Sp1 Binding Site Polymorphism at COL1A1 Gene and Its Relation to Bone Mineral Density for Osteoporosis Risk Factor Among the Sikkimese Men and Women of Northeast India

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Abstract Single nucleotide polymorphism in the first intron of Collagen type I alpha 1 (COL1A1) gene which is the binding site of specificity protein 1 (Sp1) transcription factor associated with low bone mineral density and osteoporosis. To evaluate such genetic factors among the Sikkimese population, a total of 150 cases (75 men and 75 women) with primary osteopenia and osteoporosis and 150 healthy controls (75 men and 75 women) of age range between 35 and 65 years were enrolled in this study. The COL1A1 genotypes [SS, Ss and ss] were assessed by restriction enzyme [MscI] digestion of DNA after amplification by polymerase chain reaction. There, only 2.7% women and 1.3% men cases had restriction site with heterozygous genotype (Ss) and no homozygous genotype (ss) were detected. There was no statistically significant association between low bone mass and genotypes on analysis ($\chi^2 = 1.014$, $P = 0.314$; $RR = 1.510$) that, the Sp1 binding site polymorphism at the COL1A1 gene is very rare and has no contribution in the development low bone mineral density.

Keywords Bone mineral density (BMD) · Collagen type I alpha 1 (COL1A1) · Osteoporosis · Sp1 polymorphism

Introduction

Till date, no one has reported any kind of study on COL1A1 gene polymorphism and its association with low BMD and osteoporosis among the population of Sikkim, a tiny state of India with a population of 6.11 lakhs (census 2011). Nevertheless, many studies in different countries consider the genetic factors contribute to the pathogenesis of low BMD, osteoporosis and fracture and some ethnic groups of studies show no role of genetic factors.

The combination of two chains of type I collagen called the pro-alpha1 chain produced by COL1A1 gene with one pro-alpha2 chain produced by the COL1A2 gene results cross link and make strong and rigid triple-stranded molecules that strengthen and harden the bone. The SNP at first intron (G > T) of COL1A1 gene may change an amino acid sequence of pro-alpha 1 chain forming a triple-stranded immature collagen Type-I with an increased deterioration of microarchitecture of bone leading to fragility and fracture [1]. Different polymorphisms have been described in several genes that are thought to be involved in the pathogenesis of osteoporosis [2]. Collagen type I alpha 1 (COL1A1) is one among the most prominent candidate genes, which has been consistently associated with osteoporosis in different community and populations [3–5].

Grant et al. described polymorphism in the first intron of COL1A1, a novel guanine to thymidine (G → T) that affects one of the binding sites of the transcription factor Sp1. This increase the formation of collagen type I alpha 1 protein in bone matrix in T carriers [6]. Such T allele is

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associated with low bone density, osteoporosis and increased risk of fracture [7].

A number of studies have been conducted to investigate an association of COL1A1 gene with Sp1 site polymorphism and osteoporotic fracture since the first discovery of this association. But these studies have reported conflicting results since it has been observed that lack of association and even absence of the polymorphism in many Asian studies [8–11]. Therefore, COL1A1 gene Sp1 site polymorphism as a genetic mediator of the risk of osteoporosis remains unclear. Hence the influence of COL1A1 gene needs to be explored in different population for universal confirmation. A very few study has been done in the female population of India [12]. But, so far such genetic association among males has not been studied in India. Therefore, the purpose of this study is to evaluate the association of Sp1 binding site polymorphism on COL1A1 gene and low bone mass density among men and women of the Sikkimese population of Northeast India.

Materials and Methods

Study Design

Case–control study over the period of one and half years (March 2012 to August 2013). The population of East Sikkim district is 45% of the whole Sikkim and this study may represent the population of Sikkim as a whole.

Selection of study subjects was done from those patients attending in the free BMD camps conducted in the Orthopedics departments of two major hospitals, Sir Thupton Namgyal Memorial Hospital and Central Referral Hospital, East Sikkim every 3 months after getting the approval from the Institutional Ethics committee.

Inclusion Criteria

Case group Subjects with osteopenia or osteoporosis between the age group 35–65.

Control group Subjects with the normal bone mineral density between the age group of 35–65.

Exclusion Criteria

Subjects with liver disorders, bone tuberculosis, alcoholic liver diseases, hyper/hypothyroidism, chronic renal disease, cancer, hyper/hypo para thyroidism and women on long term medication affecting the bone turn over (steroids, heparin, warfarin and hormone replacement) were strictly excluded. Subjects consuming alcohol and smokers were also excluded. None of the women in the study were pregnant or lactating.

The sample size was calculated with Genetic Power Calculator using minor allele frequency [13]. A total of 300 subjects (150 cases and 150 control) were recruited for the study. The case group consisted of 75 women (mean age 51 ± 9.9 years) and 75 men (mean age 48.4 ± 9.6 years) of age range 35–65 years with primary osteopenia and osteoporosis. The control group comprised of 75 healthy women (mean age 44.9 ± 9.4 years) and men (mean age 43.4 ± 7.4 years) of age range 35–65 years without diseases and medications that could influence bone mass and turnover. Informed consent was taken from all the subjects enrolled.

The BMD was measured at the calcaneus of right foot by QUS (Achilles Express heel ultra-densitometer) as there was no DEXA scan to access T-scores which was calculated based on WHO criteria [14]. The T-score of -1 was taken as normal, between -1 and -2.5 as osteopenia and -2.5 or lower for osteoporosis with reference to a bone density that is two and a half standard deviations below the mean of a 30-year-old man/woman.

Collagen Type I Alpha 1 Polymorphism

Genomic DNA was extracted from the samples of peripheral venous blood using salting out and method by V Iranpur M [15, 16]. The G $\rightarrow$ T polymorphism in the Sp1 binding site in the COL1A1 gene was examined by a polymerase chain reaction (PCR). A mismatched primer was used which introduces a restriction site for the enzyme *MscI* in the polymorphic alleles with the T substitution that discriminates *S* and *s* allele, which corresponding the presence of G and T respectively. Thus, the absence of the restriction site on both alleles was defined as *SS* homozygote and the presence of the restriction site *ss* homozygote whereas the combination of as *Ss* heterozygote [6]. The forward primer, 5'-TAACCTTCTGGACTATTGCGGACTTTTTGG-3' and the reverse primer, 5'-GTCCAGCCCTCATCCTGGCC-3'' were used for PCR in a final volume of 50 μ l containing 0.1–0.2 mg of genomic DNA and others Taq DNA polymerase (Ampli Taq 360 DNA Polymerase as per protocol of Applied Biosystem, USA) using standard conditions on an GENEI TC-3000 thermal cycler (Bangalore Genei, India) for 35 cycles at 94 °C (50 s), 61 °C (30 s) and 72 °C (60 s). Prior to the first cycle, initial denaturation was performed at 94 °C for 4 min and the last extension step with 5 min. The PCR products were digested with fast digest *MscI* (Thermo Scientific) according to the manufacturer's instructions and analyzed on a 3% agarose gel. The PCR product containing *s* alleles are cleaved by *MscI*, resulting in a band of 246 bp compared with the un-cleaved *S* alleles band of 264 bp.

Fig. 1 Agarose gel electrophoresis of PCR amplified product and restriction MscI-digested fragment. (Lane L is 100 bp ladder, lane 1 is PCR product with 264 bp, and lane 2, 3, 4, 5 and 7 are restricted product with SS genotype and lane 6 with Ss genotype)

Agarose gel electrophoresis of PCR amplified products and restriction MscI-digested fragment are shown in Fig. 1.

Statistical Analysis

Statistical analysis was performed using SPSS version 16. Differences in prevalence of the genotypes between cases and controls were tested using the χ^2 -test and odds ratio test was performed to calculate the relative risk, $P < 0.05$ was considered to be significant.

Results

The distribution of COLIA1 genotypes and alleles in cases and control for women and men is shown in Table 1. We observed that the polymorphic genotype was very rare in this group of the study population. The distribution of genotypes and allelic frequencies in women control group was 100% of SS types. In women cases group the distribution of genotypes was 97.3% (SS), 2.7% (Ss) and 0% (ss) whereas, the allelic frequencies 98.7% for S and 1.3% for s which differ from those reported in the European populations. Chi square test for association of low bone mass and COLIA1 genotypes were not found significant ($\chi^2 = 2.027$, $P = 0.155$; RR = 2.027). In men, control and case groups, the distribution of genotypes and allele frequencies were similar (98.7% SS, 1.3% Ss, 0% ss) and (99.3% of S and 7% of s) respectively. Chi square test for the association between the low bone mass and polymorphic gene among men were also not found significant ($\chi^2 = 0.000$, $P = 1$; RR = 1). Chi square test and odds ratio in the total subjects were also not significant ($\chi^2 = 1.014$, $P = 0.314$; RR = 1.510).

Discussion

This study shows that the distribution of genotypes and allele is not similar to the previous studies in Caucasian subjects where over representation of s allele was observed among osteoporotic patient [17]. It also contradicts with the

Table 1 Distribution of COLIA1 genotypes and alleles frequencies in cases and control

	Men		Women		All Subjects	
	Case	Control	Case	Control	Case	Control
Number	75	75	75	75	150	150
Age (mean $\pm$ SD)	48.4 $\pm$ 9.6	43.4 $\pm$ 7.4	51 $\pm$ 9.9	44.9 $\pm$ 9.4	49.6 $\pm$ 9.9	44.1 $\pm$ 8.1
COLIA1 Genotypes						
SS	74 (98.7%)	74 (98.7%)	73 (97.3)	75 (100%)	147 (98%)	149 (99.3%)
Ss	1 (1.3%)	1 (1.3%)	2 (2.7)	0 (0%)	3 (2%)	1 (0.7%)
ss	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
COLIA1 Alleles						
S	149 (99.3%)	149 (98.7%)	148 (98.7%)	150 (100%)	297 (99%)	299 (99.7%)
s	1 (0.7%)	1 (0.7%)	2 (1.3%)	0 (0%)	3 (1%)	1 (0.3%)
Chi Square (genotypes)	$\chi^2 = 0.000$		$\chi^2 = 2.027$		$\chi^2 = 1.014$	
Significance	$P = 1$		$P = 0.155$		$P = 0.314$	
Relative risk (Ss) ^a	1		2.027		1.510	
95% CI	0.248–4.036		1.722–2.887		0.848–2.690	

Genotype frequencies are shown as number (%)

^aRelative risk of low bone mineral density

study done by Monica et al. among the women group of Northwest India where a significant association between the polymorphism and low bone density were observed [12]. The genetic differences may play a role in the ethnic diversity in BMD and/or bone mineral content. In our study, the very low frequency of Ss genotype and no ss genotype were observed and there was no significant association with low bone mass. Our findings were similar to a study amongst black subjects of South Africa with a low incidence of osteoporosis [18].

The reason could be either it is very rare or maybe lack of Sp1 binding intronic sequence in this study group of the population that predominantly of Mongolian race and similar results were also observed in the study of Chinese and Korean population. It could be the one reason for the genetic difference from the Caucasian population.

It seems that several genes likely to control bone mass with modest effects, rather than a small number of genes with large effects [19]. Therefore, further study on other SNP of the COL1A1, COL1A2 genes and for any associated role of vitamin D and estrogen receptors in different ethnic populations may be of interesting studies to bring to a better conclusion.

In conclusion, the result of this study suggests that COL1A1 gene polymorphism on Sp1 binding site was found to be very rare in the Sikkimese population of Northeast Indian and this polymorphism does not seem to be responsible for genetic contribution leading to low BMD and osteoporosis.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

Ethical Approval Studies involving human participants were in accordance with the Standards of the institutional ethical committee.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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