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RFLP Report

A NOVEL SEQUENCE POLYMORPHISM IN EXON 8 OF THE HUMAN VITAMIN D-BINDING PROTEIN (GC) GENE IN AN AFRICAN POPULATION

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A novel sequence polymorphism due to a T to C transition at the third nucleotide of the codon for Cys^{283} of the vitamin D-binding protein (GC) gene assigned to chromosome 4q13-4q21.1 was revealed by sequence analysis. Population studies by single strand conformation polymorphism (SSCP) analysis showed this GC-283.3 site was polymorphic in a Black African population but monomorphic in a European population.

Key Words vitamin D-binding protein, group-specific component, sequence polymorphism, population study, PCR-SSCP analysis

DNA from 52 unrelated Black Africans from Cameroun and 58 unrelated Europeans from Southern Germany was amplified by polymerase chain reaction (PCR) followed by SSCP analysis.

Primers for PCR. E8F2: 5′-AATGCACAAACTAACCATTCG-3′ and E8RA22: 5′-GCAGCTGGCATGAAGTAAGT-3′.

Condition for PCR-SSCP. About 1 μg of genomic DNA was amplified with 50 ng of each primer in 50 μl PCR reaction mixture (10 mM Tris-HCl, pH 8.4/2.5 mM $MgCl_2$/50 mM KCl/200 μM of each dNTPs/1 unit of *Taq* polymerase). Denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 45 sec for 35 cycles. A mixture of 2 μl PCR products and 5 μl 100% formamide was heated at 95°C for 5 min followed by rapid cooling on ice. Electrophoresis was performed overnight at 300 V using 20% native polyacrylamide gel in a standard

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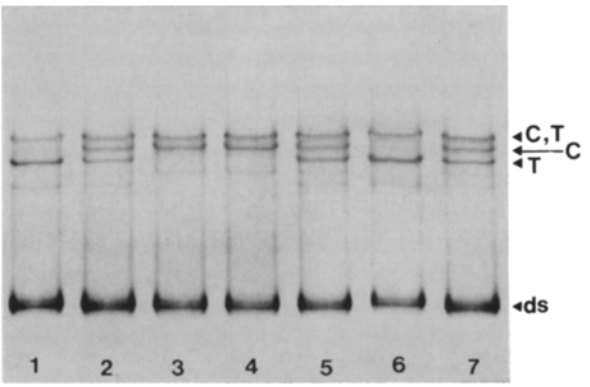

Fig. 1. SSCP analysis of GC-283.3 polymorphism in exon 8 of the human vitamin D-binding protein. Anode at bottom. C and T indicate single strands with cytosine and thymine, respectively and ds, double strands. Lanes 1 and 6, GC-283.3 T; 2, 5, and 7, GC-283.3 C/T; 3 and 4, GC-283.3 C.

Table 1. Distribution of GC-IEF and 283.3 phenotypes and allele frequencies in Black Africans.

GC-IEF phenotypes	GC-283.3				Allele frequencies
	C	C/T	T	Total	
1F	6	14	14	34	GC*1F=0.817
1F-1S	1	4	5	10	GC*1S=0.106
1S	0	0	0	0	GC*2=0.077
2-1F	1	3	3	7	
2-1S	0	0	1	1	GC-283.3*C=0.356
2	0	0	0	0	GC-283.3*T=0.644
Total	8	21	23	52	

Tris-borate-EDTA with cooling at 20°C. After electrophoresis bands were visualized by silver staining.

Polymorphism. The two alleles were detected by SSCP of the PCR products containing 29 bp of intron 7 and 122 bp of exon 8: GC-283.3*C was characterized by slow-migrating and intermediate bands and GC-283.3*T, by slow- and fast-migrating ones (Fig. 1).

Frequency. In Africans, 0.36 for GC-283.3*C and 0.64 for GC-283.3*T. PIC= 0.35. The frequency of expected and observed heterozygosity was 0.46 and 0.40, respectively. In Europeans, only GC-283.3*T was observed. This polymorphism segregated in Mendelian inheritance.

Comments. A well defined protein polymorphism of the GC has been described (GC-IEF locus), which is determined by two substitutions in exon 11 (Braun *et*

al., 1992). Table 1 shows the distribution of phenotypes and alleles of the GC-IEF and GC-283.3 polymorphisms. No association between the two markers was observed in the Africans. A few reasons for this observation are conceivable: it may be that there is a hot spot for the crossing-over between two exons or that the nucleotide substitutions in two exons is old enough to generate lots of recombinants. Anyway, each GC-IEF allele was divided into two alleles, *i.e.*, GC*1F^{C}, GC*1F^{T}, GC*1S^{C}, GC*1S^{T}, GC*2^{C}, and GC*2^{T}.

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Reference

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