The Role of Candidate Gene Polymorphisms in Postmenopausal- and Thyroid Hormone-Stimulated Bone Loss

ABSTRACT

Osteoporosis (OP) is one of the most common chronic disorders; the disabilities that result from osteoporotic fractures (OPF) worldwide have an enormous impact on the health of individuals, societies and economies. Genetic factors together with environmental factors play an important role in regulating the development of osteoporosis as well as contributing to the susceptibility of osteoporotic fractures. Our aims were to analyze some genetic factors that affected osteoporosis permitting early detection of individuals who are at risk for the disease, and allowing for early initiation of preventive therapy. We have studied the role of certain gene polymorphisms of proteins having biological effects on bone metabolism in postmenopausal and thyroid hormone stimulated bone loss. We have also looked at the possible functional contributions of these genes to the pathogenesis of hyperthyroidism in toxic adenoma (TA), as one of the major group responsible for secondary osteoporosis. Our results demonstrate that COL1A1 gene G1245T (Sp1) and CaSR gene A986S polymorphisms might cause a predisposition to postmenopausal osteoporosis and might also be a prognostic marker of the disease. We could not prove the direct clinical significance of these gene variants on bone fracture. Based on our observation we conclude that IL-1RN VNTR polymorphism may not play an essential role in the determination of BMD in postmenopausal osteoporosis; however, our results support a hypothesis that it may influence the bone fracture risk, independent of BMD. This is the first study reporting the possible functional contribution of ER alpha gene XbaI and IGF-I gene CA repeat polymorphism in the pathogenesis of toxic adenoma. Based on our data VDR gene BsmI- and IL-1RN gene VNTR polymorphisms do not appear to have an impact on the development of TA. Our results also raise the possibility of the contribution of this microsatellite repeat variant of IGF-I gene in bone loss as the consequence of TA. We have not confirmed the role of ER alpha XbaI-, VDR BsmI- and IL-1RN gene VNTR polymorphisms in predicting low BMD caused by toxic adenoma.
INTRODUCTION

Osteoporosis is the most common bone disease. It is „characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and an increase in fracture risk” (Consensus Development Conference 1993; Kanis et al. 1994). It is usually generalized, affecting the elderly, both sexes, and all racial groups. It is a major risk factor for fracture, which leads to considerable morbidity, mortality, and expense (Wolinsky et al. 1997). The most common form of osteoporosis is the primary, involutional (postmenopausal and senile) osteoporosis, however, secondary osteoporosis caused by other conditions, such as hormonal imbalances, diseases, or medications is becoming more frequent.

In spite of the fact that the medical attendance of osteoporotic patients is outstanding in our country, bone mineral density (BMD) values in Hungary have been found to be very low compared to those in other European countries. Based on the results of European Vertebral Osteoporosis Study (EVOS) at least 7-10% of our total population, currently 700 thousand -1 million people are affected, over 25-30 % of women and 20-25 % of men according to a stratified random sample of people aged 50 years and over (Poor et al. 1997, 1998). There are approximately 15-16000 hip -, 30-35000 spine -, and 10-12000 wrist fractures related to osteoporosis per annum in Hungary (Somogyi et al. 2000, 2003). Following a hip fracture there is 16% mortality over the subsequent 6 months, 40% of sufferers will be unable to take care of themselves, and only 50% of them will recover. Hip fractures are associated with increased mortality, although conditions other than the fracture itself may account for most of the deaths (Meyer et al. 2000; Center et al. 1999). Health care expenditures attributable to osteoporotic fractures were estimated by Hungarian Society for Osteoporosis and Osteoarthrology (HSOO) at 12-14 billion forints (Somogyi et al. 2003; Kricsfalasy et al. 2000).

These findings on osteoporosis and its consequences convinced us to start a project and investigate genetic factors responsible for the disease.

The pathogenesis of osteoporosis

The pathogenesis of osteoporosis depends upon the acquisition of peak bone mass reached during adolescence and the rate of bone loss during later in life (Hansen et al. 1991; Seeman et al. 1994). The bone remodeling cycle is a tightly coupled process for life. Changes in bone mass are due to a mismatch between bone resorption and bone formation (Riggs 1986, 1992). There are primary - type I and type II-, and secondary kinds of osteoporosis.
It is now widely believed that estrogen (E) deficiency is the major cause of both the early, accelerated and the late, slow phases of bone loss in postmenopausal women. In both types of osteoporosis bone resorption is higher than formation, indicating impaired compensation and osteoporosis (Riggs et al. 2002).

As many as 30% of postmenopausal women with osteoporosis have been found to have other conditions that may have contributed to their bone loss (Consensus Development Conference 1993). Hyperthyroidism is one of the most important conditions increase the susceptibility to low BMD (X. Bajnok E -Lakatos P). Overt hyperthyroidism is associated with accelerated bone remodeling (Eriksen 1986), reduced bone density, osteoporosis, and an increase in fracture rate, especially in postmenopausal women (De Menis et al. 1992; Vestergaard & Moselinde 2002; Ross 2000).

It is still unclear why falling estrogen levels, which is a universal event during the menopausal years, causes such rapid bone loss in a relatively small, 20 percentage of women. Women with type I osteoporosis have higher bone turnover and a larger remodeling imbalance, but do not have consistently lower levels of serum sex steroids as compared with nonosteoporotic control women. (Davidson et al.1983). Hyperthyroidism does not cause osteoporosis in every patient either. In the disease about 54-87 % of patients have reduced bone density (Foldes et al. 1993); the significant bone loss is estimated from 10 to 20% (Linde & Friis 1979). These differences may be modulated by hereditary conditions of thyroid and/or bone metabolism. The importance of genetic factors in primary osteoporosis is established, (Ralston 2002), its direct effect in secondary osteoporosis, such as in hyperthyroidism-related osteoporosis, is yet unclear. Results of recent studies support the association between genetic polymorphisms and secondary osteoporosis, caused by primary biliary cirrhosis (Lakatos et al. 2001); (VII. Szalay F-Bajnok E); (VI. Lakatos PL-Bajnok E); (Xl. Lakatos PL-Bajnok E).

The role of gene polymorphisms in osteoporosis

Osteoporosis is a multifactorial disorder where a number of genes each having a small additive effect, together with environmental factors, increase the individual’s susceptibility to disease (Guéguen et al. 1995). Genetic factors account for 60-80 % of the variance in BMD, the best predictor of the risk of osteoporosis (Pocock et al. 1987; Slemenda et al. 1991). The heritability of bone loss has not yet been exactly established; however, the rate of bone turnover as measured by biochemical markers has been reported to be heritable (Harris et al. 1998; Kelly et al. 1991). The heritability of fracture itself has been estimated to lie between 25 - 35% on the basis of twin and family studies (Deng et al. 2000; MacGregor et al. 2000). The increased fracture risk associated with a positive family history of fracture persists after
adjustment for BMD (Cummings et al. 1995). This indicates that a genetic susceptibility to fracture is mediated by additional factors other than only those predisposing to low bone mineral density. Genetic risk factors (certain alleles or gene variants-polymorphisms) will be transmitted from one generation to the next but the phenotypic expression of these factors will be dependent of interaction with other gene variants and with environmental factors. An allele is one of several alternative forms of a DNA sequence at a specific chromosomal location. Polymorphism is the existence of two or more alleles at a frequency of at least 1% in the population.

All of the analytical approaches to find “osteoporosis genes” are based on the observation that the genomic DNA sequence between two individuals differ in certain positions. Candidate gene studies involve identifying polymorphisms of a particular gene and relating allelic variants to BMD or osteoporotic fractures. Candidate genes are typically chosen on the basis that they have biological effects on bone metabolism or bone cell activity, genes of cytokines and growth factors that regulate bone turnover, genes that encode components of bone matrix, and genes that encode receptors for calcitropic hormones. Candidate osteoporosis gene identifies sequence variants, some of which are only polymorphic while others have functional consequences. Sequence variations can lead to alterations in the amino acid composition of the protein, changes in the 5’ promoter region leading to differences in the expression, and/or polymorphism in the 3’ region resulting in differences in mRNA degradation. Non-functional or anonymous polymorphisms (there is no known functional effect of the polymorphism to provide a direct biological explanation of the association) are also of interest, because they could be used as markers being with a truly functional polymorphism elsewhere in the gene. Candidate gene association studies are relatively easy to perform and can be powered to detect small effects. Disadvantages include the possibility of false positive (or false negative) results due to confounding factors and population stratification (Ralston 2002; Peacock et al. 2002).

The role of gene polymorphisms in the postmenopausal osteoporosis, pathogenesis of toxic adenoma and thyroid hormone stimulated bone loss

Clearly, current methods of treatment for osteoporosis are far from ideal. However, there are a number of excellent drugs available, which can reduce or prevent further bone loss, however, it is nearly impossible to restore completely. Genetic studies have major potential advantages in combating osteoporosis. Our aims were to find genetic markers for identifying individuals susceptible to osteoporosis and to gain an understanding of the disease pathophysiology. A number of unrelated, homogeneous, postmenopausal women visiting the
National Osteoporosis Center and Endocrinology Outpatient Unit of the Semmelweis University, 1st Department of Internal Medicine, Budapest representing the Hungarian population have provided source for our investigations. Taking all the factors into account – the different benefit and limitation of gene analytical approaches, the complex genetic trait of osteoporosis, and the composition of our patient population – examination of candidate genes becomes evident and the obvious choice. Using this method we could relatively easily, quickly and with less financial investigation test our hypothesis, and get a possible new predictors of osteoporosis to further improve the identification of subjects at increased risk of bone fracture.

Based on the results of former studies, our aim was to investigate the importance of vitamin D receptor (VDR) (Cooper & Umbach 1996; Uitterlinden et al. 2001), collagen type 1 (COL1A1) (Mann et al. 2001; Brown et al. 2001), calcium sensing receptor (CASR) (Cole et al. 2001; Eckstein et al. 2002), estrogen receptor (ER) alpha (Albagha et al. 2001; Ioannidis et al. 2000), interleukin-1 receptor antagonist (IL-1RN) (Keen et al. 1998; Langdahl et al. 2000), insulin-like growth factor I (IGF-I) (McCarthy et al. 1997; Rosen et al. 1998) gene polymorphism in BMD and their possible functional contribution to the susceptibility to osteoporotic fractures in Hungarian postmenopausal women.

Osteoporosis as a result of conditions that influence bone metabolism and contributes dramatically to the total number of individuals with very low BMD and/or osteoporotic fractures. One of the most important conditions is hyperthyroidism. In iodine deficient areas such Hungary, subclinical and overt hyperthyroidism is the major cause of morbidity and it is mainly due to toxic adenoma (TA) (Aghini-Lombardi et al. 1999). The molecular and genetic mechanisms that generate TA are poorly understood. Solitary hyperfunctioning thyroid adenomas are benign monoclonal tumors characterized by their capacity to grow and produce thyroxine and triiodothyronine autonomously, i.e. in the absence of thyrotropin (TSH) (Corvilain 2003). Mutations of the TSH receptor and/or in the Gs alpha gene causing permanent activation of the thyroid follicular cell adenylate-cyclase have been shown to be the most probable molecular cause of the hyperfunction and growth of TA (Derwahl 1999; Tonacchera & Pinchera 2000). However, these gene mutations alone are not sufficient to cause TAs, other factors may affect the mutated cells to promote proliferation (Derwahl 1999; Trulzsch et al. 2001).

Pleiotropic “master” genes might have a cascade of effects in several pathways and we can expect to find an association with multiple traits and disease phenotypes. The traditional role of estrogen-ER, vitamin D-VDR, IL-1/IL-1RN and IGF/IIGFBPs system in bone metabolism exert important immunomodulatory properties. The polymorphisms of this gene can promote or inhibit cell proliferation and generation of different tumors (Weiderpass et al. 2000; Breherton-Watt et al. 2001; Sehouli & Mustea 2002; Khandwala et al. 2000). We hypothesized and
investigated that the ER alpha gene XbaI, VDR gene BsmI, the IL1-RN gene VNTR- and IGF-I

gene CA repeat polymorphisms can act as secondary factors and they might mediate the
development of TA and thyroid hormone induced bone.

We investigated the role of

? The polymorphisms in the 3’ region of the VDR gene, situated between exons 8 and 9,
which are recognized by the restriction enzyme BsmI (Morrison et al. 1994).

? The G-to-T substitution polymorphism at position 1245 of the Sp1 binding site within the
first intron of COLIA1 gene (Grant et al. 1996)

? The A986S missense polymorphism at the exon 7 in cytoplasmic tail of the CaSR
gene (Cole et al. 2001)

? The genetic variants defined by restriction enzyme XbaI in the first intron of the ER gene
(Ioannidis et al. 2000)

? The number of repeats of an 86 bp polymorphism located in second intron of IL-1 RN
gene; containing three protein binding sites (Tarlow et al. 1993)

? The CA dinucleotide repeat polymorphism in the promoter region located 1 kb upstream
from the IGF-I gene transcription start site, containing specific regulatory elements (Rosen et
al. 1998)

OUR AIMS WERE

Based on the aforementioned data in the introduction we wished to study

? The impact of IL-1RN gene 86 bp VNTR polymorphism on bone mineral density in a
population of Hungarian postmenopausal women (Ia; Ib; I.c)

? The significance of the CaSR gene A986S polymorphism on BMD in a population of
Hungarian postmenopausal women (IIa; II.b)

? The importance of COL1A1 gene G1245T (Sp1), CaSR gene A986S and IL-1RN gene 86
bp VNTR polymorphisms in BMD in Hungarian postmenopausal women with serious
osteoporosis (III)

? The role of the COL1A1 gene G1245T, the CaSR gene A986S and the IL-1RN gene 86
bp VNTR polymorphisms in the susceptibility to osteoporotic fracture in Hungarian
postmenopausal women with serious osteoporosis (III)
The possible functional contribution of the ER alpha gene XbaI, VDR gene BsmI and the IL-1RN gene 86 bp VNTR polymorphism to the pathogenesis of TA

(IV.a; IV.b; IV.c)

The functional contribution of the ER alpha gene XbaI, the VDR gene BsmI and the IL-1RN gene VNTR polymorphism to the development of thyroid hormone-stimulated bone loss in TA

(IV.a; IV.b; IV.c)

The association between CA repeat polymorphism of IGF-I gene and the genesis of TA

(V.)

The possible role of the CA repeat polymorphism of IGF-I gene in osteoporosis caused by hyperthyroidism in postmenopausal women with TA

(V.)

SUBJECTS AND METHODS

Subjects: Unrelated, homogeneously of Hungarian origin, postmenopausal women in the age range of 40-70 years were selected from patients who attended the Endocrinology Outpatient Unit of the Semmelweis University, 1st Department of Internal Medicine, Budapest. Menopause was defined as the absence of menstruation for at least 12 months. Subjects with secondary causes of osteoporosis -except for TA in certain studies-, or who were taking medications likely to affect skeletal metabolism, and women with early menopause (before 40 years) were excluded. There were no differences in smoking habits, calcium intake, alcohol and caffeine consumption between the investigated C groups. The local ethics committee approved the study and all patients gave written informed consent.

Diagnosis of toxic adenoma: TA was established by a “hot” nodule on thyroid scan, suppressed TSH, elevated peripheral free thyroid hormone levels, and the lack of TRAb. The mean duration of hyperthyroidism was estimated to be 5±3 months according to the onset of subjective hyperthyroid symptoms.

PCR methods: Genomic DNA was extracted from peripheral mononuclear cells by ethanol precipitation using an established protocol (Wyman & White 1980). DNA was subjected to polymerase chain reaction for gene polymorphisms using a Hybaid TouchDown Thermocycler (Hybaid, Teddington, UK). PCR products-except IGF-I- were separated by electrophoresis and visualized by ethidium bromide staining under UV illumination (302 nm). Separation patterns were documented by Kodak 1D Electrophoresis Documentation and Analysis System (Kodak, Digital Science, Rochester, NY, USA). For IGF-I radiolabeled PCR products were screened by electrophoresis on a 6.5% polyacrylamide gel (70w; 2h 45 min) using a Kodak BioMax STS 45I (Eastman Kodak Co., Rochester, NY.) sequencing gel apparatus

BMD measurement: was measured at the lumbal spine (L2–4) and at the femoral neck by dual-energy X-ray absorptiometry (DPX-L, Lunar Corp., Madison, WI), and by SPA (NK 364,
Gamma Works, Budapest, Hungary) at the distal radius. BMD was expressed as areal density in grams per square centimeter, as well as in t scores (difference from the mean BMD value of healthy young people divided by its standard deviation) and z scores (difference from the mean BMD value of age-matched people divided by its standard deviation). OP was diagnosed if the BMD value was below a -2.5 t score (Kanis et al. 1994). Severe osteoporosis was diagnosed if the BMD value was below -4 t score at any measured site.

**Statistical analysis:** Data were expressed as mean ± SD, and p<0.05 was considered significant. Allele and genotype frequencies were compared using Chi-square or Fisher’s exact tests as appropriate. Analysis of variance (ANOVA) with appropriate post hoc tests or independent samples t-tests were used to assess a possible association between BMD and the different genotypes in the subgroups as needed. When an association was found between the genotypes and BMD, the results were also adjusted for age and body mass index using multiple regression analysis to better describe the observed association. For statistical analysis SPSS for Windows 9.0.0 was used.

**STUDIES AND RESULTS**

**The impact of IL-1RN gene 86 bp VNTR polymorphism on BMD in a population of Hungarian postmenopausal women (I.a; I.b; I.c)**

**Subjects:** Allele and genotype frequencies for the IL-1RN VNTR polymorphism were determined among 286 postmenopausal, unrelated, consecutive, Hungarian, white patients in the age range 40–65 years. From this cohort, 98 osteoporotic (OP) women (mean age 56.5 [40–65] years) were compared with 81 healthy, age-matched controls (C) (mean age 54.3 [44–64] years). BMD was determined at lumbal spine and femoral neck.

**Results:** Analysis of genotypes of 286 osteoporotic postmenopausal women showed evidence of four VNTR polymorphisms of the IL-1RN gene, in accordance with previous reports. The most common carriage rate was proved of the “A1” (72.7%) and “A2” (25.3%) alleles. Studying subjects by BMD (osteoporotic and controls), we found no difference in the distribution of IL-1RN alleles or genotypes between groups. There was correlation between alleles or genotypes and BMD neither in total population nor within subgroups.

**The significance of the CaSR gene A986S polymorphism on BMD in a population of Hungarian postmenopausal women (II.a; II.b)**

**Subjects:** Allele and genotype frequencies for the CaSR gene A986S polymorphism were determined consecutively among white postmenopausal, unrelated, Hungarian patients in the
age range of 40–70 years. 108 osteoporotic women (OP group; mean age 59.3 ± 6.5 years) were compared with 122 non-osteoporotic women (C group; mean age 57.2 ± 5.7 years). BMD was measured at lumbar spine and femoral neck.

Results: We found no difference in the distribution of alleles or genotypes of CaSR gene A986S genotypes between osteoporotic and control groups (p = 0.762). To analyze the polymorphism and its relation to BMD subjects were divided into presence (25.2%) or absence (74.8%) of “S” allele. There was no correlation between genotypes and BMD at either measured site in the 230 subjects or within the subgroups.

Our study is the first to examine the effect of the A986S polymorphism of CaSR on BMD in a homogeneous postmenopausal white population. There are several possible explanations for this negative result. It is possible, that the CaSR may not have a crucial role in the regulation of osteoblast function. The novel osteoblastic CaSR-like receptor sowed by Pi et al. (Pi et al 2000) may modify the genetic effect of the A986S polymorphism on bone metabolism. The existence of different CaSRs and/or second messengers is further corroborated by the fact that the different subtypes of familial hypocalciuric hypercalcemia caused by mutations in CaSR gene are localized to different chromosomal regions (Chou 1992, Heath 1993).

The role of COL1A1 gene G1245T (Sp1), CaSR gene A986S, and IL-1RN gene VNTR polymorphisms in serious postmenopausal osteoporosis and bone fracture in Hungarian postmenopausal women

Subjects: Allele and genotype frequencies for the COL1A1 Sp1-, CASR A986S, and IL-1RN gene VNTR polymorphisms were determined among 180 unrelated, consecutive, Caucasian women in the age range of 40-70 years. 90 severe osteoporotic postmenopausal women (sOP, mean age: 60.79 ± 8.75 years) were compared with 90 healthy, age-matched controls (C, mean age: 54.2 ± 4.83 years). Bone density was measured at the lumbar spine (L2–4), at the femoral neck and at the distal radius. Patient with osteoporosis were enrolled in the study, if the BMD value was below –4 t-score at any measured site. Prevalent fractures were estimated by obtaining a fracture history from each subject. Patient was drawn in the investigation, if the fracture was occurred after the age of 40 years, and was due to minimal trauma included at the femoral neck-, thoracolumbal vertebral region or at the wrist.

Results: According to our including criterions, BMD at the measured sites was significantly lower in patient groups with serious osteoporosis compared to the controls with normal BMD. We have found a higher rate of osteoporotic bone fracture in the sOP group compared to the control subjects (p=0.003).
**COLIA1 gene:** The distribution of the genotypes of our population was: SS: 64.4%; Ss: 32.2%; ss: 3.3%. The “ss” genotype was present more frequently in the sOP group compared to the controls (ss: 13.3%; p=0.028). BMD at the femoral neck were significantly higher in the subjects with “SS” or “Ss” genotypes compared with “ss” genotype adjusted to age and BMI. We have found no significant differences in the distributions of the Sp1 genotypes between patients with or without osteoporotic fracture in total group or within C and sOP subgroups.

Mann and his coworkers (Mann et al. 2003) have supported in their meta-analysis, that the osteoporotic fracture-predicting role of this polymorphism is largely independent of the effect on BMD. This fact is one that could explain why we were not able to confirm the role of the polymorphism in fracture susceptibility, despite of its association with BMD. Not only the multifactorial genetics but also environmental factors have an influence on bone mineral density. If the genetic effect was weak, the environmental factors may have masked the actual genetic influence of the CaSR gene in our association study.

**CaSR gene:** The distribution of the genotypes of our population was: AA: 64%; AS 34.9%; SS: 1.2%. “AS” and “SS” genotypes of CaSR gene were more frequent in patients with serious osteoporosis compared to healthy control subjects (50.6% ? 36.1%; p=0.04). We have found a correlation between genotypes and BMD values at lumbal spine - adjusted to age and BMI - in sOP group. Our positive results in this study might be interpreted as a more serious disorder of bone. Our observations suggest that in the presence of other factors being responsible for osteoporosis susceptibility the role of this polymorphism seems to be more significant. Cole et al. observed that women who were “AA” homozygous for CaSR gene had lower serum Ca concentration than “AS’ heterozygous or “SS” homozygous subjects (Cole et al. 2001). In our study BMD was found to be significantly higher in the subjects carrying the “AA” genotypes compared with “AS” or “SS” genotypes. It is possible, that mutation of the CASR gene alter the set-point for extracellular ionised calcium. To compensate the lower Ca concentration in altered AS-SS genotypes results in increased calcium release into the circulation due to the amplified bone resorption, and cause bone loss and even osteoporosis.

No clinical significance of the CaSR polymorphism on bone fracture was proved. In previous studies, others were also unable to confirm any significance of this gene variant on development of osteoporotic bone fracture (Bollerslev; 2003, Cetani; 2003).

**IL-1RN gene:** The distribution of the genotypes of our population was: A4A4+A4A3+A4A5: 58.9%; A2A4+A2A3: 34.4%; A2A2: 6.7%. There was no difference in the distribution of alleles or genotypes of IL-1RN between the C and sOP groups. No genetic polymorphism of IL-1 RN gene was significantly associated with BMD adjusted to age and
BMI at any sites in the C or sOP groups or in the total population. On the other hand, in the sOP group the „A2A2-A2A4-A2A5” genotypes were more frequent in patients with osteoporotic fracture than in the subject without fracture (61.1% ? 38.9%; p=0.05). This difference was not seen in the C group. Our result raises the possibility, that this polymorphism of IL-1RN gene or another genes are in linkage disequilibrium with might have a role in the pathogenesis of bone fracture in severe osteoporosis, independently of BMD.

_The possible functional contribution of ER alpha gene XbaI, VDR gene BsmI and IL-1RN gene VNTR polymorphism to the development of toxic adenoma and thyroid-hormone stimulated bone loss of postmenopausal women_ (IV.a; IV.b; IV.c)

**Subjects:** Allele and genotype frequencies for the ER alpha-, VDR and IL-1RN gene polymorphisms were determined among 296 unrelated, consecutive, Caucasian women in the age range of 18-79 years. The study population consisted of 107 patients with toxic adenoma before antithyroid therapy (TA group, mean age: 57.3 ± 10.6 [30-79] years), as well as 189 healthy euthyroid non-osteoporotic controls (C group, mean age: 55.2 ± 6.3 [40-70] years). BMD of the lumbar spine, femoral neck and distal radius was measured in a subgroup of the whole population: in postmenopausal control (Cpm) and postmenopausal TA (TApM) women. In the latter group, there were 71 patients with TA (TApM group, mean age: 56.08 ± 8.3 [41-70] years) and 188 control subjects (Cpm group, mean age: 55.3 ± 5.8 [44-70] years).

**Results:** The distribution of the genotypes of our population was: XX: 18.2%; Xx: 58.2%; xx: 23.63%. Analysis of genotypes of 296 subjects showed the ER alpha “xx” genotype to be less frequent in the TA group compared to the controls (4.2% ? 23.63%; p=0.0003). There was no significant difference in the distribution of the investigated VDR “BsmI” and IL-1RN VNTR genotypes between the study groups.

BMD at the measured sites was significantly lower in the TA group compared to the controls. Forty three percent of postmenopausal hyperthyroid patients were found to be osteoporotic. In the postmenopausal subgroup the ER alpha XbaI, VDR BsmI and IL-1RN VNTR genetic polymorphisms were not significantly associated with BMD at any sites in the subgroups. We have found no significant differences in genotype distributions between osteoporotic and non-osteoporotic subjects within TA group.

Several lines of evidence support the effect of estrogens on thyroid gland and on tumorgenesis (Franklyn; 1987, Banu; 2001, Egawa; 2001). ER alpha gene XbaI polymorphisms may modulate the effect of estrogens on target genes since several studies have suggested the role of ER alpha gene XbaI polymorphism in the development of different types of cancer.
(Speer; 2001, Modugno; 2001, Andersen; 1994). Results of this study indicate that genetic polymorphisms in the ER-alpha gene may play a role in the etiology of toxic adenoma. Thyroid and estrogen receptors could influence transcriptional activities of each other (Yen 2001), which might have an impact on the pathogenetic processes of hyperthyroidism, however, the exact link between ER alpha gene XbaI polymorphisms, and TA is yet unclear.

The importance of IGF-I gene CA repeat polymorphism in the development of toxic adenoma and thyroid-hormone stimulated bone loss

Subjects: Allele and genotype frequencies for the IGF-I microsatellite repeat polymorphisms were determined among 182 unrelated, consecutive, Caucasian women in the age range of 40-70 years. 68 postmenopausal patients with TA before antithyroid therapy (TA, mean age: 57.03 ± 9.15 years [40-70 years]) were compared with 114 healthy, age matched postmenopausal controls (C, mean age: 54.21 ± 5.38 year [40-68 years]). BMD of the lumbar spine, femoral neck and distal radius was measured in control and postmenopausal TA women.

Results: Analysis of genotypes of 182 postmenopausal women showed evidence of nine CA repeat polymorphisms of the IGF-I gene. We have found the allele “192” (67.03%) and “194” (18.14 %) to be the most common gene variants in our population. We have demonstrated the higher presence of the noncarrier subjects of allele “192” in the TA group compared to the controls (p =0.028).

As the expected consequence of hyperthyreoidism, BMD at the measured sites was significantly lower in TA group compared to the controls. Fifty percent of postmenopausal hyperthyroid patients were found to be osteoporotic. To analyze the possible contribution of this polymorphism to the development of TA and its relation to BMD subjects were divided into the presence or absence of the 192 allele. Studying subjects by BMD, 192/192 genotype was found to be exhibited more frequently in TA patients having normal BMD compared to TA subjects with osteoporosis and to healthy controls (p=0.0267). This polymorphism of IGF-I gene was also correlated with BMD in TA group. Z-score values at the radius, were higher in the subjects homozygous for the “192” allele compared to that be heterozygous or noncarrier of it (p=0.025).

Based on former results and on our findings it might be that the absence of the 192-bp allele characterizes subjects who are chronically exposed higher IGF-I levels and could promote the development of TA. Because there is stronger evidence that high circulating concentrations of IGF-I may predispose individuals to neoplasms (Chan et al. 2002), and it is associated with
goiter development (Miyakawa et al. 1988), it is possible that the presence of “192” allele of IGF-I gene might be protective for TA.

Because production and effects of IGF-I are regulated by complex mechanisms, it could be, that the changes in IGF-I concentrations are consequence and not a cause of disease, and this gene polymorphism might have an unknown local effect on thyroid tissue. The effects of IGF-I may be limited in hyperthyroidism due to the increases of inhibitory IGFBPs that can counteract the anabolic effects and contribute to the observed net bone loss (Lakatos et al. 2002).

Our results also raise the possibility, that this variation of IGF-I gene might promote the development of osteoporosis, principally at skeletal areas as being among the first sites affected by hyperthyroidism (Mosekilde & Melsen 1978).

In all of our studies, subjects enrolled in the investigations have significantly homogenous origin, which helps prevent getting a false positive or negative association based on the genetic difference between populations. It may provide a strong power of our results. To be aware of the pitfalls in genetic association studies, in the case of a positive association of one of the marker alleles with the phenotype of interest, one supposes than that the marker allele is in linkage disequilibrium with a truly functional polymorphism elsewhere in the gene. If the genetic effect was weak, the environmental factors may have masked the actual genetic influence. The conflicting results may also be explained with allelic heterogeneity between different populations, the small sample size or inhomogeneity of the investigated populations.
MAIN CONCLUSIONS

? Our results suggest that the IL-1RN gene alleles or genotypes are not associated with low BMD as the main factor of the fracture risk in postmenopausal women who have either moderate or serious osteoporosis. We have concluded that this polymorphism might not play an essential role in the determination of BMD in postmenopausal OP. However, our results have demonstrated that the risk of an osteoporotic fracture could be associated with this gene variants among patients with serious osteoporosis. The IL-1RN VNTR polymorphism might be one of the prevalent factors of osteoporotic fractures, independent of BMD (I.a; I.b; I.c; III).

? We have found no evidence to support a relationship between the CaSR gene A986S polymorphism and BMD in Hungarian postmenopausal women with moderate OP. On the other hand, we suppose that this polymorphism might have a predictive role in the progression of osteoporosis (II.a; II.b; III).

? We have corroborated the role of the COL1A1 gene Sp1 polymorphism in the development of OP in our population, which suggests the use of this test of this gene variant as the marker for osteoporosis susceptibility (III).

? We could not prove any direct clinical significance of COL1A1 gene Sp1 and CASR A986S polymorphisms on bone fractures (III).

? Our study is the first one investigating the association between the ER alpha gene XbaI and IGF-I gene promoter polymorphism and toxic adenoma. Our results show that the presence of the “x” allele of the ER gene and the “192” allele of the IGF-I gene might reduce the risk for development of TA (IV.a; IV.b; IV.c; V).

? In contrast to their significant role in other diseases, the VDR gene BsmI and IL-1RN gene VNTR polymorphisms do not appear to have an impact on the development of TA (IV.a; IV.c).

? ER alpha gene XbaI, VDR gene BsmI, and IL-1RN gene VNTR polymorphisms seem not to be essential in predicting BMD in hyperthyroidism caused by TA (IV.a; IV.b; IV.c).

? Our results also raise the possibility that there might be a protective effect of the “192” allele of IGF-I gene on the development of osteoporosis among TA patients. Identification of IGF-I gene variants could be useful in predicting those who are at risk for osteoporosis susceptibility (V).

? We have compared age and gender adjusted healthy control subjects to TA patients and have also confirmed that in 43-50 % of patients with hyperthyroidism have osteoporosis. However, this data has been known for a long time, this supports the extreme importance of the diagnosis and treatment of bone loss as the part of the medical attendance of hyperthyroidism (IV.a; IV.b; IV.c V).