Pedigree-based quantitative genetic analysis of interindividual variation in circulating levels of IGFBP-3

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Abstract Circulating levels of insulin-like growth factor binding protein-3 (IGFBP-3) vary greatly between normal individuals, but until now little attention has been given to the study of the genetic factors involved in IGFBP-3 variability in healthy populations. The present study investigated the extent and pattern of the possible genetic influences on plasma levels of IGFBP-3 in 91 nuclear and more complex families, totaling 396 individuals (201 males and 195 females) of Caucasian ethnic origin. The variance decomposition analysis, was performed using the FISHER statistical package. In the second stage of the analysis, we used complex segregation analysis as implemented in the statistical package MAN. Significant negative correlation was revealed between age and plasma levels of IGFBP-3 in both sexes ($r = -0.49; r = -0.23; P < 0.001$). Multivariate analysis identified age, body weight, and height as significant covariates in men, but for women only age had a considerable effect. It has been demonstrated that about $57.7\%$ of IGFBP-3 variation adjusted for significant confounding factors was attributable to genetic factors. The results of bivariate variance decomposition analysis showed no significant genetic and phenotypic correlation between the mineral density of hand bones and IGFBP-3. Segregation analysis revealed the existence of a potential major gene effect that was able to explain some $27.5\%$ of IGFBP-3 variation. Multifactorial effects, likely, unknown minor genes, contributed an additional $30\%$ to IGFBP-3 variation. The segregation analysis also provided evidence of significant genotype X sex interaction in the determination of plasma levels of IGFBP-3.

Key words IGFBP-3 · BMD · major gene · segregation analysis

Introduction

The insulin growth factor system, comprising insulin-like growth factor-1 (IGF-1) and its binding proteins (IGFBP), plays an important biological role in bone formation [1]. It, presumably, figures importantly in the maintenance of bone mass through the stimulation of osteoblast function and inhibition of collagen matrix degradation [2–4]. IGF-1 exerts a potent stimulatory effect on the synthesis of bone-specific proteins and osteoblastic proliferation in cell and organ cultures in vitro [5,6]. Most of the IGFs circulate in the blood, attached to IGF-binding proteins, whose predominant form in adult human serum is IGFBP-3 [7,8].

IGFBP-3 belongs to a family of high-affinity IGFBPs that bind to IGFs and modulate IGF action at the cellular level, through either the inhibition or potentiation of cell growth [9], and IGFBP-3 also has an intrinsic activity, independent of its binding to IGFs [10]. The stabilization and spatial distribution of IGFs depends mainly on IGFBP-3, which, by increasing the half-life of IGFs, protects them from rapid inactivation. Therefore, the measurement of IGFBP-3 in serum better reflects the steady-state levels of IGFs than does the measurement of IGF-1, which has a short half-life and is predominantly a paracrine factor [11].

It has been reported that serum levels of IGFBP-3 decrease with age after menopause and in osteoporotic (hip-fractured) women, and that it is a marker of lean body mass and muscle strength in young men [9,12–14]. Several investigators have found significantly reduced serum levels of IGF-1 and IGFBP-3 in men with idiopathic osteoporosis compared with healthy controls [6]. Furthermore, it has been reported that levels of circulating IGFs or IGFBP-3 correlated positively with bone mass and/or bone density in healthy subjects, particularly in young men with or without osteoporosis [2,14]. Moreover, multiple regression analyses revealed significant positive correlation between serum IGFBP-3 level
and midradius bone mineral density (BMD), and this was independent of age and body mass index [15]. Contrariwise, some studies have found no evidence of reduced levels of IGF-1 or IGFBP-3 in women with spinal osteoporosis; furthermore, the IGFBP-3 level was not related to age and/or bone mass index or BMD, nor to markers of bone turnover in healthy menopausal women [16,17].

Despite a growing body of literature on the physiological functions of IGFBP-3 and the wide range of interindividual variation encountered even in healthy individuals, very little is known of the role of genetic determination in such variation. Even so, the few studies to date do suggest a significant genetic effect on IGFBP-3 variation. Thus, Harrela et al. [18], who examined variations in circulating levels of IGFBP-3 in dizygotic (DZ) and monozygotic (MZ) twins of both sexes, have shown that the estimated heritability, i.e., the proportion of variance attributable to genetic effects in the component-combined model, was about 60%. Recent studies on some candidate gene polymorphisms, however, reveal that only about 8% to 9% of IGFBP-3 variation are attributable to a candidate gene effect; these studies were conducted separately on males [19] and females [20], and showed that age, alcohol consumption, and current oral contraception usage may also be statistically significant, albeit that their independent contribution is rather small (2%–3%).

Thus, it is not clear whether there are additional genetic sources for IGFBP-3 variation apart from the studied DNA polymorphisms, or rather, that the data of Harrela et al. [18] were derived from a specific population possessing high genetic heterogeneity.

The major aim of the present study was to quantitatively evaluate the contribution of the putative genetic sources of plasma variation in IGFBP-3 in an ethnically homogeneous sample of pedigrees of Caucasian origin. An additional aim was to ascertain whether phenotypic and genetic correlations do exist between the studied binding protein IGFBP-3 and radiographic hand BMD.

Subjects and methods

To detect possible genetic or environmental sources of variation in the levels of circulating IGFBP-3 in humans, we studied 91 nuclear and more complex three-generation pedigrees, totalling 396 healthy individuals, and comprising 201 males and 195 females, aged 19–75 and 18–75 years, respectively. The pertinent data were collected from several small villages in the Bashkortostan Autonomic Region, Russian Federation. The canvassed population was characterized by a demographically stable structure with traditional relations between family members. The villagers have lived at least for the last few generations under the same environmental conditions. The collected pedigrees were sampled randomly, regardless of the variables measured. The gathered information included data on different socioeconomic parameters, standard anthropometric measurements, BMD measurements, blood samples, and also data on chronic morbidity. Individuals with known bone disease (osteoporosis, osteoarthritis, etc), or amenorrhea, and those receiving hormone replacement therapy or steroid medicines were not included in the study. A more detailed description of the sample is given by us elsewhere [21,22]. The canvassed subjects signed an informed-consent document, and the study was approved by the Tel Aviv University Ethics Committee.

Hormonal and biochemical marker measurement

Blood samples were collected by standard venipuncture technique after a 12-h fast. Plasma samples were stored at −80°C until their use in immunologic analysis. Plasma levels of IGFBP-3 were measured by an immunoradiometric assay (IRMA) kit (Diagnostic System Laboratories, Webber, TX, USA). This assay is a noncompetitive radioimmunoassay in which what is to be measured is “sandwiched” between two antibodies. Samples were diluted (1:100) and incubated with 200 ml of the anti-IGFBP-3 [I125] reagent at room temperature (25°C) overnight (18–24 h). After incubation, all tubes were washed three times in deionized water and counted with a gamma counter for 1 min. The results were calculated using a log-log curve fit, and expressed as nanograms/milliliter. The coefficient of variation (CV) for most samples was less than 5%. Also measured were the plasma levels of sex hormones (testosterone and estradiol). Total testosterone (TESTO) and estradiol (ESTR) values were determined by means of a standard radioimmunoassay (RIA) procedure, using TESTO-CT2 and ESTR-US-CT RIA kits (CIS bio international, ORIS Group, GIF-SUR-YVETTE CEDEX, France). All hormones and biochemical traits were assayed in duplicate and mean values were used in further analyses.

Bone mineral density measurements

BMD was measured using plain radiographs of the second and third phalanges of the left and right hands for separate compact and cancellous bone and aluminum wedge as a control [21,22]. Roentgenographic densitometry of hand bones is a precise and accurate method, having a CV ranging from 0.6% to 2%, and a possible accuracy error of around 4% [23]; it conforms well with BMD in other parts of the skeleton [24] and is suitable for use under field conditions.