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Roles of the *Apolipoprotein E* Gene and Its Polymorphisms in the Etiopathophysiology of Type 2 Diabetes Mellitus and Its Atherosclerotic Complication in Senegalese Females

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Abstract

Lipid metabolism disorders would be among the components responsible for the risk of the onset of T2DM and its vascular complications. Apolipoprotein E plays an important role in lipid metabolism. We studied the involvement of the *APOE* gene in the onset of T2DM and its vascular complications. Clinical and biochemical parameters were assessed in each participant. *APOE* genotypes were identified by PCR-RFLP. Arterial stiffness was studied using a pOpmetre® which evaluates the pulse wave velocity (ft-PWV). Endothelial dysfunction was studied using an EndoPAT2000® which measures endothelium-dependent vasodilation (RHI). In control subjects, the $\varepsilon 3$ allele was associated with an increase in fasting blood glucose ($r = 2.36$, $p = 0.018$), and a decrease in LDL cholesterol levels ($r = -2.17$, $p = 0.03$), and $\varepsilon 4$ was associated with an increase in total cholesterol ($r = 2.59$, $p = 0.01$), LDL cholesterol ($r = 2.84$, $p = 0.004$), and No-HDL cholesterol ($r = 2.74$, $p = 0.006$). In type 2 diabetes subjects, the $\varepsilon 2$ was associated with a decrease in diastolic blood pressure ($r = -2.25$, $p = 0.02$). The $\varepsilon 3$ was associated with a decrease in ft-PWV ($r = -2.26$, $p = 0.024$) while the $\varepsilon 4$ was associated with an increase in ft-PWV ($r = 2.52$, $p = 0.012$). Carrying the $\varepsilon 2\varepsilon 3$ genotype would have in 99% a limited

risk of developing T2DM, and in event of T2DM, only 1 to 2% would have a significant risk of developing atherosclerosis, which would be severe in 17%. Of the $\varepsilon 2\varepsilon 4$ genotype, 93% had a limited or even possible risk of developing T2DM, the remaining 7% had a very high risk of developing T2DM. Diabetics carrying $\varepsilon 2\varepsilon 4$ had in 7% very high risk of developing atherosclerosis. The latter had a 20% very high risk of being very severe. Subjects carrying the $\varepsilon 3\varepsilon 4$ genotype had a 67% possible or even probable risk of developing T2DM and in the event of diabetes, there was in 34% very high risk of developing atherosclerosis which will not have even the time to evolve towards severity. For subjects carrying the $\varepsilon 3\varepsilon 3$, the risk of developing T2DM and athérosclerosis was higher than that of the $\varepsilon 2\varepsilon 3$, and $\varepsilon 2\varepsilon 4$ genotypes but lower than that $\varepsilon 3\varepsilon 4$ genotype. The physio-pathological role of the *APOE* gene and the impacts of its polymorphisms are important in the onset and progression of type 2 diabetes mellitus.

Keywords

APOE Gene, Polymorphisms, Type 2 diabetes Mellitus, Vascular Dysfunctions

1. Introduction

Diabetes mellitus, a chronic metabolic disease, affects 9.3% of adults all around the world by 2019, rising to 10.2% by 2030 [1]. Diabetes mellitus is among the top 10 causes of death in adults and was estimated to have caused four million deaths globally in 2017 [2]. Type 2 diabetes mellitus (T2DM) contributes 90% - 95% of all diabetes mellitus, it's a chronic multifactorial disease of adulthood [3]. The high morbi-mortality attributed to diabetes mellitus would be largely linked to its cardiovascular complications, which have been almost 70% responsible for death in diabetic patients [4]. Type 2 diabetes mellitus patients have a 4-fold-higher risk of having a cardiovascular event than people without diabetes [5]. By vascular disease, diabetes mellitus is said to be the major cause of blindness, kidney failure, heart attacks, stroke, and lower limb amputation [6]. T2DM results from complex interactions between multiple genetic and environmental factors [7]. Genetic susceptibility is thought to contribute to the pathogenesis of diabetes but also to its vascular complications. From the genetic point of view, the causes of diabetes can be divided into monogenic and polygenic diseases.

About a decade ago, the *Apolipoprotein E* gene (*APOE*) was suggested as a susceptible gene that could induce the pathogenesis of type 2 diabetes mellitus [8] and type 2 diabetes vascular dysfunction [9]. The *APOE* gene is polymorphic in nature and possesses three alleles namely $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$ having six genotypes three of which are homozygous ($\varepsilon 2\varepsilon 2$, $\varepsilon 3\varepsilon 3$, and $\varepsilon 4\varepsilon 4$) and three are heterozygous ($\varepsilon 2\varepsilon 3$, $\varepsilon 2\varepsilon 4$ and $\varepsilon 3\varepsilon 4$) [10]. The *APOE* gene encodes Apolipoprotein E protein (APOE) and regulates its plasma concentration and its binding capability. *APOE* protein is an essential apolipoprotein in plasma [11] and plays a vital role

in the transport, metabolism, and digestion of lipoproteins [12]. *APOE* promotes the efficient uptake of lipoproteins from the circulation and takes part in cellular cholesterol efflux and reverse cholesterol transportation [13]. The three alleles (*ε*2, *ε*3, and *ε*4) of the *APOE* gene are responsible for the production of the corresponding ApoE2 (E2), ApoE3 (E3), and ApoE4 (E4) plasma isoproteins [8] and contribute to the variation in lipoproteins concentration. In addition, the structural variations of the *APOE* protein influence its functions and its antiatherosclerotic effects [14]. So the *ε*2, *ε*3, and *ε*4 allele variants and their corresponding protein variations have been linked with differential risks of cardiovascular diseases [15].

The vascular disease would have as the initial stage endothelial dysfunction and arterial stiffness. Endothelial dysfunction is commonly described as the inability of the artery to sufficiently dilate in response to an appropriate endothelial stimulus. Impairment of vascular function, such as endothelial dysfunction and increased arterial stiffness, is closely associated with the development and maintenance of atherosclerotic conditions, leading to target organ damage and cardiovascular complications [16]. Noninvasive vascular function tests have been developed and performed for the assessment of functional vascular damage and the severity of atherosclerosis [17]. Recently authors have shown that reactive hyperemia index (RHI), an index of endothelial function, and finger-toe pulse wave velocity (ft-PWV), an index of arterial stiffness, are significant predictors of cardiovascular events independent of conventional cardiovascular risk factors [18].

Several studies on the role of the *APOE* gene in developing pathological conditions of type 2 diabetes and its vascular diseases complications have been conducted and the pathogenesis was further linked to lipid and lipoprotein metabolic abnormalities [19].

At present, there are no similar studies on the Senegalese populations. In addition, there is no study simultaneously evaluating the predictive values of RHI, ft-PWV and *APOE* gene polymorphisms in patients with type 2 diabetes.

However, as a hypothesis, we believe that the *APOE* gene polymorphism would be involved in the pathophysiological mechanisms underlying the onset of type 2 diabetes and diabetic vascular disease complications such as endothelial dysfunction and arterial stiffness. Hence, in this current study, we aimed to evaluate the hypothesis in a case-control analysis and subsequently examined the relationship between *APOE,* its polymorphisms, and the risk of type 2 diabetes and diabetic vascular complications among Senegalese subjects.

2. Methodology

2.1. Ethical Approval

All procedures were conducted in accordance with the standard of the declaration of Helsinki. It was reviewed and approved by the Ethics Committee of the University of Cheikh Anta Diop (UCAD) of Dakar in Senegal (Protocole

027512018/CERruCAD). All study participants provided signed informed consent.

2.2. Study Participants

A total of 81 healthy control and 121 type 2 diabetic subjects were included in this study. They were recruited at the physiology and biochemistry laboratory of the Cheikh Anta Diop University (Dakar, Senegal). They were HIV, HBV, and HCV negative. The groups were matched for age.

T2DM was diagnosed according to American Diabetes Association criteria [20].

Inclusion criteria were: ≥18 years old; Subjects who didn't take or had stopped taking lipid-regulating drugs at least three months were mainly included; liver and kidney functions were normal (aspartate aminotransferase [AST] and alanine aminotransferase [ALT], serum creatinine, and blood urea were in the normal range).

Subjects were excluded if they were diagnosed with other diseases that affect blood lipid levels. Pregnant and breastfeeding women were also excluded. Any ongoing treatments or medications of the study subjects were not stopped for this research.

2.3. Clinical Explorations

Health assessment for the subjects was conducted at the physiology laboratory and included interviews and physical examination. Interviews were on the basis of a questionnaire composed of the medical and family history of participants. Physical examination collected data on body weight, height, waist size, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate.

2.4. Investigations of Vascular Function

The measure of reactive hyperemia index (RHI) by EndoPAT2000® for screening endothelial dysfunction and the measure of finger-toe pulse wave velocity (ft-PWV) by pOpmetre® for screening arterial stiffness were performed by the same operator (a medical doctor). They were screened after the clinical exploration and subjects were also fasted (at least 12 hours, overnight).

2.4.1. Screening for Endothelial Dysfunction

Reactive hyperemia index (RHI) or EndoScore explores the peripheral arterial tone, a mark of endothelial vasodilator function. RHI, therefore, evaluates the endothelium-dependent vasodilation, the main mechanism inducing FMD is thought to be an increase in shear stress, leading to the release of nitric oxide (NO) from endothelial cells which causes blood vessel dilation. RHI was evaluated with the EndoPAT2000® device (Itamar-Medical, Israel). It's a device for measuring the vasodilatory response to 5 min occlusion of the brachial artery vascular flow using an inflatable cuff placed on the arm. Measurements were

performed according to the manufacturer's instructions, and it is noninvasive and not operator-dependent. Briefly, the subjects were in dorsal decubitus position for a minimum of 10 minutes before measurements, in a quiet, temperature-controlled room with dimmed lights. Each recording consisted of 5 minutes of baseline measurement, 5 minutes of occlusion measurement, and 5 minutes post-occlusion measurement (hyperemic period). On the non-dominant upper arm, the occlusion pressure was minimally 200 mmHg. Reactive hyperemia in response to the occlusion is calculated automatically by the device and translated into reactive hyperemia index (RHI) and its logarithm (LnRHI). The calculated values are normalized by measures on the contralateral arm where the blood flow is not interrupted. After each measurement, the software installed on a PC and connected to the device expresses directly the results giving RHI and LnRHI, whose normal values are respectively (1.67 to 2) and (0.51 to 0.70). RHI value below 1.67 or LnRHI value below 0.51 are categorized as endothelial dysfunction.

2.4.2. Assessing Arterial Compliance and Screening for Arterial Stiffness

The arterial compliance parameter that we chose was the finger-toe pulse wave velocity (ft-PWV). It was measured by a pOpmeter® (Axelife SAS, France). Measurements were performed according to the manufacturer's instructions. The pOpmeter® use two photodiode sensors, which were similar to pulse oximeters (one emitting diode and one receiving diode), and a cable that connects it to a computer. The sensors are positioned on the thumb finger and the first toe of the subject, at the level of the pulp arteriole, recording the pulse waves. From the software installed on the computer, the operator provides information on the age and height of the subject. The pOpmeter® measures the time that separates the acquisition of pulse waves at the finger and at the toe, which is called transit time (TT). This time difference is calculated by the device using the maximum of the second derivative method. The calculations will be made by the software and the results displayed on the computer for over 20 seconds. We did two measurements of ft-PWV for each subject and then took the mean of the two measurements. A ft-PWV over 10 m/s indicates excessive arterial stiffness whatever the age and therefore a higher cardiovascular risk.

2.4.3. Quantification of Nitrogen Monoxide

The quantitative determination of total nitric oxide and nitrate/nitrite concentrations in serum was carried out using the method of enzyme-linked immunosorbent assay (ELISA) kits (Assay Catalog Number KGE001.96 wells, R&D systems, Bio-techne, Minnesota, USA).

2.5. Biochemical Markers and Lipids Analysis

After the clinical exploration and before breakfast (at least 12 hours, overnight), fasting venous blood samples were collected into vacutainers and placed in a container filled with dry ice. The glycated hemoglobin (HbA_1c) was measured the same day by the turbidimetric method with a total blood sample on ethyle-

nediaminetetraacetic acid (EDTA) vacutainers. Serum and Plasma were separated from blood shortly after collection and stored in a freezer at −80˚C until further analysis. EDTA vacutainers containing whole blood samples were also stored at the same temperature for DNA extraction for genotyping.

The serum and plasma samples were also assayed to establish blood lipid and carbohydrate parameters.

On the fluoride tube, we measured the fasting glycemic according to the glucose oxidase method. On the heparin tube, we measured lipids parameters (Apolipoprotein A, Apolipoprotein B, total cholesterol, HDL cholesterol, and triglycerides) by chemical method. All biochemistry parameters were analyzed on an automated Abbott device (architect i1000SR, laboratories, Abbott Park, Seattle, WA, USA) according to the standard laboratory protocol.

LDL cholesterol levels were calculated according to the following equation LDL cholesterol = Total cholesterol − HDL cholesterol − Triglyceride/5 [21]. No-HDL cholesterol levels were calculated by subtracting HDL cholesterol value from total cholesterol value: No-HDL cholesterol = (Total cholesterol − HDL cholesterol) [22].

2.6. Genetic Analysis

2.6.1. DNA Extraction

Genomic DNA was extracted from peripheral blood lymphocytes using a commercial kit (REF A1125, Wizard® genomic DNA Purification kit, Promega Corporation, Madison, USA). The assays were performed with strict adherence to the protocols and instructions specified by the manufacturer.

2.6.2. PCR-Amplification

APOE genes were amplified by polymerase chain reaction (PCR). PCR was performed in a 25 µl reaction volume including 2 µl of DNA (100 ng genomic DNA) and 23 µl for Mix-PCR containing 1.5 µl for apoE-forward primer (10 µM), 1.5 µl for Apo E-reverse primer (10 µM), 12.5 µl of Green mix (GoTaq® Green Master Mix; M7122; Promega Corporation, Madison, WI, USA) and 7.5 µl nuclease-free water.

The *APOE* primers were (forward 5'-TCC AAG GAG CTG CAG GCG GCG CA-3' and reverse: 5'-GCC CCG GCC TGG TAC ACT GC-3', respectively; product length, 295 bp; Inqaba Biotec, West Africa Ltd.)

First, all samples were amplified with a ThermoCycler (Thermocycleur biometra T-personal 48 Konrad-Zuse-Strasse 107745 Jena/Germany, Allemagne). The PCR operation conditions included an initial denaturation phase at 95˚C for 5 minutes, followed by 35 cycles of denaturation at 95˚C for 30 seconds, annealing at 70˚C for 20 seconds, an extension phase at 72˚C for 20 seconds, and the final elongation at 72˚C for 10 minutes. Amplification was confirmed by 1.5% agarose gel electrophoresis stained with ethidium bromide and with a 100-bp marker (Invitrogen™ 100 bp DNA Ladder, Thermo Fisher Scientific Inc, France). A single band of about 244 base pairs (bp) in 1% agarose gel was considered

confirmatory to the *APOE* amplification.

2.6.3. Optimized PCR-RFLP

Optimized PCR restriction-fragment length polymorphism (PCR-RFLP) method using the restriction enzymes HaeII and A*fl*III. After confirmation of amplification, 10 µl of each amplified DNA with the two restriction enzymes. The HaeII digestion mixture contained 10 µl PCR products mixed with 1.5 units of HaeII (R0107L; New England Biolabs) in the buffer supplied by the manufacturer (NEB 4 buffer). Similarly, the A*fl*III digestion mixture contained 10 µl PCR products and 2.5 units of A*fl*III (R0541L; New England Biolabs, Ipswich, WI, USA) in the buffer supplied by the manufacturer (NEB 3 buffer). The two reactions were allowed to proceed for at least three hours at 37˚C. The resulting fragments were separated on a 4% agarose gel stained with ethidium bromide and migrated for 2 hours with 100 volts with a 50-bp marker (Invitrogen™ 50 bp DNA Ladder, Thermo Fisher Scientific Inc, France). The gel images were captured by an Azure CSeries gel imaging system (c600, Azure Biosystems, 6747 Sierra Court, Suites A-B, Dublin, CA 94568 USA). Several bands at different levels were identified in the 4% agarose gel after the digestion and were used to identify different *APOE* genotypes based on their presence/absence at identical bp levels. The presence of one band at 227 bp for the HaeII and two bands (at 177 and 50 bp) for A*fl*III was used to identify the $\varepsilon 2/\varepsilon 2$ genotype, and the presence of two bands (at 195 and 32 bp) for the HaeII and two bands (at 177 and 50 bp) for A*fl*III was used to identify the $\varepsilon 3/\varepsilon 3$ genotype, the presence of two bands (at 195 and 32 bp) for the HaeII and one band at 227 bp for A*fl*III was used to identify the $\varepsilon 4/\varepsilon 4$ genotype, the presence of three bands (at 227, 195 and 32 bp) for the HaeII and two bands (at 177 and 50 bp) for A*fl*III was used to identify the $\varepsilon 2/\varepsilon 3$ genotype, the presence of three bands (at 227, 195 and 32 bp) for the HaeII and three bands (at 227, 177 and 50 bp) for A*fl*III was used to identify the $\varepsilon 2/\varepsilon 4$ genotype, the presence of two bands (at 195 and 32 bp) for the HaeII and three bands (at 227, 177 and 50 bp) for A*fl*III was used to identify the $\varepsilon 3/\varepsilon 4$ genotype.

2.7. Statistical Analysis

All variables were saved in an Excel table. Quantitative variables were described using mean±standard deviation (SD) and qualitative variables using absolute values and percentages. Pairwise comparisons of the metabolic parameters between control, diabetic and diabetic with atherosclerosis were evaluated by ANOVA test (post hoc test LSD). The Chi^2 and Fisher test was used to compare the mean of the qualitative variables. Linear regression was used to assess the impacts of the *APOE* on the risk of type 2 diabetes and its vascular complications. The results were considered significant when $p \leq 5\%$.

The exploitation of the data was carried out by SPSS Statistics software version v23 × 64 (IBM, Chicago, IL, USA), and STATA version 12.0 (ISBN-13: 978-0-8400-6463-9).

3. Result

3.1. Baseline Characteristics in Control and Type 2 Diabetic Participants

The mean age of all study participants was 50.68 years ± 7.25. The general and biochemical data of participants according to the groups are shown in Table 1.

Control subjects were different from the type 2 diabetic subjects by their vascular constante (heart rate), and their parameters of carbohydrate and lipid metabolism (fasting plasma glucose, glycated hemoglobin, LDL cholesterol, and No-HDL cholesterol).

Control subjects were different from the type 2 diabetes subjects with arterial stiffness by their age, their vascular parameters (SBP, ft-PWV), their carbohydrate and lipid metabolism parameters (fasting glycemic, glycated hemoglobin, total cholesterol, LDL cholesterol, and No-HDL cholesterol).

Type 2 diabetic subjects without arterial stiffness were different from type 2 diabetic subjects with arterial stiffness by their age, vascular parameters (SBP,

Table 1. Demographic, clinical, and biochemical data of the study population.

Variable	Control *n* = 81 *a*	T2DM *n* = 90 *b*	TD2M/AS *n* = 31 *c*	p value *a & b*	p value *a & c*	p value *b & c*
Age (years)	49.09 ± 8.11	50.27 ± 6.36	54.26 ± 5.22	0.27	0.001**	0.007**
Waist size (cm)	91.35 ± 13.57	93.09 ± 12.13	90.52 ± 11.45	0.36	0.75	0.32
Body mass index (kg/m^2)	28.82 ± 6.43	28.87 ± 5.35	26.94 ± 5.50	0.96	0.12	0.11
SBP (mm Hg)	132.47 ± 21.53	134.28 ± 25.99	144.68 ± 32.90	0.64	0.02*	0.047*
DBP (mm Hg)	88.32 ± 14.20	87.11 ± 12.88	89.39 ± 18.11	0.58	0.72	0.44
Heart rate (bpm)	76.64 ± 10.81	84.03 ± 11.95	79.84 ± 14.04	<0.0001***	0.21	0.10
ft-PWV (m/s)	6.99 ± 1.44	7.34 ± 1.34	14.59 ± 5.04	0.37	<0.0001***	<0.0001***
Reactive hyperemia index	2.00 ± 0.70	1.99 ± 0.69	1.71 ± 0.39	0.96	0.33	0.27
Nitric oxide (µmol/L)	750.30 ± 250.99	770.06 ± 217.60	777.65 ± 256.42	0.59	0.60	0.88
Fasting glycemic (g/l)	0.85 ± 0.15	1.50 ± 0.80	1.88 ± 0.99	<0.0001***	<0.0001***	0.005**
Glycated hemoglobin (%)	5.24 ± 0.59	8.04 ± 2.36	8.58 ± 2.55	<0.0001***	<0.0001***	0.16
Triglyceride (g/l)	0.79 ± 0.33	0.86 ± 0.45	0.90 ± 0.44	0.23	0.19	0.65
Total cholesterol (g/l)	2.08 ± 0.43	2.21 ± 0.52	2.33 ± 0.53	0.90	0.015*	0.22
LDL cholesterol (g/l)	1.36 ± 0.39	1.59 ± 0.51	1.74 ± 0.49	0.001**	<0.0001***	0.13
HDL cholesterol (g/l)	0.59 ± 0.13	0.56 ± 0.18	0.59 ± 0.12	0.22	0.90	0.30
No-HDL cholesterol (g/l)	1.49 ± 0.37	1.65 ± 0.50	1.74 ± 0.50	0.025**	0.01*	0.33
ApoB/ApoA	0.64 ± 0.21	0.77 ± 0.64	0.65 ± 0.20	0.08	0.93	0.23

T2DM: type 2 diabetes mellitus, TD2M/AS: type 2 diabetes mellitus with arterial stiffness, SBP: systolic blood pressure, DBP: diastolic blood pressure, ApoA: apolipoprotein A, ApoB: apolipoprotein B; *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.0001$: were considered significant in the statistical analysis t-test.

ft-PWV), and carbohydrate parameters (fasting glycemic). See Table 1.

3.2. Association between Vascular Markers and Other Studied Parameters

We noted that the ft-PWV was positively correlated with age (r = 0.30, p < 0.0001), LDL cholesterol (r = 0.16, p = 0.02), fasting glycemic (r = 0.20, p = 0.004) and glycated hemoglobin (r = 0.18, p = 0.01). The RHI was negatively correlated with the total cholesterol levels (r = −0.40, p = 0.02), and the fasting glycemic (r = −0.41, p = 0.02). The nitric oxide was negatively correlated with the HDL cholesterol levels (r = −0.27, p < 0.0001).

3.3. Genotypic Distribution and Allelic Frequencies

In Table 2, we show the allelic and genotypic characteristics of the *APOE* gene according to the studied groups. The Hardy-Weinberg Equilibrium (HWE_X^2) verifies that the genotype frequencies remain the same from generation to generation. A significant variation in the genotype frequencies was observed for rs7412 (*ε*2) among the control subjects (HWE_X^2 = 39.63, p < 0.0001), the type 2 diabetic subjects (HWE_X^2 = 55, p < 0.0001), and in type 2 diabetic subjects with arterial stiffness (HWE_X^2 = 16.35, p = 0.00005). For rs429358 (*ε*4), the genotype frequencies were not significant regardless of the group considered.

Table 2. Genotype distributions and allele frequencies of *APOE* polymorphisms by subgroup.

Variables	Control *a*	T2DM *b*	T2DM/AS *c*	p value *a & b*	p value *a & c*	p value *b & c*
Allele n (%)	*n* = 162	*n* = 186	*n* = 62			
*ε*2	70 (86.4)	82 (91.1)	28 (90.3)	0.23	0.42	0.42
*ε*3	76 (84.0)	87 (84.4)	29 (83.9)	0.55	0.60	0.60
*ε*4	16 (19.8)	17 (18.9)	5 (16.1)	0.52	0.44	0.44
Genotype n (%)	*n* = 81	*n* = 90	*n* = 31			
*ε*2/*ε*3	57 (70.4)	68 (75.6)	23 (74.2)	0.28	0.44	0.44
*ε*2/*ε*4	13 (16.0)	14 (15.6)	5 (16.1)	0.55	0.60	0.60
*ε*2/*ε*2	/	/	/	/	/	/
*ε*3/*ε*3	8 (9.9)	5 (5.6)	3 (9.7)	0.22	0.64	0.64
*ε*3/*ε*4	3 (3.7)	3 (3.3)	/	0.61	0.37	0.37
*ε*4/*ε*4	/	/	/	/	/	/
HWE						
rs7412 (*ε*2)	39.63 (p < 0.0001)	55 (p < 0.0001)	16.35 (p = 0.00005)	/	/	/
rs429358 (*ε*4)	0.27 (p = 0.60)	0.43 (p = 0.51)	/	/	/	/

T2DM: type 2 diabetes mellitus; T2DM/AS: type 2 diabetes with arterial stiffness, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, ApoA: apolipoprotein A, ApoB: apolipoprotein B; *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.0001.

However, the genotype distribution and the allelic frequencies were not significantly different between the studied groups. In the study population, the overall allele frequency *APOE* gene was ε2 (43.9%), ε3 (46.8%), and ε4 (9.3%). The *APOE* genotype distribution was ε2ε3 (73.3%), ε2ε4 (15.8%), ε3ε3 (7.9%), and ε3ε4 (3%).

3.4. Variations in the Clinical and Biochemical Characteristics in Study Group Subjects Depending on the Allelic and Genomic Status

3.4.1. Variations Depending on the Allele Frequencies

In control subjects, the presence of ε3 was associated with an increase in fasting blood glucose ($r = 2.36$, $p = 0.018$), and a decrease in plasma LDL cholesterol levels ($r = -2.17$, $p = 0.03$), while the presence of ε4 was associated with an increase in total cholesterol ($r = 2.59$, $p = 0.01$), LDL cholesterol ($r = 2.84$, $p = 0.004$), and No-HDL cholesterol ($r = 2.74$, $p = 0.006$).

In type 2 diabetes subjects, the presence of ε2 was associated with a decrease in diastolic blood pressure ($r = -2.25$, $p = 0.02$). At the same time, ε3 was associated with a decrease in ft-PWV ($r = -2.26$, $p = 0.024$) while the presence of ε4 was associated with an increase in ft-PWV ($r = 2.52$, $p = 0.012$).

In type 2 diabetics with arterial stiffness, no statistically significant association was noted between the *APOE* gene alleles and the studied parameters. See Table 3.

3.4.2. Variations Depending on Their Genotype Distribution

In Table 4, we noted the influence of the *APOE* genotype on the parameters studied Among control subjects, ε2ε4 was associated with a decrease in fasting glycemic ($r = -2.36$, $p = 0.018$) and an increase in plasma LDL cholesterol levels ($r = 2.17$, $p = 0.037$), and ε3ε4 was associated with an increase in plasma levels of total cholesterol ($r = 2.13$, $p = 0.033$) and no-HDL cholesterol ($r = 2.06$, $p = 0.039$).

In type 2 diabetes subjects, ε2ε3 was associated with a decrease in the ft-PWV ($r = -2.36$, $p = 0.005$) and in the plasma adiponectin levels ($r = -2.10$, $p = 0.035$). The ε3ε3 was associated with an increase in the SBP ($r = 2.44$, $p = 0.014$).

We noted that 99% of ε2ε3 genotype carriers had a limited to possible risk of developing type 2 diabetes mellitus. For ε2ε4 genotype carriers, 93% had a limited to possible risk of developing type 2 diabetes mellitus however the remaining 7% had a very high risk of developing type 2 diabetes mellitus.

In the same wake, 13% of subjects carrying the ε3ε3 genotype and 33% of subjects carrying the ε3ε4 genotype had a probable risk of developing type 2 diabetes mellitus.

At the same time, 94% of diabetic subjects harboring the ε2ε3 genotype had a limited to possible risk of developing diabetic atherosclerosis. Among the ε2ε4 genotype carriers, 93% had a limited risk of developing diabetic atherosclerosis but it is important to note that the remaining 7% had a very high risk of developing

Table 3. Difference in the anthropometric, biochemical, and vascular parameters of the subjects according to *APOE* allele in each group.

Variables	ε2		ε3		ε4	
	coefficient	p value	coefficient	p value	coefficient	p value
Control						
Waist size (cm)	−0.15	0.88	0.89	0.37	−0.89	0.37
BMI (kg/m²)	−1.29	0.20	−0.27	0.79	0.73	0.47
SBP (mm Hg)	−0.47	0.64	0.27	0.79	−0.24	0.81
DBP (mm Hg)	−0.01	0.99	0.75	0.45	−0.71	0.48
Heart rate (bpm)	−0.51	0.61	−0.52	0.60	0.28	0.78
ft-PWV (m/s)	1.10	0.27	0.88	0.38	−1.37	0.17
Reactive hyperemia index	0.78	0.43	−0.92	0.36	0.92	0.36
Nitric oxide (µmol/l)	0.84	0.40	−0.33	0.74	−0.49	0.62
Fasting glycemic (g/l)	−1.59	0.11	2.36	0.018*	−1.49	0.14
Glycated hemoglobin (%)	1.05	0.29	0.78	0.44	−1.01	0.31
Triglycerides (g/l)	−0.33	0.74	1.14	0.25	−0.80	0.42
Total cholesterol (g/l)	−0.09	0.93	−1.71	0.09	2.59	0.01*
LDL cholesterol (g/l)	0.07	0.95	−2.17	0.030*	2.84	0.004**
HDL cholesterol (g/l)	−0.39	0.69	−0.22	0.83	0.73	0.47
No-HDL cholesterol (g/l)	0.04	0.97	−1.90	0.06	2.74	0.006**
ApoB/ApoA	0.73	0.64	−1.13	0.26	1.74	0.08
Type 2 diabetes mellitus						
Waist size (cm)	0.18	0.86	0.34	0.73	−0.97	0.33
BMI (kg/m²)	−0.86	0.39	0.60	0.55	−0.67	0.50
SBP (mm Hg)	−1.92	0.06	0.02	0.98	−0.29	0.77
DBP (mm Hg)	−2.25	0.02*	0.67	0.50	−0.50	0.62
Heart rate (bpm)	0.78	0.43	0.96	0.34	−1.60	0.11
ft-PWV (m/s)	−1.39	0.17	−2.26	0.024*	2.52	0.012*
Reactive hyperemia index	0.12	0.90	0.63	0.53	−0.88	0.38
Nitric oxide (µmol/l)	0.31	0.76	−0.65	0.52	0.63	0.53
Fasting glycemic (g/l)	−0.03	0.97	1.12	0.26	−1.32	0.19
Glycated hemoglobin (%)	1.08	0.28	0.71	0.48	−1.34	0.18
Triglycerides (g/l)	0.57	0.57	−0.75	0.46	0.63	0.53
Total cholesterol (g/l)	0.01	0.99	0.94	0.35	−0.76	0.45
LDL cholesterol (g/l)	−1.39	0.16	1.24	0.22	−0.37	0.71
HDL cholesterol (g/l)	0.67	0.50	−0.16	0.87	−0.23	0.82

Continued

No-HDL cholesterol (g/l)	−0.23	0.82	1.03	0.30	−0.70	0.48
ApoB/ApoA	0.22	0.83	−1.29	0.20	1.20	0.23
Type 2 diabetes mellitus with arterial stiffness						
Waist size (cm)	−0.72	0.47	−0.79	0.43	0.79	0.43
BMI (kg/m^2)	−0.75	0.45	−0.02	0.98	0.02	0.98
SBP (mm Hg)	−1.34	0.18	0.02	0.98	−0.02	0.98
DBP (mm Hg)	−1.24	0.21	−0.96	0.34	0.96	0.34
Heart rate (bpm)	1.07	0.29	−0.80	0.43	0.80	0.43
ft-PWV (m/s)	0.91	0.37	1.09	0.28	−1.09	0.28
Reactive hyperemia index	–	–	−0.19	0.85	0.19	0.85
Nitric oxide (µmol/l)	0.05	0.96	0.19	0.85	−0.19	0.85
Fasting glycemic (g/l)	0.47	0.64	0.59	0.56	−0.59	0.56
Glycated hemoglobin (%)	0.01	0.99	−0.67	0.50	0.67	0.50
Triglycerides (g/l)	0.19	0.85	−1.38	0.17	1.38	0.17
Total cholesterol (g/l)	−0.47	0.64	−0.64	0.52	0.64	0.52
LDL cholesterol (g/l)	−0.29	0.77	−0.78	0.43	0.78	0.43
HDL cholesterol (g/l)	0.66	0.51	0.10	0.92	−0.10	0.92
No-HDL cholesterol (g/l)	−0.66	0.51	−0.71	0.48	0.71	0.48
ApoB/ApoA	−0.20	0.84	−1.12	0.90	0.12	0.90

coefficient = r, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, ApoA: apolipoprotein A, ApoB: apolipoprotein B; *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.0001: were considered significant.

Table 4. Comparison of the clinical, biochemical, and vascular data stratified by *APOE* genotype distributions in each group.

Variables	*ε2ε3*		*ε2ε4*		*ε3ε3*		*ε3ε4*	
	coefficient	p value	coefficient	p value	coefficient	p value	coefficient	p value
Control								
Waist size (cm)	0.17	0.87	−0.35	0.73	−0.02	0.98	0.30	0.76
Body mass index (kg/m^2)	−1.19	0.24	0.27	0.79	0.86	0.39	1.00	0.32
SBP (mm Hg)	−0.13	0.89	−0.27	0.79	0.53	0.60	0.02	0.99
DBP (mm Hg)	0.60	0.55	−0.75	0.45	0.04	0.97	−0.04	0.97
Heart rate (bpm)	−1.80	0.42	0.52	0.60	0.86	0.39	−0.43	0.67
ft-PWV (m/s)	1.53	0.13	−0.88	0.38	−0.51	0.61	−1.16	0.25
Reactive hyperemia index	−0.42	0.68	0.92	0.36	−0.78	0.43	–	–
Nitric oxide (µmol/l)	0.36	0.72	0.33	0.74	0.10	0.92	−1.58	0.12
Fasting glycemic (g/l)	0.76	0.45	−2.36	0.02*	0.84	0.40	1.53	0.13
Glycated hemoglobin (%)	1.40	0.16	−0.78	0.44	−0.81	0.42	−0.64	0.52

Continued

Triglycerides (g/l)	0.67	0.50	−1.14	0.25	0.04	0.97	0.54	0.59
Total cholesterol (g/l)	−1.45	0.15	1.71	0.09	−1.37	0.17	2.13	0.033*
LDL cholesterol (g/l)	−1.72	0.09	2.17	0.03*	−1.34	0.18	1.88	0.06
HDL cholesterol (g/l)	−0.47	0.64	0.22	0.83	−0.27	0.80	1.09	0.28
No-HDL cholesterol (g/l)	−1.51	0.13	1.90	0.06	−1.49	0.14	2.06	0.039*
ApoB/ApoA	−0.37	0.71	1.13	0.26	−1.80	0.07	1.49	0.14
Type 2 diabetes mellitus								
Waist size (cm)	0.41	0.69	−0.34	0.73	0.89	0.37	−1.45	0.15
Body mass index (kg/m^2)	−0.06	0.95	−0.60	0.55	1.24	0.22	−0.25	0.81
SBP (mm Hg)	−0.69	0.49	−0.78	0.43	1.69	0.09	0.94	0.35
DBP (mm Hg)	−1.04	0.30	−0.67	0.50	2.47	0.014*	0.26	0.80
Heart rate (bpm)	1.32	0.19	−0.96	0.34	0.26	0.79	−1.56	0.12
ft-PWV (m/s)	−2.80	0.005*	−1.09	0.28	0.98	0.33	0.95	0.34
Reactive hyperemia index	0.77	0.44	−0.63	0.53	0.86	0.39	−0.63	0.53
Nitric oxide (µmol/l)	−0.35	0.73	0.65	0.52	−0.43	0.67	0.06	0.95
Fasting glycemic (g/l)	0.93	0.35	−1.12	0.26	0.54	0.59	−0.63	0.52
Glycated hemoglobin (%)	1.31	0.19	−0.71	0.48	−0.17	0.86	−1.40	0.16
Triglycerides (g/l)	−0.26	0.80	0.75	0.46	−0.60	0.55	−0.13	0.89
Total cholesterol (g/l)	0.80	0.42	−0.94	0.35	−0.20	0.84	0.25	0.80
LDL cholesterol (g/l)	0.11	0.91	−1.24	0.22	0.42	0.68	1.62	0.11
HDL cholesterol (g/l)	0.31	0.76	0.16	0.87	−0.19	0.85	−0.83	0.41
No-HDL cholesterol (g/l)	0.72	0.47	−1.03	0.30	−0.14	0.89	0.55	0.58
ApoB/ApoA	−1.06	0.29	1.29	0.20	−0.20	0.84	−0.09	0.93
Type 2 diabetes mellitus with arterial stiffness								
Waist size (cm)	−1.13	0.26	0.79	0.43	0.72	0.47	–	–
Body mass index (kg/m^2)	−0.53	0.60	0.02	0.98	0.75	0.45	–	–
SBP (mm Hg)	−0.94	0.35	−0.02	0.98	1.34	0.18	–	–
DBP (mm Hg)	−1.59	0.11	0.96	0.34	1.24	0.21	–	–
Heart rate (bpm)	0.05	0.96	0.80	0.43	−1.07	0.29	–	–
ft-PWV (m/s)	1.47	0.14	0.92	0.36	−0.91	0.37	–	–
Reactive hyperemia index	−1.19	0.85	0.19	0.85	–	–	–	–
Nitric oxide (µmol/l)	0.19	0.85	−0.19	0.85	−0.05	0.96	–	–
Fasting glycemic (g/l)	0.81	0.42	−0.59	0.56	−0.47	0.64	–	–
Glycated hemoglobin (%)	−0.56	0.58	0.67	0.50	−0.01	0.99	–	–
Triglycerides (g/l)	−1.07	0.29	1.38	0.17	−0.19	0.85	–	–

Continued

Total cholesterol (g/l)	−0.85	0.40	0.64	0.52	0.47	0.64	–	–
LDL cholesterol (g/l)	−0.85	0.39	0.78	0.43	0.29	0.77	–	–
HDL cholesterol (g/l)	0.53	0.60	−0.10	0.92	−0.66	0.51	–	–
No-HDL cholesterol (g/l)	−1.03	0.30	0.71	0.48	0.66	0.51	–	–
ApoB/ApoA	−0.24	0.81	0.12	0.90	0.20	0.84	–	–

coefficient = r, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, ApoA: apolipoprotein A, ApoB: apolipoprotein B; *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.0001: were considered significant in the statistical analysis t-test

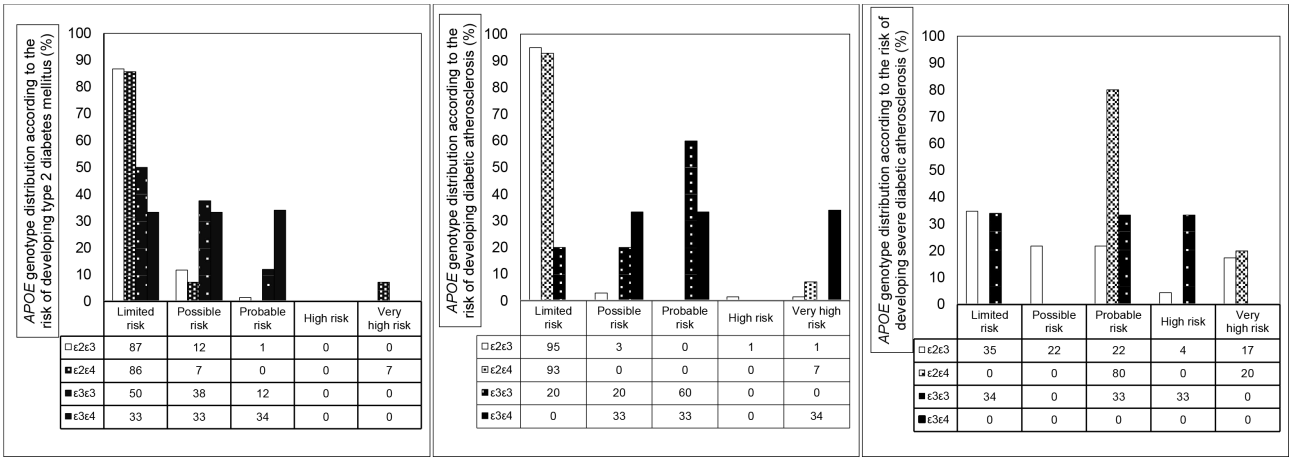

Figure 1. *APOE* genotype distribution according to the risk of developing type 2 diabetes mellitus and its vascular complications. Panel A shows the nature of the risk of developing type 2 diabetes according to the *APOE* genotype distribution. Panel B shows the nature of the risk of developing diabetic atherosclerosis according to the *APOE* genotype distribution. Panel C shows the nature of the risk of developing severe diabetic atherosclerosis according to the *APOE* genotype distribution. In each panel, the symbol without filling represents *ε*2*ε*3, the symbol withwhite background with black strokes represents *ε*2*ε*4, the symbol with a black background and white dots represents *ε*3*ε*3, and the symbol with a single filling represents *ε*3*ε*4.

diabetic atherosclerosis. All subjects carrying the *ε*3*ε*3 genotype had a limited to probable risk of developing diabetic atherosclerosis. However, 33% of diabetics carrying the *ε*3*ε*4 genotype had a very high risk of diabetic atherosclerosis.

In type 2 diabetic subjects with atherosclerosis, 17% and 20% of subjects harboring respectively *ε*2*ε*4 and *ε*2*ε*3 genotypes had a very high risk of developing a severe form of diabetic atherosclerosis, and 33% of subjects harboring the *ε*3*ε*3 genotype had a high risk of developing a severe form of diabetic atherosclerosis. See Figure 1.

4. Discussion

APOE plays an essential role in the transport, metabolism, and digestion of blood lipids and lipoproteins [12]. About a decade ago, the *APOE* gene was suggested as a susceptible gene that could induce the pathogenesis of T2DM [8]. At the same time, the *APOE* gene is involved in the development of several cardiovascular disorders such as vascular dysfunction in type 2 diabetes [9]. At present, there is no study investigating the association between the *APOE* gene and type 2 diabetes

mellitus in Senegal. Therefore, the relationships between the *APOE* gene and the risk of type 2 diabetes mellitus, diabetes atherosclerosis, and the influences of *APOE* polymorphisms in the Senegalese population were the aim of our study.

Compared to control subjects, type 2 diabetic subjects with or without vascular complications had a significant increase in heart rate ($p < 0.0001$), systolic blood pressure ($p = 0.02$), finger-toes pulse wave velocity progression ($p = 0.04$), LDL cholesterol ($p < 0.0001$), and No-HDL cholesterol ($p = 0.007$).

A ft-PWV greater than or equal to 10 m/s was considered arterial stiffness. In this study, the increase in ft-PWV would be favored by advancing age ($r = 0.30$, $p < 0.0001$), an increase in the plasma LDL cholesterol levels ($r = 0.16$, $p = 0.02$), and by chronic hyperglycemia (fasting glycemic ($r = 0.20$, $p = 0.004$) and glycated hemoglobin ($r = 0.18$, $p = 0.01$)). An RHI decreased and lower than 1.67 was considered endothelial dysfunction. A decrease in RHI would be favored by an increase in the plasma total cholesterol and glucose levels ($r = -0.40$, $p = 0.02$ and $r = -0.41$, $p = 0.02$, respectively).

In control subjects, the presence of $\varepsilon 3$ was associated with an increase in fasting blood glucose ($r = 2.36$, $p = 0.018$), and a decrease in plasma LDL cholesterol levels ($r = -2.17$, $p = 0.03$), while the presence of $\varepsilon 4$ was associated with an increase in total cholesterol ($r = 2.59$, $p = 0.01$), LDL cholesterol ($r=2.84$, $p=0.004$), and No-HDL cholesterol ($r = 2.59$, $p = 0.01$).

In type 2 diabetes subjects, the $\varepsilon 2$ presence was associated with a decrease in the diastolic blood pressure ($r = -2.25$, $p = 0.02$). At the same time, the $\varepsilon 3$ presence was associated with a decrease in ft-PWV ($r = -2.26$, $p = 0.024$) while the presence of $\varepsilon 4$ was associated with an increase in ft-PWV ($r = 2.52$, $p = 0.012$).

Among $\varepsilon 2\varepsilon 3$ genotype carriers, 99% had a limited risk of developing type 2 diabetes. In the case of type 2 diabetes, only 1% to 2% would have a significant risk of developing diabetic atherosclerosis, which would be severe in 17%. Regarding the $\varepsilon 2\varepsilon 4$ genotype carriers, 93% had a limited or even possible risk of developing type 2 diabetes, but the remaining 7% had a very high risk of developing type 2 diabetes. Diabetics carrying this genotype had in 7% of cases a very high risk of developing diabetic atherosclerosis. The latter had a 20% very high risk of being very severe.

Subjects carrying the $\varepsilon 3\varepsilon 4$ genotype had in 67% of cases a possible or even probable risk of developing type 2 diabetes and in the event of diabetes, there was in 34% a very high risk of developing atherosclerosis which will not have even the time to evolve towards severity.

For subjects carrying the reference genotype $\varepsilon 3\varepsilon 3$, the risk of developing type 2 diabetes and diabetic athérosclerosis was higher than that of the genotypes ($\varepsilon 2\varepsilon 3$, $\varepsilon 2\varepsilon 4$) but lower than that of the $\varepsilon 3\varepsilon 4$ genotype.

4.1. *APOE* Genotype Distribution & Allele Frequencies

APOE alleles were all present in the study population and their frequencies were $\varepsilon 3$ (46.8%) > $\varepsilon 2$ (43.9%) > $\varepsilon 4$ (9.3%), which corroborates data from the literature

on the general frequency distribution of *APOE* alleles [23], unlike the distribution of ε2 which was significantly higher and exceeds the frequency of the ε4 allele which was the lowest. ε3 allele, the most common of the three isoforms, is considered to be the normal form.

The *APOE* genotype distribution was ε2ε3 (73.3%) > ε2ε4 (15.8%) > ε3ε3 (7.9%) > ε3ε4 (3%), this is contrary to previous studies for which the most common genotype was ε3ε3 [24]. The ε2ε2 and ε4ε4 genotypes were totally absent in our study population of adult subjects. These results support those found in a previous study [25]. We estimate that the absence of the ε2ε2 and ε4ε4 genotypes would be justified by a relatively short life expectancy for individuals harboring these genotypes.

4.2. Association between *APOE* Gene Polymorphisms and Glucose Metabolism

In control subjects, the ε3 presence was associated with an increase in fasting blood glucose ($r = 2.36$, $p = 0.018$), but ε2ε4 genotypes carriers were associated with a decrease in fasting blood glucose ($r = -2.36$, $p = 0.018$). This suggests that the *APOE* gene and its polymorphisms may also be involved in carbohydrate metabolism.

The *APOE* protein is expressed in adipose tissue, mainly in adipocytes and in macrophages [26]. Adipose tissue is a reservoir of stored triglycerides and has important metabolic effects, including influencing glucose homeostasis. In addition, endogenous *APOE* produced by adipocytes plays an important role in normal lipid homeostasis of adipose tissue [26]. There appears to be a reciprocal regulation of *APOE* and carbohydrate-lipid homeostasis in adipose tissue. Adipose tissue *APOE* levels decrease with obesity and increase with fasting [27]. However, plasma *APOE* levels vary only slightly between fasting and non-fasting states [28].

4.3. Association between *APOE* Gene, Its Polymorphisms, and Lipid Metabolism

The *APOE* ε3 allele carriers were associated with a decrease in plasma LDL cholesterol levels ($r = -2.17$, $p = 0.03$), and ε4 allele carriers were related to the increase in plasma total cholesterol ($r = 2.59$, $p = 0.01$), LDL cholesterol ($r = 2.84$, $p = 0.004$), and No-HDL cholesterol ($r = 2.74$, $p = 0.006$). These results suggest that ε4 compared to ε3 would expose to an increase in blood lipid levels.

No particular relationship was noted between harboring the ε2 allele and plasma lipid levels. In view of previous studies, there was great heterogeneity in the comparison of the ε2 allele with the ε3 allele [29]. However, the sources of heterogeneity are still not identified.

In terms of genotypic distribution, ε2ε4 was associated with an increase in plasma LDL cholesterol levels ($r = 2.17$, $p = 0.03$), and ε3ε4 was associated with an increase in plasma total cholesterol ($r = 2.13$, $p = 0.03$) and No-HDL cholesterol ($r = 2.06$, $p = 0.039$). Thus, we found that the genotypes involving the ε4

allele are characterized by an increase in the blood level of lipids, either total cholesterol or LDL cholesterol. These findings corroborate the data in the literature, the ε4 isoform was associated with an increase in the total cholesterol and LDL cholesterol concentrations when the ε3ε3 homozygote carriers are used as references [30]. This effect has been confirmed in many healthy people [31].

APOE is structural apolipoproteins that participate in the regulation of the catabolism and uptake of lipoproteins rich in triglycerides or cholesterol.

The association between *APOE* genotypes and plasma lipid and lipoprotein levels has been repeatedly demonstrated [32]. The genetic polymorphism of *APOE* can contribute to the variation in lipoproteins concentration. In addition, the *APOE* allele polymorphism (*ε2, ε3 and ε4*) is thought to be one of the factors responsible for interindividual differences in lipid and lipoprotein levels [33].

The ε2 allele and the ε4 allele are associated with unfavorable lipid profiles. The ε2 allele is associated with lower levels of plasma cholesterol and LDL cholesterol and higher levels of triglycerides compared to the ε3 allele, whereas the ε4 allele is associated with higher levels of total cholesterol, LDL cholesterol, and triglycerides relative to the ε3 allele [32].

Due to the reduced affinity of the ε2 allele for the LDL receptor, remnants accumulate triglycerides in plasma. The ε4 allele binds preferentially with VLDL lipoproteins and LDL [34] with a lower binding affinity with serum lipids and lipoproteins compared to the ε3 and ε2 alleles [35] and would have higher levels lowest average *APOE* protein levels [36], and therefore would have a lesser capacity to eliminate dietary fats from the blood and higher plasma lipid levels than ε3ε3 homozygotes [35].

APOE polymorphism is common and it contributes substantially to the overall phenotypic variability of plasma lipid levels in the general population [37]. However at the individual level, the effect of the *APOE* polymorphism on plasma lipid levels is moderate and would be influenced by gender, possibly height, diet, alcohol consumption, obesity, hyperglycemia, diabetes, hypothyroidism, and estrogen therapy.

These influences underlie differences in the magnitude of associations between common *APOE* genotypes and plasma lipid levels between populations and subgroups within populations.

4.4. Association between *APOE* Gene, Its Polymorphisms, and the Risk of Developing Type 2 Diabetes Mellitus

Among ε2ε3 genotype carriers, 99% had a limited risk of developing type 2 diabetes mellitus. Regarding the ε2ε4 genotype carriers, 93% had a limited or even possible risk of developing type 2 diabetes mellitus. Previous studies have supported our results and had already shown that the ε2 allele and the genotypes (ε2ε3 and ε2ε4) are not associated with a risk of developing T2DM [29]. The ε3 presence was associated with an increase in fasting blood glucose ($r = 2.36$, $p = 0.018$) and a decrease in plasma LDL cholesterol levels ($r = -2.17$, $p = 0.03$). No

particular relationship was noted between harboring the ε2 allele and plasma lipid levels. Consequently, it was understandable that the genotypes involving the *ε2* allele (*ε2ε3 and ε2ε4*) had a limited or even possible risk of exposure to the onset of type 2 diabetes mellitus. The subjects harboring the ε3ε4 genotypes had in 67% of cases a possible or even probable risk of developing type 2 diabetes mellitus. The findings of our study were in accordance with the previous studies, showing that both the ε4 allele and the genotypes including ε4 alleles such as *ε3ε4* were associated with an increased risk of developing type 2 diabetes mellitus [38].

The impact of ε4 on lipid metabolism would be a risk factor for the onset of type 2 diabetes mellitus. The combination of the effects of ε3 on plasma glucose and of ε4 on lipid metabolism could explain the fact that 67% of subjects carrying the ε3ε4 genotype had a possible or even probable risk of developing type 2 diabetes mellitus.

Insulin resistance is known to be strongly associated with lipid metabolism disorder and the correlation of lipid profiles with type 2 diabetes phenotypes is significant. Therefore, the ε4 allele and the *ε3ε4* genotypes were associated with an increased risk of developing type 2 diabetes by affecting lipid metabolism. The ε4 allele has been found to be an independent risk factor for type 2 diabetes mellitus [39] and associated with the development of type 2 diabetes mellitus [40].

Several studies on the role of the *APOE* gene in developing pathological conditions of type 2 diabetes have been conducted and the pathogenesis was further linked to lipid and lipoprotein metabolic abnormalities [19].

4.5. Association between *APOE* Gene, Its Polymorphisms, and the Risk of Developing Diabetic Atherosclerosis

In type 2 diabetes subjects, among ε2ε3 genotype carriers, only 1% to 2% would have a significant risk of developing diabetic atherosclerosis whereas 7% of the subjects harboring the ε2ε4 genotype, and 34% of the subjects harboring the ε3ε4 genotype had a very high risk of developing diabetic atherosclerosis.

Based on allele frequency in type 2 diabetes subjects, the presence of ε2 was associated with a decrease in diastolic blood pressure ($r = -2.25$, $p = 0.02$). The ε3 was associated with a decrease in ft-PWV ($r = -2.26$, $p = 0.024$) while the presence of ε4 was associated with an increase in ft-PWV ($r = 2.52$, $p = 0.012$). This means that harboring the ε2 and ε3 alleles would protect against arterial stiffness while the ε4 allele would expose to arterial stiffness. An increase in ft-PWV corresponds to arterial stiffness, one of the initial characteristics of atherosclerosis. On one hand, the ε4 allele has been an independent risk factor for the onset and the development of type 2 diabetes mellitus [39] [40] and exacerbates vasculopathology during T2DM, and on another hand, the ε4 allele plays a significant role in atheromatous disease [41], while the ε2 allele would be associated with lower total cholesterol and LDL cholesterol, and it would be linked

to a reduced risk of cardiovascular disease such as high blood pressure [42].

According to the genotype distribution, the subjects harboring the ε2ε3 genotype were almost three times less likely to have arterial stiffness ($r = -2.80$, $p = 0.005$). These data support the limited risk of diabetic atherosclerosis in subjects harboring the ε2ε3 genotype.

The risk of hyperglycemia associated with the ε3 allele and the effects of the ε4 allele on lipid metabolism could explain the very high risk of diabetic atherosclerosis in subjects harboring the ε3ε4 genotype.

The findings of our study stir up the comments that the ε4 allele and the genotype ε3ε4 expose people to type 2 diabetes mellitus and its vascular complications.

The ε3 allele, being the normal form and the most common allele, would protect against arterial stiffness and therefore against atherosclerosis. The *APOE* influence on atherosclerosis is actively studied [43]. Reverse cholesterol transport and macrophage expression are one of the mechanisms by which *APOE* is atheroprotective by promoting the efflux of cholesterol from lipid-laden macrophages into the arterial wall. *APOE* is one of the main proteins secreted by macrophages [44] and has an anti-atherogenic role [45], in addition, much of *APOE* is bound to HDL cholesterol.

APOE promotes the efficient uptake of lipoproteins from the circulation and takes part in cellular cholesterol efflux and reverse cholesterol transportation [13]. The human *APOE* gene has three alleles (ε2, ε3, and ε4), which are responsible for the production of the corresponding apoE2, apoE3, and apoE4 plasma isoproteins [8], and thus change its function its anti-atherosclerotic effects [14]. Each of these isoforms has specific effects on atherosclerosis [46].

The protein *APOE* is important for lipid homeostasis in adipose tissue and arterial wall through its synthesis by adipocytes and macrophages. *APOE* gene expression in macrophages is up-regulated upon macrophage cholesterol load [45].

Additionally, non-lipid-related functions have also been attributed to ApoE. Some of the effects of the *APOE* protein have been shown to depend on its different domains, variations in its concentration, and its lipid state. Thus, this multifunctional protein has a normal and physiopathological impact at several levels [47].

The proliferation of vascular smooth muscle cells contributes to atherogenesis. *APOE* can inhibit smooth muscle cell proliferation and migration [47], by induction of the expression of the iNOS gene and the formation of nitric oxide (NO) because the increased level of NO inhibits cell cycle progression.

Furthermore, low concentrations of *APOE* also inhibit the migration of vascular smooth muscle cells. Since subphysiological plasma levels of *APOE* are able to inhibit neointimal formation after arterial injury [48], this suggests that vascular smooth muscle cell migration is likely the upstream effector of neointimal formation. *APOE* protects LDL against oxidation [49].

APOE may possess direct anti-inflammatory or immunoregulatory effects, which may also play a role in the pathogenesis of atherosclerosis [50].

4.6. Association between *APOE* Gene, Its Polymorphisms, and the Risk of Developing Severe Diabetic Atherosclerosis

In type 2 diabetic subjects with atherosclerosis, 17% of subjects harboring the $\varepsilon 2\varepsilon 3$, and 20% of subjects harboring the $\varepsilon 2\varepsilon 4$ had a very high risk of developing severe diabetic atherosclerosis.

The $\varepsilon 4$ allele plays a significant role in atheromatous disease [41], and is associated with an increased risk of cardiovascular disease. These protein variations, and their corresponding allele variants *(ε2, ε3 and ε4)*, have been linked with differential risks of cardiovascular diseases [15].

We find that subjects carrying the $\varepsilon 4\varepsilon 4$ genotype are not represented in the category of diabetic with severe atherosclerosis. This could imply a short life expectancy linked to this genotype.

5. The Strengths of the Present Study

First, we included women of different ethnicities, this allowed us to avoid bias linked to ethnicity.

Second, we had a relatively large sample size, despite the difficulties and the limited means.

Third, this study is the first study on the involvement of the *APOE* gene in the pathophysiology of type 2 diabetes and its vascular complications.

6. The Limitations of This Study

First, it is hard to exclude the effects of diet and activities on blood lipid levels, which exert certain bias.

Second, the study was carried out on an exclusively female population, so the effect of gender was not evaluated. Third, the sample is made of young adults, so the absolute absence of the $\varepsilon 2\varepsilon 2$ and $\varepsilon 4\varepsilon 4$ genotypes in the Senegalese population cannot be confirmed.

7. Conclusion

Alleles and genotypes of the *APOE* gene have divergent frequency, distribution, and effects in our study population. The physio-pathological role of the *APOE* gene and its polymorphisms in common and clinically is important in type 2 diabetes. The knowledge of this polymorphism becomes an element to be taken into consideration for the follow-up, a better therapeutic and dietary adaptation of type 2 diabetic patients, as well as the knowledge of the interindividual variations in the initiation and progression of diabetic atherosclerosis. These associations can be altered by the interaction with other factors, namely genetic, biochemical and clinical. Large-scale studies including all age groups would be needed.

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Data Origin

We declare on behalf of all the authors that all the data reported in this study were obtained internally in our various laboratories mentioned above.

Authors' Contributions

Maïmouna Touré: drafting of the project, director of the experiments, writing and submission of the article.

Fatou Diallo Agne: drafting of the project, participation in the purchase of reagents.

Amadou Dieng: participation in the statistical exploitation of data.

Rokhaya Ndiaye Diallo: owner of the laboratory and the materials used for the manipulations.

Lamine Gueye: participation in the purchase of reagents.

Abdoulaye Samb: participation in the purchase of reagents, Head of the physiology laboratory.

All authors read and approved the final manuscript.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations Ethics Approval and Consent to Participate

The study protocol was carried out according to the Helsinki recommendations of the World Medical Association of 1989. It was approved by the institutional ethics committee of the Faculty of Medicine, Pharmacy and Odonto-Stomatology of the University Cheikh Anta Diop (UCAD) (Protocol 027512018/CERruCAD). Informed written consent to participate in the study was obtained from all participants. All participants were informed of the intention to publish the results and they all consented.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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