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EXPERIMENTAL PAPER

The role of folate receptor and reduced folate carrier polymorphisms in osteoporosis development

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Summary

Introduction: Osteoporosis is a chronic metabolic disease with multifactorial etiology. One of possible osteoporosis causes may be impairment of osteoclasts function which leads to increased bone resorption. This

may be a result of many metabolic changes. It is believed that changes of folate-methionine metabolism in osteoporosis play an essential role in the etiology of this disease.

Objective: The aim of this study was to examine how polymorphisms of SLC19A1 and FOLR3 genes may play the key role in folate-methionine pathway and influence on the etiology of osteoporosis.

Results: The statistically overrepresentation of mutated GG genotype of FOLR3 (rs11235449) was observed in the control group compared to the osteopenia (34.9% in osteopenia vs. 37.8% in controls, p=0.025, OR=0.61). As to the SLC19A1 (rs3788200) polymorphism we have noted the statistically significant overrepresentation of wild-type GG genotype (35.8% vs. 26.2%, p=0.046, OR=1.57) and overrepresentation of wild-type G allele (56.9% vs. 50.2%, p=0.061, OR=1.31) in osteopenia group if compared to the controls.

Conclusions: In our study we shown the protective role of mutated GG genotype of FOLR3 (rs11235449) polymorphism to osteopenia progress and possible role of wild-type GG genotype and wild-type G allele of SLC19A1 (rs3788200) polymorphism in osteopenia development.

Key words: ***osteoporosis, osteopenia, folate-methionine metabolism, SLC19A1, FOLR3***

Słowa kluczowe: ***osteoporoza, osteopenia, metabolizm folianów i metioniny, SLC19A1, FOLR3***

INTRODUCTION

Osteoporosis is a chronic metabolic disease of multifactorial etiology. One of possible osteoporosis causes may be the impairment of osteoclasts function what leads to increased bone resorption. This may be a result of many metabolic changes.

Osteoporosis is 3-fold more often diagnosed at women what is partially caused by low peak bone mass and hormonal changes observed in postmenopausal women. In the Report of World Health Organization Scientific Group from 2003, osteoporosis was recognized as a well-defined disease or disturbance concerning 75 million of people in Europe, Japan and USA and is a reason of over than 2.3 million of breakages in Europe and USA [1].

Proper folate metabolism is strictly correlated with not disturbed development and functioning of human organism. Any perturbation in folate cycle may lead to many complications in cardiovascular system, nervous system and neoplasms. Well balanced folate cycle has a vital influence on proper functioning of hematopoetic, nervous and cardiovascular system and reduces the risk of thrombotic events.

Folic acid, due to its high biological activity, has both direct and indirect effects on the metabolism of body's cells. Deficiency or lack in the diet causes disorders in many vital metabolic processes and may lead to impaired growth and development of human organism. The rich source of folates in daily diet are plant products. The richest are greenleaf vegetables. Large amounts of folates contain legume seeds. Significant amounts of folate are also found in yeast, whole cereal grains and wheat germ. Very good sources are vegetables and fruits also containing vitamin C or β-carotene. In food, folic acid is predominantly methylated in form of L-5-methyl-THF.

It is believed that folate status could modify the risk of osteoporosis development. Variants of genes involved in proper function of folate cycle could be the candidate genes for osteoporosis and osteopenia development. One of the most important roles in folate metabolism is played by genes encoding transport proteins, they mainly maintain intracellular folate level. It is known that FOLR1 and FOLR2 are glycosyl phosphatidylinositol proteins and anchored proteins. The FOLR3 is a secreted receptor protein, SLC12A1 is a transmembrane protein, SLC19A1 gene is located on 21q22.3 chromosome and encodes one of the most important link of metabolism cycle, SLC19A1 functions as anion accepting folate cofactors and exporter of many organic anions. These gene is composed of 5 exons and contains open reading frames (ORF) [2].

FOLR3 (Folate Receptor 3) is a protein encoding gene. It encodes a folate receptor with high affinity for folic acid and other folate derivatives. It is located on chromosome 11 and has many variants.

It is believed that changes of folate-methionine metabolism in osteoporosis play an pivotal role in the etiology of this disease. On the above-mentioned we hypothesized that polymorphisms of SLC19A1 and FOLR3 genes may play the key role in

methionine-folate pathway and influence the etiology of osteoporosis [2, 3].

MATERIAL AND METHODS

Study design

The 109 unrelated postmenopausal Caucasian women (mean age: 53.24±8.12) with osteopenia and 333 patients with osteoporosis (mean age: 56.06±8.83) were enrolled into study. The control group consisted of 233 postmenopausal women with normal T-score (mean age: 53.38±8.22). Local Ethical Committee consent (No 158/06) for performing the study was given. All women were Caucasian of Polish origin. All of them were informed about the goal of study and given their consent.

All subjects passed densitometry examination at lumbar spine (L2–L4) by dual energy X-ray absorptiometry (DEXA). From each woman data about body weight, height, and years since menopause (YSM) have been collected. We have analysed body mass index (BMI) according to WHO criteria (normal BMI 18.9–24.9 kg/m^2, overweight 25.0–29.9 kg/m^2, and obesity – higher than 30.0 kg/m^2). Bone mineral density (BMD), *t*-score, z-score, the percent of young adults (YA) index, and aged matched (AM) index measured in L2–L4 were given as result of densitometric evaluation. Densitometry was performed using the LUNAR DPX 100 camera (Lunar Corp., Madison, USA).

The correct value of the BMD by DEXA is between one standard deviation from the mean with respect to the age of peak bone mass (–1 <T-score> 1). Based on these measurements, the women were classified into group with osteopenia (–2.5 <T-score ≤1), osteoporosis (T–score ≤2.5) and with the correct value of the T-score (T-score≥1). The ratio of the average bone mineral density in relation to mean value for young adult (young adults, YA) and in comparison to age (age matched, AM) was also evaluated. Furthermore, the height and weight measurements were performed and the body mass index (BMI) was calculated. During an interview with each patient, the data concerning the occurrence of disease, drugs used, age of first and last menstruation, number of pregnancies and birth weight of children were collected.

Inclusion criteria for genetic research were: menopause at least a year before, no hormone replacement therapy (HRT) and drugs affecting bone mass (selective estrogen receptor modulators SERMs, calcitonin, bisphosphonates, heparin, steroids, thyroid hormones, antiepileptic drugs, GnRH analogues, tibolone).

Patients with endocrine and metabolic disorders, hematological diseases, kidney disease, cancer, autoimmune and connective tissue disease and after bilateral ovariectomy were excluded from the study.

Expression analysis

The analysis of the FOLR3 and SLC19A1 gene polymorphism was conducted at the Department of Stem Cell and Regenerative Medicine, Institute of Natural Fibres and Medicinal Plants, Poznań, Poland. Genomic DNA was extracted from peripheral blood using QIAamp Blood Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol.

The genetic analysis of FOLR3 and SLC19A1 genes was performed using LightCycler®96 instrument. The rs11235449 polymorphism in the FOLR3 gene and the rs3788200 polymorphism in the SLC19A1 gene were performed using LightSNiP FOLR3 and SCL19A1 (TIBMolbiol), which contained the primers and probes specific for the amplified fragments. PCR was performed in 10 μl reaction mixture according to the manufacturer's protocol under the following conditions: initial denaturation at 95°C for 10 min., and 35 cycles as follows: denaturation at 95°C for 10 s, annealing at 60°C for 10 s, elongation for 15 s at 72°C, and melting step of 30 s at 95°C and 40C by 120 s. Polymorphism FOLR3 and SCL19A1 genes were observed as different melting curves of PCR products.

Statistical analysis

Data analysis was performed using SPSS Statistics 17.0 for Windows using one-way ANOVA test. The observed frequencies were compared with expected frequencies and tested for the Hardy-Weinberg equilibrium. The expected results are presented with 95% confidence intervals (CI). We also calculated the odds ratio (OR) for the genotypes and the alleles. The value of $p<0.05$ was considered as statistically significant.

RESULTS

In the investigated women with osteoporosis the statistically differences connected with T-score

(p<0.001) and Z-score (p<0.001) values as well as body weight (p=0.001), BMI (p=0.04), age (p=0.014), birth weight (p=0.005) have been observed if compared to the women with osteopenia and controls. We have not observed any differences in BMD L2-L4, BMD L2-L4 YA, BMD L2-L4 AM values between investigated subgroups of women with osteoporosis, osteopenia and controls (tab. 1).

The statistically overrepresentation of mutated GG genotype in the control group if compared to the osteopenia (34.9% in osteopenia *vs.* 37.8% in controls, p=0.025, OR=0.61) has been observed. The other genotypes (homozygous AA and heterozygous AG) as well as the alleles were in similar frequencies. In the osteoporosis group we have observed the similar frequencies of genotypes and alleles of investigated FOLR3 (rs11235449) polymorphism (tab. 2).

As to the SLC19A1 (rs3788200) polymorphism we have noted the statistically significant overrepresentation of wild-type GG genotype (35.8% *vs.* 26.2%, p=0.046, OR=1.57) and overrepresentation of wild-type G allele (56.9% *vs.* 50.2%, p=0.061, OR=1.31) in osteopenia group if compared to the controls. The other frequencies of genotypes and alleles of SLC19A1 (rs3788200) polymorphism in osteopenia as well as in osteoporosis groups were similar (tab. 3).

DISCUSSION

Several risk factors for osteoporosis development have been identified such as age, low body mass index, smoking, alcohol intake and low bone mass density. It was also already indicated that folate deficiency results in increased serum level of homocysteine which disturbs proper bone metabolism. Homocysteine probably is responsible for accumulation of collagen in bones what leads to decrease of bone strength [1]. The exact mechanisms of homocysteine influence on bone turnover still remains unclear. It is thought that increased level of homocysteine impairs proper balance of osteoclasts and osteoblasts with harmful effect for bone mineralisation [2]. Proper functioning of folate receptors is vital for well-balanced folate cycle what leads to decrease of homocysteine concentration. And homocysteine lowering favorably influences the risks and course of osteoporosis [4].

Increased level of homocysteine is also positively correlated with bone fracture risk. So probably folate supplementation at post-menopausal women may modify homocysteine serum concentration and what is more important may influence on the risk of bone fracture [5]. The aim of many present studies was to investigate the association between high serum homocysteine level, decreased bone mineral density and increased risk of bone fractures [6, 7].

Observed results of large epidemiologic researches showed correlation between increased serum homocysteine concentration and high risk of osteoporotic fractures [8-10]. Moreover, increased homocysteine level may lead to excessive bone resorption [11, 12]. One of the studies based on the National Health and Nutrition Examination Survey revealed that increased plasma homocysteine concentration and folate insufficiency are correlated with lower lumbar and overall bone mineral density [13].

The correlation between osteoporosis development, bone fracture, folate metabolism and genetic polymorphisms of MTHFR has been considered in many studies [14-17]. However to our best knowledge there are only a few studies considered the contribution of folate receptor and reduced folate carrier polymorphisms in the osteopenia and osteoporosis development. One of them is the study of Urano *et al.* performed in population of 851 Japanese postmenopausal women. The authors investigated single nucleotide polymorphism genotype of mitochondrial inner membrane folate transporter (SLC25A32) and plasma homocysteine or folate levels. In the studied group of women the AA genotype was connected with higher rate and earlier onset of incident fractures than the other genotypes. The results reveal that the SLC25A32 gene polymorphism could be a risk factor for lower folate concentration and future fracture in this group of women [18].

Facts mentioned above prove the important role of proper folate cycle in the risk, etiology and the course of osteoporosis. That is why in the presented study we aimed to estimate the role of polymorphisms of SLC19A1 and FOLR3 genes in methionine-folate pathway and their influence on the etiology of osteoporosis. And as far as we know this is the first research concerning that issue.

Obtained in our investigation results reveal the statistically significant overrepresentation of mutated GG genotype of FOLR3 (rs11235449) polymorphism in women with osteopenia what shows its protective role in this disturbance development. Additionally the statistically significant overrepresentation of wild-type GG genotype and wild-type G allele of SLC19A1 (rs3788200) polymorphism in women with osteopenia indicated its possible role in this disease development.

Probably more comprehensive research on that issue is needed to confirm these results. However,

Table 1

Characteristics of the study population (perimenopausal women with osteopenia, osteoporosis, and normal T-score)

		P	Mean	SEM	95% CI	
					Lower limit	Upper limit
T-score	Osteopenia	0.04[a]	-1.8520	0.06212	–1.9072	–1.7530
	Osteoporosis	<0001[b]	–3.1640	0.05627	–3.2757	–3.0522
	Controls		0.0779	0.11321	–0.1482	0.3040
	Total		–1.8138	0.08072	–1.9727	–1.6549
Z-score	Osteopenia	0.117[a]	–0.8448	0.08470	–1.0143	–0.6754
	Osteoporosis	<0001[b]	–3.5691	1.94626	–7.4572	0.3190
	Controls		0.6425	0.19620	0.2423	1.0427
	Total		–1.6696	0.81499	–3.2794	–0.0597
Weight [kg]	Osteopenia	0.026[a]	65.1721	0.99502	63.2022	67.1420
	Osteoporosis	0.001[b]	61.2088	0.93755	59.3462	63.0714
	Controls		68.7273	1.49288	65.7458	71.7088
	Total		64.7204	0.65801	63.4251	66.0157
Height [cm]	Osteopenia	0.08[a]	162.6311	0.45083	161.7386	163.5237
	Osteoporosis	0.01[b]	160.2527	0.52869	159.2024	161.3031
	Controls		163.0758	0.73612	161.6056	164.5459
	Total		161.9606	0.32149	161.3277	162.5934
BMI	Osteopenia	0.04[a]	24.6445	0.35747	23.9368	25.3522
	Osteoporosis	0.04[b]	23.7879	0.31784	23.1564	24.4193
	Controls		25.8802	0.55665	24.7685	26.9919
	Total		24.6574	0.23299	24.1988	25.1161
Age [years]	Osteopenia	0.54[a]	53.2377	0.73506	51.7825	54.6930
	Osteoporosis	0.014[b]	56.0643	0.74650	54.5883	57.5403
	Controls		53.3788	1.01176	51.3582	55.3994
	Total		54.4726	0.47138	53.5452	55.3999
Birth weight [g]	Osteopenia	0.026[a]	3226.7857	77.68484	3067.3896	3386.1818
	Osteoporosis	0.005[b]	3141.2500	134.07981	2855.4656	3427.0344
	Controls		3628.9474	110.29173	3397.2330	3860.6617
	Total		3326.3492	63.20235	3200.0095	3452.6889
Years of reproduction	Osteopenia	0.724[a]	36.2000	0.63682	34.9257	37.4743
	Osteoporosis	0.528[b]	35.6154	0.62160	34.3736	36.8572
	Controls		36.3750	0.94586	34.4459	38.3041
	Total		35.9936	0.40144	35.2007	36.7866
Age of first menstruation	Osteopenia	0.636[a]	13.1167	0.30908	12.4982	13.7351
	Osteoporosis	0.754[b]	12.9385	0.26843	12.4022	13.4747
	Controls		13.3750	0.33224	12.6974	14.0526
	Total		13.0955	0.17517	12.7495	13.4416
Age of last menstruation	Osteopenia	0.069[a]	49.2099	0.49588	48.2230	50.1967
	Osteoporosis	0.058[b]	48.1585	0.54804	47.0681	49.2490
	Controls		50.1707	0.68512	48.7860	51.5554
	Total		48.9804	0.32876	48.3322	49.6286
Number of pregnancies	Osteopenia	0.869[a]	1.8852	0.09821	1.6908	2.0797
	Osteoporosis	0.902[b]	1.9560	0.13525	1.6873	2.2247
	Controls		1.9394	0.14940	1.6410	2.2378
	Total		1.9211	0.07075	1.7819	2.0604
Years after menopause	Osteopenia	0.854[a]	7.1833	0.77769	5.6272	8.7395
	Osteoporosis	0.001[b]	10.6308	0.71305	9.2063	12.0553
	Controls		7.0313	0.98832	5.0156	9.0469
	Total		8.5796	0.48209	7.6273	9.5319
BMD L2–L4 [g/cm^2]	Osteopenia	0.986[a]	0.9674	0.02003	0.9276	1.0072
	Osteoporosis	0.944[b]	0.9752	0.01495	0.9456	1.0048
	Controls		0.9694	0.02186	0.9254	1.0133
	Total		0.9713	0.01066	0.9503	0.9923
BMD L2–L4 YA [%]	Osteopenia	0.965[a]	80.9022	1.71939	77.4868	84.3175
	Osteoporosis	0.982[b]	81.2783	1.24077	78.8203	83.7362
	Controls		81.0204	1.77293	77.4557	84.5851
	Total		81.0938	0.89537	79.3305	82.8570
BMD L2–L4 AM [%]	Osteopenia	0.989[a]	89.1304	1.82717	85.5010	92.7599
	Osteoporosis	0.968[b]	89.5043	1.23064	87.0665	91.9422
	Controls		89.7755	1.93765	85.8796	93.6714
	Total		89.4219	0.93156	87.5873	91.2564

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)

Table 2

Distribution of FOLR3 (rs11235449) polymorphism in patients with osteopenia and in control group

Genotype	Osteoporosis					Osteopenia					Control	
	Observed value n [%]	Expected value [%]	OR	95% CI	P	Observed value n [%]	Expected value [%]	OR	95% CI	P	Observed value n [%]	Expected value [%]
AA	52 (15.6)	20.8	1.05	0.64–1.72	0.472	19 (17.4)	17.0	1.19	0.61–2.28	0.337	35 (15.0)	14.9
AG	162 (48.6)	49.6	0.97	0.68–1.38	0.471	52 (47.7)	48.5	1.02	0.63–1.64	0.512	110 (47.2)	47.4
GG	119 (35.7)	29.6	0.92	0.64–1.32	0.342	38 (34.9)	34.5	0.61	0.38–1.00	0.025	88 (37.8)	37.7
Total	333 (100)	100.00	–	–	–	109 (100)	100.00	–	–	–	233 (100)	100.00
Alleles												
A	266 (39.9)	–	1.06	0.82–1.36	0.351	90 (41.3)	–	1.12	0.79–1.58	0.281	180 (38.6)	–
G	400 (60.1)	–	0.95	0.74–1.21	0.351	128 (58.7)	–	0.89	0.63–1.26	0.281	286 (61.4)	–
Total	666 (100.00)	–	–	–	–	218 (100.00)	–	–	–	–	466 (100.00)	–

Table 3

Distribution of SLC19A1 (rs3788200) polymorphism in patients with osteopenia and in control group

Genotype	Osteoporosis					Osteopenia					Control	
	Observed value n [%]	Expected value [%]	OR	95% CI	P	Observed value n [%]	Expected value [%]	OR	95% CI	P	Observed value n [%]	Expected value [%]
GG	99 (29.7)	29.0	1.19	0.81–1.77	0.204	39 (35.8)	32.4	1.57	0.93–2.63	0.046	61 (26.2)	25.2
GA	161 (48.3)	49.7	1.01	0.71–1.43	0.508	46 (42.2)	49.0	0.79	0.48–1.28	0.185	112 (48.1)	50.0
AA	73 (21.9)	21.3	0.81	0.54–1.22	0.169	24 (22.0)	18.6	0.81	0.45–1.43	0.271	60 (25.8)	24.8
Total	333 (100)	100.00	–	–	–	109 (100)	100.00	–	–	–	233 (100)	100.00
Alleles												
G	359 (53.9)	–	1.16	0.91–1.48	0.122	124 (56.9)	–	1.31	0.93–1.83	0.061	234 (50.2)	–
A	307 (46.1)	–	0.86	0.68–1.10	0.122	94 (43.1)	–	0.76	0.54–1.07	0.061	232 (49.8)	–
Total	666 (100.00)	–	–	–	–	218 (100.00)	–	–	–	–	466 (100.00)	–

correlation of impaired folate cycle and the risk of osteoporosis development seems to be obvious. It should be kept in mind that identification of risk factors of osteoporosis is of high importance. It would allow to recognize subjects of high risk of osteoporosis at which inexpensive and effective treatment and prophylaxis could be started.

CONCLUSIONS

In our research the protective role of mutated GG genotype of FOLR3 (rs11235449) polymorphism to osteopenia progress and possible role of wild-type GG genotype and wild-type G allele of SLC19A1 (rs3788200) polymorphism in osteopenia development was shown.

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