The UGT2B17 gene polymorphism and bone mineral density in puberty.

Isma Sohail, kull H-03, University of Oslo.

Supervisor professor/doctor Jens Petter Berg, Clinical Chemical Department, Ullevaal University Hospital.

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Summary

BACKGROUND: The uridine diphosphate-glucuronosyltransferase B17 is important for the excretion and thereby inactivation of testosterone. Testosterone is important for the bone growth in puberty. Low values of this hormone can cause male hypogonadism. Here we determine if there is any relationship between the UGT2B17 polymorphism and bone growth in puberty. Our agenda was to find if the distribution of the genotypes (insertion/insertion, insertion/deletion and deletion/deletion) were different for the groups with the highest increase in bone mass. Also we wanted to find out if there was any relationship between the UGT2B17 polymorphism and male hypogonadism. At last we studied if the polymorphism is associated with postmenopausal bone mass and loss. METHODS: The DNA samples used in this study were selected from three different groups 1) a cohort of children and adolescents recruited for studies of bone growth by Prof. Jens P Berg, Hormone Laboratory, Aker university hospital and UiO, 2) a substudy of male hypogonadism in the fourth survey of the Tromsø study by Prof. Johan Svartberg, UNN and UiTø, and 3) a study of postmenopausal bone loss (BUS-study) by Prof. Jan A. Falch, Endocrine Clinic, Aker university hospital and UiO. Genotyping was performed using primers for the insertion and deletion gene. The products were amplified using polymerase chain reaction (PCR) and separated on gel. RESULTS: There was a correlation between the gain in bone mineral density ultradistal forearm (BMDud) for boys in the puberty and the UGT2B17 polymorphism (p=0,009). The gain in BMDud was higher in boys homozygous for the UGT2B17 deletion polymorphism (0.100±0.037) compared with homozygotes for the insertion polymorphism (0.060±0.040) and heterozygotes (0.071±0.041). A statistically significant relationship was found between

the UGT2B17 polymorphism and BMD of trochanter major (p=0.017) and the BMD of total hip (p=0.016) for the whole group. The BMD of trochanter major showed higher values for the UGT2B17 deletion/deletion genotype (0.845±0.147) compared with the insertion/insertion genotype (0.786±0.115) and heterozygotes (0.767±0.119).Additionally, BMD of the total hip also showed higher values for the UGT2B17 deletion/deletion genotype (1.05±0.155) compared with the insertion/insertion genotype (0.992±0.131) and heterozygotes (0.962±0.138). The association between BMD of trochanter major and the UGT2B17 polymorphism was also found for the boys analysed separately (p=0.02). There was also a statistically significant higher value for the UGT2B17 deletion/deletion genotype (0.904±0.157) compared with the insertion/insertion genotype (0.791±0.129) and heterozygotes (0.788±0.129). Substudy 2: There was no association between male hypogonadism and the UGT2B17 polymorphism. Substudy 3: There was no association between postmenopausal bone mass and loss and the UGT2B17 gene polymorphism. DISCUSSION: The UGT2B17 gene polymorphism is playing a role in determining the bone mineral density in the puberty. The deletion/deletion polymorphism is associated with a higher serum IGF-1 value again stimulates to a higher bone mineral density in the puberty".

Introduction

Hereditity has a major contribution in the determination of bone mass. Studies have shown the importance of genetic factors for bone mass. In addition, several studies have indicated a correlation between bone mass and sex hormones [1]. Sex hormones are again dependent of genetic factors. Also genes are important contributors for determining the total amount of sex hormones. This factor again decide the age of puberty and menopause. Genes being central in the production and activation of sex hormones have previously been studied. In this study we

analysed a gene which is necessary for the inactivation of testosterone. The gene encodes for one of the uridine diphosphate-glucuronosyltransferases (UGTs), called UGT2B17.

The UGTs

Till today we are aware of 16 UGTs. The UGTs are integral membrane proteins of the endoplasmatic reticulum. Their function consists of attaching glucuronic acid to a vast number of lipophilic endobiotics and xenobiotics and thereby making them water soluble. This reaction facilitates the excretion of bilirubin, medicines and many other toxic metabolites. Glucuronidation occurs mainly in liver but also in various extrahepatic tissues, possibly affecting pharmacokinetics of drugs [2]. The UGT genes can further be divided into two primary families, which are called UGT1 and UGT2 gene families, respectively. There is also two other UGT families, UGT3 and UGT8. However, the catalytic activity of these enzymes still remain to be characterized[3]. There are seven members of the UGT2B subfamily. They have preference for glucuronidation of bile acids, steroids, fatty acids, carboxylic acids, phenols and carcinogens. The speciality of UGT2B17 is androgen glucuronidation[4]. The enzyme shows the highest activity for androsterone, testosterone and dihydrotestosterone and is mainly expressed in the prostate. In addition UGT2B15 can also glucuronidate these hormones. The genes for UGT2B15 and UGT2B17 are 95 % identical, but studies have showed that UGT2B17 has a higher biologic activity for testosterone[5]. There is described a gene deletion affecting this enzyme [6], which is related with inactivity of the enzyme. The occurrence of the gene shows ethnical variation. 70 % of Asians and 10 % of West Europeans are homozygous for the deletion.

Probably this polymorphism has an effect on the glucuronidation activity. A Swedish study [7] compared the excretion of testosterone in urine between Swedish and Korean men. The excretion was much lower for the Korean men. Furthermore, it was found that all the Koreans with lower excretion were homozygous for the deletion polymorphism. The deletion may result in a higher hormonal effect on account of the reduced inactivation of testosterone. Keeping in mind that UGT2B17 may influence the serum-testosterone level, which again plays a role for bone mass, we decided to test if there is a relationship between the UGT2B17 insertion/deletion polymorphism and bone mass. Because the UGT2B17 del/del genotype subjects had negligible levels of testosterone-glucuronide in their urine in the Swedish-Korean study, this enzyme seems to be the most important for testosterone glucuronidation and testosterone excretion *in vivo*.

Testosterone

The androgen secretion is regulated by the hypothalamic-pituitary-testicular axis. The gonadotropin-releasing hormone, GnRH, is secreted from the hypothalamus in a pulsatile fashion. The pulsatile secretion of GnRH is followed by a pulsatile secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the gonadotrophs of the anterior pituitary. The pulsatile secretion of GnRH is essential for the LH and FSH secretion. On the other hand a continuous release of GnRH has an inhibitory effect on gonadotropin release.

FSH plays a role in converting testosterone to estradiol by interacting with the receptors on the Sertoli cell membranes in the testis.

In men, LH secretion is regulated primarily by negative feedback, as normal concentrations of gonadal steroids inhibit LH secretion. Testosterone acts on the hypothalamus to slow the hypothalamic pulse generator and decrease LH pulse frequency, probably by a mechanism

involving endogenous opioids. Testosterone also appears to inhibit LH release directly at the level of the pituitary. Some of these effects may be mediated by the local conversion of testosterone to estradiol. In adult men, estrogen inhibits LH secretion both by decreasing GnRH pulse frequency at the level of the hypothalamus and by reducing the amplitude of LH pulses via decreased pituitary responsiveness to GnRH .

The synthesis and metabolism of testosterone to the active metabolites, 5-alpha-dihydrotestosterone and 17-beta-estradiol happens in the Leydig cells in the testes. Cholesterol, the precursor steroid, can either be synthesized in the Leydig cell or derived from the plasma pool via uptake of low density lipoproteins. Five enzymatic processes are involved in the conversion of cholesterol to testosterone. The first reaction is the cleavage of the side chain of cholesterol which takes place in the mitochondria of the Leydig cells, while the other four reactions happens in the endoplasmatic reticulum. The first step is converting cholesterol to pregnenolone. This is a rate-limiting process, but the rate is rather determined by the rate of delivery of cholesterol to the enzyme in the inner mitochondrial membrane by the steroidogenic acute regulatory protein (StAR). This process is the main site of action of LH.

Testosterone is transported in the plasma largely bound to albumin and sex hormone-binding globulin (SHBG, also called testosterone-binding globulin or TeBG). In normal men, approximately 2 percent of plasma testosterone is free or unbound, 44 percent is bound to SHBG, and 54 percent is bound to albumin and other proteins . Although albumin has about 1000-fold lower affinity for testosterone binding than SHBG, it binds half or more of plasma testosterone because of its high concentration. Since nearly all of the albumin-bound testosterone is available for tissue uptake, bioavailable testosterone in plasma approximates the sum of free plus albumin-bound hormone.

The physiological actions of testosterone are the result of the combined effects of testosterone itself plus its active androgenic and estrogen metabolites. The major functions of androgens in males include regulation of gonadotropin secretion by the hypothalamic-pituitary system, initiation and maintenance of spermatogenesis and formation of the male phenotype during embryogenesis. In addition it is necessary to mediate many of the differentiation, growth-promoting, and functional aspects of male sexual differentiation and virilization.

The male hypogonadism

The male hypogonadism refers to either a decrease in the male testosterone production or the sperm production. We can diagnose the deficiency by measuring the sperm quantity in semen or the testosterone level in serum. Depending on whether the defect is related to the testes or the hypothalamus-pituary system the hypogonadism is called primary or secondary. We can differentiate between these two abnormalities by measuring the level of LH and FSH. If the hypogonadism is primary the levels of these two hormones are increased as a response on the low androgen levels. In secondary hypogonadism these are reduced due to the defective production compared to the secondary hypogonadism when they are reduced. The primary hypogonadism is caused by diseases which affects the testes directly, such as viral orchitis, tumors, traumas, radiation, renal failure, Klinefelter syndrome and Prader-Willi syndrome. Prolactinomas, surgery of the pituary gland and hemochromatosis can cause secondary hypogonadism.

The clinical features depend upon whether the defect includes the testosterone production, the sperm production or both. If the spermatogenesis is impaired this will result in just two defects, infertility and reduced testicular size. On the other hand impaired testosterone production will result in several clinical features depending on its time of onset during the reproductive development. If the onset is after puberty the symptoms are decreased libido,

erectile dysfunction, reduced muscle mass and bone density, depression and anemia. Normal puberty is not possible if the testosterone deficiency is congenital.

Bone synthesis

Bone synthesis is a process starting early in the fetal life. The skeletal structure is formed by first making a pattern of cartilage and thereby replacing and modeling it with bone. This process is regulated by a large number of genes. Not only in the fetal life, but also during the adolescence and the adult life, modeling, is critical for formation of normal skeletal structures. After all this is the essential process for the growth of the skeleton. Another big part of the cellular activity related to bones is the remodeling, a process consisting of replacing and removing skeletal structures already present. This process is present already from fetal life, but it becomes the dominant form of bone cell activity after puberty.

The remodeling is a process constituting of three parts: resorption, reversal and formation. First of all the resorption takes place. This is the process where the resorptive cells, the osteoclasts, remove mineral and matrix to a limited depth on the trabecular surface or within cortical bone. High concentrations of calcium has an inhibitory role in this process. Thereby the reversal follows. In this process mononuclear cells prepare the surface for the formation cells, the osteoblasts. At last the formation happens. Here the osteoblasts fill in with bone tissue until all the resorbed bone is completely replaced.

Bone consists of two major parts, the cortical and the trabecular bone. The cortical bone is dense and compact and constitutes 80 % of the bone mass. Its major function is to give strength and protection. On the other hand the trabecular bone is found inside the long bones. This part of the skeleton is also an important contributor to mechanical support. During puberty and early adult life the trabecular thickening provides maximum skeletal mass and

strength, which is called peak bone mass. These processes are under the control of local and systemic factors.

The major systemic regulators are the calcium-regulating hormones, parathyroid hormone (PTH) and calcitriol. In addition other hormones play a role in regulation, like glucocorticoids, thyroid hormones and sex hormones. The changes in the concentration of the systemic hormones plays a role in osteoporosis. This is the process which leads to bone loss, either because the resorption is increased or the formation is decreased. Estrogen deficiency has a central role in the pathogenesis of osteoporosis in postmenopausal women. Usually estrogen inhibits bone resorption, and a lack of this effect after the menopause results in increased bone resorption and rapid bone loss. The rate of loss slows down with time after menopause.

Testosterone effects at the tissue level is to reduce bone resorption. In addition it has a modest effect on osteoblast proliferation. Testosterone increases the periosteal apposition resulting in a larger male compared to female skeleton during puberty [8]. Investigation has shown an association between bone mass and testosterone decline with age in men [9].

The loss of bone mass is not only related to the loss of endogenous testosterone with age, but also endogenous estrogens. Estrogens and testosterone use different pathways to inhibit osteclastic activity and bone resorption. Moreover data on bone health from the Framingham study were analyzed in 2006. In this study measurements of estradiol and testosterone were performed in serum in the early 80s from 793 men. The group was followed until 1999 and the incidence of hip fracture were calculated for those with low estradiol and testosterone levels. Interestingly, there was no significant increased risk for hip fracture among men with low testosterone. On the other hand there was a higher risk of hip fractures among the group

with low estradiol. The greatest risk for fractures was among the group with both low estradiol and testosterone levels [10].

Osteoporosis

Osteoporosis leads to increased risk of fractures and is defined as a skeletal disease with reduced bone mass. We call it established osteoporosis when there is sign of fractures in addition to osteoporosis. The fractures usually occur at the hip, vertebra and the forearm. The World Health Organization (WHO) has defined osteoporosis in women as a bone mineral density score 2.5 standard deviations or lower than the average for healthy, young women. The etiology is divided in primary and secondary causes. The primary causes are again divided in two major factors. Type I is postmenopausal osteoporosis associated with the postmenopausal estrogen deficiency. Type II is called senile osteoporosis, which is the age-related loss of bone and appears in both sexes. Secondary osteoporosis is related to systemic diseases, like diseases in the gastrointestinal tract, endocrine diseases, kidney problems, bone marrow diseases, psychiatric diseases and so on. Risk factors for osteoporosis are age and being a woman. After the menopause women loose 3-5 % of their bone mass per year during 5-10 years. Osteoporosis is also associated with polymorphisms in the LRP5-gene. Slim and tall persons, women entering an early menopause, inadequately nourished persons, smokers and alcohol users have a higher risk of getting osteoporosis. High values of homocystein is also a risk factor. The osteoporotic changes are greatest in the trabecular bone. We find the trabecular bone in the ends of the hollow tubular bones and in the vertebrae. Therefore this is the sites for fractures.

Aims of the study

The aim of this study was to analyse whether UGT2B17 insertion/deletion genotype plays a role for hypogonadism, bone mass and bone loss.

1. In the first substudy we hypothesized that there is a relation between the UGT2B17 polymorphism and bone mass in boys and girls in different stages of puberty and adolescence.

2. In the second substudy we wanted to investigate the allele distribution of the UGT2B17 insertion/deletion polymorphism in a group hypogonadal men compared to a control group.

3. The aim of the third substudy was to analyse any role of the UGT2B17 insertion/deletion polymorphism for postmenopausal bone mass and bone loss.

For all substudies several bone health related variables were available for comparison with the UGT2B17 genotype.

Subjects and methods

The DNA samples used in this study were selected from three different studies; 1) a cohort of children and adolescents recruited for studies of bone growth by Prof. Jens P Berg, Hormone Laboratory, Aker university hospital and UiO, 2) a substudy of male hypogonadism in the fourth survey of the Tromsø study by Prof. Johan Svartberg, UNN and UiTø, and 3) a study of postmenopausal bone loss (BUS-study) by Prof. Jan A. Falch, Endocrine Clinic, Aker university hospital and UiO.

The first study group consisted of a cohort of 144 girls and 129 boys at the age of 8.7-17.0 years at the start of the study. This population was followed for 3.8 years. At the baseline they were examined with measurements of height and weight, and blood samples for chemical and

genetic analyses were drawn. In addition measurements of body composition was performed by dual X-ray absortiometry (DXA) using a QDR 4500 device and analysed using available QDR software at the last follow-up.

In a substudy of male hypogonadism conducted by Prof. Johan Svartberg, DNA was extracted from 178 samples (70 hypogonadal men and 108 controls) collected during the fourth survey of the Tromsø study. Gonadal status was determined by measuring serum concentrations of total testosterone, free testosterone and sex hormone binding globuline (SHBG) [11]. Several variables such as height, weight, total testosterone, free testosterone, SHBG, bone mass, fat mass, waist circumference (WC), body mass index (BMI; calculated as kg/m^2), and muscle mass were measured or calculated and recorded for these individuals. Height, weight and waist circumference were measured in standing subjects wearing light clothing without shoes, waist was measured at the umbilical line according to a written protocol. Waist circumference alone was used as an integrated measure of obesity and fat distribution, on the basis of studies suggesting that WC, as compared to waist-hip ratio (WHR) correlates better with visceral body fat measured by computed tomography or magnetic resonance imaging.

The third study group consisted of 80 women who were a part of a study of bone mass estimated before and after menopause by Prof. Jan A. Falch. In addition, anthropometric (weight and height) and biochemical data were available. Bone mass measurements were done by DXA and ultrasound.

Deoxyribonucleic acid was extracted from EDTA-blood in the Tromsø study. For the other two study groups extracted DNA was available. The gene regions of interest were amplified using the polymerase chain reaction (PCR).

An insertion and deletion polymorphism of the UGT2B17-gene was studied essentially as described by (ref) [12]. Allele specific PCR amplification of the deletion allele was expected to give an 884 bp product, whereas the insertion would give a 124 bp product based on the primers selected for the PCR. After many experiments we adjusted the concentration of the insertion primers to 0.12 µl/sample while the concentration of the deletion primers still was 0.32 µl/sample. This is to balance the amplification rate of the genotypes. The PCR product of the insertion is shorter and preferentially amplified. The total concentration of one sample was 25 µl. The different concentrations were necessary for the replication on account of the different sizes of the primers. The forward and reverse primer combination for the insertion allele was 5'-GAATTCATCATGATCAACCG-3'and 5'-ACAGGCAACATTTTGTGATC-3', respectively, whereas the deletion allele was amplified by the combination of forward primer 5'-TGCACAGAGTTAAGAAATGGAGAGATGTG-3' and reverse primer 5'-GATCATCCTATATCCTGACAGAATTCTTTT-3'. The amplification was performed in the presence of 25 mM $MgCl_2$ along with 5 mU/µl Taq polymerase and a reaction buffer. The samples were initially denatured at 95 °C for 5 min, followed by 30 cycles of denaturation (95 °C for 30s), annealing (60 °C for 1 min), elongation (72 °C for 1 min) and final elongation at 72 °C for 5 min. Five µl of the PCR product was mixed with 2 µl of loading buffer, containing violet colour, prior to agarose gel electrophoresis. The samples were separated in a 1% agarose gel in Tris-acetate-EDTA-buffer, TAE-buffer, stained with ethidium bromide, visualized under UV-light and photographed. For some of the samples a 3% agarose gel also was used to improve the visualisation of the 884 and 124 bp bands for the deletion and insertion products, respectively. Subjects homozygous for the insertion alleles were indicated by 0, insertion/deletion heterozygotes by 1, and the homozygotes for the deletion by 2.

The study data were analyzed with respect to the UGT2B17 polymorphism genotype to find a correlation. This was done using the analysis of variation, ANOVA, combined with Student-Neuman-Keuls test to identify stastically different groups. SDS scores for the continous variables were estimated and then compared with the UGT2B17 polymorphism genotype.

Results

Substudy 1

In the first substudy of children and adolescents, measurements like height and weight were available as shown in table 1. All values were compared with the UGT2B17 genotype to find a relationship. The boys and girls were compared independently and together as a group. Measurements used for height and weight were from the start of the study (1992), while body fat mass and body lean mass values were measured in 1996. Gain in height and weight was found by calculating the difference between the values from 1996 and 1992. There were no statistically significant correlations between the gene polymorphism and variables like height, weight, body fat mass and body lean mass for both the whole group and boys and girls analysed independently. The gain of height for boys was greatest in the del/del group and reached almost statistically significant difference (p=0.071).

Table 1

Height, weight, body fat mass and body lean mass in substudy 1 (children and adolescents) among the three groups of UGT2B17 gene insertion/deletion polymorphism. Data are presented as mean ± SD.

	Ins/ins	Ins/Del	Del/Del	*p*
n	116	108	23	
Age baseline (yr)	12.9±2.4	12.8±2.3	13.0±2.1	0.929
Height baseline (cm)	154.4±15.2	154.2±14.0	153.7±11.7	0.978
Weight baseline (kg)	46.7±12.7	46.3±12.1	45.8±10.9	0.940

Height gain (cm)	16.0±9.3	14.8±9.3	16.0±10.3	0.606
Weight gain (kg)	15.9±8.1	15.7±7.1	16.4±9.4	0.903
Body fat mass 1996	15012.0±6604.3	14867.6±7319.2	13300.5±5699.5	0.544
Body lean mass 1996	42803.1±9591.1	42283.0±8638.0	43986.1±8155.8	0.703
Boys				
n	55	51	12	
Age baseline (yr)	12.8±2.4	12.6±2.3	12.2±1.7	0.675
Height baseline (cm)	155.5±16.5	154.3±14.0	150.1±9.3	0.519
Weight baseline (kg)	46.9±12.8	45.0±11.2	42.6±7.4	0.444
Height gain (cm)	16.6±8.5	18.7±8.0	22.3±7.7	0.071
Weight gain (kg)	18.2±7.4	18.6±5.4	22.0±7.4	0.193
Body fat mass 1996	11848.3±5716.9	10748.9±5151.9	10846.6±6122.6	0.571
Body lean mass 1996	48392.0±10359.2	47663.6±9000.8	48562.0±8127.5	0.912
Girls				
n	61	57	11	
Age baseline (yr)	12.9±2.4	12.9±2.3	13.9±2.3	0.453
Height baseline (cm)	153.3±14.1	154.0±14.1	157.6±13.1	0.643
Weight baseline (kg)	46.5±12.7	47.4±12.9	49.3±13.3	0.774
Height gain (cm)	11.8±9.4	11.4±9.0	9.2±8.4	0.68
Weight gain (kg)	13.8±8,3	13.1±7.4	10.3±7.4	0.394
Body fat mass 1996	17864.5±6061.0	18552.8±7027.6	15977.5±3888.7	0.460
Body lean mass 1996	37764.1±5028.3	37468.8±4503.2	38994.2±4597.8	0.624

Bone mineral density in the distal (BMDd) and ultradistal (BMDud) forearm among children and adolescents.

BMDd and BMDud values were measured three times (1992, 1993 and 1996). There was a correlation between the gain in BMDud for boys and the UGT2B17 polymorphism (p=0,009) (Table 2). The gain in BMDud was higher in boys homozygous for the UGT2B17 deletion polymorphism (0.100±0.037) compared with homozygotes for the insertion polymorphism (0.060±0.040) and heterozygotes (0.071±0.041). The same correlation was not found among girls (p=0.714).There were no other associations between forearm bone values and the UGT2B17 gene polymorphism neither for the whole group nor for boys and girls analysed independently.

Table 2.

Forearm bone mineral density among UGT2B17 insertion/deletion genotypes. Results are presented as mean±SD.

	Ins/Ins	Ins/Del	Del/Del	p
				p
n	116	108	23	
BMDd 1992 (g/cm2)	0.368±0.070	0.354±0.060	0.369±0.068	0.261
BMDd 1993 (g/cm2)	0.387±0.078	0.377±0.068	0.386±0.075	0.608
BMDd 1996 (g/cm2)	0.445±0.080	0.437±0.078	0.456±.0085	0.503
BMDud 1992 (g/cm2)	0.350±0.069	0.339±0.054	0.347±0.058	0.429
BMDud 1993 (g/cm2)	0.365±0.068	0.355±0.058	0.368±0.063	0.425
BMDud 1996 (g/cm2)	0.400±0.069	0.392±0.063	0.416±0.081	0.250
BMDd gain	0.078±0.050	0.083±0.058	0.087±0.064	0.676
BMDud gain	0.050±0.043	0.053±0.041	0.070±0.048	0.139
Boys				
n	55	51	12	
BMDd 1992 (g/cm2)	0.369±0.058	0.356±0.053	0.364±0.069	0.456
BMDd 1993 (g/cm2)	0.389±0.071	0.377±0.061	0.379±0.082	0.676
BMDd 1996 (g/cm2)	0.452±0.091	0.449±0.079	0.459±0.107	0.937
BMDud 1992 (g/cm2)	0.365±0.068	0.349±0.055	0.353±0.072	0.406
BMDud 1993 (g/cm2)	0.378±0.068	0.368±0.061	0.378±0.081	0.706
BMDud 1996 (g/cm2)	0.425±0.069	0.420±0.066	0.453±0.093	0.342
BMDd gain	0.083±0.050	0.093±0.066	0.095±0.067	0.606
BMDud gain	0.060±0.040	0.071±0.041	0.100±0.037	0.009
Girls				
n	61	57	11	
BMDd 1992 (g/cm2)	0.367±0.080	0.353±0.066	0.375±0.069	0.501
BMDd 1993 (g/cm2)	0.385±0.083	0.377±0.075	0.393±0.071	0.769
BMDd 1996 (g/cm2)	0.440±0.069	0.426±0.075	0.454±0.060	0.399
BMDud 1992 (g/cm2)	0.336±0.068	0.330±0.051	0.339±0.040	0.828
BMDud 1993 (g/cm2)	0.353±0.066	0.344±0.054	0.358±0.034	0.602
BMDud 1996 (g/cm2)	0.377±0.061	0.366±0.047	0.376±0.039	0.506
BMDd gain	0.073±0.051	0.073±0.049	0.078±0.062	0.946
BMDud gain	0.042±0.044	0.036±0.033	0.037±0.035	0.714

Furthermore all BMD values were compared with the UGT2B17 polymorphism (Table 3). A statistically significant relationship was found between the UGT2B17 polymorphism and BMD of trochanter major (p=0.017) and the BMD of total hip (p=0.016) for the pooled group. The BMD of trochanter major showed higher values for the UGT2B17 deletion/deletion genotype (0.845±0.147) compared with the insertion/insertion genotype (0.786±0.115) and heterozygotes (0.767±0.119).Additionally, BMD of the total hip also showed higher values for the UGT2B17 deletion/deletion genotype (1.05±0.155) compared with the insertion/insertion genotype (0.992±0.131) and heterozygotes (0.962±0.138). The association between BMD of trochanter major and the UGT2B17 polymorphism was also found for the boys analysed separately (p=0.02). There was also a statistically significant higher value for the UGT2B17 deletion/deletion genotype (0.904±0.157) compared with the insertion/insertion genotype (0.791±0.129) and heterozygotes (0.788±0.129). For girls such an association was not found, but after calculating the SDS score for BMD of the trochanter and the total hip we found a statistically significant association between the SDS score for the BMD of the total hip and the UGT2B17 polymorphism (0.042).

Table 3

Bone mineral density among the UGT2B17 insertion/deletion genotypes. Data are presented as mean ± SD.

	Ins/Ins	Ins/Del	Del/Del	*p*
n	116	108	23	
BMD spine (L1-L4) (g/cm2)	0.968±0.144	0.960±0.144	0.985±0.136	0.733
BMD femoral neck (g/cm2)	0.901±0.121	0.891±0.131	0.949±0.145	0.140
BMD trochanter (g/cm2)	0.786±0.115	0.767±0.119	0.845±0.147	0.017
BMD total hip (g/cm2)	0.992±0.131	0.962±0.138	1.050±0.155	0.016
BMD whole body (g/cm2)	1.086±0.117	1.071±0.119	1.111±0.118	0.518
SDS BMD trochanter	0.183±0.968	0.040±1.019	0.675±1.038	0.022
SDS BMD total hip	0.139±1.014	.-	0.490±1.009	0.042

		0.136±1.009		
Boys				
n	55	51	12	
BMD spine (L1-L4) (g/cm2)	0.935±0.155	0.935±0.149	0.949±0.137	0.954
BMD femoral neck (g/cm2)	0.908±0.133	0.919±0.149	0.997±0.172	0.154
BMD trochanter (g/cm2)	0.791±0.129	0.788±0.129	0.904±0.157	0.020
BMD total hip (g/cm2)	0.998±0.146	0.987±0.155	1.092±0.188	0.104
BMD whole body (g/cm2)	1.096±0.128	1.086±0.133	0.124±0.146	0.664
SDS BMD trochanter	.-0.024±1.002	.-0.012±0.975	0.910±1.063	0.011
SDS BMD total hip	0.064±0.959	.-0.001±0.989	0.763±1.177	0.056
Girls				
n	61	57	11	
BMD spine (L1-L4) (g/cm2)	0.998±0.128	0.982±0.137	1.024±0.130	0.582
BMD femoral neck (g/cm2)	0.895±0.110	0.866±0.107	0.879±0.090	0.299
BMD trochanter (g/cm2)	0.781±0.101	0.747±0.106	0.781±0.109	0.195
BMD total hip (g/cm2)	0.987±0.118	0.940±0.117	1.001±0.096	0.056
BMD whole body (g/cm2)	1.077±0.106	1.058±0.104	1.099±0.083	0.396
SDS BMD trochanter	0.370±0.904	0.087±1.064	0.418±0.995	0.253
SDS BMD total hip	0.207±1.065	.-0.257±1.026	0.112±0.726	0.042

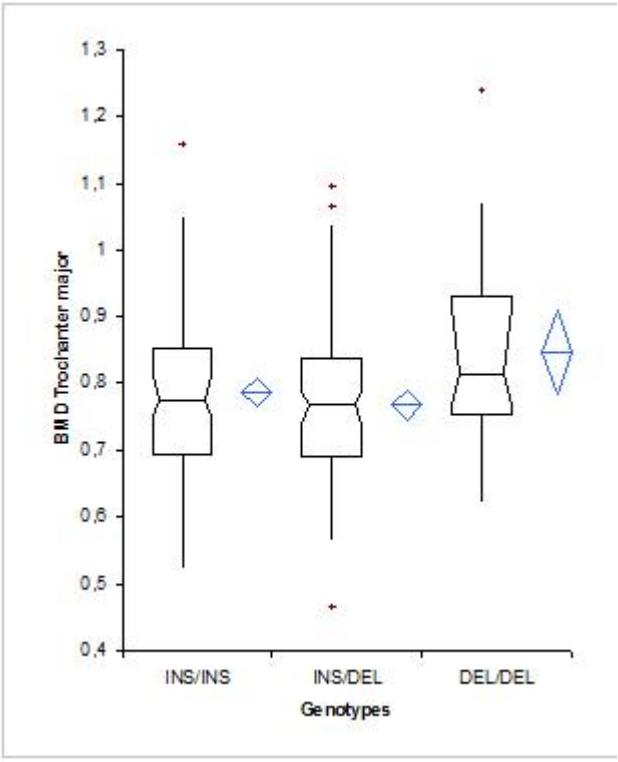

Figure 1.

Box-plot of BMD values for trochanter major in the three different genotypes for boys and girls. The values for the BMD is higher for the deletion/deletion genotype.

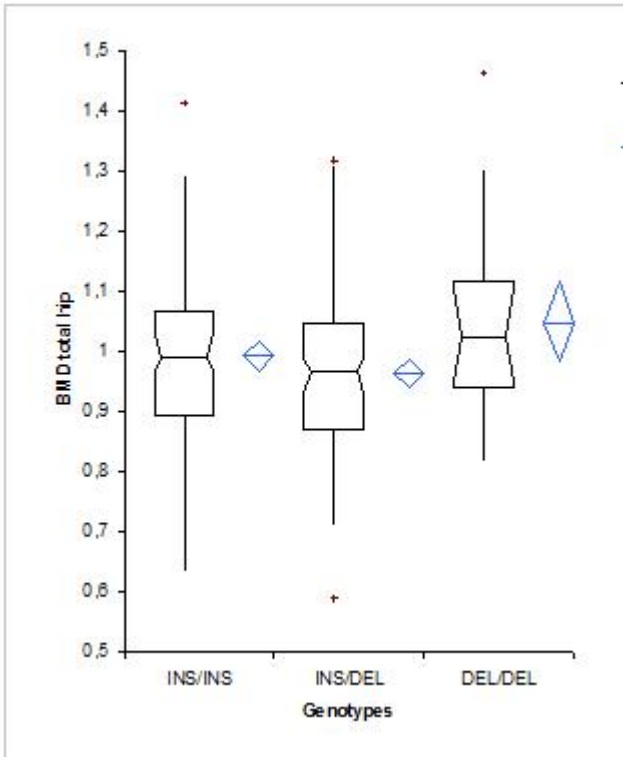

Figure 2.

Box-plot of BMD values for total hip in the three different genotypes for boys and girls. The values for the BMD is higher for the deletion/deletion genotype.

Serum-IGF-1, serum-testosterone and the UGT2B17 gene polymorphism

The relationship between serum-IGF-1 values and serum testosterone was analysed for the males in the first substudy (Fig 3). Serum-IGF-1 values were statistically significantly higher among the deletion/deletion genotype (p=0.001, One Way ANOVA and Student-Neuman Keuls test) compared to the groups with insertion/deletion and insertion/insertion genotypes.

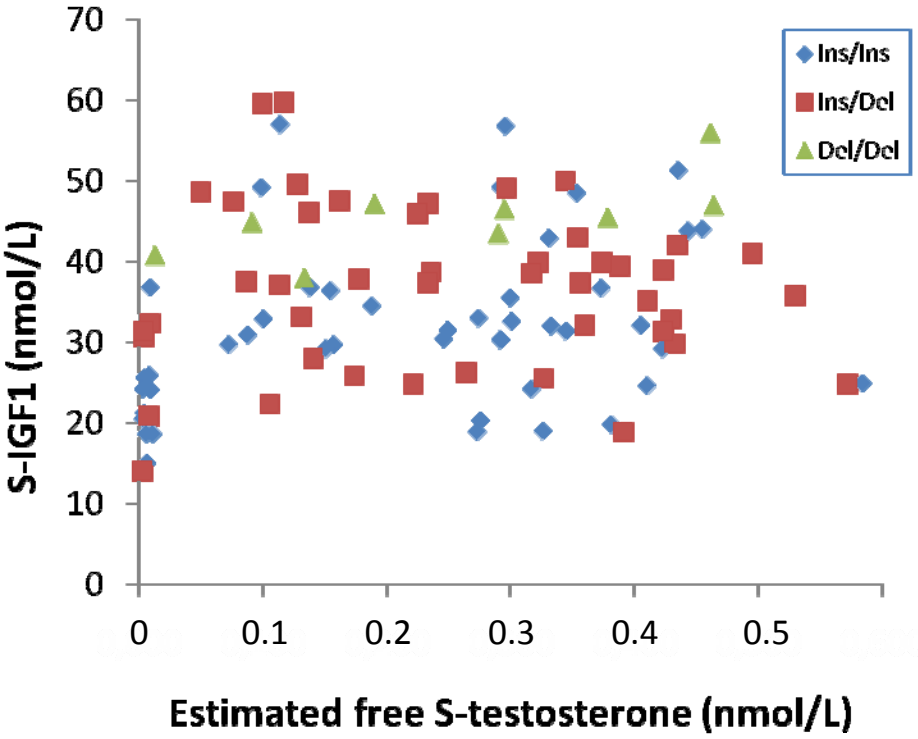

Figure 3.

Scatter-plot of estimated free serum-testosterone and serum-IGF1 levels among UGT2B17 insertion/deletion gentotypes in boys.

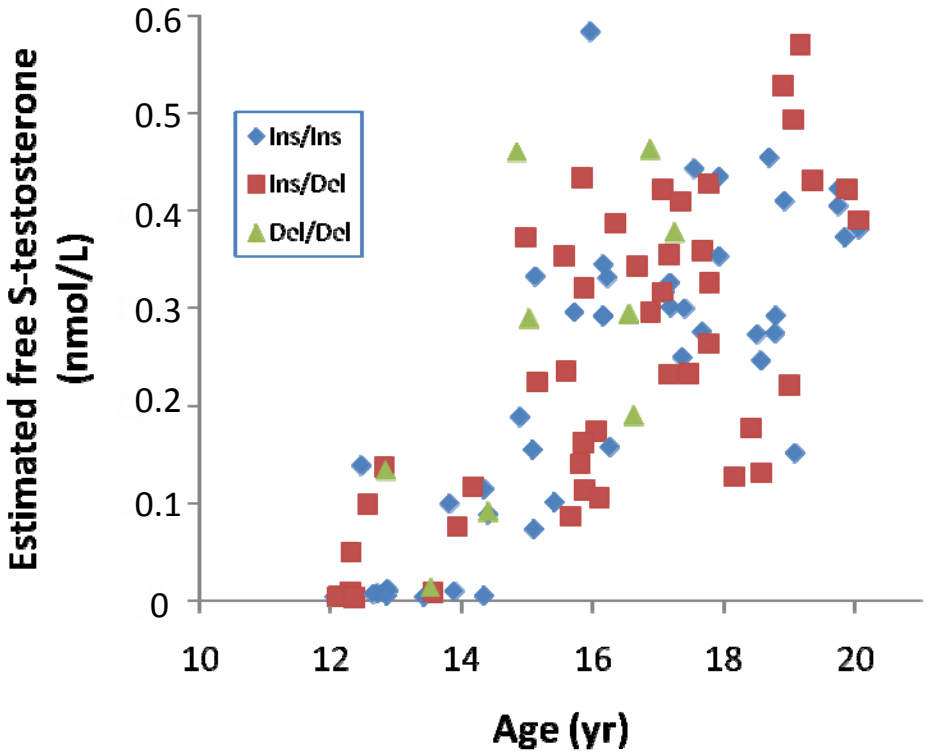

Figure 4.

Estimated free serum testosterone in boys according to age among different UGT2B17 insertion/deletion genotypes.

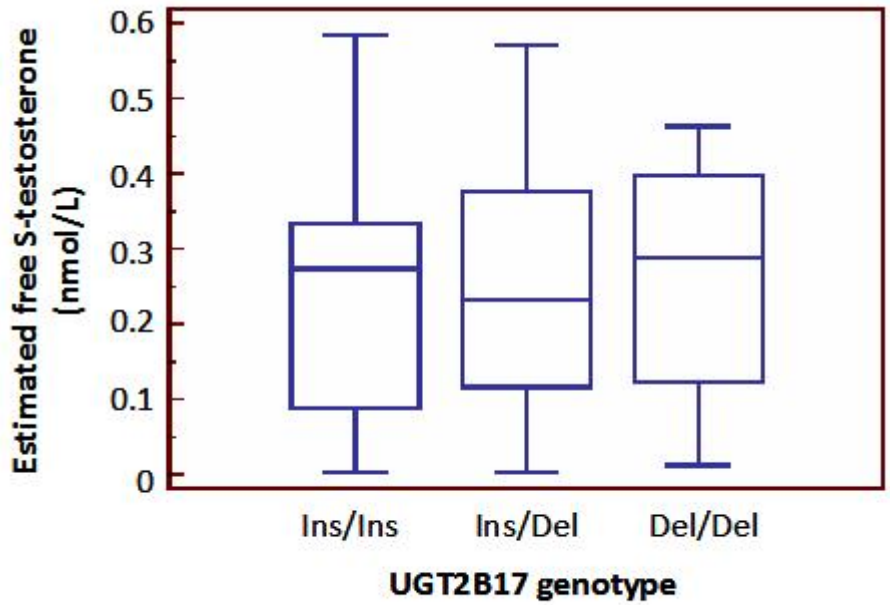

Figure 5

Box plot of estimated free serum testosterone in boys according among the different UGT2B17 insertion/deletion genotypes. There is no statistically significant difference among the groups (p=0.744).

Substudy 2

Measurements of height, weight and BMD were also available for the the UGT2B17 analysis of the study of male hypogonadism in the Tromso study. There were no statistically significant associations were between the UGT2B17 insertion/deletion polymorphism and the anthropometric variables. The genotype distribution among subjects with hypogonadism and controls was similar.

Substudy 3

In the study of postmenopausal bone mass and bone loss in the BUS-project no association was found between UGT2B17 insertion/deletion genotype and measurements of height, weight or BMD. In addition serum values from the BUS-project were available. By ANOVA we found a statistically significant lower serum calcium levels measured in 1995 for the deletion/deletion genotype compared to the insertion/insertion and insertion/deletion genotypes (Table 4). Also the decrease in serum calcium from 1993 to 1995 was greater for this group.

Table 4

Total serum calcium values in postmenopausal women measured in 1993 and 1995 and the decrease in total serum calcium among different UGT2B17 insertion/deletion genotypes. Values are in mmol/L.

The mean values

n	*32*	*23*	*12*	
	INS/INS	INS/DEL	DEL/DEL	*p*
S-calcium 93	2,3000	2,3100	2,2850	0,7320
S-calcium 95	2,3200	2,3300	2,2600	0,0450
Delta S-calcium 93-95	-0,0106	-0,0300	0,0475	0,0150

Another interesting finding was the reduction in FSH for these women. Using data from 1993 and 1995 we found a statistically significant difference (p=0,046) between the genotypes for

the reduction in FSH, which was greatest among the deletion/deletion polymorphism (Table 5).

Table 5

Serum FSH values in postmenopausal women measured in 1993 and 1995 and the change in FSH among different UGT2B17 insertion/deletion genotypes. Values are in mIU/L.

	The mean values			
n	32	23	12	*p*
	INS/INS	INS/DEL	DEL/DEL	
S-FSH 93	63,4220	55,6700	63,9250	0,3660
S-FSH 95	63,7870	58,2090	54,0170	0,4000
Delta FSH 93-95	-0,3500	-4,4000	5,2500	0,0460

Discussion

The relationship between serum-IGF-1 levels and the genotype of the UGT2B17 in boys was a quite interesting finding. Until now there is not any study indicating a role of the UGT2B17 genotype for the serum IGF-1 concentration. IGF-1 is important for skeletal growth. Thereby the genotype may play an indirect role for the growth of the skeleton. This association to the skeleton was also shown by the correlation with BMD of the forearm. The genes effect on

bone mass could be a combined result of the effect on IGF-1 and on testosterone excretion, but there was no difference in serum testosterone levels.

We did not find any association between the gene polymorphism and measurements of height, weight, body fat mass, and body lean mass. On the other hand studies have shown that growth hormone combined with an anabolic steroid gives a greater height gain than treatment with growth hormone alone [13]. This was also shown in a study of two children who entered puberty with a short stature. This indicates a correlation between sex steroid hormone levels and height. It was surprising to not find an association between the gene polymorphism and height. Maybe an association could have been found if the study group had been bigger. There is not described any relationship between UGT2B17 and height and weight earlier.
Another study has shown that UGT2B17 genotypes are predictors of fat mass in men[14]. In our study there is no relation between the childrens fat mass and UGT2B17. There is normally a decrease in testosterone levels with age. It can be speculated that the influence of the UGT2B17 genotype on serum testosterone and fat mass is greater in older men compared to boys.

The correlation between BMD for total hip and trochanter major in children compared with the Tromsø study and the BUS study is surprising. It is also necessary to have in mind that the BMD of the total hip and trochanter major are two depending factors. The BMD of the total hip contains the BMD of the trochanter. We suggest that the cause of such a relation only for this group indicate that the polymorphism in the UGT2B17 gene plays a role for the BMD in puberty, but not for the final BMD. In the other two groups we find people who have come to a steady state or decline of BMD, which may preclude an association. Probably the UGT2B17 gene polymorphism has an effect for the gain in bone mass during puberty.

There was an association between BMD of trochanter major and the UGT2B17 polymorphism for the boys, while the values for girls were not statistically significantly different. The UGT2B17 gene may play a bigger role for boys. Several studies have indicated that testosterone increases bone mass. Therefore it is not so surprising that UGT2B17 also plays a role in determining bone mass[15]. Still there is a lack of studies indicating the relationship between this gene and bone mass. It can not be excluded that repeated testing of associations may give false statistically significant p-values and that the observed association is spurious. Another drawback of this study was the lack of power. The group of children was small and with a greater amount of children we had had the chance to find more associations.

For all the significant values like BMDud, BMD of trochanter major and BMD of total hip we found higher values for the deletion/deletion polymorphism. As mentioned in the introduction the lack of testosterone inactivation in this genotype may result in higher local effects of testosterone and thereby higher bone mineral density.

There is also a point to be noted that there was no association between the UGT2B17 polymorphism and male hypogonadism. None of the polymorphisms were overrepresented in this group compared to controls. Male hypogonadism is a condition defined by a low serum testosterone value. Our result indicate that decreased production of testosterone is more important for serum testosterone levels than genetic variation in the UGT2B17 gene.

Neither was there any association between BMD in postmenopausal women and the gene polymorphism. This indicates also that there the genotype has no relationship to postmenopausal bone loss. On the other hand we found a decrease in serum calcium and

serum FSH for this group. The decrease in calcium was greater for the deletion/deletion genotype. These differences among the genotypes seem not to be of clinical importance.

In the beginning it was difficult to amplify both the gene for insertion and deletion with a concentration of 0.32 µl primer for each sample. Allele specific PCR amplification of the deletion allele was expected to give an 884 bp product, whereas the insertion would give a 124 bp product. The PCR product indicating deletion consisted of 884 bp while the insertion product only consisted of 124 bp. In the heterozygotes the gene for insertion competed out the gene for deletion in the initial experiments and the products seemed like homozygotes for insertion. After many experiments we adjusted the concentration of the insertion primer to 0.12 µl/sample while the concentration of the deletion primer still was 0.32 µl/sample. 76 of the Tromsø samples were still difficult to amplify. For these samples parallel PCR were run, one with separate primer sets for the insertion alone and one for the deletion alone. Under these circumstances both the genes had optimal conditions to be replicated.

To summarize this study we have found some associations between the UGT2B17 deletion/deletion genotype and measurements of bone mass, and serum levels of IGF1, total calcium and FSH. Specifically, there was a correlation between the genotypes and BMD in the trochanter major, total hip, and the gain in BMDud among boys. The deletion/deletion genotype was associated with increased serum IGF1 levels independent of estimated free testosterone, and seemed to give a higher bone mineral density and to be important for growth in the puberty. Details about this relationship remains to be characterized. Our study shows that there is no relationship between male hypogonadism and the UGT2B17 genotypes. Furthermore, the genotype does not seem to play a role for postmenopausal bone loss.

Acknowledgement

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