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Assessment of the Genetic Susceptibility to Chronic Inflammation in MetS through an IL-6 and IL-6R Genes Polymorphism Survey in the Congolese Black African Population

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Abstract

Background: Chronic inflammation appears to be a pivotal mechanism underlying the pathophysiology of metabolic syndrome (MetS). Increasing evidence highlights the role of cytokines and their receptors gene polymorphisms in the susceptibility to the chronic inflammatory state observed in MetS. Since we recently showed combination of hs-CRP and IL-6 dosages improved MetS diagnosis accuracy and provided that IL-6/IL-6R gene polymorphisms appear to play a role in susceptibility to MetS, we aimed at investigating the role of some IL-6 and IL-6R receptor SNPs in the genetic susceptibility to chronic inflammation in this debilitating syndrome. **Methods:** A total of 319 Congolese adults (93 with MetS and 226 without MetS) were recruited for this population-based cross-sectional study. The NCEP-ATPIII criteria were used to define MetS. DNA extracted from whole blood was used for genotyping IL-6 (-174G/C, -597G/A) and IL-6R (+48867A/C) gene polymorphisms by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. Serum hs-CRP and IL-6 levels were measured using the immunoturbidimetric and ELISA methods. **Results:** To our knowledge, our study is the first one to assess the genetic susceptibility to chronic inflammation in

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MetS through an IL-6 and IL-6R genes polymorphism survey in a black African population. In men, IL-6 (-174G/C) heterozygous genotype GC was found to be significantly associated with MetS but very interestingly in women, IL-6R (+48867A/C) polymorphism was, on the contrary, linked to the pathology with a strong association for heterozygous AC genotype. Finally, the IL-6 (-174G/C, -597G/A) and IL-6R (+48867A/C) CAC haplotype were indistinctly associated with MetS. This shows that observed gender differences in MetS susceptibility might at least partially be related to differing gender responses to inflammation gene polymorphism. **Conclusion:** For the first time here, our findings unravel a possible association between IL-6R (+48867A/C) polymorphism and MetS in the adult Congolese population. More interestingly, the IL-6 (-174G/C, -597G/A) and IL-6R (+48867A/C) CAC haplotype appears to be related to MetS genetic susceptibility, highlighting the contribution of this SNPs combination in the settlement and/or development of this inflammatory condition possibly alongside with others factors.

Keywords

Inflammation, Interleukin-6, Interleukin-6 Receptor, Polymorphisms, MetS

1. Introduction

Chronic inflammation appears to be a pivotal mechanism underlying the pathophysiology of metabolic syndrome (MetS), a complex medical condition consisting of visceral obesity, high blood pressure, decreased HDL-cholesterol, and increased triglycerides and glucose levels [1]-[4]. Substantial progress has been made over the last decade in the identification of gene variants related to chronic inflammation [5]-[7]. Genetic variations, especially in genes encoding molecules of the host defense system, such as cytokines and cytokine receptors, influence susceptibility to chronic inflammation [8]. Among them, several candidate single-nucleotide polymorphisms (SNP) of interleukin-6 (IL-6) and its receptor (IL-6R) have been shown to be associated with MetS in different ethnic populations [9]-[13]. The human IL-6 gene is located on the short arm of chromosome 7 p15.3 and contains five exons and four introns. There are four promoter SNPs mapping at positions -174G/C, -373A/G, -572G/C and -597G/A [14]. Located in the long arm of chromosome 1 q21.3, the human IL-6R gene is composed of ten exons and eleven introns [15]. IL6R gene encodes a subunit of an IL-6R protein complex that exists in two forms: mIL-6R and sIL-6R, resulting in different signal transduction mechanisms known as IL-6/IL-6R classic signaling and trans-signaling respectively [16]. Seven SNPs are described in the IL-6R gene at positions -183G/A, +29753G/A, +42700C/T, +48869T/A, +24013G/A, +48867A/C and +59818C/T [17]. Although the IL-6 (-174G/C), IL-6 (-597G/A) and IL-6R (+48867A/C) polymorphisms have been best studied in the MetS, no IL-6/IL-6R polymorphisms study has been performed in black African popula-

tion. Variations in the frequencies of inflammatory pathway SNPs may help to explain racial disparities in disease risks and such interracial disparities have been scarcely documented [18], although the prevalence of MetS in African populations is now well recognized [19]. In this regard, considering the singularity of the Congolese ethnic group, we wondered if combinations of certain inflammatory pathways SNPs could predispose or contribute to MetS in this population. Multiple studies showed that variations in the IL-6 gene promoter at rs 1800796 (572 G > C), rs 1800795 (174 G > C) and rs 1800797 (597 G > A) are related to IL-6 serum levels as well as the occurrence, prevalence and development of several diseases including sepsis, chronic obstructive pulmonary disease, and hepatocellular carcinoma [20] [21]. The contribution of IL-6 polymorphisms in the severity of MetS is unclear, though, and very few IL-6/IL-6R polymorphism studies have been performed in specific ethnic groups. The hypothesis is that sequence polymorphisms of inflammation-related IL-6 and its receptor could impact genetic susceptibility to MetS and, that sorts of SNPs co-evolution could take place between them and drive this above-mentioned susceptibility. We therefore aimed at characterizing the inflammatory IL-6/IL-6R pathway SNPs variations in the adult Congolese population, which, to our knowledge, has never been achieved before.

2. Patients and Methods

2.1. Study Design

A cross-sectional study was designed and carried out from July to December 2021 in Brazzaville. The study was approved by the Ethical Committee of Health Research Sciences.

2.2. Study Population

The study population consisted of type 2 diabetic patients and obese non-diabetic subjects.

2.2.1. Diabetic Patients

All type 2 diabetic patients diagnosed by physicians on the WHO guidelines, men and women aged at least 30 years, were recruited in Diabcare clinic during the survey period.

2.2.2. Obese Subjects

Obese subjects were selected from Mère-Enfant Blanche Gomes Specialist Hospital. All of them had no clinical or laboratory evidence of diabetes and men and women were aged ≥ 30 years.

Clinical and anthropometric data were recorded for the 319 participants included in this study who had signed a consent form. MetS was defined as the presence of ≥3 out of 5 modified National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) criteria as previously described [22].

2.3. Biochemical and Immunoassay Analysis

Blood sampling was performed on all participants after an overnight fast. The fluoride sodium anticoagulated tubes were used to collect plasma for the quantification of glucose while dry tubes were used to collect serum for lipid parameters (CT, TG, HDL, LDL) and CRP quantification. Glucose and lipid parameters were measured by an enzymatic colorimetric method using a Cobas c111 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). CRP was quantified using the immunoturbidimetric method, allowing the detection of high-sensitivity C-reactive protein (hs-CRP). IL-6 in serum from participants was quantified using a commercial ELISA kit (SL1001Hu, Sunlong Biotech Co., Ltd., Hangzhou, China) according to the manufacturer's instructions. The absorbance was read at 450 nm using SK202 Elisa reader (Sinothinker Technology Co, Ltd., China).

2.4. DNA Extraction and Genotyping

Blood samples were obtained from participants in EDTA anticoagulated tubes, aliquoted and stored at −20˚C. DNA extractions were performed using Blood DNA isolation Mini Kit (Norgen Biotek Corp, Ontaria, Canada). For each sample, DNA concentration and purity were determined by NanoDrop 3300 Fluorospectrometer (Thermo Fisher Scientific, Massachusetts, USA). The PCR amplification of IL-6 (-174G/C and -597G/A) and IL-6R (+A48867C) gene polymorphisms were done using primers as presented in Table 1. The PCR final reaction volume of 25 µL contained 12.5 µL of HotStart Taq PCR Master Mix (cat #KT202, Tiagen Biotech, Beijing, China), 0.4 µM of each primer and 100 ng of DNA template. PCR was carried out on a T100 Thermal Cycler (BIORAD, USA) using the cycling conditions as follows: 95˚C for 5 min, 35 cycles of (95˚C for 30 sec; 56˚C for 30 sec; 72˚C for 30 sec), and final extension of 72˚C for 10 min. A volume of 10 µL of the PCR products were digested with 5 units Hin1II, 10 units FokI and 20 units HinfI (Thermo Fisher Scientific, Massachusetts, USA) at 37˚C overnight. Digested products were run in 2, 5% agarose gel, stained with ethidium bromide and viewed under ultra-violet light. The primers used for amplification and restriction enzymes were those previously described [23] [24].

Table 1. Primers and restriction enzymes used for PCR-RFLP.

SNP	Primer sequence	PCR product	Restriction enzymes	Digest products
IL-6 (-174G/C)	F: 5'-GGAGTCACACACTCCACCT-3' R: 5'-CTGATTGGAAACCTTATTAAG-3'	525 pb	Hin1II	**GG: 327 + 169 pb** **CC: 327 + 122 + 47 pb** **GC: 327 + 169 + 122 + 47 pb**
IL-6 (-597G/A)			FokI	**GG: 525 pb** **AA: 468 + 57 pb** **GA: 525 + 468 + 57 pb**
IL-6R (+48867A/C)	F: 5'AAGGTTCCTTTGAGGCTTTT-3' R: 5'-CCATAAATTTCAGAATGGGC-3'	290 pb	HinfI	**CC: 290 pb** **AA: 172 + 118 pb** **AC: 290 + 172 + 118 pb**

2.5. Statistical Analysis

The data analyses were performed using R software version 4.2.1 and the online software SNPStats (https://www.snpstats.net/start.htm) [25]. Results are expressed as mean ± SD for numerical variables or as percentages for categorical variables. Deviation from Hardy–Weinberg equilibrium (HWE) of each studied SNP was assessed using the χ^2 goodness-of-fit test. The association between different genotypes and alleles with MetS was assessed using rapport between exposure odds in cases and exposure odds in controls (odds ratio: OR) with 95% confidence interval (CI). Linkage disequilibrium (LD) analysis between the three SNPs was verified. Logistic regression model was used for the haplotype analysis association. The value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Genotype and Allele Frequencies of the IL-6 (-174G/C, -597G/A) and IL-6R (+48867A/C) SNP

The distribution of genotype, allele frequencies and Hardy-Weinberg equilibrium (HWE) of each single-nucleotide polymorphism are presented in **Table 2**. Results show no significant differences in genotype and allele distribution frequencies for IL-6 (-174G/C and -597G/A) gene polymorphisms among patients with MetS compared to those without MetS. The IL-6 (-174G/C) homozygous genotype CC was used as a reference to estimate the risk for dominant (GG vs CC+GC), and recessive (CC vs GG+GC) models respectively. Likewise, the IL-6 (-597G/A) homozygous genotype AA was used as a reference to estimate the risk for dominant (GG vs AA+GA), and recessive (AA vs GG+GA) models, respectively. No significant association between each of the IL-6 (-174G/C or -597G/A) genotypes and MetS was observed. IL-6R (+48867A/C) homozygous genotype AA was used as a reference to estimate the risk for dominant (CC vs AA + AC), and recessive (AA vs CC + AC) models. In this case, there was a significant association of the IL-6R (+48867A/C) heterozygous genotype AC with MetS (OR: 1.75, 95% CI: 1.02 - 2.99, $p = 0.0397$). There was also a significant association of the dominant model AA vs AC + CC) of IL-6R (+48867A/C) with MetS (OR: 1.67, CI: 1.46 - 2.79, $p = 0.049$).

Considering the reported gender differences in MetS settlement and/or outcomes and in order to tackle the possible influence of sex type on MetS susceptibility according to genotype, we also ran an intra-sex analysis (**Table 3**) for SNPs distribution. The results confirmed and showed an even stronger IL-6R (+48867A/C) SNP association with MetS in females (OR: 2.07, CI: 1.05; 4.05). Surprisingly, the IL-6 (-174G/C) heterozygous genotype GC appeared to be significantly associated with MetS only in males (OR: 0.38, CI: 0.16; 0.91). These results could actually account for the above-mentioned initial reports. **Figure 1** represents the PCR-RFLP analysis of the IL-6 (-174G/C) using the Hin1II restriction enzyme (B) and the IL-6R (+48867A/C) using the HinfI restriction enzyme (C) respectively.

Table 2. Distribution of genotype and allele frequencies of IL-6 and IL-6R.

Polymorphisms	Genetic models	with MetS	without MetS	p-value	OR [95% CI]
		N (%)	N (%)		
IL-6 (-174G/C)	CC	6 (6.45)	11 (4.87)	-	1.000
	GC	37 (39.78)	68 (30.09)	0.996	1.01 [0.32; 2.93]
	GG	50 (54.76)	147 (65.04)	0.372	1.62 [0.52; 4.54]
	GG vs CC + GC	43 (46.24)	79 (34.96)	0.059	0.63 [0.38; 1.03]
	CC vs GG + GC	87 (93.55)	215 (95.13)	0.566	0.74 [0.27; 2.07]
	C	49 (26.34)	90 (19.91)	-	1.000
	G	137 (73.66)	362 (80.09)	0.074	1.44 [0.96; 2.14]
IL-6 (-597G/A)	AA	7 (7.53)	17 (7.52)	-	1.000
	GA	32 (34.41)	65 (28.76)	0.719	0.85 [0.30; 2.20]
	GG	54 (58.06)	144 (63.72)	0.844	1.11 [0.40; 2.75]
	GG vs AA + GA	39 (41.93)	82 (36.28)	0.344	0.79 [0.48; 1.29]
	AA vs GA + GG	86 (92.74)	209 (92.48)	0.998	0.99 [0.40; 2.49]
	A	46 (24.73)	99 (21.90)	-	1.000
	G	140 (75.27)	353 (78.10)	0.438	1.17 [0.78; 1.74]
IL-6R (+48867A/C)	AA	35 (37.63)	60 (26.55)	-	1.000
	AC	45 (48.39)	135 (59.73)	0.039	1.75 [1.02; 2.99]
	CC	13 (13.98)	31 (13.72)	0.400	1.38 [0.65; 3.07]
	AA vs AC + CC	58 (62.36)	166 (76.45)	0.049	1.67 [1.46; 2.79]
	CC vs AA + AC	80 (86.02)	195 (86.28)	0.063	1.58 [0.97; 2.57]
	A	115 (61.82)	255 (56.41)	-	1.000
	C	71 (38.17)	197 (43.58)	0.208	1.25 [0.88; 1.78]

MetS: Metabolic syndrome; OR: Odds ratio; CI: Confidence interval.

Table 3. Distribution of genotype and allele frequencies of IL-6 and IL-6R according to sex.

Genotypes	Males			Females		
	MetS +	MetS -	OR [95% CI]	MetS +	MetS -	OR [95% CI]
IL-6 (-74G/C)						
GG	13 (36.1)	39 (60.9)	1.00	37 (64.9)	108 (66.7)	1.00
GC	20 (55.6)	23 (35.9)	0.38 [0.16; 0.91]	17 (29.8)	45 (27.8)	0.91 [0.46; 1.81]
CC	3 (8.3)	2 (3.2)	0.17 [0.03; 1.20]	3 (5.3)	9 (5.5)	0.82 [0.21; 3.25]
IL-6 (-97G/A)						
GG	19 (52.8)	38 (59.3)	1.00	35 (61.4)	106 (65.4)	1.00
GA	13 (36.1)	21 (46.6)	0.74 [0.30; 1.82]	19 (33.3)	44 (27.2)	0.75 [0.38; 1.46]
AA	4 (11.1)	5 (11.1)	0.61 [0.14; 2.63]	3 (5.3)	12 (7.4)	1.33 [0.34; 5.15]
IL-6R (+48867A/C)						
AA	10 (27.8)	17 (26.5)	1.00	25 (43.9)	43 (26.2)	1.00
AC	20 (55.5)	41 (64.1)	1.27 [0.49; 3.33]	25 (43.9)	94 (57.3)	2.07 [1.05; 4.05]
CC	6 (16.7)	6 (9.4)	0.54 [0.13; 2.19]	7 (12.2)	27 (16.5)	1.92 [0.71; 5.15]

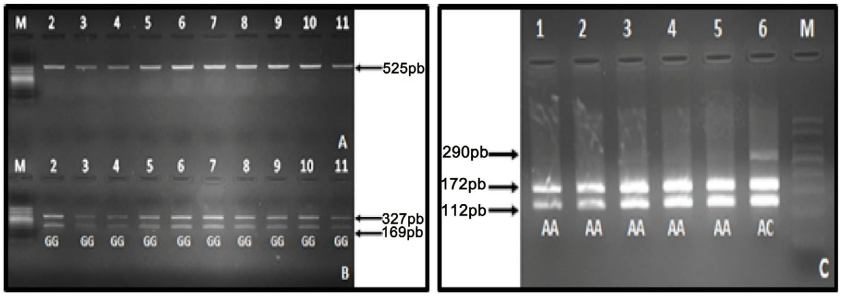

Figure 1. Genotyping result by PCR RFLP of IL6 and IL6R gene polymorphisms.

3.2. Linkage Disequilibrium Analysis

Multiple linkage disequilibrium analysis was performed among the investigated candidate SNP for IL-6 and IL-6R. D, D' and r statistics as well as *p*-values, were calculated (Table 4). Although not highly significant, SNP1 and 2 tend to show a slight linkage disequilibrium, being co-inherited in 7.35% of the cases with a *p*-value close to significance of 0.07. This could potentially indicate a functional co-evolution that deserves to be further tackled. SNP3 inheritance didn't show any linkage disequilibrium, although its LD score was implemented by the 10% inter-chromosomal correction factor [26].

Table 4. Linkage disequilibrium analysis among SNPs.

SNP1	SNP2	D	D'	r	*p*-value
−174G/C	−597G/A	0.0124	0.0735	0.0715	0.0708
−174G/C	+48867A/C	0.0101	0.0802	0.0497	0.2089
−597G/A	+48867A/C	−0.0012	0.0122	−0.0056	0.8872

3.3. Haplotypes Analysis

Frequency occurrence of 8 different haplotypes of the three candidate SNPs in patients with or without MetS was analyzed. As shown in Table 5, the association of one haplotype CAC (OR = 0.14, 95% CI: 0.02; 0.84, $p = 0.033$) was found to be significantly associated with MetS.

Table 5. Haplotypes association analysis with the MetS.

Haplotypes	Frequency (%)	OR [95% CI]	*p*-value
GGA	0.3591	1.00	-
GGC	0.2582	1.52 [0.81; 2.85]	0.19
GAA	0.1037	1.08 [0.49; 2.39]	0.85
CGA	0.0883	0.58 [0.27; 1.25]	0.17
CGC	0.067	2.66 [0.75; 9.42]	0.13
GAC	0.0611	1.80 [0.54; 6.00]	0.34
CAC	0.0337	0.14 [0.02; 0.84]	0.033
CAA	0.0288	2.62 [0.47; 14.60]	0.27

OR: Odds ratio.

3.4. Serum hs-CRP and IL-6 Levels According to -174G/C, -597G/A and +48867A/C Genotypes

The distribution of serum hs-CRP and IL-6 levels according to genotype polymorphisms (IL-6 (-174G/C, -597G/A) and IL-6R (+48867A/C)) in patients with or without MetS is shown in Table 6. Results indicate that both hs-CRP and IL-6 levels differed between the GG, GC and CC genotypes of -174G/C polymorphism. Likewise, hs-CRP and IL-6 levels differed between GG, GA and AA genotypes of -597G/A polymorphism. Although both hs-CRP and IL-6 levels differed between IL-6R (+48867A/C) AA, AC and CC genotypes, no significance was observed.

Table 6. Genotypes association of -174G/C, -597G/A and +48867A/C with serum CRP and IL6.

	Genotypes	hs-CRP (mg/L) (Mean ± SD)	IL-6 (pg/mL) (Mean ± SD)
	IL-6 (-174G/C)		
with MetS	GG	7.57 ± 5.1	22.6 ± 3.94
	GC	7.1 ± 6.04	22.5 ± 4.31
	CC	4.66 ± 3.36	22.8 ± 5.69
without MetS	GG	7.1 ± 4.42	21.8 ± 3.81
	GC	6.44 ± 3.72	22.6 ± 3.79
	CC	6.27 ± 2.68	21.7 ± 3.43
***p*-value**		0.307	0.419
	IL-6 (-597G/A)		
with MetS	GG	7.85 ± 5.8	22.7 ± 4.0
	GA	6.48 ± 5.04	22.4 ± 4.38
	AA	5.47 ± 3.19	22.6 ± 4.92
without MetS	GG	7.11 ± 4.21	22.1 ± 3.46
	GA	6.28 ± 4.15	22.0 ± 4.38
	AA	7.01 ± 3.61	21.5 ± 4.26
***p*-value**		0.212	0.876
	IL-6R (+48867A/C)		
with MetS	AA	8.0 ± 5.44	22.9 ± 4.46
	AC	7.09 ± 5.19	22.7 ± 3.91
	CC	5.41 ± 4.03	21.0 ± 4.1
without MetS	AA	6.32 ± 4.68	21.9 ± 4.35
	AC	7.39 ± 4.55	22.0 ± 3.74
	CC	5.62 ± 2.54	22.0 ± 2.86
***p*-value**		0.0706	0.714

hs-CRP: high sensitivity C-reactive protein, IL-6: interleukin-6, SD: standard deviation.

4. Discussion

Chronic inflammation promotes the pathogenesis of several diseases and disorders including type 2 diabetes, coronary heart disease, cancer and metabolic syndrome [27]. Therefore, the identification of genetic factors underlying inter-individual variation in circulating levels of inflammatory biomarkers may allow better prediction of individual disease risk and prognosis. We have recently shown that circulating hs-CRP and IL-6 levels in adult Congolese subjects with MetS are significantly higher than in those without MetS in an age-dependent manner. In the same study, an ROC curve analysis allowed us to show that combined serum hs-CRP and IL-6 dosages delivered improved discriminatory power to predict MetS [22]. In the present study, we investigated whether IL-6 (-174G/C and -597G/A) or its receptor IL-6R (+48867A/C) gene polymorphisms could predict or explain chronic inflammation susceptibility in MetS. In the codominant model, no association was found between the -174G/C or -597G/A polymorphisms in the IL-6 promotor gene and MetS risk in our Congolese cohort. Similarly, Suazo *et al.* (2014) found no significant association between genetic polymorphisms of IL-6 and MetS in obese Chilean children [28]. Contrary to our recessive model (CC vs GG+GC) results for IL-6 -174G/C polymorphism, the Zafar *et al.* (2019) study in Pakistani population showed that CC homozygous genotype was associated with increased risk of MetS (OR = 3.32, CI: 1.15 - 4.71; $p = 0.016$) [12] and our results showed a significant GC genotype enrichment in males with MetS. However, these contradictory results with the Pakistani one are conflicting with another study by Maintinguer et *al.* (2018) in the Brazilian population that established a relationship between CC homozygous genotype of -174G/C polymorphism and MetS risk as dominant model (CC vs GG+GC, OR = 1.88, CI: 1.08 - 3.26) [29]. These inconsistencies might be explained by the variations in allelic frequencies among different ethnic groups, thus pinpointing the interest in performing such experiments on different ethnic groups. Moreover, the present study did not notice any association between GG homozygous genotype of -597G/A polymorphism and MetS risk under the dominant model (GG vs GA + AA) [30]. Our results also showed a statistically significant association between the heterozygous AC genotype of the +48867A/C IL-6R polymorphism and MetS risk under codominant model, which is not in accordance with other studies [23] [27]. This observation was even stronger in females. We found that the homozygous AA genotype of +48867A/C polymorphism was significantly associated with MetS risk under the dominant model (OR = 1.67, CI: 1.46 - 2.79). Moreover, the respective relationships between serum hs-CRP or IL-6 levels and genotypes showed no statistical significance in our study as well as for any potential co-distribution of the various stutied SNPs. Although a slight trend of both IL-6 SNPs to co-distribution was suspected, we did not manage to show any strict sign of mechanistic coevolution in between these markers. Several limitations to our study need to be addressed. First, the lack of circulating levels of soluble interleukin-6 (sIL-6) dosage did not allow us to investigate its association with the three candidate polymorphisms in the studied

population. Second, our moderate sample size and its cross-sectional design need to be scaled up. Third and above all, since we previously showed the combined dosage of both IL-6 and hs-CRP improved MetS diagnosis accuracy, testing the association of cytokines cascade SNPs with this above-mentioned combined dosage inside genders deserves to be further achieved. To our knowledge, our study is the first one to assess the genetic susceptibility to chronic inflammation in MetS through an IL-6 and IL-6R genes polymorphism survey in a black African population. Our findings unravel a possible association between IL-6R (+48867A/C) polymorphism and, more interestingly, the IL-6 (-174G/C and -597G/A) and IL-6R (+48867A/C) CAC haplotype with MetS in adult Congolese population highlighting their contribution in genetic susceptibility of this medical condition possibly alongside with others factors. More specifically, our study unravels sex differences in the inflammatory response to IL-6 and its receptor gene polymorphism. Sex differences in MetS settlement and/or outcomes have been documented [31] but scarce studies have tackled their molecular mechanisms. For instance, studies have shown increased and/or accelerated expression levels of IL-6 and its receptor in females compared to males following inflammatory stimuli depending on sexual hormones [32] [33]. Very interestingly, our study partially fits with the reported increase in IL-6 levels in the IL-6 G > C, rs 1800795 polymorphism [34] but it is the first time that these three polymorphisms are considered together in MetS and, above all, in a sex-dependant manner. Our work thus introduces the potential role of IL-6/IL-6R SNPs in combination with sex-dependent factors that could impact MetS development, detection, and healthcare management.

Authors' Contribution

All authors have contributed to the achievement of this work and to the drafting of the manuscript.

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Conflicts of Interest

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

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