Distribution Of *CCR5-Δ32*, *CCR2-64I*, and *SDF-1-3'A* Alleles Among Jordanians

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Abstract

Entry of HIV virus into cells is mediated by chemokine receptors. Genetic variations in chemokine receptors have been shown to modulate susceptibility to HIV infection and disease course. In this study, the frequencies of *CCR5* (*CCR5-Δ32*), *CCR2* (*CCR2-64I*), and *SDF-1* (*SDF-1-3'*) gene polymorphisms were determined in a Jordanian population. A total of 540 subjects were randomly selected from different regions of Jordan (South, Middle, and North). Six individuals were found to carry the *CCR5-Δ32* allele (0.6%) and only in the heterozygous genotype. The frequencies of *CCR2-64I* and *SDF1-3'A* were 17.5% and 34.2%, respectively. In addition, no significant difference in the distribution of the examined polymorphisms among different regions of Jordan was detected. In conclusion, the *CCR5-Δ32* allele is rare, whereas the *CCR2-64I* and *SDF1-3'A* alleles are common among Jordanians.

Introduction

VARIATIONS IN SUSCEPTIBILITY TO infections are well known among individuals of the same population. This has been attributed to differences between individuals regarding genetic background and/or environmental factors such as diet and life style.^{1–3} Genetic variations in certain chemokines and their receptors have been reported to be responsible for resistance to HIV-1 infection, delayed progression to AIDS, response to the treatment, and mother-to-child transmission.⁴

Chemokine receptors are proteins found on the cell surface.⁵ The entry of HIV-1 virus to the host cells is primarily mediated by the CD4 receptor and at least one coreceptor from multi-CC and CXC chemokine receptors.⁶ Previous studies have identified the *CCR5* chemokine receptor as the coreceptor for macrophage-tropic strain (R5)^{5,7,8} and CXCR4 chemokine receptor for the X4 strain.⁸ In addition, *in vitro* studies showed that *CCR2*, *CCR3*, *CCR8*, and *CCR9* chemokine receptors may act as coreceptors.⁷

Among the polymorphisms that modulate HIV infection is deletion of 32 bp in the coding region of the *CCR5* receptor (*CCR5-Δ32*, rs333). The homozygous genotype of the *CCR5-Δ32* allele gives strong resistance against R5 strains. On the other hand, the heterozygous genotype gives limited resistance and/or delays the progression to AIDS.^{7,9–11} Another important variation that modulates HIV infection is *CCR2-64I* polymorphism (rs1799864). Individuals with one or two copies of the 64I variant have reduced risk for progression to

AIDS after infection. This risk reduction is 58% in the first 4 years after HIV seroconversion, followed by a 22% reduction in the next 4 years with no reduction after that.^{7,10} Finally, the *SDF-1-3'A* polymorphism (Rs1801157) at the 3' untranslated region of the *SDF-1* gene, which codes for the unique ligand of the CXCR4 receptor, has been shown to be associated with delayed progression to AIDS in homozygous mutant individuals,^{7,10,12} and prenatal transmission of HIV-1 in heterozygous pregnant women.¹³

The above mutations have been widely studied and their global distribution was found to be variable among different populations. For example, the *CCR5-Δ32* is highly prevalent in Europeans (ranges from 10% to 20%) and Ashkenazi Jews (20.93%).^{10,14} The aim of the present study was to determine the frequencies of *CCR5-Δ32*, *CCR2-64I*, and *SDF-1-3'A* polymorphisms in the Jordanian population.

Materials and Methods

Subjects

A total of 540 healthy Jordanian subjects were randomly selected from all the geographic areas of Jordan. Subjects were divided into three groups according to their region: (1) South, with 176 subjects, includes Al-Aqaba, Ma'an, Al-Tafehla, and Al-Karak provinces, (2) Middle, with 212 subjects, includes Amman, Zarqa, Al-Balqa, and Madaba provinces, and (3) North, with 152 subjects, includes Irbid, Ajloun, Jerash, and Al-Mafraq provinces. Written informed consents were

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obtained from all participants in accordance with the requirements of the Institutional Review Board (IRB) of the Jordan University of Science and Technology.

DNA extraction

The genomic DNA isolation kit (Promega, Madison, WI) was used to extract DNA from EDTA blood samples as described by the manufacturer. The concentration of extracted DNA was measured using a spectrophotometer. DNA samples were stored at -20°C until used.

Genotyping of genes polymorphisms

To analyze the CCR5- $\Delta 32$ polymorphism, a polymerase chain reaction (PCR) was carried out to amplify the target DNA using the following pair of primers: forward 5'-ACC AGA TCT CAA AAA GAA GGT CT-3' and reverse 5'-CAT GAT GGT GAA GAT AAG CCT CAC A-3' as previously described¹⁰ in a 25 μl reaction that contains GoTaq green master mix (Promega), 0.5 μM of each primer, 5 ng of genomic DNA, and nuclease free water. The reaction mix was subject to 35 cycles: initial denaturation at 94°C for 3 min, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The PCR product was then separated on 3% agarose gel stained with ethidium bromide (Sigma-Aldrich Corp.) and visualized under UV light. Positive samples were sequenced as previously described¹⁵ using an ABI Prism 3.1 automated sequencer (Applied Biosystems, Foster City, CA).

For genotyping of CCR2-64I, the PCR restriction fragment length polymorphism method (PCR-RFLP) was used as previously described¹⁰ using *FokI* restriction enzyme (Fermentas, Germany). The digested product was separated on 2% agarose gel stained with ethidium bromide and visualized under UV light; the wild-type allele remained as one undigested 379-bp fragment while the mutant allele gave two fragments of 164 bp and 215 bp.

SDF1 was also genotyped by using the PCR-RFLP method as described previously¹⁰ using the *MspI* restriction enzyme (Fermentas, GmbH, St. Leon-Rot, Germany). The wild-type allele gives two fragments of 205 bp and 106 bp while the mutant allele remains as one undigested 311-bp fragment.

Statistical analysis

Data analysis was carried out using the statistical package for social studies (SPSS version 17.0, Chicago, IL). Genotype distribution and allele frequencies were evaluated using

the Chi square test. A p value < 0.05 was considered to be statistically significant.

Results

A total of 540 subjects volunteered in the study from different regions of Jordan. The distribution of subjects was 36.5% from the south, 39.3% from the middle, and 28.3 from the north of Jordan. Of the 540 participants, 100% were born in Jordan and 51.8% were males. The mean age of sample was 30.5 ± 8.5 years. No significant differences in the demographic variables were observed among groups from different regions (data not shown).

Table 1 shows genotypes and allele frequencies of the CCR5- $\Delta 32$ polymorphism in the studied sample. The frequency of the CCR5- $\Delta 32$ allele was 0.6% and was present in the heterozygous genotype only. In addition, no significant difference was found in the distribution of this variant among different geographic regions of Jordan (Table 1). To confirm the deletion, all heterozygote samples were sequenced. A 100% correlation was noticed between the results of the two methods. The CCR5- $\Delta 32$ genotypic distribution was found to be consistent with Hardy-Weinberg equilibrium ($p = 0.955$).

Genotypes and allele frequencies of the CCR2-64I polymorphism among Jordanians are illustrated in Table 2. The G/G genotype refers to the homozygous wild-type genotype (CCR2+/+) while G/A and A/A are for the heterozygous (CCR2 +/64I) and homozygous (CCR2 64I/64I) genotypes, respectively. The frequency of the G allele among Jordanians was 82.5% compared to 17.5% for the A allele. When data were analyzed according to different regions, the A allele was more abundant in the middle region (20.8%) compared to other regions (15.1–15.8%). However, the difference in the distribution of this variant among different regions was not statistically significant ($p > 0.05$, Table 2). In Hardy-Weinberg assay, both alleles were in equilibrium in the total population ($p = 0.550$).

The *SDF1*-3'A polymorphism is highly enriched in the Jordanian population with a frequency of 34.2% of the mutant A allele and 65.8% of the G wild-type allele (Table 3). The allelic frequencies of this variant are similar in the different regions of Jordan (range: 33.5–35.5%, $p > 0.05$). Similar to other variants, the distribution of *SDF1*-3'A genotypes is consistent with Hardy-Weinberg equilibrium in the total population.

Discussion

Following the reporting the CCR5- $\Delta 32$, CCR2-64I, and *SDF1*-3'A polymorphisms and their roles in HIV infection,

TABLE 1. FREQUENCIES OF GENOTYPES AND ALLELES OF THE CCR5- $\Delta 32$ POLYMORPHISM IN THE STUDIED SAMPLE

Geographic area	CCR5: +/+		CCR5: +/ $\Delta 32$		CCR5: $\Delta 32/\Delta 32$		CCR5: + allele		CCR5: $\Delta 32$ allele	
	N	%	N	%	N	%	N	%	N	%
Middle	209	98.6	3	1.4	0	0	421	99.3	3	0.7
South	175	99.4	1	0.6	0	0	351	99.7	1	0.3
North	150	98.7	2	1.3	0	0	302	99.3	2	0.7
Total	534	98.9	6	1.1	0	0	1074	99.4	6	0.6
p value						0.702 ^a				0.703 ^b

^aCalculated p value for genotype frequency.

^bCalculated p value for allele frequency.

Person chi square test.

TABLE 2. FREQUENCIES OF GENOTYPES AND ALLELES OF THE CCR2-64I POLYMORPHISM IN THE STUDIED SAMPLE

Geographic area	G/G		G/A		G/A		G allele		A allele	
	N	%	N	%	N	%	N	%	N	%
Middle	128	60.4	80	37.7	4	1.9	336	79.2	88	20.8
South	128	72.7	43	24.4	5	2.8	229	84.9	53	15.1
North	107	70.4	42	27.6	3	2.0	256	84.2	48	15.8
Total	363	67.2	165	30.6	12	2.2	891	82.5	189	17.5
<i>p</i> value						0.058 ^a				0.075 ^b

^aCalculated *p* value for genotype frequency.^bCalculated *p* value for allele frequency.

Person chi square test.

progression to AIDS, and treatment response, a number of studies were carried out to find their frequencies worldwide.¹⁶ Therefore, this study came to comprehensively investigate the frequencies of these polymorphisms in Jordan.

The polymorphism CCR5- Δ 32, which has a protective effect against HIV in homozygous mutant form and a delaying effect in heterozygous form, is enriched in Europe.¹⁷ In Arabic countries, the CCR5- Δ 32 allele frequency is very low. For example, the reported prevalence of CCR5- Δ 32 is <1% in Syria and was absent in Lebanon.^{5,18,19} In countries such as Bahrain, Iraq, Kuwait, and Saudi Arabia, the prevalence is approximately equivalent with a distribution of CCR5- Δ 32 between 2% and 3%.⁵ The results of the current study showed that the frequency of CCR5- Δ 32 in Jordan is 0.6%. In a previous report that considered 50 Jordanians, CCR5- Δ 32 was not detected in the Jordanian population.¹⁹ Our results rank Jordan with Egypt with 0.6%⁵ at the lowest frequencies among Arabic countries. Thus, the result presented in this study did not reveal any deviation from what was reported in Arabic countries.

The enrichment of the CCR5- Δ 32 allele in European populations suggests that this polymorphism most likely originated in Europe and then spread globally under natural positive selection.¹⁷ Based on this hypothesis, Lebanon, Syria, and Jordan are supposed to have high frequencies compared to other Arabic populations since the admixture with European was very high in these countries. The fact that the CCR5- Δ 32 allele is rare in Lebanon, Syria, and Jordan, provides evidence against this hypothesis. The unequal CCR- Δ 32 global distribution suggests other unidentified protective mutations in the CCR5 gene among populations in which the CCR- Δ 32 allele is absent or has a very low frequency. Identification of such

variants will be of great importance both scientifically and clinically.

The chemokine receptor CCR2, on the other hand, modulates HIV infection by delaying disease progression rather than affecting the initial HIV transmission. Although this polymorphism, which substitutes Val with Ile (CCR2-64I), does not change the CCR2 structure and function, both homozygous and heterozygous states are associated with the delay in disease progression. The allele is distributed all over the world and is never inherited together with CCR5- Δ 32 because they occur on separate haplotypes.²⁰ The distribution of the CCR2 polymorphism in Arabic countries including Jordan is still lacking. In the present study the frequency of the CCR2-64I allele among Jordanians was 17.5%. This is higher than that reported in Bahrain (8.9%) and is close to that reported in Asians (25%).²⁰

The third gene in this study is the SDF-1, which codes for the ligand of the orphan CXCR4 chemokine receptor.²¹ The effect of this variant occurs during the advanced stages of AIDS when the virus shifts strains from R5 to X4. During disease progression, the X4 strain utilizes CXCR4 as the main coreceptor and the excess amount of SDF-1 competes with the virus on the receptors,^{10,22} thus delaying its effect. Analysis of the allele frequencies in our sample revealed that the prevalence of SDF-1-3'A among Jordanians is 34.2%. In other populations such as Brazilian, Hungarian, Chinese, Polish, and Indian, the distribution of this variant ranges between 14% and 40%.²³⁻²⁸ Thus, distribution of this variant in the Jordanian population is within the range described in other countries.

Jordan is a small country located in Southwest Asia with total population of 6.13 million as of the 2010 census. The

TABLE 3. FREQUENCIES OF GENOTYPES AND ALLELES OF THE SDF1-3'A POLYMORPHISM IN THE STUDIED SAMPLE

Geographic area	G/G		G/A		A/A		G allele		A allele	
	N	%	N	%	N	%	N	%	N	%
Middle	86	40.6	110	51.9	16	7.5	282	66.5	142	33.5
South	54	30.7	119	67.6	3	1.7	227	64.5	125	35.5
North	55	36.2	92	60.5	5	3.3	202	66.4	102	33.6
Total	195	36.1	321	59.4	24	4.4	711	65.8	369	34.2
<i>p</i> value						0.10 ^a				0.811 ^b

^aCalculated *p* value for genotype frequency.^bCalculated *p* value for allele frequency.

Person chi square test.

population is predominantly Arab (98%) and is originally from Jordan and Palestine with roughly equal percentages. Since Jordanians and Palestinians share a common origin, we do not expect a significant change in the population homogeneity due to migration of Palestinians to Jordan in 1948. The participants of the current study were randomly recruited from different parts of Jordan irrespective of their parental origin. The results did not show significant variations in the allelic and genotypic distribution of the examined polymorphisms among different regions of Jordan (South, Middle, and North). Thus, these results support the homogeneity of the Jordanian population.

It is worth mentioning that the role of the CCR5-Δ32, CCR2-64I, and SDF1-3'A polymorphisms in HIV resistance and progression to AIDS might vary among different populations. For example, a positive association between the CCR5-Δ32 polymorphism and HIV resistance and/or progression to AIDS has been reported in Italian and Australian populations.^{29,30} On the other hand, lack of association with this polymorphism has been reported in Polish and Spanish populations.^{31,32} Similarly, an association between the CCR2-64I polymorphism and progression to AIDS has been shown in Brazilian and Chinese populations^{23,33} but not in Georgian and Kenyan populations.^{34,35} For SDF1-3'A, controversial associations with this polymorphism have been reported.³⁶ Thus, associations between examined polymorphisms and HIV infection and progression of the disease might have a population-specific component, being affected by the population-specific gene pool as well as by gene-environment interactions.

In conclusion, the CCR5-Δ32 allele is rare whereas the CCR2-64I and SDF1-3'A alleles are common among Jordanians. The role of these variants in HIV infection and progression to AIDS in the Jordanian population needs more investigation.

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Author Disclosure Statement

No competing financial interests exist.

References

- Garantziotis S, Hollingsworth JW, Zaas AK, and Schwartz DA: The effect of toll-like receptors and toll-like receptor genetics in human disease. *Annu Rev Med* 2008;59:343–359.
- Qu HQ, Fisher-Hoch SP, and McCormick JB: Knowledge gaining by human genetic studies on tuberculosis susceptibility. *J Hum Genet* 2011;56:177–182.
- Chatzidaki V, Kouroumalis E, and Galanakis E: Hepatitis B virus acquisition and pathogenesis in childhood: Host genetic determinants. *J Pediatr Gastroenterol Nutr* 2011;52:3–8.
- de Souza PR, Arraes LC, de Lima Filho JL, Brunessa D, Milanese M, and Crovella S: CCR5 promoter polymorphisms and HIV-1 perinatal transmission in Brazilian children. *J Reprod Immunol* 2006;69:77–84.
- Salem AH and Batzer MA: Distribution of the HIV resistance CCR5-Delta32 allele among Egyptians and Syrians. *Mutat Res* 2007;616:175–180.
- Cicala C, Arthos J, and Fauci AS: HIV-1 envelope, integrins and co-receptor use in mucosal transmission of HIV. *J Transl Med* 2011;9(Suppl 1):S2.
- Apostolakis S, Baritaki S, Krambovitis E, and Spandidos DA: Distribution of HIV/AIDS protective SDF1, CCR5 and CCR2 gene variants within Cretan population. *J Clin Virol* 2005;34:310–314.
- Paxton WA and Kang S: Chemokine receptor allelic polymorphisms: Relationships to HIV resistance and disease progression. *Semin Immunol* 1998;10:187–194.
- de Silva E and Stumpf MP: HIV and the CCR5-Delta32 resistance allele. *FEMS Microbiol Lett* 2004;241:1–12.
- Ma L, Marmor M, Zhong P, Ewane L, Su B, and Nyambi P: Distribution of CCR2-64I and SDF1-3'A alleles and HIV status in 7 ethnic populations of Cameroon. *J Acquir Immune Defic Syndr* 2005;40:89–95.
- Suzuki S, Miyagi T, Chuang LF, Yau PM, Doi RH, and Chuang RY: Chemokine receptor CCR5: Polymorphism at protein level. *Biochem Biophys Res Commun* 2002;296:477–483.
- Alagarasu K, Selvaraj P, Swaminathan S, Raghavan S, Narendran G, and Narayanan PR: CCR2, MCP-1, SDF-1a & DC-SIGN gene polymorphisms in HIV-1 infected patients with & without tuberculosis. *Indian J Med Res* 2009;130:444–450.
- John GC, Rousseau C, Dong T, *et al.*: Maternal SDF1 3'A polymorphism is associated with increased perinatal human immunodeficiency virus type 1 transmission. *J Virol* 2000;74:5736–5739.
- Faure E and Royer-Carenzi M: Is the European spatial distribution of the HIV-1-resistant CCR5-Delta32 allele formed by a breakdown of the pathocenosis due to the historical Roman expansion? *Infect Genet Evol* 2008;8:864–874.
- Khabour OF, Mesmar FS, Al-Tamimi F, Al-Batayneh OB, and Owais AI: Missense mutation of the EDA gene in a Jordanian family with X-linked hypohidrotic ectodermal dysplasia: Phenotypic appearance and speech problems. *Genet Mol Res* 2010;9:941–948.
- Galvani AP and Novembre J: The evolutionary history of the CCR5-Delta32 HIV-resistance mutation. *Microbes Infect* 2005;7:302–309.
- Lucotte G and Dieterlen F: More about the Viking hypothesis of origin of the delta32 mutation in the CCR5 gene conferring resistance to HIV-1 infection. *Infect Genet Evol* 2003;3:293–295.
- Stephens JC, Reich DE, Goldstein DB, *et al.*: Dating the origin of the CCR5-Delta32 AIDS-resistance allele by the coalescence of haplotypes. *Am J Hum Genet* 1998;62:1507–1515.
- Voevodin A, Samilchuk E, and Dashti S: A survey for 32 nucleotide deletion in the CCR-5 chemokine receptor gene (deltacr-5) conferring resistance to human immunodeficiency virus type 1 in different ethnic groups and in chimpanzees. *J Med Virol* 1998;55:147–151.
- Berger EA, Murphy PM, and Farber JM: Chemokine receptors as HIV-1 coreceptors: Roles in viral entry, tropism, and disease. *Annu Rev Immunol* 1999;17:657–700.
- Tiensiwakul P: Stromal cell-derived factor (SDF) 1-3'A polymorphism may play a role in resistance to HIV-1 infection in seronegative high-risk Thais. *Intervirology* 2004;47:87–92.
- Nookhai S, Ruxrungtham K, Phanuphak P, and Oelrichs R: Prevalence of CCR2-64I, SDF1-3'A and CCR5-Delta32 alleles in healthy Thais. *Eur J Immunogenet* 2000;27:153–157.
- Vieira VC, Barral MF, Mendoza-Sassi RA, Silveira JM, Soares MA, and de Martinez AM: The effect of combined polymorphisms in chemokines and chemokine receptors on

- the clinical course of HIV-1 infection in a Brazilian population. *Mem Inst Oswaldo Cruz* 2011;106:408–415.
24. Juhasz E, Beres J, Kanizsai S, and Nagy K: The consequence of a founder effect: CCR5-32, CCR2-64I and SDF1-3'A polymorphism in Vlach Gypsy population in Hungary. *Pathol Oncol Res* 2012;18:177–182.
  25. Su QJ, Zhou P, Bi ZY, *et al.*: [Distribution of HIV-1 subtype and prevalence of HIV-1 drug resistance in Liuzhou and Nanning]. *Bing Du Xue Bao* 2010;26:290–294.
  26. Xiao J, Hu F, Xu H, *et al.*: Provincial distribution of three HIV-1 resistant polymorphisms (CCR5-Delta32, CCR2-64I, and SDF1-3' A) in China. *Sci China C Life Sci* 2000;43:16–20.
  27. Verma R, Gupta RB, Singh K, *et al.*: Distribution of CCR5delta32, CCR2-64I and SDF1-3'A and plasma levels of SDF-1 in HIV-1 seronegative North Indians. *J Clin Virol* 2007;38:198–203.
  28. Lewandowska M, Franciszkiewicz K, Prokop J, Ofori H, and Jagodzinski PP: Distribution of two HIV-1-resistant polymorphisms (SDF1-3'A and CCR2-64I alleles) in the Polish population. *J Hum Genet* 2002;47:585–589.
  29. Stewart GJ, Ashton LJ, Biti RA, *et al.*: Increased frequency of CCR-5 delta 32 heterozygotes among long-term non-progressors with HIV-1 infection. The Australian Long-Term Non-Progressor Study Group. *AIDS* 1997;11:1833–1838.
  30. Romiti ML, Colognesi C, Cancrini C, *et al.*: Prognostic value of a CCR5 defective allele in pediatric HIV-1 infection. *Mol Med* 2000;6:28–36.
  31. Vidal F, Peraire J, Domingo P, *et al.*: Lack of association of SDF-1 3'A variant allele with long-term nonprogressive HIV-1 infection is extended beyond 16 years. *J Acquir Immune Defic Syndr* 2005;40:276–279.
  32. Wasik TJ, Smolen J, Kruszynski P, Bratosiewicz-Wasik J, and Beniowski M: [Effects of CCR5-delta32, CCR2-64I and SDF-1-3'A polymorphic alleles on human immunodeficiency virus 1 (HIV-1) infection in the Polish population]. *Wiad Lek* 2005;58:500–507.
  33. Xu L, Qiao Y, Zhang X, *et al.*: CCR2-64I allele is associated with the progression of AIDS in a Han Chinese population. *Mol Biol Rep* 2010;37:311–316.
  34. Brouwer KC, Yang C, Parekh S, *et al.*: Effect of CCR2 chemokine receptor polymorphism on HIV type 1 mother-to-child transmission and child survival in Western Kenya. *AIDS Res Hum Retroviruses* 2005;21:358–362.
  35. Karchava M, Nelson KE, Gochitashvili N, Dvali N, and Tsertsvadze T: Distribution of HIV-1 resistant polymorphisms among HIV infected patients in Georgia. *Georgian Med News* 2008;165:28–33.
  36. Poropatich K and Sullivan DJ: Human immunodeficiency virus type 1 long-term non-progressors: The viral, genetic and immunological basis for disease non-progression. *J Gen Virol* 2011;92:247–268.

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