

Research Article

Analysis of the Vitamin D Receptor (VDR) Start Codon Polymorphism (Rs2228570) in Hemodialysis Patients from Abidjan, Côte d'Ivoire

Koné-Dakouri Yékayo Bénédicte^{1, 2, *} , **Yao-Yapo Carine Mireille¹** ,
Kouacou Kadio Morel¹ , **Koné-Koné Fatoumata¹** , **Kouamé Dia Yah Chantal¹** ,
Yayo Sagou Eric¹ , **Kacou-N'Douba Adèle²** ,
Hauhouot-Attoungbre Marie Laure¹ 

¹Unité Pédagogique de Biochimie, Et de Biologie Moléculaire, UFR Sciences Pharmaceutiques et Biologiques, Université Félix Houphouët Boigny, Abidjan, Côte d'Ivoire

²Unité de Biologie Moléculaire du Service de Biologie Médicale du Centre Hospitalier Universitaire (CHU) d'Angré, Abidjan, Côte d'Ivoire

Abstract

In patients with chronic kidney disease (CKD), particularly those undergoing hemodialysis, vitamin D deficiency is highly prevalent. Individual sensitivity to vitamin D can be influenced by genetic variations in the vitamin D receptor (VDR) gene. Among these, the rs2228570 polymorphism, located in exon 2 and commonly referred to as the FokI polymorphism, produces two protein isoforms with differing transcriptional activity. Although this polymorphism has been widely studied in other populations, data on its distribution in West African populations, particularly among individuals with altered vitamin D metabolism, such as hemodialysis patients, remain scarce. The aim of this study was to investigate the frequency of the rs2228570 (FokI) polymorphism in the VDR gene among hemodialysis patients in Abidjan, Côte d'Ivoire. In a cross-sectional study, genomic DNA from fifty adult patients with CKD were extracted from whole blood samples. The DNA region spanning the SNP of interest was amplified by PCR. The amplified DNA was subjected to the action of FokI restriction enzymes. Genotyping was performed by analysis of the length of restriction fragments by 3% agarose gel electrophoresis. The mean age of the study population was 40 ± 17 years, with a sex ratio of 1.9. Genotyping was successfully performed in forty-three of the fifty patients included. Genotype and allele frequencies were calculated, and their distribution was assessed. The FF (CC) genotype was the most frequent (79%), followed by the Ff (CT) and ff (TT) genotypes at 16% and 5%, respectively. Our findings highlight a high frequency of the FF genotype of the VDR gene in hemodialysis patients in Abidjan. Future research is needed to investigate the functional impact of this genotype on vitamin D status and its potential clinical implications in this population.

Keywords

Vitamin D Receptor Gene, Rs2228570, FokI Polymorphism, Genotyping, Hemodialysis, Abidjan, Côte d'Ivoire

*Corresponding author: bene.yekayo@yahoo.fr (Koné-Dakouri Yékayo Bénédicte)

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1. Introduction

Vitamin D is essential for maintaining phosphocalcium homeostasis, ensuring skeletal integrity, and modulating immune function [1]. Its biological activity is mediated by the vitamin D receptor (VDR), a nuclear receptor encoded by the VDR gene, which harbors several polymorphic variants [2]. Among these, the rs2228570 single nucleotide polymorphism (SNP) has received considerable attention due to its potential functional implications [3, 4]. The rs2228570 polymorphism, identifiable by the FokI restriction enzyme, is located within exon 2 of the VDR gene. It arises from a T>C substitution (ATG to ACG) at the first potential translation initiation codon, resulting in the production of two distinct VDR protein isoforms with differing lengths and potentially altered functional activity. Specifically, the shorter isoform, associated with the C allele, lacks the first three amino acids and has been reported to exhibit higher transcriptional activity compared to the longer variant encoded by the T allele [3, 5, 6]. Numerous studies have linked the VDR-FokI polymorphism to inter-individual differences in serum vitamin D levels, as well as to susceptibility to various pathologies associated with vitamin D deficiency, including osteoporosis, autoimmune disorders, cardiovascular diseases, and certain cancers [7-10]. This association is particularly relevant in individuals with chronic kidney disease (CKD), where hypovitaminosis D is highly prevalent and contributes to the pathophysiology of various complications [11, 12]. While the impact of this polymorphism has been investigated in various populations [13-15], data remain scarce for sub-Saharan African cohorts. Notably, allele frequencies and the associated clinical implications can differ significantly across ethnic groups [16, 17], underscoring the need for population-specific studies. The objective of this study is to determine the frequency of the VDR-FokI polymorphism among hemodialysis patients in Abidjan, Côte d'Ivoire. This investigation aims to provide preliminary data on the genetic profile of this population, with potential implications for understanding vitamin D metabolism and guiding personalized therapeutic strategies in chronic kidney disease.

2. Materiel 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 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 50 (fifty) 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 °C.

2.2.2. Determination of rs2228570 Polymorphisms of the Vitamin D Receptor Gene

Primers pairs: the primers used for the amplification of the VDR target DNA are those described by Mishra et al.: (Forward: 5'-GATGCCAGCTGGCCCTGGCACTG-3' and Reverse: 5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3') [18].

Extraction and storage of extracts: Following the manufacturer's instructions, genomic DNA extraction was carried out using the Gene JET Whole Blood Kit (thermoscientific, Ref. K0781) after the whole blood had thawed at room temperature. After being measured using a Qubit™ fluorimeter, the extracted DNA was stored for subsequent use at -20 °C.

Amplification of the extract: Using the LightCycler®480z thermocycler (Roche Diagnostic), the conventional PCR method was used to amplify a 273 bp segment of the VDR gene that spans the rs2228570 polymorphism. The PCR conditions were as follows: 5 minutes of initial denaturation at 96 °C, 40 cycles of 94 °C for 45 s, 30 s of annealing at 59 °C, and 30 s of elongation at 72 °C [18]. About 50 ng of genomic DNA were used in a 25 µL reaction for every patient. 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

Designation	Initial concentration	Final concentration	Volume / Sample	Volume for 50 Samples
Reverse Primer	100 μ M	0.5 μ M	0.125 μ L	6.25 μ L
DreamTaq DNA Polymerase	500 U	2 U	0.1 μ L	5 μ L
H ₂ O			11.65 μ L	582.5 μ L
DNA			10 μ L	

The size of the amplified fragments (amplicons) was verified by electrophoresis on 3% agarose gel against GeneRuler 50bp DNA Ladder (ThermoFisher DNA ladder). Migration was performed for 1 hour at 50 volts. The gel was post-stained with GelRed DNA stain for 30 minutes.

Enzymatic digestion and polymorphism research: The PCR products were then digested with the restriction enzyme FokI fast digest (Thermo Fisher), at 37 °C for 5 min, to enable polymorphism analysis. Fragments or digestion products were separated on a 3% agarose gel, against GeneRuler 50bp DNA Ladder (ThermoFisher DNA ladder). The gel was stained with GelRed DNA Gel Stain. Migration was performed for 1 hour at 50 volts.

2.3. Ethical Considerations

The study was approved by all competent scientific authorities of the participating health centers. The research protocol was submitted to the National Ethics Committee for Life Sciences and Health of Côte d'Ivoire (CNESVS) and received ethical approval under reference number 0011125/MSHPCMU/CNESVS. This approval authorized the collection and analysis of participants' biological and genetic data.

3.3. Biological Parameters

2.4. Data Analysis

SNPstat software (<http://bioinfo.iconcologia.net/snpstats>) was used to perform statistical analyses of the genotypic data, including Hardy-Weinberg equilibrium testing. Quantitative variables were expressed as mean $\pm$ 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 40 $\pm$ 17 years, extreme values (18-83 years). The majority of patients were male with a sex ratio of 1.9.

3.2. Clinical Features

Approximately 1/3 of patients (32%) had been on dialysis for more than 5 years (60 months). The median duration of dialysis was 36 months with an interquartile range of (24 - 72) months. CKD was associated in 86% of cases with high blood pressure.

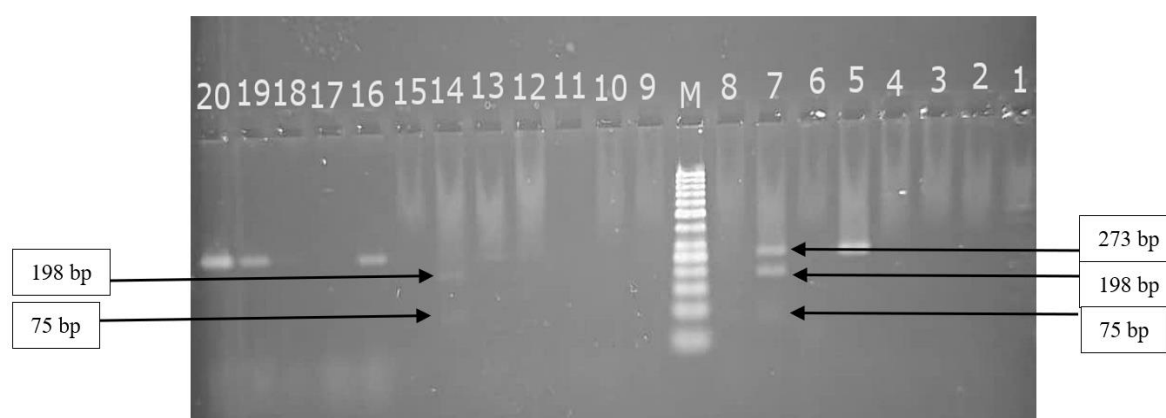


Figure 1. Agarose gel electrophoresis of FokI digestion products for rs2228570 genotyping. Lane M: DNA molecular weight marker (50 bp ladder); Lanes 7: heterozygous Ff genotype showing three bands (273 bp, 198 bp, and 75 bp); Lanes 5, 13, 16, 19, 20: homozygous FF genotype with a single undigested 273 bp band; Lanes 14: homozygous ff genotype showing two bands (198 bp and 75 bp). The presence of the f allele introduces a FokI restriction site, leading to digestion of the 273 bp PCR product into 198 bp and 75 bp fragments.

Genotyping was carried out by analyzing the fragment sizes generated after digestion of the 273 bp PCR amplicon with the FokI restriction endonuclease. The C allele of the rs2228570 polymorphism in the VDR gene, referred to as the f allele, introduces a recognition site for the FokI restriction enzyme. In contrast, the T allele, or F allele, abolishes this site. Consequently, digestion of the 273 bp PCR product with FokI yields two fragments of 198 bp and 75 bp in the presence of

the f allele. [Figure 1](#) illustrates the electrophoretic migration pattern of the digestion products.

Genotyping was successfully performed in 43 (forty-three) participants. The study population exhibited all three expected genotypes: FF, Ff and ff. [Table 2](#) shows the distribution of genotypes frequencies. The ff genotype was the least frequent (5%).

Table 2. Genotypes distribution of rs2228570 polymorphism.

Polymorphism	Genotypes	Number (n)	Frequency%
Rs2228570	Homozygous ff (TT)	2	5
	Homozygous FF (CC)	34	79
	Heterozygous Ff (CT)	7	16

Allelic frequencies were calculated from the genotypic distribution data. The results are presented in [Table 3](#).

Table 3. Alleles distribution of rs2228570 polymorphism.

Polymorphism	Alleles	Number (n)	Frequency%
Rs2228570	f (T)	11	13
	F (C)	75	87

4. Discussion

In the context of growing interest in the genetic determinants of vitamin D status, this preliminary study aimed to evaluate the genotype distribution of the rs2228570 polymorphism in the VDR gene, which encodes the vitamin D receptor, in hemodialysis patients in Abidjan. Understanding the frequency of this variant may help lay the groundwork for future investigations into its potential impact on vitamin D status in this particular population. Genotyping was performed using PCR-RFLP technique. The genotyping results revealed the presence of all three expected genotypes: FF, Ff, and ff, with a low frequency of the homozygous ff genotype, found in only 5% of participants.

The Hardy-Weinberg exact test showed no significant deviation from genetic equilibrium ($p = 0.12$), indicating that the genotype distribution in the study population is consistent with expected proportions under Hardy-Weinberg assumptions. This suggests that the sample is genetically representative of the broader population, and that there were likely no major genotyping errors, selection biases, or

non-random mating patterns affecting the distribution of the rs2228570 polymorphism.

The genotypic distribution observed in our study (FF = 79%, Ff = 16%, and ff = 5%) aligns with the genetic profiles commonly reported in African populations. The FF genotype is generally the most prevalent among African populations and those of African descent, although the exact frequencies may vary depending on the country and ethnic group. For instance, a study conducted in Sudan reported CC (FF) frequencies of 50.1%, with CT (Ff) and TT (ff) genotypes accounting for 39.7% and 10.2%, respectively [16]. In Ugandan, the genotypes frequencies in pulmonary *Mycobacterium tuberculosis* patients were FF: 87.8%, Ff: 7.3% and ff: 4.8% (ff). In the same study, for normal healthy subjects the frequencies were FF: 92.6%, Ff: 2.4% and ff: 4.8% [19]. In South Africa, the frequency of the CC (FF) genotype in black populations was estimated at 68%, compared with just 37% in white participants in the same study [20]. Similarly, in a premenopausal women cohort in the United States, the distribution of the VDR-FokI genotypes differed significantly by race: 4% of blacks versus 18% of whites were ff and 65% of blacks versus 37% of whites were FF [21], illustrating notable inter-ethnic differences.

Comparative analysis with other ethnic groups reveals significant interpopulation variability in the frequency of the rs2228570 polymorphism, which may reflect underlying genetic diversity and evolutionary influences [3]. These findings underscore the importance of conducting population-specific studies to better understand the genetic determinants of vitamin D status.

Asian populations generally show higher frequencies of the minor f allele, whereas Caucasian populations tend to display a more balanced distribution between the F and f alleles [22, 23].

In Caucasian cohorts, genotypic frequencies reported in the United Kingdom were 48% for FF, 41% for Ff, and 11% for ff [24]. Similar distributions were found in France, with frequencies of 43% (FF), 47% (Ff), and 10% (ff) [25]. In East Asia, a Japanese population exhibited a higher proportion of heterozygotes and minor allele carriers, with 37% FF, 51% Ff, and 12% ff [26]. In South Asia, frequencies vary by region: among Indian women, the FF genotype was observed in 59% of individuals [15], while in a cohort of Pakistani women, the ff genotype reached a notably high frequency of 42% [13]. Middle Eastern populations also show distinct patterns; for example, in the United Arab Emirates, the distribution was 31% FF, 42% Ff, and 27% ff, with the f allele (51.96%) being more frequent than the F allele (48.04%) [23]; a trend opposite to that observed in most other regions.

The high prevalence of the FF genotype (associated with the ancestral C allele of the VDR gene polymorphism rs2228570) observed in African populations may be attributed to a combination of evolutionary and environmental factors. From an evolutionary point of view, this ancestral allele is considered to be the original form of the vitamin D receptor [6]. It encodes a three-amino-acid shorter VDR protein, which may be more active in terms of transcriptional signaling [3].

Individuals with darker skin produce vitamin D less efficiently in the skin due to higher melanin content [27]. As a result, Black individuals typically have lower circulating levels of total 25-hydroxyvitamin D compared to White individuals. However, despite these lower levels, they tend to have greater bone mineral density (BMD) and a reduced risk of fragility fractures relative to Whites [28, 29]. This can be partially explained by the high prevalence of the FF genotype in Black individuals. Indeed, the presence of the more active form of the vitamin D receptor (FF genotype) may have conferred a selective advantage by enhancing the biological response to lower circulating levels of 25-hydroxyvitamin D, suggesting that increased VDR activity may compensate for reduced vitamin D availability.

5. Conclusions

At the end of this study, it was found that the FF genotype is more frequent among hemodialysis patients in Abidjan. To understand its functional impact on vitamin D status in these

patients and its potential clinical implications in this population further studies are needed.

Abbreviations

BMD	Bone Mineral Density
CKD	Chronic Kidney Disease
CNESVS	National Ethics Committee for Life Sciences and Health
DNA	Deoxy Ribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
VDR	Vitamin D Receptor

Author Contributions

Kon éDakouri Y ákayo B áédicte: Conceptualization, Methodology, Writing - original draft

Yao-Yapo Carine Mireille: Investigation

Kouacou Kadio Morel: Investigation

Kon éKon éFatoumata: Formal Analysis

Kouam éDia Yah Chantal: Investigation

Yayo Sagou Eric: Formal Analysis, Supervision

Kacou-N'Douba Adèle: Visualization

Hauhouot-Attoungbre Marie Laure: Validation

Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Field

Kon éDakouri Y &ayo B á ádicte: 1. Genetic susceptibility to diseases; 2. Evaluation of molecular biology techniques; 3. Biochemical assay methods assessment; 4. Biochemical parameters to monitoring CKD in Africans; 5. Genetic variability in response to drug therapy

Yao-Yapo Carine Mireille: 1. Chronic kidney disease in African; 2. Determination of glomerular filtration rate; 3. Mineral-Bone Disorders; 4. Bone biomarkers in Africans; 5. Reference values in biology

Kouacou Kadio Morel: 1. Bone biomarkers and chronic kidney disease; 2. Method analysis and validation; 3. Genetic variability of chronic kidney disease patients in Africa; 4. Cardio renal syndrome in africans; 5. Reference values of biochemical parameters