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BONE MINERAL DENSITY AND THE 570A>T POLYMORPHISM OF THE BONE MORPHOGENETIC PROTEIN 2 (*BMP2*) GENE IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE: A CROSS-SECTIONAL STUDY

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Finding genetic predictors of osteoporosis and fractures in patients with inflammatory bowel disease (IBD) may provide incentives for non-pharmacological actions and so improve the long-term prognosis of the patients. We analysed the incidence of *BMP2* 570A>T polymorphic variants and their association with bone mineral density (BMD) and the incidence of fractures in patients with IBD. The study comprised 198 IBD patients (100 with Crohn's disease (CD), and 98 with ulcerative colitis, (UC)) and 41 healthy controls. Bone densitometric analysis was carried out using the DXA method. The 570A>T polymorphisms in the *BMP2* gene were genotyped using RFLP. We found significant differences in the BMD and T-scores of the lumbar spine (L2-L4) and femoral neck between the three groups. In controls and CD patients, the highest L2-L4 BMD was found in carriers of the AA variant of the *BMP2* gene, while among UC patients it was the case of TT carriers. In both femoral neck and lumbar spine among UC patients, the highest BMD was observed in carriers of the TT variant of the *BMP2* gene. Among patients with CD and in the control group, the highest L2-L4 BMD was found in carriers of the AA variant, whereas in UC patients, it was the case of TT homozygotes. Within the femoral neck, there were no significant differences in BMD for the carriers of individual variants of *BMP2* gene polymorphism. We conclude that the 570A>T polymorphism of the *BMP2* gene, no statistically significant relationship was observed between the polymorphic variant and bone mineral density or the incidence of fractures in IBD patients.

Key words: *inflammatory bowel disease, crohn's disease, ulcerative colitis, osteoporosis, bone morphogenetic protein 2 (BMP2), 570A>T BMP2 gene polymorphism, bone mineral density*

INTRODUCTION

Inflammatory bowel disease (IBD) comprise ulcerative colitis (UC) and Crohn's disease (CD). Both diseases affect primarily inhabitants of developed countries in Europe and North America, and rarely occur in Africa, South America or Asia (1). The global incidence of UC varies from 0.5 to 24.5 per 100,000 inhabitants and that of CD from 0.1 to 16 per 100,000 inhabitants (1). A multicentre study conducted in 1991 – 1993 in twelve Western European countries and Israel estimated the incidence of UC and CD at 10.4 and 5.6 per 100,000 inhabitants, respectively (2, 3). In Eastern Europe, the incidence of IBD increased considerably in the late 1980s and in the 1990s. Detailed data on the incidence on Crohn's disease in Poland have been available from 2005, when the National Register of Crohn's disease was established. In 2008, the number of patients was estimated for 5000. The disease affected more frequently individuals between 16 and 40 years of age, with a secondary or university education, living in cities. In the 2015 year, 6114 new cases of Crohn's disease in Poland were registered (4, 5). There are no detailed epidemiological data as to the incidence of ulcerative colitis in the Polish population.

One of the many clinical consequences of these diseases is the development of secondary osteoporosis and an increased risk of bone fractures. Osteoporosis is defined as a bone architecture disorder with a reduction in the bone mass (a reduction in the bone mineral density, BMD) leading to weakening of bones and an increased risk of fractures. In 2010 in Europe, osteoporosis occurred in 22 million women and 5.5 million men, and the number of deaths due to osteoporotic fractures amounted to 43,000 (6). Osteoporotic fractures are a particular complication of osteoporosis of the spine (about 560,000 per year), of the proximal end of the femur (about 300,000 per year) and of the forearm (about 250,000), and constitute one of the most common causes of disability and a major factor contributing to the increasing cost of medical care (7, 8). One in two women and one in four men over the age of 50 suffer from an osteoporotic fracture during their lifetime. The risk of fractures in female

Caucasians aged 50 years is about 40%. The prevalence in men is less frequent and is approximately 13% (9). It should be emphasised that there are other BMD-independent risk factors that are also important. These include: age, previous fractures, premature menopause, family history of femoral neck fracture and the use of oral corticosteroids (10). However, if physicians can identify patients at risk for fracture, prevention programs may be initiated to reduce the number of fractures sustained. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture (11, 12).

The pathogenesis of bone loss in patients with IBD is complex and incompletely understood. The pathophysiology of IBD-related osteoporosis is multifactorial; however, risk factors such as steroid treatment, calcium and vitamin D deficiencies, malnutrition and genetic factors are known to be involved (13-17). On the other hand, it is also believed that one of the etiological factors of bone loss in IBD is a systemic effect of chronic inflammation. This is driven mainly by several pro-inflammatory mediators, like for example tumor necrosis factor-α (TNF-α), which was showed to be elevated in the presence of several extraintestinal manifestations both in CD and UC (18).

There have been few population-based cohort studies evaluating the prevalence of metabolic bone disease in IBD and on the specific risk factors for low BMD in IBD. Several studies have implicated the presence of a low BMD in patients with IBD whereas others have not (13, 17, 19-22). Therefore, studies linking the common pathogenesis of IBD and osteoporosis are of particular importance. Secondary causes of osteoporosis, which include IBD, are the reason for the occurrence of the disease in younger age groups, often with a higher incidence in men. The stereotypical perception of osteoporosis as a disease of older women leads to a delay in the diagnosis of the disease. Therefore, it is difficult to implement prophylaxis and prevent low-energy fractures. Finding a genetic predictor of osteoporosis and fractures in this unique group may increase incentives for non-pharmacological actions and improve long-term prognosis. The occurrence of IBD during the building of peak bone mass may affect its quality. Regulation of increase in BMD and peak bone mass depend on genetic factors. Osteoporosis is, therefore, a chronic disease of polygenic inheritance, in which the effects of many genes over a lifetime are modified by a number of environmental factors and chronic diseases. Development of osteoporosis is attributed in 40 – 80% to genetic factors (23). BMP2 belongs to the family of transforming growth factors (TGF-β) and is secreted by osteoblasts (24). It is involved in bone tissue metabolism and is essential for the proper formation of the skeleton. The *BMP2* gene is located on the short arm of chromosome 20 (20p12) and is composed of three exons. The functional protein is coded by exons 2 and 3 (25). The promoter region of the gene contains the shear stress response element (SSRE), which induces the *BMP2* mRNA expression in response to mechanical stress (26). BMP2, in an inactive form, is produced as a polypeptide precursor of pro-BMP2 (27). The active form of BMP2 participates in bone tissue metabolism, after binding with a specific serine/threonine kinase receptor on the surface of the target cell (28). BMP2 has an effect on the bone and cartilage, stimulating their formation especially in young people during the peak bone mass formation period. The impairment of this process, especially in young IBD patients, may be of significance in the development of the peak bone mass. BMP2 is responsible for processes relating to the metabolism of bone tissue: osteogenesis, bone and cartilage morphogenesis, and the regeneration and mineralisation of the bones (29-31). Any changes in the synthesis of BMP2, its structure, stability, function, activity or receptor affinity due to polymorphisms may affect the mineral density of the bone and its spatial structure, and therefore cause an increased susceptibility to fractures. Polymorphisms of the BMP2 gene is potentially considered to be a predisposing factor for the development of osteoporosis (32). Among all the studied human bone morphogenetic proteins, BMP2 has the highest osteoinducing potential (33-35). It is very much involved in the process of the morphogenesis of limb buds and the formation of the entire skeletal system (36). BMP2 primarily affects the process of bone formation and osteoblast differentiation, as well as the attainment of the peak bone mass (37). A functional BMP2 is essential for osteogenesis (36). It was also reported that BMP2 can be used as a treatment to accelerate bone growth following fractures and especially in surgical osteotomy for the treatment of bone defects (34, 35, 38-40). Genetic testing in osteoporosis is one of the means to asses individual risk of the disease progression and fractures, and may be a way of seeking suitable therapy in the future. Genetic profile identifying the risk of low bone mass and susceptibility to fractures, although difficult to identity, may become a premise for use or intensification of therapy and greater motivation for preventive actions. Besides, recent studies have shown that BMP plays an important role in the process of osteogenesis by the induction of the differentiation and maturation of osteoblasts and modulation of the ossification process (41, 42). Knowledge of the role of the bone morphogenetic protein 2 (BMP2) in bone metabolism was a premise for the search of the relationship between polymorphic variants of *BMP2*, bone mass values and the incidence of hip fractures in patients with IBD we conducted in this study.

MATERIALS AND METHODS

Aim of the study

The objective of this study was to determine the incidence of polymorphic variants of the *BMP2* gene in Polish IBD patients and a possible relationship between the polymorphisms of the *BMP2* gene and bone mineral density, and the incidence of fractures in these patients.

Study subjects

Study was approved by the local Bioethical Committee and all patients gave their written consent to participate in the genetic testing.

The study group consisted of 198 patients with IBD including 100 patients with CD, and 98 patients with UC. The control group (CG) consisted of 41 healthy volunteers with no signs of osteoporosis (as estimated from BMD) and reporting no other health problems, coming from the same region as the patients and matched by age. The inclusion criteria were as follows: age between 18 and 60 years, diagnosis of IBD based on cross-sectional imaging and/or endoscopy with histopathological confirmation, disease duration over 1 year, lack of any other condition (e.g. rheumatoid arthritis, chronic renal failure). History was collected with regards to low-energy (osteoporotic) fractures in the patients and their parents; physical examination included height and weight measurements. Densitometric measurements of the lumbar spine (L1-L4) and femoral neck (FN) of the patients were carried out using the dual energy X-ray absorptiometry (DXA) with the Lunar DPX-Plus instrument. The following densitometric parameters were recorded and then taken into account in statistical analyses: bone mineral density (BMD), Z-score and T-score. Z-score is the difference between the obtained BMD measurement and mean BMD matched by age, divided by standard deviation in the general population. T-score is the difference between the obtained BMD measurement and mean BMD for young adults, divided by standard deviation for young adults.

DNA extraction and BMP2 variants' analysis

DNA was extracted from whole blood leukocytes using guanidine isothiocyanate and phenol-chloroform, dissolved in 1×TE buffer and stored at –20°C until use. The *BMP2* c.570A>T polymorphism (p.Arg190Ser, rs235768) genotyping was performed using restriction fragment length polymorphism (RFLP - Restriction Fragments Length Polymorphism) technique as described elsewhere (43, 44). In brief, exon 3 of the *BMP2* gene was amplified by PCR using the following primers:

sense 5'-CCCCACGGAGGAGTTTATCAC and antisense 5'-CCGGGGGAGCCACAATC (PCR product length 524 bp, Tm = 59°C).

Amplification products were then digested with the BseNI restriction enzyme (New England Biolabs). After the incubation, the sizes of the restriction fragments were estimated following their separation in a 1.6% agarose gel with ethidium bromide. Polymorphic *BMP2* c.570A variant has no restriction site for the BseNI enzyme (fragment size: 524 bp). Polymorphic variant c.570T generates a BseNI restriction site, giving two digestion products: 374 bp and 150 bp long.

Example of an agarose gel images for RFLP analysis for 24 different samples that represent the 3 polymorphic variants homozygous AA, heterozygous AT and homozygous TT were presented below. Due to results verification we used controls for each genotype, which were determined at the beginning of our studies by Sanger sequencing.

Statistical analysis

For each of the groups studied, the concordance of genotype distribution with the expected distribution according to the Hardy-Weinberg equilibrium law was analysed. An analysis of the allele dose, recessive interaction, and dominant interaction effect was carried out using the Chi-squared test of independence. In order to compare the sizes of the analysed parameters between the individual *BMP2* gene polymorphisms in the study groups, the uni-factorial analysis of variance (ANOVA) along with the Tukey post-hoc test were used. In the event of non-concordance of data with the normal distribution (as verified by Shapiro-Wilk tests) or in case of lack of homogeneity of variances (Levene's test), the Kruskal-Wallis test and the Dunn's post-hoc test were used. All analyses were performed using the STATISTICA 10.0 software (StatSoft) and the calculator on http://ihg.gsf.de/cgi-bin/hw/hwa1.pl website. P-values lower than 0.05 were considered as indicative of a statistical significance.

Table 1. Characteristics of the study subjects.

	CD patients	**UC patients**	**Controls**	**P-values**
Gender (F/M) (n,%)	(50,50%)/(50,50%)	(51,52%)/(47,48%)	(20,49%)/(21,51%)	P = 0.9519
n	100	98	41	
Age (years)	35.59 ± 12.79[1]	39.46 ± 14.69	30.37 ± 8.58	*P < 0.01 ***P < 0.01
Body mass (kg)	63.39 + 13.71	68.38 + 14.83	74.63 ± 14.07	**P* < 0.01 ***P* < 0.01 ***ns
Height (cm)	171.17 ± 10.19	171.01 ± 9.25	173.05 ± 9.25	ns
BMI (kg/m^2)	21.51 ± 3.72	23.29 ± 4.28	24.79 ± 3.51	**P* < 0.001 ***P* < 0.01 ***ns
L2-L4 BMD (g/cm^2)	1.11 ± 0.18	1.16 ± 0.14	1.23 ± 0.08	*ns **P < 0.001 ***P < 0.05
L2-L4 T-score	–0.90 + 1.45	–0.42 ± 1.15	0.12 ± 0.69	*ns **P < 0.001 ***P < 0.05
L2-L4 Z score S.D.	–0.12 ± 1.18	–0.12 ± 1.18	0.09 ± 0.64	*ns **P < 0.05 ***ns
FN BMD (g/cm^2)	0.94 ± 0.18	0.98 ± 1.18	1.08 ± 1.16	*ns **P < 0.001 ***P < 0.05
FN T-score	–0.64 ± 1.30	–0.31 ± 1.22	0.44 ± 1.02	*ns **P < 0.001 ***P < 0.01
FN Z-score	–0.25 ± 1.11	0.08 ± 1.06	0.38 ± 0.97	*ns **P < 0.01 ***ns
Bone fractures (n)%	(26) 25.24%	(29) 27.62%	(0) 0.00%	*ns **P < 0.001 ***P < 0.001

[1]All results are presented as means with standard deviations (S.D.), ns: non-significant. The number of asterisks denotes the groups included in the comparison as follows: * CD versus UC, ** CD versus controls, ***UC versus controls.

RESULTS

The characteristics of the study group are presented in *Table 1*.

Patients (mean age: 37.48 ± 13.85) included CD patients (age: 35.59 ± 12.79), among which 50 were women in their middle age (40.08 ± 14.21) and 50 were men in their middle-age (31.10 ± 9.35), as well as UC patients: (39.46 ± 14.69), including 51 middle-aged women (38.86 ± 14.53) and 47 middle-aged men (40.16 ± 15.02). Controls (mean age: 30.37 ± 8.58) included 20 middle-aged women (33.75 ± 10.83) and 21 middle-aged men (27.14 ± 3.65). Study groups were heterogeneous in terms of age. The age of the CG is statistically significantly younger than UC group.

The distribution of *BMP2* polymorphic variants among patients with Crohn's disease and ulcerative colitis as well as among controls was concordant with Hardy-Weinberg equilibrium (*Table 2*).

Table 3 shows the results of the analysis of the association of the *BMP2* gene 570A>T polymorphism with bone mineral density as well as T-score and Z-score indicators in the L2-L4 section of the spine and the proximal end of the femur (femoral neck).

There were no statistically significant differences in BMD, T-scores, and Z-scores between carriers of different *BMP2* polymorphic variants, neither among controls, nor among IBD patients. An analysis using the Kruskal-Wallis test showed differences depending on the *BMP2* polymorphic variants between CD and UC patients. A univariate analysis showed significant differences between subgroups for BMD, lumbar spine (L2-L4 level) T-score and BMD, and T-score for femoral

Table 2. Concordance of the *BMP2* polymorphic variants' distribution in ulcerative colitis (UC) patients, Crohn's disease (CD) patients and controls with Hardy-Weinberg equilibrium.

	***BMP2* gene 570A>T genotypes**			Chi^2 test P-value
	AA	AT	TT	
CD (n = 100)	10 (10%)	48 (48%)	42 (42%)	0.487
UC (n = 98)	14 (14%)	41 (42%)	43 (44%)	0.411
Controls (n = 41)	6 (15%)	14 (34%)	21 (51%)	0.176

Table 3. An association between BMD, T-score and Z-score in the L2-L4 section of the spine and proximal end of the femur (femoral neck), and *BMP2* 570A>T polymorphism in patients with Crohn's disease (CD) and ulcerative colitis (UC) as compared to healthy controls.

Group	L2-L4 BMD (g/cm^2)	L2-L4 T-score	L2-L4 Z-score	Femoral neck BMD (g/cm^2)	Femoral neck T-score (g/cm^2)	Femoral neck Z-score (g/cm^2)
CD (n = 100)	1.100 ± 0.180[1]	–0.930 ± 1.440	–0.490 ± 1.290	0.940 ± 0.170	–0.660 ± 1.280	–0.260 ± 1.100
AA (n = 48)	1.146 ± 0.048	0.690 ± 0.390	–0.330 ± 0.370	0.993 ± 0.054	–0.220 ± 0.380	0.100 ± 0.340
AT (n = 35)	1.074 ± 0.022	–1.140 ± 0.180	–0.580 ± 0.170	0.910 ± 0.024	–0.920 ± 0.170	–0.400 ± 0.150
TT (n = 17)	1.130 ± 0.024	0.740 ± 0.190	0.420 ± 0.180	0.961 ± 0.025	–0.480 ± 0.190	–0.170 ± 0.160
P-value	0.1497	0.2533	0.7123	0.3392	0.2058	0.4988
UC (n = 98)	1.170 ± 0.140	–0.420 ± 1.160	–0.150 ± 1.180	0.980 ± 0.150	–0.310 ± 1.190	0.070 ± 1.060
AA (n = 21)	1.148 ±0.041	0.560 ± 0.330	0.110 ± 0.310	0.946 ± 0.044	–0.580 ± 0.320	–0.12 ± 0.290
AT (n = 59)	1.157 ± 0.024	0.520 ± 0.190	0.150 ± 0.190	0.975 ± 0.026	-0.370 ± 0.190	0.100 ± 0.170
TT (n = 18)	1.182 ± 0.024	–0.290 ± 0.190	–0.170 ± 0.180	1.002 ± 0.025	–0.170 ± 0.180	0.110 ± 0.160
P-value	0.5116	0.4798	0.9818	0.5919	0.7441	0.9344
Controls (n = 41)	1.230 ± 0.080	0.120 ± 0.690	0.090 ± 0.640	1.080 ± 0.160	0.440 ± 1.020	0.380 ± 0.970
AA (n = 9)	1.263 ± 0.062	0.360 ± 0.500	0.190 ± 0.480	1.094 ± 0.067	0.520 ± 0.490	0.450 ± 0.440
AT (n = 22)	1.219 ± 0.041	0.040 ± 0.330	0.170 ± 0.310	1.054 ± 0.044	0.410 ± 0.320	0.480 ± 0.290
TT (n = 10)	1.222 ± 0.033	0.110 ± 0.270	0.010 ± 0.260	1.093 ± 0.036	0.440 ± 0.260	0.310 ± 0.230
P-value	0.4429	0.5847	0.4208	0.8339	0.7734	0.4777

[1]All results are presented as means with standard deviations (S.D.); P-value of Kruskal-Wallis test[1].

Table 4. An association between BMD. T-score and Z-score in the L2-L4 section of the spine and proximal end of the femur (femoral neck) in patients with Crohn's disease (CD), ulcerative colitis (UC) and healthy controls (CG) in three age categories (up to 30 years. 30 – 50 years and over 50 years).

age	L2-L4 BMD (g/cm^2)	L2-L4 T-score	L2-L4 Z-score	Femoral neck BMD (g/cm^2)	Femoral neck T-score (g/cm^2)	Femoral neck Z-score (g/cm^2)
CD up to 30 y. n = 37	1.113 ± 0.199	–0.899 ± 1.588	–0.53 ± 1.457	0.974 ± 0.189	–0.412 ± 1.377	–0.218 ± 1.255
UC up to 30 y. n = 40	1.176 ± 0.133	–0.282 ± 1.189	–0.091 ± 1.164	1.044 ± 0.163	0.196 ± 1.197	0.287 ± 1.129
CG up to 30 y. n = 26	1.212 ± 0.08	0.009 ± 0.683	–0.012 ± 0.543	1.096 ± 0.172	0.535 ± 1.141	0.414 ± 1.097
P-value	ns	P = 0.016 **P = 0.0203	ns	P = 0.0265 **P = 0.0298	P = 0.0119 **P = 0.0153	ns
CD 30 – 50 y. n = 45	1.127 ± 0.165	–0.723 ± 1.311	–0.578 ± 1.152	0.951 ± 0.16	–0.619 ± 1.161	–0.322 ± 1.067
UC 30 – 50 y. n = 32	1.179 ± 0.16	–0.386 ± 1.239	–0.278 ± 1.213	0.968 ± 0.134	–0.387 ± 1.06	–0.084 ± 0.999
CG 30 – 50 y. n = 12	1.269 ± 0.069	0.434 ± 0.652	0.352 ± 0.82	1.054 ± 0.108	0.283 ± 0.695	0.327 ± 0.696
P-value	P = 0.0105 **P = 0.0077	P = 0.0078 **P = 0.0055	P = 0.0190 **P = 0.0146	ns	P = 0.0244 **P = 0.0197	ns
CD more than 50 y. n = 18	1.052 ± 0.159	–1.244 ± 1.322	–0.063 ± 1.089	0.833 ± 0.148	–1.318 ± 1.166	–0.178 ± 0.798
UC more than 50 y. n = 26	1.127 ± 0.131	–0.684 ± 0.938	0.064 ± 1.17	0.909 ± 0.153	–0.98 ± 1.154	0.017 ± 1.049
CG more than 50 y. n = 3	1.132 ± 0.076	–0.57 ± 0.612	–0.24 ± 0.076	0.934 ± 0.976	–0.39 ± 0.751	0.09 ± 0.587
P-value	ns	ns	ns	ns	ns	ns

[1]All results are presented as means with standard deviations (S.D.). P-value of Kruskal-Wallis test[1].

neck. Post-hoc analyses confirmed the differences in BMD and T-score of the L2-L4 spine and in T-score of the femoral neck between ulcerative colitis patients and controls (*Table 1*).

The highest lumbar spine (L2-L4 level) bone mass among controls and CD patients was found in carriers of the AA *BMP2* variant, while in UC patients, the TT *BMP2* variant was associated with the highest lumbar spine bone mass. Within the proximal end of the femur (femoral neck), as well as in the lumbar vertebral bodies, the highest bone mass was observed in UC patients who were carriers of the TT variant of the *BMP2* gene.

Differences in bone metabolism existing between individuals during the peak bone mass formation as well as between pre- and postmenopausal women, and men were the premise for the analysis of an association between densitometric parameters in both locations and the polymorphic *BMP2* variant in these subgroups. The analyses were carried out for each of the patient groups in three age categories (up to 30 years, 30 – 50 years, over 50 years) and separately for women and men distribution of women and men (*Table 4*). Differences in BMD, T-score and Z-score in the L2-L4 spine or femoral neck for carriers of individual polymorphic variants of the *BMP2* gene were found no statistically significant.

Low-energy fractures are a clinical manifestation of osteoporosis. An analysis of their occurrence was carried out in carriers of various polymorphic variants of the *BMP2* gene in all the studied groups (CD patients, UC patients, controls), regardless of age and sex, as well as taking them into account. The dose effect (by separately comparing the incidence of fractures in AA, AT and TT groups of carriers) and dominance effect (incidence of fractures in the carriers of AA and AT compared with TT) and recessive effect (the incidence fractures in carriers of AA compared with AT and TT) were analysed. No statistically significant *BMP2* genotype-dependent differences were observed in any of these analyses in IBD patients.

DISCUSSION

The long-term asymptomatic course of osteoporosis contrasts with its fatal consequences. Search for determinants of the disease is aimed at identifying individuals who are particularly susceptible to develop the disease. However, it is difficult to define these determinants, considering the complexity of factors influencing bone metabolism and its

remodelling (16, 45) This is of particular importance in case of diseases which are a potential cause of secondary osteoporosis, one of them being inflammatory bowel disease which often affects young patients and progresses more dynamically. This requires faster identification of high-risk patients and more vigorous implementation of measures to prevent fractures. BMP2 protein is essential for the regulation of bone metabolism and any changes in the sequence of the *BMP2* gene that would cause a change in the structure of the protein may affect its activity and function.

In this study, based on 198 IBD patients and 41 controls, we have not found a significant association of the *570A>T BMP2* polymorphism with osteoporosis. However, one must be cautious excluding this polymorphism from the group of factors that may potentially contribute to the development of osteoporosis. One of the potential bias sources in our study may be a heterogeneity of the population or relatively small sizes of analysed subgroups. In previous studies, a multipoint analysis of common alleles showed a relationship of the chromosomal *BMP2* location with BMD or the incidence of fractures (37, 46). Of extreme importance is also the effect of BMP2 on the expression of osteoprotegerin in osteoblasts (47), the bone tissue protective function of which is undeniable. The studies of the 570A>T *BMP2* polymorphism itself have been rare to date, and no association between the allelic variants of the gene and the development of osteoporosis has been reported. However, linkage analysis carried out in Irish families have shown a link of phenotypes characteristic for osteoporosis with chromosomal location 20p12.3 (multi-spot analysis of common LOD 5.1 alleles). Still, despite the frequent occurrence of the polymorphism in the studied population, its statistically significant association with osteoporosis was not demonstrated (37). Similarly, in the Dutch population, the association of polymorphism 570A>T with BMD, risk of fractures or the loss of bone mass was not demonstrated (48). These results were also confirmed by studies carried out on a group of healthy Americans (46), and a study carried out on a group of Caucasian men, in whom there was no significant effect of the polymorphism on BMD of the lumbar spine, BMD of the hip and the incidence of fractures (32). Other studies, including studies carried out on a Dutch population (48) and in the United States (46), demonstrated a lack of the association of this polymorphism with a typical osteoporosis phenotype. In a study of *BMP2* gene polymorphism in the Polish population (714 people, including 674 women and 40 men), no statistically significant association with bone mineral density and the occurrence of hip fractures was observed (49).

Inflammatory bowel disease leads to the development of secondary osteoporosis (50). The formation and maintenance of the bone mass is influenced by many genetic and environmental factors. In the course of inflammatory bowel diseases, the effects of involutional processes overlap with the effect of pro-inflammatory cytokines (inflammation), dietary restrictions, absorption disorders, effects of surgical treatment and decreased physical activity. Moreover, several administered drugs seem to decrease BMD. One of the pharmacological agents with the strongest potential to promote osteoporosis are steroids. The molecular mechanisms leading to low BMD during the therapy with steroids are complex and include e.g. direct influence on osteoclasts and osteoblasts, dysregulation of calcium turnover, and modulation of several hormonal (mainly gonadal sex steroids) and other signalling pathways (for example endocannabinoid pathway) (51).

The above discussed factors determine, that osteoporotic lesions occur in younger age groups and affect mostly men in IBD. The BMP2/TGF-beta metabolic pathway is of particular importance during the formation of the peak bone mass, up to 30 years of age (37). The lack of significant differences in the Z-score for carriers of different variants of the *BMP2* polymorphism, however, undermines the importance of this polymorphism for the bone mass in patients with IBD because it can be assumed that the differences in BMD and T-score in L2-L4 observed in this study resulted not only from the genetic diversity of patients but also from the heterogeneity of the study groups with regards to age. The study participants were between 18 and 60 years of age, which means that they included individuals in their bone modelling period with still unfinished formation of the peak bone mass (20 – 30 years of age), during the relative stability period of 30 – 50 years of age and after 50 years of age, when processes of bone resorption dominate. In addition, drawing definite conclusions is limited by the overall consideration of the effect of the examined polymorphism in women and men. The occurrence of IBD in the study group, however, did not allow for clear age distinction as in the population of patients with primary osteoporosis because the physiological processes of modelling, remodelling and involution of bones had been disrupted by the chronic disease process and its consequences. The lack of clear differences in bone mass and results of laboratory investigations of carriers of the various *BMP2* polymorphism variants in patients with IBD, does not mean that the tested polymorphism does not have effect on the development of osteoporosis in these patients. In assessing the progress of osteoporosis, an assessment of the clinical effect being the occurrence of low-energy fracture is of greater importance than an assessment of the bone mass. The patients analysed were relatively young subjects, whereas age is the most important fracture risk factor (the risk of fracture doubles every five years after the age of 65). A limitation of this research is a study group size, which should be possibly large in association analysis, but in case of IBD patients with osteopenia or osteoporosis is limited by a frequency of this comorbidities. The calculation power was 80% but with 10% probability of type I error.

It can therefore be assumed that a longer follow-up of studied patient groups in future will allow for a verification of the conclusions reached. Perhaps the analysis of particular age groups and at the same time increasing the number of the youngest patients in particular, would allow to draw definitive conclusions. Recently, more and more doubts have been raised concerning basing decisions on the results of the bone mineral density test. Among patients with Crohn's disease and in healthy controls, the highest bone mass within the trabecular bone (L2 – L4 level) was found in carriers of the AA genotype, whereas in ulcerative colitis patients it was the highest in TT homozygotes. Within the cortical bone (neck), there were no differences in bone mass between the carriers of the two *BMP2* polymorphic variants. Patients with CD and CG have a tendency to association of AA haplotype with elevated BMD in the L2-L4 level. In contrast, in UC patients, the higher BMD in the L2-L4 level was associated with haplogenotypene TT. Differences in the described genotypes may be due to different predispositions for osteoporosis in both groups of IBD. In addition, the different percentage of spongy bone tissue in relation to bone mass in both analyzed sites undoubtedly influences the different susceptibility of these areas to bone loss. The results of several analyzes suggest that genetic factors have a greater impact on bone mass and loss in the spinal region, and environmental factors such as exercise play a greater role in regulating bone mass and bone loss in the hips region (52-54). For the 570A>T polymorphism of the *BMP2* gene, no statistically significant relationship was observed between the polymorphic variant and bone mineral density or the incidence of fractures in IBD patients. Further studies are needed to confirm our results on a larger cohort of patients.

Authors' contribution: I. Krela-Kazmierczak designed the study. A. Wawrzyniak and I. Krela-Kazmierczak prepared the

paper; M. Kaczmarek-Rys, M. Skrzypczak-Zielinska and N. Drweska-Matelska contributed to the experimental and laboratory work; I. Krela-Kazmierczak, A. Szymczak, P. Eder, L. Lykowska-Szuber were responsible for patients' clinical examination and qualification to the study; M. Michalak was responsible for statistical analysis of the data; W. Horst-Sikorska and K. Linke (in memoriam) were the originators of research. R. Slomski coordinated the whole research. All authors revised the paper critically for intellectual content and approved the final version.

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