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COLLAGEN I α 1 AND VITAMIN D RECEPTOR GENE POLYMORPHISMS IN SOUTH AFRICAN WHITES, BLACKS AND INDIANS

P.J. Ojwang, MBChB, FRC Path (UK), FC Path(SA), R.J. Pegoraro, BSc, PhD (Natal), L. Rom, Dipl. Clin. Path and P. Lanning, Nat. Higher Dipl. Med. Tech., School of Pathology and Laboratory Medicine, Department of Chemical Pathology, Nelson R Mandela School of Medicine University of Natal, Durban, South Africa.

Request for reprints to: Professor P.J. Ojwang, School of Pathology and Laboratory Medicine, Department of Chemical Pathology, Nelson R Mandela School of Medicine, University of Natal, Private Bag 7, Congella 4013, Durban, South Africa.

COLLAGEN I α 1 AND VITAMIN D RECEPTOR GENE POLYMORPHISMS IN SOUTH AFRICAN WHITES, BLACKS AND INDIANS

P.J. OJWANG, R.J. PEGORARO, L. ROM and P. LANNING

ABSTRACT

Objective: To determine whether polymorphic differences exist between black, white and Indian South Africans in genes associated with bone mineral density and osteoporosis.

Design: Genes selected were the vitamin D receptor (Apa I and Taq I polymorphisms) and collagen (Sp I transcription factor polymorphism) using standard molecular biology techniques.

Setting: Department of Chemical Pathology, Nelson R Mandela School of Medicine, University of Natal, Durban, South Africa.

Subjects: Healthy male and female blood donors living in the Durban metropolitan region, South Africa. The group comprised black Africans (n=264), white Caucasians (n=247) and Asians of Indian origin (n=194).

Results: No significant differences in genotypes were seen between white and Indian subjects. Blacks had a significantly higher frequency of the TT Taq I genotype and a significantly lower frequency of the Ss Sp I genotype. No ss genotype was detected in blacks.

Conclusion: The very low frequency of the collagen Sp I s allele and higher frequency of the VDR T allele in blacks may be associated with the lower incidence of osteoporosis in this ethnic group.

INTRODUCTION

Studies undertaken in the 1960s have clearly shown that there are ethnic differences in the prevalence of osteoporosis. In South Africa, it was established that black women have a lower incidence of this condition than their white counterparts; they also have a lower incidence of osteoporotic hip fractures(1,2). Ethnic differences in the incidence of osteoporosis were also reported from the USA and other countries(3,4). It was postulated at the time that these differences might be explained by differences in the peak bone mass attained in early adulthood and it was subsequently confirmed that blacks have a higher peak bone mass than whites(5-7). Peak bone mass as measured by bone mineral density is now recognised as a major determinant of the risk of osteoporosis.

Whilst the pathogenesis of osteoporosis is considered multifactorial, several studies have focussed on the genetic influence on the basic determinants of bone mineral density(8-10). Amongst these are the vitamin D receptor (VDR) and the collagen type I alpha 1 (COLIA1) genes.

In an Australian study in 1994, it was reported that common allele variants of the VDR gene accounted for up to 75% of the total genetic effect on bone density in healthy male and female twins(11). It was also shown that in homozygotes, the more common b allele, as defined by the Bsm I restriction enzyme, was associated with higher bone density, and the less common B allele with the lower bone

density(11). In addition it was demonstrated that the TT genotype defined by Taq I restriction enzyme, was associated with the lowest decline in bone mineral density compared to the Tt or tt genotypes. The TT genotype was shown to be in 90-100% concordance with the bb genotype.

In another study on American girls of Mexican descent, Sainz *et al*(12) demonstrated that the bb genotype and aa genotype (defined by Apa I restriction enzyme) had higher femoral bone densities than those with BB or AA genotypes.

These results have not been confirmed in subsequent studies, some of which found no association between VDR gene polymorphisms and bone mass or risk of hip fractures in osteoporosis(13-17). Thus, the association between VDR gene polymorphisms and bone mineral density remains controversial.

The possible association of the type I collagen encoding gene on bone density has also been investigated, given that type I collagen comprises 80% of bone protein. A guanidine to thymidine polymorphism in the Sp I binding site of the COLIA1 gene was found to be associated with low bone density and increased osteoporotic vertebral fractures(18). Women with Ss genotypes had lower bone mineral density at the femoral neck and lumbar spine than those with the SS genotypes. The lowest bone mineral density was found among those with the ss genotypes. Other studies have reported an over-representation of the Ss and ss genotypes among women with osteoporotic fractures(19).

More recent reports have however shown conflicting results. In a group of 133 Danish women followed for 18 years, no association of the COLIA1 Sp I polymorphism with the rate of bone loss was detected(20). Neither was this polymorphism shown to have any influence on bone mass or bone turnover rate in a Finnish study on early postmenopausal women(17).

Whilst no clear association of VDR and COLIA1 gene polymorphisms and bone mineral density has emerged in these studies on Caucasian populations, the differing susceptibility to osteoporosis shown by various ethnic groups still suggests a genetic basis to the pathogenesis of this disorder. In this study, we have determined the allele and genotype frequencies of the VDR (Taq I and Apa I) and COLIA1 (Sp I) gene polymorphisms in South African whites, blacks and Indians.

MATERIALS AND METHODS

Subjects: The subjects used in this study were obtained retrospectively from a cross-sectional cohort of healthy male and female blood donors living in the metropolitan area of Durban, South Africa. The group comprised black Africans (n=264), white Caucasians (n=247) and Asians of Indian origin (n=194). DNA was isolated from peripheral venous blood according to standardised procedures.

Vitamin D receptor polymorphisms: Detection of the Apa I and Taq I polymorphisms in intron 8 and exon 7, respectively, of the vitamin D receptor gene was determined by PCR based restriction analysis. The primers used were: 5'-GGCAGTGGTATCACCGGTCAGC - 3' (forward) and 5'-CTGGAAGGAGAGGCAGCGGTAC-3' (reverse).

Collagen polymorphism: The G→T polymorphism at the first base of a consensus site for the transcription factor Sp1 in intron 1 of the COLIA1 gene was detected by allele specific PCR amplification(18). The primers used were: 5'-CACCTGCCAGGGGAATGG-3' (forward) and 5'-GAGGGCGGGGAGAATA-3' (reverse) for detection of the G allele, and 5'-TGACCTGGAGGCATTGGT-3' (forward) and 5'-CAGCCCTCATCCCGCCA-3' (reverse) for detection of the T allele.

Statistics: Statistical significance of the genotype and allele frequency differences between the three racial groups was tested for by χ^2 . Differences were considered significant when $p < 0.05$.

RESULTS

The results for allele and genotype frequencies of the VDR and COLIA1 genes polymorphisms are shown in Table 1. There was no marked difference between the white and Indian subjects in any of the polymorphic alleles examined. All differences observed were in the black subjects.

With respect to the VDR gene, both the Apa I and Taq I homozygous genotype (AA and TT) were significantly higher in blacks ($p < 0.05$ and $p < 0.001$ for Apa I and Taq I, respectively). The lowest frequency of the tt genotype was also seen among the blacks (4%) which differed significantly from the 13% seen in whites and 11% seen in Indians ($p < 0.001$).

Table 1

Allele and genotype frequencies for the Vitamin D receptor and collagen gene polymorphisms in different racial groups

		Blacks (n=181)	Whites (n=238)	Indians (n=175)
Vitamin D receptor				
<i>Apa I</i>				
Genotype	AA	87 (48%)*	75 (32%)	64 (37%)
	Aa	74 (41%)	124 (52%)	81 (46%)
	aa	20 (11%)	39 (16%)	30 (17%)
Allele	A	248 (0.69)	274 (0.58)	209 (0.60)
	a	114 (0.31)	202 (0.42)	141 (0.40)
<i>Taq I</i>				
Genotype	TT	(n=287) 186 (65%)**	(n=239) 87 (36%)	(n=175) 61 (35%)
	Tt	89 (31%)	121 (51%)	94 (54%)
	tt	12 (4%)	31 (13%)	20 (11%)
Allele	T	461 (0.80)	295 (0.62)	216 (0.62)
	t	113 (0.20)	183 (0.38)	134 (0.38)
Collagen				
Genotype	SS	(n=264) 261 (99%)**	(n=247) 198 (80%)	(N=194) 163 (84%)
	Ss	3 (1%)	40 (16%)	28 (14%)
	ss	0	9 (4%)	3 (2%)
Allele	S	525 (0.99)	436 (0.88)	354 (0.91)
	s	3 (0.01)	58 (0.12)	34 (0.09)

* = $p < 0.05$; ** = $p < 0.001$

For the COLIA1 gene polymorphism, the S allele was predominant in all three racial groups (Table 1). The frequency of the s allele was significantly lower in blacks, at only 1% as against 12% and 9% ($p < 0.001$) in whites and Indians, respectively. There was complete absence of the ss genotype in blacks in contrast to four per cent in whites and two per cent in Indians. The homozygous SS genotype approached universality in blacks (99%) compared to 80% in whites and 84% in Indians ($p < 0.001$). The heterozygous Ss genotype was significantly more frequent in whites (16%) and Indians (14%), compared to one per cent in blacks ($p < 0.001$).

DISCUSSION

In South Africa, osteoporosis is predominantly a disease of Caucasians and the incidence of osteoporotic fractures is significantly higher in whites than in blacks(1,2,21).

Several studies have linked specific gene defects to metabolic bone disorders which result in osteoporosis. Loss-of-function mutations in the VDR gene was found to be responsible for vitamin D resistant rickets(22,23). Similarly, mutations in the oestrogen receptor gene are thought to cause osteoporosis(18), while young girls with loss-of-function mutations in the aromatase P450 gene have been shown to be osteoporotic and deficient in oestrogens(19). In pedigree studies of osteoporosis-pseudoglioma syndrome, characterised by juvenile onset blindness and osteoporosis, the loci for 'high peak bone density' and osteoporosis pseudoglioma syndrome were

found to be located in the same region on chromosome 11, suggesting the presence of a gene directly affecting bone density(24,25).

In studies to evaluate the genetic determinants of bone density and susceptibility to osteoporosis, the genes encoding VDR and COLIA1 have been investigated. Reports from earlier studies indicated a possible association between the polymorphisms of these genes and bone mineral density(11,12), while recently there has been evidence provided that the Sp I COLIA1 polymorphism is a functional genetic variant that predisposes to osteoporotic fractures by mechanisms involving a reduction in bone quality and quantity(26). The genetic mechanisms that lead to ethnic differences in bone mineral density continue to be explored. In this study, we have investigated three polymorphic allele and genotype frequencies in the VDR and COLIA1 genes in white, black and Indian South Africans.

Our data show a clear similarity between the genotype distribution in whites and Indians. The allele and genotype frequencies for the VDR gene were almost identical in both population groups. In blacks, the Apa I polymorphism frequency was higher but achieved only marginal significance compared to that observed in Whites and Indians. The 65% frequency of the TT genotype found in blacks was however significantly higher than that found in whites (36%) or Indians (35%) ($p < 0.001$). While it is not clear whether the TT genotype is directly linked to the relatively high bone mineral density, it is interesting to note that in a recent study, black South African women were found to have significantly higher bone mineral density at the femur and calcaneum (but not lumbar spine) independent of age and body mass index when compared to 'coloureds' and whites(27).

The TT genotype frequencies in the South African whites and Indians in this study (36 and 35%, respectively) was also lower than the 54% found in American girls of Mexican descent in which the relatively high TT genotype frequency was associated with a trend towards higher values for vertebral and femoral bone density. This study has also demonstrated a marked similarity between whites and Indians for the allelic frequencies of COLIA1 polymorphism. Our data revealed a predominance of the S allele in all three groups but with a significantly higher prevalence in blacks (99%) compared to 88% in whites and 90% in Indians ($p < 0.001$). The results also show a complete absence of the ss genotype in the black group with only one per cent for the heterozygote Ss. These results are in agreement with those from a previous study in the Gambia, West Africa, which showed a low frequency of the s allele among the Gambian blacks compared to Caucasian subjects from France, United Kingdom and Denmark(28). Since it has been postulated that bone mineral density is highest in those with the SS genotype and lowest in the ss genotype(19), the significantly higher frequency of the SS genotype accompanied by the absence of the ss genotype in the South African black population

may be partly responsible for the higher bone mineral density and lower susceptibility to osteoporosis found in this group.

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