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The ACE 2 G8790A and IL-22 Gene Polymorphisms and their Association with Susceptibility to COVID-19 in Yaounde, Cameroon

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Abstract: Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) is an enveloped positive-stranded RNA virus that replicates in the cytoplasm, and uses envelope spike projections as a key to enter to human cells with the receptor. Host susceptibility upon exposure to the virus might be as a result of genetic polymorphisms observed on genes that encode for molecules in the immune system such as ACE 2, as well as IL-22 which influences the immune response. This study was aimed at investigating the association between the Single Nucleotide Polymorphisms (SNPs) of ACE2 G8970A and IL-22 (rs1179521) with COVID-19 susceptibility in Yaounde, Cameroon. A case-control study was performed on 331 conveniently collected blood samples, spotted on Whartman N° 3-filter paper from which DNA was extracted by the chelex-100 DNA extraction method. Genotyping of the ACE2 G8970A and IL-22 SNPs were performed using Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP). The Chi-square test (X^2) was used to establish associations. A P-value of <0.05 was considered significant. The most predominant genotype for ACE2 G8790A, was the homozygous wild type GG genotype (75.23%, 249/331) with no homozygous mutant (AA genotype) observed amongst the study participants, whereas for IL-22 rs1179251, it was the heterozygous GC (47.43%, 157/331). The mutant C allele for IL-22 rs1179251 was most predominant (58%). In the same manner, the wild type G allele for ACE 2 was predominant (88%). No statistical significance was found in the gene and genotype frequencies of ACE2 G8790A and IL-22 rs1179251 between the COVID-19 infected group and healthy controls. The GG genotype for the IL-22 rs1179251 was a protective factor against the presentation of clinical features of COVID-19 (OR=0.430; P=0.003) whereas, the C allele was a risk factor (OR=2.324; P=0.003). In conclusion, no association was

found between the SNPs of ACE2 G8790A and IL-22 rs1179251, and COVID-19, but an association was found between the SNP of IL-22 rs1179251 and COVID-19 clinical features. The combined SNPs of ACE2 G86790A and IL-22 rs1179251, showed statistical significance between the symptomatic and asymptomatic groups when the wildtype allele (G) for ACE2 G8790A the mutant allele (C) for IL-22 rs1179251 were combined amongst study participants, with participants possessing the resultant genotype (GC), 2 times likely to present clinical features of COVID-19 (GC; OR=2.324, P=0.003).

Keywords: ACE2, IL-22, COVID-19, Susceptibility, Gene Polymorphism, Immune Response

1. Introduction

The Severe Acute Respiratory Syndrome 2 (SARS-CoV-2) responsible for the COVID-19 pandemic is a new coronavirus of the subgenus Sarbecovirus [1] and the third zoonotic disease by coronavirus to affect humans, following Severe Acute Respiratory Syndrome (SARS) and Middle-East Respiratory Syndrome (MERS) [2] and spreads faster than its two ancestors, but has lower fatality. SARS-CoV-2 is an enveloped positive-stranded RNA virus that replicates in the cytoplasm, and uses envelope spike projections as a key to enter human airway cells with the receptor [3]. A total of 16 298 556 confirmed cases had been reported globally [4]. In the WHO African region, the number of coronavirus disease 2019 (COVID-19) cases has continued to decrease in, a total of 34564 new confirmed COVID-19 cases and 1173 new deaths were reported from 45 countries between 2 and 8 September 2020 [4]. In Cameroon, as of the 25 June 2020, there have been over 12,825 confirmed COVID-19 cases, with 7,774 recoveries and 331 deaths (fatality rate: 2.6%). Cases have been reported in all ten regions of the country though the majority remain in Central and Littoral regions [5]. Despite all the barrier and diagnostic measures put in place, the novel COVID-19 is still a major public health burden in the world with the morbidity and mortality of global community due to this disease dramatically increasing from time to time and the spread and severity of the virus has been dramatically increasing across the world [6].

Coronaviruses (along with viral forms of pneumonia), tend to see reduced ACE2 expression and upregulation of angiotensin II. However, SARS-CoV-2 reduces ACE2 expression, thus leading to lung injury by a cascade of inflammation [7]. The Renin-Angiotensin-Aldosterone System (RAAS) seems to play an important role in the pathogenesis of COVID-19 [7, 8]. Recently, SARS-CoV-2 was reported to be a human angiotensin I converting enzyme 2 (ACE2)-tropic virus [9] able to bind the alveolar pneumocytes which express ACE2 at their surface. ACE2 hydrolyzes Ang-II into Ang-1–7. Ang-II binds to the AT1-receptor driving vasoconstriction, fibrosis, inflammation, thrombosis, among other responses; while Ang-1–7 binds to the AT2-receptor with increased vasodilation and reduced fibrosis, inflammation, and thrombosis [10]. Therefore, it is not surprising that initials reports suggested that hypertension, diabetes and cardiovascular diseases were the most frequent comorbidity in COVID-19 disease [11]. High levels of Ang II leads to cytokine induced organ failure with the increase production of IL-22 by Th 22 and other inflammatory cytokines [12] that increases inflammatory activities. Interleukin-22 was first described in 2000 by Dumoutier *et al* [13], it is a member of the IL-10 family and is produced by special immune cell populations, including specific T and NK cells, and lymphoid tissue inducer cells [13, 14]. IL-22 is a cytokine produced by immune cells and is important for the regulation of tissue cell responses during inflammation and infection. Results from studies shows a significant association between ACE 2 polymorphic variants and SARS CoV-2 host susceptibility [15] with the ACE 2 G8790A (rs2285666) polymorphism, having a strong association with the development of hypertension, kidney disease and diabetes mellitus [16]. In the same manner, SNPs loci of IL-22 (rs2227484, rs2227491, rs2227485, rs1179251) has been shown to play a part in host resistance to viral infections (HIV) and hypertension and other inflammatory diseases [13, 17–19]. However, few studies have been done on the association of the SNPs in the ACE 2 and IL-22 with COVID-19 host susceptibility. Thus, this study was aimed at investigating the SNPs of G8790A (rs2285666) and (rs1179251) within the ACE 2 and IL-22 gene respectively, and their association with COVID-19 susceptibility in Yaounde, Cameroon, for better diagnosis, management and treatment of the disease.

2. Materials and Methods

2.1. Study Setting and Location

The study was carried out in Yaoundé, the capital of the Centre Region in Cameroon (3°51′ N 11°29′ E) the second largest city of Cameroon with a population of more than 4 million. It has a surface area of about 180 km² and it lies in the Centre Region of the nation at an elevation of about 800 meters above sea level and surrounded by many hills with an average annual rainfall of 1628.3mm per year. It contains people from all works of life and people from all other 10 regions of the country. The city is located within the Congo-Guinean phytogeographic zone characterized by a typical equatorial climate with two rainy seasons extending from March to June and from September to November [20].

2.2. Study Population and Sampling

A total of 331 participants were recruited in this study, including 157 COVID–19 infected patients and 174 healthy individuals as a case–control study after obtaining their informed consent. Inclusion criteria were: positive for COVID–19 after diagnosis for case group and a negative COVID–19 test for the control group, with individuals in both

groups exposed to the virus. Nasopharyngeal swabs were collected from study participants and a COVID–19 Antigen Test (RDT) (LOT N°: QCO3021003C/Sub: C–2) was carried out to confirm diagnosis. Positive results were later confirmed by a returning Real Time Polymerase Chain Reaction (qPCR) test results, carried out at the National Public Health Laboratory in Yaounde, Cameroon. Venus blood samples were also collected from study participants, from which dried blood spots were made using the Whartman N° 3-filter paper, air dried, labelled, sealed in a sterile individual zip lock bags and stored at room temperature for further Molecular analysis.

2.3. DNA Extraction

Human genomic DNA was extracted from study participants using the Chelex–100 method as previously described [21]. With the aid of sterile scissors, the blood spot on the filter paper was excised and placed into a 1.5ml eppendorf tube containing 1ml 0.5% saponin and incubated overnight at 4°C. The spot was then rinsed the next day with 1X PBS for 15–30 minutes at 4°C and then the introduced into 50µl of hot 20% Chelex and 150µl of DNase free water at 100°C in a heating block. The mixture was then vortexed 3 times during the incubation period for 15–20 seconds and later centrifuged at 10000 rpm for 2 minutes and supernatant stored in a sterile 1.5ml eppendorf tube and stored at –20°C.

2.4. Genotyping of ACE2 G8790A and IL–22 Polymorphisms

Genotyping of the ACE2 and IL–22 genes was done using the Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR–RFLP) technique, adapted from that of the research [16, 22] respectively. The primers used for the amplification of ACE2G8790Awas as follows: Forward (ACE2–F): 5'–CATGTGGTCAAAAGGATATCT–3' and Reverse (ACE2R): 5'–AAAGTA AGGTTGGCAGACAT–3'. A total reaction mixture of 20µl, consisting of 7 µl of nuclease free water, 10 µl of One Taq® Hot Start 2X Master Mix with standard buffer (New England Biolabs, MA, USA.), 0.5 µl of each primer (0.4µM) and 2µl of DNA template made up the master mix. Amplification of target gene was performed using a T3 Thermocycler (Biometra, UK. The PCR protocol was as follows: pre-denaturation (95°C for 5mins), denaturation (94°C for 30secs), annealing (50.6°C for 30secs), elongation (72°C for 45secs), amplified for 34 cycles and a final elongation of 72°C for 7mins to terminate all reactions. The amplicons after amplification if not immediately used was stored at 4°C. Digestion of PCR product with AluI restriction enzyme, was performed as previously described [16]. Products were run on a 2% agarose stained with Ethidium bromide and visualized under UV light with the undigested being the wild type producing bands at 485bp (Figure 1).

To amplify the IL–22 gene (rs1179251), the primers used was as follows: Forward (IL–22–251F): 5'–CAGAAATTAGCCCTATATGC–3'and Reverse (IL–22–251R): 5'–GAAAAAGGTAGGTAGGACTGATAAC–3'. A total reaction mixture of 20µl, consisting of 6 µl of nuclease free water, 10µl of One Taq® Hot Start 2X Master Mix with standard buffer (New England Biolabs, MA, USA.), 1 µl of each primer (0.5µM) and 2 µl of DNA template made up the master mix. Amplification of target gene was performed using a T3 Thermocycler (Biometra, UK). The PCR protocol was as follows: pre-denaturation (95°C for 5mins), followed by 35cycles of denaturation (95°C for 30secs), annealing (60°C for 30secs), elongation (72°C for 45secs) and a final elongation for 72°C for 7mins to terminate all reactions. The amplicons after amplification if not immediately used was stored at 4°C. Digestion of PCR product with AlwNI restriction enzyme, was performed as previously described [22]. Products were run on a 2% agarose stained with Ethidium bromide and visualized under UV light with the undigested being the wild type producing bands at 560bp (Figure 2).

2.5. Statistical Analysis

Data were analyzed using the IBM SPSS biostatistics version 20.0 software (SPSS, Chicago, IL). Chi Square test (X^2 test) was used to establish associations between variables. Unadjusted Odds Ratios (ORs) were calculated with 95% Confidence Intervals (CI). Variable differences with P–values less than 0.05 were considered as statistically significant.

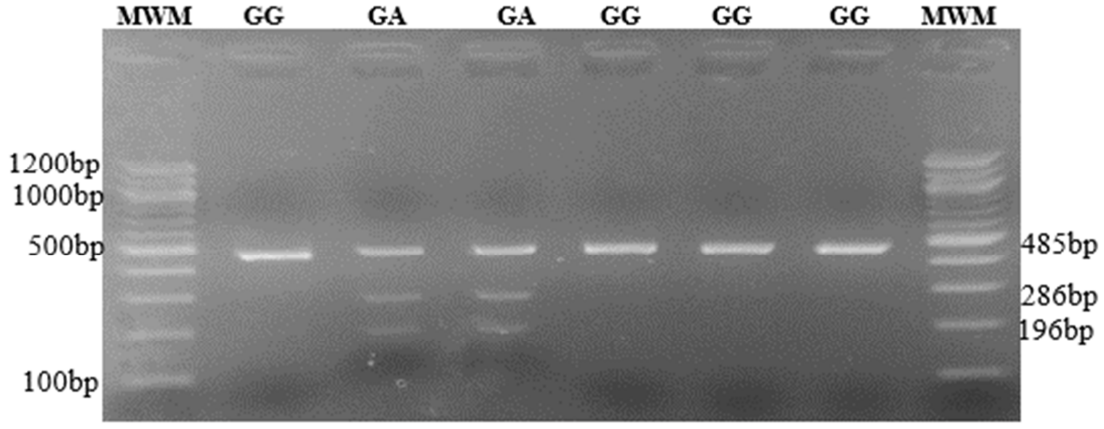

Figure 1. *Electrophoresis of ACE2 gene G8790A polymorphisms with AluI enzyme. GG; homozygote wildtype, GA; homozygote and with no homozygote mutant AA. Both ends of the gel picture is the DNA size markers (MWM) 100bp.*

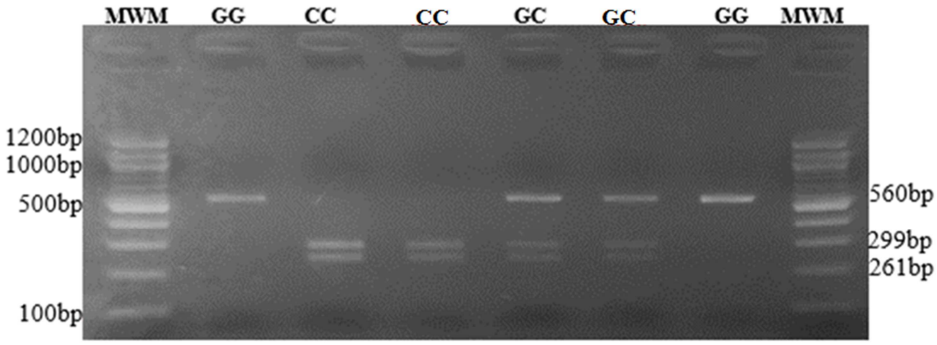

Figure 2. *Digestion patterns of IL–22 gene SNP rs1179251 with AlwNI enzyme. GG; homozygote wildtype, CC; homozygote mutant and GC; heterozygote. Both ends of the gel picture is the DNA size markers (MWM) 100bp.*

3. Results

3.1. Distribution of Demographic Characteristics of the Study Population

Out of the 331 participants enrolled in this study, 195 (58.91%) were women and 136 (41.09%) were men. In the COVID–19 infected group, 64 were male (40.76%), and 93 were female (59.24%), on the other hand, in the control group 72 individuals were male (41.38%), and 102 were female (58.62%). Again, based on the clinical features of the disease, in the symptomatic group, 65 were male (35.33%), and 119 were female (64.67%), and on the other hand, in the asymptomatic group, 71 individuals were male (48.30%) and 76 were female (51.70%). The mean age was 35.45±14.97 years, that of the patients was 34.64 years, and controls were 36.19 years (Table 1).

3.2. Genotype and Allele Frequency of the ACE2 G8790A and IL–22 rs1179251 Gene Polymorphisms

Results from genotyping were as follows: the ACE2 G8790A single nucleotide polymorphism revealed the distribution of GG (120, 76.43%), and GA (37, 3.57%) in the COVID–19 infected group and GG (129, 74.14%), and GA (45, 25.86%) in the controls group. No AA genotype was observed amongst the study participants in both groups (Table 2). The IL–22 rs1179251 single nucleotide polymorphism revealed the distribution of GG (*25*,15.92%), GC (78, 49.68%), and CC (54, 34.40%) in the COVID–19 infected group and GG (37, 21.27%), GC (79, 45.40%), and CC (58, 33.33%) in the controls group (Table 3).

Table 1. *Demographic Distribution of Study Population.*

Variables		Population N=331	COVID-19 Positive N=157	Controls N=174	Symptomatic N=184	Asymptomatic N=147
Age (Mean±SD)		35.45±14.97	34.64±15.17	36.19±14.79	36.15±15.12	34.59±14.80
Gender	Male	136 (41.09%)	64 (40.76%)	72 (41.38%)	65 (35.33%)	71 (48.30%)
	Female	195 (58.91%)	93 (59.24%)	102 (58.62%)	119 (64.67%)	76 (51.70%)

SD= Standard Deviation

Table 2. *Association between ACE2 G8790A gene polymorphism and susceptibility to COVID-19.*

ACE2 G8790A SNP	COVID-19 Positive N=157	Controls N=174	OR	95%CI	P-Value
Genotypes					
GG	120 (76.43%)	129 (74.14%)	1.131	0.686-1.867	0.629
GA	37 (23.57%)	45 (25.86%)	0.884	0.536-1.459	0.629
Alleles					
G	277 (88.22%)	303 (87.07%)	1.000	-	-
A	37 (11.78%)	45 (12.93%)	0.884	0.536-1.459	0.629

SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, CI= confidence interval, P- value=statistical significance level

Table 3. *Association between rs1117251 polymorphism of IL-22 gene and susceptibility to COVID-19.*

IL-22 (rs1179251) SNP	COVID-19 Positive N=157	Controls N=174	OR	95%CI	P-Value
Genotypes					
GG	25 (15.92%)	37 (21.27%)	0.701	0.400-1.229	0.214
GC	78 (49.68%)	79 (45.40%)	1.187	0.770-1.829	0.436
CC	54 (34.40%)	58 (33.33%)	1.048	0.665-1.654	0.838
Alleles					
G	128 (40.76%)	153 (43.97%)	0.954	0.605-1.504	0.838
C	186 (59.24%)	195 (56.03%)	1.426	0.814-2.499	0.214

SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, CI= confidence interval, P- value=statistical significance level

Table 4. *Association between ACE2 G8790A gene polymorphism and Clinical features of COVID-19.*

ACE2 G8790A SNP	Symptomatic N=184	Asymptomatic N=147	OR	95%CI	P-Value
Genotype					
GG	143 (77.72%)	106 (72.11%)	1.349	0.818-2.225	0.240
GA	41 (22.28%)	41 (27.89%)	0.741	0.449-1.223	0.240
Allele					
G	327 (88.86%)	253 (86.05%)	1.000	-	-
A	41 (11.14%)	41 (13.95%)	0.741	0.449-1.223	0.240

SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, CI= confidence interval, P- value=statistical significance level

Table 5. *Association between rs1117251 polymorphism of IL-22 gene and Clinical features of COVID-19.*

IL-22 (rs1179251) SNP	Symptomatic N=184	Asymptomatic N=147	OR	95%CI	P-Value
Genotype					
GG	24 (13.04%)	38 (25.85%)	0.430	0.244-0.758	0.003*
GC	94 (51.09%)	63 (42.86%)	1.393	0.900-2.154	0.136
CC	66 (35.87%)	46 (31.29%)	1.228	0.775-1.947	0.382
Allele					
G	142 (38.59%)	139 (47.28%)	0.814	0.514-1.291	0.382
C	226 (61.41%)	155 (52.72%)	2.324	1.320-4.094	0.003*

SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, CI= confidence interval, P- value=statistical significance level

From the genotyping results of this study, no statistical significance was found between the COVID-19 infected and the healthy controls for SNPs of ACE2 G8790A and IL-22 rs1179251 (Tables 2 and 3). Statistical significance was observed in the gene and genotype frequencies for the SNP of IL-22 rs1179251 and clinical features of COVID-19 between the symptomatic and asymptomatic groups (Table 5).

Table 6. *Combined Single Nucleotide Polymorphisms of ACE2 G8790A and IL-22 rs1179251, and association with COVID-19.*

SNPs	Alleles	Genotype	COVID-19	Controls	OR	95% CI	P-Value
ACE 2 G8790A + IL-22 rs1179251	G*+G	GG	103	116	0.954	0.605-1.504	0.838
	G*+C	GC	132	137	1.426	0.814-2.499	0.214
	A+G	AG	33	35	1.057	0.620-1.802	0.839
	A+C	AC	19	28	0.718	0.383-1.344	0.299

SNPs= Single Nucleotide Polymorphisms, G*= Wild type allele ACE G8790A, OR= Odds Ratio, CI= confidence interval, P- value=statistical significance level

Table 7. *Combined Single Nucleotide Polymorphisms of ACE2 G8790A and IL-22 rs1179251, and association with clinical features of COVID-19.*

SNPs	Alleles	Genotype	Symptomatic	Asymptomatic	OR	95% CI	P-Value
ACE 2 G8790A + IL-22 rs1179251	G*+G	GG	118	101	0.814	0.514-1.291	0.382
	G*+C	GC	160	109	2.324	1.320-4.094	0.003*
	A+G	AG	37	31	0.942	0.551-1.609	0.826
	A+C	AC	22	25	0.663	0.357-1.231	0.191

SNPs= Single Nucleotide Polymorphisms, G*= Wild type allele ACE G8790A, OR= Odds Ratio, CI= confidence interval, P- value=statistical significance level

A statistical significance between the symptomatic and asymptomatic groups was observed, when the wildtype allele (G) for ACE2 G8790A and the mutant allele (C) for IL-22 rs1179251 were combined amongst study participants, and participants possessing the resultant genotype (GC) were 2 times likely to present with clinical features of COVID-19 (GC; OR=2.324, P=0.003) (Table 7).

4. Discussion

The aim of this study was to detect the genetic polymorphisms of ACE2 G8790A and IL-22 (rs1179251) and to establish a possible association with susceptibility to COVID-19 in Yaounde, Cameroon. The ACE2 receptor plays a crucial role in cellular invading of SARS-CoV-2 as well as various pathophysiological conditions of the COVID-19 [23]. The spike protein of the SARS-CoV-2 binds to ACE2 receptors and facilitates the internalization of the virus [24]. Findings from a study carried out by Mateo *et al.,* showed that there were 2 SNPs that were able to potentially affect the interaction of ACE2 with the SARS-CoV-2 spike [25]. A study carried out in 2017, showed that the ACE2 G8790A polymorphism in Type 2 Diabetes Mellitus patients was correlated with Cerebral Stroke, and that the A allele might be a risk factor of Type 2 Diabetes Mellitus combined with Cerebral Stroke [16].

Results from this study showed no association between the ACE2 G8790A SNP and COVID-19. The AA genotype was not found among the study participants. Findings from this study are contrary to that carried out in China by Wu *et al.,* in 2016, who found the ACE2 G8790A polymorphism in Type 2 Diabetes Mellitus to be correlated with cerebral stroke [16]. Little or no information is known about the possible association of this polymorphism and disease susceptibility in

Cameroon and other African countries, but a study carried out in Brazil in 2019 [26], showed no association between the ACE2 G8790A polymorphism and the risk of developing Severe Arterial Hypertension development [26]. The differences observed in the studies, can be attributed to environmental conditions, ethnicity, nutrition which can lead to mutations since our findings showed a low prevalence of the mutant A allele. Studies carried out on ACE 2 gene polymorphisms have brought conflicting results, and some of them have found absent or limited association for ACE2 [27, 28] polymorphisms, as in this study. It still remains possible that ACE2 gene polymorphisms, human ACE2 mRNA expression and human ACE2 protein polymorphisms influence SARS-CoV-2 susceptibility and COVID-19 outcome [29].

Elevated ACE2 expression may be compensatory in patients with cardiovascular disease and heart failure. Indeed, influenza and coronaviruses (along with viral forms of pneumonia), tend to see reduced ACE2 expression and upregulation of angiotensin II. However, SARS-CoV-2 reduces ACE2 expression, thus leading to lung injury by a cascade of inflammation [7]. Host genetic background is a crucial factor which regulates cytokine responses, and this maybe link with inflammation, viral clearance, or disease progression [22]. The association between single nucleotide polymorphisms and various clinical diseases such as Ulcerative colitis, gastric cancer, and autoimmune diseases or infectious diseases including HBV infection have been studied and proved in several different surveys [30, 31]. Recently, IL-22 has emerged as a potential factor in the chronic inflammatory diseases [18, 32] and studies have evaluated the association between IL-22 polymorphisms in the progression of chronic inflammatory diseases [13].

Results from this study, showed no association between the SNP of IL-22 rs1179251 and COVID-19. These findings are similar to that of a study carried out in 2014 by Quin *et al.,* on gastric cancer in China [30]. Marquet *et al.,* also found similar results with the IL-22 (rs1179251) and development of cerebral malaria amongst Nigeria and Malian children, but found a significant difference in rs1012356, rs2227476, and rs2227473 between children with cerebral malaria and healthy controls. They claimed that SNP rs2227473 is placed in a vital production regulator position of IL-22. Which also seems that Individuals with T allele of rs2227473 express higher levels of IL-22 than those without this allele [33]. The similarities observed might be due to the fact that individuals with the mutant C allele expresses low levels of IL-22 than individuals without that allele [22]. Ethnic differences may be a plausible explanation for the lack of an association between IL-22 (rs1179251) polymorphisms and COVID-19 as compared to other studies.

This study shows a statistically significant difference in the rs1179251 of the IL-22 gene with clinical features of COVID-19 between the symptomatic and asymptomatic groups. The C allele was found to be an increased risk (OR= 2.324; P=0.003) to COVID-19 clinical features. Results also shows that, individuals possessing the GG genotype for the IL-22 rs1179251 SNP, where at decreased risk to COVID-19 clinical features (OR=0.430; P=0.003). These results are contrary to findings from a study carried out in 2016 [19] who found no association with the SNPs of IL-22 (rs2227485, rs2272478, rs2227491). Combined SNP of the ACE2 G8790A and IL-22 rs1179251, showed statistical significance between the symptomatic and asymptomatic groups when the wildtype allele (G) for ACE2 G8790A and the mutant allele (C) for IL-22 rs1179251 were combined amongst study participants with participants possessing the resultant genotype (GC), at increased risk to present the clinical features of COVID-19 (GC; OR=2.324, P=0.003). Interestingly, the influence of some of the genetic factors may have a geographic distribution [19]. In other words, one specific SNP may predispose individuals in a given ethnic group, whereas in a different group, it may neither predispose to nor protect against COVID-19 clinical features.

5. Conclusion

This study showed no association between the ACE2 G8790A and IL-22 (rs1179251) gene polymorphisms with COVID-19. An association was found between the SNP of IL-22 rs1179251 and COVID-19 clinical features. An association was also found between the combined SNP of the ACE2 G8790A and IL-22 rs1179251 and COVID-19 clinical features. However, the limitation of the small sample size should be taken into consideration for lack of associations with COVID-19. Thus, a further study with larger sample size should be conducted to better conclude our findings.

Authors Contributions

Study design, Wilfred Fon Mbacham, Calvino Fomboh Tah, Akindeh Mbuh Nji, Jean Paul Kengne Chedjou Methodology, Wilfred Fon Mbacham, Calvino Fomboh Tah, Akindeh Mbuh Nji, Jean Paul Kengne Chedjou, Carine Nguefeu Nkenfou-Tchinda, Cyrille Mbanwi Mbu'u and Lesley Ngum Ngum. Sample collection, Calvino Fomboh Tah, Palmer Masumbe Netongo, MacDonald Bin Eric, Lesley Ngum Ngum, Carine Nguefeu Nkenfou-Tchinda, Tatiana Tchakote Wami, and Rhoda Bongshe Laban. Data analysis, Calvino Fomboh Tah, Akindeh Mbuh Nji, Jean Paul Kengne Chedjou and Olivier Wilfried Ngandjeu Tchamdjeu. Molecular analysis, Calvino Fomboh Tah., Rhoda Bongshe Laban., Tatiana Tchakote Wami., and Marie-Claire Vernyuy Fonyuy. Writing of original manuscript, Calvino Fomboh Tah, Jean Paul Kengne Chedjou. All authors contributed in the revision of the manuscript and approved the final version of the manuscript prior to submission.

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Conflicts of Interest

The authors declare no conflict of interest.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and was approved by the Cameroonian Regional Ethics Committee for Research in Human Health (CRECRHH) under the ethical clearance document number 2173/CRERSHC/2021 on the 21 July 2021.

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