Osteoporosis in Klinefelter’s syndrome

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Submitted on December 24, 2009; resubmitted on March 22, 2010; accepted on March 24, 2010

ABSTRACT: Hypogonadism represents one of the most important causes of male osteoporosis. Testosterone regulates male bone metabolism both indirectly by aromatization to estrogens and directly through the androgen receptor (AR) on osteoblasts, promoting periosteal bone formation during puberty and reducing bone resorption during adult life. Early onset of testosterone deficiency, as observed in Klinefelter’s syndrome (KS), is an important risk factor for precocious osteoporosis. Osteoporosis is present in up to 40% of subjects with KS and has usually been attributed to low testosterone levels. However, reduced bone mass might be present also in KS men with normal testosterone levels and testosterone replacement therapy does not always restore bone density in KS patients. Possible new determinants for osteoporosis in KS might be related to the AR function and insulin-like factor 3 (INSL3) levels. The CAG length and inactivation pattern of the AR in KS have been related to osteoporosis, but definitive proof is lacking. INSL3 has an anabolic role on bone metabolism by acting on osteoblasts and INSL3 levels are low in KS. Therefore, low INSL3 concentrations might represent a possible new pathogenic mechanism for reduced bone mass in KS.

Key words: Klinefelter’s syndrome / bone mass / hypogonadism / androgen receptor / INSL3

Osteoporosis in men

Osteoporosis, which is defined as reduced bone density and strength predisposing to increased risk of fracture, is an underestimated, under-diagnosed and undertreated condition in men (Liu et al., 2008), and most cases (40%) are classified as idiopathic (Khosla et al., 1994; Liu et al., 2008). Osteoporosis is defined according to measurement of bone mineral density (BMD) using dual-energy X-ray absorptiometry (DEXA). The commonly used BMD-based operational definition of osteoporosis has been validated for white post-menopausal women only and there is no consensus regarding a BMD-based definition of osteoporosis in men. However, it is generally accepted that a bone density T-score at or below 2.5 standard deviations (SD) below normal peak values for young adults defines osteoporosis, whereas a T-score of between −1 and −2.5 SD defines osteopenia, (Table I). For younger men both T-score and Z-score could be used for the diagnosis of low BMD, with Z-scores <−2 SD below the gender- and age-specific population mean defining osteoporosis (Leib et al., 2004; Table I).

It was estimated that, in the USA, up to 13 million of men older than age 50 years were affected by low bone mass and 2 million had osteoporosis (Looker et al., 1997). Also in the USA, the prevalence of osteoporosis and osteopenia is estimated to be, respectively, about 6 and 47% (Kanis et al., 2001). It has been reported that 13% of men older than 50 years of age has an osteoporotic fracture during life and about 30% of all hip fractures occur in men (Cooper and Melton, 1992). Although there is increasing recognition of the problem of male osteoporosis, there remains considerable gaps in knowledge regarding this disorder as well as in the care of these patients.

Generally, men loose less bone mass than women during lifetime (Bilezikian, 1999), and are protected against bone loss and osteoporosis, having a lifetime risk for fragility fractures of 15% compared with 40% in women (Vanderschueren et al., 2008). During puberty, young men and women present the same peak vertebral bone density, but bone width is greater in men, and therefore they have greater bone strength (Seeman, 2001). Bone loss in men appears later in life compared with women (Cauley, 2006).

Male osteoporosis is a heterogeneous entity, with multiple underlying causes and risk factors. Male osteoporosis is generally divided into primary causes (age-related and idiopathic osteoporosis) and secondary causes. Many systemic diseases can affect bone mass in males (Table II), directly or indirectly (Dupree and Dobs, 2004; Cauley, 2006). The most important risk factors for low BMD mediated fractures in males are age >70 years, low body weight (body mass index <20), weight loss (>10%), physical inactivity, prolonged corticosteroid use, previous osteoporotic fractures and hypogonadism/androgen deprivation therapy (castration or therapy with gonadotrophin-releasing hormone agonists; Liu et al., 2008).

Androgens, hypogonadism and bone

Androgens have an important role in bone metabolism and different conditions associated with hypogonadism are associated with low
bone mass. Hypogonadism is found in about 15% of men with osteoporosis, although this proportion varies both across populations and with the definition of hypogonadism. From an epidemiological point of view the most common cause of reduced testosterone levels is late-onset hypogonadism (Wang et al., 2008). In normal male population there is a gradual decline in serum testosterone between 0.4 and 2.6% per year after age 40, with a prevalence of hypogonadism (total testosterone < 10.4 nmol/l; Bhasin et al., 2006) from 10 to 25% in elderly men (Araujo et al., 2007). In elderly men there is also a concomitant increase in sex-hormone-binding globulin concentration, with a consequent decline of free testosterone—the biologically active testosterone—and estradiol. The other main causes of hypogonadism are Klinefelter’s syndrome (KS), orchitectomy, primary testiculopathies and treatment with GnRH agonists.

Testosterone regulates male bone metabolism both indirectly by aromatization to estrogens and directly on osteoblasts through the androgen receptor (AR). The net effect of testosterone is to promote periosteal bone formation mostly during puberty (Jackson et al., 1987) and to reduce bone resorption mostly during adult life (Dupree and Dobs, 2004). The final effect of androgens on the bone is to maintain cancellous bone mass and to increase bone size by stimulation of both longitudinal and radial growth. This leads to higher bone size and bone strength compared with women.

The AR pathway is particularly effective in the trabecular bone where androgens preserve or increase trabecular numbers, suppress trabecular resorption and reduce trabecular spaces therefore increasing trabecular bone volume (Marcus et al., 2000). AR knockout mice have prevalent decrease in trabecular bone (Callewaert et al., 2008; Wiren et al., 2008) and patients with AR mutations show a reduced bone mass (Bertelloni et al., 1998; Marcus et al., 2000; Danilovic et al., 2007). On the other hand, cortical bone is affected when both AR and estrogen receptor disruption occur (Callewaert et al., 2008; Wiren et al., 2008), whereas bone loss from estrogen deficiency is mostly evident at the cortical level (Khosla et al., 2008). Supporting these data, studies suggested that 1 year after castration in men, vertebral BMD is reduced by at least 5–10% (Vanderschueren et al., 2008).

Testosterone is fundamental in a critical stage of bone maturation to reach the peak bone mass at the end of puberty and to keep it during adult life. A deficiency in testosterone production during puberty is an important risk factor for precocious male osteoporosis. In fact, premature male osteoporosis is usually associated with hypogonadism, as observed in KS, idiopathic hypogonadotropic hypogonadism or delayed puberty and in hyperprolactinemia (Arisaka et al., 1995).

A positive correlation between BMD and testosterone levels has been demonstrated in normal men, osteoporotic men and in KS (Foresta et al., 1983; Horowitz et al., 1992; Seo et al., 2007). It has also been reported that both serum levels of testosterone and luteinizing hormone (LH) show a significant association with osteoporosis or fractures (Stanley et al., 1991; Moyad, 2002; Yeh et al., 2002). Interestingly, LH was directly related to a positive influence on bone metabolism in men: LH receptors are present on osteoblasts and LH receptor knockout animals showed age dependent bone loss (Yarram et al., 2003).

Moreover, there are connection between testosterone and the vitamin D pathway. It is well known that vitamin D is an important factor in bone metabolism and vitamin D levels < 62.5 nmol/l are associated with an increased risk of hip fracture in men older than 65 years (Looker and Mussolino, 2008). Testosterone acts indirectly on the parathyroid hormone-vitamin D axis, because testosterone

<table>
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<th>Table I</th>
<th>Diagnostic criteria for osteoporosis in men.</th>
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<tr>
<td>Age</td>
<td>Criteria</td>
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<tr>
<td>&lt;50</td>
<td>The diagnosis should be made on the basis of both T-score and Z-score. Some Authors recommend to using only the Z-score (low BMD when Z-score ≤ −2 SD, osteopenia if Z-score ≤ −1 SD)</td>
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<tr>
<td>50–64</td>
<td>T-score ≤ −2.5 SD at both spine and hip plus risk factors for fracture</td>
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<tr>
<td>≥65</td>
<td>T-score ≤ −2.5 SD</td>
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<tr>
<td>Any age + secondary causes of low BMD</td>
<td>T-score &lt; −1 SD</td>
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SD, standard deviation.

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<th>Table II</th>
<th>Systemic diseases that can affect bone mass in males.</th>
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<td>Genetic disorders</td>
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<td>Aneuploidies (KS)</td>
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<td>Hemochromatosis</td>
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<td>Haemophilia</td>
<td>Hyperparathyroidism</td>
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<td>Idiopathic scoliosis</td>
<td>Hyperthyroidism</td>
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<td>Osteogenesis imperfecta</td>
<td>Hypovitaminosis D</td>
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<td>Thalassaemia</td>
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<td>Hypophosphataemia</td>
<td>Hypercortisolism or glucocorticoid therapy</td>
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<td>Malignancies</td>
<td>Other disorders</td>
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<td>Leukaemia</td>
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<td>Lymphoma</td>
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<td>Multiple myeloma</td>
<td>Malabsorption</td>
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<td>Inflammatory bowel diseases</td>
<td>Sickle cell anaemia</td>
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<td>Rheumatoid arthritis</td>
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deficiency is related to a reduction in renal 1α-hydroxylase activity with a subsequent decrease in 1,25-hydroxy vitamin D concentration, the active form of vitamin D (Francis et al., 1986).

Bone turnover in men is influenced also by estrogens and a large part of testosterone action on bone metabolism is mediated by its aromatization to estrogens. Estrogens are important enhancer of bone mass during growth and maturation, they thicken bone cortices, maintain BMD, retard bone loss and are essential in periosteal bone expansion during puberty. As testosterone, estrogens gradually decrease in men during aging (Khosla et al., 2001) and rare causes of isolated hypoestrogenism in men or estrogen receptor disorders associated with bone loss have been described (Smith et al., 1994, 2008).

Osteoporosis in KS

Patients with KS have a high risk of developing osteoporosis and osteopenia and an increased risk of fractures due to osteoporosis and osteopenia (Bojesen et al., 2006a; Aksglaede et al., 2008). KS is associated with decreased bone mass in 25–48% of cases (Breuil and Euller-Ziegler, 2001) and with osteoporosis in 6–15% (Van den Bergh et al., 2001) and is due to both reduced bone formation and higher bone resorption (Luissetto et al., 1995). The annual decrease in bone mass rate in KS has been calculated in 1.18 ± 0.53% at the lumbar level and 1.03 ± 0.43% at the femoral neck level (Stepan et al., 2003).

Young KS subjects have normal bone density in childhood and at the beginning of pubertal development (Aksglaede et al., 2008). During the later stages of puberty, KS subjects develop a progressive testicular failure leading to primary hypogonadism. Such a deficiency in testosterone production during puberty represents the most important risk factor for reduced bone mass and osteoporosis in KS. Actually, decreased bone mass in KS has usually been attributed to hypogonadism and supporting this hypothesis testosterone plasma levels have been shown to positively correlate with BMD in these subjects (Foresta et al., 1983; Kubler et al., 1992; Stepan et al., 2003; Seo et al., 2007). Similarly to that observed in hypogonadal non-KS patients, bone histology of KS subjects demonstrated loss of cancellous tissue, profound depression of osteoblast activity, decreased osteoid seam width and slowing of the apposition rate (Delmas and Meunier, 1981). These findings have not been documented in KS subjects with normal testosterone levels who have a normal cortical bone mass (Foresta et al., 1983).

However, several studies showed that testosterone replacement in KS men with low testosterone levels and low BMD does not reverse the decreased bone mass (Wong et al., 1993). This was more evident when testosterone replacement therapy was started after puberty, also after many years of therapy (Van den Bergh et al., 2001). On the contrary, other studies showed that androgen replacement therapy starting in young age (i.e. before 20 years) can lead to normal BMD (Kubler et al., 1992). Of particular interest is the finding of reduced bone mass also in KS subjects with normal testosterone levels (Seo et al., 2007), suggesting that bone loss in KS might be, at least in part, independent from the presence of hypogonadism. It is well known that testosterone levels can be normal in a high proportion (40–50%) of KS subjects (Lanfranco et al., 2004). However, it should be considered that testosterone levels in KS might not truly define hypogonadism. In fact, signs of hypogonadism might be present also in men with normal testosterone levels, and LH is invariably high and disproportionately high with respect to testosterone levels in these patients. In fact, some authors suggested that hypogonadism might be better defined using a bivariate LH and testosterone chart (Aksglaede et al., 2007). Unfortunately, there are no definitive data in the literature clearly showing the prevalence of osteoporosis in KS subjects with low or normal testosterone levels or with/without other signs of hypogonadism.

There are other possible hormonal modulators of bone metabolism in KS. Even if estradiol levels are generally normal or high in this syndrome, low estrogen levels have been related to decreased bone mass in these patients in some studies (Eulry et al., 1993; Choi et al., 1995) and estradiol levels are inversely related to the rate of bone loss (Stepan et al., 2003). However, these data have not been replicated and conclusions on this possible pathogenic mechanism cannot be made. Similarly, only one study examined the vitamin D levels in KS. The authors showed that 25-hydroxy and 1,25-hydroxy vitamin D are in the low-normal range in KS subjects and that the rate of gain of BMD in the femoral neck after androgen therapy was inversely related to 25-hydroxyvitamin D, highlighting the importance of vitamin D insufficiency or deficiency in the response to therapy (Stepan et al., 2003).

Another possible mechanism involved in the development of bone loss in KS might be related to the unfavourable fat/muscle ratio caused by increased fat mass and reduced muscle mass (Bojesen et al., 2006a, b; Ishikawa et al., 2008). However, it is not clear whether such an altered ratio is caused exclusively by the low testosterone levels or by other mechanisms related to the genetic defect. In fact, recent studies suggested that the unfavourable fat/muscle ratio is already present in young adolescents, whereas bone mass defects appear in late puberty or later (Aksglaede et al., 2008).

Principles of therapy

There are some areas of uncertainty about therapy for osteoporosis in men, because few prospective, randomized trials of osteoporosis therapies have been performed specifically in men (Kaufman and Goemaere, 2008). General measures include lifestyle and dietary recommendations: exercise, a balanced diet and sun exposure should be encouraged, whereas excessive alcohol intake and smoking should be discouraged. Men with hypogonadism, including KS, should receive substitutive testosterone treatment, although there have not been controlled trials assessing the long-term bone effects of testosterone substitution in these men. Observational data suggested beneficial effects of testosterone supplementation on BMD in hypogonadal non-Klinefelter men, in particular adolescents: 6 months with testosterone gel therapy led to an improvement in cortical and trabecular bone mass (8.9% increase at spine, 7.5% L2–L4 and 5% in femoral trochanter region, 2% in vertebrae; Snyder et al., 1999; de Laet et al., 2002; Wang et al., 2004). Furthermore, long-term androgen replacement therapy (9.2 ± 8.2 years) has been demonstrated to reduce the incidence of vertebral and femoral fractures (Van den Bergh et al., 2001). There are no data on the dose–effect relationship for testosterone action on bone, therefore in clinical practice testosterone substitution aims at achieving testosterone levels in the mid-physiological range for young adults.
On the other hand, there are currently no indications for testosterone substitution in men with low BMD and normal testosterone levels. More research is needed in this field in the light of the high LH levels and clinical signs of hypogonadism that are quite invariably present in KS subjects despite testosterone levels in the low-normal range. Current recommendations in men with normal testosterone levels suggest the use of bisphosphonates as primary therapy. Although no conclusive data can be drawn from the limited number of prospective, randomized trials in men, these drugs seem to have rather comparable effects to those previously shown to substantially reduce fracture risk in post-menopausal women (Kaufman and Goemaere, 2008). Alendronate, risedronate and zoledronic acid are the most common bisphosphonates used. Pamidronate, can be administrated if the other three agents are not tolerated (Romeo and Ybarra, 2007). In subjects with low testosterone levels testosterone replacement therapy should be associated with an oral bisphosphonate that helps in achieving and maintaining the increase in BMD (Romeo and Ybarra, 2007).

Although there are conflicting results on benefits of calcium and vitamin D supplementation, a calcium intake of 1000–1200 mg per day (1200–1500 if osteoporosis is present) and vitamin D supplementation (800 IU or calcitriol 0.50 µg per day) should be recommended, above all in men with or at high risk for deficiencies. This supplementation reduces osteoporotic fractures by 12% in both men and women ≥50 years old (Ebeling et al., 2001; Tang et al., 2007). The goal is to maintain serum levels of 25-hydroxy vitamin D at least at 30 ng/ml (Dawson-Hughes et al., 2005).

Finally, therapy with parathyroid hormone (i.e. teriparatide, parathyroid hormone) could be considered in both hypogonadal and eugonadal men to increase BMD of the spine reducing the risk of vertebral fractures, even if data about effects on non-vertebral fractures in men are lacking (Orwoll et al., 2003). This treatment is recommended only for severe osteoporosis and in those who do not tolerate or have not an adequate response to bisphosphonates (Ebeling, 2008). Few and not consistent data are available about calcitonin treatment in men and no data are available about strontium ranelate therapy or estrogen therapy in men (Khosla et al., 2008).

Although evidence-based data for the treatment of osteoporosis in KS are sparse, Fig. 1 schematically shows a possible diagnostic and therapeutic approach to osteoporosis in KS.

**New developments**

Although osteoporosis in KS might have different causes and risk factors, low testosterone levels are generally considered the main etiological mechanism. However, as outlined above, clinical signs in KS, including reduced BMD, are very heterogeneous and highly variable even among patients with similar levels of testosterone, and reduced BMD is quite frequent also in KS men with normal or low-normal
testosterone levels. Furthermore, testosterone substitution therapy in those with hypogonadism does not unequivocally increase BMD. Recent research suggested other possible pathogenic mechanisms of reduced BMD in KS, related in particular to the AR function and insulin-like factor 3 (INSL3) levels.

The first exon of the AR gene encodes for the transactivator domain of the AR protein. It contains the highly polymorphic CAG repeat, the length of which is inversely correlated with androgen sensitivity (Chamberlain et al., 1994). Although the length of CAG repeat has been associated with different disorders (male hypogonadism, cryptorchidism, prostate cancer, testicular cancer), conflicting data have been published on the relation between it and bone metabolism. The CAG polymorphism of the AR has been reported to be negatively and independently associated with BMD, in particular in young men (Zitzmann, et al., 2001; Zitzmann and Nieschlag, 2003). On the contrary, another study found on the contrary a positive relation, explained with the negative feed-back of CAG related AR sensitivity on testosterone concentrations and thus on higher estrogen levels, with a global positive effect on BMD (Stiger et al., 2008).

A certain degree of androgen resistance has previously been reported in KS (Grigorescu et al., 1983; Breuil and Fuller-Ziegler, 2001), with a decreased activity of bone 5α-reductase (Gautier and Bauduceau, 1990) and a lower peripheral AR expression on lymphocytes (Meurer et al., 2007) and smooth muscle cells (Kotula-Balak et al., 2004). However, the AR expression in the bone has never been studied in KS.

Another important aspect of AR is that the AR gene is located on the X chromosome (and therefore present in double copy in KS) and there is evidence of non-random X inactivation in men with more than one X chromosome (Iitsuka et al., 2001). In KS the CAG polymorphism length depends on the inactivation rate of the two X chromosomes by methylation. Therefore, the effective CAG repeat value in heterozygous KS men for the CAG polymorphism of the AR gene is calculated after the analysis of the methylation rate in the two X chromosomes in order to obtain a X-weighted biallelic mean, not an arithmetic mean (Zitzmann et al., 2004). In this way, a relation with different clinical outcome and response to testosterone therapy was found, with a statistically significant negative correlation between bone density evaluated by phalangeal ultrasound and the X-weighted biallelic mean of CAG repeats (Zitzmann et al., 2004), as previously shown in normal men (Zitzmann et al., 2001). Moreover, a higher inactivation rate of shorter alleles has been described, thus determining a less functional AR (Zitzmann et al., 2004).

This was the first important finding suggesting that reduced testosterone levels are not the only cause of decreased bone mass in KS subjects and that additional factors related to the androgenic status might contribute to the altered bone metabolism in subjects with KS. A non-random X inactivation and lower androgen function could therefore be, at least in part, responsible for or contribute to decreased bone mass in KS, particularly evident in those patients with normal testosterone concentration. Thus, this mechanism could explain not only the high prevalence of decreased BMD in eunuchoidal KS patients, but also the frequent ineffectiveness of testosterone replacement therapy in improving BMD in KS.

Another important aspect related to testicular failure and bone metabolism in KS is the circulating levels of INSL3. INSL3 is a protein hormone produced almost exclusively by pre- and post-natal Leydig cells of the testis (Foresta et al., 2004; Ferlin et al., 2009; Ivell and Anand-Ivell, 2009). The major known endocrine role of INSL3 is related to the regulation of testicular descent during fetal development by acting on gubernaculum via its specific receptor RXFP2 (Relaxin Family Peptide 2; Nef and Parada, 1999; Zimmermann et al., 1999; Overbeek et al., 2001; Gorlov et al., 2002). As a consequence, Ins3 and Rxfp2 knockout mice have bilateral cryptorchid testes (Nef and Parada, 1999; Zimmermann et al., 1999; Overbeek et al., 2001; Gorlov et al., 2002) and mutations in the INSL3 and RXFP2 genes have been associated with testis maldevelopment also in humans (Gorlov et al., 2002; Ferlin et al., 2003, 2006a, 2008a; Bogatcheva et al., 2007; Foresta et al., 2008). In addition to the pre-natal role for INSL3, further possible endocrine and paracrine actions in adult males have recently gained particular attention (Foresta et al., 2004, 2009; Bay et al., 2005; Bay et al., 2006; Ferlin et al., 2006b). These studies showed that INSL3 is produced constitutively but in a differentiation-dependent manner by the Leydig cells under the long-term Leydig cell differentiation effect of LH. On this basis INSL3 has been proposed as a specific marker of Leydig cell differentiation status (Foresta et al., 2004; Bay et al., 2005; Ivell and Anand-Ivell, 2009). Testosterone and INSL3 provide different information on the status of the Leydig cells: testosterone better reflects the steroidogenic activity that is acutely sensitive to LH, whereas INSL3 seems to be uncoupled from this rapid stimulation of steroidogenesis and better reflects the differentiation status and general wellness of the Leydig cells (Foresta et al., 2004; Bay et al., 2005; Sadeghian et al., 2005; Bay et al., 2006; Ferlin et al., 2006c).

The dynamic of circulating levels of INSL3 is very similar to that of testosterone. After birth, INSL3 increases at about 3 months of age under the increased levels of LH (mini-puberty) (Bay et al., 2007). Soon after, INSL3 declines to undetectable levels and remains low during infancy (Bay et al., 2007) and then progressively increases throughout puberty (Ferlin et al., 2006c). Finally, INSL3 levels in adulthood decline steadily throughout life and at 75–80 years INSL3 concentrations are reduced by about 40% with respect to levels found at 35–40 years (Anand-Ivell et al., 2006a). Reduced plasma concentrations of INSL3 are seen in situations of undifferentiated or altered Leydig cell status or reduced Leydig cell number, such as in anorchid men, men with hypogonadism, infertility or obesity (Foresta et al., 2004; 2009; Bay et al., 2005; Anand-Ivell et al., 2006a).

Although the exact role of post-natal INSL3 is not fully understood, the general hypothesis is that reduced INSL3 activity (caused by altered testicular function, INSL3 or RXFP2 gene mutations) could cause or contribute to some symptoms and signs of hypogonadism, such as reduced BMD, currently attributed to testosterone deficiency. RXFP2 is expressed in many tissues besides the gubernaculum, including kidney, skeletal muscle, thyroid, pituitary gland, brain and bone marrow (Overbeek et al., 2001; Hsu et al., 2002; Foresta et al., 2004), and paracrine roles for INSL3 have been suggested in the testis (Kawamura et al., 2004; Anand-Ivell et al., 2006b), ovary (Kawamura et al., 2004), thyroid (Hombach-Klonisch et al., 2003) and mammary gland (Hombach-Klonisch et al., 2000). Most importantly, we showed that human and mouse osteoblasts express the INSL3 receptor and that young adult men carrying the T222P mutation of the RXFP2 gene and with normal testosterone levels are at significant risk of reduced bone mass and osteoporosis (Ferlin et al., 2008b). Consistent with the human phenotype, bone
histomorphometric and μ CT analyses at the lumbar and femoral sites of Rxfp2−/− mice showed decreased bone volume, alterations at the trabecular bone, reduced mineralizing surface, bone formation and osteoclast surface (Ferlin et al., 2008b). These data suggested a functional osteoblast impairment causing a negative balance between bone formation and bone resorption in mice knockout for Rxfp2 and in humans with mutations in RxFP2.

Only one study examined INSL3 levels during puberty in boys with KS, showing a normal increase in serum INSL3 at initial stages of puberty and then a levelling off (Wikström et al., 2006). Few studies examined INSL3 in adult men with KS. We reported that adult KS with reduced testosterone levels had also very low levels of INSL3. We examined INSL3 in adult men with KS. We reported that adult KS with normal testosterone levels. Future patients. Unresolved questions are related to the choice of treatment during young adulthood could therefore represent the pathogenic mechanism. Table III summarizes the possible mechanisms contributing to osteoporosis in KS. These data have been confirmed in another study (Bay et al., 2005), which showed also that KS patients with the highest INSL3 levels were those who were not in need of testosterone substitution.

These preliminary data suggested that INSL3 could be a valuable marker of Leydig cell function in KS. In particular, these data highlighted that men with KS invariably have low INSL3 levels, independently from the presence of low or normal testosterone levels (Foresta et al., unpublished). Taken together these findings, although preliminary, would suggest that: (i) INSL3 seems more appropriate than testosterone to assess the Leydig cell function in KS; (ii) the low INSL3 levels observed from mid-puberty onward in KS could have a role in the reduced bone density and osteoporosis in these subjects; (iii) the limited efficacy of testosterone replacement therapy in KS subjects with osteoporosis could be explained by this alternative pathogenic mechanism. Table III summarizes the possible mechanisms involved in reduced bone mass in KS.

### Implications

New exciting areas of research on reduced bone mass and osteoporosis in KS involve androgen (in)sensitivity and the exact role of INSL3. Differences in mechanisms in subjects with KS can contribute to reduced bone mass and osteoporosis, which have a precocious onset and are detected in up to 40% of subjects, irrespective of testosterone levels. Androgen sensitivity, X chromosome inactivation, and INSL3 levels seem to co-operate and modulate the effect of testosterone on the bone in these subjects. In progress studies on these topics are therefore opening new exciting areas of research on the pathophysiology and treatment of bone loss in KS.

## Summary

Different mechanisms in subjects with KS can contribute to reduced bone mass and osteoporosis, which have a precocious onset and are detected in up to 40% of subjects, irrespective of testosterone levels. Androgen sensitivity, X chromosome inactivation, and INSL3 levels seem to co-operate and modulate the effect of testosterone on the bone in these subjects. In progress studies on these topics are therefore opening new exciting areas of research on the pathophysiology and treatment of bone loss in KS.

### References


