.18)	−0.35	(± 1.31)	NS
M3	−0.12	(± 1.13)	−0.34	(± 1.09)	NS
M2	−0.59	(± 1.14)	−0.75	(± 0.98)	NS
P5	0.02	(± 1.12)	−0.24	(± 1.29)	NS
P4	0.14	(± 0.94)	−0.12	(± 1.25)	NS
P3	0.11	(± 0.94)	−0.09	(± 1.07)	NS
P2	−0.07	(± 0.99)	−0.27	(± 1.23)	NS
P1	−0.24	(± 1.11)	−0.70	(± 1.13)	NS
ME5	−0.18	(± 1.02)	−0.09	(± 1.10)	NS
ME4	−0.26	(± 0.92)	−0.14	(± 0.97)	NS
ME3	0.00	(± 0.96)	−0.01	(± 0.86)	NS
ME2	−0.11	(± 0.96)	−0.06	(± 1.06)	NS
ME1	0.17	(± 0.86)	−0.17	(± 0.89)	NS
Mean Z	−0.22	(± 0.66)	−0.28	(± 0.47)	NS
σ_z	0.92	(± 0.38)	0.82	(± 0.25)	NS

NS = Not statistically significant; σ_z = Pattern variability index; BD = Bone density (Z score); D = Distal phalanx (Z score); *n* = Number of participants; M = Middle phalanx (Z score); P = Proximal phalanx (Z score); ME = Metacarpal (Z score)

Discussion

Infantile hypophosphatasia is a severe autosomal recessive inherited metabolic bone disorder. Affected infants are usually stillborn or die in the neonatal period [8]. Radiologic features include markedly decreased bone density and rachitic like changes in the metaphyses [1]. There are milder forms of hypophosphatasia i.e. juvenile and adult forms. Juvenile hypophosphatasia is characterized by onset of symptoms after six months of age consisting of early loss of deciduous teeth, craniosynostosis, rachitic like changes and fractures. Adult hypophosphatasia presents in adult life with recurrent fractures secondary to "osteomalacia" and early loss of the adult teeth. We, however, are not aware of any other study demonstrating radiologic abnormalities in asymptomatic HOPS carriers.

Various biochemical abnormalities have been documented in HOPS carriers by our group as well as by others [2, 8, 9, 10]. These abnormalities include decreased ALP, increased PEA, increased Pi and increased pyridoxal-5'-phosphate. Carrier assignment based on these parameters is extremely accurate [2]. As there is a very high incidence of infantile hypophosphatasia in the Mennonite communities of Manitoba and Saskatchewan and since an accurate test for carrier status is available, we were able to identify populations of carriers and non-carriers to assess potential phenotypic differences between the two groups. In this study we have now documented that there is lower bone density in carriers than in non-carriers. Although most individual bone density Z-scores were within the normal range (i.e. −2 to +2), the mean bone density was significantly lower in the carriers. Similarly we have now shown that the lengths of 6 bones of the hand are significantly greater in carriers. It is not surprising to find that a disease which causes markedly decreased bone density in the homozygous state may cause a less striking but significant decrease in bone density in heterozygotes. It is unclear, however, why carriers should have longer hand bones. Height would not be the explanation as there were no significant differences seen in the heights of carriers as compared with non-carriers. There are some other diseases (eg. homocystinuria) where osteoporotic bone changes are seen in combination with arachnodactyly [11]. The underlying mechanism for the increased bone growth is unclear.

Garn et al. have reported one patient with hypophosphatasia who had a high σ_z (i.e. 1.979) suggesting a markedly dysmorphogenic hand [6]. We find no evidence of dysmorphogenesis in the heterozygotes as there is no difference in σ_z between the heterozygotes and controls.

We are unable to detect any bias which might have accounted for our results. Because the radiographs were analyzed blindly to knowledge of carrier status, observer or measurement bias should not account for the differences. That is, any inaccuracy or imprecision due to the technique of measurement should affect both carriers and non-carriers equally. The sex distribution does not seem to be a significant factor. It is possible, however, that the unequal age distribution among males may have skewed our results somewhat. It is difficult to imagine how as the same trends were seen in both males and females. The normal values for bone lengths and density used for comparison were based on the Fels group of Ohio Whites [4, 5]. The fact that our study and control populations are from a different ethnic group should not affect the results although the actual values for bone length or density Z-scores may differ in a different reference population, the comparison between the carriers and non-carriers group should remain valid as they are from the same ethnic population.

Individuals who are heterozygotes for autosomal recessive conditions often show some deviation from normal [10]. Carrier tests based on these deviations have been developed. In most cases, these tests are based on hematological (e.g. thalassemia) or biochemical (e.g. Tay-Sachs) parameters. We have now shown that there is potential for using other types of parameters (i.e. bone density) as a carrier test for autosomal recessive conditions such as this metabolic bone disease. Unfortunately, in this situation the overlap between heterozygotes and normals is too great to make carrier testing based on radiographs practical.

From a clinical point of view we would not recommend that hand radiographs be used as part of an organized screening program designed to detect HOPS carriers. The best combinations of sensitivity and specificity that can be obtained from these tests are much lower than those based on biochemical tests [2]. We have already shown that a screening program based on a single blood test which measured ALP and Pi can have a potential sensitivity of 100% and a specificity of 96% (2). We do, however, feel that the radiologist should consider the possibility of the

HOPS carrier state when a hand radiograph shows decreased bone density even if the bone lengths are not increased. This would be especially true if other factors (eg. age, sex or clinical symptoms) were not consistent with idiopathic osteoporosis or if the patient was from a population with a high incidence of hypophosphatasia (e.g. the Mennonite Communities of Manitoba and Saskatchewan). Further studies of bone density measurements involving the spine and hip are planned in these two groups of individuals. Should similar bone density differences be seen in carriers vs non-carriers, radiologists and clinicians should be aware that heterozygotes for infantile hypophosphatasia may present with findings similar to patients with osteoporosis.

The technique that we have described can be used by others who wish to compare large groups of individuals from two different populations, i.e. those with a particular disease compared to those without. Improvements in the technique we have described can still be made. Although the use of these computer programs allowed measurements and calculations on 116 radiographs to be performed much more quickly than could have been done by hand, there was still a great deal of effort required to trace the bony outlines onto paper. Cost considerations precluded the use of the contact prints as suggested by Coupland et al. [3]. This also introduced a potential source of error. The different sources of error in this technique are reflected in the large standard deviation shown on Tables 1–3. As stated earlier, this error should not have affected the comparisons between the two groups. We have recently become aware of the development of a translucent graphics tablet that would enable the radiograph to be placed directly on the tablet, and the measurements could then be taken directly from that radiograph [11]. Such a tablet has been developed for use with IBM compatible computers, but would require rewriting of the programs so that they would also be IBM compatible.

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