

Genetic and environmental factors in human osteoporosis from Sub-Saharan to Mediterranean areas

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Abstract The aim of this study was to determine the prevalence of known gene polymorphisms associated with osteoporosis in postmenopausal normal women from Burkina Faso and Sicily, compared to postmenopausal Sicilian women with osteoporosis, and to establish the weight of environmental factors in the mechanism of osteoporosis. Bone mass density (BMD) was measured by phalangeal quantitative ultrasound (QUS) in Burkinabe woman and by the dual X-ray absorptiometry at the femoral neck in Sicilian women. The polymorphisms of the vitamin D receptor (VDR) gene, estrogen receptor (ESR) gene, calcitonin receptor (CTR) gene and COL1A1 collagen gene were characterized by PCR. The social

characteristics of studied women were evaluated by a specific questionnaire. The observed percentages of single specific polymorphisms did not differ from that expected with exception of VDR B allele and ESR X and P allele in Burkinabe and Sicilian women, respectively. Association analyses and multivariate two-step regression model of social and molecular parameters, demonstrated that in comparison to the VDR, ESR, CTR polymorphisms, physical activities and healthy diet, associated with outdoor work are the best favourable prognostic factors for osteoporosis. A diet rich in calcium, other minerals and vitamin D in association with physical activity represents the most effective way to maintain not only a healthy bone structure but also an acceptable BMD. This is particularly true for Sub-Saharan women.

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Introduction

Osteoporosis is a reduction in skeletal mass associated with bone micro-architectural deterioration, which results in increased fracture risk [1]. Moreover, it is considered a bone disorder of postmenopausal women, even though younger women can also be affected [2, 3]. The risk of bone fractures for a 50-year-old Caucasian-American woman is almost 40%, which is the double of the risk of African-American woman [4, 5]. Indeed, Caucasian- and Asian-Americans are more susceptible to develop osteoporosis than African and Hispanic-Americans, even though it has also been demonstrated that they have a significant risk to develop pathologic fractures as well [6].

Osteoporosis is a multi-factorial disease and many genetic and non-genetic risk factors contribute to the development of osteoporosis [7–9]. Recently, non-genetic factors have been widely investigated [10] as well as several genes have been identified as osteoporosis cause that remains clearly a polygenic disorder. In this context, the contribution of several gene polymorphisms controlling bone mass (and its mineral content) and/or bone turnover have been recently recognized [7–11]. Four primary areas of human genome seem to forge the link between genetics and osteoporosis.

In 1994, Morrison et al. [12] reported their pioneering work looking at the close relationship between VDR genotype and bone mass, generating a considerable amount of interest and conflict. In fact other studies have shown no effect of polymorphism of the vitamin D receptor (VDR) gene on bone mineral density (BMD) [13], where in some population groups [14] there exists a natural variation (polymorphism) in the VDR gene associated with low BMD [15]. Furthermore, the contribution of a gene to achieve low bone mass is always polyphasic. If low BMD suggests an increased tendency toward osteoporosis fractures [5], it has been observed that the association between VDR polymorphism and susceptibility to fractures are independent from the BMD. For example, estrogen-receptor gene polymorphisms may show varied importance in the different stage of women's life span. The estrogen levels play a major role in pre- and post-menopausal women; therefore mutation of the estrogen receptor (ESR) gene may be also important in some cases of osteoporosis.

Polymorphism of the ESR gene has been correlated with BMD in some populations [16, 17], even though other studies did not confirm this effect [18]. The discovery that inactivation of the ESR1 gene is associated with low BMD indicated ESR1 as a candidate gene for susceptibility to osteoporosis. Becherini et al. genotyped 610 postmenopausal women for three ESR1 gene polymorphisms [19]. The authors observed a statistically significant difference in the number of (TA)_n repeats between 73 analyzed women with a vertebral fracture compared to the non-fracture group, that is equivalent to a 2.9-fold increased fracture risk in women with a low number of repeats. They concluded that in their large sample the (TA)_n polymorphism in ESR1 accounts for part of the heritable component of BMD and may prove useful in the prediction of vertebral fracture risk in postmenopausal osteoporosis.

Calcitonin inhibits bone resorption via receptors located on the osteoclasts. Two calcitonin receptor gene (CTR) alleles exist: a proline (CCG) at 447 position changes to a leucine (CTG) [20]. Proline/leucine heterozygotes show higher bone densities than either proline or leucine homozygotes. Data on the CTR genotypes are compatible

with an advantage of heterozygotes in having larger bone hardness [21].

The COL1A1 and COL1A2 genes encode type I collagen, a key bone protein, which may play a role in the genetic control of bone mass [22]. Mutations in coding regions of the two genes lead to the formation of abnormal collagen and, accordingly, abnormal bone strength and architecture [23]. Identification of BMD-related polymorphism of the COL1A1 gene has been proven to be an important factor. Indeed, Liu et al. have demonstrated that reduced bone density and osteoporosis were associated with a polymorphic Sp1 binding site in the COL1A1 gene [24].

The complexity of these different determinants, such as genetic (polymorphism of candidate genes) and non-genetic factors (habitual intake of food, intention of labor and sunray exposure), could interact each other to exacerbate or ameliorate the problem [25]. The equilibrium among genetic and non-genetic determinants could be modified through the immigration process from undeveloped (Africa, Asia etc.) to developed countries.

The aim of this study is to determine the prevalence of some gene polymorphisms associated with osteoporosis in two groups of post-menopausal women from Sub-Saharan to Mediterranean regions, different in term of lifestyle, compared to a third group of Mediterranean women with documented osteoporosis. Since differences in genotypes of selected candidate gene are not always connected to differences in BMD, the weight of environmental factors in the pathogenesis of osteoporosis was also considered.

Materials and methods

Inclusion and exclusion criteria

African women

One hundred of the 360 postmenopausal women living at the Centre Delwende of Tanghin (Ouagadougou, Burkina Faso) were enrolled using a random population-based sampling in a period between July and October 2004. They were in the 50–100-year-old range (mean 66.5 ± 8.9) and all were considered in menopause because their interruption of menstrual cycle. All subjects enrolled were from Ouagadougou or nearby villages. Their diet was typical of the country: millet flower with vegetable sauce, cereals, pork (rarely or no more than once a week), and local seasonal fruits. The diet of all subjects was hypocaloric, averaging between 1,200 and 1,400 kcal/day. No vitamin supplementation was given. The women carried out simple household chores inside Centre Delwende of Tanghin.

Sicilian women

According to the Eurage Senieur Protocol criteria, 100 normal postmenopausal women were selected according randomly population-based recruitment at Institute of Medical and Environmental Research (IRMA), Acireale (CT), Italy and 100 osteoporosis Sicilian women were also selected randomly at the same institution. All performed a BMD measurement at IRMA Laboratory and the osteoporosis group was selected on the basis of T-score considering a cutoff of less than -2.5 . Their age was 53.4 ± 4.4 (45–65) and 49.9 ± 3.1 (55–70) years old, respectively. They went in menopause for the absence of menstrual cycle between 45 and 52 years old. The 88% of them lived at home and were engaged in domestic work. Of this group, 12% had a regular employment in a public administration, and actually were retired. All of them had a sedentary lifestyle. None of them used alcoholic drinks and a few of them were smokers. They were married with 0–3 sons and their diet was typical of Mediterranean habits.

Clinical evaluations

In African and Sicilian women weight, height, blood pressure, heart (radial artery) and ventilation rate were evaluated using a standard protocol. The body mass index (BMI) was calculated in all subjects using the weight/height² formula. All subjects involved in this study gave a full social and medical history and underwent a physical examination.

Exclusion criteria

A weight over the 97th percentile, systolic pressure greater than 2 SD per age, cardiomyopathy, clinical evidence of endocrine disorders (thyroid, surrenal or pancreas) influencing the bone metabolism, renal, lung, stomach, intestine or liver diseases, prolonged use of steroids, antacids and anticonvulsant medications, which are all known to be risk factors for osteoporosis, were used as exclusion criteria.

The study was approved by the Ethical Committee of CMSC of Ouagadougou, Africa and IRMA of Acireale (CT), Italy. All women gave their informed consent for this study.

Bone mass density measurement

In Burkinabe women the BMD was measured with Sonic Bone Profiler Instrument (IGEA SRL) using bone ultrasonography of the distal metaphysis of phalanx (II–V finger). The T-score value was calculated using Eq. 1

$$\text{T-score} = \frac{(\text{AD} - \text{SoS}_{\text{measured}} - 2124)}{70} \quad (1)$$

where the AD-SoS (amplitude dependent speed of sound) corresponds to speed of sound measured in the patient. 2124 m/s corresponds to reference value for young healthy subjects and 70 m/s represents the standard deviation of healthy young population. A T-score value less than -3.2 , for AD-SoS at the phalanx, was considered to be abnormal [26].

In Sicilian women the BMD measurement was focused on the proximal femur with dual energy X-ray absorptiometry (DXA) and the T-score was obtained. T-score was calculated by reference tables showing mean and standard deviation for age according the formula:

$$\text{T-score} = \frac{\text{subject's BMD value} - \text{mean young normal BMD value}}{\text{young normal BMD standard deviation}}$$

A T-score cutoff for DXA of less than -2.5 was considered significant for osteoporosis.

Collection, processing and storage of blood samples

Blood samples of peripheral blood (5 ml in a plain tube and 5 ml in EDTA) were collected from both African and Sicilian women in the morning after overnight fast. Blood in EDTA-containing tubes was immediately centrifuged at $1500 \times g$ (the centrifugation started within 2 min after the collection) for 10 min at 4°C . Plasma was collected and stored at -80°C . The remaining leukocytes and packed red cells were stored at -80°C for DNA analysis. Blood in tubes containing no additive was left at room temperature for 30 min. Then, serum was separated by centrifugation and stored at -80°C .

Routine haematological studies

Clinical tests of African women were performed at the laboratory of CMSC of Ouagadougou-Africa and those of Sicilian women at IRMA, Acireale (CT), Italy, using the same standard methods. A value of $123.16 \mu\text{mol/l}$ was considered as the upper reference limit for serum creatinine. Moreover, we used 2 SD from the normal value as limit for other laboratory parameters.

The determination of Hcy and other thiols within the plasma was carried out by HPLC at the Clinical Biochemistry Laboratory of Catholic University, Rome, Italy.

Other biochemical studies

Folate level $<11.33 \text{ nmol/l}$ was considered hypofolatemia, serum values $<20.23 \text{ nmol/l}$ and $<115.86 \text{ pmol/l}$ for B6 and B12, respectively were considered low.

Cystatin C values ranging from 0.69 to 2.30 mg/l were considered normal. All the above parameters were evaluated at the Clinical Biochemistry Laboratory of Catholic University, Rome, Italy.

Molecular studies

DNA extraction

DNA was purified, according to the QIAamp DNA blood kit instruction (Qiagen—www.qiagen.com), from peripheral leucocytes and DNA quantification was performed by a spectrophotometer method (OD260/OD280).

Polymerase chain reaction

PCR amplifications were performed, by DNA Thermal Cycler PTC 200 MJ Research, with two different primer pairs corresponding to the internal region of VDR receptor [27], ER receptor [28], CTR receptor [20], COL1A1 collagen [29].

Enzymatic digestion of PCR products and genotyping

After amplification, 10 µl of PCR products for COL1A1 and CTR, 20 µl of PCR products for ESR-X and ESR-P and 8 µl of PCR products for VDR were digested with 5 U of *BsmI* (VDR), 2.5 U of *XbaI* (ESR-X), 5 U of *PvuII* (ER-P), 3 U of *AluI* (CTR) and 2.5 of *Fnu4HI* (COL1A1) restriction endonucleases, at 37°C for 1 h for CTR and VDR, 2 h for ESR-X, ESR-P and up to 3 h for COL1A1.

Gel electrophoresis

The digested products were electrophoresed on polyacrylamide gel in 10% X TBE at 180 V for 30 min, stained with GelStar nucleic acid gel stain (Cambrex) and investigated at 312 nm. The obtained products were: 339 bp (VDR), 1300 bp (ESR), 230 bp (CTR), 257 bp (COL1A1). Polymorphisms, related restriction fragments and their clinical significance are summarized in Table 1.

Statistical methods

Laboratory data are presented as mean values \pm standard deviation. Statistical comparisons were performed using the Student *t*-test, when appropriate. Allele and genotype frequencies were compared between African and Sicilian postmenopausal women with and without osteoporosis, using either Fisher's exact probability test or the χ^2 test (SPSS-10 program for Windows, SPSS Inc, Chicago, USA). Carriage rates for alleles were calculated as the portion of individuals with at least one copy of the allele.

Table 1 Polymorphisms, related restriction fragments and their clinical significance

Gene	Proteins	Polymorphism	Restriction fragments
VDR	Vitamin D receptor	BB	339
		Bb	339 + 260 + 79
		bb	260 + 79
ESR (X)	Estrogen receptor	XX	1,300
		Xx	1,300 + 900 + 400
		xx	900 + 400
ESR (P)	Estrogen receptor	PP	1,300
		Pp	1,300 + 850 + 450
		pp	850 + 450
CTR	Calcitonin receptor	CC	339
		CT	339 + 260 + 79
		TT	260 + 79
COL1A1	Collagen type 1 α -chain	SS	180 + 54 + 23
		Ss	180 + 77 + 54 + 23
		ss	180 + 77

Each gene locus was also examined for the allele dosage effect, by comparing the numbers of heterozygous and homozygous individuals among Africans and Sicilians with or without osteoporosis. An association analyses was performed to examine the relation between genotypes and BMD. The relationship among the variables in a multivariate analysis was performed by a dependence perspective, to verify the influence of social factors (physical and work activity, sun exposition, nutrition habit) and biological parameters (VDR, ESR (X), ESR (P), CTR, COL1A1) on BMD. We analyzed by a two-step regression model the β -coefficients and, the significance by a Student *t*-test with SPSS-10 program for Windows (SPSS Inc, Chicago, USA) was carried out. Expected genotype frequencies were estimated from each woman group using the Hardy–Weinberg equilibrium model and results were compared with observed frequencies using χ^2 analyses. The power of samples calculated at a significance limit <0.05 with Statmate 2 program for Windows (GraphPad Prism v. 4, La Jolla, USA) was $>60\%$. A *P*-value <0.05 was selected for significance in all the statistical tests.

Results

Table 2 shows both clinical and social characteristics of the studied women. The age of African and Sicilian women were comparable. There was a significant difference in the number of deliveries and the educational level was clearly poorer in African women when compared to Sicilians. African women had houses without electricity, private water supply nor toilets and they spent all the day outside,

Table 2 Clinical and social parameters for African, Sicilian and Sicilian women with osteoporosis

Parameter	African women (<i>n</i> = 100) (A)	Sicilian women (<i>n</i> = 100) (B)	Sicilian osteoporotic women (<i>n</i> = 100) (C)
Age (years)	67 (50–100) 66.5 ± 8.9*	55 (45–65) 52.39 ± 4.38 [^]	64 (55–70) 49.91 ± 3.08
BMI (kg/m ²)	24.2 ± 2.6**	25.3 ± 2.4 ^{^^}	24.5 ± 2.6
Number delivery	1–9	1–3	0–3
Maternal education (%)			
None	100*	0	0
Primary school	0	100	100
Secondary school	0	70	60
Higher level	0	5	1
Woman's occupation (%)			
None	0	0	0
Domestic work	100	70	88
Employment in office	0	30	12
Seniority pension	0	30	12
Homes with (%)			
No electricity	100*	0	0
No refrigerator or freezer	100*	0	0
No private water supply	100*	0	0
No private toilets	100*	0	0
Radio set	0	100	100
Television set	0	100	100

A → B: * $P < 0.0001$, ** $P = 0.0022$; B → C: [^] $P = 0.0001$, ^{^^} $P = 0.0249$

engaged in domestic works. The 30% of the Sicilian women were employed in a public office and actually were retired. The remaining 70% were housewives. About 12% of women with osteoporosis had a dependent work in public office and actually were retired. The remaining 88% were housewives.

Table 3 shows the laboratory parameters of studied women. The most striking differences were found in the blood pressure, serum nitrogen, serum triglycerides, serum creatinine, plasma Hcy, and folate levels which were significantly lower in Africans than in Sicilians. Other parameters, such as serum cholesterol, cystatin C, B6 and B12 vitamins, were comparable.

Table 4 summarizes the most relevant information about the lifestyle of women enrolled in this study.

African women were used to exercise intense physical activity outside, and the sun exposition was continuous in the light hours. Their alimentation is poor and rarely the caloric intake reached a maximum of 1,400 calories. Also proteins are introduced in limited quantities (10–15 g/day). Sicilian women worked in public service or at home and rarely were exposed to direct sun. They eat regularly a Mediterranean diet and their caloric intake is about 1,700–1,900 kcal. The T-score for AD SoS gave values significantly negative for osteoporosis in Burkinabe women

(-0.12 ± 0.10). The T-score measured by DXA in Sicilian women with osteoporosis was significantly altered (-2.66 ± 0.75), thus confirming the bone fragility, while in healthy Sicilian women was -0.72 ± 0.39 .

Table 5, Figs. 1 and 2 report the genotypes and allele frequencies of the five polymorphisms associated with osteoporosis.

The statistical analysis of allele frequencies showed that the allele B of the VDR gene was significantly lower in African women when compared with normal and osteoporosis Sicilian women ($P < 0.01$ and $P < 0.001$, respectively). The allele frequency of VDR B was also significantly lower in Sicilian healthy women with respect to osteoporosis Sicilian women ($P < 0.05$). The ESR X allele frequency was significantly lower in both Africans and Sicilians compared to Sicilian women with osteoporosis ($P < 0.01$ and $P < 0.001$, respectively). The ESR P allele was significantly lower in healthy Sicilian women with respect to Sicilian women with osteoporosis ($P < 0.01$). The CTR T was significantly lower in both African and Sicilian healthy women compared to Sicilian women with osteoporosis ($P < 0.001$). The COL1A1 polymorphism of collagen did not show any statistical significant difference among the three groups of studied women. Based on the Hardy–Weinberg model, the

Table 3 Laboratory parameters for African, Sicilian, and Sicilian women with osteoporosis

Parameters	African women (n = 100) (A)	Sicilian women (n = 100) (B)	Sicilians osteoporotic women (n = 100) (C)
Blood nitrogen (mmol/l)	2.2 ± 0.33**	3.3 ± 0.39	3.2 ± 0.43
Serum cholesterol (mmol/l)	4.51 ± 0.82	4.64 ± 0.84	4.60 ± 0.83
Serum triglycerides (mmol/l)	0.97 ± 0.10**	1.19 ± 0.15 [^]	1.13 ± 0.15
Serum creatinine (mmol/l)	69.83 ± 14.14**	88.7 ± 12.4	87.8 ± 12.5
Cystatin C (mg/l)	0.86 ± 0.24	0.89 ± 0.21	0.88 ± 0.23
Hcy (mmol/l)	12.2 ± 5.7**	16.4 ± 5.2	16.2 ± 4.7
Serum folate (nmol/l)	13.37 ± 5.1**	24.3 ± 6.5 [^]	21.6 ± 5.7
Serum Vit B ₆ (nmol/l)	24.7 ± 12.9	27.9 ± 13.0	26.3 ± 12.5
Serum Vit B ₁₂ (pmol/l)	527.7 ± 277.8	587.4 ± 255.6	547.5 ± 245.3
Systolic blood pressure (mm Hg)	139.9 ± 22.5*	146.3 ± 23.2	145.8 ± 24.3
Diastolic blood pressure (mm Hg)	79.5 ± 13.6*	83.8 ± 13.9	83.2 ± 12.4

A → B and A → C: * P < 0.05, ** P < 0.0001; B → C: [^] P < 0.01

Table 4 Life style in African, Sicilian and Sicilian women with osteoporosis and values of T-score calculated with AD SoS and DXA, respectively

Life style	African women (n = 100) (A)	Sicilian women (n = 100) (B)	Sicilian women (osteoporosis) (n = 100) (C)
Physical activity	Elevated (++++)	Moderate (+++-)	Moderate (++--)
Sun exposition	++++	++--	++--
Energy intake (kcal/day)	1,200–1,400 [^]	1,800–1,900	1,700–1,800
Protein (g/kg per day)	0.22 ± 0.10 [^]	0.75 ± 0.15	0.72 ± 0.14
T-score (AD SoS)	-0.12 ± 0.10	-	-
T-score (DXA)	-	-0.72 ± 0.39 [^]	-2.66 ± 0.75

A → B → C: [^] P < 0.0001

Table 5 Allele frequencies of gene polymorphisms associated with osteoporosis in African, Sicilian and Sicilian women with osteoporosis

Polymorphisms of osteoporosis alleles	African women allele frequency (%) (A)	Sicilian women allele frequency (%) (B)	Sicilian women (with osteoporosis) allele frequency (%) (C)
VDR B	22.8** +++	43 [^]	57.5
VDR b	77.2	57	42.5
ESR X	43.5 ⁺⁺	33.5 ^{^^}	65
ESR x	56.5	66.5	35
ESR P	52.6	40 ^{^^}	60
ESR p	47.4	60	40
CTR C	59.5	66	32.5
CTR T	40.5 ⁺⁺⁺	34 ^{^^}	67.5
COL1A1 S	64.7	73.5	62.5
COL1A1 s	35.3	26.5	37.5

A → B: * P < 0.05, ** P < 0.01, *** P < 0.001; A → C: ⁺ P < 0.05, ⁺⁺ P < 0.01, ⁺⁺⁺ P < 0.001; B → C: [^] P < 0.05, ^{^^} P < 0.01, ^{^^^} P < 0.001

observed percentages of single specific polymorphisms did not differ from that expected with the exception of VDR (b) and ESR (X) in healthy African women (P < 0.001) and ESR (P) in healthy Mediterranean women (P < 0.001) (Table 6).

The association analyses that takes into account social characteristics and the polymorphisms of genes involved in the pathogenesis of osteoporosis, demonstrated that the physical activities and the healthy diet, associated with a outside work are the best favorable prognostic factors for

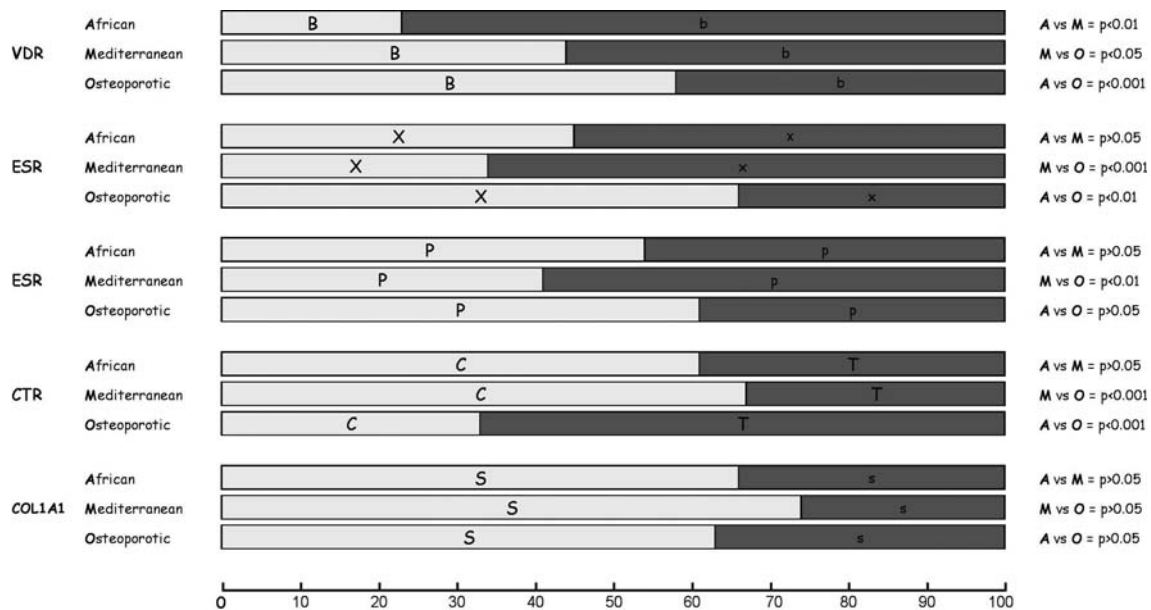


Fig. 1 Allele frequencies of some osteoporosis-related gene polymorphisms in African women from Sub-Saharan area and in Mediterranean women without and with osteoporosis

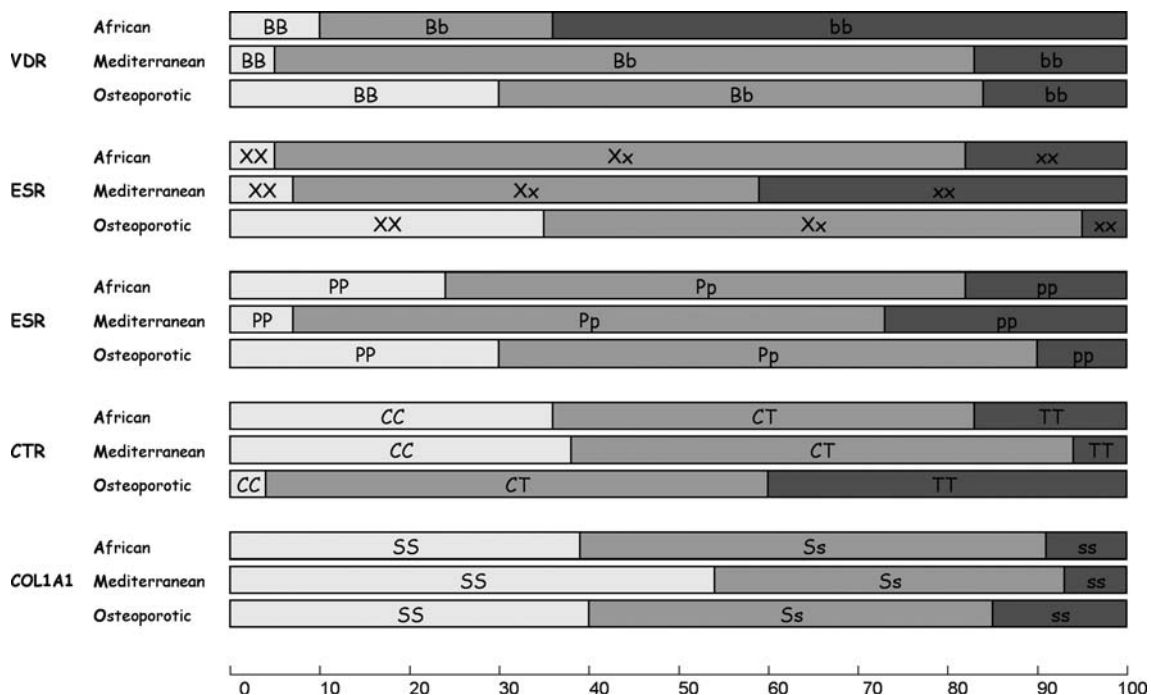


Fig. 2 Distribution of genotypes for some osteoporosis-related gene polymorphisms in African women from Sub-Saharan area and in Mediterranean women without and with osteoporosis

this severe diseases. In fact, a more significant difference was found in social parameters between African and Sicilian women with respect to the polymorphism frequencies (Table 7).

For the dependence analysis, the significant β -coefficients with P -value <0.05 in the multivariate two-step

regression model showed that both physical activity and sun exposition (standardized coefficients 0.485 and 0.264, respectively), as well as CTR, VDR and ESR (X) (standardized coefficients -0.091 , 0.085 and 0.104 , respectively) influence the BMD of African and Sicilian women (Table 8).

Table 6 Comparison between observed and expected genotype frequencies on the basis of the Hardy–Weinberg equilibrium

Genes	Series of women	Genotypic frequencies						χ^2 (*) vs. (**)
		Observed (*)			Expected (**)			
		BB	Bb	bb	BB	Bb	bb	
VDR	African	0.1	0.26	0.64	0.05	0.35	0.59	$P < 0.05$
	Sicilian	0.13	0.6	0.27	0.18	0.49	0.32	$P = \text{N.S.}$
	Osteoporosis	0.3	0.55	0.15	0.33	0.49	0.18	$P = \text{N.S.}$
		XX	Xx	xx	XX	Xx	xx	
ESR	African	0.06	0.76	0.18	0.19	0.49	0.31	$P < 0.001$
	Sicilian	0.07	0.53	0.4	0.11	0.45	0.44	$P = \text{N.S.}$
	Osteoporosis	0.35	0.6	0.05	0.42	0.46	0.12	$P < 0.01$
		PP	Pp	pp	PP	Pp	pp	
ESR	African	0.24	0.58	0.18	0.28	0.5	0.22	$P = \text{N.S.}$
	Sicilian	0.07	0.66	0.27	0.16	0.48	0.36	$P < 0.001$
	Osteoporosis	0.3	0.6	0.1	0.36	0.48	0.16	$P = \text{N.S.}$
		CC	CT	TT	CC	CT	TT	
CTR	African	0.36	0.47	0.17	0.35	0.48	0.16	$P = \text{N.S.}$
	Sicilian	0.38	0.56	0.06	0.44	0.45	0.12	$P = \text{N.S.}$
	Osteoporosis	0.05	0.55	0.4	0.11	0.44	0.46	$P < 0.05$
		SS	Ss	ss	SS	Ss	ss	
COL1A1	African	0.39	0.52	0.09	0.42	0.46	0.12	$P = \text{N.S.}$
	Sicilian	0.54	0.39	0.07	0.54	0.39	0.07	$P = \text{N.S.}$
	Osteoporosis	0.4	0.45	0.15	0.39	0.47	0.14	$P = \text{N.S.}$

Discussion

The reason for the use of peripheral bone ultrasonography in Burkinabe women, while the BMD (DXA) measurement was made only in Sicilian, was due to the less burden of a portable apparatus in such a remote part of world. Even though ultrasound is not used to diagnose osteoporosis, evidence [30, 31] lends support to their use for the assessment of fracture risk in elderly women [32], with results comparable to BMD (DXA). In fact, Soballa et al. have shown that the detection level of pathological changes in osteoporosis are similar between AD SoS and BMD (DXA) [33].

The results of this study show a significant reduction of VDR polymorphisms in African population, which fits well with the low prevalence of osteoporosis among African postmenopausal women. Vitamin D is involved in the phosphate re-absorption from the renal tubules and in

the intestinal calcium absorption. African women introduce low quantities of calcium with aliments [34]. Nevertheless, they have the advantage of better utilizing the calcium having higher production of vitamin D due to the sunray exposure [35]. Therefore, the differences in genotype of the selected candidate genes may not be simply connected to the difference in BMD. It is known that African women reach the bone mass peak (BMP) earlier and are rarely affected by osteoporosis in the postmenopausal period, and the fracture rate is lower in the African than in the Caucasian population [36]. However, this observation is not consistent with the data of Aspray et al. [37], who measured bone mineral content (BMC) and bone mineral density (BMD) in rural Gambian women and compared these results with Caucasian women of same age. BMC in Gambians was lower than in Caucasian women. Nevertheless, trauma fractures are rare in this African population and these results challenge

Table 7 Association analyses of lifestyle and osteoporosis polymorphisms versus the presence of osteoporosis in African and Sicilian women

	Physical activity	Sun light	Work activity	Nutrition style	VDR	ESR (X)	ESR (P)	CTR	COL1A1
African women (n = 100)	$P < 0.0001$	$P < 0.001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	N.S.	$P < 0.001$	N.S.
Sicilian women (n = 100)	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.0047$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	N.S.

Table 8 Multivariate two-step regression model with BMD as dependent variable in African, Sicilian and Sicilian women with osteoporosis

Model 1	Unstandardized coefficients		Standardized coefficients Beta	<i>t</i> SE	Significance Beta
(Constant)	-3.553	0.690		-5.152	<0.0001
Physical activity	0.748	0.166	0.485	4.501	<0.0001
Sun exposition	0.467	0.228	0.264	2.047	0.042
Work activity	0.134	0.167	-0.048	-0.806	0.421
Nutrition style	0.044	0.065	0.029	0.675	0.500
CTR	-0.163	0.070	-0.091	-2.324	0.021
VDR	0.147	0.069	0.085	2.134	0.034
COL1A1	-0.028	0.068	-0.015	-0.406	0.685
ESR (X)	0.209	0.083	0.104	2.514	0.013
ESR (P)	0.010	0.075	0.005	0.135	0.892
Age	-0.008	0.011	-0.025	-0.687	0.493

the concept of BMC as a primary determinant of fracture risk in African population. Another study involving Gambian women living in the UK [38] demonstrated that they did not show significant differences in bone mineral content (BMC), bone mineral density (BMD) and compared to Caucasian woman, without significant differences in bone turnover and calcitropic hormone levels. Also this study revealed few ethnic differences in osteoporosis fracture rates when Africans and Caucasians live in the same western country.

When African women migrate from undeveloped to developed countries modifying their lifestyle, the hypothetical advantage of genetic characteristics (low prevalence of VDR polymorphisms) may disappear, overriding the role of environmental factors. It is known that African women living in western countries, lose the traditional diet, reduce their physical activity [39] and can show osteoporosis.

The difference of the VDR polymorphism found in this study between Sicilian women with osteoporosis and healthy Sicilian women seems to sustain the role of VDR (B) in the osteoporosis.

However, it was surprisingly to observe the absence of any difference in the ESR (X) allele prevalence between healthy African and Sicilian women, while we observed a difference comparing both groups with Sicilian women with osteoporosis. Moreover, the ESR (P) in African women did not show statistically significant differences versus Sicilian women with osteoporosis. In addition, only a negligible statistically difference was found between Sicilian women with osteoporosis and healthy Sicilian women. This result could suggest that the weight of ESR (P) allele in African women may be compensated by ESR (x) allele. In fact, among African women the concomitant presence of the PPXX genotype was demonstrated only in five women, while the same genotype was present in ten Sicilian women with osteoporosis.

The CTR (T) prevalence in both African and Sicilian women are consistent with the previously published data [40]. The CTR (T) allele at the homozygote is associated with higher bone fragility in women with osteoporosis [20, 41], while the CC genotype is associated with a higher bone strength [20]. No statistical differences in the CT genotype prevalence were found in the present study, while a significant difference in the osteoporosis group was noted (<0.05).

The COL1A1 polymorphism does not differ in the three studied groups, indicating that the bone collagen structure does not interfere with the bone strength [10]. These observations were also confirmed by other studies performed in different ethnic groups [42], such as South African whites, blacks and Indians.

Significantly the association analysis demonstrated that the healthy lifestyle hinders the appearance of osteoporosis. African women have a poorer diet, especially in protein compared to Sicilian women and have more elevated physical activity. Consequently, laboratory parameters (blood nitrogen, serum triglycerides, serum creatinine) and plasma Hcy are significantly lower than those observed for Sicilian postmenopausal women. In our previous study [43], we found that plasma Hcy levels in African postmenopausal women were relatively elevated, but lower than Caucasian postmenopausal women. This could represent another protective factor, since an association of Hcy, MTHFR genotype, folate, vitamin B12 and vitamin D B6 levels with low BMD, in postmenopausal British women, has been recently demonstrated [44]. In addition the African women live outside, exposing their body to sunrays. It is important to consider that the immigration process represents a radical change in life habits for individuals coming from equatorial countries and conditions such as obesity, diabetes, cardiovascular diseases, hypertension are more frequent after immigration, due to the diet choices and to inactivity [45, 46].

The conclusion of this study remains that both genetic and environmental factors are important in the osteoporosis pathogenesis. However, the importance of lifestyle and environmental factors were particularly relevant, especially for immigrants where a life habit change can tip the weight of genetic component thus increasing their related healthy costs.

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