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The importance of polymorphic variants of collagen 1A2 gene (COL1A2) in the development of osteopenia and osteoporosis in postmenopausal women

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ABSTRACT

Objectives: 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. The aim of this study is to determine whether *Eco*RI, *Del*38 and *Pvu*II polymorphisms of *COL1A2* are associated with the development of osteoporosis and osteopenia in postmenopausal Polish women. Moreover, analysis of relationship between frequency of *COL1A2* gene polymorphic variants and clinical parameters of bone turnover and degree of osteoporosis was performed.

Material and methods: The study group comprised of women with osteoporosis (n = 90), osteopenia (n = 56) and healthy individuals (n = 56). The *Eco*RI, *Del*38 and *Pvu*II polymorphisms in *COL1A2* gene were detected by PCR-RFLP method.

Results: In women with osteoporosis the TT genotype of *Eco*RI polymorphism had the lowest Z-score value compared to other genotypes (p = 0.034). In case of *Del*28 polymorphism, there was a statistically significant correlation between lower BMI values and the DD genotype in women with osteopenia (p = 0.041). There was no statistically significant correlation between polymorphic variants of *Del*28 polymorphism and clinical parameters of women with osteoporosis. The analysis of *Pvu*II polymorphism showed that in women with osteopenia the CC genotype had the lowest body weight compared to other genotypes (p = 0.039). *Pvu*II polymorphism and clinical parameters in the group of women with osteoporosis had no statistically significant correlations.

Conclusions: The analyzed COL1A2 polymorphisms seem to be related to osteoporosis development and their particular clinical parameters. Hence, the *COL1A2* polymorphism may be a genetic risk factor related to the development of osteoporosis.

Key words: *COL1A2*, polymorphism, osteoporosis, osteopenia, genetic marker

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INTRODUCTION

Understanding the mechanism responsible for a low bone mass as a main risk factor for fractures, and establishing the genetic framework that determines the specific anatomic features of bones may help explain its role in the development of osteoporosis. Clinical studies, gene polymorphism analysis, genome-wide association studies (GWAS) and whole-exome sequencing, all suggest that osteoporosis has a strong genetic component [1].

The difficulty lies in the multifactorial character of osteoporotic changes because genetic factors, environmental factors and gene-environment interactions contribute to the risk of developing an osteoporotic fracture [2]. Many researchers and physicians try to find the most important polymorphic variants of selected genes that are responsible for bone mineral density and deterioration of bone quality.

Collagen alters in the course of bone disease and is involved in the pathogenesis of osteoporosis [3]. The collagen network plays an important role in bone toughness and in age-related changes in bone quality [4]. 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 [5]. It has been shown that *COL1A1* and *COL1A2* candidate genes are important factors in osteopenia and osteoporosis development and may influence bone metabolism [6].

COL1A2 gene encodes the pro-alpha2 chain of type I collagen, which is a major component of bone extracellular matrix. The results of molecular genetic studies suggest that mutations in this gene are associated with osteogenesis imperfecta types I-IV, Ehlers-Danlos syndrome type VIIB, recessive Ehlers-Danlos syndrome classical type, idiopathic osteoporosis, and atypical Marfan syndrome [7].

The aim of this study is to assess the frequency of *Eco*RI, *Del*38 and *Pvu*II polymorphisms of *COL1A2* gene in the studied groups of women. Additionally, the analysis of relationship between the studied polymorphic variants of *COL1A2* gene and the clinical parameters of bone turnover and the stage of osteoporosis development is performed.

MATERIAL AND METHODS

Study groups

This study comprises 236 unrelated Caucasian postmenopausal women from Poland. Patients were divided into three subgroups given the t-score value: 90 osteoporotic women (t-score ≤ –2.5, mean age: 57.55 ± 7.99 years), 90 women with osteopenia (t-score between –2.5 and –1, mean age: 54.39 ± 7.76 years), and 56 healthy women (t-score > –1, mean age: 55.49 ± 8.15 years). Having been in menopause for at least one year was an eligibility criterion. The study included the clinical parameters such as: T-score, Z-score, weight [kg], height [cm], BMI [kg/m^2], age, birth weight, reproductive years, age of first menstruation, age of last menstruation, number of pregnancies, years after menopause, BMD L2–L4 [g/cm^2], BMD L2–L4 YA [%] and BMD L2–L4 AM [%]. Bone mineral density was estimated from the lumbar spine L2 to L4 vertebrae using DEXA scan method (Dual Energy X-ray Absorptiometry). Densitometry was performed using the LUNAR DPX 100 camera (Lunar Corp., Madison, USA). The exclusion criteria include hormone replacement therapy and therapies that affect the bone mass (such as selective estrogen receptor modulators, calcitonin, bisphosphonates, heparin steroids, thyroid hormones, antiepileptic drugs, GnRH analogue, tibolone). Other exclusion criteria are bilateral ovariectomy, endocrine and metabolic disorders, cancer and autoimmune diseases. The clinical data of postmenopausal women are presented in Table I.

Table 1. Characteristics of the study population (postmenopausal women with osteopenia, osteoporosis and normal T-score)

		P	Mean	SEM
T-score	Osteopenia		–1.831	0.043
	Osteoporosis	< 0001[b]	–3.166	0.052
	Controls		0.139	0.114
	Total		–1.874	0.081
Z-score	Osteopenia	0.102[a]	–0.804	0.075
	Osteoporosis	< 0001[b]	–3.032	1.847
	Controls		0.644	0.175
	Total		–1.310	0.817
Weight [kg]	Osteopenia	0.036[a]	65.171	0.975
	Osteoporosis	0.001[b]	60.208	0.943
	Controls		68.723	1.432
	Total		64.724	0.657
Height [cm]	Osteopenia	0.07[a]	162.800	0.422
	Osteoporosis	0.01[b]	159.911	0.543
	Controls		163.107	0.712
	Total		161.771	0.343
BMI	Osteopenia	0.03[a]	24.592	0.342
	Osteoporosis	0.04[b]	23.626	0.334
	Controls		25.971	0.367
	Total		24.552	0.254
Birth weight [g]	Osteopenia	0.046[a]	3216.153	75.484
	Osteoporosis	0.005[b]	3141.250	134.081
	Controls		3633.333	100.173
	Total		3321.333	53.235
Reproductive years	Osteopenia	0.644[a]	36.433	0.682
	Osteoporosis	0.438[b]	35.622	0.660
	Controls		37.107	0.986
	Total		36.283	0.404

Table 1 (cont.). Characteristics of the study population (postmenopausal women with osteopenia, osteoporosis and normal T-score)

		P	Mean	SEM
Age of first menstruation	Osteopenia	0.536[a]	13.178	0.308
	Osteoporosis	0.644[b]	13.244	0.143
	Controls		13.303	0.224
	Total		13.233	0.117
Age of last menstruation	Osteopenia	0.053[a]	49.688	0.588
	Osteoporosis	0.068[b]	48.189	0.504
	Controls		50.607	0.612
	Total		49.334	0.326
Number of pregnancies	Osteopenia	0.745[a]	1.867	0.081
	Osteoporosis	0.643[b]	1.922	0.225
	Controls		1.964	0.140
	Total		1.911	0.065
Years after menopause	Osteopenia	0.654[a]	8.600	0.769
	Osteoporosis	0.001[b]	10.322	0.615
	Controls		8.964	0.832
	Total		9.343	0.309
BMD L2-L4 [g/cm^2]	Osteopenia	0.686[a]	0.974	0.013
	Osteoporosis	0.874 [b]	0.817	0.012
	Controls		1.221	0.021
	Total		0.973	0.011
BMD L2-L4 YA [%]	Osteopenia	0.735[a]	81.144	1.532
	Osteoporosis	0.682[b]	68.255	1.241
	Controls		102.125	1.353
	Total		81.207	0.743
BMD L2-L4 AM [%]	Osteopenia	0.587[a]	89.544	1.717
	Osteoporosis	0.568 [b]	78.622	1.064
	Controls		110.500	1.765
	Total		90.35	0.536

a — Comparison between the groups with osteopenia and normal T-score (one-way ANOVA)
b — Comparison between the groups with osteoporosis and normal T-score (one-way ANOVA)

The study was approved by Local Bioethical Committee of Poznan University of Medical Sciences (1415/03, 158/06). All women were informed about the aim of conducted research and gave their written consent.

Detection of *COL1A2* gene polymorphism by PCR-RFLP

The genetic analysis of *COL1A2* gene polymorphism was conducted in the Laboratory of Experimental Pharmacogenetics at Chair and Department of Clinical Pharmacy and Biopharmacy, Poznan University of Medical Sciences. Peripheral blood samples (5 mL) were collected in tubes with EDTA and stored at -20°C. A commercial set QIAamp DNA Blood Mini Kit (Qiagen, USA) was used to isolate peripheral blood leukocyte DNA. To detect *COL1A2* gene polymorphism an allele-specific PCR procedure was performed using the following primers: for *Del*: 5'- TCA GTG TAT GTT GCT ATC AG-3' and 5-ATT CCA CAG TCA ACA TCA AC-3'; for *EcoR*1: 5'-GGA CTA TGA GAG TCT GTG A-3' and 5'-TGT TTG ACC TGG AGT TCC AT-3'; for *Pvu*II: 5'-GGG ATA TAA GGA TAC ACT AGA GG-3' and 5'-GAA ATA TCG GCC CCG CTG GAA-3'. In order to examine the *Eco*RI and *Pvu*II polymorphisms in *COL1A2* gene, the restriction enzymes such as *EcoR*1 and *Pvu*II were used. The products of PCR-RFLP reaction were subjected to electrophoretic separation in 2.75% agarose gel. The analysis of digestion products was performed by visualization in UV light using documentation and computer image analysis system UVI-KS4000/Image PC manufactured by Syngen Biotech Molecular Biology Instruments.

Statistical analysis was performed using SPSS 17.0 PL program. We used the Hardy-Weinberg equilibrium to calculate the expected genotype frequency for each polymorphism, which was compared with the observed values using the chi-square test. The expected results are presented with 95% confidence intervals (CI). We also calculated the odds ratio (OR) for the genotypes and the alleles. Next, we evaluated the effect of the *COL1A2* polymorphism on T-score, Z-score, L2L4AM, L2L4YA, L2L4BMD, BMI, and other clinical parameters. Correlation analysis between genotypes and clinical parameters using one-way ANOVA test was performed. Value of $p < 0.05$ was considered statistically significant.

RESULTS

The distribution of genotype frequency was consistent with the Hardy-Weinberg equilibrium for *COL1A2 Eco*RI and *Pvu*II polymorphisms (Tables 2 and 3). When analyzing *Eco*RI polymorphism it was observed that the CT genotype was more frequent in women with osteoporosis than in women with normal T-score values (55.6% *vs*. 44.6%, OR = 2.06, CI: 0.97-4.38, p = 0.03). In addition, the CC genotype was more frequent in women with normal T-score (39.3%) compared to women with osteoporosis (39.3% *vs*. 32.2%, OR = 0.73, CI: 0.34-1.56, p = 0.24).

Furthermore, the analysis of *COL1A2 Del*38 polymorphism showed that the II genotype was more frequent in women with normal T-score values (57.1%) than in women with osteopenia (51.1%) or osteoporosis (46.7%). Similarly, the genotype DD was more frequent (14.3%) in postmenopausal women with normal T-score values than in women with osteopenia (8.9%) or osteoporosis (6.7%) (Tables 2 and 3). Increased OR values in heterozygotes for the *Del*38 polymorphism were noted in both: women with osteopenia (OR = 1.67, CI: 0.77-3.67, p = 0.11) and osteoporosis (OR = 2.18, CI: 1.02-4.79, p = 0.02).

In case of the *COL1A2 Pvu*II polymorphism, it has been observed that the AC genotype was less frequent in women

Table 2. Frequency of the genotype and allele of the *COL1A2 EcoR*1, *Del38*, *PvuII* polymorphisms in women with osteopenia and controls (Mann-Whitney U test)

Genotype/Allele	Osteopenia		Controls		OR	95% CI	P
	Observed value n (%)	Expected value [%]	Observed value n (%)	Expected value [%]			
*EcoR*I							
CC	36 (40.00)	40.11	22 (39.30)	37.96	1.03	0.49–2.16	0.54
CT	42 (46.70)	46.44	25 (44.60)	47.30	1.08	0.52–2.24	0.47
TT	12 (13.30)	13.45	9 (16.10)	14.74	0.80	0.28–2.33	0.41
Total	90 (100)	100	56 (100)	100	–	–	–
C	114 (63.33)	–	69 (61.61)	–	1.07	0.64–1.80	0.43
T	66 (36.67)	–	43 (38.39)	–	0.93	0.55–1.56	0.43
Total	180 (100)	–	112 (100)	–	–	–	–
Del38							
II	46 (51.10)	50.57	32 (57.14)	51.02	0.78	0.37–1.62	0.29
ID	36 (40.00)	41.09	16 (28.58)	40.82	1.67	0.77–3.67	0.11
ID	8 (8.90)	8.34	8 (14.28)	8.16	0.58	0.17–1.92	0.23
Total	90 (100)	100	56 (100)	100	–	–	–
I	128 (71.11)	–	80 (71.43)	–	0.98	0.56–1.71	0.53
D	52 (28.89)	–	32 (28.57)	–	1.01	0.58–1.78	0.53
Total	180 (100)	–	112 (100)	–	–	–	–
PvuII							
AA	38 (42.20)	43.71	27 (48.20)	44.84	0.78	0.25–2.15	0.29
AC	43 (47.80)	44.81	21 (37.50)	44.24	1.52	0.73–3.20	0.14
CC	9 (10.00)	11.48	8 (14.30)	10.92	0.67	0.21–2.14	0.29
Total	90 (100)	100	56 (100)	100	–	–	–
A	119 (66.11)	–	75 (66.96)	–	0.96	0.56–1.63	0.49
C	66 (33.89)	–	37 (33.04)	–	1.04	0.61–1.77	0.49
Total	180 (100)	–	112 (100)	–	–	–	–

with normal T-score values (37.5%) compared with women with osteopenia (47.8%) or osteoporosis (46.7%), while the CC genotype was more frequent in women with normal T-score values (14.3%) compared to women with osteopenia or osteoporosis (Tables 2 and 3).

Additionally, the genotype analysis for *COL1A2 EcoR*I polymorphism found no statistically significant correlations with clinical features between the women with osteopenia and the controls. There was a statistically insignificant tendency towards higher Z-score values in women with the CC genotype (–7.6) for *EcoR*I polymorphism compared to individuals with the CT (–8.1) and TT (–9.1) genotypes. In case of *Del*28 polymorphism, there was a statistically significant correlation ($p = 0.041$) between lower BMI values (22.9 kg/m^2) in women with the DD genotype compared to the ID (25.6 kg/m^2) and the II (24.1 kg/m^2) genotypes. Furthermore, the analysis of *Pvu*II polymorphism showed that women with the CC genotype had the lowest body weight (59.6 kg) compared with the AC (67.5 kg) and the AA (63.8 kg) genotypes ($p = 0.039$).

Furthermore, in women with osteoporosis, there were statistically significant correlations between polymorphic variants of *EcoR*I polymorphism of *COL1A2* gene and Z-score values ($p = 0.034$). The TT genotype patients had the lowest Z-score values compared to other genotypes (TT: –2.76 *vs*. CT: –1.7 and CC: –1.6, $p < 0.05$). Other correlations between *EcoR*I polymorphism and diagnostic parameters were not statistically significant.

Moreover, the correlations between the *Del*28 polymorphic variants and clinical parameters in women with osteoporosis were also not statistically significant. However, a tendency was observed for lower Z-score values in patients with ID variant (-4.66) compared to the DD (–1.95) and the II (–1.56) genotypes. There was also a statistically insignificant correlation between the length of reproductive period and the distribution of genotypes for *Del*28 polymorphism (DD: 39 years *vs*. ID: 35 years and II: 35 years).

Also, no statistically significant correlations were observed for *Pvu*II polymorphism of *COL1A2* gene and clinical parame-

Table 3. Frequency of the genotype and allele of the *COL1A2 EcoR*1, *Del38*, *PvuII* polymorphisms in women with osteoporosis and controls

Genotype/Allele	Osteoporosis		Controls		OR	95% CI	P
	Observed value n (%)	Expected value [%]	Observed value n (%)	Expected value [%]			
EcoRI							
CC	29 (32.20)	36.00	22 (39.30)	37.96	0.73	0.34–1.56	0.24
CT	50 (55.60)	48.00	25 (44.60)	47.30	2.06	0.97–4.38	0.03
TT	11 (12.20)	16.00	9 (16.10)	14.74	0.72	0.25–2.15	0.33
Total	90 (100)	100	56 (100)	100	–	–	–
C	108 (60.00)	–	69 (61.61)	–	0.94	0.56–1.56	0.44
T	72 (40.00)	–	43 (38.39)	–	1.07	0.64–1.79	0.44
Total	180 (100)	–	112 (100)	–	–	–	–
Del38							
II	42 (46.70)	49.00	32 (57.14)	51.02	0.65	0.32–1.35	0.14
ID	42 (46.70)	42.00	16 (28.57)	40.82	2.18	1.02–4.79	0.02
ID	6 (6.60)	9.00	8 (14.28)	8.16	0.42	0.12–1.51	0.11
Total	90 (100)	100	56 (100)	100	–	–	–
I	126 (70.00)	–	80 (71.43)	–	0.93	0.53–1.62	0.45
D	54 (30.00)	–	32 (28.57)	–	1.07	0.62–1.87	0.45
Total	180 (100)	–	112 (100)	–	–	–	–
PvuII							
AA	42 (46.70)	48.00	27 (48.20)	44.84	0.94	0.45–1.94	0.49
AC	42 (46.70)	42.00	21 (37.50)	44.24	1.45	0.70–3.06	0.18
CC	6 (6.60)	9.00	8 (14.30)	10.92	0.43	0.11–1.51	0.11
Total	90 (100)	100	56 (100)	100	–	–	–
A	126 (70.00)	–	75 (66.96)	–	1.15	0.67–1.96	0.33
C	54 (30.00)	–	37 (33.04)	–	0.87	0.51–1.49	0.33
Total	180 (100)	–	112 (100)	–	–	–	–

ters in the group of women with osteoporosis. Analysis showed a tendency towards lower Z-score values in women with the AA genotype compared to other genotypes (AA: –4.71 *vs.* AC: –1.56 and CC: –1.67). The highest birth weight was noted in women with the CC genotype (CC: 3400 g *vs.* AC: 3197 g and AA: 2978 g). Moreover, the longest reproductive period was observed in women with the CC genotype (38 years) compared to patients with AC and AA genotypes (35 years).

DISCUSSION

The analysis of polymorphic variability of protein coding genes, which take part in the processes of bone turnover and in the stabilization of the skeletal system is an important aspect of research concerning osteoporosis and bone disease. Many polymorphic sequence variants in the type I collagen gene (*COL1A1* and *COL1A2*) has been found and described so far [8, 9]. Additionally, researchers have described the implication that the variants' have in changes of the skeletal system and in the development of osteogenesis imperfecta or osteoporosis [6, 7, 10].

In this study we have analyzed the relationship between the *Eco*RI, *Pvu*II and *Del*38 polymorphism of *COL1A2* gene and clinical and bone parameters, as well as we have estimated the incidence rate of osteoporosis.

In case of the *Eco*RI polymorphism we have shown a relationship between heterozygous TC, homozygous TT variants, decreased bone mineral density and the development of osteoporosis. The results obtained by our research team were consistent with the Hardy–Weinberg equilibrium but were not statistically significant. However, statistically significant correlation for this polymorphism was obtained for pregnancy-related clinical parameters. The observed decreased bone mass in the carriers of T allele may suggest its correlation with the development of osteoporosis, which requires a statistically significant confirmation on a larger study group. So far, very few studies were performed in the field of influence of *Eco*R1 of the *COL1A2* gene on the creation of bone mass in postmenopausal women leaving its influence on the bone mass not confirmed [11]. This polymorphism was analyzed intensively and was used as

an anthropogenic marker in the search for ethnicity-based inter-population variance. Pepe et al. have used genotyping of *COL1A2* with *EcoR*I enzyme to differentiate ethnically American population living in Cayapa region: the indigenous from the Asian descendants [12]. In a later study, same authors have differentiated ethnically four West African and two Asian populations [13]. As a result, it was shown that *EcoR*1 polymorphism of the *COL1A2* gene was a highly distinctive population marker making it possible to differentiate ethnically human populations. Population analysis based on this polymorphism was used to differentiate human populations in Asia, Africa and America by other researchers as well [14, 15, 16]. In case of Europeans in contrast, the analyses based on *EcoR*I polymorphism, among others, has shown a high genetic homogeneity [17]. Our study on the influence of *EcoR*I polymorphism of the *COL1A2* gene on the osteoporotic changes is one of the first studies on its relationship with osteoporosis.

Mechanisms underlying the influence of individual *Pvu*II polymorphic sequence variants in the *COL1A2* gene on the risk of fractures remain unknown. Changes in the sequence from CpA to CpC described as a transversion in exon 25 had no effect on the coding of the rest of proline in position 392 but a relationship has been found between this polymorphism and osteogenesis imperfecta or osteoporosis [18, 19]. The analysis of *Pvu*II polymorphism carried out by our research team showed that the AA (PP) genotype was correlated in a statistically significant way with a lower value of Z-score in women with osteoporosis and with lower birth weight of these women. Numerous studies have shown that a lower birth weight was associated with a predisposition to many diseases in the adult life, e.g. hypertension, insulin resistance, ischemia and lowered bone mass [20-*23]*. AA (PP) homozygotes were also evident to have a shorter reproduction period. That must have led to negative changes in hormonal balance and in skeletal system in the carriers, since a long reproductive period is known to have a protective factor in osteoporosis development *[24]*. These observations were confirmed in a study carried out on a population of adolescent females. Girls with PP genotype had a 4.9-fold higher relative risk of fracture than girls with pp (CC) genotype ($p = 0.015$) [25].

The study on the *Del*38 polymorphism presented in this paper and the search for a relationship between individual polymorphic variants, bone mass and incidence of osteoporosis fills a gap in the literature, since available studies concentrate on this polymorphism mainly in light of population biomarker or liver cancer development [26, 27]. In our study we have observed that genotype II was more common in healthy women as opposed to DD genotype, which was associated with osteopenia and osteoporosis. It has to be emphasized that our search for association between the development of osteoporosis and *Del*38 polymorphism, whether in Polish, European or world population, is one of the very first of its kind.

CONCLUSIONS

In our study we report some significant associations between the *EcoR*I, *Pvu*II and *Del38* polymorphisms of *COL1A2* gene and clinical and bone parameters together with the incidence rate of osteoporosis.

Based on the presented data, it is probable that the *COL1A2* polymorphism has a positive association with osteoporosis development. We conclude that the relationship between the studied *COL1A2* polymorphisms and clinical and bone parameters with incidence rate of osteoporosis requires a statistically significant confirmation on a larger study group.

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