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# The VNTR polymorphism of the CLEC4M gene and susceptibility to HIV-1 infection in Han Chinese population

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ABSTRACT

C-type lectin domain family 4, member M (CLEC4M, also known as DC-SIGNR) is a C-type lectin that functions as a transreceptor for human immunodeficiency virus-1 (HIV-1). The relationship between variable number tandem repeat (VNTR) polymorphism of the DC-SIGNR gene and susceptibility to HIV-1 infection has been under debate. In the present study, a cohort of 287 HIV-1 seropositive patients and 388 ethnically age-matched healthy controls from Han Chinese population were enrolled in order to determine the influence of host genetic factors on HIV-1 infection. A total of 11 genotypes and 5 alleles were found in our population. A cross-sectional comparison between HIV-1 seropostive patients and healthy controls did not reveal significant differences with regards to DC-SIGNR genotype distribution, allele frequencies and homozygotes proportion. In addition, previous studies showed that DC-SIGNR might play different roles in different HIV infection routes. We stratified the patients into two subgroups: sexual contact patients and intravenous drug abuser/blood transfusion patients. Our results showed the frequencies of DC-SIGNR genotypes/alleles in these two subgroups were similar. To our knowledge, this is the first study performed in Northern Chinese. Our findings suggested that DC-SIGNR neck region VNTR polymorphism was not directly associated with hosts' predisposition for HIV-1 infection and not associated with the HIV-1 routes of infection. By lack of HIV-1 exposed seronegative (HESN) individuals and relative small sample size in present study made our conclusions not strong enough. In addition, the role of the DC-DIGNR neck region in different HIV-1 infection routes remains open for future study.



## 1. Introduction

The incidence of acquired immunodeficiency syndrome (AIDS) has increased over the past few decades. Up to now, nearly 34 million people suffered from human immunodeficiency virus-1 (HIV-1) infection, and an estimated 1.8 million people die every year from HIV/AIDS (http://www.who.int/features/factfiles/hiv/facts/en/index3.html). But the natural course of HIV-1 infection is highly heterogeneous among individuals (Cao et al., 1995; Pantaleo, 1997). Polymorphisms in host genes involved in HIV-1 virus replication or immune regulation play an important role in resistance to HIV-1 infection and in the rate of disease progression (Tang and Kaslow, 2003). Chemokine receptors act with CD4 as HIV-1 coreceptors to mediate the first step in cell entry. Being necessary coreceptors for HIV-1 entry, chemokine receptors have already attracted much attention in HIV-1 related researches. Up to now, many polymorphisms of chemokine receptors and their natural ligand genes have been reported to modify HIV-1 transmission and disease progression, such as CCR5, CCR2, SDF-1, RANTES and CXCR4 (Kristiansen et al., 1998; O'Brien and Moore, 2000). Among them, the most investigated variation is the 32-bp deletion in the chemokine receptor-5 (CCR5 Δ32) gene, which was shown to confer resistance to HIV-1 infection in homozygous carriers, and its role has been investigated in a clinical context (Kasten et al., 2000; Liu et al., 1996). However, it could only account for a small fraction of the hosts' resistance to HIV-1 infection especially in China since the CCR5 Δ32 allele is rare among Asians (Jiang et al., 1999; Martinson et al., 1997). Thus the possible genetic mechanism underlying the resistance presently remains elusive.

Dendritic cell-specific intracellular adhesion molecular-3-grabbing nonintegrin (DC-SIGN /CD209) is a prototype C-type lectin and is abundantly expressed primarily on dendritic cells (DC). As a receptor on dendritic cells (DC), it can bind the HIV-1 gp120 surface protein with high affinity at mucosal sites and then enhancing trans-infection of CD4+ T cells in regional lymph nodes (Geijtenbeek et al., 2000; Liu et al., 2005). C-type lectin domain family 4, member M (CLEC4M, also known as DC-SIGNR, or CD209L/

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CLEC4M/DC-SIGN2/L-SIGN/CD299) is a homologue of DC-SIGN with 77% amino acid identity and preferential expression in liver and lymph node epithelial cells. DC-SIGNR functions similarly to DC-SIGN in capturing HIV-1 and enhancing HIV-1 infection of T cells (Pohlmann et al., 2001).

Both DC-SIGN and DC-SIGNR are organized into 3 domians: (1) an N-terminal cytoplasmic region with a di-leucine motif for internalization followed by a transmembrane domain, (2) a C-terminal extracellular domain with a C-type carbohydrate recognition domain (CRD) involved in pathogen binding, and (3) a neck-region containing variable number tandem repeat (VNTR) of conserved subunit of 23-amino-acid sequence, that connects the CRD to the transmembrane region. The neck-region is involved in assembling the lectin into a tetrameric protein conformation on the cell surface, which is believed to be required for efficient recognition of multivalent ligands, such as HIV-1. And the length variation of this neck-region had been proposed to affect the pathogen-binding properties of the CRD of these proteins (Feinberg et al., 2005; Soilleux et al., 2000). Although the VNTR in DC-SIGN is highly conserved (7 repeats is found in >90% in the population), DC-SIGNR neck-region exhibits a higher level of heterozygosity due to presence of 4- to 9-repeats in substantial frequencies in the populations. Since the highly polymorphic of VNTR of the neck-region in DC-SIGNR gene, some studies have been done to detect the associations between VNTR polymorphism and hosts' susceptibility to various infectious diseases, such as HIV-1, SARS and Hepatitis C, though conflicting results were found (Chan et al., 2006; Li et al., 2008; Nattermann et al., 2006; Tang et al., 2007; Zhao et al., 2008b; Zhi et al., 2007). Among them, the association between this polymorphism and host genetic predisposition to HIV-1 infection was the most widely studied and carried out in different ethnic worldwide populations (Boily-Larouche et al., 2009; Chaudhary et al., 2008; Li et al., 2012; Lichterfeld et al., 2003; Liu et al., 2006; Rathore et al., 2008; Wang et al., 2008, 2010; Wichukchinda et al., 2007; Xu et al., 2010; Zhao et al., 2008a, 2008b). Yet, the conclusions are controversial and unclear at present.

Chinese account for more than one fifth of the world's population, and the emerging HIV-1 epidemic in China is rapidly increasing. Although several studies have been conducted in China populations to detect the association between DC-SIGNR VNTR polymorphism and host's susceptibility to HIV-1 infection (Wang et al., 2008, 2010; Xu et al., 2010; Zhao et al., 2008a, 2008b), the results are incomprehensive and the studied populations are confined to the Southern Chinese. In addition, since the DC-SIGNR transcripts of spliced isoforms were detected in the genital and rectal mocosa and the DC-SIGNR on DCs derived from PBMC was expressed at lower levels, we proposed that the DC-SIGNR might play different roles in different HIV-1 infection routes. Thus, in this study, a cohort of 287 HIV-1 seropositive patients and 389 matched healthy individuals from Anhui province of Han China population were recruited. We determined the distribution of DC-SIGNR neck region VNTR polymorphism and analyzed the relationship between this polymorphism and host susceptibility to HIV-1 infection in Han China population. Meanwhile, we also divided the HIV-1 seropostive patients into two subgroups according to their different HIV-1 routes of infection to test the relationship between this polymorphism and the HIV-1 routes of infection in Han China population.

## 2. Materials and methods

### 2.1. Subjects

Two hundred and eighty-seven HIV-1 seropositive patients were enrolled from the drug rehabilitation center and prison of Anhui Province. HIV-1 infection status was determined by ELISA and confirmed by Western blot analysis. Three hundred and eighty-nine age-matched controls were healthy blood donors from Anhui Blood Center. As Chinese is a heterogeneous population, the population stratification can easily cause us to draw false-positive or false-negative conclusions in population-based association study. All the subjects, including patients and controls, were Chinese Han ethnicity and selected from the same source population, Anhui province of China. The demographic parameters of the HIV-1 seropositive patients and control group were listed in Table 1. All participants signed informed consent after they had been given a clear explanation of the potential risk of the study according to the Declaration of Helsinki. The research has been approved by the Ethics Committee on human experimentation of Southeast University.

### 2.2. Genotyping and statistical methods

Genomic DNA was extracted from peripheral blood using DNA extraction kit according to the manufacturer's instruction (Omega, USA). The VNTR polymorphism of the neck-region was genotyped as described in Li et al. (2008). The genotype was determined by separating the PCR products in 3% agarose gel with ethidium bromide staining. To validate the genotyping results, 10% of the samples were re-genotyped by duplicated genotyping experiments. Genotyping of CCR5 Δ32 deletion was done as described in Kristiansen et al. (2001).

The Hardy–Weinberg equilibrium (HWE) test for VNTR in the HIV-1 seropositive patients and control groups was performed by GENEPOP software (http://wbiomed.curtin.edu.au/genepop/index.html). Statistical analysis of genotype distribution, homozygote proportions and allele frequencies were performed by a chi-square (for $n > 5$) or Fisher's exact test (for $n < 5$) (SPSS for windows 19.0, SPSS Inc.). The $p$ value is two tailed and the cut off value is 0.05. For correction of multiple tests, when $p < 0.05$, it is subjected to Bonferroni's correction.

## 3. Results and discussion

We genotyped the VNTR polymorphism of DC-SIGNR neck region among 287 HIV-1 seropositive patients and 389 matched healthy controls in Han Chinese. The homozygote proportion, genotype and allele frequencies of the VNTR were listed in Table 2. At the same time, genotyping of CCR5 Δ32 deletion was done to rule out any possibility of protection conferred by CCR5 Δ32 deletion in all subjects. One individual from healthy control group who was heterozygous for CCR5 Δ32 deletion was excluded for further analysis. Five alleles were observed in our cohorts, and these different alleles form 11 genotypes. The allele 7 was detected frequently with the frequency of 64.95%, followed by allele 5 of 18.56%, allele 9 of 10.45% and allele 6 of 3.61%. The most common genotype was the 7/7 repeat, followed by 5/7 and 7/9 repeats, which were comparable to the distributions for China population that have been reported elsewhere (Li et al., 2009). Both HIV-1 seropositive patients and control groups did not deviate from the Hardy–Weinberg

**Table 1**
Demographic and clinical characteristics of the study cohorts.

| Parameters | Healthy Individual | HIV+ seropostive patiemts |
|---|---|---|
| Number | 388 | 287 |
| Age (median, range) | 36 years (20–59) | 32 years (24–61) |
| Sex (M/F ratio) | 3.2/1 | 3.0/1 |
| Mode of infection | | |
| Sexual contact | – | 180 |
| Intravenous drug abuser | – | 75 |
| Blood transfusion | – | 8 |
| Unknown | – | 24 |