

Association between Genetic Polymorphisms of COL1A2 Gene (Rs412777) and the Development of Osteoporosis

Suroor Mohammed Ali¹, Mohammed Abdullah Jebor^{2*}

^{1,2}Department of Biology, College of Sciences, Babylon University, Iraq

* Corresponding author: drmoh_abdullah62@yahoo.com

ABSTRACT

Osteoporosis is a polygenic disorder and has been demonstrated to be associated with ~30 candidate genes, the majority of which have also been implicated in the regulation of bone mineral density (BMD). Collagen type I plays an important role in the bone matrix and is encoded by COL1A2 (collagen type I alpha 2) gene that may be a potential candidate for osteoporotic fracture. This study aimed to determine the distribution of COL1A2 gene (rs412777) polymorphisms using a RFLP in osteoporosis patients. The present results revealed there were significant differences in COL1A2 genotype (rs412777) frequencies between the cases and control. The genotypic frequency of heterozygous A/C with homozygous A/A (OR=0.2298, 95% CI= 0.0613 -0.8614, P= 0.0292). and the genotypic frequency of homozygous variant C/C with the homozygous A/A (OR=0.9375, 95% CI=0.1326 -6.6285, P= 0.9484). There were no significant differences in C allele frequencies in patients as compared to the controls in the COL1A2 gene (rs412777) polymorphism (OR=0.8759, 95% CI=0.4911 -1.5621, P=0.6536).

Keywords:

Osteoporosis; COL1A2 polymorphism; rs412777; COL1A2 PvuI; PCR-RFLP.

Introduction

Osteoporosis is a systemic bone disease mostly occurring in elderly individuals. In this disease, disturbance in bone remodeling (bone resorption and formation) leads to a bone mass reduction, bone fragility, and eventually, to fracture. Osteoporotic fracture may cause disability, decreased quality of life, and ultimately, mortality – it affects all aspects of the patient's life [1]. There have also been studies reporting the rate of this disease in a local region; for example, in 2009, an Iranian multi-center study indicated that 70% of women and 50% of men aged ≥ 50 years suffered from osteoporosis or osteopenia [2]. Peak bone mineral density (BMD) as a major determinant of bone strength achieved in early adulthood plays an important role in the prediction of osteoporotic fracture in later life. In addition to many confirmed factors, such as race, sex, age, nutrition, hormonal status, menopausal state, smoking, alcohol intake, and physical activity, there are many studies that support the remarkable influence of genetic factors on bone strength. Studies show that up to 80% of BMD variation is attributable to genetic factors [3,4]. Collagen alters in the course of bone disease and is involved in the pathogenesis of osteoporosis [5]. The collagen network plays an important role in bone toughness and in age-related changes in bone quality [6]. It is observed that the age-related changes in bone tissue result in a decreased resistance to fractures, a lowered bone strength and flexibility, as well as an impaired functioning of collagen fiber networks [7]. It has been shown that COL1A1 and COL1A2 candidate genes are important factors in osteopenia and osteoporosis development and may influence bone metabolism [8]. COL1A2 polymorphism may be a genetic risk factor related to the development of osteoporosis [9]. Over 85% of osteogenesis imperfecta (OI) cases associates to mutations in procollagen type I genes (COL1A1 or COL1A2), however, no hot spots were linked to particular clinical phenotypes [10].

Material and Methods

Ethical statement

Every volunteer has informed written consent. The ethics committee of the MOH and MOHSE in Iraq's ethical approval for scientific research has accepted this research.

Study Population

The study subjects comprised from 64 patients selected from Imam Al-Hussein Medical-City all were female as patients group with age range (20-82 years). The control group study included 36 people apparently healthy that also were females with age range (20–71)years. All subjects in this study were taken written consent before participation in this study .

DNA extraction and genotyping

DNA of blood was extracted and purified using extraction and purification kit from Geneaid company (UK). The genotyping of the study groups was performed using the PCR-RFLP technique after DNA extraction from blood samples.

The targeted sites of DNA were amplified using design specific primers which used to identify COL1A2(rs412777), obtained from Macrogen company/south Korea . Forward primer: 5'- GTTTCATCCGTGGCAGCATC -3', and reverse primer: 5'- GACTGGACTGATTTCGCAGGA -3'.PCR was carried out in 20 µl reaction volumes containing 1 µl of each forward and reverse primer, 12.5 µl of Green Master Mix, 3 µl of Genomic DNA, and 2.5 µl of nuclease-free water to bring the reaction volume up to 20 µl . Amplification was conducted in a thermocycler (Biometra, Germany) with the following settings: 5 min pre-denaturation at 95°C; 30 cycles with denaturation for 20 seconds at 95°C, annealing for 30 seconds at 59°C, extending for 30 seconds at 72°C; and a final extension of 5 min .

PCR products were electrophoresed in 1 percent agarose at 75 V using gel electrophoresis (cleaver science – UK) and visualized with ethidium bromide. A gel documentation system (Cleaver Scientific –UK) was used to take photos.

polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP).

For COL1A2(rs412777) the PCR product was digested with 2 units of one of the specific endonucleases (PvuI) for 5-15min at 37°C , according to manufacturer's instructions(Biolabscompany) .PCR and digestion products were analyzed by electrophoresis in 1 percent agarose at 75 V using gel electrophoresis (cleaver science – UK) and visualized with ethidium bromide.

Statistical analysis

All the statistical analyses were done with the SPSS statistical software (version 23; SPSS Inc., Chicago, IL), $p < 0.05$ was considered statistically significant

Results and Discussion

The genomic DNA (Fig.1) was extracted from the blood samples as a first step to amplify the target region of COL1A2 (rs412777) gene.

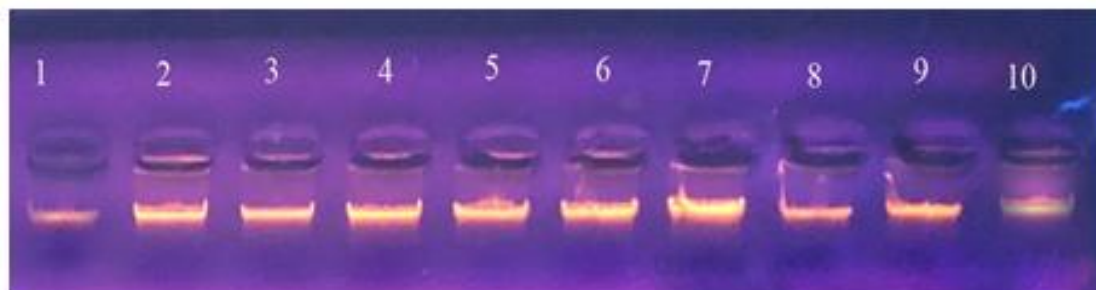


Figure 1. The electrophoresis pattern of genomic DNA extracted from blood samples of osteoporosis patients and healthy control groups.

(Lane 1 -lane 10 refers to genomic DNA from blood samples; Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h, stained with ethidium bromide).

Genotyping of COL1A2 (rs412777) Gene Polymorphisms

For COL1A2 (rs412777) genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal conditions. The results revealed that the presence a single band (249bp) of the target sequence of COL1A2 (rs412777) gene in agarose gel (Fig.2).

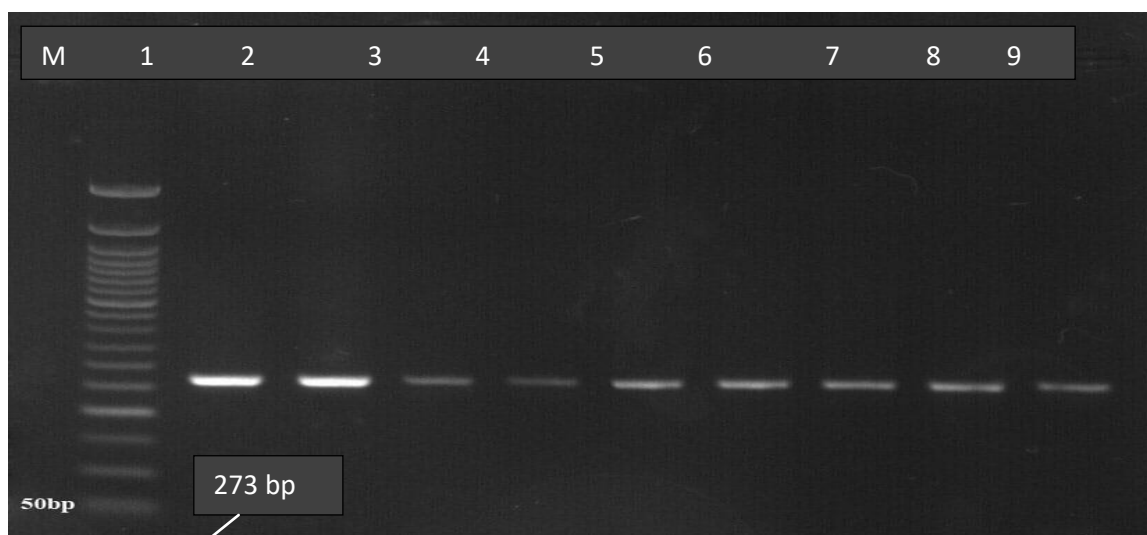


Figure 2. Agarose gel electrophoresis of an amplified product patterns of COL1A2 (rs412777) with specific primer.

M: refers to DNA size marker; lanes 1 - 9 refer to PCR products of *COL1A2* (rs412777) (273 bp) of osteoporosis patients and healthy control groups. Electrophoresis conditions: 1% agarose concentration 1%; 75 V, 20 mA for 120 min. Staining method; precast ethidium bromide.

After that, the PCR products of the *COL1A2* (rs412777) target sequences were digested with *PvuII*(5'CAG⁺CTG 3')restriction enzyme (Fig.3) to detect the rs412777 SNP in *COL1A2* gene (Fig. 3). The results of PCR-RFLP showed that the presence of three different genotypes as in figure (3). the first A1/A1 (CC) homozygous, presents the expected 273bp fragment, the second A1/A2 (AC) demonstrated 273, 148 & 125 bp fragment. While the third A2/A2 (AA) demonstrated 148 & 125 bp fragments.

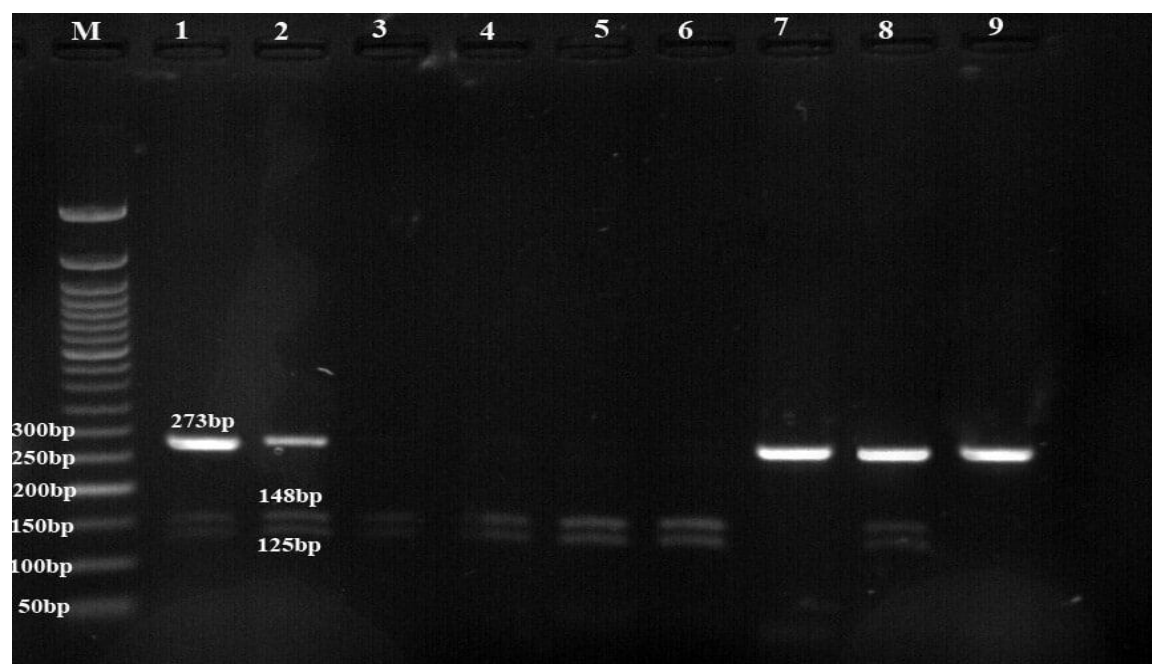


Figure 3. Electrophoresis patterns of allelotyping of *COL1A2* (rs412777) gene of osteoporosis patients and healthy control groups using *PvuI*enzyme by PCR-RFLP method

M: DNA ladder (50 bp); Lanes 3,4,5&6 refer to a homozygous allele (AA) had a two bands with 148&125 bpmolecular size; Lanes 1,2&8 refer to a heterozygous allele (AC) had 3 bands with 273,148&125 bp; Lanes 7,9 refer to a homozygous allele (CC) had a single band with 273 bp molecular size.

The Genotypes Distribution of rs412777 Polymorphisms with Allele Frequency in Control and Case Groups.

The distribution observed in *COL1A2* (rs412777) genepolymorphism in cases group and control group are showed in Table (1). The highest genotype in control group was heterozygote genotype AC (86.1%) followed by AA homozygote genotype (8.3%), CC homozygote genotype (5.6%). In

breast cancer disease , the highest genotype was heterozygote genotype AC (59.3%) followed by AA homozygote genotype (25%), CC homozygote genotype (15.7%). Analyses showed significant differences between cases and controls in the COL1A2 (*PvuI*) polymorphism (AC vs AA: OR=0.2298,95% CI= 0.0613 -0.8614, P= 0. 0292). and no significant differences between cases and controls in the COL1A2 (*PvuI*) polymorphism (CC vs AA: OR=0.9375, 95% CI=0.1326 -6.6285, P= 0.9484).

Table 1. Genotype distribution and odd ratio of *Rs412777* polymorphisms between the patients vs healthy control

Genotype of rs412777	Patients No.(%)	Control No.(%)	Significance level	O.R	CI (95%)
AA ^a	16 (25%)	3 (8.3%)			
AC	38 (59.3%)	31 (86.1%)	0.0292	0.2298	0.0613 - 0.8614
CC	10 (15.7%)	2 (5.6%)	0.9484	0.9375	0.1326 - 6.6285
Total No.	64	36			
Allele	Frequency	Frequency			
A	0.54	0.51			
C	0.46	0.49	0.6536	0.8759	0.4911 - 1.5621

$P \leq 0.05$; OR=(95%CI); ^a reference

The relationship between COL1A2 genotypes and osteoporosis is less well characterized than that between COL1A1 genotypes and osteoporosis. A number of studies have found an association

between GT-repeat polymorphisms, Msp I, Pvu II, and EcoR I of the COL1A2 gene and BMD [11].Lindahl et al.,[11] discovered interactions between the COL1A2 gene and BMD in older men from Sweden, the United Kingdom and Hong Kong (n=2004). Lau et al.,[12]found that Pvu II and EcoR I in the COL1A2 gene was associated with BMD in elderly men in Hong Kong, but they did not find any association between these two SNPs and BMD in postmenopausal women. This study also did not find associations between SNPs in COL1A1 and COL1A2 genes and osteoporotic fracture or BMD [13].

The analysis of PvuII polymorphism showed that in women with osteopenia the CC genotype had the lowest body weight compared to other genotypes ($p = 0.039$). PvuII polymorphism and clinical parameters in the group of women with osteoporosis had no statistically significant correlations. , the COL1A2 polymorphism may be a genetic risk factor related to the development of osteoporosis [9].Over 85% of osteogenesisimperfecta (OI) cases associates to mutations in procollagen type I genes (COL1A1 or COL1A2), however, no hot spots were linked to particular clinical phenotypes. In COL1A2 one mutation was identified in exon 22. Mutations of deletion type in COL1A1 that resulted in OI type I an effect neither on collagen type I secretion nor its intracellular accumulation were detected. Also, a missense mutation in COL1A2 changing Gly>Cys in the central part of triple helical domain of the collagen type I molecule caused OI type III. It affected secretion of heterotrimeric form of procollagen type I,Mutation in COL1A2 affected its incorporation to procollagen type I [10]. No significant difference was observed between fracture and control groups with respect to allele frequency or genotype distribution in 9 selected SNPs and haplotype. No significant association was found between fragility fracture and each SNP or haplotype. The results remained the same after additional corrections for other risk factors such as weight, height, and bone mineral density,Our results show no association between common genetic variations of COL1A1 and COL1A2 genes and fracture, suggesting the complex genetic background of osteoporotic fractures [14].

No statistically significant overall effect was found from the meta-analyses of any genetic model or SNP ($p > 0.06$) [15]. The two studies that provided data for the COL1A2 PvuII (rs412777) analysis presented conflicting results, with one reporting that the “PP” genotype halved fracture risk [16] , and the other suggesting that the P allele (either PP or Pp genotype) increased fracture risk [17]. In the combined analysis performed herein, these contradictory results lead to null effects, which found no significant overall effect of the COL1A2 PvuII (rs412777) SNP with fracture risk. The results of the studies may differ if two different proximal PvuII sites in the COL1A2 gene have been assessed, only Blades et al. (2010) report the specific reference SNP number (rs412777); or if the intervention of the study on calcium and vitamin D supplementation, Significant sub-group heterogeneity was observed between sexes in the effect of the COL1A1 Sp1 (rs1800012), COL1A2 PvuII (rs412777), and ESR1 PvuII (rs2234693) SNPs, highlighting the potential for sex-specific associations. Epidemiological data suggest that fracture incidence is greater in males between the age 18 and 49 than females in the general population [18].

Conclusion

Based on the present statistical analysis, our study shows significant association of COL1A2 (rs412777) gene with the development of osteoporosis.

References

- [1] Ichikawa S, Koller DL, Johnson ML.(2006). Human ALOX12, but not ALOX15, is associated with BMD in white men and women. *J Bone Miner Res.*;21:556–564.
- [2] Rahnavard Z, Zolfaghari M, Hossein-Nezad A, VahidDastgerdi M.(2009). The incidence of osteoporotic Hip fracture: Iranian Multicenter Osteoporosis Study (IMOS). *Res J Biol Sci.*;4:171–173.
- [3] Harsløf T, Husted LB, Nyegaard M.(2011). Polymorphisms in the ALOX12 gene and osteoporosis. *Osteoporos Int.*;22:2249–2259.
- [4] Xiao WJ, Ke YH, He JW.(2012). Polymorphisms in the human ALOX12 and ALOX15 genes are associated with peak bone mineral density in Chinese nuclear families. *Osteoporos Int.*;23:1889–1897.
- [5] Saito M, Marumo K(2010). Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. *Osteoporos Int.*; 21(2): 195–214.
- [6] Wang X, Bank RA, TeKoppele JM, (2001). The role of collagen in determining bone mechanical properties. *J Orthop Res.*; 19(6): 1021–1026,
- [7] Wang X, Shen X, Li X, (2002). Age-related changes in the collagen network and toughness of bone. *Bone.*; 31(1): 1–7, indexed in Pubmed:12110404.
- [8] Majchrzycki M, Bartkowiak-Wieczorek J, Wolski H,(2015). Polymorphisms of collagen 1A1 (COL1A1) gene and their relation to bone mineral density in postmenopausal women. *Ginekol Pol.*; 86(12): 907–914,
- [9] Majchrzycki M, Bartkowiak-Wieczorek J, Bogacz A, Szyfter-Harris J, Wolski H, Klejewski A, Goch M, Drews K, Barlik M, Ożarowski M, Kamiński A, Gryszczyńska A, Seremak-Mrozikiewicz A. (2017). The importance of polymorphic variants of collagen 1A2 gene (COL1A2) in the development of osteopenia and osteoporosis in postmenopausal women. *Ginekol Pol.*;88(8):414-420.
- [10] Augusciak-Duma A, Witecka J, Sieron AL, Janeczko M, Pietrzyk JJ, Ochman K, Galicka A, Borszewska-Kornacka MK, Pilch J, Jakubowska-Pietkiewicz E. (2018). Mutations in the COL1A1 and COL1A2 genes associated with osteogenesis imperfecta (OI) types I or III. *Acta Biochim Pol.*;65(1):79-86.
- [11] Lindahl K, Rubin CJ, Brandstrom H, Karlsson MK, Holmberg A, Ohlsson C.(2009). Heterozygosity for a coding SNP in COL1A2 confers a lower BMD and an increased stroke risk. *Biochem Biophys Res Commun.*;384:501–5.
- [12] Lau EM, Choy DT, Li M, Woo J, Chung T, Sham A. (2004). The relationship between COL1A1 polymorphisms (Sp1) and COL1A2 polymorphisms (Eco R1 and Puv II) with bone mineral density in Chinese men and women. *Calcif Tissue Int.*;75:133–7.
- [13] Richards JB, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N, Wilson SG.(2008). Bone

mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet*.;371:1505–12.

- [14] Hu, W. W., He, J. W., Zhang, H., Wang, C., Gu, J. M., Yue, H., Ke, Y. H., Hu, Y. Q., Fu, W. Z., Li, M., Liu, Y. J., & Zhang, Z. L. (2011). No association between polymorphisms and haplotypes of COL1A1 and COL1A2 genes and osteoporotic fracture in postmenopausal Chinese women. *ActapharmacologicaSinica*, 32(7), 947–955.
- [15] Ryan-Moore E, Mavrommatis Y, Waldron M. (2020). Systematic Review and Meta-Analysis of Candidate Gene Association Studies With Fracture Risk in Physically Active Participants. *Front Genet*. Jun 16;11:551.
- [16] Blades, H. Z., Arundel, P., Carlino, W. A., Dalton, A., Crook, J. S., Freeman, J. V. (2010). Collagen gene polymorphisms influence fracture risk and bone mass acquisition during childhood and adolescent growth. *Bone* 47, 989–994.
- [17] Suuriniemi, M., Mahonen, A., Kovanen, V., Alén, M., and Cheng, S. (2003). Relation of Pvu II site polymorphism in the COL1A2 gene to the risk of fractures in prepubertal Finnish girls. *Physiol. Genom.* 14, 217–224.
- [18] Curtis, E. M., van der Velde, R., Moon, R. J., van den Bergh, J. P. W., Geusens, P., de Vries, F., et al. (2016). Epidemiology of fractures in the United Kingdom 1988-2012: variation with age, sex, geography, ethnicity and socioeconomic status. *Bone* 87, 19–26.