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SHORT COMMUNICATION

Stromal Cell-Derived Factor (SDF) 1-3'A Polymorphism and Sequences in Thais

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SUMMARY **Stromal cell derived factor (SDF) 1-3'A polymorphism in Thai subjects was determined by restriction fragment length polymorphism (RFLP) of polymerase chain reaction (PCR) products using a restriction enzyme *Msp* 1 cleavage. The allelic frequency of SDF1-3'A in this population was 0.289. SDF1-3'A genotyping showed 9.52% SDF1-3'A/3'A, 38.89% SDF1-wt/3'A, and 51.59% SDF1-wt/wt. Two clones of Thai-SDF genes, BD41 and BD42, were isolated and sequenced. Wild type Thai-SDF1 (BD42), a 278 bp sequence was identically aligned to human pre B cell stimulating factor homoloque (SDF1-b) (GenBank accession number L-36033) from nucleotides 788 to 1,065. Homologous Thai SDF1-3'A (BD41), a 277 bp sequence differed by single nucleotide with adenine substitution to guanine at position 880 of the SDF1-b, is the first evidence of SDF1-3'A polymorphism in Thais.**

Chemokines are potent activators and chemoattractants for leukocytes. Their actions are mediated by binding to 7-transmembrane G-protein coupled receptors. There are 4 groups of the chemokine receptors; CXC, CX_3C, CC and C chemokine receptors. The groupings are base on the number of the intervening amino acids between the first two closely paired and highly conserved cysteines. Presently, there are 18 human chemokine receptors that have been identified.[1] The chemokine receptors function as coreceptors in addition to CD4 receptor for HIV-1 infections. CC chemokine receptors, CCR5 in particular, function as coreceptors for nonsyncytium inducing HIV-1 (R5 strain) in primary infection.[2,3] Whereas CXC-chemokine receptor, namely CXCR4, functions as coreceptor for syncytium inducing HIV-1, T-lymphocytotropic (X4 strain) in the late stage of HIV-1 infection.[4] Dual tropic HIV-1 can use either CCR5 or CXCR4 in the infection.[5]

A CCR5 polymorphism with 32 bases deletion (CCR5-Δ32) was found to play role in resisting HIV-1 infection.[6] This deleted mutant predominates in Caucasians with high allelic frequency but rarely occurs in Asians. In addition to the CC-chemokine receptor mutant, its corresponding chemokines such as regulated upon activation, normal T-cell expressed and secreted [RANTES], macrophage inflammatory protein-1-alpha [MIP-1α] and MIP-1β play role in inhibition of HIV-infection. These ligands block the infection by competition for CCR5 binding and activating cytotoxic T-cells.[7] On the other hand, CXC chemokine receptor mutant has not been reported, however, its only one ligand, stromal cell derived factor (SDF)-1α has been shown to inhibit the replication of T-cell tropic HIV.[8,9] Variant at 3' UTR of the SDF-1 (SDF1-3'A) was described and the homologous state associated with inhibition of HIV-infection and delaying disease progression.[10-12] This mutation was found to be a substitution of adenine to

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guanine at the nucleotide 801 from ATG start codon.[10] It causes up-regulation of SDF production and down-regulation of CXCR4 expression. The increased SDF, a ligand, can compete with gp120 of HIV-1 subtype T-tropic (X4-strain) in binding to CXCR4 coreceptor, and in turn inhibit the HIV-1 infection. A number of seronegative high-risk of HIV-1 infection, as well as serodiscordant couples have been observed among the Thai population (unpublished observation).

To elucidate the prospective resisting mechanism in Thais, we have investigated an allelic frequency of SDF1-3'A and sequenced this mutant isolated from normal blood donor Thai subjects compared to the known sequence from the GenBank.

MATERIALS AND METHODS

Study subjects

Cross-sectional analysis was made on heparinized blood from 126 donors aged 20-45 years old, 63 males and 63 females. The peripheral blood mononuclear cells (PBMC) were partially purified by density gradient centrifugation using Histopaque (Sigma Chemical Company, USA) and suspended in 10% dimethyl sulfoxide (DMSO) in 30% fetal calf serum (FCS) in Dulbecco's modified Eagle medium. The cells were frozen slowly and kept in liquid nitrogen until use.

SDF1-3'A genotyping

Crude genomic DNA was prepared by lysis of cryopreserved PBMC at 10^6 cells in 0.1 ml proteinase-K lysis buffer. SDF1-3'A genotyping was performed by PCR amplification with suitable control for 40 cycles as described previously.[13] Primers used were SDF-3'-UTR-F (sense) 5'-CAG TCA ACC TGG GCA AAG CC-3' and SDF-3' UTR-R2 (antisense) 5'-CCT GAG AGT CCT TTT GCG GG -3'.[11]

Restriction fragment length polymorphism (RFLP)

Amplification products were analyzed by restriction fragment length polymorphism. Briefly, the PCR product in a volume of 10 µl was mixed with an equal volume of *Msp* 1 restriction enzyme solution containing 0.5 µl *Msp* 1 (10 U/µl; Promega, USA), acetylated BSA (0.2 µl), restriction enzyme buffer B (2 µl) and 7.3 µl distillated water. The reaction mixtures was incubated in 37°C water bath for 4 hours after which, they were analyzed in 3% agarose electrophoresis in TAE buffer, pH 8.5. Ethidium bromide stained agarose gels were documented on Polaroid film.

SDF Sequencing

Pools of PCR products BD41 (SDF1-3'A/3'A) and BD42 (SDF1-wt/wt) were purified by an

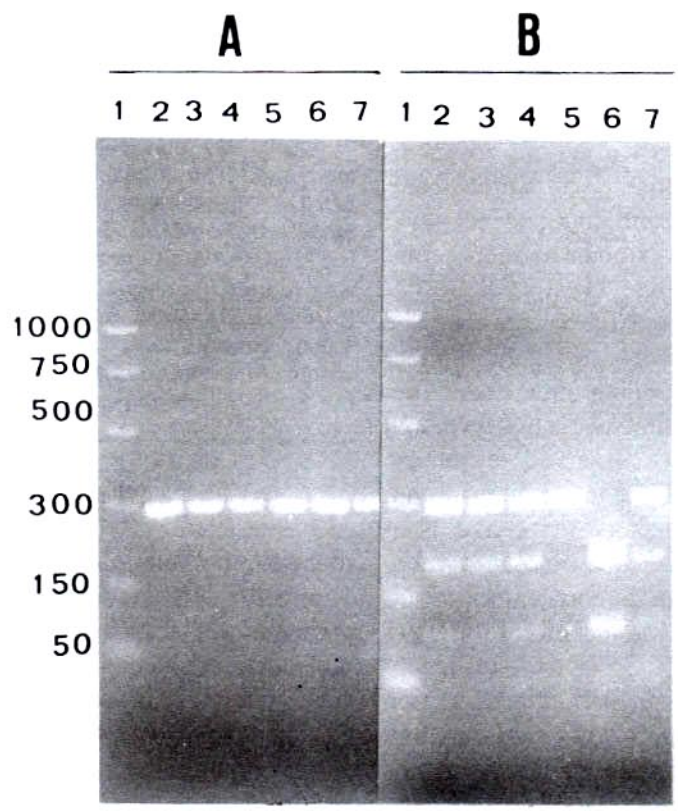

Fig. 1 Polymerase chain reactions for SDF1-3'A. Panel A: PCR products without restriction enzyme *Msp* 1 cut; Panel B: PCR products with restriction enzyme *Msp* 1 cut. Lane 1, PCR markers 1,000, 750, 500, 300, 150, and 50 bp; lanes 2, 3, 4, 7, SDF1-3'A Heterozygotes, lane 5, SDF1-3'A Homozygote, lane 6, SDF1- wild type.

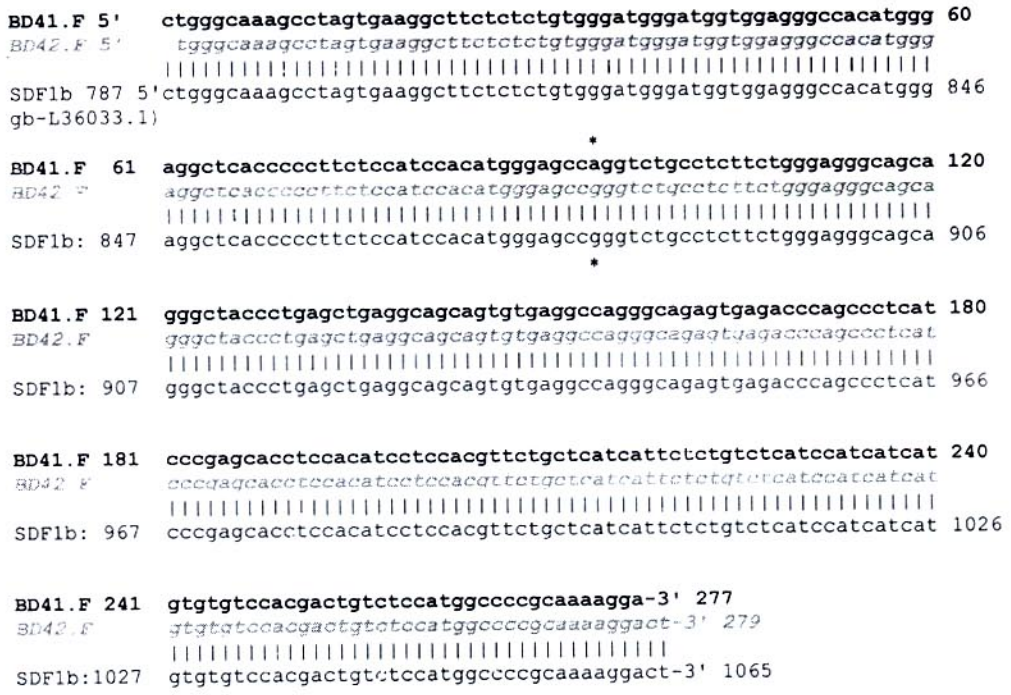

Fig. 2 Sequences of SDF1 clones BD41.F and BD42.F aligned to SDF1b. BD41.F, Thai-SDF1-3'A /3'A, homozygous mutant; BD42.F, Thai-SDF1 +/+, Wild type; SDF1b, human pre-B cell stimulating factor homoloque (SDF1b; GenBank accession number L36033).

ion exchange resin, Wizard DNA prep (Promega). They were verified for purity by an agarose gel electrophoresis prior to subject of DNA sequencing by ABI-377 sequencer and Big Dye Terminator reagents (Applied Biosystems). Sequences of BD41 and BD42 were aligned to GenBank in NIH genetic sequence data base by program blastn in Basic Local Alignment Search Tool (BLAST 2.0).

RESULTS

Genotyping of SDF1-3'A

The PCR products were determined for SDF1-3'A genotypes by RFLP using *Msp* 1 restriction enzyme cut. The restriction enzyme cleaves specific sequence of 5'--C▼CGG--3'. The SDF1-wild type, having this sequence at the nucleotides 878 to 881 as shown in SDF1b clone (Fig. 2), would be cut at the specific site to generate 2 fragments of approximately 200- and 100-bp as shown in Fig. 1 (panel B, lane 6). Whereas, the SDF1-3'A homozygote, having adenine (a) substitution to guanine (g) at nucleotide 880 (Fig. 2) would not be cut, resulting a single band approximately 300 bp as shown in Fig. 1 (panel B, lane 5) identical to its original products without restriction enzyme cut (panel A, lane 5). On the other hand, the SDF1-3'A heterozygote, would be cleaved for only one strand resulting in 3 fragments of approximate 300-, 200-, and 100-bp as seen in Fig. 1 (panel B, lanes 2, 3, 4, 7). Results of SDF1-3'A genotyping in 126 blood donor samples were 65 (51.59%) wild types (SDF1 +/+), 12 (9.52%) homozygotes (SDF1 3'A/3'A), and 49 (38.89%) heterozygotes (SDF1 +/3'A).

Nucleotide sequences of SDF1

Two clones of SDF1 were purified and sequenced by DNA sequencer. BD41 clone, a homozygous SDF1-3'A had 277 bp sequenced. BD42 clone, a wild type SDF1 had 279 bp sequenced. They were aligned to human pre B-cell stimulating factor homoloque (SDF1b), a GenBank accession number L36033. As shown in Fig. 2, Thai SDF1-wild type (BD42) was identically aligned to nucleotides 788 to 1065 of the referenced SDF-1b gene. Whereas Thai SDF1-

3'A homologous was aligned to nucleotides 787 to 1063 with single base mismatched by having an adenine substitution to guanine at the nucleotide 880 of the referenced SDF-1b gene.

DISCUSSION

This study provides the first evidence for SDF-3'A polymorphism in Thais. The allelic frequency of SDF1-3'A in the population is 0.289 which is higher than that reported in Caucasian (0.211), Hispanic (0.160), and African-American (0.057).[10] The SDF1-3'A allelic frequency in Thais is even higher than that reported in Japanese (0.234) but is same frequency reported in Chinese (0.286).[14,15] Our results of SDF1-3'A genotyping show 9.52% homozygotes and 38.89% heterozygotes which are similar to that found in Japanese (10.5% homozygotes and 37.1% heterozygotes).[14] Thai SDF1-3'A is higher in homozygosity than that found in Chinese (4.8% homozygotes and 47.6% heterozygotes).[15] Since the homozygous state of SDF1-3'A plays an important role in HIV infection by delaying the onset of AIDS and prolonged survival after AIDS diagnosis,[10,11] the unique high percentage of homologous SDF1-3'A and allelic frequency may imply increased resistance to HIV in Thais.

We have isolated 2 clones of SDF genes, one is a SDF1-3'A/3'A (BD41) and another is a SDF1-wt/wt (BD42). They have been sequenced and aligned to SDF1-b, a referenced human pre B-cell stimulating factor homoloque from GenBank, accession number L36033. The Thai SDF1 wild type (clone BD42) is 278 bp sequence and identically aligned to SDF1-b (gb-L36033) from nucleotides 788 to 1,065. Whereas the Thai SDF1-3'A homozygote is 277 bp sequence and aligned to nucleotides 787 to 1063 of the SDF1-b. The Thai SDF1-3'A homozygote is different from its Thai SDF1 wild type by one nucleotide having adenine substitution to guanine at the nucleotide 880 reading from the referenced clone. The Thai SDF1-3'A is probably the same as the SDF1-3'A polymorphism designed SDF1-3'UTR-801-G-A, previously described by Winkler *et al.*,[10] which has adenine substituted guanine at nucleotide 801 counting from ATG start codon. Winkler *et al.*[10] counted from ATG start codon which is nucleotide number 79 in the GenBank while we used exact number of nucleotide in the GenBank.

Our reported sequences, BD41 and BD42 in Thais support that SDF gene is highly conserved in humans by identical alignment on to the GenBank data base. The high allelic frequency of SDF1-3'A in Thais has prompted us to investigate the SDF1-3'A polymorphism in our seronegative, high risk of HIV infection patients. The short coming results may help us to elucidate the resistant mechanisms of HIV infection in Thais.

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