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Polymorphism characteristics of HIV-1 gp120 and 5 hypervariable regions

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Background/aim: To investigate polymorphism characteristics of HIV-1 gp120 and its 5 hypervariable regions.

Materials and methods: Length polymorphism, potential number of N-linked glycosylation sites (PNGSs), and sequence characteristics of nearly all available global gp120 and its 5 hypervariable regions from HIV-1 subtypes A, B, C, D, G, and H were analyzed.

Results: We found that the majority of HIV-1 gp120s have 496–515 amino acids and 21–30 PNGSs, suggesting that a gp120 with this length might be a good virus candidate for vaccine development. Among 5 hypervariable regions, the V3 regions had the lowest levels of length polymorphism and heterogeneity and less PNGSs, while V1 and V4 regions had high levels of length polymorphism and heterogeneity and more PNGSs. These results suggest that reducing the polymorphism, heterogeneity, and PNGSs of the 4 hypervariable regions should be taken into account in AIDS vaccine development for effectively eliciting immune response. Except for subtype D, other subtypes have the consensus V3 sequences with R5 tropism, implying that the majority of HIV-1 strains are R5 strains.

Conclusion: The results suggest that CCR5 antagonists may be extremely efficient for AIDS treatment and R5 strains should be used as candidates for AIDS vaccine development.

Key words: HIV-1, gp120, hypervariable region, length polymorphism, N-linked glycosylation sites, CCR5 antagonists

1. Introduction

The human immunodeficiency virus type 1 (HIV-1) is a virus with high diversity due to low replication fidelity of viral reverse transcriptase (RT), and it is classified into 4 groups: M (major or main), N (non-M, non-O), O (outlier), and P (1). The M group that has led to the global AIDS epidemic is further classified into 11 subtypes (including subtypes A1, A2, B, C, D, F1, F2, G, H, J, and K), 49 circulating recombinant forms (CRFs), and more than 200 unique recombinant forms (URFs) (2). High mutation rates allow HIV-1 to easily escape from the immune attack and/or drug pressure (3–5).

Env encodes surface glycoprotein gp120 and transmembrane glycoprotein gp41 and has the highest mutation rate within the HIV-1 genome. gp120 is not only responsible for virus entry by recognizing and binding to cellular receptors (CD4 and CCR5 or CXCR4), but also serves as the major target of host immune systems (6–9). gp120 consists of 5 hypervariable regions (V1–V5) that are interspersed with 4 relatively conserved regions (C1–C4) (10). Among these hypervariable regions, the V3 region consists of approximately 35 amino acid residues flanked by 2 cysteines and forms a loop structure by a disulfide bond. It plays a dominant role in the determination of vial coreceptor usage (cell tropism) by direct interaction with the coreceptors (11–13). Apart from the V3 loop, other hypervariable regions, such as V1/V2 and V4, also influence the coreceptor usage (14–16).

On the other hand, these hypervariable regions are the important target of neutralizing antibodies. The V1/V2 domains can act as targets for neutralizing antibodies, and variability in these regions may contribute to immune escape from neutralization (16–18). V4 and V5 participate in CD4 binding and contribute to autologous neutralization (19,20). The functional interactions between the V1–V5 hypervariable regions especially facilitate viral escape from the host immune responses (5). Recent research showed that V1/V2 length polymorphism is associated with the epidemiology of HIV transmission within the subtype B epidemic, and increased V1/V2 length facilitates the adaptation of subtype B viruses to host immune responses (21). These imply that the

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diversity and sequence characteristics of gp120, especially the 5 hypervariable regions, play a crucial role in HIV-1 pathogenesis and immune escape, which influence and determine HIV-1 vaccine development.

In this study, we analyzed length polymorphism, the potential number of N-linked glycosylation sites (PNGSs), and sequence characteristics of nearly all available global gp120 and V1–V5 sequences from HIV-1 subtypes A, B, C, D, G, and H. We found that V3 loops had the lowest level of length polymorphism, sequence heterogeneity, and PNGSs, whereas V1 and V4 regions had very high levels of length polymorphism, heterogeneity, and PNGSs. The polymorphism characteristic of HIV-1 gp120 hypervariable regions will provide new insight into AIDS vaccine development.

2. Materials and methods

2.1. Data sets

All available global HIV-1 gp120 and V1–V5 sequences were retrieved from the HIV sequence database (http://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html) in October 2010 (Table). A total of 246,032 sequences were divided into 35 data sets according to gp120 hypervariable regions V1–V5 and HIV subtypes (A, B, C, D, G, or H).

2.2. Length polymorphism and sequence characteristic analyses

After being translated into amino acid sequences, the length polymorphisms of 35 data sets (Table) were analyzed by counting the length of each sequence. To investigate the sequence characteristic of each data set, the amino acid sequences of each data set were aligned using Clustal W implemented in MEGA4.0.2. The residue frequency at each position of the sequence alignment was calculated. To display the consensus sequence of each hypervariable region, the sequence logos were obtained using WebLogo (http://weblogo.berkeley.edu/logo.cgi) (22). In addition, because V3 can determine the coreceptor tropism of HIV-1, we compared the consensus V3 sequences of 6 analyzed subtypes and predicted the coreceptor tropism based on consensus V3 sequences using HIV-1 PhenoPred (http://yjxy.ujs.edu.cn/R5-X4%20pred.rar) (23).

2.3. Potential N-linked glycosylation sites

To investigate the PNGSs of each sequence, a program that predicts PNGSs based on the motif "Asn-Xaa-Ser/Thr" was developed. To avoid errors, a few low-qualified sequences (i.e. too short and too long) were discarded from the data sets.

3. Results

3.1. Length polymorphism of gp120

Length polymorphisms of gp120 of different HIV-1 subtypes are shown in Figure 1A. The length scopes of gp120 were 484–535, 476–545, 476–540, 487–524, and 484–531 amino acids in subtypes A, B, C, D, and G, respectively. The sequence length of 68.6% of subtype A and 55.8% of subtype D sequences focused on the scope of 501–510 amino acids, showing a relatively lower polymorphism. For other subtypes, most sequences focused on larger length scopes. About 80.3% of subtype B and 73.5% of subtype C gp120 sequences had 501–520 and 496–515 amino acids. For subtype G, 75.8% of sequences had 496–515 amino acids, and 14.7% had 486–490 amino acids. Among 5 analyzed subtypes, subtype B had relatively longer gp120 lengths, whereas subtype G had relatively shorter gp120 lengths (Figure 1A).

Table. Data sets used in this study.

Subtype	gp120	gp120 hypervariable regions				
		V1	V2	V3	V4	V5
A	694	1670	2238	4894	2329	1201
B	9893	20,079	20,272	62,124	37,509	30,529
C	4321	5813	6385	11747	8291	6512
D	471	585	741	2300	1656	1206
G	95	229	249	959	553	259
H	–	6	6	105	71	40
Total	15,474	28,382	29,891	82,129	50,409	39,747

The number of sequences in each data set is shown.

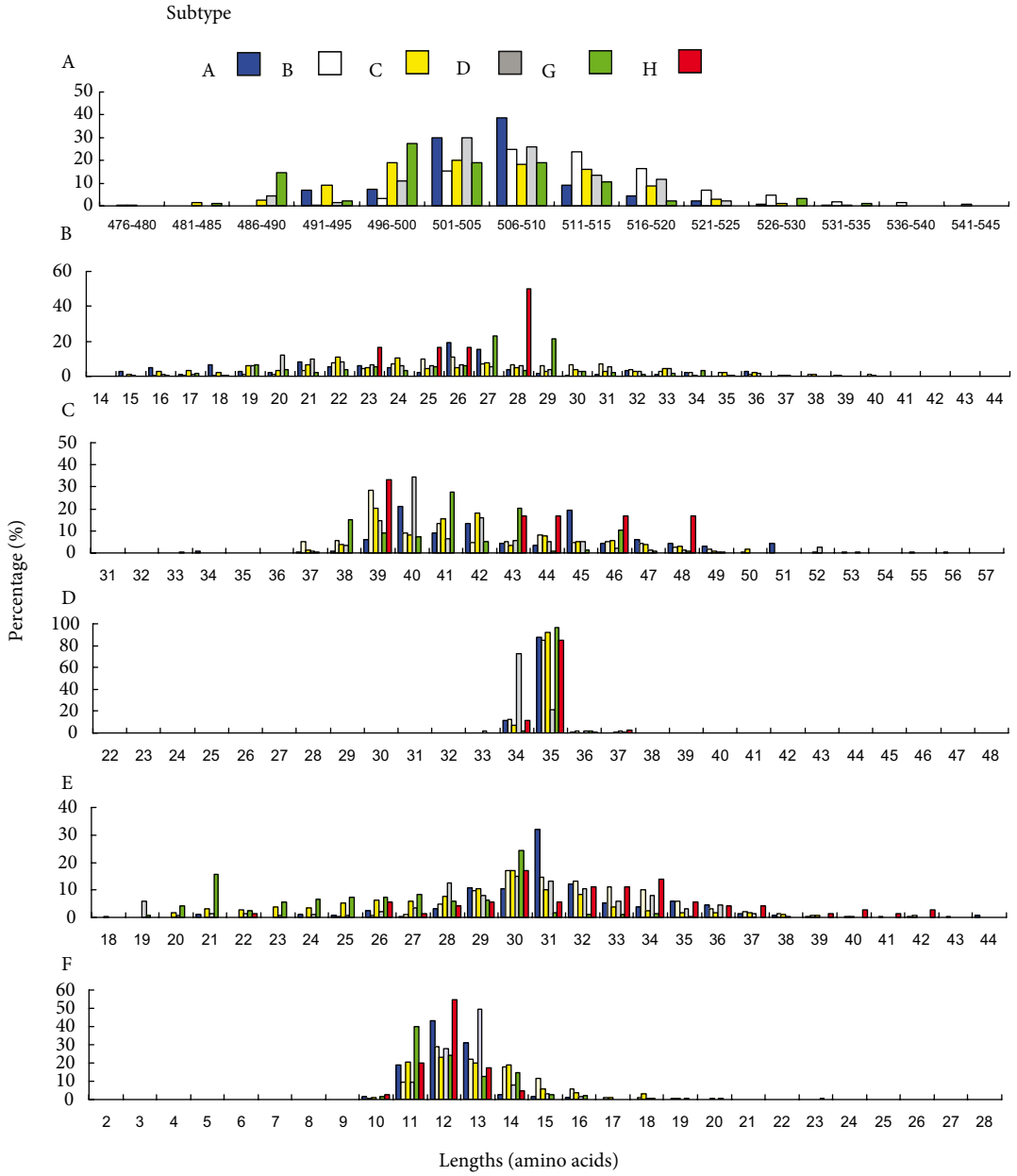

Figure 1. Length distribution of HIV-1 gp120 and its 5 hypervariable regions with different subtypes. A) gp120, B) V1 region, C) V2 region, D) V3 region, E) V4 region, F) V5 region.

3.2 Length polymorphism of 5 gp120 hypervariable regions

Length polymorphisms of 5 gp120 hypervariable regions are shown in Figures 1B–1E. The average lengths of V1–V5 were 27 (from 15 to 40), 42 (from 33 to 57), 35 (from 32 to 38), 31 (from 18 to 44), and 13 (from 10 to 23) amino acids, respectively. Different subtypes showed very similar length polymorphisms for all hypervariable regions with the exception of the V3 region. Most V3 loops from subtypes A (87%), B (85%), C (92%), G (96%), and H (85%) had 35 amino acids, whereas most (73%) of subtype D had 34 amino acids. The length distributions of 5 hypervariable regions showed that V1, V2, and V4 had higher length polymorphism and the V3 loops were the highest conserved in length (Figures 1B–1E). The conservation of V3 length may be ascribed to its importance in HIV-1 infection and pathogenesis.

Furthermore, we compared consensus V3 sequences of 6 HIV-1 subtypes. Subtypes A, B, C, G, and H had almost identical consensus V3 sequences except for 2 mutations occurring at sites 2 (T/A) and 18 (Q/R) of subtype B and 1 mutation (R/H) occurring at site 13 of subtypes B and

H (Figure 2), supporting the conservation of V3 loops. Compared with other subtypes, V3 loops of subtype D contained a deletion of glycine (G) at site 24, and some unique mutations including a D/K mutation at site 25. Previous research showed that amino acid shifts at V3 sites 11 and 25 from neutral and/or acidic amino acid to basic amino acid determine the co-receptor tropism of HIV-1. We found that, although all subtypes had serine (S) at site 11 of V3 loops, a change from acidic (asparagine) to basic (lysine) amino acid at site 25 appeared in subtype D (Figure 2), implying a X4 tropism (CXCR4-using) of most subtype D sequences. We then predicted the coreceptor tropism based on these consensus V3 sequences using a bioinformatics software R5/X4-pred (23). The result showed that except for subtype D having a X4 tropism, all other subtypes exhibited an R5 tropism (CCR5-using), not only supporting previous observations that most of subtypes B and C belonged to R5 (24), but also implying that R5 strains played a crucial role in HIV-1 transmission and pathogenesis (25,26).

3.3 PNGSs of 5 gp120 hypervariable regions

HIV-1 gp120 is a highly glycosylated protein and more than half of its molecular mass is from N-linked carbohydrates. Glycosylation not only participates in the processing and maturation of gp120, but also reduces the immunogenicity of gp120, thereby minimizing neutralizing antibody response. Therefore, we investigated the PNGSs of gp120 and its 5 hypervariable regions. The results showed that the distribution of the PNGSs of gp120 was very consistent among different subtypes, and the majority (91.9%–98.9%) of gp120s from the 5 analyzed subtypes had 21–30 PNGSs (Figure 3A). Interestingly, 5 hypervariable regions had different PNGSs (Figures 3B–3F). Among 5 hypervariable regions, 86.5%–98.2% of V4 regions possessed 3–6 PNGSs, showing the most PNGSs (Figure 3E), and 79.1%–96.1% V3 loops had 1 PNGS, exhibiting the least PNGSs (Figure 3D). In addition, it was very surprising that 46% of subtype C V5 regions did not possess PNGSs and 47.4% had 1 PNGS. For other subtypes, 90.9%–97.5% of V5 regions had 1–2 PNGSs (Figure 3F), slightly more than those of V3 loops. Lastly, 82.5%–100% of V1 regions and 86.3%–100% V2 regions had 2–4 PNGSs (Figures 3B and 3C), slightly less than those of V4 regions.

3.4 Sequence characteristics of 5 gp120 hypervariable regions

To display the sequence characteristics of V1–V5 regions, the sequence logos of each hypervariable across subtypes were generated. For each hypervariable region, different subtypes contained not only relatively conserved motifs, but also some highly variable regions (Figure 4). These highly variable regions may be ascribed to the genetic divergence of different subtypes and represent subtype-associated sequence characteristics. V1 and V4, in particular, appeared to be more variable than other regions, possibly due to the importance of both regions in immune escapes. The V3 regions appeared to be the most conserved hypervariable regions, consistent with the observation in length polymorphism (Figure 1). The crown motif GPGQ/R can be found in almost all V3 loops. On V2 and V5 loops, conserved motifs ALFY and ETFRP were also identified. These conserved motifs may imply strong function constraint in evolution, suggesting that they play a crucial role in gp120 function.

4. Discussion

HIV-1 gp120 expresses on the surface of virions and is responsible for viral entry into host cells (11). It is the most important target of neutralizing antibodies (6–9). The high heterogeneity and high glycosylation site occupancy of gp120 increase the difficulty of AIDS vaccine development by continuously changing epitopes and minimizing antigenicity, respectively (5). By analyzing all available global HIV-1 gp120s, we found that the majority of HIV-1 gp120s have 496–515 amino acids and 21–30 PNGSs (Figures 1A and 3A). This suggests that HIV-1 gp120s with 496–515 amino acid lengths might be a good virus candidate for vaccine development.

gp120 includes 5 hypervariable regions. Among them, the V3 regions were very highly conserved in loop length and had a high level of sequence homogeneity, implying

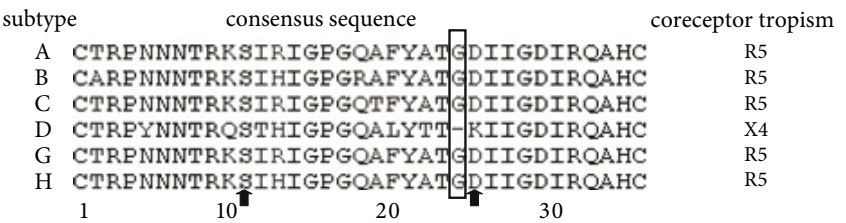

Figure 2. Consensus V3 sequences of different HIV-1 subtypes. Two sites in V3 loop that determine HIV-1 tropism are highlighted using arrows. A frame shows a V3 site where subtype D consensus sequence has a gap. The coreceptor tropism was predicted using a bioinformatics tool of HIV-1 PhenoPred.

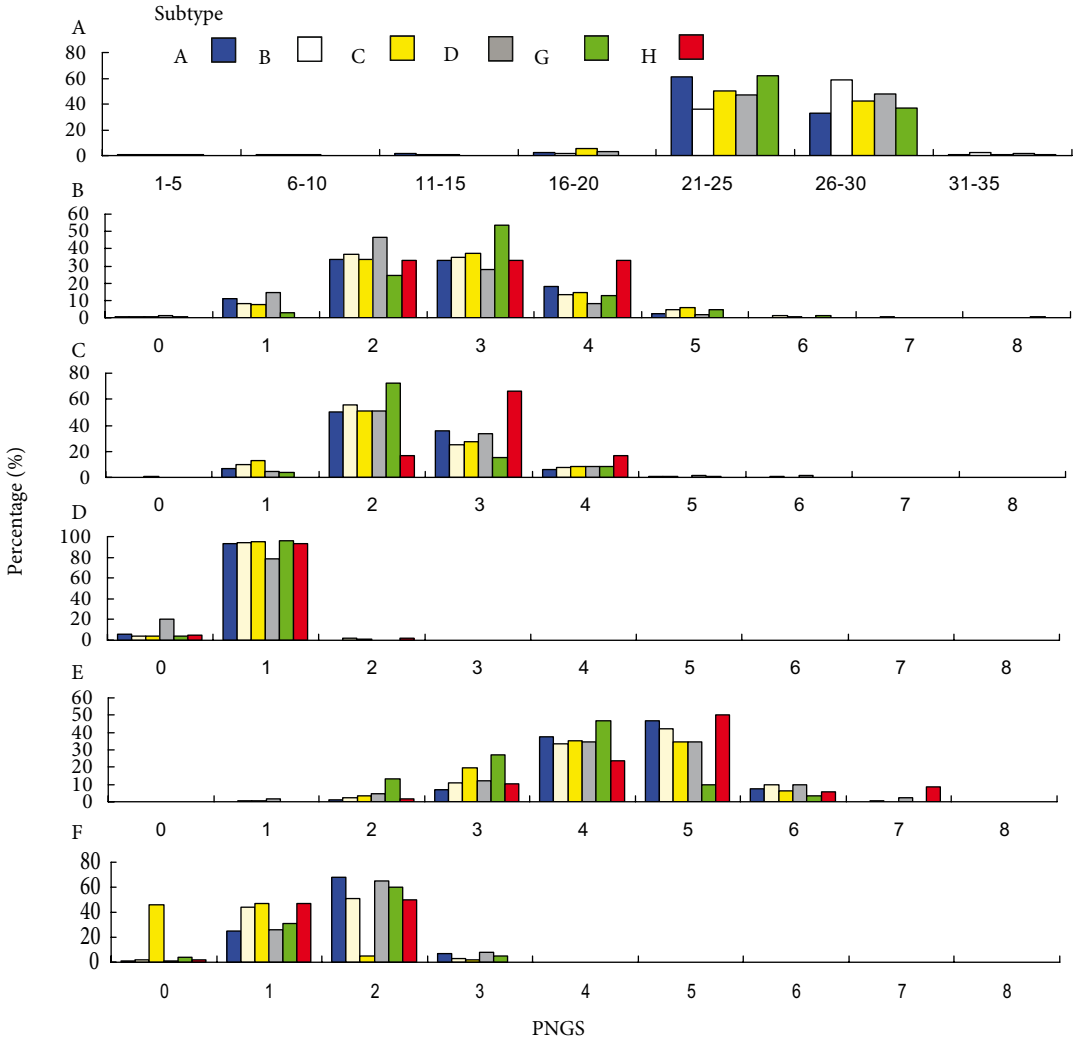

Figure 3. Distribution of N-glycosylation sites in HIV-1 gp120 and its 5 hypervariable regions with different subtypes. A) gp120, B) V1 region, C) V2 region, D) V3 region, E) V4 region, F) V5 region.

a strong function constraint (21,27). This might be due to the fact that the V3 loop is one of the most important factors for viral survival by determining the cellular tropism of HIV-1 (25). In addition, V3 loops had the least PNGSs, which can increase its accessibility to the coreceptor (CCR5 or CXCR4). HIV-1 R5 strains can often be detected during the early stages of HIV-1 infection, as well as throughout the infection (28,29). They can result in the breakdown of the immune system directly or by evolving into X4 strains (26,30,31). Except for subtype D, the consensus V3 loop sequences of all other subtypes exhibited R5 tropism (Figure 2), possibly implying that the majority of HIV-1 strains are R5 strains (24). This suggests that CCR5 antagonists may be extremely efficient for AIDS treatment and R5 strains should be used as candidates for AIDS vaccine development. In fact, a number of CCR5 antagonists have been developed and some have been approved by the FDA for the treatment of HIV and AIDS.

CCR5 is an important coreceptor for HIV infection and transmission. During the course of HIV infection, R5 variants dominate the viral quasispecies early in and even throughout infection, whereas X4 variants evolve from R5 variants relatively late and coexist with R5 viruses (32). R5 viruses were significantly less sensitive to neutralization by neutralizing antibodies than newly emerged (R5)X4 viruses (33). Therefore, to induce sustained neutralizing responses against R5 viruses might be a key point for future AIDS vaccine development.

The V1/V2, V4, and V5 regions are located outside enwrap V3 and are accessible to immune attack (20). The V1/V2, V4, and V5 regions have been demonstrated to contribute to viral immune escape from autologous

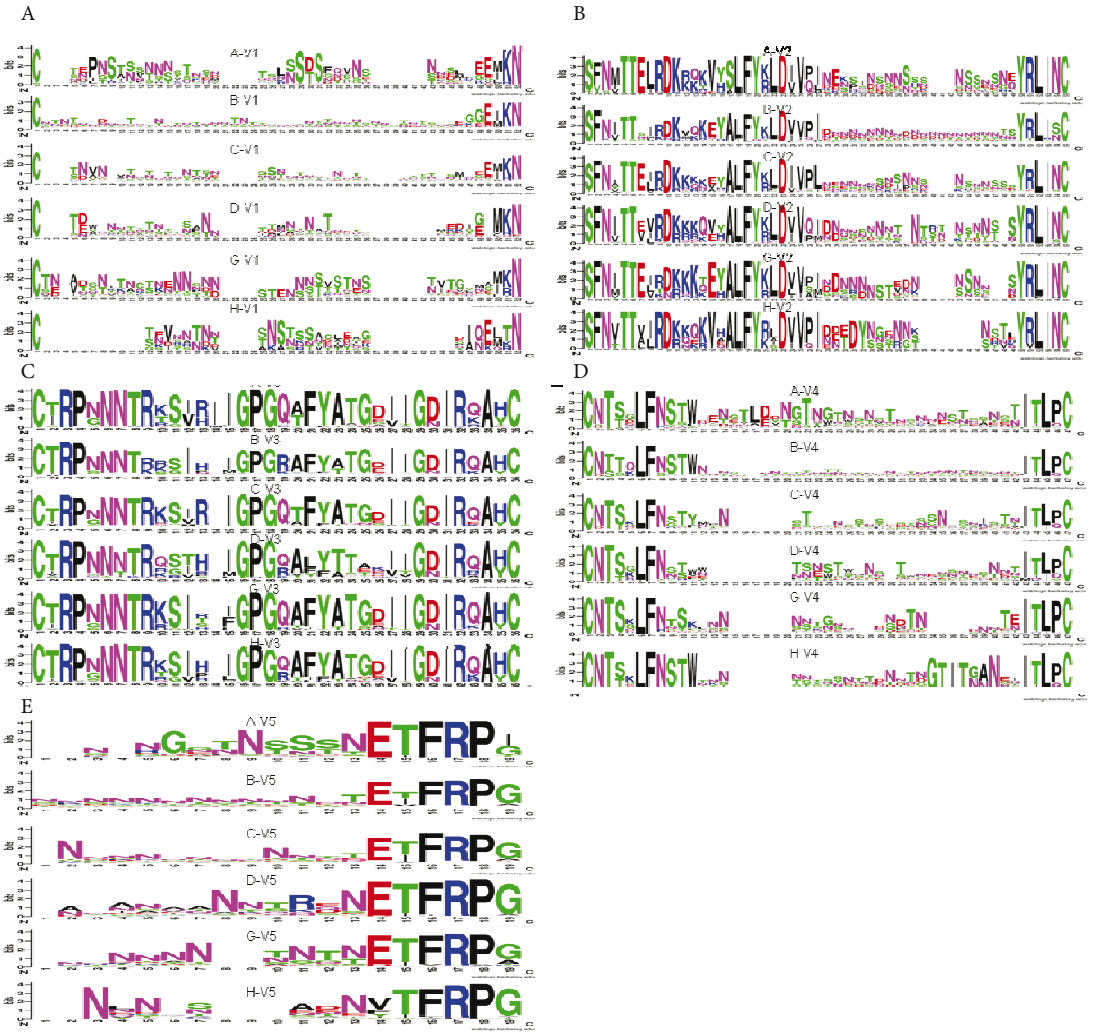

Figure 4. Sequence logos of 5 gp120 hypervariable regions with different subtypes. A) V1 region, B) V2 region, C) V3 region, D) V4 region, E) V5 region.

neutralization (34). To counter the immune selection, V1 and V4 regions of some HIV-1 subtypes indeed underwent strong diversifying selection pressure (35). Our results showed that the V1 and V4 regions had high levels of length polymorphism and heterogeneity and more PNGSs, and V2 and V5 exhibited moderate levels of length polymorphism, heterogeneity, and PNGS (Figures 1, 3, and 4). As another consequence of adaptive evolution, the high levels of length polymorphism, heterogeneity, and PNGSs of the 4 hypervariable regions not only facilitate viral escape from immune attacks, but also protect V3 from immune attacks (21). These results suggest that new strategies to reduce the polymorphism, heterogeneity, and PNGSs of the 4 hypervariable regions should be developed to effectively elicit immune response in AIDS vaccine development.

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