Familial High Serum Concentrations of the Carboxyl-Terminal Propeptide of Type I Procollagen

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We describe a family with an apparently autosomal-dominant trait that caused extremely high circulating concentrations of the carboxyl-terminal propeptide of type I procollagen (PICP). All family members examined had normal values for other biochemical markers of bone formation and degradation and no related clinical abnormalities. Furthermore, their serum concentrations of the amino-terminal propeptide of type I procollagen (PINP) were normal. Although PINP and PICP are released from the same precursor molecule, PINP is cleared from the circulation via the scavenger receptor in liver endothelial cells, whereas PICP is cleared via the mannose receptor of these cells. We thus hypothesize that the clearance of circulating PICP is compromised in the affected subjects of this family, the result of either a defective mannose receptor function or an abnormal molecular structure of their PICP.

Indexing Terms: bone metabolism/growth/heritable disorders/liver endothelial cells/mannose receptors/procollagens

Type I collagen is a major structural protein both in mineralized bone and in soft tissues. During its synthesis, two proteins known as the propeptides of type I procollagen are cleaved off and released into the interstitial fluid and blood: the carboxyl-terminal (PICP) and the amino-terminal (PINP) propeptides (1–4). The concentration of PICP in tissue fluids correlates with the rate of local synthesis of type I collagen (5), and the concentration in serum correlates with the rate of bone formation (1, 2, 4). PICP has been accepted as a new clinical tool in monitoring growth (6, 7) and in assessing bone formation and turnover (1–4, 6, 7).

An exceptionally high serum concentration of PICP was reported in a healthy individual (8), but its pathogenetic mechanism is not known. Here we report a family with an inherited trait associated with exceptionally high concentrations of PICP in the blood and with no other abnormalities in the biochemical markers of bone turnover or type I collagen metabolism.

Case Report

A 17-year-old boy was treated with inhaled glucocorticoids for bronchial asthma. He had grown and developed normally. He had sustained two bone fractures, i.e., a radial fracture due to a fall while skating at age 13, and a metatarsal stress fracture in basketball training at age 17. Recovery was normal after both fractures. To detect any possibly adverse effects of glucocorticoids, we measured, among other variables, serum PICP. The value found was very high, 2920 μg/L; an even higher value (5860 μg/L) had been measured 2 years earlier, during the growth spurt of the boy (Fig. 1). Results of routine laboratory tests were normal for age, as were those for serum total and bone-specific alkaline phosphatase, osteocalcin (Fig. 1), total and ionized calcium, phosphate, and intact parathyroid hormone (data not shown).

The high serum PICP values without related clinical abnormalities prompted us to investigate the boy's sister and parents. PICP concentrations very high for age were also found in the mother and subsequently in several other maternal relatives (Fig. 1). The mode of inheritance of the high values appeared to be autosomal-dominant. All the subjects with the high PICP concentrations were healthy, were normal in height and weight, and had grown and developed normally. They had not sustained fractures or bone diseases. No biochemical abnormalities were found in routine laboratory tests or in common tests for bone metabolism (Fig. 1).

Materials and Methods

Informed consent was obtained from all the individuals participating in the study. The information given to the patient and his relatives, as well as the procedures for collecting case histories, clinical data, and blood samples, were accepted by the local ethical committee and were in accordance with the Declaration of Helsinki II.

Radioimmunoassays of PICP, PINP, and the cross-linked carboxyl-terminal of type I collagen (ICTP). The serum concentration of PICP was measured with an equilibrium RIA of the human protein (Orion Diagnostica, Espoo, Finland) (3). The reference range, based on 25 healthy adult women, was 60–160 μg/L; the manufacturer's reference range for adults is 50–170 μg/L. The intraassay CV was 2.9% in the reference range (127
increase of the serum concentration of PICP or of any other propeptide extension of procollagens. Theoretically, the increase probably results from either increased synthesis or decreased clearance, or both. To assess the first possibility we also measured the serum concentrations of PINP, which is also cleaved from the type I procollagen molecule during type I collagen synthesis and is released into the blood. The PINP concentrations assayed with a new method (Melkko et al., submitted) were normal for age in all subjects, whether affected or not—which argues against an increased synthesis of type I procollagen. Moreover, serum concentrations of ICTP, a novel serum marker of bone collagen degradation (4, 9) (Fig. 1), and the amino-terminal propeptide of type III procollagen, a serum marker of soft connective tissue synthesis and growth (6, 11) (data not shown), were both within the reference intervals.

Thus, the very high serum PICP in this family seems hypothetically to be due to decreased clearance without abnormalities in bone collagen turnover. In the rat, liver endothelial cells remove PINP from the circulation by the scavenger receptor (12), but PICP is removed mainly by the mannose receptor (13). In humans, extrhepatic mannose receptors seem to have a major role in the elimination of PICP (K. Bentsen et al., unpublished). A genetic defect in the mannose receptor hypothetically might explain the present findings in this family. Because mannose receptors also remove other ligands from the serum, e.g., β-hexosaminidase (14), we measured the serum concentration of this mainly lysosomal enzyme in five of the subjects with high serum PICP and in three normal subjects; results were normal in all. Nonetheless, a receptor defect might influence ligand affinities differently. Because mannose receptors are phylogenetically primitive structures and essential for life, it seems reasonable to speculate that major defects in their function would be lethal. Yet another hypothetical explanation for the high serum PICP would be that supranormal concentrations of other ligands (or other circulating substances) competitively inhibit the binding of PICP to entirely normal receptors.

An abnormality in the molecular structure of PICP, such that its binding to receptors and (or) subsequent catabolic steps are affected, could also explain the high serum PICP. Neither mutations nor a defect of any kind in mannose receptor function has been reported so far. And no inherent confounding factors are known that interfere with the immunoassay itself. Given that the assays for serum PICP and also for serum PINP and ICTP use a similar first antibody–second antibody separation technique, it is unlikely that, e.g., heterophile antibodies against rabbit immunoglobulins would be the cause of an apparent increase in PICP.

Whatever the nature of the underlying abnormality, it appears to be a biological feature not hitherto characterized. The discovery of this family raises several questions: how common is this genetic trait, and could there be polymorphism, i.e., do other families exist with normal bone turnover and intermediate PICP concen-

μg/L), and 4.9% and 3.5% below and above the reference range, respectively (41 and 223 μg/L). The interassay CV was 6.2–8.0%.

PINP was purified from type I procollagen isolated from the cell culture media of human MG-63 osteosarcoma cells and digested with highly purified bacterial collagenase (grade CLSPA; Worthington Biochemicals, Freehold, NJ). The radioimmunoassay, which uses polyclonal antibodies to PINP and a kaolin-IgG solid-phase precipitation system, will be described in detail elsewhere (Melkko et al., submitted). A preliminary reference interval for PINP in adults is 10–79 μg/L. ICTP (9) was measured with reagents from Orion Diagnostica, Oulunsalo, Finland. The intrassay CV was 4.9–7.3% and the interassay CV 4.7–6.9%; the reference range in the laboratory was 1.3–4.2 μg/L for adults.

Other assays. Serum alkaline phosphatase activity was measured according to the Scandinavian recommendation (diethanolamine buffer, 37°C). To determine bone-specific alkaline phosphatase activity, we precipitated the bone isoform with lectin (Iso-ALP; Boehringer Mannheim, Mannheim, Germany). Serum β-hexosaminidase concentrations were determined spectrophotometrically (10). Serum osteocalcin concentration was measured with a competitive RIA in which bovine osteocalcin was the tracer, immunogen, and standard (CIS Bio International, Gif-sur-Yvette, France). The amino-terminal propeptide of type III procollagen was also assayed by RIA (II; Orion Diagnostica, Espoo).

**Discussion**

In the affected subjects (Fig. 1), serum PICP values exceed the age-specific means by 10- to 10s of SDs (6, 7). There are no previous reports on a familial

Fig. 1. The proband’s family: pedigree and markers of bone turnover in the subjects investigated.

Circles for females, squares for males; solid for affected, open for unaffected; crossed, square for deceased and data unobtainable. The arrow indicates the proband. AP, alkaline phosphatase; BAP, bone-specific alkaline phosphatase; OC, osteocalcin.
trations between extremely high and normal? After being released to the blood, PICP is waste to be catabolized for amino acid recycling, with no known physiological function in serum. Nor does the extremely high concentration itself or the underlying defect result in any clinically apparent deleterious consequences in this family. Further, the genetic trait does not seem to impair survival or reproduction, i.e., cause any selective disadvantage. Thus, it might not be extremely rare.

From the clinical point of view, even if the trait should prove to be common, the familially high serum PICP values far beyond those found in clinical situations (1-4, 6, 7) do not diminish the usefulness of serum PICP as a biochemical marker of bone metabolism. In cases in which other markers of bone turnover are discordant, extremely high concentrations of serum PICP should not give rise to confusion and complex, prolonged clinical investigations. In such a situation an obvious next step would be to measure serum PICP in the patient’s first-degree relatives.

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References