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Measurement of Tartrate-Resistant Acid Phosphatase and the Brain Isoenzyme of Creatine Kinase Accurately Diagnoses Type II Autosomal Dominant Osteopetrosis but Does Not Identify Gene Carriers

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Autosomal dominant osteopetrosis type II (ADO2) is typically diagnosed from radiographs, which demonstrate the pathognomonic findings of osteosclerosis and endobone formation. Individuals with ADO2 also have elevated serum levels of tartrate-resistant acid phosphatase (TRAP) and the BB isoenzyme of creatine kinase (CK-BB). In the current study, we tested the utility of these enzymes in making or refuting a diagnosis of ADO2. Furthermore, because ADO2 has incomplete penetrance, we examined whether TRAP and CK-BB were helpful in identifying gene carriers. We studied eight families, measured serum levels of TRAP and CK-BB in 52 affected individuals and 12 obligate gene carriers, and compared their values with age-matched controls. Our results demonstrate that affected patients have significantly elevated levels of both TRAP and CK-BB. In contrast, gene carriers have values that are not different from controls. Furthermore, in our study population, TRAP and CK-BB have a high diagnostic sensitivity and specificity, particularly in children. From this large study of ADO2 patients and carriers, we conclude that: 1) TRAP and CK-BB are significantly elevated in patients with ADO2, 2) obligate carriers cannot be adequately identified by measurement of these analytes, and 3) TRAP and CK-BB are highly sensitive and specific diagnostic tests that can efficiently and effectively screen high-risk individuals who have not had previous radiographic assessment. (*J Clin Endocrinol Metab* 87: 2212–2217, 2002)

AUTOSOMAL DOMINANT OSTEOPETROSIS type II (ADO2) is a metabolic bone disorder that results from ineffective osteoclast-mediated bone resorption. First described in 1904 by the German radiologist Heinrich Albers-Schönberg (1), it is the major type of autosomal dominant osteopetrosis. Although ADO2 can be asymptomatic, complications frequently arise from the lack of normal bone turnover and may include fractures, osteomyelitis, cranial neuropathies secondary to narrowing of neural foramina, and rarely bone marrow failure because of diminution of the medullary space (2–4).

The diagnosis of ADO2 is ordinarily made via radiography. In addition to osteosclerosis, which may be subtle, the pathognomonic radiographic feature of ADO2 is the presence of endobones (bone-within-a-bone appearance), most commonly noted in the vertebrae, pelvis, and at the ends of long bones (5) (Fig. 1). The appearance of endobones and/or end plate thickening in the vertebrae is often described as a rugger-jersey spine or sandwich vertebrae.

Histologic evaluation of bone taken from ADO2 patients reveals an increased number of large osteoclasts that demonstrate no signs of active bone resorption (6–8). Individuals with ADO2 also have elevated serum levels of tartrate-resistant acid phosphatase (TRAP) (2, 9, 10) and the BB isoenzyme of creatine kinase (CK-BB) (10–14). Because these enzymes are closely associated with the osteoclast (15–21), one explanation for the elevated serum levels may be the significantly increased numbers of osteoclasts observed in ADO2 (10). Other than for being markers of the disorder, TRAP and CK-BB do not currently play a role in the clinical management of patients and families with ADO2.

ADO2 is a disorder of incomplete penetrance, with a maximum estimated penetrance rate of 75% (2, 4, 22). It is feasible that ADO2 gene carriers may have subtle abnormalities in TRAP and CK-BB that would allow detection of these asymptomatic individuals, who are at risk for having affected children. Identification of gene carriers is desirable because it would permit more accurate genetic counseling and would facilitate positional cloning studies designed to identify the disease gene. We previously tested one ADO2 carrier, who had no demonstrable biochemical abnormalities (23).

TRAP and CK-BB are rarely significantly elevated in human serum. Consequently, the measurement of these enzymes may serve as an invaluable tool in the diagnosis of ADO2, thereby obviating the need to obtain multiple radiographs in individuals who are genetically at risk. This would permit more focused radiographic screening, resulting in cost savings and minimizing unnecessary radiation exposure. Therefore, we evaluated the utility of TRAP and CK-BB in diagnosing ADO2 in a large group of affected individuals. Moreover, given that gene carriers have normal radiographs, we also studied whether measurement of these analytes was useful in identifying this population.

Abbreviations: ADO2, Autosomal dominant osteopetrosis type II; CK-BB, BB isoenzyme of creatine kinase; TRAP, tartrate-resistant acid phosphatase.

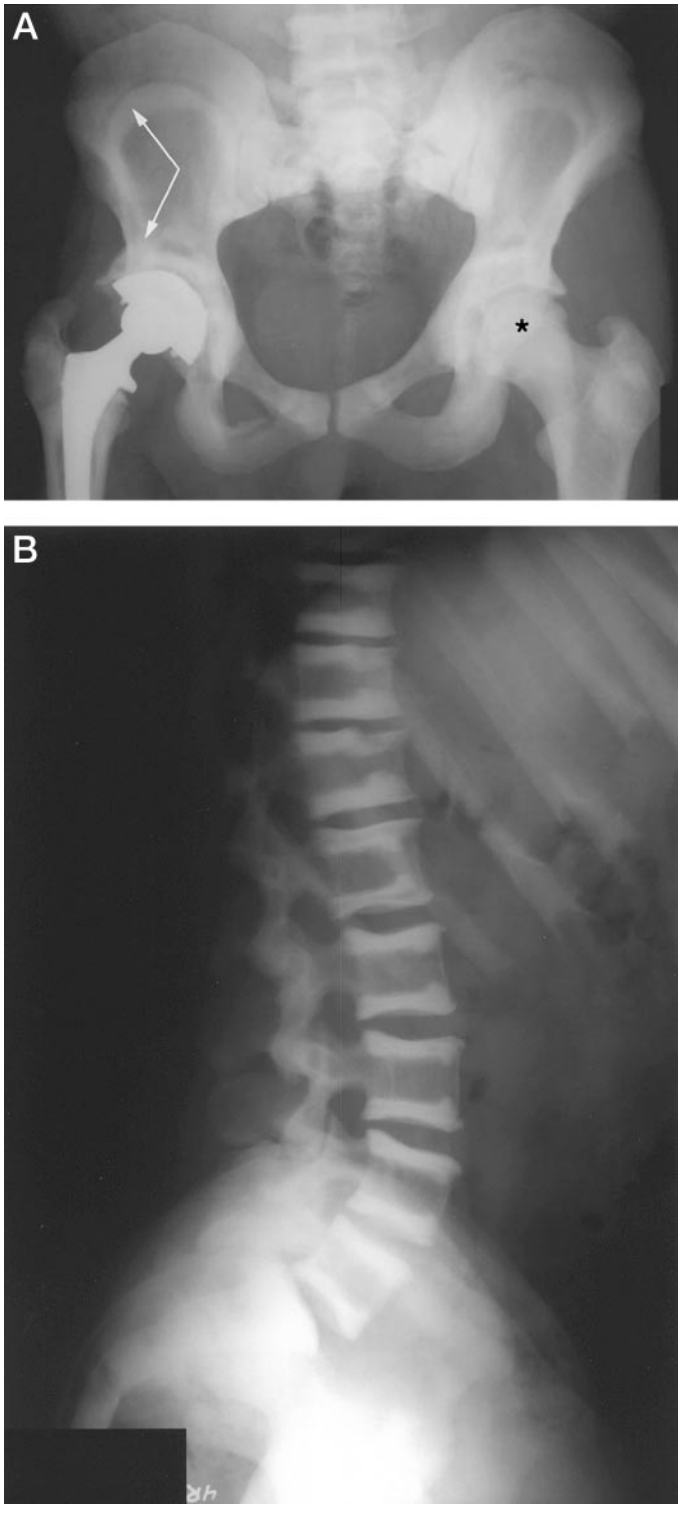

FIG. 1. Classic radiographs of ADO2. A, Pelvic radiograph in a 27-yr-old patient. Note the typical findings of osteosclerosis and endobones within the pelvis (*arrows*) and proximal femur (*asterisk*). B, Lateral x-ray of the lower spine in a 47-yr-old patient that demonstrates the appearance of a rugger-jersey spine.

Materials and Methods

Study subjects and research methods

Between April 1998 and August 2001, we collected serum samples, obtained clinical histories, and reviewed radiographs from the members of eight osteopetrosis families, all of which demonstrated an inheritance pattern consistent with autosomal dominant transmission. Sporadic cases of osteopetrosis were not included for analysis.

All available radiographs of the study participants were blindly reviewed, with the reader (K.A.B.) having no knowledge of the patient's clinical phenotype, genotype, or relationship to the other study subjects. The diagnosis of ADO2 was made if there was diffuse osteosclerosis and/or the pathognomonic findings of endobones in any portion of the visualized skeleton. A study subject was considered unaffected if these features were absent. An obligate carrier was defined as an individual with normal radiographs who has a child or grandchild with ADO2 in addition to another family member with the disorder.

Radiographs without evidence of osteopetrosis were considered informative only if they included a view of the spine, pelvis, and/or a long bone. Because the findings of osteopetrosis are invariably seen in these locations (our unpublished observations), the possibility of not identifying a case was minimized by ensuring that at least one of these areas was visualized. Except for spouses, who are not genetically related to the families and therefore at minimal risk of having ADO2, all participants in the control group were required to have at least one negative informative radiograph for their data to be included for analysis. For the current report, the control group also included unaffected subjects who met the inclusion criteria but who were from families other than those reported here. These families either had sporadic cases of osteopetrosis or had been referred for a possible diagnosis of osteopetrosis, which was subsequently excluded. Radiographs were not available in five of the study subjects, including three affected individuals. In these cases, written documentation of radiographic findings was accepted as long as the films obtained were of one of the sites mentioned above.

There were 178 subjects who participated in this research. Each subject was placed into one of three main groups based on his/her radiographs and family history: clinically affected, obligate gene carrier, and control subject. The study participants were further stratified into two groups, based on age (Table 1). The pediatric group (age $<$18 yr) included 17 affected children (aged 9 months to 17 yr) and 22 controls (aged 18 months to 11 yr). In the adult group (age $>$18 yr), there were 35 affected subjects (aged 22–79 yr), 12 obligate carriers (aged 30–56 yr), and 92 control subjects (aged 18–80 yr). Results from affected patients and obligate carriers were compared with controls in the same age group. Given the definition used in this study, all obligate carriers were older than age 18 yr and were, therefore, compared with the same control group as the adult affected subjects.

The research protocol was approved by the Indiana University Institutional Review Board. All subjects or their parents (in the case of children under age 18 yr) gave written informed consent before participating in the study.

Laboratory tests

Serum samples were obtained at the Indiana University Medical Center (either in the metabolic bone clinic or in the General Clinical Research Center) or at family reunions that were organized for this purpose. For those family members geographically distant or unable to attend their family reunion, participation was facilitated by the overnight shipping of their blood samples. There were 13 affected, 2 obligate carriers, and 10 control subjects who participated in this manner. Serum was separated from clot and kept frozen at −80 C until tested. At family reunions, the serum was placed on dry ice and kept frozen until storage at −80 C was available. For those participants who sent blood overnight, the serum was separated immediately upon arrival and frozen as described above. Separate studies indicated that this treatment did not significantly alter the test results (data not shown). Five study subjects (two affected, one carrier, and two controls) had serum studies measured on two separate occasions during the course of the study. The mean of their values was used in the data analysis.

Serum values of calcium, phosphorus, creatinine, and alkaline phosphatase were determined using standard automated techniques (Cobas Mira, Roche Diagostics Systems Laboratories, Inc., Branchburg, NJ).

TABLE 1. Demographic and biochemical data from ADO2 patients, obligate carriers, and normal controls

	Affected <18 yr	Controls <18 yr	Affected >18 yr	Controls >18 yr	Obligate carriers
No.	17	22	35	92	12
Age (yr)	8.6 ± 4.2	6.7 ± 2.6	45.9 ± 17.0	45.1 ± 16.2	41.3 ± 9.8
Male/female	11/6	13/9	16/19	44/48	4/8
Calcium (mmol/liter)	2.55 ± 0.20	2.50 ± 0.10	2.50 ± 0.13[b]	2.43 ± 0.15	2.40 ± 0.23
Phosphorus (mmol/liter)	1.68 ± 0.23	1.55 ± 0.39	1.39 ± 0.26[a]	1.10 ± 0.23	1.10 ± 0.23
Alkaline phosphatase (U/liter)	219 ± 89	262 ± 64	84 ± 22	81 ± 25	74 ± 21
Creatinine (μmol/liter)	53 ± 9	53 ± 9	88 ± 27	97 ± 27	88 ± 9
TRAP (U/liter)	79.6 ± 17.0[a]	21.2 ± 4.5	50.0 ± 18.9[a]	9.7 ± 2.8	11.3 ± 5.4
Total CK (U/liter)	305 ± 117[a]	115 ± 40	154 ± 81[b]	117 ± 78	108 ± 33
Absolute CK-BB (U/liter)	177.8 ± 92.9[a]	1.7 ± 3.2	61.3 ± 47.2[a]	0.0 ± 0.0	0.8 ± 2.6

[a] $P < 0.0001$ *vs.* age-matched controls.
[b] $P < 0.03$ *vs.* age-matched controls.

Reference ranges for these tests are as follows: calcium 2.2–2.65 mmol/liter; phosphorus 0.78–1.42 mmol/liter (adults); creatinine 53–141 μmol/liter; and alkaline phosphatase 65–400 U/liter (0–18 yr) and 17–142 U/liter (>18 yr). Note that the values for alkaline phosphatase are inclusive of all normal ranges for the ages in the two study groups. The serum TRAP assay was performed in the Indiana University General Clinical Research Center laboratory as previously described (24). The reference ranges for TRAP are 4.3–21.1 U/liter (5–18 yr) and 3.5–9.1 U/liter (>18 yr). Assays for total CK and CK isoenzymes were performed by the Indiana University Hospital laboratory (Clarian Health). Total CK activity was measured at 37 C using a Vitros CK slide (Ortho-Clinical Diagnostics, Inc., Rochester, NY) and isoenzymes were quantified after electrophoretic separation on agarose gels using the REP CK isoenzyme procedure (Helena Laboratories, Beaumont, TX). The reference range for total CK is 50–180 U/liter. In adults, CK-BB is normally immeasurable (detection limit 3 U/liter), although it may be detected as high as 10–20 U/liter in children (25–27). Absolute CK-BB was calculated as the product of the total CK and the percentage of the BB isoenzyme activity detected in serum.

Statistical analysis

All data are presented as the mean ± 1 SD. Comparisons among study groups were made using the two-sample *t* test. A *P* value less than 0.05 is considered to be statistically significant.

Results

Affected patients

Table 1 displays the laboratory results of the various study groups. Mean serum levels of calcium, phosphorus, alkaline phosphatase, and creatinine were within the normal range for all affected patients. However, in the adult group, statistically significant differences in calcium and phosphorus values were observed between affected and control subjects. The differences among the groups are small and of doubtful clinical significance. In both age groups, patients with ADO2 had significantly higher mean levels of both TRAP and CK-BB ($P < 0.0001$) than controls. Of note, the mean CK value was also significantly different between affected subjects and controls. In fact, the elevation of CK-BB levels was so pronounced in the pediatric affected group that the mean total CK measurement was increased to above the normal range.

Obligate carriers

Obligate carriers did not show any significant differences in TRAP or CK-BB levels, compared with controls ($P > 0.3$). In fact, of the 12 carriers tested, only one had any significant elevation in both TRAP and absolute CK-BB (24.2 U/liter and 9.1 U/liter, respectively). Without this patient's data, the mean values of TRAP and CK-BB for the obligate carrier group were 10.12 ± 3.54 U/liter and 0.0 U/liter, respectively. The patient in question did not have classic findings of endobones on radiographs of the lateral spine, hands, and right upper humerus. However, there was some mild sclerosis noted in the x-ray of the upper humerus. Whether this patient has a very mild expression of ADO2 (without classic radiographic findings) or represents instead the rare obligate carrier who has subtle abnormalities in TRAP and CK-BB remains unknown. However, because the subject fulfilled only the criteria for the definition of an obligate carrier, his data were analyzed as part of this group.

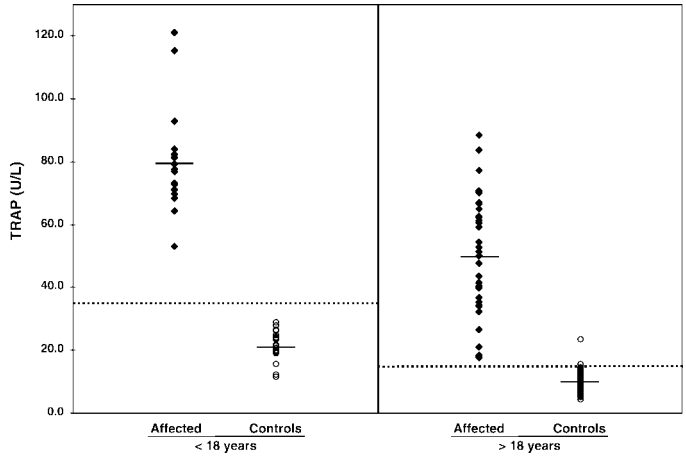

FIG. 2. TRAP levels in affected subjects (*black diamonds*) *vs.* their age-matched control groups (*open circles*). Duplicate results are represented by one symbol only. Each *dotted line* represents the diagnostic cutoff (35 U/liter age <18 yr; 15 U/liter age >18 yr) used in the determination of sensitivity and specificity. Each *short solid line* represents the sample mean.

TRAP and CK-BB in the diagnosis of ADO2

The usefulness of any diagnostic test lies in its ability to distinguish affected subjects from normal individuals. In particular, a test with a high diagnostic sensitivity is preferable so that all individuals with a disease can be identified. By assessing the distribution of individual laboratory values in our study population, one can see clear demarcations between affected and control subject values (Figs. 2 and 3). Based on these data, empiric cutoff values were determined to maximize the separation between affected and control subjects.

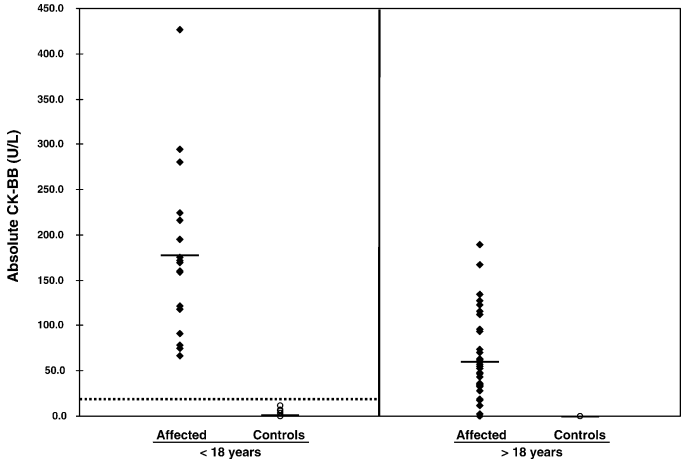

FIG. 3. Absolute CK-BB levels in affected subjects *(black diamonds) vs.* their age-matched control groups (*open circles*). Duplicate results are represented by one symbol only. The *dotted line* is the diagnostic cutoff (20 U/liter) used in the determination of sensitivity and specificity for the pediatric group. It is not shown in the subjects >18 yr because the cutoff value used in this population is 0 U/liter. Each *short solid line* represents the sample mean.

In the pediatric group, TRAP is 100% sensitive and specific if a diagnostic cutoff of 35 U/liter is used. In adults, there was more overlap among the study groups. Using a diagnostic cutoff of 15 U/liter, TRAP has a sensitivity of 100% and a specificity of 98% in this population. In the current study, every child with ADO2 had markedly elevated levels of CK-BB. Although occasional mild elevations of CK-BB were observed in normal control children (n = 6 or 27%), the two groups remained clearly distinct. If 20 U/liter is used as the diagnostic cutoff, absolute CK-BB proves to be 100% sensitive and specific in determining the radiographic phenotype in children. None of the control adults had detectable levels of CK-BB, giving the test 100% specificity at a cutoff of 0 U/liter. However, unlike the pediatric affected group, in which all children had marked elevations of CK-BB, there were three adults with undetectable CK-BB levels, despite having radiographically proven cases of ADO2. All of these patients had no significant clinical signs of the disorder, and their radiographs revealed minimal osteosclerosis and classic endobones. Interestingly, these three patients did have mild but significant elevations of TRAP (range 17.7–21.1 U/liter), which would have raised concern independent of CK-BB. Therefore, in the adult population, CK-BB has a sensitivity of 91%. Finally, if TRAP and CK-BB values are concordant (*i.e.* both levels are above or below the designated cutoff levels), the diagnostic sensitivity and specificity becomes 100% in both adult and pediatric subjects.

Discussion

Despite reports of elevated levels of TRAP and CK-BB in ADO2 (2, 9–14), the clinical utility of these enzymes in the diagnosis of the disorder has not previously been demonstrated. In the current study, TRAP and CK-BB prove to be highly sensitive and specific in the diagnosis of ADO2, particularly in the pediatric population, in which there is no overlap between affected and control values. Although TRAP is not readily available in hospital laboratories, CK isoenzymes are ubiquitously offered and rapidly available. Our results demonstrate that CK-BB can be used alone in the diagnosis of ADO2. However, the interpretation of CK-BB values requires that one recognize the differences between the adult and pediatric population as delineated in the *Results* section. In short, a significantly elevated CK-BB (>0 U/liter in adults and >20 U/liter in children) ascertained all persons with classic radiographic findings of ADO2 in the current study. Conversely, an undetectable CK-BB likely rules out the diagnosis in a child but may occasionally miss the adult with a mild expression of ADO2. Finally, measurement of these enzymes is not useful in identifying obligate gene carriers, who are at risk of having an affected child.

Given the current study design, it is inevitable that unidentified carriers were included in the control group. However, it is unlikely that the inclusion of these individuals altered the outcome of the study, given the normal results observed in obligate gene carriers. Furthermore, because a full skeletal survey was not obtained on study subjects, it is also possible that mild cases of osteopetrosis were missed and inadvertently placed in the control group. However, because there were strict radiographic criteria for inclusion, it is unlikely that this occurred to a degree to significantly alter the test results.

The usefulness of these blood tests lies in their ability to screen high-risk populations without ordering a multitude of radiographs to determine the diagnosis. This proves to be particularly valuable in the pediatric patient, who is less likely to have had a radiograph and in whom one would like to limit unnecessary radiation exposure. In the patient being screened for ADO2, an abnormal test result should prompt the clinician to obtain radiographs, namely of the pelvis and lateral spine. In the patient with classic radiographic features of ADO2, there is no need to obtain these tests to confirm the diagnosis. However, in someone who has limited radiographs or whose radiographic findings are unclear, measurement of TRAP and CK-BB is an invaluable adjunct in confirming or refuting the diagnosis.

TRAP is an isoenzyme of the nonspecific acid phosphatases that is expressed by both erythrocytic and macrophagic cells. In bone, it is highly associated with the osteoclast (8, 20, 21, 28). It is postulated that most of the serum activity of TRAP is derived from osteoclasts (29), and physiologic increases in serum levels are observed in children, presumably secondary to increased osteoclastic activity related to bone growth (30). Besides osteopetrosis, pathologically elevated TRAP levels are observed in the lysosomal storage disorder, Gaucher's disease (31), and conditions of increased bone resorption (24, 29, 32).

CK-BB is one of three isoenzymes of creatine kinase, an enzyme that catalyzes the reversible transfer of phosphate (ADP + creatine phosphate ↔ ATP + creatine) (33). It is primarily found in brain cells but is also widely distributed in other human tissues (34, 35). In bone, CK-BB appears to be associated with the osteoclast (16, 35). An osteoclastic origin is also supported by its close direct correlation with TRAP in patients with ADO2 (10). Although CK-BB is a normal component of human serum, it is rarely detectible via currently used techniques, except in children, who can normally have mild elevations of the enzyme (25–27). The current study

corroborates the previous reports that minimal elevations of CK-BB are a normal finding in the pediatric population. Clinical disorders other than osteopetrosis that may demonstrate elevated levels of CK-BB include metastatic bone lesions (36), giant cell tumor of the bone (37), acute brain injury (38), and various carcinomas (33, 39–41). Importantly, outside these clinically evident disorders, an elevated CK-BB is specific for osteopetrosis and is not merely a consequence of osteosclerosis (12, 14).

Although clearly linked to the osteoclast, TRAP and CK-BB remain enigmatic in regards to their exact roles in osteoclast physiology. CK-BB does appear to be necessary for cartilage formation at the growth plate (17, 18), but its function, if any, in bone resorption remains to be determined. TRAP may be important for the removal of pyrophosphate, a natural inhibitor of bone resorption (28), or it may play a more direct role by producing reactive oxygen species and destroying endocytosed bone degradation products (42). Disruption of TRAP activity decreases bone resorption *in vitro* (28, 43), and knockout of the TRAP gene leads to mild osteopetrosis in the mouse model (44). Therefore, regardless of its specific action, TRAP does appear to play a significant role in bone resorption. Furthermore, *in vitro* models have been created in which TRAP levels are dramatically elevated with inhibition of bone resorption (45). These experiments, reminiscent of the human osteopetrotic condition, suggest that TRAP, although necessary, is not sufficient for normal bone resorption.

The exact etiology for the elevated serum TRAP and CK-BB in ADO2 remains elusive. Osteoclast numbers and size are significantly increased in bone biopsies obtained from ADO2 patients (6–8). One explanation, therefore, is that the increased osteoclast population leads to elevated serum levels (9, 10). However, the percentage rise in serum TRAP and CK-BB levels appears much larger than the percentage increase in osteoclast numbers (9, 10). Therefore, it is also possible that TRAP and CK-BB are overexpressed by the osteoclast in response to the primary defect in bone resorption or that the elevated serum levels are derived from other tissues affected by the underlying gene mutation.

In conclusion, the current study represents the largest number of ADO2 patients and carriers tested for abnormalities in serum TRAP and CK-BB. These enzymes are significantly elevated in cases of radiographically proven osteopetrosis. Although these analytes have poor sensitivity for detecting asymptomatic carriers of the ADO2 gene, they are excellent markers for the disorder and have high diagnostic sensitivity and specificity, particularly in children. Therefore, measurement of TRAP and CK-BB can effectively screen individuals at risk for ADO2 and diminish the need to obtain multiple radiographs to determine the skeletal phenotype.

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