Hum Genet (1992) 89:401–406

Human Genetics

Molecular analysis of the gene for the human vitamin-D-binding protein (group-specific component): allelic differences of the common genetic GC types

Andreas Braun, Regina Bichlmaier, and Hartwig Cleve

Institut für Anthropologie und Humangenetik der Universität, Richard-Wagner-Strasse 10/I, W-8000 München 2, Federal Republic of Germany

Received October 1, 1991 / Revised November 26, 1991

Summary. DNA sequence analysis of the polymerase chain reaction products, including the coding region for amino acids 416 and 420, of the vitamin-D-binding protein (DBP, group-specific component, GC) shows allele-specific differences. The GC2 and GC1F phenotypes have an aspartic acid residue at amino acid position 416, whereas the GC1S phenotype has a glutamic acid at this position. In the GC2 phenotype, amino acid 420 is a lysine residue, and in the both common GC1 phenotypes, it is a threonine residue. The nucleotide exchanges involve a *Hae*III (position 416) and a *Sty*I (position 420) restriction site: the *Hae*III restriction site is specific for the GC*1S allele and the *Sty*I restriction site is specific for the GC*2 allele. We have tested 140 individual genomic DNA samples for the *Hae*III site and 148 samples for the *Sty*I site by restriction fragment length polymorphism (RFLP) analysis with a DBP-specific direct genomic DNA probe, and have compared these findings with the GC phenotype classification, by isoelectric focusing (IEF) of the corresponding plasma. The results of the *Hae*III RFLP analysis and the IEF typing were in complete agreement. By using our DNA probe, we could disclose, in addition to the *Sty*I site at amino acid position 420, two further *Sty*I site downstream: one was specific for the GC*1S allele and another for the GC*1F allele. In 147 samples, there was agreement between the IEF GC typing and the analysis of the *Sty*I restriction sites. In a single case, the observed result of the *Sty*I-digest differed from the result expected after IEF classification: homozygous GC 1F-1F by IEF and heterozygous by *Sty*I RFLP analysis. We discuss this finding as a recombination event or a possible silent allele in IEF typing. The GC polymorphism revealed by Southern blot analysis of *Sty*I-digests provides an informative DNA marker system for chromosome 4q11–q13.

Introduction

The group-specific component (GC, vitamin-D-binding protein, DBP) is a polymorphic plasma protein discovered by Hirschfeld et al. (1959). There are three common alleles (GC*2, GC*1F, and GC*1S) and more than 120 variant alleles of the GC/DBP system in the human population (Cleve and Constans 1988). GC is a globular protein of the α_2-fraction of human serum with a molecular mass of 51200 kDa (Schoentgen et al. 1986). Only part of the GC*1S and GC*1F allele product is o-glycosylated with a terminal sialic acid residue (Svasti and Bowman 1978). Therefore, the GC1S and GC1F types are characterized by a double-band pattern as analyzed by isoelectric focusing (IEF), whereas the non-glycosylated GC2 type is present as a single band (Cleve and Patutschnik 1979). The interaction of GC with four other biomolecules results in four apparently independent biological functions. First, GC binds vitamin D_3 and its natural derivatives, with the highest affinity for 25-(OH)-vitamin D_3, the inactive precursor of the 1,25-$(OH)_2$-vitamin D_3. In this function, GC is a transparent protein for vitamin D_3 and its derivatives (Daiger et al. 1975; Haddad and Walgate 1976). Secondly, GC stabilizes monomeric G-actin in plasma, thereby preventing the spontaneous polymerization of G-actin to F-actin and, thus, possible damage to the capillary blood system (Van Baelen et al. 1980, 1988). Thirdly, GC binds to immunoglobulin G and could therefore play a role in immunological responsiveness (Constans et al. 1981; Petrini et al. 1985). Lastly, GC can serve as a co-chemotaxin for C5a and C5a des Arg, thereby enhancing the chemotactic activity and restoring the activity of these complement components (Perez et al. 1988; Kew and Webster 1988).

At present, the molecular basis for the biochemical differences of the three common alleles is known only in part. Two published GC cDNA sequences have revealed apparent nucleotide differences at 7 sites (Yang et al. 1985; Cooke and David 1985). Six differences are caused by single nucleotide exchanges, of which three lead to amino acid exchanges (positions 152, 416, and 420). The seventh difference concerns amino acid positions 310 and 311. Four nucleotides appear to be inverted resulting in an amino acid exchange at position 311. The derived amino acid sequence from the cDNA sequence of Yang et al. (1985) is identical with the amino acid sequence determined for the homozygous GC type 2-2

Correspondence to: A. Braun

(Schoentgen et al. 1986). Therefore, it seems possible that the other cDNA sequence could be specific for the GC*1S or the GC*1F allele.

Reynolds and Sensabaugh (1990) have examined three of the four different amino acid positions (311, 416, and 420) with the help of the polymerase chain reaction (PCR). The DNA sequences for amino acid positions 310 and 311 were identical for the three common GC alleles. At position 416, they found, for the GC*2 and GC*1F alleles, the codon for aspartic acid and, for the GC*1S allele, the codon for glutamic acid. The nucleotide exchange in the GC*1S allele results in a *Hae*III restriction site. The codon for threonine in the GC*1S and GC*1F alleles and for lysine in the GC*2 allele lies at position 420. The corresponding nucleotide exchange results in a *Sty*I restriction site for the GC*2 allele. The threonine residue is the possible site for the o-glycosylation of the GC*1 allele products (Svasti et al. 1979; Viau et al. 1983).

In this study, we confirm, by DNA sequence analysis, the nucleotide exchanges characteristic for the three common alleles as observed by Reynolds and Sensabough (1990). In addition, we have examined samples of 144 and 148 individuals by RFLP analysis for the *Hae*III and *Sty*I restriction sites, respectively, in order to test the uniformity of these allele-specific nucleotide exchanges. We have constructed a genomic DNA probe by the PCR method; it covers the nucleotide sequence comprising the positions for the amino acids at 416 and 420.

Materials and methods

Preparation of genomic DNA

Genomic DNA was prepared from 10 ml EDTA-treated blood samples that were collected from unrelated healthy blood donors from the blood bank of the Bavarian Red Cross at Munich, Bavaria. After centrifugation of the whole blood sample, the plasma supernatant was preserved for GC-typing by IEF. The erythrocytes were lysed twice in ice-cooled isotonic ammonium buffer (155 m*M* NH_4Cl, 10 m*M* $KHCO_3$, and 0.1 m*M* EDTA, pH 7.4). The white blood cells were resuspended in 5 ml 75 m*M* NaCl, 25 m*M* EDTA, pH 8.0, lysed with 250 µl of a 20% SDS solution, and incubated with 150 µg pronase E (Sigma, Munich, FRG) at 37°C for 14 h. On the next day, the sample was vortexed with 1.5 ml 5 *M* NaCl for 10 s and centrifuged for 10 min at 3000 *g*. The supernatant DNA was precipitated with 20 ml 100% ethanol and washed in 10 ml 70% ethanol. The precipitated DNA was resuspended in 0.5 ml 10 m*M* TRIS/HCl, 1 m*M* EDTA, pH 8.0.

PCR

For PCR, we used two exon-specific oligonucleotids spanning the coding region 1201–1220 (5′-GACAAGGGACAAGAACTATG-3′) for the 5′end, and 1371–1390 (5′-AATCACAGTAAAGAG-GAGGT-3′) for the 3′end. The *Taq* DNA polymerase was purchased from Boehringer Mannheim, FRG. The total reaction volume of 40 µl included about 1 µg genomic DNA, 50 ng of each primer, 1.25 U *Taq* DNA polymerase, 200 µmol of each dNTP, and 1.5 m*M* magnesium chloride. Each sample was subjected to the following 30 amplification cycles: 1 min at 94°C for denaturation, 1 min at 53°C for annealing, and 2 min at 72°C for extension.

Ligation, subcloning and sequencing of the allelic PCR products

The allelic PCR products were blunt-ended with T4-DNA polymerase (Boehringer, Mannheim, FRG) by elevating the magnesium concentration after the PCR reaction to 10 m*M* and adding 0.5 U of the enzyme. After incubation at 37°C, the PCR fragments were ready for ligation in the *Hin*dII site of pUC19 (BRL, Eggenstein, FRG). Ligation was performed with 1 U T4-DNA ligase (Boehringer, Mannheim, FRG) at 15°C for 14 h. Recombinant plasmids were subcloned in *E. coli* MC 1061 and purified with Quiagen midi-kit (Diagen, Düsseldorf, FRG) according to the manufacturer's instructions. DNA sequencing was performed by the dideoxy chain termination method (Sanger et al. 1977) using the T7 polymerase sequencing kit supplied by Pharmacia (Freiburg, FRG). Sequencing electrophoresis was carried out with a 6% acrylamide 6 *M* urea gel in a DNA sequencing electrophoresis unit (21 × 50 cm) from Biorad (München, FRG). The running conditions were 2000 V at 50°C for 2 h.

Restriction-digest and agarose electrophoresis

A 7.5-µg sample of genomic DNA or 10 µl of each PCR sample were digested with 10 U *Sty*I or *Hae*III (both restriction endonucleases were purchased from Boehringer, Mannheim, FRG) at 37°C for 1 h. The genomic DNA digests were separated in 0.8% (*Sty*I) or 1.3% (*Hae*III) agarose gel with 0.35 µg/ml ethidium bromide and 35 m*M* TRIS, 30 m*M* NaH_2PO_4, 1 m*M* EDTA as the electrophoresis buffer for 14 h at 25 V. The digested and non-digested PCR products were separated in a 1% agarose gel with 0.35 µg/ml ethidium bromide and 85 m*M* TRIS, 90 m*M* boric acid, 2 m*M* EDTA as the electrophoresis buffer at 100 V for 1.5 h. The separation was directly visualized by UV fluorescence. For all agarose gels, the 1 kb ladder supplied from BRL (Eggenstein, FRG) was used as the size standard.

Southern blotting and hybridization

Before blotting, the agarose gels with the separated genomic digests were soaked twice in 0.25 *M* HCl for 10 min, twice in 0.4 *M* NaOH and 1.5 *M* NaCl for 20 min, and once in 0.4 *M* NaOH for 5 min. The following DNA transfer on Hybond N^+ membrane (Amersham, Braunschweig, FRG) was performed by diffusion blotting in 0.4 *M* NaOH for 14 h. The membranes were washed twice in 0.3 *M* NaCl, 30 m*M* sodium citrate, pH 7.2, for 5 min.

Radioactive probes were obtained by labeling the purified PCR fragment with α-[^{32}P]dATP according to the random priming procedure (Feinberg and Vogelstein 1983). Hybridization was performed at a concentration of 5×10^6 cpm labeled fragment per ml of hybridization solution (0.5 *M* sodium phosphate, pH 7.2, 7% (w/v) SDS, and 1% (w/v) bovine serum albumin (Fraction V, Sigma, Munich, FRG) for 14 h. The membranes were subsequently washed three times in 40 m*M* sodium phosphate, pH 7.2, with 1% (w/v) SDS for 10 min. Autoradiography was performed on X-ray 90 over 2–4 days.

IEF and immunoprinting

IEF for disclosing GC types was carried out in polyacrylamide gels (0.5 mm thick) with a pH gradient between 4.5 and 5.4 on a Multiphore II unit from LKB (Freiburg, FRG). Saccharose (2 g) was dissolved in 2.8 ml 30% acrylamide stock solution [29.1 g acrylamide (Serva, Heidelberg, FRG) and 0.9 g bisacrylamide (Serva, Heidelberg, FRG) in 100 ml twice-distilled water]. To this was added 1.2 ml pharmalytes, pH 4.5–5.4 (Pharmacia, Freiburg, FRG) and 0.3 ml ACES stock solution [18 mg ACES (Serva, Heidelberg, FRG) in 5 ml twice-distilled water]. Hereafter, the gel solution was degassed for 5 min. Polymerization was started after addition of 5 µl TEMED (Serva, Heidelberg, FRG) and 0.5 ml 1% ammonium peroxodisulfate solution (Merck, Darmstadt, FRG). The gel solution was immediately transferred to a 0.5-mm casting chamber

(LKB, Freiburg, FRG). Polymerization was allowed to take place for 1 h at room temperature and overnight at 4°C. The electrode buffers were: for the anode 1.0 *M* H_3PO_4, and for the cathode 0.2 *M* NaOH. The running conditions were: 1900 V, 25 mA, and 20 W at 8°C.

Whole plasma samples were applied to Whatman 3 mm filter paper (3 × 5 mm). The filter papers were soaked in whole plasma and positioned at the top of the gel at a distance of 1 cm from the cathode.

The GC-specific bands were detected by immunofixation. For this, cellulose acetate sheets (Biotec-Fischer, Reiskirchen, FRG) were soaked in a 1:3 dilution of rabbit anti-human GC antiserum (Dakopatts, Hamburg, FRG) and placed on top of the gel for 3 min. After being rinsed in water for 30 min, the immunoprint was stained with 1.8 g Coomassie R 250 (Serva, Heidelberg, FRG) in 1 l ethanol/acetic acid/water (25/8/67) for 10 min and destained in ethanol/acetic acid/water alone.

Results

Characterization of the allelic DBP PCR products

By using the two DBP-specific exon primers described above, we could amplify a PCR product of about 2.0 kb. Sequence analysis showed that the PCR fragment included 62 bp from the 3′ end of exon 10, intron 10, and the 128 bp from the 5′ end of exon 11 in which the differences between the three common DBP alleles GC*2, GC*1F, and GC*1S reside (genomic human DBP sequence, A. Braun et al., in preparation). Restriction-digests of the allelic PCR products disclose a *Hae*III site specific for the GC*1S allele (Fig. 1A) and a *Sty*I site specific for the GC*2 allele (Fig. 1B). Furthermore, there are two *Hae*III sites in intron 10; these are common for all tested DBP alleles.

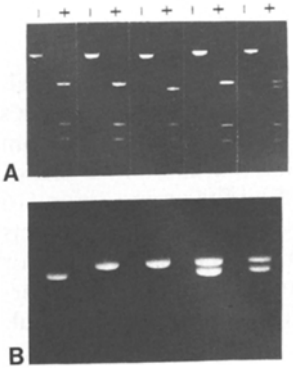

Fig. 1A, B. *Hae*III (**A**) and *Sty*I (**B**) restriction-digests of the allelic PCR products by using DBP-specific oligonucleotides that amplify the region coding for amino acids 416 and 420. −, Not digested; +, digested, in **A**

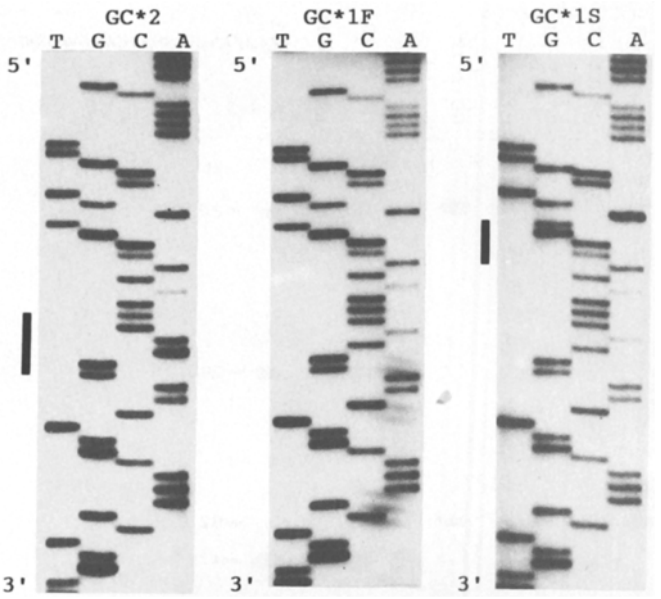

Fig. 2. Sequence analysis of the three common DBP alleles in the region coding for amino acids 416 and 420: GC*2, GC*1F, and GC*1S. The *Hae*III restriction site (5′-GGCC-3′) is marked *(vertical bar left)* in the GC*1S allele, and the *Sty*I restriction site (5′-CCAAGG-3′) is marked *(vertical bar left)* in the GC*2 allele

We subcloned the PCR products of the three different alleles in pUC 19 and sequenced them in the region of interest, i.e., at the nucleotide position coding for amino acids 416 and 420. Figure 2 shows the different nucleotide sequences of the three common DBP alleles. This result confirms the *Hae*III restriction site for the GC*1S allele at amino acid position 416, and the *Sty*I restriction site for the GC*2 allele at amino acid position 420.

HaeIII and StyI RFLPs

We tested, in this study, 144 individual DNA preparations for the existence of the *Hae*III restriction site in the DBP gene region coding for amino acid position 416. The hybridization of *Hae*III-digested Southern blots with the described RFLP probe disclosed two variant DNA bands. For the GC*2 and GC*1F alleles, the band lay at about 2.0 kb, and for the GC*1S allele at about 0.95 kb. In all cases, the RFLP pattern was concurrent with the IEF-determined DBP plasma protein phenotype. Figure 3A shows the *Hae*III Southern blot of all six common DBP phenotypes and two rare variants (1S-2A9 and 2-1C3). Table 1 summarizes the allele frequencies of the DBP IEF phenotypes and the *Hae*III RFLPs from our study in Southern Germany.

For the *Sty*I RFLP (DBP gene region coding for amino acid 420), we digested 148 individual DNA preparations. The result was surprising. In these digests, all three common DBP alleles could be differentiated by Southern blot analysis with our DBP-specific genomic DNA probe. For the GC*2 allele, a 2.5-kb band was detected, for the GC*1S allele a 4.75-kb band, and for the GC*1F allele a 6.2-kb band (Fig. 3B). We could ascribe the difference

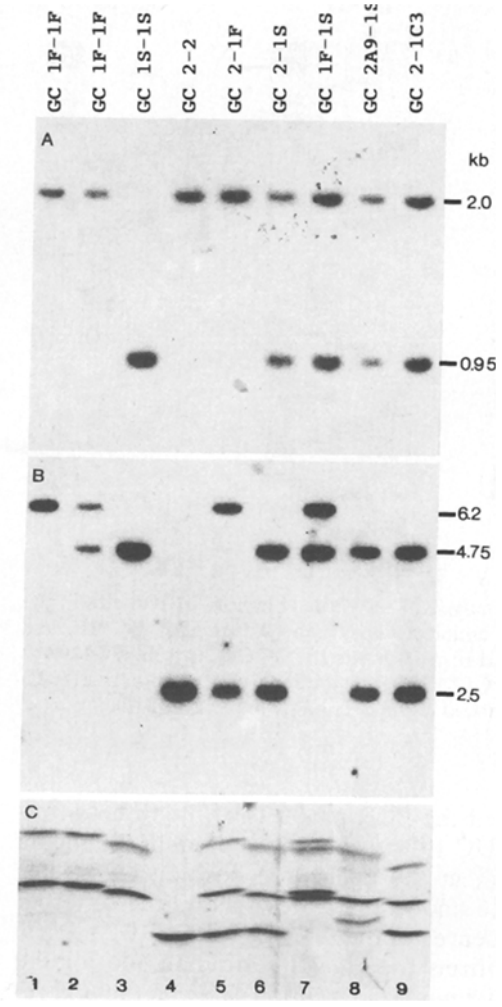

Fig. 3. A Southern blot of *Hae*III restriction-digest and **B** Southern blot of *Sty*I restriction-digest with a direct DBP-specific genomic DNA probe. **C** IEF of the appropriate plasma and immunoprinting with a polyclonal anti-GC antiserum (Dako, Hamburg, FRG). *Lane 1* GC 1F-1F; *lane 2* GC 1F-1F; *lane 3* GC 1S-1S; *lane 4* GC 2-2; *lane 5* GC 2-1F; *lane 6* GC 2-1S; *lane 7* GC 1F-1S; *lane 8* GC 1S-2A9; *lane 9* GC 2-1C3

between the GC*1S and the GC*1F alleles to the 3′downstream region of the triplet coding for amino acid 420, since the next *Sty*I site in the 5′upstream direction was sequenced in exon 9 and was present in all three common alleles. The distance from this site to the site coding for amino acid 420 is about 2.5 kb, which corresponds to the length of the restriction fragment of the GC*2 allele in the genomic Southern blot. A schematic representation of the allele-specific *Sty*I site is given in Fig. 4.

In one sample, the result of the *Sty*I-digest and the IEF plasma protein phenotype did not correspond. In this case, we disclosed the GC 1F-1F phenotype by IEF (Fig. 3C, lane 2) and the heterozygous allele bands for GC*1F/GC*1S by *Sty*I RFLP analysis (Fig. 3b, lane 2). However, in the *Hae*III RFLP analysis, we could not see the 0.95-kb band that is specific for the GC*1S allele (Fig. 3A, lane 2). In 147 samples, there was correspondance between the DBP plasma protein phenotypes and

Table 1. Allele frequencies of the DBP IEF phenotypes and the *Hae*III RFLPs in Southern Germany

*Hae*III in RFLP			GC IEF phenotypes		
Bands in kb	*n* observed	*n* expected	Types	*n* observed	*n* expected
0.95	44	45.2	1S-1S	44	45.2
2.0	25	26.1	1F-1F	5	4.6
2.0/0.95	71	68.7	2-2	9	8.8
			2-1S	41	39.8
			2-1F	11	12.7
			1F-1S	30	29.0
Total	140	140.0	Total	140	140.1
Allele frequencies			Allele frequencies		
*Hae*III * 0.95	0.5679		GC*1S	0.5679	
*Hae*III * 2.0	0.4321		GC*1F	0.1821	
			GC*2	0.2500	
Total	1.0000		Total	1.0000	

Fig. 4. Schematic representation of the *Sty*I restriction sites that can be evaluated with our DBP-specific genomic DNA probe. The *Sty*I[1] site is present in all common DBP alleles. The *Sty*I[2] site is involved in the region coding for amino acid 420 and is specific for the GC*2 allele. The *Sty*I[3] site is specific for the GC*1S allele, but we cannot test for the presence of this site in the GC*2 allele, with our probe. The *Sty*I[4] site is specific for the GC*1F allele, but we cannot test for the presence of this site in the GC*2 or GC*1S alleles, with our probe.

the analysis of the *Sty*I-digests. Table 2 summarizes the allele frequencies of the DBP IEF phenotypes and of the *Sty*I RFLPs.

Discussion

The two published GC cDNA sequences (Yang et al. 1985; Cooke and David 1985) show several differences when compared. Using PCR and RFLP analysis from Southern blots of genomic DNA, we examined two of these nucleotide differences coding for amino acids 416 and 420. The sequence analysis of the PCR products from this region obtained from donors homozygous for the three common GC alleles revealed the same differences as the published GC cDNA sequences (Yang et al. 1985; Cooke and David 1985), thereby confirming the results of Reynolds and Sensabaugh (1990). Thus, at amino acid position 416, the triplet GAT codes for an aspartic acid residue in the GC2 and GC1F phenotypes, whereas the GC1S phenotype has a glutamic acid residue at this position determined by the codon GAG. Amino acid 420 is a lysine residue in the GC2 phenotype and is coded by AAG, whereas a threonine residue in

Table 2. Allele frequencies of the LBP IEF phenotypes and the *Sty*I RFLPs in Southern Germany

*Sty*I RFLP			GC IEF phenotypes		
Bands in kb	*n* observed	*n* expected	Types	*n* observed	*n* expected
4.75	48	48.8	1S-1S	48	48.2
6.2	4	4.2	1F-1F	5	4.4
2.5	10	9.8	2-2	10	9.8
2.5/4.75	44	43.7	2-1S	44	43.4
2.5/6.2	12	12.8	2-1F	12	13.1
6.2/4.75	30	28.7	1F-1S	29	29.1
Total	148	148.0	Total	148	148.0
Allele frequencies			Allele frequencies		
*Sty*I * 4.75	0.5743		GC*1S	0.5709	
*Sty*I * 6.2	0.1689		GC*1F	0.1723	
*Sty*I * 2.5	0.2568		GC*2	0.2568	
Total	1.0000		Total	1.0000	

both GC1 phenotypes is coded by ACG. Threonine at position 420 is the putative site for the o-glycosylation (Svasti et al. 1979; Viau et al. 1983). These nucleotide exchanges result in new restriction sites for the endonucleases *Hae*III and *Sty*I, respectively. Their restriction-digests confirm the sequencing data and exclude a *Taq* polymerase failure manifested by the subcloning procedure. The digestion of the PCR products was also performed with two heterozygous individual DNA preparations (GC 2-1F and GC 2-1S) and led to the expected result for both restriction sites.

When 144 individual genomic samples were tested with respect to the *Hae*III RFLP, the results corresponded without exception to the GC classification in plasma by IEF followed by immunoprinting. The disadvantage in the RFLP *Hae*III system is that one cannot discriminate between the GC*2 and GC*1F alleles. On the other hand, a discrimination between all three common GC alleles is possible by *Sty*I RFLP analysis with our DBP-specific DNA probe. The 2.5-kb band of the GC*2 allele is marked by two *Sty*I sites in exon 9 and exon 11. This band includes intron 9 and intron 10 (genomic DBP DNA sequence; A. Braun et al., in preparation). With our DBP-RFLP probe, we cannot test the next 3′ downstream *Sty*I^3 site (Fig. 4), which is present in the GC*1S but not in the GC*1F allele. Moreover, we cannot test for the GC*2 and GC*1S alleles at the *Sty*I^4 site (Fig. 4), which is specific for the GC*1F allele in our RFLP study. Nevertheless, the use of this genomic DNA probe permits the differentiation of the three common alleles at the genic level.

The analysis of the RFLPs of the *Sty*I-digests corresponded to the GC plasma protein polymorphisms in all cases except one. In 147 out of 148 samples, *Sty*I analysis permitted the classification of the six common GC types. In the incompatible case, the homozygous type GC 1F-1F was found by IEF, and a heterozygous type 4.75 kb/6.2 kb was observed by *Sty*I RFLP analysis (Fig. 3, lane 2). We believe that another GC*1F allele might have occurred by a recombination event between the normal GC*1F allele and a GC*1S allele: this event might have taken place between the site coding for amino acid 416 and the *Sty*I site specific for the GC*1S allele in our RFLP study. If the GC*2 allele has the *Sty*I^3 site (Fig. 4), it could also represent a recombination event between the site coding for amino acid 420 and the *Sty*I^3 site of the GC*2 allele. Another possibility is that the DNA is from an individual heterozygous for GC*1F and a silent allele in the IEF.

The RFLP analysis of the rare variant GC*1C3 allele results in a 0.95-kb *Hae*III band and a 4.75-kb *Sty*I band (Fig. 3, lane 9). Both fragments are indistinguishable from those of the GC*1S allele. Therefore, it is possible that this GC variant has originated from a GC*1S allele by mutation. For the variant GC*2A9 allele, we obtained a 2.0-kb *Hae*III band and a 2.5-kb *Sty*I band (Fig. 3, lane 8). These fragments were indistinguishable from the fragments determined by the GC*2 allele. In this case, it is possible that the GC*2A9 allele is descended from the common GC*2 allele.

The conclusion of our study is not that the GC classification by IEF of the plasma protein polymorphisms should be replaced by analysis at the DNA level: additional differentiation of GC mutants may be made in rare circumstances, as evidenced by our observation of a GC1F-1S heterozygote typed as GC1F-1F by IEF. On the other hand, the vast majority of the less common GC variants as depicted by IEF will be missed by RFLP analysis of the *Sty*I-digest. We should point out, however, that the classification of the three common GC alleles by Southern blot analysis provides an informative DNA marker system for chromosome 4q11–q13 (Cooke et al. 1986).

Acknowledgements. This study was supported by a grant from the Deutsche Forschungsgemeinschaft, Bad Godesberg, FRG (CL 27/14-2), for which we are grateful. We thank Mr. G. Honold for the excellent preparations of the various oligonucleotides and Ms. A. Brandhofer for the technical assistance. We are grateful to Mrs. Berndt from the Bavarian Red Cross Blood Bank at Munich for her help with the collection of the blood samples.

References

Cleve H, Constans J (1988) The mutants of the vitamin D binding protein: more than 120 variants of the Gc/DBP system. Vox Sang 54:215–225

Cleve H, Patutschnick W (1979) Neuraminidase treatment reveals sialic acid differences in certain genetic variants of the GC system (vitamin D binding protein). Hum Genet 47:193–198

Constans J, Oksman F, Viau M (1981) Binding of the apo and holo forms of the serum vitamin D-binding protein to human lymphocyte cytoplasm and membrane by indirect immunofluorescence. Immunol Lett 3:159–162

Cooke NE, David EV (1985) Serum D-binding protein is a third member of the albumin and alpha fetoprotein gene family. J Clin Invest 76:2420–2424

Cooke NE, Willard HF, David EV, George DL (1986) Direct regional assignment of the gene for vitamin D binding protein (Gc-globulin) to human chromosome 4q11–q13 and identification of an associated DNA polymorphism. Hum Genet 73:225–229

Daiger SP, Schanfield MS, Cavalli-Sforza LL (1975) Human group-specific component (Gc) proteins bind vitamin D and 25-hydroxy vitamin D. Proc Natl Acad Sci USA 72:2076–2080

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Haddad JG, Walgate J (1976) 25-hydroxy vitamin D transport in human plasma: isolation and partial characterization of calcifidiol binding protein. J Biol Chem 251:4803–4809

Hirschfeld J, Jonsson B, Rasmuson M (1959) Immuno-electrophoretic demonstration of qualitative differences in human sera and their relation to the haptoglobins. Acta Pathol Microbiol Scand 47:160–168

Kew RR, Webster RO (1988) Gc-globulin (vitamin D-binding protein) enhances the neutrophil chemotactic activity of C5a and C5a des Arg. J Clin Invest 82:364–369

Petrini M, Galbraith RM, Emerson DL, Nel AE, Arnaud P (1985) Structural studies of T lymphocyte Fc receptors. Association of Gc protein with IgG binding to Fc_γ. J Biol Chem 260:1804–1810

Perez HD, Kelly E, Chenoweth D, Elfman F (1988) Identification of the C5a des Arg cochemotaxin. Homology with vitamin D-binding protein (group-specific component globulin). J Clin Invest 82:360–363

Reynolds RL, Sensabough GF (1990) Use of the polymerase chain reaction for typing Gc variants. In: Polesky HF, Mayr WR (eds) Advances in forensic haemogenetics, vol 3. Springer, Berlin Heidelberg New York, pp 158–161

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-termination inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Schoentgen F, Metz-Boutigue M-H, Jolles J, Constans J, Jolles P (1986) Complete amino acid sequence of human vitamin D-binding protein (group-specific component): evidence of a three-fold internal homology as in serum albumin and alpha-fetoprotein. Biochim Biophys Acta 871:189–198

Svasti J, Bowman BH (1978) Human group-specific component. Changes in electrophoretic mobility resulting from vitamin D binding and from neuraminidase digestion. J Biol Chem 253:4188–4194

Svasti J, Kurosky A, Bennett A, Bowman BH (1979) Molecular basis for the three major forms of human serum vitamin D binding protein (group-specific component). Biochemistry 18:1611–1617

Van Baelen H, Bouillon R, De Moor P (1980) Vitamin D-binding protein (Gc-globulin) binds actin. J Biol Chem 255:2270–2272

Van Baelen H, Allewaert K, Bouillon R (1988) New aspects of the plasma carrier protein for 25-hydroxycholecalciferol in vertebrates. Ann NY Acad Sci 538:60–68

Viau M, Constans J, Debray H, Montreuil J (1983) Isolation and characterisation of the o-glycan chain of the human vitamin-D binding protein. Biochem Biophys Res Commun 117:324–331

Yang F, Brune JL, Naylor SL, Cupples RL, Naberhaus KH, Bowman BH (1985) Human group-specific component (Gc) is a member of the albumin family. Proc Natl Acad Sci USA 82:7994–7998