C-Type Natriuretic Peptide (CNP) Levels Are Altered in Boys with Klinefelter Syndrome

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Context: B-type natriuretic peptide (BNP) expression in vitro is up-regulated by the protein coded by the short stature homeobox gene (SHOX). C-type natriuretic peptide (CNP) is a paracrine regulatory factor of the growth plate that plays a key role in endochondral growth and shares clearance pathways with BNP. We explored the possibility that alterations in natriuretic peptide regulation may play a role in the overgrowth of boys with Klinefelter syndrome.

Objective: The objectives of the study were to document the blood levels of amino-terminal propeptide of B-type natriuretic peptide (NTproBNP), CNP, and its amino-terminal propeptide (NTproCNP) in boys with Klinefelter syndrome and compare values with age- and height-matched control subjects.

Design: This was a prospective, case-controlled, observational study.

Subjects: Participants were 24 healthy boys with Klinefelter syndrome between 4 and 14 yr of age. Data from sex-, age-, and height-matched healthy controls were obtained from subjects participating in a previously described study.

Results: Plasma levels of NTproBNP and CNP were lower, whereas levels of NTproCNP were higher in boys with Klinefelter syndrome compared with published reference ranges. In addition, CNP levels were lower and NTproCNP levels higher than in sex-, age-, and height-matched controls.

Conclusions: In contrast to plasma NTproBNP, CNP production and clearance are increased in boys with Klinefelter syndrome. Together these findings argue against an interaction between BNP with CNP in the growth plate. Why CNP peptide levels are altered in Klinefelter syndrome remains to be explored. (J Clin Endocrinol Metab 97: 4206–4211, 2012)

Klinefelter syndrome (XXY syndrome) is a common disorder, occurring in about 1:500–1000 boys and men (1) and is widely underdiagnosed (2). The characteristic features of Klinefelter syndrome are hypogonadism, infertility, learning disabilities, and tall stature. Zeger et al. (3) noted boys with Klinefelter syndrome aged 2.0–14.6 yr had a mean height SD score (SDS) score of 0.9 ± 1.3 (mean ± SD, n = 55). The authors noted a negative correlation between upper to lower segment ratio and age, suggesting that, over time, leg growth exceeded trunk growth in these boys. These findings confirmed those of others (4), showing that the tall stature of Klinefelter syndrome is due primarily to increased leg length.

Height has a positive correlation with the number of sex chromosomes. Anthropometric studies of subjects with sex chromosome abnormalities suggested the presence of a gene in the pseudoautosomal regions of the short arms of the X and Y chromosomes regulating height in a gene dose-dependent manner. Subjects with haploinsufficiency (such as Turner syndrome or large scale deletions) have...
short stature with disproportionately short limbs, whereas subjects with overdosage (such as those with karyotypes 47, XXX; 47, XXX; 48, XXXY; or those with duplications of this region) have tall stature (reviewed in Ref. 5). Subsequently, the short stature homeobox-containing gene (SHOX) was identified as the gene on the pseudoautosomal regions of the X and Y chromosomes responsible for growth abnormalities (6, 7).

The protein encoded by SHOX is a transcription factor. However, how it regulates growth remains unknown. In the human embryo, SHOX is expressed exclusively in the limbs and in the first and second pharyngeal arches (8). In postnatal tissues, SHOX mRNA and protein is expressed in the growth plate, primarily in hypertrophic chondrocytes (9). Marchini et al. (10), cloned the human SHOX gene and inserted it into human osteosarcoma and osteoclasts (9). Marchini et al. (10), cloned the human SHOX gene and inserted it into human osteosarcoma and osteochondroma cell lines. Using microarray technology, they determined that the gene most highly up-regulated was natriuretic peptide precursor B (NPPB), coding for B-type natriuretic peptide (BNP). Immunohistochemistry documented co-localization of SHOX protein and BNP in hypertrophic chondrocytes in an adolescent (human) growth plate, and the authors speculated that SHOX regulates growth through interaction with the natriuretic peptide system in the growth plate.

The natriuretic peptides are a family of three peptides, atrial natriuretic peptide (ANP), BNP, and C-type natriuretic peptide (CNP) (11). The natriuretic peptides interact with two guanylate cyclase membrane receptors, natriuretic peptide receptor-A, which binds ANP and BNP; and natriuretic peptide receptor-B (NPR-B) which binds CNP. A third membrane receptor binds and internalizes all three peptides and acts as a clearance receptor (12). Clearance of the natriuretic peptides from the extracellular space also occurs through the action of several specific endopeptidases (12). Biosynthetic processing of each of the natriuretic peptides releases a bioinactive aminoterminal propeptide [amino-terminal propeptide of A-type natriuretic peptide, amino-terminal propeptide of B-type natriuretic peptide (NTproBNP), and amino-terminal propeptide of B-type natriuretic peptide (NTproCNP)], which are not subject to these clearance pathways but rather are cleared by renal filtration. With respect to CNP, plasma NTproCNP reflects natriuretic peptide production more accurately than levels of the active peptide in the setting of normal renal function (13).

C-type natriuretic peptide is produced in the growth plate and acts in a paracrine manner. In rodents and humans, CNP is a potent positive regulator of linear growth (reviewed in Ref. 14). Three children have been described with tall stature, long limbs, and arachnodactyly (a Marfanoid body habitus) and having chromosomal translocations involving 2q37.1 (15, 16). In each case, the breakpoint was upstream of the CNP gene, and all three children showed high blood levels of CNP and/or NTproCNP. A family with an activating mutation of NPR-B had a similar phenotype (17). Conversely, heterozygous inactivating mutations of NPR-B are a cause of idiopathic short stature (18), and homozygous mutations cause acromesomelic dysplasia, Maroteaux type, a severe form of short-limbed dwarfism (19). Although overexpression of the BNP gene in rodents results in skeletal overgrowth (20), disruption of the genes for BNP (21) or natriuretic peptide receptor-A (the BNP receptor) (22) does not affect postnatal growth in rodents, suggesting that the overgrowth in the BNP transgenic mouse is an indirect effect. Overexpression of BNP (as in states of SHOX excess) could potentially increase skeletal growth by displacing CNP from clearance receptors or by cross-activating NPR-B, if local concentrations were high enough.

Accordingly, we hypothesized that excess SHOX copy number up-regulates BNP in the growth plate and that BNP, by competing with CNP in clearance pathways, decreases CNP clearance. The excess CNP could be then be the cause of the overgrowth seen in Klinefelter syndrome. An alternative hypothesis (that high rates of local BNP production in growth plates maybe sufficient to cross-activate NPR-B and raise circulating concentrations of NTproBNP) was also considered.

We conducted a prospective study of boys with Klinefelter syndrome to determine whether they have an alteration in plasma NTproBNP or in CNP production and clearance.

Materials and Methods

Subjects

Subjects were healthy boys with karyotype-confirmed Klinefelter syndrome. Twenty-one of the subjects were recruited for and participated in a separate study (23). None of the subjects had a mosaic karyotype. This study was approved by the Nemours Florida Institutional Review Board. All subjects had written parental permission obtained.

Data from sex-, age-, and height-matched healthy control subjects were drawn from a pool of healthy children that participated in a separate study (24). For each case subject, a control subject was identified by matching sex (boys), age (±0.3 yr), and height (±4 cm).

Study procedures

This was a prospective study. Boys were seen in the Pediatric Endocrinology clinics at Thomas Jefferson University (Philadelphia, PA) or at Nemours Children’s Clinic (Jacksonville, FL). Anthropometrics were done, including height by Harpenden stadiometer and weight by electronic scale. A physical examination was performed and included Tanner staging. Blood was drawn.
Assays

Blood was drawn into EDTA tubes and stored at 4°C until processed. Blood was centrifuged at 4°C and plasma aliquoted and frozen at −80°C until assayed.

NTproBNP was assayed by a commercially available non-competitive electrochemiluminescent immunoassay (Elecsys ProBNP, Roche Diagnostics, Indianapolis, IN).

The RIA used for CNP was as previously described (24). The limit of detection for this assay is 1.0 pm (0.2 pm after sample concentration). Within- and between-assay coefficients of variation of the assay are 4.9 and 8.9%, respectively, at 2.1 pm. Cross-reactivity with ANP in this assay is less than 0.004%. Cross-reactivity with human BNP (at 100 pm) is less than 0.07%.

The RIA used for NTproCNP was as previously described (24). The detection limit of this assay is 1.2 pm (0.4 pm after sample concentration). Within- and between-assay coefficients of variation are 6.8 and 8.4%, respectively, at 14 pm. Cross-reactivity with ANP propeptide in this assay is less than 0.07% and with human BNP propeptide is less than 0.4%.

Statistical analysis

Height and body mass index (BMI) SDS were calculated using the Centers for Disease Control and Prevention 2000 growth charts (25). NTproBNP SDS were calculated using data from Albers et al. (26) and those for CNP, NTproCNP, and the CNP to NTproCNP ratio from our previous study (24).

Data are summarized as full range or median and interquartile range (25th to 75th percentiles). Comparisons of the SDS data with the general population (mean = 0, SD = 1) were made using one-sample Student’s t tests. Comparisons between the subjects and matched controls were made using a paired Student’s t test. Statistics were calculated using Excel software (version 2007; Microsoft, Redmond, WA). The significance was assumed for P < 0.05.

Results

The characteristics of the subject population are summarized in Table 1. Twenty-four boys were studied, with ages ranging from 4.0 to 13.7 yr. As expected, the boys with Klinefelter syndrome trended taller than the general population (median height SDS of 0.5), although in this sample the difference did not reach statistical significance. The boys also trended heavier (median BMI SDS of 0.5), but this difference was also not significant. Twenty-one of the subjects were prepubertal and three had reached pubic hair Tanner stage II. None of the subjects had been treated with testosterone.

Compared with the general population, plasma NTproBNP levels were lower (median SDS of −0.7 with an interquartile range of −1.6 to 0.1, P = 0.006) in boys with Klinefelter syndrome (Fig. 1A). CNP levels were also lower (median SDS of −1.4 with an interquartile range of −1.9 to −0.8, P = 0.00009, Fig. 1B). In contrast, levels of NTproCNP were significantly elevated (median SDS of 0.5 with an interquartile range of 0.0–1.3, P = 0.009, Fig. 1C). The ratio of CNP to NTproCNP is a marker of CNP clearance. This ratio was profoundly reduced in the boys with Klinefelter syndrome (median SDS of −2.1 with an interquartile range of −2.9 to −0.9, P = 0.00001, Fig. 1D).

To reduce the potential influence of taller stature in the boys with Klinefelter syndrome, we used data from a pool of healthy controls to identify a sex-, age-, and height-matched control for each boy with Klinefelter syndrome. In this pair-wise (case control) analysis, the differences in CNP, NTproCNP, and CNP to NTproCNP persisted (Table 2).

Discussion

It is clear from recent studies that SHOX is an important regulator of growth. It is expressed in the growth plate and genetic studies have shown mutations or deletion of this gene cause short stature, whereas an excess in gene copy number is associated with tall stature. However, the mechanism through which this transcription factor regulates growth remains unknown. The discovery that the over-expression of SHOX up-regulates BNP in chondrocytes in vitro was the first evidence to implicate natriuretic peptides. The possibility that this hormonal system could be involved in the skeletal abnormalities associated with SHOX disorders is an important issue that could affect future diagnostic or treatment opportunities. We first sought evidence for increased BNP production by looking at products of BNP production in plasma, but none was found. The NTproBNP assay used in this study has higher imprecision in the low concentration range. Although levels were statistically lower in the subjects, this imprecision leads us to conclude only that levels are not elevated in

TABLE 1. Subjects

<table>
<thead>
<tr>
<th>Result</th>
<th>P^α</th>
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<tr>
<td>Number</td>
<td>24</td>
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<tr>
<td>Age (yr)</td>
<td>4.0 to 13.7</td>
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<tr>
<td>Height SDS</td>
<td>0.5 (−0.6 to 0.7)</td>
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<tr>
<td>BMI SDS</td>
<td>0.5 (−1.0 to 1.1)</td>
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<tr>
<td>proBNP (pm)</td>
<td>5.1 (2.6–6.9)</td>
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<tr>
<td>proBNP SDS</td>
<td>−0.7 (−1.6 to 0.1)</td>
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<tr>
<td>CNP (pm)</td>
<td>1.1 (1.0–1.3)</td>
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<tr>
<td>CNP SDS</td>
<td>−1.4 (−1.9 to −0.8)</td>
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<tr>
<td>NTproCNP (pm)</td>
<td>41.5 (36.6–46.4)</td>
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<tr>
<td>NTproCNP SDS</td>
<td>0.5 (0.0–1.3)</td>
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<td>CNP to NTproCNP</td>
<td>0.025 (0.020–0.034)</td>
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<tr>
<td>CNP to NTproCNP SDS</td>
<td>−2.1 (−2.9 to −0.9)</td>
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Data are full range or median (intraquartile range). Numbers in bold are significant.

^α Compared with the general population, by one-sample Student’s t test.
boys with Klinefelter syndrome. BNP is primarily produced in the heart ventricles and is regulated by cardiovascular signals. As a result, BNP and NTproBNP in the circulation are primarily of cardiac origin (27). Although growth plate tissue concentrations of natriuretic peptides in SHOX disorders will eventually answer some of these questions, our finding that plasma levels of NTproBNP were not elevated suggests that any increase in growth plate BNP is modest and unlikely to reach a level sufficient to cross-activate the CNP receptor.

Unlike BNP, skeletal tissues contribute significantly to circulating levels of CNP and NTproCNP (27). We have shown that levels of NTproCNP are elevated in growing children and levels correlate with height velocity in healthy children at all ages (24). We show here that NTproCNP levels are elevated in boys with Klinefelter syndrome. Circulating levels of NTproCNP are a function of its rate of appearance and its rate of clearance. For NTproCNP, clearance occurs via renal filtration. We also demonstrate that the CNP levels are decreased in boys with Klinefelter syndrome. For CNP, circulating levels are a function of rate of appearance, and its rate of clearance (cellular uptake by natriuretic peptide receptor-C, and proteolysis by membrane metalloendopeptidase and insulin degrading enzyme) (12). Equimolar secretion of CNP and NTproCNP occurs as both are cleavage products of a single precursor. If we assume renal filtration (and hence the rate of NTproCNP clearance) is similar in the subject and

![Graph A](image1)

**FIG. 1.** Natriuretic peptide levels are altered in boys with Klinefelter syndrome. Plasma levels of natriuretic peptides were determined. In each graph, points show the results from individual subjects; heavy lines, the median; and light lines, the fifth and 95th percentile of the healthy population. A, NTproBNP levels. B, CNP levels. C, NTproCNP levels. D, The ratio to CNP to NTproCNP, an indicator of CNP clearance.

<table>
<thead>
<tr>
<th>Table 2. Comparison between boys with Klinefelter syndrome and age- and height-matched healthy boys</th>
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<tbody>
<tr>
<td><strong>Klinefelter</strong></td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>CNP (pM)</td>
</tr>
<tr>
<td>NTproCNP (pM)</td>
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<tr>
<td>CNP to NTproCNP</td>
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</table>

*Data are median (intraquartile range).*

*Data are mean ± sd.*

*By paired Student’s t test, numbers in bold are significant.*
control groups, and then the ratio of CNP to NTproCNP is a function primarily of CNP clearance, which may occur at the tissue source or in the circulation. Our hypothesis was that CNP clearance would be reduced in boys with Klinefelter syndrome. The data presented here show that CNP to NTproCNP levels are markedly decreased, suggesting that CNP clearance is increased in these boys. Conversely, NTproCNP levels are elevated, demonstrating an increase in the CNP production. Whether these increases in CNP production and clearance are a cause or an effect of the overgrowth in Klinefelter syndrome remains to be seen.

Although the CNP and NTproCNP differences between subjects and controls were strongly statistically significant, there was considerable overlap, and the majority of subjects had levels within the reference range. Clearly these tests will have little value as screening tools for Klinefelter syndrome.

In addition to regulating endochondral growth, CNP has vasorelaxant and antifibrotic effects in the cardiovascular system. It is also found in gonadal tissues and in seminal fluid at high concentrations, although its role here is unknown. We now show that boys with Klinefelter syndrome have altered regulation of CNP metabolism. How this may affect cardiovascular or reproductive health in these subjects remains to be determined.

We conclude that the CNP system is somehow involved in the growth abnormalities of the SHOX disorders; however, mechanisms through which SHOX affects the CNP system remain to be explored.

Acknowledgments

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Disclosure Summary: T.C.R.P. and E.A.E. have a patent filed entitled “Assessment of skeletal growth using measurements of NT-CNP peptides.” The other authors have nothing to disclose.

References


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