



## REVIEW ARTICLE

# Genetic variation in the chemokine receptor 5 gene and course of HIV infection; review on genetics and immunological aspect

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Received 15 February 2020; received in revised form 4 April 2020; accepted 12 April 2020

Available online 18 April 2020

## KEYWORDS

Acquired immunodeficiency syndrome; Chemokine and chemokine receptors; Human immune deficiency viruses; Single nucleotide polymorphism

**Abstract** Chemokines are small protein molecules associated with various physiological events precisely in immune modulation via chemokine receptors. The chemokine receptors are G-protein coupled receptors express mainly on the cell surface of immune cells. Retroviruses, including HIV in the early stage of infection, primarily target chemokines receptors and get internalized easily into immune cells; T cell and escape from immune surveillance. HIV glycoprotein selectively develops an affinity for the extracellular domain of chemokines receptors and allows the pathogen to internalize via CCR-5. Now, CCR-5 remains a crucial signaling pathway that can be translated into the therapeutic target by changing the receptor protein environment. Many populations have a mutation in coding and promoter regions of CCR-5, tuning a resistance for HIV infection. Natively, there are several mechanisms where the human genome remains in the dynamic state by changing its composition and acquiring variations. Single nucleotide polymorphism is spontaneous phenomenon responsible for precise and point mutation at the genome. Several studies have demonstrated that European and African American populations are enriched in significant CCR5 promoter SNP (CCR5 $\Delta$ 32) in the coding and promoter region as well. Now, such SNP can be an early-stage biomarker in studying HIV and other similar infections. Here, in this study, we have elucidated the role of SNP (both the promoter and coding region) and the fate of HIV infections. We also empathized with the genetics of such SNPs, mostly frequency and its immunological impact.

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Peer review under responsibility of Chongqing Medical University.

## Introduction

Over the last 40 years since the discovery of HIV and associated disease, i.e., AIDS in the 1980s, the death rate remains high in developing countries, and more than 32 million people died worldwide. Among retroviruses, human immune deficiency virus (HIV) remains a major global health issue and infects millions of people worldwide annually.<sup>1</sup> HIV selectively target immune cells; T cells (CD4 T helper cell) and reside there for a long time; as a result, innate immune surveillance mechanism fail to identify infection and pathogen entry.<sup>2</sup> The unique ability of HIV to bypass immune surveillance entirely relies on glycoprotein mimic ligand for CCR-5 (early stage) and CXCR in advance stage.<sup>3,4</sup> In the advanced stage, HIV starts copying itself using reverse transcription as it comprises of RNA as genetic material. HIV-infected CD-4 T cell undergoes a rapid cell death via programmed cell death mechanism and at the same time decreased the production of immune cells further weakens immunity.<sup>5</sup> However, HIV loaded CD 4 T cell programmed cell death mechanism remains unclear. Still, several reports have demonstrated that such cells are much prone to programmed cell death via an indirect mechanism, i.e., the formation of syncytia and apoptosis subsequently.<sup>6,7</sup> Hence, the gradual decrease in CD4 T cells in untreated HIV infection patients affects both adaptive and acquired immunity leading to severe malignancy called AIDs.<sup>8</sup> In a chronic condition, patients become prone to other infections, including bacterial and fungi, as a pathological outcome from decreased CMI.<sup>9</sup>

HIV is a retrovirus, and its genome is comprised of RNA. Retroviruses also contain an enzyme called reverse transcriptase, which allows the retrovirus's genetic material to imitate the host cell's genetic material.<sup>10,11</sup> Human immunodeficiency virus (HIV) grouped to the genus lentivirus within the family of Retroviridae, subfamily Orthoretrovirinae. Epidemiologic and phylogenetic analyses currently available imply that HIV was introduced into the human population around 1920 to 1940.<sup>12,13</sup> HIV-1 evolved from nonhuman primate immunodeficiency viruses from Central African chimpanzees (SIVcpz) and HIV-2 from West African sooty mangabeys.<sup>14</sup> The HIV genome is nearly 9 kb (9.2 kb in HIV-1 and 9,6 kb in HIV-2) with significant genes within the genome of HIV lies in between LTR (3' and 5') are polymerase (RT), gag (group-specific antigens) and env (genes coding for envelope).<sup>15</sup> Further, HIV also possesses *tat* (trans-activation of transcription), *rev* (regulator of virion), *nef* (negative factor), *vpr* (viral protein r), *vif* (viral infectivity factor), and *vpu* (viral protein u).<sup>16</sup> The HIV genes are highly specific and robust, encode several proteins essential for viral replication and infection. The pol gene encodes three proteins; protease, RT, and integrase, while gag is responsible for condign all the protein for capsid.<sup>17</sup> Additionally, HIV acquired extra protection by encoding protective lipids by env.

## Chemokines receptors and physiological significance

Chemokines are small protein molecules with immense physiological significance precisely in immunomodulatory

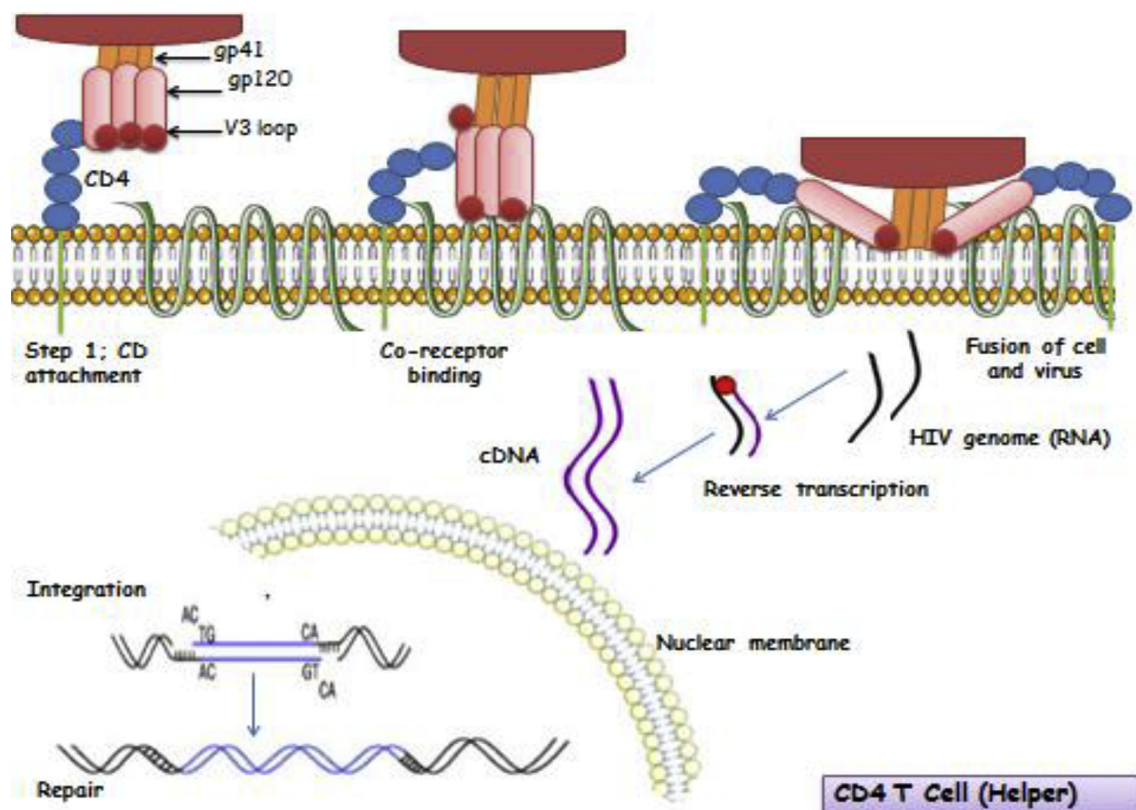
activities.<sup>18</sup> Chemokine receptors are G-protein coupled receptor containing seven transmembrane domains. These seven domains span plasma membrane where C-terminal is attached to G protein (cytosolic), and the N-terminal region lies in the extracellular region.<sup>19</sup> The chemokines receptors are primarily express over the cell surface of macrophages, dendritic cells, and memory T cells. However, these receptors are also typical in the endothelium, epithelium, vascular smooth muscle and fibroblasts, and microglia, neurons, and astrocytes in the central nervous system.<sup>20</sup> Here, the unique pattern of Asp-Arg-Tyr (DRY motif) plays critical roles in the determination of receptor conformations for signaling and intracellular trafficking of GPCR by forming intermolecular interactions.<sup>21</sup> The C-terminal domain of the Chemokine receptor contains serine and threonine acts as a potential site for phosphorylation during receptor regulation.<sup>22</sup> Further, the N-terminal domain acts as a ligand-binding site to target by many pathogens, including retroviruses such as HIV. Its cysteine defines chemokines and classified as C, CC, CXC, and CX3C, while chemokines receptors are denoted with additional R with a chemokine.<sup>23</sup>

G Protein-Coupled Receptor (GPCR) represents one of the most crucial signal transduction receptor families associated with many physiological processes. Chemokines receptor is one of a group of GPCR family, i.e., rhodopsin-like GPCR.<sup>24</sup> Chemokines receptors are further subclassified, including CCR1, CXCR1, CCR5, XCR1, etc. Over the last few decades, nearly 20 chemokines receptors have been identified and characterized for their involvement in different physiological signaling pathways.<sup>25</sup> If we examine proteomics of chemokines, it's a single peptide of 352 amino acids with a molecular weight of 40.6 kDa.<sup>26</sup> Considering chemokines receptor signaling, it purely depends on G proteins in the cytosolic region. A few retroviruses, including HIV, selectively utilized the unique pattern of CCR-5 for its internalization into T cells.<sup>27</sup> The HIV two glycoprotein receptors, gp120 and gp41 (designated gp160), possess a high affinity for CCR-5 and allow pathogen binding and internalization<sup>28,29</sup> (Fig. 1).

## Chemokine receptor-mediated signaling

HIV finds an opportunity to get internalize via chemokines receptor (CCR 5 in early-stage and CXCR4 in advance stage) in conjugation with CD4.<sup>30</sup> In the course of the first infection, the human macrophage expressing CCR5 and CXCR4 are primarily targeted by HIV. The primary interaction between viral envelope surface glycoprotein gp120, the cell-surface protein CD4, and a chemokines receptor CCR5 or CXCR4 start the complex process involved in the internalization of the pathogen in the cell.<sup>31</sup> It has been demonstrated that HIV-1 gp120 and chemokines activate ion channels in primary macrophages through CCR5 and CXCR4 stimulation.<sup>32</sup> The HIV gp120, a dynamic envelope protein utilizes chemokines receptors as co-receptor for pathogen binding, and several conformational changes occur during the interaction.<sup>33</sup> The gp120 and CD4 interaction lead to conformational changes in the viral envelope and facilitate membrane fusion.<sup>34</sup> Further, gp120 binds to integrin  $\alpha_4\beta_7$  activating LFA-1, the central integrin involved in the





**Figure 2** The figure demonstrates the detailed mechanism and the role of chemokine signaling in HIV infection. Here, a chemokine receptor that acts of co-receptor for binding of HIV envelope using gp120 and V3 loop mainly stabilizes interaction. While the CD4 molecule act as primarily attachment molecule present on the cell surface of T cells (T helper cells).

specific single nucleotide polymorphism (<http://ranger.uta.edu/~heng/Longitudinal/>). Likewise, several online tools and software are available to identify polymorphism in the genome and their link for particular diseases (<https://omictools.com/statistical-analysis-category>).

## Significance of SNPs

The SNP is a key biomarker in the early diagnosis of various diseases and metabolic disorders. As reported in the study, SNP leads to change in disease outcome as rs333 (Decreased susceptibility), rs1799987 (Fast progression), rs1800023 (Slow progression), rs1800024 (Slow progression), rs2734648 (Slow progression) and rs1799988 (Slow progression).<sup>47</sup> Many SNPs have also been shown to affect the development and predisposition of disease.<sup>48</sup> The site of SNP remains crucial; one if SNPs in the coding region alters mRNA transcription and amino acid in translating protein, which could be a marker for identifying a disease in the early stage.<sup>49</sup> Second and most important, SNP in the promoter region has a significantly larger impact on protein expression, either up-regulation and or down-regulation.<sup>50</sup> The SNPs which are associated with diseases precisely are key markers brought to pharmacogenomics studies for therapeutic interventions.<sup>51</sup> The functional genomics largely utilizes SNPs as a tool for the study of drug response (pharmacodynamics studies). There is a preponderance of data suggesting the role of SNPs in cancer (all major cancer

and tumor including breast, colon, colorectal, myeloma, thyroid and lung etc.), vascular disorders, immunological disorders and metabolic disorders.<sup>52–54</sup> Considering intensive research findings in the last decade, SNPs have a direct impact on the pathophysiology of various infectious diseases, including hepatitis, leprosy, tuberculosis, AIDs, malaria, and meningitis etc.<sup>55–57</sup> As a result, the SNPs database has been built after complete genome analysis demonstrate the role of SNPs in the onset of disease and metabolic disorders in human.<sup>58</sup>

## SNPs in chemokine gene

Within human genome, two significant clusters of chemokine receptor genes were identified on chromosome 3 encoding all major chemokine receptor proteins including CCR 2, CCR 3, CCR 4, CCR 5, CCR 7, CCR 8, CCR 10, CCR 11, CCR 12, CCR 20 and CCR 21.<sup>59</sup> Among these genes, CCR 5 reported as a significant co-receptor in HIV infection. The CCR 5 gene contains four exon and two introns with two promoters; one-week promoter (upstream) and one strong promoter (downstream).<sup>60</sup> The most phenomenon genetic variation in CCR5 gene; polymorphism at CCR5 promoter region as Delta 32 ( $\Delta 32$ ) mutation was reported (rs333) (CCR5 $\Delta 32$ ) protect the individual group.<sup>61,62</sup> In both cases, either SNP lies at the promoter and or at the coding region of the CCR5 gene, the expression of the receptor protein (CCR5) will be altered and or inhibited completely.<sup>63</sup> The

altered protein (CCR5) will not be active ultimately and simultaneously, affect (minimize) HIV infections.<sup>64</sup> Looking more precisely, SNPs in the coding region (A77G, G316A, T532C, C921T, and G668A) (mutation) will primarily change protein structure (due to the incorporation of wrong amino acid) that further affects localization, transport, chemokines binding and signaling of the CCR5 receptor.<sup>65,66</sup> However, if polymorphism will be reported in the promoter region, it primarily affects binding DNA transcription factors and aberration in mRNA formation.<sup>67</sup> In both cases, binding of HIV gp120 to the host cell will be altered based on the site of polymorphism.

The CCR 5 single nucleotide polymorphism remains high throughput research concern worldwide parallel with HIV infections.<sup>59</sup> Over a period of time, several racial and ethnic populations were profiled for CCR5 polymorphism. These studies include polymorphism in the coding and promoter region of CCR5.<sup>68</sup> Gonzalez, E *et al*, 2001 have conducted a global survey for genetic variations in CCR5 and possible ligands, more precisely RANTES, and MIP-1alpha. Their finding suggested a different distribution of RANTES haplotypes (AC, GC, and AG).<sup>69</sup> They have identified an association of RANTES haplotype in the selected population (European Americans) with an increased risk of acquiring HIV-1 as well as accelerated disease progression in European Americans. However, African Americans were reported in acquiring resistance to HIV-1 infection due to a particular AC haplotype. Further, the Japanese cohort, with an AC haplotype, has shown a distinct phenotype associated with delayed HIV-1 infection. Similarly, Mikawa *et al*, 2002 studied Brazilian populations for CCR5 polymorphism and plasma  $\beta$  Chemokines in HIV-1 infection.<sup>70</sup> Their finding differs from Gonzalez *et al*, in correlating a link between plasma  $\beta$  Chemokines (RANTES, and MIP-1alpha). Such variations could be due to multifactorial HIV infections and dependence on different racial and ethnic populations.

India is the third-largest home for HIV-1 patients and has significant HIV growth within the Indian population. The increased HIV-1 infection and subsequent AIDS linked genetics as driving force.<sup>71</sup> In a study, Gupta and Harish (2015) profiled CCR5 and SDF1 genetic variations in the Indian population and analyzed links in HIV-1 infections.<sup>72</sup> In the study, Gupta and Harish targeted SDF1-3'A, CCR5 $\Delta$ 32, and CCR5 promoter polymorphism at selected positions, including 58934 G/T, 59029 G/A, 59353 T/C, 59356 C/T, 59402 A/G and 59653 C/T within Indian population. They conclude Indian people probably more susceptible to HIV-1 infection as the most common CCR $\Delta$ 32 mutation was utterly absent. Further, nine haplotypes with more than 1% frequency identified were not significant in their protective role against HIV-1.<sup>73</sup> However, within Indian populations, southern India has a comparative larger impact of HIV-1 infection. Four states, including Tamil Nadu, Andhra Pradesh, Telangana, and Karnataka, collectively account for half (50%) cases of HIV -1 infection in India.<sup>74</sup> There are limited studies in the Indian population precisely looking haplotype screening of CCR5 and role in the onset and progression of HIV-1 infection. The similar patterns of CCR5 polymorphism were reported in native African people.

Joshi *et al*, 2017 studied CCR 5 promoter activity in different haplotypes under polymorphism, considering CD 4 T cell apoptotic activity in HIV infected patients.<sup>75</sup> The

study concludes a relative CCR 5 promoter activity (CRPA) in HIV patients regulates CD4 cell loss. Gonzalez, E. *et al*, 1999 has identified 8 CCR5 promoter haplotypes as a result of single nucleotide polymorphisms in CCR 5 promoter region.<sup>76</sup> They have estimated CCR5 haplogroup frequencies in different racial and ethnic groups, including African, African American, Asian Caucasian, and Hispanic Americans considering HIV infected and uninfected patients. The major CCR5 haplotypes reported are HHA, HHC, HHD, HHE, HHF1, HHF 2, HHG1, and HHG2. Now, the study demonstrates all these haplotypes varies in different race and ethnic populations towards HIV infections.<sup>77</sup> In general, haplogroup C (HHC) and haplogroup E (HHE) were identified comparatively higher in HIV infected patients in all the races, and ethnic populations studied. These findings further support Joshi *et al*, work concludes different CCR5 promoter transcriptional activity in different haplotypes.

### CCR5 $\Delta$ 32 mutations and HIV resistance

CCR5 delta 32 SNPs and association with the HIV susceptibility remain a key area of research and scientific debates. Grown scientific findings and conclusive outcomes suggest SNPs/mutations CCR5 delta 32 differ in the human ethnic population. In a meta-analysis, Ni *et al* 2018 demonstrated that CCR5 delta 32 homozygous offer resistance against HIV infection over heterozygous genetic orientation.<sup>78</sup> The CCR5 delta 32 mutations vary in different human populations. Novembre *et al* 2005 studied patterns and frequency of CCR5 delta 32 HIV resistance alleles in different populations.<sup>79</sup> The study also concludes such mutations are more common in Europe and western Asia. Homozygous carriers of the Delta32 mutations remain associated with higher resistance against HIV infections with lower expression of CCR5 chemokine receptors. The expression of CCR5 chemokine receptor and SNP/mutations in CCR5 Delta 32 are closely associated. It is also important to note here mutations are random, and selection of such mutations among various human populations defines resistance against HIV. Adojaan *et al* 2007 investigated the higher frequency of CCR5Delta32 HIV-resistance mutations in the northern European population.<sup>80</sup> Targeting CCR5 chemokine receptors has shown a promising result in HIV infection management. Hutter *et al*, 2015 have demonstrated that allogeneic transplantation of CCR5-delta 32 homozygous stem cells in HIV patients offers a practical and robust approach to control HIV infection and disease burden.<sup>81</sup> This could be achieved via either using CCR5 negative stem cells and or CCR5 knockout cells. However, such studies require validation in larger populations of different human ethnic groups. The second approach altering CCR5 availability for HIV gp120 protein attachment via inhibitors and or antibodies might not be feasible as CCR5 associated with several vital cellular processes.<sup>82</sup>

### Genetics and immunological attributes of polymorphism in CCR5

The CCR5 $\Delta$ 32 allele containing a homozygous individual completely lacks a functional CCR5 receptor and hence

provides resistance to HIV infection.<sup>83</sup> On the contrary, heterozygous individuals will have a moderate activity of HIV infection as lower expression and activity of CCR 5. The Delta 32 variant of rs333 is also associated with a higher risk of abdominal aortic aneurysm (AAA).<sup>84</sup> The population genetic studies have demonstrated that CCR5Δ32 is a universal and most predominant genetic variation in different population groups acquire and develop resistance towards HIV infection. The encoded CCR 5 protein (functional CCR5) N-terminal region is enriching in tyrosine and acidic amino acids that play an essential role in both HIV interaction and chemokines binding.<sup>85</sup> A polymorphism in the CCR5 coding region will have a more substantial impact not only in binding with HIV HIV-1 gp120 but also natural ligand (MIP-1alpha, RANTES).<sup>86</sup> Now, under polymorphism at CCR5 will be unable to bind its ligands and disable several critical immunological events such as recruitment of leukocytes into inflammatory sites.<sup>87</sup> The patients containing such phenotype will be unable to activate granulocytes (neutrophils, eosinophils, and basophils).<sup>88</sup> Such phenotype will also be unable to synthesis and release of other pro-inflammatory cytokines such as IL-1, IL-6, and TNF- $\alpha$  from fibroblasts and macrophages.<sup>89</sup>

## The diagnostic and therapeutic relevance

Genomic instability has been characterized as a critical trigger toward the onset and progression of several human diseases and metabolic disorders.<sup>90</sup> Further, it is the signature patterns of a sequence such as alterations, frequency, site (regulatory, coding, and or noncoding region), etc. resulting in an allelic imbalance. Several reports have demonstrated how useful SNPs are in correlating with a particular phenotype. Erichsen and Chanock, 2002 confirmed risk assessment in breast cancer based on SNPs analysis, and subsequent meta-analysis further validated their findings.<sup>91</sup> The presence of CCR5 promoter haplotype can be used as the first biomarker for susceptibility and resistance to HIV-1 infection. However, finding a therapeutic relevance of CCR5 promoter polymorphism may need a large scale population study to ensure the exact pattern of haplotype and common conserved haplotypes.<sup>59</sup> There are growing pieces of evidence that the use of natural CCR5 ligand mimics could find an alternate approach provided immunological functions remain unaffected. Such competitive natural CCR5 ligand inhibitors effectively compete with HIVgp120 for receptors with the least immune damage.

## Conclusions

Chemokines signal are vital for many physiological events, including immune and inflammatory responses, wound healing, and angiogenesis. The chemokine receptors with appropriate ligand play a crucial role in the activation and mobilization of immune cells. HIV pathogenesis is multifactorial, involving both CD4 and CCR5 as a coreceptor for the infection. The single nucleotide polymorphism results in the aberrations in CCR 5 receptor protein disable

binding of gp120 and hence the entry of the virus into the host cell. It's noteworthy to understand the effective inhibition of HIV entry relies on the site single nucleotide polymorphism. Several approaches have been made over some time to utilize such genomic stability (polymorphism) as a potential target against HIV infections. It is evident considering extensive studies on population genetics that the rise in CCR5Δ32 mutations at the promoter region of CCR 5 gene offers resistance against HIV infection. It is evident that CCR5Δ32 mutations differ in the human population, and the selection of such mutations can lead to an HIV infection resistant population. On the contrary, these studies required validation on a large scale to find a close relation between CCR5Δ32 mutations and resistance to HIV infection. Research finding has demonstrated CCR5 knockdown CCR5 stem cells could be a possible cure for HIV. There is growing concern and emphasis on developing CCR5 deficient stem cells and clinical uses.

## Conflict of Interests

The author declares no conflict of interest.

## Acknowledgements

The author would like to acknowledge the Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India for the kind support, and providing a facility for the proposed study.

## Abbreviations

AIDs	Acquired Immunodeficiency Syndrome
HIV	Acquired immune deficiency viruses
CCR	Chemokines receptor
G-PCRs	G-protein coupled receptor
gp	Glycoprotein
SNPs	Single nucleotide polymorphism
CMI	Cell-mediated Immunity
HI	Humoral Immunity
RT	Reverse transcriptase
CD	Cluster of differentiation
CRPA	CCR5 Relative Promoter Activity
RANTES	Macrophage inflammatory protein (MIP)-1alpha (MIP) and Regulated on Activation Normal T Cell Expressed and Secreted
rSNPs	Random SNPs
gSNPs	gene-associated SNPs
coding SNPs	(cSNPs) and phenotype-relevant SNPs (pSNPs)

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