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Research Article

Polymorphisms rs7041 (c.1296T>G) and rs4588 (c.1307C>A) and Distribution of Gc Variants in a Population of Hemodialysis Patients in Abidjan

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Abstract

Background: Vitamin D deficiency is associated with chronic kidney disease (CKD). Renal failure patients are routinely supplemented with vitamin D to compensate for this deficiency. The response to vitamin D supplementation can vary according to variants in the Gc (Vitamin D-binding protein) gene. The combination of two single-nucleotide polymorphisms (SNPs), rs7041 (c.1296T>G) and rs4588 (c.1307C>A), in the Gc gene forms three variants, namely Gc1f (c.1296 T, c.1307C), Gc1s (c.1296G, c.1307C), Gc2 (c.1296T, c.1307A), which result in six vitamin D-binding protein (DBP) phenotypes. Significant variations in variant frequency are reported in different populations. Objectif: The aim of our study was to determine the distribution of Gc genotypes and variants in a population of haemodialysis patients. Methods and Results: Genomic DNA from forty-eight blacks Africans adults with CKD were extracted from whole blood samples. The DNA region spanning the two SNPs of interest was amplified by PCR. The amplified DNA was subjected to the action of restriction enzymes, StyI and HaeIII in two different reactions. Genotyping was performed by analysis of the length of restriction fragments by 2.5% agarose gel electrophoresis. The mean age of the study population was 42 ±12 years, with a sex ratio of 1.6. The C/C genotype of rs4588 (c.1307C>A) was the most frequent, followed by the T/T genotype (90.6%) of rs7041 (c.1296T>G). Three DBP phenotypes, Gc1f-1f (c.1296T, c.1307C/p.432Asp, p.436Thr): 89.6 %, Gc1s-1s (c.1296 G, c.1307C/p.432Glu, p.436Thr): 8.3 %, and Gc1f/Gc1s: 2,1% were identified. Conclusion: Finally, the Gc1f variant was the most frequent. Our results suggest the need for vitamin D testing to establish the correlation between the observed Gc genotypes/variants and vitamin D status in the study population.

Keywords

Chronic Kidney Disease, rs7041(c.1296T>G), rs4588 (c.1307C>A), VDBP

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1. Introduction

Chronic kidney disease (CKD) is characterized by the persistence for more than three months of a decrease in glomerular filtration rate (GFR) below 60 ml/min/1.73m2, whether or not associated with proteinuria [1, 2]. CKD is a public health problem with an estimated prevalence of over 10% of the world's population [3]. In sub-Saharan Africa, in Ivory Coast, this was 7% of patients admitted to the internal medicine department in 2018 [4]. The evolution of CKD is marked by various disturbances, including mineral-bone disorders, which are one of the disease's frequent and serious complications [5].

These bone complications are related to a deficiency in vitamin D, a hormone that regulates phosphocalcic metabolism [6, 7]. Thus, hemodialysis patients are regularly supplemented with vitamin D to compensate for this deficit. However, the response to vitamin D supplementation varies considerably from one individual to another. These variations can be explained by demographic, genetic and environmental factors [8, 9].

Genetic factors include polymorphisms in the Gc (group-specific component) gene of the vitamin D-binding protein (VDBP) [10]. The Gc gene, located on chromosome 4q11-q13, codes for vitamin D-binding protein (DBP), a 58-kDa glycoprotein of 458 amino acids synthesized in the liver [11]. The Gc gene is well known for its single-nucleotide polymorphisms (SNPs), the most common of which are rs7041 (c.1296T>G) and rs4588 (c.1307C>A), located in exon 11 of the gene [12]. The combination of these two polymorphisms forms three VDBP variants entitled Gc1f (rs7041T, rs4588C), Gc1s (rs7041G, rs4588C) and Gc2 (rs7041T, rs4588A), which are partly responsible for the variable efficacy of vitamin D supplementation [10].

There are ethnic differences in the frequencies of Gc gene variants. The Gc1f variant is more frequent in individuals of African descent, while the Gc1s variant is more frequent in Caucasians [13]. Most studies of Gc gene polymorphisms have been conducted in Caucasian and African American populations. In a study of healthy African children from five countries (Kenya, Uganda, Gambia, Burkina Faso and South Africa), the distribution of Gc variants was 83.3% (Gc1f), 8.5% (Gc1s) and 8.2% (Gc2) overall, and varied by country [14]. In Ivory Coast, the distribution of different Gc gene variants remains unknown in the general population. Given the importance of these variants in the response to vitamin D supplementation, the aim of this study was to determine the distribution of Gc variants in a population of haemodialysis patients.

2. Materials and Methods

2.1. Material, Population and Study Sites

The study involved a population of subjects suffering from CKD on dialysis, followed in the National Center for Prevention and Treatment of Renal Failure in Abidjan (CNPTIR). The participants recruited in this study were all blacks Africans. The molecular biology unit of the Medical Biology Department of the Angré University Hospital was used for all the molecular biology analyses.

2.2. Methods

2.2.1. Collection and Storage of Samples

The cross-sectional and descriptive study recruited 48 (forty-eight) adult patients with end-stage renal disease followed for more than 3 months at the time of their annual check-up. Whole blood sample was taken from each patient in a purple-capped tube (EDTA). Whole blood was distributed in aliquots and stored at -20 ℃.

2.2.2. Determination of rs7041 and rs4588 Polymorphisms of the Vitamin D Binding Protein Gc gene

Primers pairs: the primers used for the amplification of the VDBP target DNA are those described by Blanton et al.: (Forward: 5'-CAAGTCTTATCACCATCCTG-3' and Reverse: 5'-GCCAAGTTACAATAACAC-3') [15].

Extraction and storage of extracts: After thawing whole blood at room temperature, genomic DNA extraction was performed using the Gene JET Whole Blood Kit (Thermoscientific, Ref. K0781) according to the protocol described by the manufacturer. The extracted DNA was quantified by fluorimetry (Qubit™ fluorimeter) and then stored at -20 ℃ for one week.

Amplification of the extract: Using the LightCycler®480z thermocycler (Roche Diagnostics®, Mannheim, Germany), an 809 bp fragment of the Gc gene spanning the two polymorphisms rs7041 and rs4588 was amplified using the classical PCR method. PCR conditions consisted of initial denaturation at 95 ℃ for 5 min, followed by 34 cycles of 95 ℃ for 30 s, annealing at 58 ℃ for 30 s and elongation at 72 ℃ for 30 s, with a final extension step of 72 ℃ for 10 min. For each patient, approximately 50 ng of genomic DNA was used in a 25 µL reaction. The volumes and concentrations of reagents, nucleic acids and buffers are reported in Table 1.

Table 1. Volumes and concentrations of each constituent element of the reaction mix.

Designation	Initial concentration	Final concentration	Volume / Sample	Volume for 50 Samples
DreamTaq Green Buffer	10 X	1 X	2.5 µL	125 µL
dNTP Mix	10 mM	200 µM	0.5 µL	25 µL
Forward Primer	100 µM	0.5 µM	0.125 µL	6.25 µL
Reverse Primer	100 µM	0.5 µM	0.125 µL	6.25 µL
DreamTaq DNA Polymerase	500U	1.25 U	0.0625 µL	3.125 µL
H_2O			16.69 µL	834.5 µL
DNA			5 µL	

The size of the amplified fragments (amplicons) was verified by electrophoresis on 2% agarose gel, against GeneRuler 50bp DNA Ladder (ThermoFisher DNA ladder). The gel was stained with SYBR Safe DNA Gel Stain (Invitrogen). Migration was performed for 45 minutes at 100 volts.

Enzymatic digestion and polymorphism research: After PCR, the amplicons were digested with the appropriate restriction enzyme (endonuclease) (Table 2).

Table 2. Endonucleases used for digestions [15].

Designation	Restriction site	Target
HaeIII	(GG/CC)	809 bp fragment of the Gc gene (rs7041)
StyI	(C/CWWGG)	809 bp fragment of the Gc gene (rs4588)

The HaeIII (GG/CC) enzyme was used to digest the 809 bp PCR product at 37 ℃ for 2 hours followed by enzyme inactivation at 80 ℃ for 20 min.
The StyI enzyme (C/CWWGG) was used for digestion of the 809 bp PCR product at 37 ℃ for 2 h followed by enzyme inactivation at 65 ℃ for 20 min.

Fragments or digestion products were separated on a 2.5% agarose gel, against GeneRuler 50bp DNA Ladder (ThermoFisher DNA ladder). The gel was stained with SYBR Safe DNA Gel Stain (Invitrogen). Migration was performed for 45 minutes at 100 volts.

2.3. Ethical Considerations

The study was approved by all competent scientific authorities of the participating health centers. Informed consent was obtained from patients before their inclusion in the study, and each could withdraw from the study at any time.

2.4. Data Analysis

SNPStats software was used to estimate allele, genotype, and haplotype frequencies. SNPStats software is a web application. The software main page is available online at http://bioinfo.iconcologia.net/SNPstats. Anonymous use is guaranteed and data are treated as confidential. Quantitative variables were expressed as mean ± standard deviation. Qualitative variables were expressed as numbers (n) and percentages (%).

3. Results

3.1. Socio-demographic Characteristics

The mean age of the study population was 42 ± 12 years, extreme values (18-83 years). The majority of patients were male with a sex ratio of 1.6.

3.2. Clinical Features

Approximately 1/3 of patients (28.26%) had been on dialysis for more than 5 years (60 months). The mean duration of dialysis was 49 months, or almost 4 years, with extremes ranging from 6 months to 240 months (about 20 years). CKD was associated in 88% of cases with high blood pressure.

3.3. Biological Parameters

After digestion of the 809bp PCR product by HaeIII enzyme (GG/CC) and StyI enzyme (C/CWWGG) separately, identification of alleles was performed by fragment size analysis of digestion products. The rs7041 G allele introduces a HaeIII restriction site (GG/CC) generating 578bp and 231bp fragments, while the rs4588 A allele introduces a StyI restriction site (C/CWWGG) and produces 585bp and 224bp fragments. Figure 1 shows the electrophoretic migration of HaeIII (GG/CC) restriction enzyme digestion products.

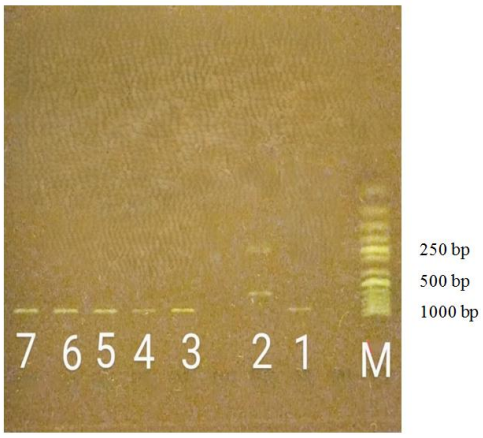

Figure 1. *Result of electrophoresis of HaeIII restriction enzyme digestion products (GG/CC). The numbers 1, 2, 3, 4, 5, 6, 7 correspond to the patient samples. The letter M corresponds to Generuler 50bp DNA ladder. Patients 1, 3, 4, 5, 6 and 7 are homozygous T/T (one single fragment of 809bp) and patient 2 is homozygous G/G (one fragment of 578 bp and one fragment of 231bp).*

The frequency of each allele was estimated using SNPStats software. Tables 3 and 4 show the distribution of different alleles of rs7041 and rs4588 variants and the genotypes respectively. The rs7041 variant showed a G mutant allele frequency of 9.4%. However, the A allele of rs4588 was not found in the study population, this variant remained 100% wild type after the action of the Styl enzyme (Table 3).

Homozygosity (T/T) of rs7041(c.1296T> G), and (C/C) homozygosity of rs4588 (c.1307C>A) were found to be the most common genotypes in the study population (Table 4). Of the three phenotypes, Gc1f-1f (p. 432Asp, p. 436Thr) was the most frequent. Finally, the Gc1f variant (c.1296 T, c.1307C), was the predominant variant in our study, followed by the Gc1s (c.1296G, c.1307C) variant (Table 5).

Table 3. *Alleles distribution of rs4588 and rs7041 polymorphisms.*

Polymorphims	Alleles	Number (n)	Frequency %
rs4588 (c.1307C>A)	A	0	0
Style (C/CWWGG)	C	96	100
rs7041 (c.1296T> G)	T	87	90.6
HaeIII (GG/CC)	G	9	9.4

Table 4. *Genotypes distribution of rs4588 and rs7041 polymorphisms.*

Polymorphims	Genotypes	Number (n)	Frequency %
rs4588 (c.1307C>A)	HomozygousA/A	0	0
	Homozygous C/C	48	100
	Heterozygous C/A	0	0
rs7041 (c.1296T> G)	Homozygous T/T	43	89.6
	Homozygous G/G	4	8.3
	Heterozygous G/T	1	2.1

Table 5. *Gc variants and haplotypes distribution.*

rs7041 (c.1296T> G)	rs4588 (c.1307C>A)		Number (n)	Frequency (%)
		Variants		
T	C	Gc1f	87	90.6
G	C	Gc1s	9	9.4

rs7041 (c.1296T> G)	rs4588 (c.1307C>A)		Number (n)	Frequency (%)
T	A	Gc2	0	0
		Haplotypes		
T/T	C/C	Gc1f-1f	43	89.6
G/G	C/C	Gc1s-1s	4	8.3
T/G	C/C	Gc1f-1s	1	2.1
T/T	A/A	Gc2-2	0	0
T/T	C/A	Gc1f-2	0	0
T/G	C/A	Gc1s-2	0	0

4. Discussion

The aim of this preliminary study was to determine the frequency of the main Gc genotypes and variants in CKD patients undergoing dialysis. Genotyping of two SNPs (rs7041 (c.1296T>G) and rs4588 (c.1307C>A) of the vitamin D-binding protein Gc gene was performed using the PCR-RFLP technique. The results showed that the major homozygous genotypes rs7041 (T/T) and rs4588 (C/C) were predominant. The frequency of the Gc1f variant was highest, in agreement with Braithwaite et al. who reported a similar distribution in a rural population in Gambia, with the Gc1f variant at 86% and the Gc1s variant at 11% [16]. The distribution of these variants was also consistent with that observed in children in sub-Saharan Africa: 83.3% Gc1f, 8.5% Gc1s and 8.2% Gc2 [14]. Previous studies have also shown that the Gc1f variant predominates in Africans, however, some exceptions have been reported in Northeast African populations, where Gc1s frequency were higher (>50%) than Gc1f frequency [17-20].

It has also been found that Gc1f and Gc1s variants, generally separate, genetically, two distinct populations, namely Caucasians and Africans [17]. Thus, in the study by Powe et al., Gc1f frequency was dominant in African-Americans, while Gc1s frequency was elevated in Caucasian subjects. [21]

We did not observe the Gc2 variant in our study population, as did Constans et al., in the Tuareg population in Mali [17]. However, Lefranc et al., recorded the Gc2 variant in the Tunisian population at a frequency of 2.1% [19]. Consistent with the absence of the Gc2 variant in our study population, several works have reported a rather abundant Gc2 frequency in Caucasians but a rare frequency in Africans [13, 16, 22].

At the same time, an association between Gc alleles, genotypes and variants and response to vitamin D supplementation was assessed. Thus, among Danes receiving vitamin D-fortified bread and milk or ultraviolet B treatment, carriers of the A rs4588 allele of the Gc gene showed the smallest increase in serum 25(OH)D concentration [23]. In a Saudi population supplemented with vitamin D, Gc1s subjects showed the greatest increase in serum 25(OH)D levels, with the lowest increase observed in Gc1f subjects [10]. Their results highlight the need to know the genetic profile of the patient with CKD before any vitamin D rehabilitation.

5. Conclusions

At the end of this study, it was found that the Gc1f variant is more frequent in haemodialysis patients in Abidjan. However, additional studies taking vitamin D dosage into account are needed to establish the correlation between the Gc genotypes and variants observed and vitamin D status in the population studied.

Abbreviations

CKD	Chronic Kidney Disease
GFR	Glomerular Filtration Rate
Gc	Group Specific Component Gene
VDBP	Vitamin D-binding Protein
SNP	Single-nucleotide Polymorphism
CNPTIR	National Center for Prevention and Treatment of Renal Failure in Abidjan
EDTA	Ethylenediaminetetraacetic Acid
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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