ORIGINAL ARTICLE

CCR5 Polymorphisms and its Relationship with HIV Susceptibility, Viral Load and CD4 Count in Early Antiretroviral Therapy among HIV Patients in Selangor and Terengganu

Irma Izani Mohamad Isa[1,2], Suhaili Abu Bakar[1], Umi Rufaidah Zolraimi[1], Nurfhaezah Khairil Wahidin[1], Nur Sakinah Matnor[1], Zulkefley Othman[1]

[1] Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor
[2] Royal College of Surgeons in Ireland, School of Medicine. Perdana University, Suite 9.2, Level 9, Wisma Chase Perdana, Changkat Semantan, Damansara Heights 50490 Kuala Lumpur, Malaysia

ABSTRACT

Introduction: Early studies have suggested the role of C-C chemokine receptor type 5 (*CCR5*) polymorphisms in influencing HIV pathogenesis and phenotypes, including the protection against HIV infection and delaying disease progression to AIDS. This study aimed to further determine the impact of *CCR5* variants (*CCR5-Δ32* and *CCR5-R223Q*) on HIV susceptibility, viral load suppression and CD4 recovery during highly active antiretroviral therapy (HAART) among Malaysian HIV patients. **Methods:** This cross-sectional study involved 182 HIV-infected who were recruited from three out-patient clinics, and 150 non-HIV subjects from Malay, Chinese and Indian ethnicities. CD4 count and viral load data at 4-6 months (t1) and 8-12 months (t2) after starting HAART were gathered from hospital records. Chi-square test was used to analyse the correlation between *CCR5* variants with dependent variables. **Results:** Heterozygous *CCR5-Δ32* and *CCR5-R223Q* occurred in a percentage of 0.5% (1/182) and 1.7% (3/182) among HIV patients respectively, while none of homozygous mutant for *CCR5-Δ32* and *CCR5-R223Q* were found. *CCR5-R223Q* was found more frequently in non-HIV as compared to the HIV group (P=0.018). However, both polymorphisms were not found to be correlated with CD4 recovery to ≥500 cells/mm^3 (P>0.05) and viral load suppression ≤50 copies/mL (P>0.05). **Conclusion:** *CCR5-R223Q* and *CCR5-Δ32* alleles probably have no modifying effects on HIV susceptibility virological and immunological recoveries in the first 12 months of HAART, partially due to the low prevalence of these mutations in the studied population.
Malaysian Journal of Medicine and Health Sciences (2023) 19(1):3-9. doi:10.47836/mjmhs19.1.2

Keywords: *CCR5*, HIV, CD4 count, viral load, HAART

Corresponding Author:
Suhaili Abu Bakar, PhD
Email: suhaili_ab@upm.edu.my
Tel: +603-97692306

INTRODUCTION

C-C chemokine receptor type 5 (*CCR5*) is one of the co-receptors used by the human immunodeficiency virus (HIV) to infect host cells, particularly at the early stage of HIV-1 infection (1–3). CCR5 binds a few C-C chemokines including the CCL3 and CCL4 (4). It is mainly found on immune cells such as macrophages and monocytes and functions in signal stimulatory for T-cell activation (5). CCR5 has also been an important modality in the treatment of HIV infection and one of the highly active antiretroviral therapy (HAART) drugs, known as Maraviroc, acts by blocking the CCR5 receptor from HIV binding (6). In finding the cure for HIV, CCR5 receptor serves as a the potential target for gene therapy (7). *CCR5* genome editing had been performed on the 'Berlin patient', who had received a stem cell transplantation from human leukocyte antigen (HLA)-matched, homozygous *CCR5-Δ32* donor. This transplantation had produced undetectable viral load for a duration of 20 months post-transplantation in the absence of the antiretroviral therapy (8).

The *CCR5* gene is the gene that encodes for the CCR5 receptor which is very pleomorphic (9). Early studies had demonstrated the effect of *CCR5-Δ32* mutation in reducing HIV susceptibility and in delaying the development of AIDS in HIV-1 patients (10,11). Individuals who are homozygous for 32 basepair (bp) deletion at the open reading frame of *CCR5*, also known as *CCR5-Δ32*, are conferred full resistance to the macrophage-tropic strains of HIV-1, by producing a non-functional receptor which is not expressed on the cell surface (10,12). Nonetheless, this mutation occurs very rarely or can be absent in some Asians population (13,14). Another *CCR5* variant, known as *CCR5-R223Q*, is more typically found in the Asian populations,

particularly among the Chinese and Japanese (15–18). A study had found the *CCR5-R223Q* as the highest occurring mutation in the open reading frame region of *CCR5* in Chinese population, with a frequency of 0.046 (18). Despite this, it had been reported that the *CCR5-R223Q* polymorphism to be conservative in nature where it does not influence cell-surface expression of *CCR5* receptor nor chemokine binding (19–21).

Besides the impact of *CCR5* polymorphisms on HIV susceptibility, this polymorphism had also been reported on several different aspects of HIV infection, including disease progression, viral load, and immune recovery (11,22–24). For example, *CCR5* polymorphisms had been shown to have HIV-1 disease-modifying effects, which could be either delaying or acceleration the progression of AIDS depending on African Americans or Caucasians races (22). In addition, the *CCL3L1-CCR5* complex had been associated with higher immune reconstitution upon the initiation of HAART (25).

Nonetheless, there are still limited number of studies that investigate the role of *CCR5* polymorphisms on the variation of individual responses to HAART particularly after the introduction of HAART. Moreover, since *CCR5* exhibits a race-specific effect on HIV disease, this also further emphasize the need for population- or ethnic-specific disease association studies involving *CCR5* polymorphisms (22). Therefore, this study aims to identify the association of the two selected mutations in the *CCR5* gene (*CCR5-Δ32* and *CCR5-R223Q*) on HIV susceptibility and the outcomes of HAART, which are viral load suppression and CD4 recovery among the HIV-infected population in Malaysia.

MATERIALS AND METHODS

Study population and data collection

This cross-sectional study involved 182 HIV-infected subjects from three Infectious Disease clinics of Hospital Sungai Buloh, Selangor, Hospital Sultanah Nur Zahirah, Terengganu and Hospital Kajang, Selangor as well as 150 non-HIV subjects. Both HIV and non-HIV subjects consisted of Malay, Chinese or Indian ethnic groups, aged 18 and above. Subjects were recruited by convenient sampling. A 170 subset of the HIV subjects who were on the standard first-line regimen of HAART and had CD4 count and viral load monitored every 4-6 months during HAART, were further analysed for the association between *CCR5* polymorphisms and the outcome of HAART. Ethical approvals were obtained for non-HIV (Ref: UPM/TNCPI/RMC/1.4.18.1 (JKEUPM)/F2) and HIV (Ref: NMRR-16-705-29043 IIR).

The recruitment of HIV subjects was carried out from August 2016 to May 2017 after informed consent was performed. Following that, a self-administered questionnaire related to socio-demographics and medical history was filled-up. A minimum of 1 mL of respondents' blood samples were also collected for genotyping. CD4 count and viral load data for the HIV subjects were retrieved from hospital record in the period of November 2007 to December 2018. Pre-treatment HAART (t0) and another two measurements of CD4 count and viral load (t1 and t2) were taken at every four to six months during HAART. CD4 count ≥500 cells/mm3 and viral load ≤50 copies/mL were chosen as the target CD4 count and viral load, respectively.

Genotyping of *CCR5* polymorphisms

DNA from the blood samples was extracted using the QIAamp DNA Maxi Kit (QIAGEN, Germany). The amplification of *CCR5-Δ32* and *CCR5-R223Q* (also referred to as *CCR5-G668A*) loci were carried out by using 10-30 ng/μL of DNA, 10uM of each forward (5′-GCT GTC GTC CAT GCT GTG TTT-3′) and reverse primers (5′-CAA CCT GTT AGA GCT ACT GCA ATT-3′) (26), Prime Taq Premix (Genet Bio, Korea), and ultra-pure water to make a final volume of 20 μL. PCR cycling conditions were set at the following: 95 °C for10 minutes, 95 °C for 30 seconds, 58 °C for 30 seconds and 72 °C for 30 seconds for 30 cycles and a final extension at 72 °C for 10 minutes. For *CCR5-R223Q* genotyping, ten μL of the PCR product was further incubated with five units of TspRI restriction enzyme (New England Biolabs, USA), CutSmart restriction enzyme buffer (New England Biolabs, USA), and ultra-pure water to produce a final volume of 25 μL.

The amplicons were then separated by using gel electrophoresis. The expected product size for the wild type and the *CCR5-Δ32* alleles are 441 bp and 409 bp, respectively. *CCR5-R223Q* allele was identified by the presence of two bands at 289 bp and 152 bp, while the wild type allele produced one band at 441 bp. Additionally, few samples were sent for Sanger sequencing for validation of the genotyping results by using ABI PRISM 3730xl Genetic Analyzer (Applied Biosystem, USA) and analysed by using Sequence Scanner 2.0 (Thermo Fisher, USA).

Statistical analysis

The association of both *CCR5-Δ32* and *CCR5-R223Q* with HIV susceptibility were analysed by using the Chi-square test (or the Fisher's exact test). The same test was used to analyse the association of *CCR5-Δ32* and *CCR5-R223Q* with CD4 count (≥500 cells/mm3 or lower) and viral load (≤50 copies/mL or higher) in HIV patients at two different time points (t1 and t2). All statistical analyses were carried out using SPSS version 22. $P<0.05$ was considered as statistically significant.

RESULTS

Prevalence of CCR5-Δ32 and *CCR5-R223Q* and their associations with HIV susceptibility

Fig. 1 shows the image of gel electrophoresis for the *CCR5-Δ32* and *CCR5-R223Q*. A few selected samples

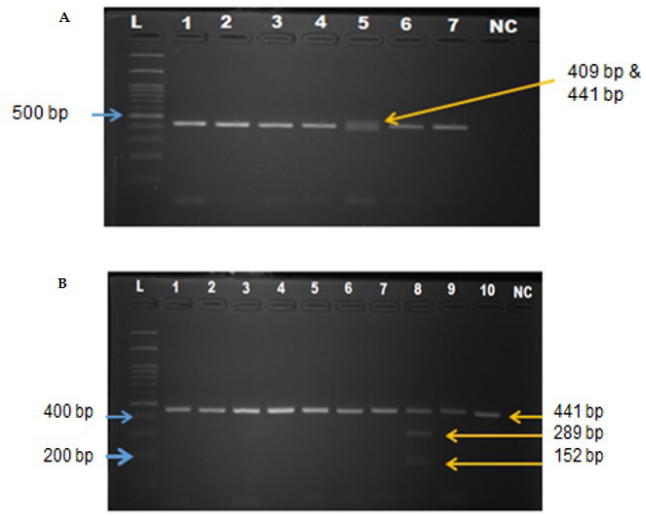

Figure 1: Gel electrophoresis images showing CCR5-Δ32 (A) and CCR5-R223Q (B). A) Lane 5 indicates heterozygous CCR5-Δ32 which yielded both at 441 bp and 409 bp fragments while the other lanes show wild type. B) Lane 8 indicates heterozygous CCR5-R223Q that produced bands at 441 bp, 289 bp and 152 bp while the other lanes show wild type. L: 100 bp DNA ladder, NC: negative control.

were sent for validation by using DNA sequencing to confirm the gel electrophoresis results. The genotype frequencies of the two polymorphisms in the HIV subjects were also shown to be in Hardy-Weinberg equilibrium (*CCR5* Δ32- χ^2=0.001, P=0.970; *CCR5-R223Q*- χ^2=1.846, P=0.174).

Table I presents the frequencies of the *CCR5-Δ32* and *CCR5-R223Q* variants in the HIV and non-HIV subjects according to ethnicity. For the *CCR5-Δ32*, all of the HIV subjects had the wild-type genotype (99.5%) (homozygous WT/WT) except for one Malay subject that showed the heterozygous genotype (WT/Δ32). This showed that heterozygous *CCR5-Δ32* occurred in a percentage of 0.5% (1/182) with the frequency of *CCR5-Δ32* allele as 0.003 in group that combined all the subjects, similar to a previous report (14). Meanwhile, heterozygous *CCR5-R223Q* occurred in 1.7% (3/182) among HIV patients to give *CCR5-R223Q* allele frequency of 0.008 in group that combined all the subjects. All three heterozygous *CCR5-R223Q* was found in Chinese ethnic, thus giving the minor allele frequency of 0.024 in the Chinese HIV subjects. Besides, none of the sample showed homozygous deletion for *CCR5-Δ32* allele (Δ32/Δ32) and R223Q allele (R223Q/R223Q) individuals (Table I).

In Table I, the results showed no association between the *CCR5-Δ32* and HIV susceptibility by using Chi-square test (P>0.05). This indicates the absence of impact of *CCR5-Δ32* allele in protecting population of study from HIV infection. On the other hand, the *CCR5-R223Q* genotype was found to be distributed in a slightly higher frequency in the non-HIV group (6.8%) than the HIV groups (1.7%). In addition, this allele was significantly associated with HIV susceptibility in pooled samples (P=0.018).

Association of CCR5-Δ32 and *CCR5-R223Q* with CD4 recovery

Chi-square test was used to determine the association of *CCR5-Δ32* and *CCR5-R223Q* with CD4 count recovery to ≥500 cells/mm3, as shown in Table II. It was

Table I: Association of CCR5-Δ32 and CCR5-R223Q with HIV serostatus (HIV Susceptibility)

Ethnic groups	HIV serostatus	*CCR5-Δ32* genotype frequency (%)			*32* allele freq	*P*	*CCR5-R223Q* genotype frequency (%)			*R223Q* allele freq	*P*
		WT/ WT	**WT/Δ32**	**Δ32/Δ32**			**WT/WT/**	**WT/R223Q**	**R223Q/R223Q**		
All subjects n=332	HIV n=182	181 (99.5)	1 (0.5)	0 (0.0)	0.003	>0.999	179 (98.3)	3 (1.7)	0 (0.0)	0.008	*0.018*
	Non-HIV n=150	150 (100)	0 (0.0)	0 (0.0)	0.000		138 (93.2)	10 (6.8)	0 (0.0)	0.033	
Malay n=137	HIV n=86	85 (98.8)	1 (1.2)	0 (0.0)	0.006	>0.999	86 (100)	0 (0.0)	0 (0.0)	0.000	0.137
	Non-HIV n=51	51 (100)	0 (0.0)	0 (0.0)	0.000		49 (96.1)	2 (3.9)	0 (0.0)	0.020	
Chinese n=115	HIV n=63	63 (100)	0 (0.0)	0 (0.0)	0.000	-	60 (95.2)	3 (4.8)	0 (0.0)	0.024	0.058
	Non-HIV n=52	52 (100)	0 (0.0)	0 (0.0)	0.000		42 (84.0)	8 (16.0)	0 (0.0)	0.077	
Indian n=80	HIV n=33	33 (100)	0 (0.0)	0 (0.0)	0.000	-	33 (100)	0 (0.0)	0 (0.0)	0.000	-
	Non-HIV n=47	47 (100)	0 (0.0)	0 (0.0)	0.000		47 (100)	0 (0.0)	0 (0.0)	0.000	

Note: p-values determined by the Chi-square test or the Fisher's exact test
Abbreviations: freq=frequency; WT=wildtype

Table II: Association of CCR5-Δ32 and CCR5-R223Q with CD4 recovery

Genotype	t_1			t_2		
	CD4 count <500cells/mm³ (%)	CD4 count ≥500cells/ mm³ (%)	*P*	CD4 count <500cells/ mm³ (%)	CD4 count ≥500cells/ mm³ (%)	*P*
CCR5-Δ32						
WT/WT	120 (75.0)	39 (24.4)	0.250	110 (69.6)	47 (29.7)	0.304
WT /Δ32	0 (0.00)	1(0.6)		0 (0.0)	1 (0.6)	
Δ32/Δ32	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
CCR5-R223Q						
WT/WT	118 (73.8)	40 (25.0)	>0.999	108 (68.4)	47 (29.7)	>0.999
WT/ R223Q	2 (1.3)	0 (0.0)		2 (1.3)	1 (0.6)	
R223Q/ R223Q	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	

Note: *p*-values determined by the Fisher's exact test
Abbreviations: t_1=4-6 months of HAART; t_2=8-12 months of HAART; WT= wildtype

found that the *CCR5-Δ32* and *CCR5-R223Q* were not associated with CD4 recovery to ≥500 cells/mm3 at t1 (P>0.05) and t2 (P>0.05).

Association of CCR5-Δ32 and *CCR5-R223Q* with viral load suppression

As shown in Table III, both *CCR5-Δ32* and *CCR5-R223Q* variants were not associated with viral load suppression to ≤50 copies/mL at t1 and t2 (P>0.05).

DISCUSSION

This study sought to determine the association of *CCR5-Δ32* and *CCR5-R223Q* on HIV resistance or susceptibility. It was shown that *CCR5-Δ32* was not associated with HIV susceptibility. In this study, it was found that *CCR5-Δ32* is a rare mutation in the population of study with allele frequency of 0-0.003. Therefore, it is likely that the very low prevalence of the *CCR5-Δ32*polymorphism in this population could be the reason for the absence of relationship between this polymorphism and HIV susceptibility. Of note, the minor alleles with the frequency of <1-5% had been excluded in several genome-wide association studies (27,28). This indicates that the prevalence of a minor allele in a population is a prerequisite for a genetic association study. Interestingly, one individual with heterozygous *CCR5-Δ32* was found in HIV-infected group. However, this finding cannot exclude the role of *CCR5-Δ32* in protecting HIV infection as previously reported (10).

In pooled sample, *CCR5-R223Q* polymorphisms was associated with HIV susceptibility, probably due to more heterozygous genotype in Chinese ethnic group. This finding suggested that *CCR5-R223Q* allele could potentially protect individuals from HIV infection. However, the association was not significant (P>0.05) in ethnic-specific analysis for Malay, Chinese and Indian. The reason for this could possibly be explained by the low sample number available after segregating the subjects into to their specific ethnicities. Therefore, the result on the protective effect of *CCR5-R223Q* in reducing of risk HIV infection in this study needs to be further validated. Although the mechanism of action that could explain this finding is still uncertain, this *CCR5* variant had been reported to produce a consistent *CCR5* expression or chemokine binding as the wild type *CCR5* (18,20).

The present study also determined the association of

Table III: Association of CCR5-Δ32 and CCR5-R223Q with viral load suppression

Genotype	t_1			t_2		
	VL ≤50 copies/mL (%)	VL >50 copies/mL (%)	P	VL ≤50 copies/mL (%)	VL >50 copies/mL (%)	P
CCR5-Δ32						
WT/ WT	134(80.2)	32 (19.2)	>0.999	149 (91.4)	13(8.0)	>0.999
WT / 32	1 (0.6)	0 (0.0)		1 (0.6)	0 (0.0)	
32/ 32	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
CCR5-R223Q						
WT/WT	132(79.0)	32(19.2)	>0.999	148 (90.8)	12(7.4)	0.222
WT/R223Q	3 (1.8)	0 (0.0)		2 (1.2)	1 (0.6)	
R223Q/R223Q	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	

Note: *p*-values determined by the Fisher's exact test
Abbreviations: t_1=4-6 months of HAART; t_2=8-12 months of HAART; VL=viral load; WT= wildtype

the *CCR5-Δ32* and *CCR5-R223Q* on the two outcomes of HAART, namely CD4 recovery and viral load suppression. However, it was found that the *CCR5-Δ32* was not associated with CD4 recovery in the first year of HAART. This result seems to be consistent with other research which found that the lack of predictive effect *CCR5* genotypes on HIV disease course (29). That study found the effect of *CCR5* genotypes on HIV disease course had diminished after controlling for other factors, including initial CD4 count, initial viral load and the virus phenotype factors (29). In addition, previous studies on the influence of *CCR5-Δ32* on CD4 recovery during HAART have shown some contradiction with positive (25) and negative findings obtained (29,30). These conflicting findings could be related to the different geographical location or study population, for example Caucasians versus Thais population (30). Apart from that, the insignificant result could have been due to the rare occurrence of the *CCR5-Δ32* allele in this study population as mentioned previously.

The *CCR5-R223Q* also showed no association with CD4 recovery in the first year of HAART. To date, no study has reported the association of this variant with CD4 count recovery previously. One study found that *CCR5-R223Q* allele to be distributed in higher frequency in asymptomatic than symptomatic HIV patients (21). However, it was uncertain whether this finding is related to the immune recovery or not since the majority of the long-term non-progressors have the wild-type *CCR5* gene, instead of *CCR5* variants (21). Based on that, it is possible that *CCR5-R223Q* is not an important genetic factor that mediates the CD4 recovery during HAART.

In our attempt to determine the association of the *CCR5* variants with viral load suppression, the viral load ≤50 copies/mL was used as the desired viral load suppression level since the target of HAART is to achieve a complete virological suppression (31,32). Surprisingly, neither *CCR5-Δ32* or *CCR5-R223Q* had the influence on viral load suppression in the first 12 months of HAART. A possible explanation for this might be due to the different parameters used in previous studies. While many studies had reported the impact of *CCR5* on baseline viral load or viral set point (11,33), this study focused on viral load suppression during HAART as the outcome.

Notably, there is only one study that had reported a slightly higher viral load reduction among individuals with a combination of minor alleles of *CCR5-Δ32*, *CCR5-59029A/G, CCR2-641* at 24 weeks the after the HAART (34). However, the *CCR5* mutations of interest are different from this study. Nonetheless, another study had found several other factors that are more superior than the *CCR5* genetic factors in regulating the expression of the *CCR5* receptor and possibly HIV-related outcomes, including DNA methylation and T-cell activation (35). Furthermore, there are other factors that had been associated with delayed viral load suppression, for example non-adherence and poor retention in care (36). The absence of the association of CD4 recovery and viral load suppression implies the effectiveness of HAART in treating HIV infection.

Nevertheless, the results of this study need to be supported with a larger sample size analysis. For example, the significant findings in ethnic-specific analyses need to be interpreted with caution due to the low sample number. Unfortunately, for this study, it was difficult to obtain a larger sample size due to the difficulty in finding subjects with closely monitored CD4 count and viral load to meet the inclusion criteria. Besides, it is also suggested that a longer follow-up of CD4 count and viral load during HAART for three to five years would provide a clearer picture of the effects of the genotypes on the responses to HAART. Finally, further studies are needed to evaluate other common polymorphisms as well as epigenetic factors that could explain the variation in immunological response to HAART particularly.

CONCLUSION

The present study has provided a preliminary findings on the absence of the effect of the *CCR5-Δ32* and *CCR5-R223Q* genetic variants to the CD4 recovery and viral load suppression during early HAART on the samples from three hospitals only. Future study with a larger samples size from different states in Malaysia is needed to support this current finding and to validate the lack of impact of the *CCR5-Δ32* and *CCR5-R223Q* genetic variants in causing variation in immunological response to HAART and other HIV-related outcomes. Besides, it is recommended to evaluate other possible *CCR5* host genetic and epigenetic factors that could influence CD4 count recovery and viral load suppression during HAART, which could potentially provide the insights for precision medicine in HIV treatment.

ACKNOWLEDGEMENTS

We acknowledge Dr. Ahmad Kashfi Ab. Rahman (Hospital Sultanah Nur Zahirah, Terengganu), Dr. Azureen Azmel (Hospital Sungai Buloh), and Dr. Siti Dalila Adnan (Hospital Kajang) for their help in providing HIV subjects for this study. We also thank all medical assistants and staff nurses involved during blood and data collection.

REFERENCES

1. Brelot A, Chakrabarti LA. *CCR5* Revisited: How Mechanisms of HIV Entry Govern AIDS Pathogenesis. Journal of Molecular Biology. 2018 Aug 17;430(17):2557–89. doi: 10.1016/j.jmb.2018.06.027.
2. Barmania F, Pepper MS. C-C chemokine receptor type five (*CCR5*): An emerging target for the control of HIV infection. Applied & Translational

Genomics [Internet]. 2013;2:3–16. doi: 10.1016/j.atg.2013.05.004.
3. Deng HK, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, et al. Identification of a major co-receptor for primary isolates of HIV-1. Nature. 1996;381(6584):661–6. doi: 10.1038/381661a0.
4. Hughes CE, Nibbs RJB. A guide to chemokines and their receptors. The FEBS Journal [Internet]. 2018 Aug 1 [cited 2018 Sep 6];285(16):2944–71. doi:10.1111/febs.14466
5. Molon B, Gri G, Bettella M, Gymez-Moutyn C, Lanzavecchia A, Martнnez-A C, et al. T cell costimulation by chemokine receptors. Nature Immunology [Internet]. 2005 May 10 [cited 2018 Sep 6];6(5):465–71. doi: 10.1038/ni1191.
6. Arts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. Cold Spring Harbor Perspectives in Medicine. 2012;2(4). doi:10.1101/cshperspect.a007161
7. Wang CX, Cannon PM. The clinical applications of genome editing in HIV. Blood. 2016;127(21):2546-2552. doi:10.1182/blood-2016-01-678144
8. Hütter G, Nowak D, Mossner M, Ganepola S, Mьssig A, Allers K, Schneider T, Hofmann J, Kьcherer C, Blau O, Blau IW, Hofmann WK, Thiel E. Long-term control of HIV by *CCR5* Delta32/Delta32 stem-cell transplantation. N Engl J Med. 2009 Feb 12;360(7):692-8. doi: 10.1056/NEJMoa0802905
9. Hoover KC. Intragenus (Homo) variation in a chemokine receptor gene (*CCR5*). PLOS ONE, 2018;13(10):e0204989. doi:10.1371/journal.pone.0204989
10. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, MacDonald ME, Stuhlmann H, Koup RA, Landau NR. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell. 1996 Aug 9;86(3):367-77. doi: 10.1016/s0092-8674(00)80110-5
11. Tang J, Shelton B, Makhatadze NJ, Zhang Y, Schaen M, Louie LG, et al. Distribution of chemokine receptor CCR2 and *CCR5* genotypes and their relative contribution to human immunodeficiency virus type 1 (HIV-1) seroconversion, early HIV-1 RNA concentration in plasma, and later disease progression. Journal of virology. 2002;76(2):662–72. doi: 10.1128/jvi.76.2.662-672.2002.
12. Naranbhai V, Carrington M. Host genetic variation and HIV disease: from mapping to mechanism. Immunogenetics. 2017;69(8–9):489–98. doi: 10.1007/s00251-017-1000-z
13. Stephens JC, Reich DE, Goldstein DB, Shin HD, Smith MW, Carrington M, et al. Dating the Origin of the *CCR5-Δ32* AIDS-Resistance Allele by the Coalescence of Haplotypes. The American Journal of Human Genetics [Internet]. 1998;62(6):1507–15. doi:10.1086/301867
14. Solloch U V., Lang K, Lange V, Bцhme I, Schmidt AH, Sauter J. Frequencies of gene variant *CCR5-Δ32* in 87 countries based on next-generation sequencing of 1.3 million individuals sampled from 3 national DKMS donor centers. Human Immunology. 2017 Nov 1;78(11–12):710–7. doi: 10.1016/j.humimm.2017.10.001
15. Liu Y, Liu F-LL, He Y, Li L, Li S, Zheng Y-TT, et al. The genetic variation of CCR5, CXCR4 and SDF-1 in three Chinese ethnic populations. Infection, Genetics and Evolution. 2012;12(5):1072–8. doi: 10.1016/j.meegid.2012.03.009
16. Liu H, Nakayama EE, Theodorou I, Nagai Y, Likanonsakul S, Wasi C, et al. Polymorphisms in CCR5 chemokine receptor gene in Japan. International Journal of Immunogenetics. 2007;34(5):325–35. doi: 10.1111/j.1744-313X.2007.00694.x
17. Ansari-Lari MA, Liu XM, Metzker ML, Rut AR, Gibbs RA. The extent of genetic variation in the CCR5 gene. Nature Genetics. 1997;16(3):221–2. doi: 10.1038/ng0797-221.
18. Zhao XY, Lee SS, Wong KH, Chan KCW, Ng F, Chan CCS, et al. Functional analysis of naturally occurring mutations in the open reading frame of CCR5 in HIV-infected Chinese patients and healthy controls. Journal of Acquired Immune Deficiency Syndromes. 2005;38(5):509–17. doi: 10.1097/01.qai.0000151004.19128.4a.
19. Carrington M, Kissner T, Gerrard B, Ivanov S, O'Brien SJ, Dean M. Novel Alleles of the Chemokine-Receptor Gene CCR5. The American Journal of Human Genetics. 1997;61(6):1261–7. doi: 10.1086/301645.
20. Capoulade-Mйtay C, Ma L, Truong LX, Dudoit Y, Versmisse P, Nguyen N V., et al. New CCR5 variants associated with reduced HIV coreceptor function in southeast Asia. AIDS. 2004;18(17):2243–52. doi: 10.1097/00002030-200411190-00004.
21. Zheng BJ, Zhao XY, Zhu NS, Chan CP, Wong KH, Chi-Wai Chan K, et al. Polymorphisms of CCR5 gene in a southern Chinese population and their effects on disease progression in HIV infections. AIDS. 2002;16(18):2480–2. doi: 10.1097/00002030-200212060-00016.
22. Gonzalez E, Bamshad M, Sato N, Mummidi S, Dhanda R, Catano G, et al. Race-specific HIV-1 disease-modifying effects associated with CCR5 haplotypes. Proceedings of the National Academy of Sciences of the United States of America. 1999;96(21):12004–9. doi: 10.1073/pnas.96.21.12004.
23. Mangano A, Gonzalez E, Dhanda R, Catano G, Bamshad M, Bock A, et al. Concordance between the CC chemokine receptor 5 genetic determinants that alter risks of transmission and disease progression in children exposed perinatally to human immunodeficiency virus. The Journal of infectious diseases. 2001;183(11):1574–85. doi: 10.1086/320705.
24. Catano G, Chykarenko ZA, Mangano A, Anaya

JM, He W, Smith A, et al. Concordance of CCR5 genotypes that influence cell-mediated immunity and HIV-1 disease progression rates. Journal of Infectious Diseases. 2011;203(2):263–72. doi: 10.1093/infdis/jiq023.
25. Ahuja SK, Kulkarni H, Catano G, Agan BK, F J, He W, et al. CCL3L1-CCR5 genotype influences durability of immune recovery during antiretroviral therapy of HIV-1–infected individuals. Nature medicine. 2009;14(4):413–20. doi: 10.1038/nm1741.
26. Bhatnagar I, Singh M, Mishra N, Saxena R, Thangaraj K, Singh L, et al. The Latitude Wise Prevalence Of The CCR5- Δ 32-HIV Resistance Allele In India. Balkan Journal of Medical Genetics. 2009;12(2):17–27. doi:10.2478/v10034-010-0001-0
27. McLaren PJ, Coulonges C, Bartha I, Lenz TL, Deutsch AJ, Bashirova A, et al. Polymorphisms of large effect explain the majority of the host genetic contribution to variation of HIV-1 virus load. Proceedings of the National Academy of Sciences of the United States of America. 2015;1–6. doi: 10.1073/pnas.1514867112.
28. Fellay J. Host genome influences on HIV-1 disease. 2010;14(6):731–8. doi: 10.3851/IMP1253
29. Taylor JMG, Wang Y, Ahdieh L, Chmiel JS, Detels R, Giorgi J V., et al. Causal pathways for CCR5 genotype and HIV progression. Journal of Acquired Immune Deficiency Syndromes. 2000 Feb 1;23(2):160–71. doi: 10.1097/00126334-200002010-00008.
30. Ammaranond P, Sanguansitthianan S, Phaengchomduan P, Sae-Lee C, Mardkhumchan S. Impact of CCR2 and SDF1 Polymorphisms on Disease Progression in HIV-Infected Subjects in Thailand. Journal of Clinical Laboratory Analysis. 2013;27(1):38–44. doi: 10.1002/jcla.21559
31. Medical Development Division. Malaysian Consensus Guidelines on Antiretroviral Therapy 2017. Ministry of Health Malaysia. Ministry of Health Malaysia; 2017. Available from: https://www.moh.gov.my/moh/resources/auto%20download%20images/589d71c4dd799.pdf
32. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. 2nd ed. Geneva: World Health Organization; 2016. PMID: 27466667. Available from: https://pubmed.ncbi.nlm.nih.gov/27466667/.
33. Malhotra R, Hu L, Song W, Brill I, Mulenga J, Allen S, et al. Association of chemokine receptor gene (CCR2-CCR5) haplotypes with acquisition and control of HIV-1 infection in Zambians. Retrovirology. 2011;8(1):22. doi: 10.1186/1742-4690-8-22
34. O'Brien TR, McDermott DH, Ioannidis JP, Carrington M, Murphy PM, Havlir D V, et al. Effect of chemokine receptor gene polymorphisms on the response to potent antiretroviral therapy. AIDS (London, England). 2000;14(7):821–6. doi: 10.1097/00002030-200005050-00008.
35. Gornalusse GG, Mummidi S, Gaitan AA, Jimenez F, Ramsuran V, Picton A, et al. Epigenetic mechanisms, T-cell activation, and CCR5 genetics interact to regulate T-cell expression of CCR5, the major HIV-1 coreceptor. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(34):E4762-71. doi: 10.1073/pnas.1423228112.
36. Mugavero MJ, Amico KR, Westfall AO, Crane HM, Zinski A, Willig JH, et al. Early retention in HIV care and viral load suppression: Implications for a test and treat approach to HIV prevention. Journal of Acquired Immune Deficiency Syndromes. 2012;59(1):86–93. doi: 10.1097/QAI.0b013e318236f7d2.