International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 03 Issue 04 April 2023

Page No: 162-169

DOI: https://doi.org/10.47191/ijpbms/v3-i4-02, Impact Factor: 6.858

Polymorphisms in the CCR5 and CCR2 genes and their Frequencies in the Middle Eastern Population

Murtadha M-Hussein A-Kadhim

Department of Genetic engineering, College of Biotechnology, AL-Qasim Green University, Ministry of Higher Education and Scientific Research, Iraq.

ABSTRACT

Background: The expression of proteins, and consequently the course of HIV-1 illness, can be influenced by the polymorphisms that can be detected in the CCR5 gene's regulatory region. As a result of this major role, variants in this gene have been subjected to diverse pressures, which has led to differences in the frequency at which they occur among human populations. There is a strong correlation between polymorphisms in the CCR2V64I mutation and CCR5 gene. As a consequence of their long history as merchants who dominated large areas within and around the Indian Ocean, the people who currently live in the Middle East have a diversified genetic makeup.

Methods: In this particular piece of research, we investigated the CCR2-CCR5 haplotypes that are found in the Middle East and compared the genetic diversity patterns of these haplotypes to those found in other populations.

Results: A total of one hundred adults from the Middle East had blood samples taken from them, and their genomic DNA was analyzed to look for polymorphism locations in the CCR5 gene as well as the CCR2V64I mutation. The frequencies of CCR5-2554T was 49% and CCR5-2086G 46%, while the frequencies of CCR5-2459A and CCR5-2135C were 36%.

Conclusions: These alleles displayed a modest degree of heterozygosity, which is an indication that balancing selection was acting upon them. On the other side, the well-known allele CCR532 was far less prevalent than expected. Eleven different haplotypes were discovered, with four of them being particularly prevalent: HHC, HH, HHA, and HHF*2. (46%, 20%, 14% and 12%) respectively.

KEYWORDS: CCR5, CCR2 genes, Polymorphisms, Frequencies

ARTICLE DETAILS

Published On:
12 April 2023

Available on:
https://ijpbms.com/

1. INTRODUCTION

1.1 Polymorphism and Human Diseases

In genetics, polymorphism refers to the variation of a single DNA sequence into at least two distinct forms. Within a population, it is said that a gene is polymorphic if there is the presence of more than one allele at the locus of that gene. The variation that occurs at a single base pair is by far the most prevalent form of polymorphism [1]. The presence of two or more phenotypes that are distinguishable from one another within the same population of the same species is known as polymorphism. Occurs when the gene pool has two alleles and one allele is gradually displacing the other. A genetic variety known as a polymorphism is one that is found in at least one percent of a population (e.g., the human ABO blood groups, the human Rh factor, and the human major histocompatibility complex). The variation that occurs at a single base pair is by far the most prevalent form of polymorphism [2]. It is possible for there to be polymorphisms in genes in any part of the genome. The vast majority of polymorphisms are what are known as "silent," which means they do not affect the way a gene works or how it is expressed (3). There are obvious examples of polymorphism. For instance, in animals such as dogs, the E locus can have any one of five distinct alleles, which are referred to as E, Em, Eg, Eh, and e. These alleles are arranged alphabetically. Different permutations of these genes are responsible for the distinct coloration and patterns that may be observed in dog coats. The best example of human

polymorphism can be found in human blood types, the human Rh factor, and the human major histocompatibility complex [1,3]. This anomaly, which may either cause disease or be linked with it, may be caused by a polymorphic variant of a gene. This variation can result in altered gene expression or the production of an abnormal version of the protein. For example, a polymorphisms variant of the gene that codes for the enzyme CYP4A11, in which thymidine substitutes cytosine at nucleotide position 8590 of the gene, generates a CYP4A11 protein that replaces phenylalanine with serine at amino acid position 434 of the protein. An alternative form of the gene is called a CYP4A11 variant. [4,5] This mutant protein has a lower enzyme activity, meaning that it takes longer to convert arachidonic acid into the eicosanoid known as 20-hydroxyeicosatetraenoic acid, which regulates blood pressure. An elevated risk of hypertension, ischemic stroke, and coronary artery disease was found in persons who had this mutation in one or both of their CYP4A11 genes [4,5]. On the basis of the aforementioned scientific findings, we came to the conclusion that it was necessary to shed light on the nature of the connection between the phenomenon of genetic variation (polymorphism) and AIDS, a disease that poses a significant threat to the human race and is of critical importance to its survival.

1.2 Acquired Immuno-Deficiency Syndrome (AIDS)

HIV, or the human immunodeficiency virus, is a virus that weakens the body's immune system. If HIV is not treated, it can lead to AIDS, or acquired immunodeficiency syndrome (6).

1.2.1 HIV/AIDS Epidemiology

Inadequate information prevents us from drawing a clear picture of the HIV/AIDS pandemic at this time. To track the epidemic's progress at the national level, researchers must rely on the CDC's AIDS case reporting system, which is the sole source of its kind. Counting the number of AIDS cases is merely the clinical tip of the iceberg when it comes to the impacts that HIV infection can have, but data can be used to assess the frequency and incidence of a disease. Although HIV seroprevalence surveys are useful for characterizing the magnitude of the epidemic, they are not as good at tracking its development since they include people whose infection dates are unknown. When trying to understand how far along in its development the HIV epidemic currently is, incidence data is by far the most useful indicator. Nonetheless, these statistics have limited generalizability to other specialized communities or to the total U.S. population because HIV infection is not recorded in all states and most HIV studies do not contain representative samples. Information can also be gleaned from HIV monitoring data, but it is of little help in foreseeing the course of the epidemic. Some have claimed (e.g., Centers for Disease Control and Prevention, 1994a; Turner et al., 1989) that a more comprehensive epidemic monitoring system should include AIDS and HIV infection precursors to overcome this shortcoming. Information on potential transmission sites and the likelihood of further spread could be gleaned from improved behavioral epidemiology data on known risk behaviors (such as sexual activity and drug use, for example). In spite of these drawbacks, the most recent epidemiologic data offer some extremely helpful insights into the HIV/AIDS pandemic that is sweeping the United States. In this part, the aforementioned data are discussed, with a primary focus on the part played by injection drug users. In spite of this, the panel felt that it was extremely important to present the reader with a concise assessment of the existing knowledge of the underlying biological mechanisms that are involved in the transmission of the virus before they moved on to examine these facts. Understanding the nuances of these behaviors and processes is essential to gaining a proper grasp for the multifaceted nature of the problems at hand.

2. HUMAN IMMUNODEFICIENCY VIRUS (HIV) PATHOGENICITY

The CD4 receptor, which is ordinarily a receptor for major histocompatibility complex class II (MHC-II) molecules, and a specific region in the gp120 envelope glycoprotein work together to allow HIV-1 to bind to cells. This process is known as the attachment mechanism (figure 1). Despite its prominence as a receptor, CD4 alone is insufficient for HIV-1 entry into cells. One of the earliest signs that there might be other receptors was the failure of HIV-1 to multiply in animal cells modified to express human CD4 (7). This process has been better understood because to the discovery of chemokine receptors such as CCR2, CCR3, CXCR4 and CCR5, all of which are coreceptors for the entry of the HIV-1 virus (8).

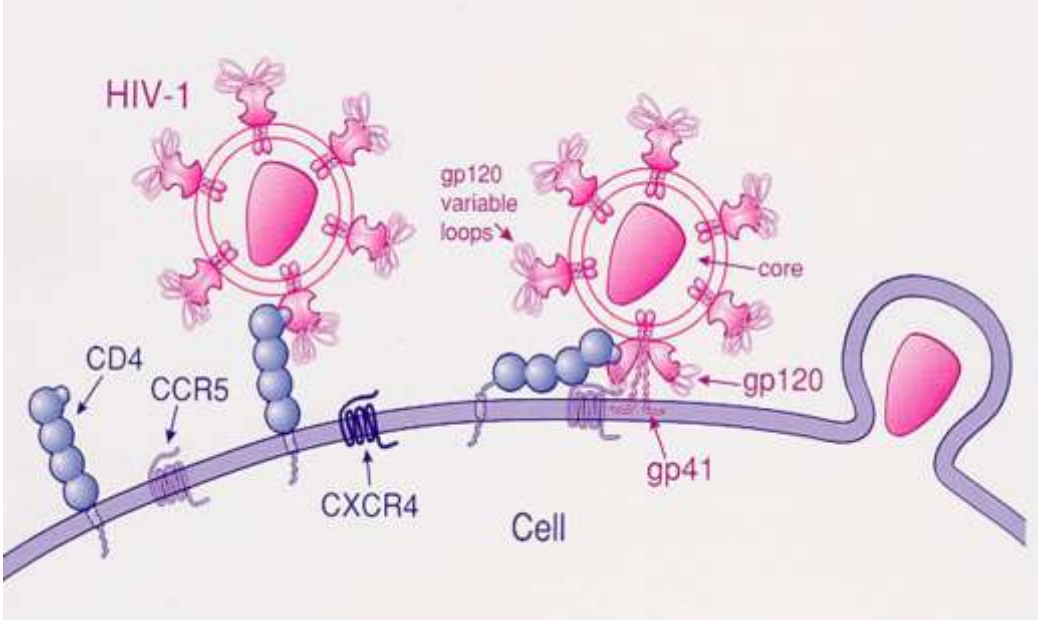


Figure 1. Infection by HIV begins with the virus binding to a CD4+ T-helper cell. Attachment of the viral gp120 protein to CD4 (1).

The second step is for the gp120 variable loop to bind to a co-receptor, like CCR5 or CXCR4. Thirdly, HIV is able to infiltrate the cell.

Physiologically speaking, chemokine receptors are responsible for directing T cells and phagocytes in the direction of sites of inflammation (9). When a ligand binds to a receptor, the receptors are able to transduce an intracellular signal, which ultimately leads to the fast mobilization of calcium within the cell. There are a total of eight known chemokine receptors, and each one of them is a G protein with its own unique ligand-binding pattern (9,10). The receptor CCR5, which also plays a significant role as a coreceptor for the HIV-1 virus that is macrophage-tropic. MCP-1, 2, 3, 4, and 5 are all different types of monocyte chemoattractant protein, and these are the ligands that bind to CCR2. CCR2 also acts as a modest co-receptor for HIV-1 entrance (11,12). In point of fact, several strains of HIV-1 have varied cell tropisms. Viruses that target T-cells make use of the CXCR4 co-receptor, whilst viruses that target selective macrophage monocytes make use of the CCR5 or CCR2 co-receptors (8,13). In macrophage-tropic isolates, the main infection is often caused by the strain that employs the CCR2 or CCR5 coreceptors expressed on mucosal dendritic cells. More cytopathic (SI) T-cell-tropic strains emerge during an infection and use the CXCR4 receptor; these strains cause rapid T-cell depletion and contribute to the development of AIDS. During the course of an infection, HIV-1 strains known as "dual tropic" develop; These strains may exist as transitional forms between macrophage-tropic and T-cell-tropic populations. This change in cell tropism, which involves adaptation by the virus and makes possible the use of a wider variety of coreceptors, may be a crucial stage in the path to AIDS (14). One such realization that resulted from the finding of coreceptors was the realization that some people are immune to HIV-1 infection, despite having had multiple sexual encounters with high-risk partners who were infected with the virus (15,16). It has been demonstrated that the CD4+ T-cells of these individuals have a high level of resistance in vitro to the infection caused by primary macrophagetropic viruses, but they are easily susceptible to infection by T-cell line-adapted viruses that have been converted (17). After this finding, a deletion of 32 base pairs, denoted by the symbol 32, was found in the CCR5 gene of some Caucasian individuals (18,19). This mutant allele causes the CCR5 co-receptor that is found on the surface of lymphoid cells to become truncated. This shortened allele, in its homozygous state, is associated epidemiologically with the ability of certain persons who have been highly exposed to HIV-1 to avoid being infected. It has also been observed that a correlation exists between CCR5 32 and a slower course of the disease (20). It has been estimated that different ethnic groups each have a different frequency of the allele CCR5 32. (20). In Caucasians, around 15% of individuals have the heterozygote genotype, while only 1% have the homozygote genotype. On the other hand, the allele that was eliminated is extremely uncommon in people of African, Asian, and Hispanic heritage (20). The CCR2 gene has polymorphisms, one of which is a point mutation that leads to a change in the transmembrane protein at position 64 from valine to isoleucine and is denoted by the notation CCR264I. CCR2B 64I polymorphisms were reported to have a link with the course of disease in one big investigation (20), but these results were not replicated in a second study (21). Despite this, there is no evidence that CCR2B 64I polymorphisms offer any protection against HIV-1 infection (20,21) Interracial marriage is very popular in Brazil, which contributes to the nation's perception of itself as an ethnically diverse country. It's possible for a Brazilian to have European, African, Asian, or even indigenous American origin. We decided to look into the CCR5 and CCR2B polymorphisms

in this population after making the determination to do so. In this molecular epidemiology study, we used a design called a cross-sectional study to determine the prevalence of individuals who were either homozygous or heterozygous for the mutation coding for the 32-pair deletion in the CCR5 chemokine receptor gene (CCR5 32), as well as for a valine to isoleucine switch in the transmembrane domain I of CCR2B. We also determined the prevalence of individuals who had both mutations (CCR2 64I).

3. MATERIALS AND METHODS

Pathology sample, Outpatient clinics in several Middle Eastern countries provided 100 HIV1-positive samples. In addition, as a control group, we evaluated one hundred samples from females whose HIV-1 status was unknown. These samples were collected in a manner that was compliant with the ethical requirements established by the Ethics Committee of the World Health Organization in the Middle East. DNA from frozen blood can be extracted and purified using a QIAamp Blood Kit (manufactured by QIAGEN Inc. in Santa Clarita, California) was utilized, and the extraction procedure that was followed was that which was specified by the manufacturer.

3.1. Genotyping and Nucleotide Sequence Analysis

QIAmp DNA micro kits (Qiagen, Germany) used and following the methodology provided by the manufacturer, genomic DNA was isolated from blood samples taken from the participants' peripheries after being treated with ethylenediaminetetraacetic acid (EDTA) to prevent excessive blood clotting. Amplification of genomic DNA by polymerase chain reaction (PCR), followed by sequencing of the DNA, was carried out in order to cover the CCR5 polymorphic sites 2733, forward: GCTGACAATACTTGAGATTT, reverse: TCCAGGATCCCCCTCTAC, sites 2459, forward: CCCGTGAGCCCATAGTTAAAACTC, reverse: CCTTACTGTTGAAAAGCCCTGTGA, site2132, forward: ATGT GCAATGTGCAAATTATTCA is the reverse of TGAGACATCCGTTCCCC and CCR2 V64I. The forward notation is TTGTGGGCAACATGCTGG, while the reverse notation is GCAATGTGCAAATTATTCA. This numbering method is based on the identification of the first nucleotide of the CCR5 translational start point as 1 and the nucleotide upstream of that as 1. This concept was first explained by (23). In addition, each sample was analyzed to determine whether or not the CCR532 and CCR2V64I variations exist in the respective coding areas of these two genes.

3.2. PCR Amplification

Each PCR mixture of 50 l contained the following components: 5 l of blood sample; a 0.1 M concentration (each) of primers specific for CCR5 polymorphic sites 2733, 2459, and 2086 and CCR2 V64I; a 0.2 M concentration of HotStarTaq master mixture (Qiagen, Hilden, Germany), which consisted of 2.5 U of HotStarTaq DNA polymerase, After an initial activation step of 15 minutes at 95 ° C. to activate the Hot Star Taq DNA polymerase, the reaction continued with 30 cycles of denaturation at 94 ° C for one minute, annealing at 55 ° C. for one minute, and extension at 72 ° C. for one minute, followed by one cycle of 10 minutes at 72 ° C.. After amplification was complete, 25 l of the amplicon was mixed with 5 l of a gel loading buffer containing 50% glycerol and 0.8 mg of bromphenol blue per ml. The mixture was then electrophoresed for one hour at 150 V in 0.5 TBE (Tris-borate-EDTA) containing 0.05 mg of 1.5% pronarose D1 gel (SphaeroQ, Burgos, Spain) containing 1.5% pronarose D1 (positive and negative controls were included in each set of amplifications). To determine the relative size of the molecules, a 1000-base pair DNA ladder from Invitrogen was utilized.

3.3. Statistical analysis:

Utilizing the GenAlEx6.41 tool, the allele frequencies of each SNP in the research sample were estimated (24), which was then followed by a test to determine whether or not there was a significant departure from the Hardy-Weinberg equilibrium (HWE). A calculation was made of the sample group's heterozygosity, which is an estimate of the genetic variety present in the group. On the basis of the expectation-maximization (EM) approach, the Arlequin v.3.5 package (25) was applied in order to get an estimate of the linkage disequilibrium (LD) that exists between all of the various pairs of alleles found at the various loci. The chi-square test was used to evaluate the statistical significance of the linkage disequilibrium (LD) between pairs of SNPs. DnaSP version 5 was utilized for the construction of haplotypes (26). In order to re-construct the evolutionary relationships that are present between the CCR2-CCR5 haplotypes, the median joining (MJ) technique that is included in the Network 4.6 program was applied. These links were found to exist between the haplotypes (27). The unrooted neighbor-joining (NJ) tree was created with the POPTREE software by using the corrected pairwise FST distances from CCR2-CCR5 haplotype frequencies determined in 1000 bootstrap replicates to evaluate the bootstrap values. This allowed for an accurate evaluation of the bootstrap values (28).

4. RESULTS

4.1. Frequency Of Alleles at The CCR2 – CCR5 Gene Locus

A 100 adult samples from the Middle East region were successfully genotyped at four distinct variable locations, including one in the CCR2 gene and three in the CCR5 gene. The frequencies of alleles as well as the genotypes are presented in Table 2. The overall genotype pattern did not deviate noticeably from the ratios predicted by the Hardy-Weinberg theory ($p > 0.05$). Only one individual possessed the CCR532 gene. For the CCR264I allele, the minor allele frequency (MAF) was reported to be 10%. while the majority of carriers (15.5%) were found to be heterozygous. CCR5-2086G was the minor allele that occurred at a frequency of

43.5%, making it the most common variant. CCR5-2459A was detected at a frequency of 34.2%, which was lower than expected. In order to gain a deeper understanding of the level of genetic variation present at the CCR2-CCR5 gene locus within the research population, we calculated the anticipated heterozygosity of each SNP (He). There was a significant amount of heterozygosity at the loci 2459 and 2086. (0.400, and 0.398, respectively).

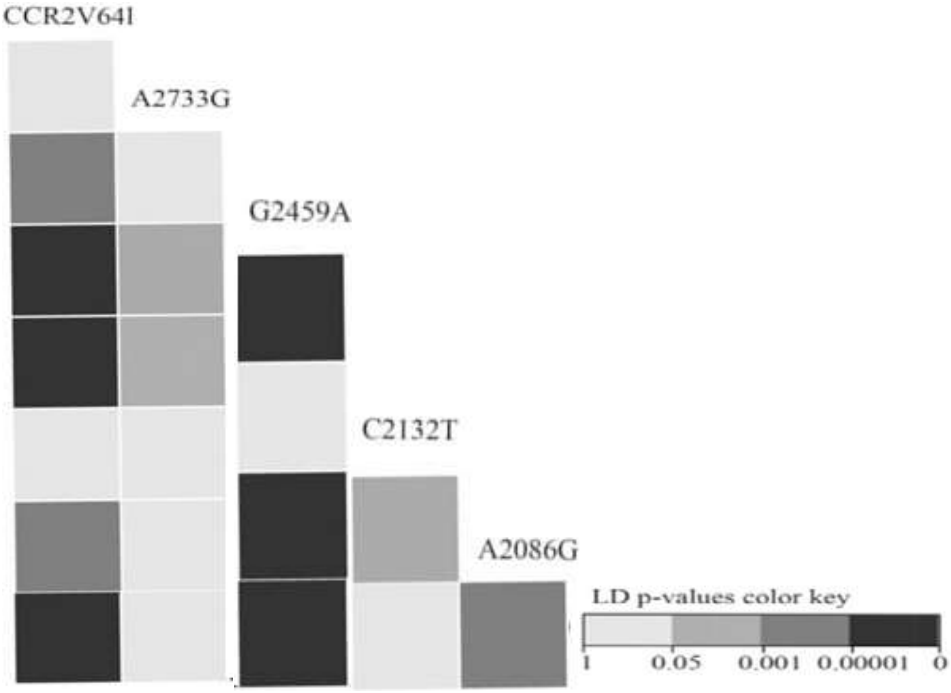
Table 2: The sample population's allele frequency (%), Hardy-Weinberg equilibrium (HWE), estimated heterozygosity (He), and genotype distribution for all loci investigated.

Allele	Site	frequency of alleles (%) (N = 100)	HWE p value	He	Genotype	frequency of a genotype (%)
CCR2(V64I)						
rs1799864	64V	85.0			VV	75.0
	64I	12.0	0.389	0.230	VI	19.0
				II	2.7	
CCR5 pro						
rs2856758	2733 A	95.2			AA	91.5
	2733 G	2.5	0.771	0.050	AG	5.0
					GG	0.0
rs1799987	2459 G	60.9			AA	15.0
	2459 A	36.1	0.415	0.463	AG	43.5
					GG	40.6
rs1800023	2086 A	51.5			AA	29.2
	2086 G	45.5	0.430	0.495	AG	44.3
					GG	21.5

The first nucleotide of the CCR5 translational start site, denoted as 1, serves as the basis for the SNP's position in the promoter region of the gene. Tm: annealing temperature.

4.2. The Linkage Between Different Snp Pairs

provides an overview of the findings from the LD tests carried out on each paired combination of SNPs. There was a significant degree of linkage disequilibrium between the distal CCR2V64I and the majority of the CCR5 SNPs that were discovered in the promoter region. It was discovered that the SNPs detected internally at positions -2459 and -2135 were in a high degree of linkage disequilibrium with one another (p 0.0001).



SNPs show pairwise linkage disequilibrium. The significant levels of the chi-square test are indicated by colored boxes (light grey p > 0.05; grey: 0.001 p 0.05; dark grey: 0.0001 p 0.001 and black: p 0.0001).

4.3. Diversification of Populations

Through the use of the network analysis, each haplotype was sorted into one of two primary categories. The human haplogroups **HHA, HHC, and HHD** were included in the first

cluster. The second cluster comprised HHE, (HHF1 & HHF2) and (HHG1 & HHG2) respectively. The evolutionary-based classification of CCR5 that had been previously published was unable to classify three recently identified haplotypes that were observed in three heterozygote individuals (Mummidi et al., 2000). This issue was made worse by the fact that there was an alternate nucleotide combination at position 2459, which was discarded during the categorization process. For example, the recently discovered haplotypes HHF1A and HHF2A both included guanine at position 2459, but the previously discovered haplotypes HHF1 and HHF2 carried adenine at that location. The new haplotypes were given names in accordance with their connections to the haplotypes of their descendants. In the population that was the focus of this research, the haplotype HHC made up 45.0% of the population, while HHE made up 19.0%. Among the haplotypes that were discovered less frequently were HHA (13.2%) and HHF2 (11.0%). Two different haplotypes, HHD and HHG1, were discovered to have a frequency of 2.4% each. 0.3% of people carried the haplotypes HHF1 and HHG2, which are known to carry the CCR532 gene. There was not a single individual who carried the HHB haplotype among the subjects (Table 3).

Table 3: Population-level CCR5 haplotype frequency distribution (%).

Haplotype	Pygmy[1]	Non-pygmy[1]	African American[1]	European[1]	Indian[1]	Asian[1]	Middle east 2
HHA	71	24	21	10	18	5	13.2
HHB	3	5	1	0	0	0	0
HHC	1	9	15	35	36	42	45
HHD	0	17	20	1	1	0	2.8
HHE	13	20	19	32	29	25	19
HHF*1	6	8	4	1	2	3	0.4
HHF*2	6	16	15	8	18	5	11
HHG*1	1	2	4	4	1	0	2.0
HHG*2	0	0	3	8	0	1	0.3

References: [1.] Gonzalez et al. (2001). [2.]This study.

Classification of the attendees The HHA haplotype is more common in some populations than others, with the highest frequency among African populations (Pygmy, non-Pygmy and African American). Asians and Europeans both possess two unique haplotypes. These are the HHC and HHE forms. The HHC haplotype is most common in people of Asian ancestry, specifically Thais (62%) (Nguyen et al., 2004), followed by people from the Middle East (45%) and Indians (35%), and finally people from European populations (34%). When compared to other populations, Europeans have the highest frequency of the HHE haplotype, which is 30% of the population. Black Americans (at 20%) reported a much higher prevalence of the HHD haplotype than people in the Middle East (2.8%). (Table 3) The HHG*2 haplotype was significantly less common than other haplotypes because it was uncommon in Asians and Africans but prevalent in Europeans (8%). The HHB haplotype was a rare one that could be found in only Africans and not in any other population on the planet.

5. DISCUSSION

The primary aims of this study were to collect baseline data on CCR2-CCR5 polymorphisms and to determine the haplotype profile of the Middle Eastern population. Our findings confirmed previous studies by demonstrating that the CCR532 allele is exceedingly rare in Asian societies (Martinson et al., 1997; Su et al., 2000). CCR264I, CCR5-2459, CCR5-2135 and CCR5-2086 have been identified as four significant allele sites (MAF > %). Due to the high level of LD between the SNPs in the CCR2-CCR5 gene locus, 11 distinct haplotypes were able to be used to assess the genetic diversity present at these two loci. When compared to other Asian communities such as Thais, the significant number of haplotypes discovered in this study, of which three were completely unique, revealed that the Middle East population possessed a higher level of genetic diversity (Clark and Dean, 2004; Nguyen et al., 2004). This study's analysis of the CCR2-CCR5 gene locus demonstrates that the Middle Eastern population is heterogeneous and matches patterns observed in previous analyses of Asian sub - populations. This finding lends credence to the hypothesis that the Middle East is home to multiple distinct subpopulations. There was a moderately high level of heterozygosity seen at three different allele locations (2459, 2135, and 2086). This heterozygosity is the outcome of selection favoring the existence of these alleles to produce an excess of intermediate frequencies, which, if seen, should have the effect of reducing population difference (Bamshad et al., 2002; Ramalho et al.,

2010). The discovery of eleven distinct haplotypes at the CCR2-CCR5 gene locus demonstrates the diversity of the Middle Eastern population. This diversity, according to the researchers of these studies, is the result of a sequence of demographic happenings in this region. These incidents took place in part because of the strategic location of the Middle East as a route between Africa and Eurasia. Depending on the adjusted FST distances, our phylogenetic analysis of the CCR2-CCR5 haplotypes placed the Middle East population near other Asians, but not too far from Europeans. These results are consistent with our haplotype phylogenetic analyses. To corroborate these findings, further research with a larger sample size would be required. This is the first study of its sort to describe the most prevalent CCR2-CCR5 haplotypes in the Middle East. In the examined population, HHC and HHE were determined to be the most frequent haplotypes. Additional study is required to examine the functional significance of the three newly discovered haplotypes, particularly their relevance in the pathophysiology of HIV-1 infection and disease development in HIV-1-infected persons residing in the Middle East. The results of this study reveal that the genetic makeup of the Omani people is quite diverse. Future research should investigate the pattern of genetic differences that exist between the CCR2-CCR5 gene loci in the north and south of the country.

REFERENCES

I. Pei Yu, Bingmei Liu, Siyu Hao, Ronggui Xing, and Yuzhen Li. A New Risk Polymorphism rs10403848 of CARD8. Significantly Associated with Psoriasis Vulgaris in Northeastern China. BioMed Research International Volume 6, 2020, Article ID 2867505, 7 pages.

II. Guo-wen Ding, Xu-yu Gu , Zhe Dai , Hui-wen Pan , Xiao-yan Wang , Heng Zhang , and Yu Fan. Association of Genetic Polymorphisms in FOXA1 with the Progression of Genetic Susceptibility to Gastric Cancer. Gastroenterology Research and Practice,Volume 4, 2020, Article ID 3075837, 8 pages.

III. Ngoufack MN, Nkenfou CN, Atogho Tiedeu B, Mouafo LCM, Dambaya B, Ndzi EN, Kouanfack C, Nguefack-Tsague G, Mbacham WF, Ndjolo A. CCR2 Genetic Polymorphism And Its Potential Effect On HIV Acquisition In A Population Of Children Living In The Northern Region Of Cameroon. Journal of The Application of Clinical Genetics, Volume 12, 2019:12 Pages 229 - 234.

IV. Cheryl L. Laffer, James V. Gainer, Michael R. Waterman, Jorge H. Capdevila, Michal Laniado-Schwartzman, Alberto Nasjletti, Nancy J. Brown, and Fernando Elijovich. The T8590C Polymorphism of CYP4A11 and 20-Hydroxyeicosatetraenoic Acid in Essential Hypertension. Hypertension, 2008 Mar; 51(3): 767–772.

V. Arthur P. Ruiz, David O. Ajasin, Santhamani Ramasamy, Vera DesMarais, Eliseo A. Eugenin & Vinayaka R. A Naturally Occurring Polymorphism in the HIV-1 Tat Basic Domain Inhibits Uptake by Bystander Cells and Leads to 21 ReducedNeuroinflammation.Scientific, Reports,2019;9:3308https://doi.org/10.1038/s41598-019-39531-5.

VI. Global HIV & AIDS statistics UNAIDS 2021.

VII. Simon J.H., Somoza C., Schockmel G.A., et al. Rat CD4 mutant containing the gp120-binding site mediates human immunodeficiency virus type 1 infection. J. Exp. Med 1993; 177:949-54.

VIII. Clapham P.R., Blanc D., Weiss R.A. Specific cell surface requirements for the infection of CD4-positive cells by human immunodeficiency virus types 1 and 2 and by Simian immunodeficiency virus. Virology 1991; 181:703-15.

IX. Horuk R. Molecular properties of the chemokine receptor family. Trends Pharmacol Sci 1994; 15:159-65.

X. Schall T.J. Biology of the RANTES/SIS cytokine family. Cytokine 1991; 3:165-8.

XI. Samson M., Labbe O., Mollereau C., et al. Molecular cloning and functional expression of a new human CC-chemokine receptor gene. Biochemistry 1996; 35:3362-7.

XII. D"Souza M.P., Harden V.A. Chemokines and HIV-1 second receptors. Confluence of two fields generates optimism in AIDS research. Nat Med 1996; 2:1293-300.

XIII. Koot M., Van„t Wout A.B., Kootstra N.A., et al. Relation between changes in cellular load, evolution of viral phenotype, and the clonal composition of virus populations in the course of human immunodeficiency virus type 1 infection. J Infect Dis 1996; 173:349-54 22

XIV. Paxton W., Martin S.R., Tse D., et al. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high risk sexual exposure. Nat Med 1996; 2:412-7.

XV. McLaren PJ, Fellay J. HIV-1 and human genetic variation. Nat Rev Genet. 2021 Jun 24:1–13. doi: 10.1038/s41576-021-00378-0. Epub ahead of print. PMID: 34168330; PMCID: PMC8223526.

XVI. Liu R., Paxton W.A., Choe S., et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply exposed individuals. Revista 1996.

XVII. Rowland-Jones S., Sutton J., Ariyoshi K., et al. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. Nat Med 1995; 1:50-64.

XVIII. Wilkinson D.A., Operskalski E.A., Busch M.P., et al. A 32-bp deletion within the CCR5 locus protects against transmission of parenterally acquired human immunodeficiency virus but does not affect progression to AIDS-defining illness. J Infect Dis 1998; 178:1163-6.

XIX. Zimmerman P.A., Buckler-White A., Alkhatib G., et al. Inherited resistance to HIV-1 conferred by an inactivating mutation in CC chemokine receptor 5; Studies in populations with contrasting clinical phenotypes, defined racial background, and quantified risk. Mol. Med 1997; 3:23-36.

XX. Smith M.W., Dean M., Carrington M., et al. Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Science 1997;15;277(5328):959-65. 23

XXI. Ronsard L, Sood V, Yousif AS, Ramesh J, Shankar V, Das J, Sumi N, Rai T, Mohankumar K, Sridharan S, Dorschel A, Ramachandran VG, Banerjea AC. Genetic Polymorphisms in the Open Reading Frame of the CCR5 gene from HIV-1 Seronegative and Seropositive Individuals From National Capital Regions of India. Sci Rep. 2019 May 20;9(1):7594. doi: 10.1038/s41598-019-44136-z. PMID: 31110236; PMCID: PMC6527560.

XXII. Mummidi S, Bamshad M, Ahuja SS, Gonzalez E, Feuillet PM, Begum K, Galvis MC, Kostecki V, Valente AJ, Murthy KK, et al. Evolution of human and non-human primate CC chemokine receptor 5 gene and mRNA. Potential roles for haplotype and mRNA diversity, differential haplotype-specific transcriptional activity, and altered transcription factor binding to polymorphic nucleotides in the pathogenesis of HIV-1 and simian immunodeficiency virus. J Biol Chem. 2000; 275:18946–18961.

XXIII. Peakall ROD, Smouse PE. Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 2006; 6:288–295.

XXIV. Excoffier LG, Schneider S. An integrated software package for population genetics data analysis. Evol Bioinform Online. 2005; 1:47–50.

XXV. Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet. 2003; 73:1162–1169.

XXVI. Bandelt HJ, Forster P, Röhl A. Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol. 1999; 16:37–48.

XXVII. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4:406–425.

XXVIII. J.H. Abramson, P.M. Gahlinger, Computer Program forEpidemiologists, PEPI version 3, Brixton Books, London,1999.