Osteoporosis associated selected single nucleotide polymorphisms frequency in HIV-infected and non-infected Polish population

Częstość wybranych polimorfizmów pojedynczych nukleotydów związanych z osteoporozą u Polaków zakażonych i niezakażonych HIV

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Abstract

Introduction: Osteoporosis poses significant risk for HIV infected subjects in the era of the long-term antiretroviral treatment. For this study frequency of the selected 11 single nucleotide polymorphisms (SNP single nucleotide polymorphism) previously associated with osteoporosis risk in HIV infected and uninfected cohorts was analysed. Association with the SNP variation and the risk of osteoporosis in the entire study and in HIV-infected cases was investigated.

Material and methods: The study included 568 patients (226 women and 342 men): 315 HIV-infected patients and 253 anti-HIV negative cases. Osteoporosis was confirmed using dual energy absorptiometry ionizing radiation (DXA) in eight HIV infected patients and three controls [odds ratio (OR): 7.66, 95% CI: 1.98–29.6; p = 0.001; relative risk (RR): 7.04, 95% CI: 1.98–25.97, p = 0.0019]. SNP assays performed for collagen type 1 (COL1A1) rs1800012, parathyroid hormone (PTH) rs9630182, estrogen receptor gene (ER1) rs2077647, rs3020314 and rs1884051, Vitamin D receptor (VDR) rs1544410 and rs731236, Osteoprotegerin rs4355801, LDL receptor protein (LRP5) rs3736228, RANK rs3018362 and CYP19A1 (aromatase) rs700518 using TaqMan SNP Genotyping Assay (Applied Biosystems) according to the manufacturer's protocol. For statistics Statistica 12 software was used.

Results: Majority of allele frequencies for the studied polymorphisms were consistent with the Hardy-Weinberg equilibrium. CC homozygotes for ER1 rs2077647 were notably more common in HIV (+) cases compared to controls [OR: 2.29, 95% CI: 1.25–4.19, p = 0.003; RR: 2.11, 1.22–3.68, p = 0.0072]. Also GG homozygotes for ER1 rs1884051 were more common both in HIV (+) [OR: 2.57, 1.33–4.94, p = 0.0016; RR: 2.37, 1.29–4.36, p = 0.002] and in all patients with osteoporosis [OR 5.04, 1.24–20.4, p = 0.025; RR 3.94, 1.38–11.24, p = 0.043] . Additionally, in HIV (+) patients parathyroid hormone rs9630182 T allele was notably more common [OR: 1.4, 1.0–1.97, p = 0.024; RR: 1.15, 0.99–1.33, p = 0.029].

Conclusions: Genetic variability for the osteoporosis-associated SNPs was similar in HIV-infected patients and uninfected persons. ER1rs1884051 variants may be associated with the increased osteoporosis risk, but increased incidence of osteoporosis in HIV-infected compared to uninfected people seems to be weakly associated with investigated single nucleotide polymorphisms. Variation in the gene for the estrogen receptor ER1rs1884051 was significantly more frequent in patients with osteoporosis and more common in HIV infection.
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Key words: *polymorphism, SNP, osteoporosis, HIV*

Streszczenie

Wstęp: Osteoporoza staje się istotnym zagrożeniem dla pacjentów zakażonych HIV, szczególnie w erze długotrwałego leczenia antyretrowirusowego. W badaniu oceniono częstość wybranych 11 polimorfizmów pojedynczych nukleotydów (*single nucleotide polymorphism*, SNP) związanych z osteoporozą u pacjentów zakażonych HIV i bez tego zakażenia. Zbadano, czy zmienność genetyczna wybranych SNP wpływa na ryzyko osteoporozy w całej badanej populacji i u zakażonych HIV.

Materiał i metody: Do badania włączono 568 osób (226 kobiet i 342 mężczyzn): 315 pacjentów zakażonych HIV i 253 osoby anty-HIV ujemne. Osteoporozę potwierdzono w badaniu za pomocą absorpcjometrii podwójnej energii promieniowania jonizującego (*Dual-energy X-ray absorptiometry*, DXA) u 8 pacjentów zakażonych HIV i 3 z grupy kontrolnej [iloraz szans (*odds ratio*, OR): 7,66; 95% Cl: 1,98–29,6; p = 0,0010; ryzyko względne (*relative risk*, RR): 7,04; 95% Cl: 1,98–25,97; p = 0,0019]. Zbadano SNP dla genów: kolagenu typu I (COLIA1) rs1800012, parathormonu (PTH) rs9630182, receptora estrogenów typu 1 (ER1) rs2077647 rs3020314 i rs1884051, receptora witaminy D (VDR) rs1544410 i rs731236, osteoprotegeryny (OPG) rs4355801, białka receptorowego dla LDL (LRP5) rs3736228, RANK rs3018362 i aromatazy (CYP19A1) rs700518 przy użyciu zestawów TaqMan SNP Genotyping Assay (AppliedBiosystems) zgodnie z protokołem producenta. Wyniki uzyskanych badań opracowano statystycznie w programie Statistica 12.

Wyniki: Częstości alleli większości badanych polimorfizmów były zgodne z prawem Hardy-Weinberga. Analizując występowanie poszczególnych genotypów, stwierdzono istotnie częstsze występowanie u zakażonych HIV w porównaniu z grupą kontrolną homozygoty CC ER1 rs2077647 (OR: 2,29; 1,25–4,19; p = 0,003; RR: 2,11; 1,22–3,68; p = 0,0072). Także częściej występowała homozygota GG ER1 rs1884051 u zakażonych HIV (OR: 2,57; 1,33–4,94; p = 0,0016; RR: 2,37; 1,29–4,36; p = 0,002) oraz u pacjentów z osteoporozą (OR: 5,04; 1,24–20,4;

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p = 0,025; RR: 3,94; 1,38–11,24; p = 0,043). Dodatkowo u pacjentów zakażonych HIV istotnie częściej (p = 0,047) występował allel T genu parathormonu rs9630182 (OR: 1,4; 1,0–1,97; p = 0,024; RR: 1,15; 0,99–1,33; p = 0,029).
Wnioski: Zmienność genetyczna pojedynczych nukleotydów związanych z występowaniem osteoporozy była podobna u pacjentów zakażonych HIV i osób niezakażonych. Warianty ER1 rs1884051 mogą mieć związek ze zwiększonym ryzykiem osteoporozy, ale wyższe ryzyko osteoporozy u pacjentów zakażonych HIV w porównaniu z osobami niezakażonymi wydaje się mieć niewielki związek z badanymi polimorfizmami pojedynczych nukleotydów. Zmienność w genie dla receptora estrogenów ER1rs1884051 istotnie częściej wystąpiła u pacjentów z osteoporozą i u zakażonych HIV. **(Endokrynol Pol 2017; 68 (4): 542–549)**

Słowa kluczowe: *polimorfizm, SNP, osteoporoza, HIV*

Introduction

Osteoporosis is a chronic, multifactorial metabolic disease manifesting in the decreased bone mineral density. It was defined by WHO as the decrease of the bone mineral density in the dual energy absorptiometry ionizing radiation (DXA) scanning by more than 2.5 standard deviations from the mean for the reference population (BMD — bone mineral density: T-score > –2.5). Consequences include increased frequency of bone fractures, decreased quality of life and higher likelihood of death.

Decrease in the bone density is a natural consequence of aging. It was estimated, that from the second half of the third decade of life an average individual would lose from 0.5 to 1% of a body mass per year. Most active research on this important issue was performed on the post-menopausal women: in this period of life tempo of bone demineralization is accelerating due to estrogen deficiency. It was also proven, that the other risk factors such as wasting, age > 65 years, calcium absorption disturbances, vitamin D3 deficiency, excessive coffee, alcohol or coca-cola consumption, as well as cigarette smoking, long term immobilization, steroid treatment of > 3 month duration, family history of low-energy fractures, etc., play a key role in the pathogenesis of this disease. Osteoporosis as a secondary disease may be associated with hyperparathyroidism, hyperthyroidism, diabetes, and higher risk of neoplasm development. It has been reported, that HIV infection is an independent risk factor of osteoporosis, especially in the era of usually lifelong, antiretroviral therapy (ART) [1–4]. HIV infection might be perceived as a generalized, long-term inflammatory process with loss of T helper lymphocytes of a CD4 phenotype. Clinical symptoms of advanced immunodeficiency among HIV infected patients manifest by an array of opportunistic infections and neoplasms, defining an acquired immunodeficiency syndrome (AIDS). Since the beginning of HIV pandemics increased prevalence of osteopenia and/or osteoporosis among infected individuals has been observed [1, 4, 5]. Prior to the antiretroviral treatment era, observations of this phenomenon had been performed on small groups of survivors, with osteoporosis diagnosed in a low percentage of patients, mainly young men. From the time of introduction of the combined antiretroviral treatment (cART) in 1996, osteoporosis or osteopenia has been described in an increasing proportion of treated patients. Prevalence of osteopenia was defined in as many as 60%, while osteoporosis in 10–15% of cART treated individuals living with HIV today [4]. Pathomechanism of this phenomenon remains unclear and is most likely multifactorial. Several hypotheses exist, however the most important include association between HIV infection of osteoblast and osteoclast precursors and decrease in its population, chronic activation of T lymphocytes as well as increase in the concentration of the pro-inflammatory cytokines such as TNF and IL-6 stimulating bone remodeling, hormonal disturbances — mainly decreased secretion of the anabolic hormones, malnutrition and nutritional deficiencies — such as vitamin D3 and calcium deficiency related to the malabsorption and chronic gastrointestinal infections among individuals with immunodeficiency and adverse effects of the antiretroviral therapy [3].

Bone is a living tissue, constantly undergoing resorption and synthesis. Bone mineral density is dependent on the balance between these two processes. Bone remodeling is directly associated with osteoclastogenesis and osteoblastogenesis. In this process genetic variability influencing various steps of this pathway seems to be of a vital importance [6, 7]. There is a well studied association between the hereditary marble bone disease (Albers — Schönberg disease) and deletion in the region coding for the NFκB transcription factor or associations between several single nucleotide polymorphisms (SNPs) such as the ones in the OPG gene (rs4355801) and LRP-5 gene (rs3736228) with decreased bone mineral density and increased osteoporosis risk, including osteoporotic bone fractures, in three cohorts in the Western Europe [6]. Osteoporosis was found to be a multilocus disease, as no single gene was found to be associated with this abnormality [8–12]. It is widely considered that an array of genetic factors influences both bone

synthesis and its metabolism [13–16]. Genetic laboratories in Europe and the USA have formed a consortium for the study of the genetic factors associated with osteoporosis — GEFOS — Genetic Factors For Osteoporosis Consortium. As a result, in 2009 a list of 20 SNPs associated with osteoporosis and increased risk of fractures was published [17]. Several groups of candidate genes related to the increased risk of osteoporosis were identified so far (presented below according to the type of coded protein):

— genes coding for interleukins: interleukin-6 and interleukin-1;
— genes coding for growth factors: tumor necrosis factor — TNF-α (chromosome 3), colony stimulating factor — CSF-1 (chromosome 12);
— genes coding for components of the protein matrix: collagen type I (chromosome 17);
— genes coding for calcitropic hormones and their receptors;
  • vitamin D3 receptor (chromosome 12),
  • estrogen receptor (chromosome 16),
  • parathyroid hormone (chromosome 7);
— Genes coding for osteoprotegerin (chromosome 8), NFκB (RANK) receptor activator, LDL receptor protein (LRP5) (chromosome 11) etc.

This genetic variability, associated with increased osteoporosis risk in various populations and ethnicities has been described in several SNP databases, however no uniform haplotype for osteoporosis have been defined yet. In Poland results of several studies on such variability have been published so far — COLIA1 investigating collagen genetic variants as well as studies for the vitamin D receptor [18, 19]. Research on association between genotypic data and the phenotype is of the highest value e.g. studies on the genetic variability of the receptor, hormone levels and bone mineral density [20–35]. Of note, no such research was performed in HIV(+) population.

The aim of this study was to analyse the frequency of osteoporosis of the well defined cohort of HIV-infected patients in comparison to the healthy controls and to investigate the genetic risk associated with osteoporosis/ /osteopenia in the analysed cohorts based on the array of selected SNPs.

Material and methods

The study included two groups of adults:

Group 1 — cross-sectional cohort of 315 study individuals with confirmed HIV infection, followed-up at the Department of Infectious Diseases and Hepatology, Pomeranian Medical University, Szczecin, Poland. To reflect current demographics of HIV infection in Poland the study group included 91 (29%) women and 224 (79%) men.

Group 2 — controls — which included 253 persons: 115 (45%) women and 118 (55%) men, with HIV infection excluded in the screening test (rapid HIV test of a single blood sample: TOYO Anti- HIV1/2 test (Turklab Tibbi Malzemeler San. Turkey; sensitivity for European population of 99.8%, specificity of 99.9%).

All patients provided a formal written consent prior to the inclusion into the study.

The study procedures included the following:

1. Medical history and examination, body mass index (BMI) calculation.
2. Substudy of the bone mineral density assessment (BMD) for the lumbar spine and femoral neck using a LUNAR Prodigy ADVANCE scanner equipped with ENCORE 2007 software v.11.40.004 (GE Healthcare, Great Britain) was performed in 87 HIV infected persons and in 230 cases from control group.
3. The genetic analyses were performed in all participants of study (568 pts) at the laboratory of Infectious Diseases and Hepatology, Pomeranian Medical University, Szczecin. For the DNA extraction samples of full blood, collected into the tubes containing EDTA coagulant were used. To ensure data safety every sample was coded by the unique number. DNA was extracted using QIAamp DNA blood mini kit isolation columns produced by QIAgen (Hilden, Germany) according to the manufacturer's protocol. All work with unprocessed blood samples was performed under the laminar flow hood to ensure maximum personal safety and reduce the risk of contamination. Genomic DNA was suspended in a buffer included in the kit and stored for analyses at 4°C.

For association studies included in this project eleven single nucleotide polymorphisms were selected, as presented in the Figure 1 and Table I (in the table common name of the coded variant is presented, GenBank accession number for the SNP (rs) as well as catalogue assay number. TaqMan SNP (Life Technologies, USA) genotyping assays were used according to the manufacturer's protocol with real-time PCR technology on the StepOne thermal cycler (Applied Biosystems/Life Technologies, Foster City, CA). Genotypes were called using TaqMan Genotyper Software v1.0.1 (Applied Biosystems/Life Technologies, Foster City, CA), calculation of Hardy-Weinberg equilibrium for each analysed set of genotypes was performed by this software.

For statistics Statistica12 software (Statsoft, Poland) was used. Statistical testing included the analyses of the differences in the frequency of the investigated genetic variants in HIV infected individuals and healthy control and association between the genotypes and haplotype and the osteoporosis risk were performed using Chi--square tests.

Table I. *Single Nucleotide Polymorphisms selected for the study of association between human genetic variability and osteoporosis*

Tabela I. *Polimorfizmy pojedynczych nukleotydów wybrane na podstawie badań nad związkiem zmienności genetycznych z osteoporozą*

Gene name	rs	Assay ID (APPLIEDBIOSYSTEMS)
COL1A1	rs1800012	C___7477170_30
VDR	rs1544410	C___8716062_10
VDR	rs731236	C___2404008_10
ER1	rs2077647	C__11414978_10
ER1	rs3020314	C__11555860_10
ER1	rs1884051	C__11918415_10
TNFRSF11B (OPG)	rs4355801	C__11869235_10
PTH	rs9630182	C__26485235_10
LRP5	rs3736228	C__25752205_10
RANK	rs3018362	C__15763310_10
CYP19A1 (aromatase)	rs700518	C___8794675_30

Results

Demographic characteristics and selected clinical study groups are presented in Table II. The study participants were of Caucasian origin and of the young age. As the control group enrollment focused on the individuals < 35 years of age to reflect the characteristics of the HIV-infected population in Poland the ultimate age of the control group was significantly lower, and the BMI higher compared to the people infected with HIV. Antiretroviral treatment (ARV) was initiated in 79% HIV infected cases prior to enrollment to the study. Bone mineral density assessment was scheduled for all participants in the study but performed only in the subset of cases who consented: 87 (28%) HIV-infected patients and 230 (91%) control subjects. Osteoporosis with confirmed in DXA T-score value < –2.5 was noted in 8 (9%) HIV-infected patients and 3 (1%) control group cases. The relative risk of osteoporosis in HIV-infected patients was 7-fold higher compared to the control group (RR = 7.04, p = 0.0019).

Genetic testing was performed in all study participants: 568 patients (315 HIV-infected and 253 control subjects). The frequencies of the genotypes tested were consistent with the Hardy-Weinberg equilibrium (HWE) except for the type 1 collagen rs1800012 and parathyroid hormone genotypes and parathyroid hormone rs9630182 with the expected frequencies notably different from the ones found in the analysed cohorts. However, the frequencies of minor allele (MAF — minor allele frequency) for individual SNPs were similar in the analyzed groups and when compared to a reference
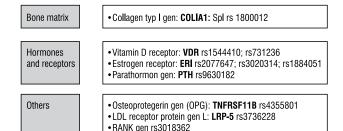
Bone matrix	• Collagen typ I gen: **COLIA1**: Spl rs 1800012
Hormones and receptors	• Vitamin D receptor: **VDR** rs1544410; rs731236 • Estrogen receptor: **ERI** rs2077647; rs3020314; rs1884051 • Parathormon gen: **PTH** rs9630182
Others	• Osteoprotegerin gen (OPG): **TNFRSF11B** rs4355801 • LDL receptor protein gen L: **LRP-5** rs3736228 • RANK gen rs3018362 • Aromatase gen: **CVP19A1** rs700518

Figure 1. *Single Nucleotide Polymorphisms selected for the study of association between human genetic variability and osteoporosis*

Rycina 1. *Polimorfizmy pojedynczych nukleotydów wybrane na podstawie badań nad związkiem zmienności genetycznych z osteoporozą*

Table II. *Demographic and clinical characteristics of study groups*

Tabela II. *Charakterystyka demograficzna i kliniczna badanych*

Character		HIV-infected persons n = 315	Non-infected control group n = 25
Age Me (IQR) yrs		40 (35–48)	37 (24–52) p = 0.0007
Sex	male n (%)	224 (71)	118 (55)
	female n (%)	91 (29)	135 (45)
BMI Me (IQR) [kg/m²]		24 (21–26)	25 (22–28) p = 0.002
Osteoporosis n (%)		8 (9)*	3 (1)#
odds ratio (OR); 95% CI; p		7.66; 1.98–29.6; p = 0.0010	
relative risk (RR); 95% CI; p		7.04; 1.98–25.97; p = 0.0019	
on ARV n (%)		249 (79)	Not related

*DXA in 87 persons; #DXA in 230 persons

frequencies according to the NCBI SNP database (The National Center for Biotechnology Information, US National Library of Medicine) [36]. The results are summarized in Table III.

Analyzing the differences in the prevalence of various genotypes and alleles among HIV-infected versus the control group and in patients with confirmed or excluded osteoporosis statistically significant differences associated with the studied SNPs were found for the ER1, PTH and COL1A1 (borderline significant). The results are shown in Table IV.

In HIV patients genotype CC of the ER1 gene (rs2077647) proved to be significantly more frequent: OR = 2.29, p = 0.003. Also ER1(rs1884051) GG genotype or T allele of the PTH (rs9630182) were notably more common among HIV-1 infected cases compared to controls: OR = 2.57, p = 0.0016 and ; OR = 1.4,

Table III. *Results of genotypic assay (SNP, single nucleotide polymorphism) and minor allele frequency (MAF)*

Tabela III. *Wyniki badań genetycznych (single nucleotide polymorphism, SNP) oraz częstość alleli mniejszościowych (minor allele frequency, MAF)*

SNP (rs)	Gene	Chromosome	Allele frequency n (%)	MAF-all	MAF-HIV(+)	MAF-controls	MAF dsSNP*
rs1800012	COL1A1	17	n = 557 AA 1 (0.2) AC 173 (31) CC 383 (68.8) A 175 (16) C 939 (84)	A = 0.1570 p = 0.63 ^ HWE# p < 0.001	A = 0.1515	A = 0.164	A = 0.0911
rs1544410	VDR1/BsmI	12	n = 563 TT 63 (11) CT 267 (47 CC 233(42) T 393 (35) C 733 (65)	T = 0.3490 p = 0.17	T = 0.3742	T= 0.3182	T = 0.2959
rs731236	VDR1/TaqI	12	n = 552 GG 60 (11) AG 262 (47) AA 230 (42) G 382 (35) A 722 (65)	G = 0.3460 p = 0.14	G = 0.3738	G = 0.3127	G = 0.2766
rs2077647	ER1	6	n = 559 CC 57 (10) CT 246 (44) TT 256 (46) C 360 (32) T 758 (68)	C = 0.3220 p = 0.14	C = 0.3480	C = 0.2905	C = 0.4665
rs3020314	ER1	6	n = 560 TT 147 (26) CT 260 (47) CC 153 (27) T 554 (49) C 566 (51)	T = 0.4946 p = 0.55	T = 0.5065	T = 0.4802	T = 0.3838
rs1884051	ER1	6	n = 564 GG 51 (9) AG 246 (44) AA 267 (47) G 348 (31) A 780 (69)	G = 0.3085 p = 0.13	G = 0.3344	G = 0.2767	G = 0.4908
rs4355801	OPG (TNFRSF11B)	8	n = 562 GG 109 (19) AG 289 (52) AA 164 (29) G 507 (45) A 617 (55)	G = 0.4511 p = 0.85	G = 0.4469	G = 0.4562	G = 0.2756
rs9630182	PTH	11	n = 562 TT 64 (11) CT 265 (47) CC 233 (42) T 393 (35) C 731 (65) HWE	T = 0.3496 p = 0.28 p < 0.05	T = 0.3694	T = 0.3254	T = 0.4609
rs3736228	LRP5	11	n = 555 TT 6 (1) CT 100 (18) CC 449 (81) T 112 (10) C 998 (90)	T = 0.1009 p = 0.47	T = 0.0945	T = 0.1089	T = 0.1160
rs3018362	RANK	18	n = 534 AA 115 (22) AG 240 (45) GG 179 (33) A 470 (44) G 598 (56)	A = 0.4401 p = 0.88	A = 0.4433	A = 0.4362	A = 0.3750
rs700518	CYP19A1	15	n = 558 TT 121 (22) CT 261 (47) CC 176 (31) C 613 (55) T 503 (45)	C = 0.5493 p = 0.20	C = 0.5863	C = 0.514	C = 0.3259

*www.ncbi.nlm.nih.gov/SNP/; ^ for MAF-HIV(+) and MAF-controls; #HWE — Hardy-Weinberg Equilibrium

Table IV. *SNPs results in all patients, HIV-infected (HIV(+)) persons and healthy control and association with osteoporosis*

Tabela IV. *Wyniki badań SNPs u wszystkich pacjentów, zakażonych HIV [HIV(+)] oraz grupy kontrolnej (Controls) oraz ich związek z osteoporozą*

SNPs Gene/rs/genotype	All n (%)	HIV(+) n (%)	Controls n (%)	Osteoporosis* n (%)	Without osteoporosis n (%)
COL1A1/rs1800012	n = 557	n = 307	n = 250	n = 11	n = 300
AA	1 (0.2)	0 (0)	1 (0.4)	0	0
AC	173 (31)	93 (30)	80 (32)	6 (55)	92 (31)
CC	383 (68,8)	214 (70)	169 (67,6)	5 (45)	208 (69)
		p = 0.48		p = 0.2461	
A	174 (31)	93 (30)	81 (32)	6 (55)	92 (31)
nA	383 (69)	214 (70)	169 (68)	5 (45)	208 (69)
		p = 0.59		p = 0.0940	
VDR1/ rs1544410	n =563	n = 310	n = 253	n = 11	n = 304
TT	63 (11)	42 (14)	21 (8)	1 (9)	30 (10)
CT	267 (47)	148 (48)	119 (47)	4 (36)	146 (48)
CC	233 (42)	120 (39)	113 (45)	6 (55)	128 (42)
		p = 0.0984		p = 0.7061	
T	332 (58)	190 (61)	142 (56)	5 (45)	176 (58)
nT	231 (42)	120 (39)	111 (44)	6 (55)	128 (42)
		p = 0.232		p = 0.8081	
VDR2/rs731236	n = 552	n = 301	n = 251	n = 11	n = 300
GG	60 (11)	39 (13)	21 (8)	1 (9)	28 (9)
AG	262 (47)	147 (49)	115 (46)	4 (36)	142 (47)
AA	230 (42)	115 (38)	115 (46)	6 (55)	130 (44)
		p = 0.0898		p = 0.7491	
G	322 (58)	186 (62)	136 (54)	5 (45)	170 (57)
nG	230 (42)	115 (38)	115 (46)	6 (55)	130 (43)
		p = 0.0709		p = 0.4615	
ER1/rs2077647	n = 559	n = 306	n = 253	n = 11	n = 303
CC	57 (10)	41 (13)	16 (6)	3 (27.5)	25 (8)
CT	246 (44)	131 (43)	115 (46)	3 (27.5)	136 (45)
TT	256 (46)	134 (44)	122 (48)	5 (45)	142 (47)
		p = 0.0222		p = 0.0798	
C	303 (54)	172 (56)	131 (52)	6 (55)	161 (53)
nC	256 (46)	134 (44)	122 (48)	5 (45)	142 (47)
		p = 0.29533		p = 0.9266	
ER1/rs3020314	n = 560	n = 307	n = 253	n = 11	n = 303
TT	147 (26)	88 (29)	59 (27)	4 (36)	64 (21)
CT	260 (47)	135 (44)	125 (23)	6 (55)	151 (50)
CC	153 (27)	84 (27)	69 (50)	1 (9)	88 (29)
		p = 0.3024		p = 0.2623	
T	407 (73)	223 (73)	184 (73)	10 (91)	215 (71)
nT	153 (27)	84 (27)	69 (27)	1 (9)	88 (29)
		p = 0.09		p = 0.1492	

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Table IV cont. *SNPs results in all patients, HIV-infected (HIV(+)) persons and healthy control and association with osteoporosis*

Tabela IV. cd. *Wyniki badań SNPs u wszystkich pacjentów, zakażonych HIV [HIV(+)] oraz grupy kontrolnej (Controls) oraz ich związek z osteoporozą*

SNPs Gene/rs/genotype	All n (%)	HIV(+) n (%)	Controls n (%)	Osteoporosis* n (%)	Without osteoporosis n (%)
ER1/rs1884051	n = 564	n = 311	n = 253	n = 11	n = 303
GG	51 (9)	38 (12)	13 (5)	3 (28)	21 (7)
AG	246 (44)	132 (43)	114 (45)	4 (36)	133 (44)
AA	267 (47)	141 (45)	126 (50)	4 (36)	149 (49)
		p = 0.014		p = 0.0441	
G	297 (53)	171 (55)	126 (50)	7 (64)	154 (51)
nG	267 (47)	140 (45)	127 (50)	4 (36)	149 (49)
		p = 0.2574		p = 0.4036	
OPG/rs4355801	n = 562	n = 311	n = 251	n = 11	n = 302
GG	109 (19)	58 (19)	51 (20)	2 (18)	63 (21)
AG	289 (52)	162 (52)	127 (51)	8 (73)	154 (51)
AA	164 (29)	91 (29)	73 (29)	1 (9)	85 (28)
		p = 0.8775		p = 0.30002	
G	399 (71)	220 (71)	178 (71)	10 (91)	217 (72)
nG	164 (29)	91 (29)	73 (29)	1 (9)	85 (28)
		p = 0.9700		p = 0.1643	
PTH/rs9630182	n = 562	n = 310	n = 252	n = 11	n = 302
TT	64 (11)	36 (12)	28 (11)	1 (9)	31 (10)
CT	265 (47)	157 (50)	108 (43)	7 (64)	136 (45)
CC	233 (42)	117 (38)	116 (46)	3 (27)	135 (45)
		p = 0.1272		p = 0.4608	
T	329 (58)	193 (62)	136 (54)	8 (73)	167 (55)
nT	233 (42)	117 (38)	116 (46)	3 (27)	135 (45)
		p = 0.047		p = 0.25277	
LRP5/rs3736228	n = 555	n = 307	n = 248	n = 11	n = 300
TT	6 (1)	5 (2)	1 (0.4)	0 (0)	1 (0.3)
CT	100 (18)	48 (16)	52 (20.6)	1 (9)	62 (20. 7)
CC	449 (81)	254 (83)	195 (79)	10 (91)	237 (79)
		p = 0.1132		p = 0.6287	
T	106 (19)	53 (17)	53 (21)	1 (9)	63 (21)
nT	449 (81)	254 (83)	195 (79)	10 (91)	237 (79)
		p = 0.2211		o = 0.3372	
RANK/rs3018362	n = 534	n = 291	n = 243	n = 9	n = 296
AA	115 (22)	61 (21)	54 (22)	2 (22)	69 (23)
AG	240 (45)	136 (47)	104(47)	4 (45)	135 (46)
GG	179 (33)	94 (32)	85 (35)	3 (33)	92 (31)
		p = 0.6578		p = 0.9914	
A	357 (67)	199 (68)	158 (65)	7 (78)	204 (69)
nA	177 (33)	92 (32)	85 (35)	2 (22)	92 (31)
		p = 0.4108		p = 0.5749	
CYP19A1/rs700518	n = 558	n = 308	n = 250	n = 11	n = 300
TT	121 (22)	58 (19)	63 (25)	2 (18)	70 (23)
CT	261 (47)	144 (47)	117 (47)	5 (46)	144 (48)
CC	176 (31)	106 (34)	70 (28)	4 (36)	86 (29)
		p = 0.1118		p = 0.8381	
C	437 (78)	250 (81)	187 (75)	9 (82)	230 (77)
nC	121 (22)	58 (19)	63 (25)	2 (18)	70 (23)
		p = 0.069		p = 0.69	

*DXA in 87 HIV(+) persons and 230 healthy control (total 317)

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Table V. *Statistically significant differences between allels in studied groups*
Tabela V. *Statystycznie istotne różnice występowania poszczególnych alleli w badanych grupach*

Group	Gen/rs/genotype or allele	OR (95% CI); p	RR (95% CI); p
HIV group	ER1/rs2077647/CC	2.29 (1.25–4.19); p = 0.003	2.11 (1.22–3.68); p = 0.0072
Osteoporosis	ER1/rs2077647/CC	4.17 (1.04–16.72); p = 0.038	3.31 (1.17–9.31); p = 0.064
HIV group	ER1/rs1884051/GG	2.57 (1.33–4.94); p = 0.0016	2.37 (1.29–4.36); p = 0.002
Osteoporosis	ER1/rs1884051/GG	5.04 (1.24–20.4); p = 0.025	3.94 (1.38–11.24); p = 0.043
HIV group	PTH/rs9630182/T allele	1.4 (1.0–1.97); p = 0.024	1.15 (0.99–1.33); p = 0.029
Osteoporosis	COL1A1/rs1800012/A allele	2.71 (0.8–9.11); p = 0.059	1.77 (1.01–3.13); p = 0.092

p = 0.024, respectively, see Table IV. In cases with confirmed osteoporosis the following genotypes were significantly more common: CC genotype for the ER1 (rs2077647): OR = 4.17, p = 0.038 and ER1 rs1884051 GG genotype: OR = 5.04, p = 0.025. Additionally, A allele of the COL1A1 rs1800012 was more frequent in people with osteoporosis, but of borderline statistical significance: OR = 2.71, p = 0.059. The results of analysis of the odds ratio and relative risk for major genetic variations are summarized in Table V. Estrogen ER1 rs2077647 and rs1884051 SNPs were more common both among cases with osteoporosis, and independently in HIV(+) cases.

Discussion

Increased incidence of osteoporosis in HIV-infected persons remains a significant concomitant health issue. In investigation on the causes of this phenomenon, alongside with the well-defined classic risk factors for osteoporosis, or immune activation pathways affecting bone metabolism, genetic factors must be considered. It should be noted, however, that no genetic factors associated with decreased bone mineral density in this group were clearly identified so far.

Previous studies on the genetic osteoporosis risk factors focused mainly on the post-menopausal women. For example, in the Polish study the impact of gene polymorphism of collagen type 1 — COL1A1 on bone mineral density in postmenopausal women was negligible. In our analysis, we have found the relationship between the COL1A1 rs1800012 A allele and osteoporosis, although our result was of borderline statistical significance (p = 0.094). In addition, the frequency of minority allele in the cited study was 5.5%, while our data indicate the frequency of only 0.2%. The reason for such discrepancies may be linked to the selection of the control group: previous studies included mostly women, while in our groups there was a predominance of men.

Despite numerous studies suggesting the influence of vitamin D receptor (VDR), osteoprotegerin (OPG), receptor for the LDL: LRP5, RANK or aromatase

(CYP19A1) genetic variation on the bone mineral density, no such association was confirmed in our study. Lack of this association may be linked to the small number of patients with confirmed osteoporosis. Significant link between osteoporosis with genetic variability was obtained for 2 SNPs of the estrogen receptor type 1 — ER1: rs2077647 and rs 1884051.

Common presence of multiple genotypes associated with increased risk of lower bone mineral density in HIV infected patients and cases with osteoporosis may confirm the link between this variability and osteoporosis. It should be noted, however that majority of patients with osteoporosis in our study were HIV patients: 8 out of 11 confirmed cases of osteoporosis; therefore the risk genotypes may be significant for this group.

Higher frequency alleles related to the increased risk of osteoporosis among HIV-infected cases, especially coding for the hormone receptors — estrogen or parathyroid hormones may be of notable clinical significance for these patients. Estrogen receptor gene variability was described not only in the context of osteoporosis, but also bone remodeling, bone reconstruction or resistance to injury.

Limitations of our study was associated to the smaller number of DXA scans in a group of HIV-infected than in the control group which limited the association study. Also, to some extent the dominance of men in the group of people infected with HIV was a limiting factor, ideally similar study should be performed in the group of postmenopausal HIV-1 infected cases, however access to such a group is limited.

Conclusions

Frequency of allelic variation for the analysed SNPs associated with osteoporosis was similar in HIV-infected patients and uninfected persons. Distribution of several genotypes was notably different between the studied populations. Significantly higher frequency was noted among HIV infected patients for the genotype CC of ER1 rs2077647, GG of ER1 rs1884051 and PTH rs9630182

T allele. Genotypes GG of ER1 rs1884051 and CC of ER1 rs2077647 were also notably more common among cases with osteoporosis. Increased incidence of osteoporosis in HIV-infected compared to uninfected people are associated only with a limited relationship with the investigated single nucleotide polymorphisms, although the variation in the estrogen receptor ER1 rs1884051 and ER1 rs2077647 was significantly associated with osteoporosis and more common in HIV infection.

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