Clinical Study

Genetic Influence of Candidate Osteoporosis Genes in Saudi Arabian Population: A Pilot Study

Mir Sadat-Ali[1] and Haifa A. Al-Turki[2]

[1] *Department of Orthopaedic Surgery, College of Medicine, University of Dammam and King Fahd University Hospital, AlKhobar 31952, Saudi Arabia*

[2] *Department of Obstetrics and Gynecology, College of Medicine, University of Dammam and King Fahd University Hospital, AlKhobar 31952, Saudi Arabia*

Correspondence should be addressed to Mir Sadat-Ali, drsadat@hotmail.com

Background and Objectives. The purpose of the present study is to find the genes and SNP that influence BMD and postmenopausal Saudi women. *Material and Methods*. Two-hundred ethnic Saudi Arabian women with a diagnosis of postmenopausal osteoporosis were the subjects of this study. Baseline blood hematology, biochemistry, and bone panel were done. Blood was collected, and three TaqMan-MGB probes were used to analyze SNP variants in ALOX15 (rs7220870), LRP5 (C 25752205 10), and TNFRSF11B (C 11869235 10). *Results*. The variant of ALOX15 17p13 showed that the BMD of the spine was lower in the AA allele (P value < 0.002) and fractures were highest at 50% compared to CC allele. In the TNFRSF11B gene, BMD of the hip and spine was significantly higher in the GG allele and the history of fractures was significantly higher in GG group. With regard to the LRP5 (C 25752205 10) gene, there was no significant difference between allele groups. *Conclusion(s)*. This study shows that the genetic influence of osteoporosis in the Caucasian and Saudi Arabians population is similar. We believe that the same genetic markers that influence osteoporosis in the Caucasian race could be used for further studies in the Saudi Arabian population.

1. Introduction

Postmenopausal osteoporosis (PMO) has become a major epidemic of the last millennium and is expected to be a problem for health care providers in the present millennium as well. Osteoporosis is a disease in which the net loss of bone exceeds bone formation, and it occurs in women after estrogen loss in postmenopause [1–3]. Postmenopausal osteoporosis (PMO) is a major public health epidemic worldwide. Most women with PMO present with a fracture as the first indication of the disease [4]. It is estimated that, in USA, 25 million women suffer from osteoporosis, and the cost to treat osteoporosis-related fractures (ORFs) exceeds $10 billion a year [5, 6]. The prevalence of PMO in Saudi Arabia and the recognition of the problems associated with PMO were only realized in the last decade. Only few studies about PMO in Saudi Arabian women have been reported [7–10].

The most serious complication of osteoporosis is hip fracture, which increases patients' morbidity and mortality rates. The incidence and costs of these fractures and their sequelae will continue to rise as the population ages; by year 2025, costs related to osteoporotic fractures are projected to reach $25.3 billion in the United States alone [11]. In an assessment of the annual cost of ORF in Saudi Arabia, Bubshait and Sadat-Ali (2007) [12] found that SR 4.27 billion is spent yearly to treat osteoporosis-related femoral fractures. Osteoporotic hip fracture usually requires hospitalization and surgery and may result in lengthy or permanent disability or even death. Within the first year after injury, a patient with a hip fracture has a 1015% greater chance of dying than others of the same age. Men, though suffering fewer hip fractures than women, are 25% more likely than women to die within one year of the injury [13–15].

From family histories, twin studies, and molecular genetics, it is quite evident now that some of a patient's predisposition for osteoporosis can be inherited. Genetic control of osteoporosis is polygenic; enumeration of the specific genes involved is in the initial phases [2, 16–18]. There is no study, as yet, about the genetic influence on osteoporosis and related fractures in Saudi Arabian women. This study is conducted to find the influence of known genes on osteoporosis among Saudi Arabian women with and without fractures.

2. Material and Methods

After approval of the ethical and review board of the University of Dammam and written consent from the patients, two-hundred ethnic Saudi Arabian women with diagnosis of postmenopausal osteoporosis by DEXA machine on the basis of T and Z scores, as described by WHO, were the subjects of this study, which was done between January 2009 and June 2010. History and clinical examination were done to rule out secondary osteoporosis. Baseline blood hematology, biochemistry, and bone panel were also done. For the genetic analysis, 5 mL of whole blood was collected. Out of several candidate osteoporosis genes which have been reported in the Caucasian race to influence BMD and fragility fractures, we did genotyping of 3 polymorphisms in three genes as a pilot study in ethnic Saudi Arabian postmenopausal women. The three genetic polymorphisms have been convincingly shown to affect the BMD and fragility fractures in the white race. Three TaqMan-MGB probes (two predesigned and one on design assays) have been used to analyze SNP variants in the requested genes: Alox15 (rs7220870), LRP5 (C_25752205_10), and TNFRSF11B (C_11869235_10). DNA from $100\,\mu L$ blood samples has been extracted using the DNeasy Tissue Extraction kit from Qiagen. Positive DNA control of known genotype (wild type, heterozygote, and mutant) was added to the PCR as well as negative control (no DNA). Genotyping was visualized on Taqman 7000 (Applied Biosystems). The data of the patients including age, history of fractures, and hip and spine BMD was entered and analyzed using SPSS Inc.'s Statistical Package for Social Sciences (SPSSs), version 14.0 (Chicago, IL, USA). Data is expressed as mean and standard deviation (SD). Statistically significant differences between groups were determined with a Student t test. P values less than .05 and a CI of 95% were used to indicate statistical significance.

3. Results

The average age of the patients was 62.5 ± 5.9 years. The variant of ALOX15 17p13 (rs7220870), C > A SNP where C is the ancestral allele (freq. 0.7–0.9), and the A allele is the variant allele (freq. 0.1–0.3). Majority (69%) of the women were CC (homozygous for the C allele); even though BMD in the hip region was significantly lower ($P < 0.001$, CI 0.017 to 0.06), history of fractures were lower than the other two alleles ($P < 0.0002$, CI 3.4 to 15.7) Table 1(a). The number of women in the homozygous for A allele was

4% with a higher BMD. A history of fractures was found in 50% of the patients ($P < 0.001$) (Table 1(b)). Regarding the analysis of the LRP5 rs3736228, the alleles were C > T SNP where C is the ancestral allele (freq. 0.7–0.9) and the T allele is the variant allele (freq. 0.1–0.3). Table 2 gives the data of the LRP5 gene. Over 96% of the patients exhibited the CC and CT alleles. In women with the homozygous T allele the BMD was significantly higher at the hip and spine and there were no fragility fractures. The statistical analysis of the LRP5 gene and SNP is given in Table 2(b). In the TNFRSF11B (C_11869235_10) gene, the majority of patients belonged to the AA and AG alleles, which was homozygous and heterozygous for the A allele, and only 3% belonged to the GG allele. BMD of the hip and spine was significantly higher in the GG allele, but the history of fractures was more common in the GG group $P < 0.001$ (CI 59.85 to 60.146) (Tables 3(a) and 3(b)). In the A allele group, there was risk of low BMD and higher risk for osteoporosis without fractures, whereas, in patients with the G allele, there was higher BMD of both hip and spine and higher prevalence of fractures.

4. Discussion

Our study provides evidence that earlier reported SNPs in the ALOX 15, LRP5, and TNFRS11B, which influences BMD, osteoporosis, and fragility fractures in Caucasians, also influences the condition in ethnic Saudi Arabian females. Tranah et al. [19] found that the T/T genotype of ALOX15 had a 33% higher rate of osteoporosis fractures. In this study we found that although only 4% of the women carried the genotype, the fracture rate in those who carried it was 50%. Contrary to our finding and that of Tranah et al. [19], Mullin et al. [20] reported that polymorphisms in ALOX15 are not associated with influencing BMD in white men and women. Low-density lipoprotein receptor-related protein 5 (LRP5) was reported to influence BMD and risk of fractures. Variations of LRP5 have been linked to BMD and susceptibility to osteoporosis, but Koller et al. [21] suggested that LRP5 was not a major influence on attainment of peak BMD of the hip and spine in white women. Van Meurs et al. [22] reported that their findings indicate that LRP5 does influence BMD and fracture risk throughout life in the general population. Mizuguchi et al. [23] found that Japanese women with the C/C genotype had higher adjusted BMD (AdjBMD) value compared to those with C/T and T/T ($P = 0.022$), but in Saudi women the C/C genotype had the lowest BMD in the hip and spine, and women with the TT allele had no fragility fractures compared to CC and CT variants. Brixen et al. [24] meanwhile, found no significant difference in BMD between the three alleles in white men. The distribution of CC, CT, and TT alleles in this study was 73.5%, 23%, and 3.5% and similar frequency. The CC, CT, and TT genotypes were found in 75.6%, 21.8%, and 2.6% of the participants, respectively [25].

Richards et al. [26] reported strong evidence of association with SNP in rs4355801 on chromosome 8, near TNFRSF11B, along with BMD and increased risk of osteoporosis. They found that the G allele at rs4355801 was

Table 1: (a) Demographic data of ALOX15 17p13 (rs7220870) of CC, CA, and AA alleles. (b) Statistical analysis of the ALOX15 17p13 (rs7220870) 3 alleles.

(a)

Parameter	CC (138)	CA (54)	AA (8)
Age years	62.3 ± 5.7	59.94 ± 6.9	61.4 ± 11.5
BMD hip g/cm^2	0.429 ± 0.1	0.468 ± 0.11	0.581 ± 0.26
T score	-2.8 ± 0.6	-2.6 ± 0.9	-2 ± 1.8
Z score	-1.6 ± 0.7	-1.1 ± 0.9	-0.74 ± 1.5
BMD spine g/cm^2	0.680 ± 0.08	0.712 ± 0.12	0.662 ± 0.22
T score	-3.25 ± 0.9	-3.07 ± 0.7	-2.54 ± 1.22
Z score	-2.02 ± 0.68	-1.81 ± 0.93	-2 ± 0.7
No. of fractures (%)	39 (28.2)	23 (42.6)	4 (50)

(b)

Parameter	Alleles	P value (CI)
Age	CC versus CA	0.002 (2.092–2.62)
	CC + CA versus AA	0.8
BMD hip	CC versus CA	0.001 (0.017–0.06)
	CC + CA versus AA	0.001 (0.111–0.159)
BMD spine	CC versus CA	0.002 (0.024–0.04)
	CC + CA versus AA	0.01 (0.045–0.012)
History of fractures	CC versus CA	0.0002 (3.4–15.7)
	CC + CA versus AA	0.001 (60.8–61.4)

Table 2: (a) Demographic data of LRP5 (C_25752205_10) of CC, CT, and TT alleles. (b) Statistical analysis of the LRP5 (C_25752205_10) 3 alleles.

(a)

Parameter	CC (147)	CT (46)	TT (7)
Age years	62 ± 10.9	60.4 ± 8.9	60 ± 12
BMD hip g/cm^2	0.443 ± 0.15	0.445 ± 0.14	0.539 ± 0.06
T score	-2.7 ± 1.1	-2.65 ± 0.8	-2.05 ± 0.7
Z score	-1.5 ± 3.6	-1.1 ± 0.7	-0.9 ± 0.5
BMD spine g/cm^2	0.687 ± 1.4	0.70 ± 0.13	0.9 ± 0.5
T score	-3.16 ± 0.97	-3.26 ± 1.02	-2.35 ± 0.28
Z score	-1.8 ± 0.9	-2.07 ± 1.3	-1.75 ± 0.6
No. of fractures (%)	51 (34.2)	15 (32.6)	0

(b)

Parameter	Alleles	P value (CI)
Age	CC versus CT	0.7
	CC + CT versus TT	0.6
BMD hip	CC versus CT	0.8
	CC + CT versus TT	0.001 (0.065–0.107)
BMD spine	CC versus CT	0.2
	CC + CT versus TT	0.001 (0.166–2.540)
History of fractures	CC versus CT	0.01 (35.8–36.20)
	CC + CT versus TT	0.001 (32.8–33.14)

associated with higher BMD, and the A allele with low BMD, while in our patients the BMD, was significantly higher with G alleles as compared to AA or AG alleles ($P < 0.001$; CI < -0.1966) and lower in AA or AG alleles. Even though the risk of osteoporosis was higher in patients with the A allele,

the fracture prevalence was not increased. Our results concur with the findings of Richards et al. with regard to the risk of fractures with the A allele.

Our study has limitations and certain strengths. The number of osteoporotic patients was small, compared to

TABLE 3: (a) Demographic data of TNFRSF11B (C_11869235_10) of AA, AG, and GG allele. (b) Statistical analysis of the TNFRSF11B (C_11869235_10) 3 alleles.

(a)

Parameter	AA (114)	AG (80)	GG (6)
Age years	61.6 ± 10.9	61.2 ± 9.8	69.5 ± 12
BMD hip g/cm^2	0.461 ± 0.15	0.443 ± 0.16	0.726 ± 0.55
T score	-2.7 ± 1.27	-2.75 ± 0.96	-1.1 ± 2.5
Z score	-1.33 ± 0.96	-1.21 ± 0.92	-0.15 ± 2.7
BMD spine g/cm^2	0.686 ± 0.13	0.689 ± 0.11	0.915 ± 0.53
T score	-3.2 ± 1.0	-3.14 ± 0.96	-1.1 ± 2.7
Z score	-1.92 ± 1	-1.72 ± 1.4	-2.35 ± 0.5
No. of fractures (%)	38 (33.3)	25 (31.25)	3 (50)

AA: homozygous for A allele, AG: heterozygous allele, and GG: homozygous for G allele.

(b)

Parameter	Alleles	P value (CI)
Age	AA versus AG	0.2
	AA + AG versus GG	0.001(8.191–8.808)
BMD hip	AA versus AG	0.232
	AA + AG versus GG	0.001(0.213–0.333)
BMD spine	AA versus AG	0.77
	AA + AG versus GG	0.001 (0.16–0.291)
History of fractures	AA versus AG	0.009 (12.94–13.059)
	AA + AG versus GG	0.001 (59.85–60.146)

many studies on genetic analysis. Since this is a pilot and comparative study, one can draw reasonable conclusions about the genetic influence on osteoporosis among the ethnic Saudi Arabian population. As many researchers resort to GWAS to get minimal new data, we opted to study the influence of reported target osteoporotic genes upon the white race.

In conclusion, there is strong similarity of the SNPs in the genes that influence BMD, osteoporosis, and fragility fractures among the reported SNPs and genes in the Caucasian race and the ethnic Saudi population. These may not be the only SNPs which influence osteoporosis risk, but it gives a direction to study the reported targeted genes among the Saudi population.

Acknowledgment

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