TURKISH JOURNAL OF SCIENCE
VOLUME IV, ISSUE I, 1-14
ISSN: 2587-0971

http://dergipark.gov.tr/tjos

Osteoporoz Duyarlılık Risk Analizi

Osteoporosis Susceptibility Risk Analysis

Naim UZUN[1,*] and Ahmet KIZILTUNÇ[2]

[1]*Ağrı İbrahim Çeçen University, Faculty of Pharmacy, Department of Biochemistry, Agri, Turkey*
[2]*Atatürk University, Faculty of Medicine, Department of Biochemistry, Erzurum, Turkey*
**Sorumlu yazar / Corresponding Author*: naimuzunn@hotmail.com

Geliş Tarihi / Received Date: 26 January 2019
Kabul Tarihi / Accepted Date: 12 March 2019

Öz: Bu çalışmada osteoporoz için risk teşkil eden bazı gen lokuslarında polimorfik yapıları araştırmayı amaçladık. Seçtiğimiz genler kemik sağlığı için gerekli olan proteinleri kodlayan genlerdir. Bu proteinler Kollajen Tip 1, Estrojen Reseptörü, D Vitamini Reseptörü, Laktaz, Osteoprotegerin ve İnterlökin 6'dır. Polimorfik yapı analizi bu altı molekülü kodlayan genlerin sekiz farklı noktasında yapıldı. Analizi yapılacak tam kan örnekleri Atatürk Üniversitesi Fizik Tedavi ve Rehabilitasyon kliniğine başvuran hastalardan alındı. Kemik Mineral Yoğunluğu hasta seçiminde kullandığımız temel kriterdi. Polimorfik Yapı Analizleri Atatürk Üniversitesi Biyokimya Anabilim Dalı Moleküler Laboratuvarında yapıldı. Genomik DNA tam kan örneklerinden manuel metot ile izole edildi. DNA miktar analizi UV spektrofotometre ile yapıldı. Primerler spesifik gen sekanslarının amplikasyonu ve etiketlenmesi Multiplex PCR'da yapıldı. PCR aplikasyonu sonucu elde edilen numuneler Agaroz Jel Elektroforeze uygulandı. DNA sekans amplikasyonları spesifik allel hibridizasyon ile Array Tüpte belirlendi. Hasta genotipi sonuçları Array Tüp okuyucu bir sisteme sahip Solas 1 Reader'den alındı. 150 gönüllüden alınan tam kan numunelerden elde edilen 1200 sonucun istatistiği yapıldı. Artan polimorfik yapı sayısı ile azalan kemik yoğunlukları arasında korelasyon olduğu gözlendi. Polimorfik yapıların osteoporoz risk oranları belirlendi. Analizi yapılan genlerdeki polimorfik yapılar osteoporoz için bir risk taşımaktaydı.Osteoporozun güçlü genetik bileşene sahip olduğu ancak diğer genetik olmayan faktörlerle birlikte değerlendirilmesi gerektiği sonucuna varıldı.

Anahtar Kelimeler — Osteoporoz, osteocheck, polimorfizim, risk analizi.

Abstract: In this study, we aimed to investigate polymorphic structures in some gene loci which are risk for osteoporosis. The genes we chose are genes that encode the proteins necessary for bone health. These proteins are collagen type 1, estrogen receptor, vitamin D receptor, lactase, osteoprotegerin and interleukin 6. Polymorphic structure analysis was performed at eight different points of the genes encoding these six molecules.

The whole blood samples to be analyzed were taken from the patients who applied to the Physical Therapy and Rehabilitation Clinic of Atatürk University. Bone Mineral Density is the basic criterion we use in patient selection. Polymorphic Structure Analysis was done at Atatürk University, Department of Biochemistry, and Department of Molecular Laboratory. Genomic DNA was isolated from whole blood samples by the manual method. DNA quantity analysis was performed with UV spectrophotometer. Primers specific gene sequences were amplified and labeled in Multiplex PCR. The samples obtained after the PCR applications were subjected to Agarose Gel Electrophoresis. DNA sequence amplifications were identified in the Array Tuple by specific allele hybridization. Patient genotype results were obtained from Solas 1 Reader with an Array Tube reader system. A total of 1200 results were obtained from whole blood samples taken from 150 volunteers.

It was observed that there was a correlation between increasing number of polymorphic structures and decreasing bone densities. The risk ratios of osteoporosis of polymorphic structures were determined. The polymorphic structures in the analyzed genes pose a risk for osteoporosis. It was concluded that osteoporosis has a strong genetic component but should be evaluated together with other non-genetic factors.

Keywords — Osteoporosis, osteocheck, polymorphism, risk analysis.

INTRODUCTION

Osteoporosis is a polygenetic skeletal system disease characterized [Bonnie 2006] by prevalence [Long 2004], progressive [Murray 2004, Anderson 1993], low bone density [Uitterlinden 1997], bone microarchitecture disruption [Fordham 2006], and increased brittleness [Raltson 2005]. The major outcomes of osteoporosis are increased rates of brittleness [Raltson 2005] and rising economic costs, high morbidity and mortality. [Raisz 2005]

Risk factors for osteoporosis include age [Ueland 2007], gender [Fordham 2006], hormonal balance [Murray 2004], nutrition [Guyton 2001], physical activity [WHO 2007], other diseases [Raltson 2005], drugs [Delmas 2000; Hannon 2000] and genes [Choi 2005; Wang 2006]. Age-related osteoporosis is an important cause of hip fracture that leads to injury and death. [Akçay 2000; Ganong 2002] Osteoporosis is more common in women than in men, and is more common in light-skinned individuals than in dark-skinned. [Sen 2005] Factors supporting osteoporosis include endocrine, metabolic and mechanical factors, parathyroid hormone and calcitonin secretion abnormalities, insufficient vitamin D and calcium intake, postmenopausal hormonal status, pregnancy, nutritional diseases, inactivity and drugs such as cortisol. [Ginaldi 2005] The risk of osteoporosis in postmenopausal women is higher than in other woman. [Sen 2005] Osteoporosis is often postmenopausal or develops slowly during menopause. A few cases are associated with mutations in Collagen 1 alpha 1 (COL1A1), Collagen 1 alpha 2 (COL1A2) and Vitamin D Receptor (VDR) [Murray 2004]. There is an increased risk of osteoporosis in inadequate and unbalanced nutritional status. The lack of adequate protein matrix due to malnutrition is important for osteoporosis. Parameters such as nutrition, exercise, smoking and alcohol consumption are risk factors for osteoporosis [WHO 2007].

Increased Parathyroid Hormone (PTH) values have been reported to be associated with increased mortality in subtle-poor ages. [Raisz 2005] Although genetic factors are effective on body shape, development in the mother's womb and childhood nutrition play a role in the pathogenesis of osteoporosis. [Fordham 2006] It has been reported that overweight may protect against osteoporosis either by increasing burden or by leptin hormone [WHO 2007]

With decreased physical activity and a sedentary lifestyle there is an increased risk of osteoporosis. Throughout their lives, the bodies of physically active people increase bone turnover in response to physical stress and have a lower risk of osteoporosis. The most effective physical activity type is weight lifting exercises. The puberty has been reported to be the most effective period to strengthen bone density. In adults, physical activity may help maintain bone mass, but the increase in bone mass is about 1-2%. Excessive exercise can lead to progressive damage to the bone. In women, heavy exercise causes menstrual cycle suppression associated with decreased estrogen levels. Bedridden people are at significantly higher risk of osteoporosis [WHO 2007].

There are many inflammatory, gastrointestinal, endocrine and genetic diseases (these include diseases such as Rheumatoid Arthritis, Chronic Liver Disease, Hypogonadism, Osteogenesis Imperfecta, Myeloma) that pose a are at risk of osteoporosis. In addition, corticosteroids, GNRH antagonists, thyroxine, aromatase inhibitors, anticonvulsants, anticoagulants, sedatives are associated with osteoporosis and increase the risk of osteoporosis [Murray 2004].

Three mechanisms have been proposed for osteoporosis, a multifactorial disease 3 resulting from the complex interaction between bone turnover, bone mass, skeletal geometry, and genetic and environmental factors affecting fall risk; inadequate bone formation, inability to reach peak bone mass, and excessive bone loss. All factors affecting the bone tissue are involved in the pathogenesis of osteoporosis through one, two, or all three of these

mechanisms. Genetic factors are confirmed to be interactions with these three mechanisms [WHO 2007].

In the literature, more than one gene name participates in the pathogenesis of osteoporosis. Some of them have a low effect on the formation of osteoporosis, but some have a high effect. For example, it has been reported that some base changes of genes encoding the Collagen 1 alpha 1 (COL1A1), Estrogen receptor (ESR), Vitamin D receptor (VDR), Osteoprotegerin (OPG), Interleukin 6 (IL-6) and Lactase (LCT) have a risk of osteoporosis. COL1A1 is the most important component of bone and connective tissue whereas ESR is an important molecule for estrogen hormone activity. VDR is an important regulator for vitamin D and calcium metabolism. While OPG has an important link between the bone and the vascular system, IL-6 inflammation is important for continuing bone health as a symptom. LCT is one of the digestive system enzymes involved in lactose breakdown in the milk [Osteocheck 2006].

In our study, we performed polymorphic analysis at eight different points on six different genes (COL1A1, ESR, VDR, OPG, LCT, IL-6). We aimed to investigate the risk ratios of these polymorphic structures to osteoporosis.

MATERIALS AND METHODS

Those with systemic disease and continuous drug use were not included in the study. 150 volunteers were included in this study and majority of the participants were woman. They were divided into groups 3 groups (osteoporosis, osteopenia, healthy) using dual energy x-ray absorptiometry (DEXA) measurements, the gold standard for the diagnosis of voluntary osteoporosis. All volunteers were examined at the Atatürk University Physical Therapy and Rehabilitation Clinic and sent to Radiology Department for DEXA measurements.

Whole Blood Specimens taken from the veins of all volunteers were sent to the Biochemistry Molecular Analysis Laboratory of the same hospital. DNA was isolated and amplified by PCR multiplex method using specific primers. The amplicons were subjected to microchip hybridization with oligonucleotide probes, and polymorphism analysis was performed in eight different gene regions.

Osteocheck microarray systems are molecular biochemical methods used to determine genetic polymorphism. Osteocheck microarray systems include the following polymorphisms: COL1A1 Sp1 G2046T polymorphism, ESR Xbal A351G polymorphism, ESR Pvull T397C polymorphism, VDR b / B INT 8C → T BsmI polymorphism, OPG G209A polymorphism, OPG T245G polymorphism, LCT T13910C polymorphism, IL-6 G174C polymorphism.

The following protocols were followed: The sampling and storage of the sample was done according to the method of Osteocheck and Invisorb [Osteocheck 2006; Invisorb 2004]. Materials required for manual DNA isolation were monitored using the Invisorb protocol. A standard registered commercial kit (Invictek's registered trademark, Invisorb) was used to determine the genomic DNA [Invisorb 2004]. Quantitative analysis of isolated DNA samples was carried out on a UV spectrophotometer at a wawelength of 260/280 [Osteocheck 2006; Invisorb 2004]. The Osteocheck protocol was followed for multiplex PCR [Osteocheck 2006]. Materials required for PCR were identified based on invisorb kit content [Invisorb 2004]. The materials required for electrophoresis were prepared according to the manual protocol. The genomic DNA was electrophoresed by the method of analysis by Agarose Gel Electrophoresis. [Sarıkaya 2004]. Array Tube Hybridization Protocol was applied for hybridization. DNA sequencing was performed by an automated method [Osteocheck 2006]. Routine Ogham Solas 1 system was used for analysis of both normal and mutant genes. 'Ogham Solas 1' was loaded on the laboratory instrument 'PrimoLas' and the results were obtained from my computer system.

A control DNA sample was used to confirm the results. The results were reported by loading it into a computer and hospital automation system in the laboratory. The SPSS Statistic program (version 15.0) was used to analize the data. Significance values were determined at p <0.05 using Pearson Chi-Square Test. The ethical approval of the study was given by the Ethics Committee of Atatürk University Medical Faculty (26/04/2007, Jan.1, 2007). The kits used for the study were received by the hospital. To all volunteers were given Osteoporosis Susceptibility Risks Analysis report. There is no the interest relationship between the parties in the study.

RESULTS

150 volunteers were included to work. Volunteers were divided into three groups, according to DEXA measurements. Polymorphic structure analysis was performed at the gene locus of COL1A1 Sp1 G2046T, ESR Xbal A351G, ESR Pvull T397C, VDR b / B INT 8C? T BsmI, OPG G209A, OPG T245G, LCT T13910C, IL-6 G174C. Polymorphic structure was detected in all gene loci analyzed. A total of 1200 polymorphic results were obtained for each volunteer. The results were reported as Wildtype, Heterozygote and Homozygote. In Table 1, the percentages of DEXA and polymorphic structures are given. There was no significant correlation between bone mineral densities and polymorphic structures (Pearson Chi-Square Test (p <0,486) (Table 1).

Table 1: Bone Mineral Density and polymorphic structure percentages

	Normal	Osteopenia	Osteoporosis
Wildtype	53,44%	51,25%	49,58%
Heterozigot	26,25%	26,50%	27,92%

Homozigot	20,31%	22,25%	22,50%
	100,00%	100,00%	100,00%
Pearson Chi-Square Test (p< 0,486)			

[Percentage values of normal, osteopenia and osteoporosis, which are voluntary base groups according to DEXA measurements, and wildtype, heterozygote and homozygote percentage values according to polymorphic analysis results.]

The most common genotype (wildtype) and 4 polymorphic structures were detected in each volunteer (35,33%). The polymorphic structure was observed at 5 points (19,33%), 3 points (17,33%), 2 points (14,67%) and 6 points (9,33%) respectively (Table 2).

Table 2: Polymorphic structure frequency

	GENE								
	1 gene	**2 gene**	**3 gene**	**4 gene**	**5 gene**	**6 gene**	**7 gene**	**8 gene**	
%	***2,67***	**14,67**	**17,33**	**35,33**	**19,33**	**9,33**	**1,34**	**0**	**100**
Total	4	**22**	**26**	**53**	**29**	**14**	**2**	**0**	**150**

[The table gives the number of normal genotypes at eight points studied. Or, if the gene is not a normal genotype, it means polymorphic structure. 4 (2,67%) with 1 polymorphic structure, 22 (14,67%) with 2 polymorphic structures, 26 (17,33%) with 3 polymorphic polymorphic structures, 53 (35,33%), 29 (19,33%) with 5 polymorphic structures, 14 (9,33%) with 6 polymorphic structures, and 2 (1,34%) with 7 polymorphic structures were detected].

R^2 values were obtained by plotting the graphs showing the relationship between wildtype numbers and bone mineral density of individuals with the wildtype genotype. The relationship between the number of wildtype genotypes and normal bone mineral densities in an individual is shown in Figure 1, the relationship between those with osteoporosis in Figure 2 and the relationship between those with osteopenia in Figure 3.

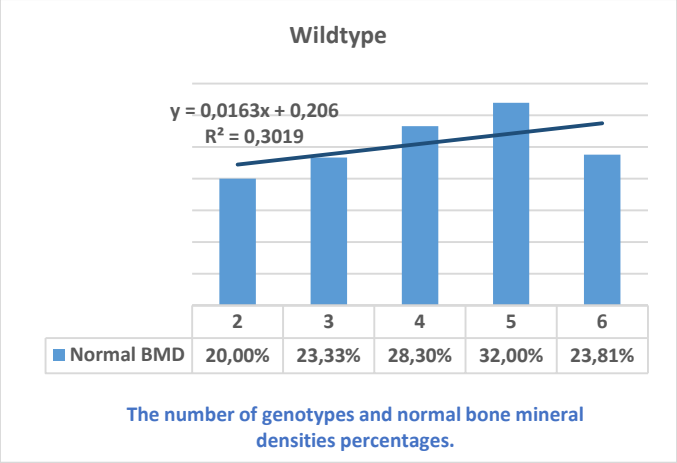

Figure 1: Wildtype; The number of genotypes and normal bone mineral densities percentages. Numbers 2, 3, 4, 5 and 6 give the number of wildtype in eight points analyzed in one person. Accordingly, the number of wildtype residues increases in proportion to having normal bone density. The percentage of individuals with normal bone mineral density having 2 wildtype genotypes was 20.00%

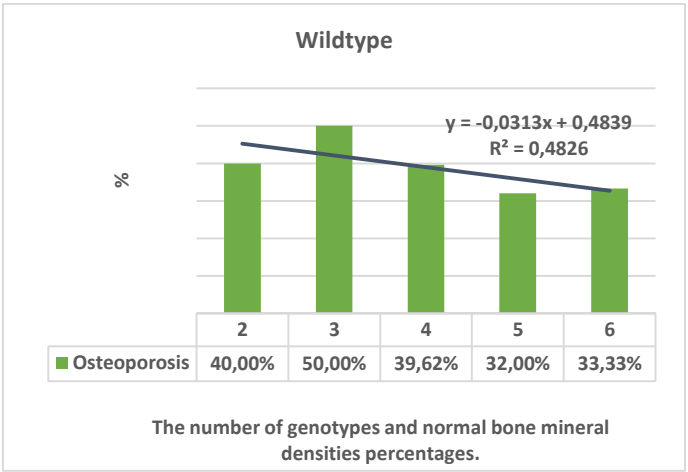

Figure 2: Wildtype; The number of genotypes and osteoporosis percentages. Numbers 2, 3, 4, 5 and 6 give the number of wildtype in eight points analyzed in one person. According to the figure, the percentage of osteoporosis is decreasing while the number of wildtypes is increasing.

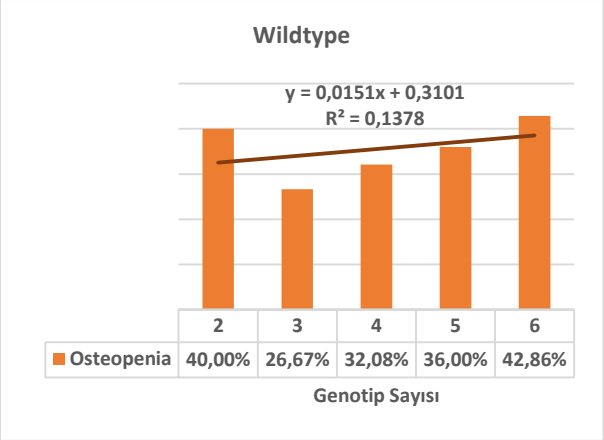

Figure 3: Wildtype; The number of genotypes and osteoporosis percentages. Numbers 2, 3, 4, 5 and 6 give the number of wildtype in eight points analyzed in one person. According to the figure, the percentage of osteopenia is increasing while the number of wildtypes is decreasing.

According to the study done between normal bone mineral density, the wildtype genotype percentage of normal bone density was higher than heterozygous and homozygous cases (Figure 4), and the wildtype percentage was lower than that of heterozygous and homozygous ones in osteoporosis cases (Figure 5). Those with osteopenia had the same genotype results as those with normal bone density (Figure 6).

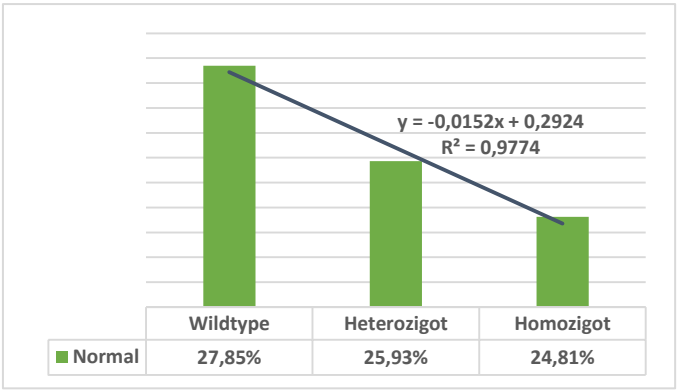

Figure 4: In those with normal bone density, the percentage of wildtype is highest (27.85%) and the percentage of homozygotes is lowest (24.81%).

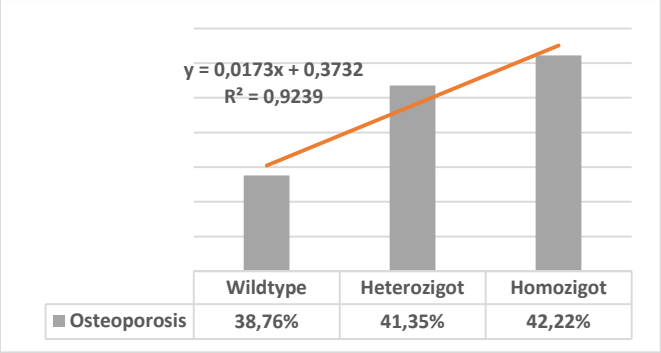

Figure 5: Genotype percentages in those with osteoporosis. In the cases of osteoporosis, the percentage of wildtype is lowest (38.76%) and the percentage of homozygote is highest (42.22%).

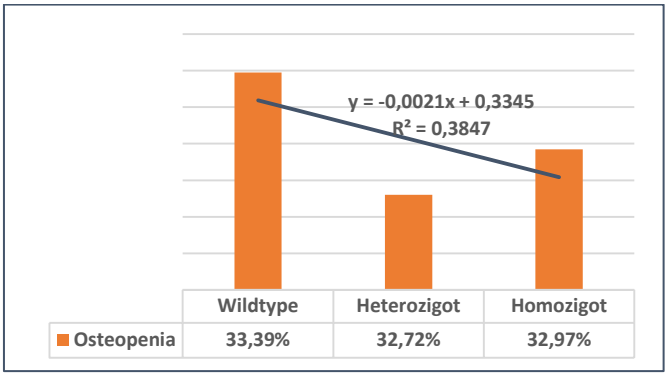

Figure 6: Genotype percentages in those with osteopenia. In osteopenic subjects, the percentage of wildtype was highest (33.39%), the percentage of heterozygotes was lowest (32.72%)

DISCUSSION

Osteoporosis is one of the diseases with great social and economic burden. The number of people affected by gives statistics the disease is also increasing in comparison with the increasing elderly population in the world. For this reason, genetic risk analysis tests performed for many different situations are performed for osteoporosis, which increases the susceptibility to disease. The desire for a healthier aging is acceptable to reduce the incidence of osteoporosis.

Polymorphisms in several genes are associated with different mass and bone fragility. It is now even more probable that osteoporosis can be predicted by these polymorphisms, the calculation of fracture risk and the approach of treatment [Raisz 2005].

A polymorphic structure every 500 nucleotides are normally expected. These polymorphic structures contribute to people being different. These differences can sometimes be neutral, sometimes positive, and sometimes negative [Hannon 2000]. Risk analysis tests conducted to detect nucleotide changes in the genes of healthy bone development and persistent direct and indirectly related molecules are drawn to bone health by providing us with information on this topic.

The role of transcriptional factors for polymorphisms in osteoporosis has not yet been elucidated [Raisz 2005]. There was no significant association between polymorphic structures and age, as seen in figure 1 in our study. With increasing age, the increased risk of osteoporosis was associated with no significant association with bone density of polymorphic structures.

In general, the interaction between polymorphisms and osteoporosis is associated with moderate effects [Uitterlinden 2004]. According to the results shown in Table 1, there was no significant difference in the incidence of polymorphic structure among the groups.

In recent years, intensive research on genetic markers has reported that several genetic polymorphisms are associated with osteoporosis. These polymorphisms are associated with decreased bone turnover and a high risk of osteoporosis [Osteocheck 2006]. The polymorphic structure of the entire gene locus of COL1A1 Sp1 G2046T, ESR Xbal A351G, ESR Pvull T397C, VDR b/B INT 8C/T BsmI, OPG G209A, OPG T245G, LCT T13910C and IL-6 G174C in the assay was determined. But these polymorphic structures were at different points in different individuals. In all groups 35.33% of polymorphic structures were detected in at

least four points (Table 2). Polymorphic structure was observed in 5 points, 3 points, 2 points and 6 points respectively. Bone mass is under the control of many genes [Murray 2004; Brandi 2001]. In different societies different polymorphic structures and their different effects have been observed [Brandi 2001]. According to the results obtained in our study, there was no one who did not have any polymorphic structure. Only at one point is the polymorphic structure number / percentage very low (2,67%). However, polymorphic structures were found to be the most common at 4 points. Polymorphic structure was observed in 5 points, 3 points, 2 points and 6 points respectively (Table 2).

Our data provide valuable rewards for the literature regardless of the age and gender about the regional polymorphic structure frequency. According to DEXA results, there was no significant relationship between osteoporosis, osteopenia and the groups that we normally formed and the incidence of polymorphic structure. If the relationship between DEXA and the polymorphic structure were made in the same age, sex, diet, and physical activity, the data would be more tangible.

It is known that genetic factors play a role in the micro-architectural properties of the bone. It is even reported that genetic factors account for 70-80% of changes in bone phenotype. In addition, osteoporosis in the family history indicates that the person has genetic background [Hannan 2000]. It has been stated that daughters of mothers with osteoporotic fractures have low bone density [Albrand 2003].

Despite the fact that the number of polymorphic structures seen and the percentage of osteoporosis figures are open to debate in many respects, it is obvious that the probability of osteoporosis increases as the polymorphic structure frequency increases in a person.

The greater the number of wildtype genotypes in a person, the greater the percentage of having normal bone density (figure 1), whereas the lower the number of wildtypes, the greater

the percentage of osteopenia (figure 2) and osteoporosis (figure 3). In those with normal bone density, the percentage of wildtype is highest (27.85%) and the percentage of homozygotes is lowest (24.81%) (Figure 4). In osteoporosis cases, the percentage of wildtype was lowest (38.76%) and the percentage of homozygotes was highest (42.22%) (Figure 5). In osteopenic subjects, the percentage of wildtype was highest (33.39%) and the percentage of heterozygotes was lowest (32.72%) (Figure 6).

Bone mineral density is known to be a corporate result of environmental and genetic factors. Likewise, our data show that it has a corporate effect on genetic factors (Figures 1, 2, 3, 4, 5, and 6). It was determined that polymorphic structures at eight different points analyzed have osteoporosis contribution, but this contribution is not statistically significant. It is understood that this contribution is quite large when considering hundreds of proteins participating in the mechanisms of bone formation and destruction.

REFERENCES

Osteocheck. Instruction Manual for Multiplex-PCR and ArrayTupe hybridization for solas 1. Version 2006/03. Ogham diagnostics GmbH 2006.

Akçay G. Metabolik Kemik hastalıkları. Akçay G, Akçay MN, Akarsu E. Endokrin ve metabolizma hastalıkları. Aktif Yayıncılık. 2000; 21: 245-58

Raltson S.H, Kleerekoper M. Osteoporosis. Çev: Arasıl T, Seçkin Ü. Osteoporoz. Ankara. Öncü basımevi. 2005; 13-74.

Murray Granner, Mayes Rodwell. Harper Biyokimya. İstanbul. Nobel Tıp Kitabevleri. 2004; 695-714.

William F. Ganong. Tıbbi Fizyoloji. Çeviri Türk Fizyolojik Bilimler Derneği. Nobel Tıp Kitabevleri. 2002; 369-383.

Anderson SC. Clinical chemistry. W. B. America. Saunders Company. 1993; 531-2.

Fordham John. Your Questions Answered Osteoporosis. Çev: Gökçe-Kutsal Yeşim. Osteoporoz Sorularınıza Cevaplar. Ankara. Öncü basımevi. 2006; 49.

Uitterlinden AG, H Burger, Q Huang, E Odding, C M Duijn, A Hofman, J C Birkenhäger, J P van Leeuwen, and H A Pols. Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J Clin Invest. 1997; 15; 100(2): 259-263.

Raisz LG. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J Clin Invest. 2005; 1; 115(12): 3318-25.

Brandi ML, Luigi Gennari, Marco Matucci Cerinic, Lucia Becherini, Alberto Falchetti, Laura Masi, Carlo Gennari, and Jean-Yves Reginster. Genetic markers of osteoarticular disorders: facts and hopes. Arthritis Res. 2001; 3(5): 270-280.

Sen Shuvayu S, PhD, Vincent P Rives, PharmD, Osvaldo D Messina, MD, Jorge Morales-Torres, MD, Gregorio Riera, MD, Juan M Angulo-Solimano, MD, João FM Neto. A Risk Assessment Tool (OsteoRisk) for Identifying Latin American Women with Osteoporosis. J Gen Intern Med. 2005; 20(3): 245-250.

Ginaldi Lia, Maria Cristina Di Benedetto and Massimo De Martinis. Osteoporosis, inflammation and ageing. Immun Ageing. 2005; 2: 14.

Guyton AC, Hall JE. Medical physiology. Çavuşoğlu H. Tıbbi fizyoloji. İstanbul. Yüce Yayınları ve Nobel Tıp Kitabevleri. 2001; 10. basım.

Albrand G, Munoz F, et al. Independent predictors of all osteoporosis- related fractures in healthy postmenopausal women: the OFELY study. Bone. 2003: 32: 78-85.

Uitterlinden AG, Pascal P. Arp, Bryan W. Paeper, Patrick Charmley, Sean Proll, Fernando Rivadeneira, Yue Fang, Joyce B. J. van Meurs, Theresa B. Britschgi, John A. Latham, Randall C. Schatzman, Huibert A. P. Pols, Mary E. Brunkow. Polymorphisms in the Sclerosteosis/van Buchem Disease Gene (SOST) Region Are Associated with Bone-Mineral Density in Elderly Whites. Am J Hum Genet. 2004 December; 75(6): 1032-1045.

Bonnie J. Deroo, Kenneth S. Korach. Estrogen receptors and human disease. J Clin Invest. 2006: 1; 116(3): 561-570.

Long Ji-Rong, Lan-Juan Zhao, Peng-Yuan Liu, Yan Lu, Volodymyr Dvornyk, Hui Shen, Yong-Jun Liu, Yuan-Yuan Zhang, Dong-Hai Xiong, Peng Xiao, Hong-Wen Deng. Patterns of linkage disequilibrium and haplotype distribution in disease candidate genes. BMC Genet. 2004; 5: 11.

Ueland T, Bollerslev J, Wilson SG, Dick IM, Islam FM, Mullin BH, Devine A, Prince RL. No associations between OPG gene polymorphisms or serum levels and measures of osteoporosis in elderly Australian women. Bone. 2007; 40(1):175-81.

Choi JY, Shin A, Park SK, Chung HW, Cho SI, Shin CS, Kim H, Lee KM, Lee KH, Kang C, Cho DY, Kang D. Genetic polymorphisms of OPG, RANK, and ESR1 and bone mineral density in Korean postmenopausal women. Calcif Tissue Int. 2005; 77(3); 152-9.

Wang YB, Guo JJ, Liu YJ, Deng FY, Jiang DK, Deng HW. The human calcium-sensing receptor and interleukin-6 genes are associated with bone mineral density in Chinese. 2006; 33(10):870-80.

Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J. The use of biochemical markers of bone turnover in osteoporosis. Osteoporos Int. 2000; 6: 2-17.

Hannon R and Eastell R. Prenalytical variability of biochemical markers of bone turnover. Osteoporos Int 2000; 6: S30-44.

Invisorb. 2004. Spin Blood Mini kit for DNA extractions from 1-200 µl blood. Invitek.

Sarıkaya AT. DNA'nın izolasyonu ve analizi. Temizkan G, Arda N. Moleküler Biyolojide Kullanılan Yöntemler. İstanbul. Nobel Tıp Kitapevleri. 2004: 68-72.

World Health Organization. 2007. Scientıfıc group on the assessment of osteoporosıs at prımary health care level.