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Spatial Genetic Structure of Two HIV-I-resistant Polymorphisms (CCR2-64Ⅰ and SDF1-3'A) Alleles in Population of Shandong Province, China[1]

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Objective To explore the spatial genetic structure of two HIV-I-resistant polymorphisms (CCR2-64Ⅰ and SDF1-3'A) alleles in the population of Shandong Province, China. **Methods** Using the techniques of spatial stratified sampling and spatial statistics, the spatial genetic structure of the locus (CCR2-64Ⅰ and SDF1-3'A), which was shown to be important co-receptor for HIV infection, was quantified from the populations of 36 sampled counties of Shandong Province, and a total of 3147 and 3172 samples were taken for testing CCR2-64I and SDF1-3'A respectively from individuals without known history of HIV-I infection and AIDS symptoms. **Results** There were significantly spatial genetic structures of the two alleles at different spatial distance classes on the scale of populations, but on the scale of individuals, no spatial structure was found in either the whole area of Shandong Province or the area of each sampled county. Although the change of frequencies of the two alleles with geographic locations in Shandong Province both showed gradual increase trends, their changing directions were inverse. The frequency of CCR2-64I allele gradually increased from the southwest to the northeast, while the frequency of SDF1-3'A allele gradually increased from the northeast to the southwest. However the *RH* to AIDS of combined types of their different genotypes did not represent obvious geographic diversity on the whole area of the Province. **Conclusion** The frequency of allele usually has some spatial genetic structures or spatial autocorrelation with different spatial distance classes, but the genotypes of individuals have random distribution in the same geographic area. Evaluating spatial distribution of the genetic susceptibility of HIV (AIDS) to CCR2-64I and SDF1-3'A alleles, should focus on the frequencies of combined genotypes of CCR2 and SDF1 based on the two-locus genotypes of each individual rather than the frequencies of CCR2-64I and SDF1-3'A alleles.

Key words: Spatial genetic structure; Chemokine receptors; HIV-I; Resistant polymorphism; Relative hazard

INTRODUCTION

Chemokine receptors CXCR4, CCR5, and CCR2B play an important role in the fusion of human immunodeficiency virus (HIV) to target CD4 cells. This discovery has accelerated the search for different genetic factors that might influence HIV infection and the onset of acquired immune deficiency syndrome (AIDS)[1-5]. In addition, the expression of the stromal-derived factor (SDF1), the ligand of CXCR4, may inhibit transmission of T cell line-tropic HIV strains[3,6]. It has been reported that mutations in the genes encoding the aforementioned chemokine receptors (CCR5 and CCR2) and ligand (SDF1) in natural populations are linked to HIV-1 resistance in cohort studies[4-5, 7-13]. Homozygous individuals with a 32-bp deletion in CCR5 (CCR5-Δ32) are resistant to HIV infection[5,7]. Heterozygotes for CCR5-Δ32 mutation exhibit a delayed progression to disease. The CCR5-Δ32 mutation results in a truncated protein removing the receptor from cells[5]. The benefit of carrying the deletion mutation is thought to result from decreased expression levels of the receptor in the patients, which lead either to resistance to virus infection for the homozygotes or to delayed disease progression for the heterozygotes[7]. SDF1 and CCR2 mutant alleles (SDF1-3'A and CCR2-64I) also might be involved in the progression to AIDS[4,14]. SDF1-3'A

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mutation is found in the un-translated region of the transcript, while the CCR2-64I allele results from the transition mutation G190A, which cause a substitution of valine to isoleucine[4]. The molecular mechanism of the effect of these mutations in the SDF1 and CCR2 genes is not yet understood. The SDF1-3'A allele has been implicated in the mobilization of $CD34^+$ progenitor cells into peripheral blood in humans as well as in the early onset of type I diabetes mellitus, while the CCR2-64I allele confers a lower risk for the development of sarcoidosis[15-16].

The distribution of the CCR5-Δ32, SDF1-3'A and CCR2-64I alleles has been surveyed in healthy natural population by many studies. These investigations usually only focus on describing the distribution of the CCR5-Δ32, SDF1-3'A and CCR2-64I frequency in different ethnic groups or different geographic areas, especially in a larger region or area[17-23]. These studies did not quantify the spatial genetic structure of the loci (CCR5-Δ32, SDF1-3'A and CCR2-64I). In fact, genetic diversity is constrained by factors operating in geographic space. Spatial genetic structure can be studied on various scales from continental to strict local. The idea behind most studies of spatial genetic structure and its diversity is that one can proceed from the observed spatial genetic structure to the underlying evolutionary process[24]. However, the methods of spatial genetic structure can be used not only in the area of evolution, but also in the field of medicine[25], including epidemiology of genetic disease and study of individual susceptibility to disease and to drugs[26]. Using the techniques of spatial statistics, the spatial genetic structure of the loci (CCR5-Δ32, SDF1-3'A and CCR2-64I) can be quantified. It is important to predict the spatial distribution of susceptibility to HIV (or AIDS), to estimate the relative hazard (*RH*) to HIV (or AIDS) in the population and to constitute preventive measures of AIDS in a special geographic area, especially in a small geographic area.

In this present study, the spatial genetic structure of the loci (SDF1-3'A and CCR2-64I) in Shandong Province, situated in the eastern part of China (Fig. 1), was quantified using the techniques of spatial statistics. As the frequency of CCR5-Δ32 is very low in Chinese Han population (only about 0.16%)[20], and most people are of Han population in Shandong Province, the spatial genetic structure of the locus CCR5-Δ32 was not detected. To identify the spatial genetic structure of the loci (SDF1-3'A and CCR2-64I), the method of spatial stratified sampling was set up (Fig. 1). To quantify their spatial genetic structure exactly, two steps were carried out in the whole study. Firstly, the genetic distograms[27-28], which represented graphs where mean genetic distances between all pairs of populations (or individuals) belonging to a spatial distance class (S_q) were plotted against the spatial distance classes, were used for analyzing their spatial genetic structure at both populations and individuals scale. Secondly, the Kriging technique[29], a geostatistics method for the environment sciences, was used to predict and estimate the spatial distribution of the allele frequency (SDF1-3'A or CCR2-64I). The inverse distance-weighted method[30] was used to predict and estimate the spatial distribution of the values of *RH* on SDF1-3'A and CCR2-64I to AIDS.

MATERIALS AND METHODS

Spatial Stratified Sampling

Shandong Province is located between 34°23'N-38°24'N latitude and 114.48°48'E-122°42'E longitude, in lower area of the Yellow River and along Chinese eastern coast as a communication hub (Fig. 1). The total area of Shandong Province is 156 000 square kilometers, and the total population is over 90 million in all 128 counties administered by the Province. According to its geographic characteristic and the spatial distribution of the population, 36 counties were sampled for the first spatial stratified scale, whose names are displayed in Fig. 1 and listed in Table 2，and 100 individuals without any known history of HIV-I infection were sampled in each sampled county for the second spatial stratified scale. The 100 sampled individuals in each sampled county must be the native of their villages at least for three generations, and the spatial distribution of the 100 sampled individuals should cover the geographic districts of the sampled county. The total samples were from 3607 individuals, but about 500 of them could not be genotyped successfully because of the problems during the genotyping, so the effective samples were from 3147 and 3172 individuals for testing CCR2-64I and SDF1-3'A, respectively, and their spatial distribution is showed in Fig. 1.

Genetyping

Genotyping of mutant CCR2-64I and SDF1-3'A alleles was performed, using PCR/RFLP assays according to previous reports[13,31-33]. The PCR primers for CCR2-64I and SDF1-3'A mutations were: CCR2-64I forward： 5'-CTC GGA TCT TGT GGG CAA CAT GAT GG-3'，reverse：5'-CTG TGA ATA ATT TGC ACA TTG C-3'; SDF1 forward: 5'-CAG TCA ACC TGG GCA AAG CC-3', reverse：5'-AGC

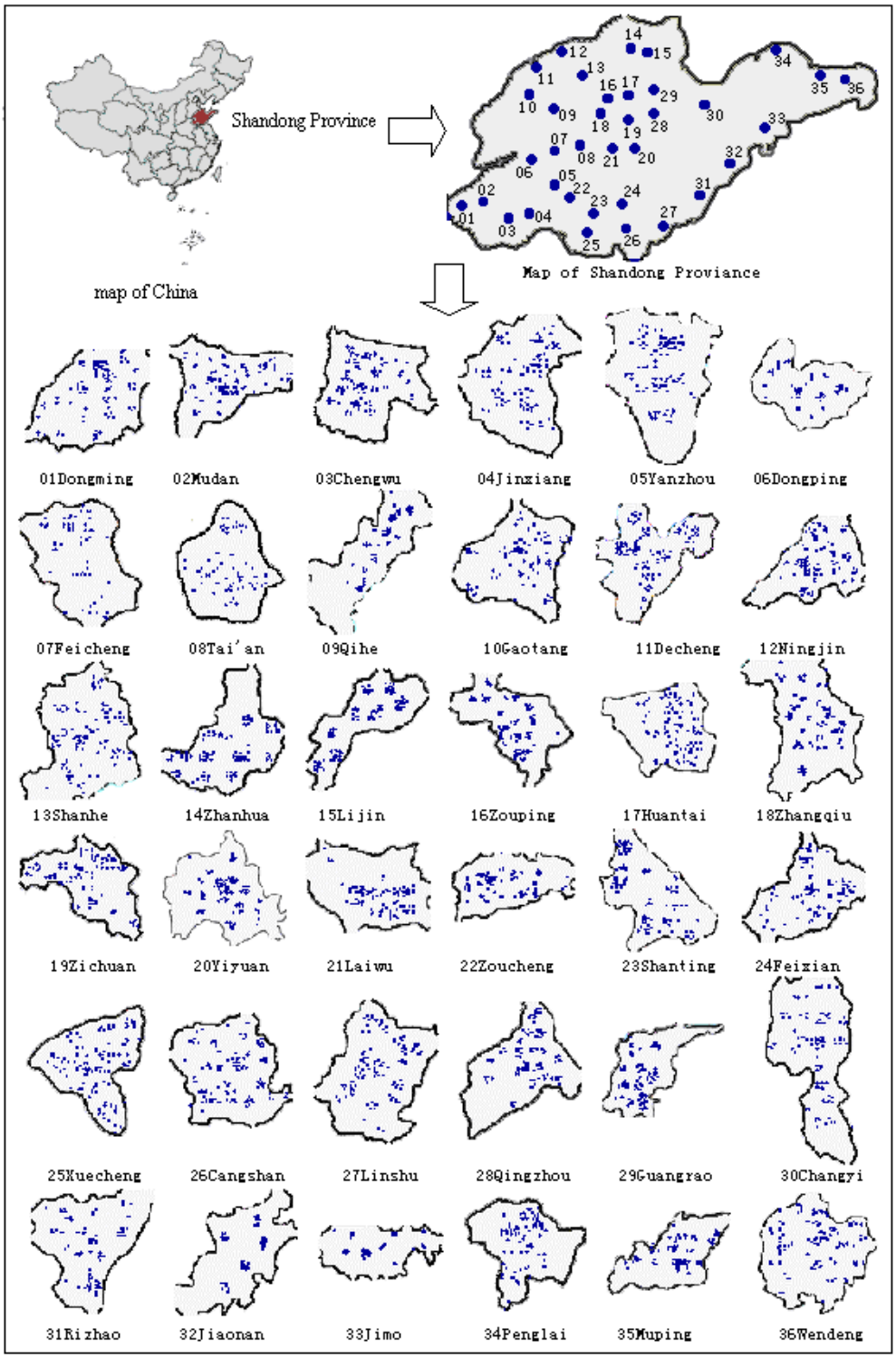

FIG. 1. Map of Spatial Stratified Sampling.

TTT GGT CCT GAG AGT CC-3'. The amplification conditions were the same for all the tests. Briefly, 1× PCR buffer, $Mg^{[2+]}$ 3 mmol/L, dNTP mix 200 μmol/L, primers 0.5 μmol/L, 1. 0 unit of Ampli-Taq Gold DNA polymerase, and 500 ng of purified genomic DNA, were placed in an eppendof tube and dd-H_2O was added to a total volume of 50 μL. Each PCR amplification was performed in a Perkin-Elmer

system 9600 for one cycle of initial denaturation at 94℃ for 5 minutes, followed by 30 cycles at 94℃ for 1 min, at 55℃ for 30 seconds and at 72℃ for 1 min, then a final extension at 72℃ for 10 min. For analysis of the CCR2-64I and SDF1-3'A alleles, PCR amplified products were digested with restriction endonuclease (Bsa BI and Msp I) and then separated by 2.0% agarose-gel electrophoresis[13,33]. Based on the characteristics of different genotypes of CCR2-64I and SDF1-3'A alleles, the expected banding patterns were as follows.For the CCR2-64I, the genotype of the wild-type (wt) homozygote (wt/wt) was one band of 191 bp, the genotype of the mutation-type (mt) homozygote (mt/mt) was two bands of 165 bp, 26 bp, and the genotype of the heterozygote (wt/mt) was three band of 191 bp ,165 bp and 26 bp (Fig. 2). For the SDF1-3'A, the genotype of the wild-type (wt) homozygote (wt/wt) was two bands of 202 bp and 100 bp, the genotype of the mutation-type (mt) homozygote (mt/mt) was one band of 302 bp, and the genotype of the heterozygote (wt/mt) was three bands of 302 bp ,202 bp, and 100 bp (Fig. 2).

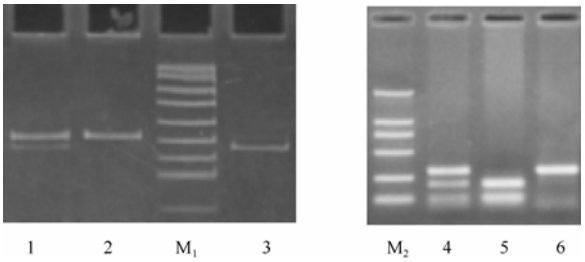

FIG. 2. Typical genetyping of CCR2-64I and SDF1-3'A alleles. M_1: markers for CCR2-64I; 1: 191 bp, 165 bp 26 bp, CCR2-64V/ CCR2-64I heterozygote; 2: 191 bp , CCR2-64V/ CCR2-64V homozygote; 3: 165 bp, 26 bp, CCR2-64I/ CCR2-64I heterozygote; M_2: markers for SDF1-3'A; 4: 302 bp, 202 bp, 100 bp, SDF1-3'G / SDF1-3'A heterozygote; 5: 202 bp, 100 bp, SDF1-3'G / SDF1-3'G homozygote; 6: 302 bp, SDF1-3'A / SDF1-3'A homozygote.

Statistical Methods

Spatial genetic structures analysis on scale of populations or individuals For data sets of CCR2-64I or (and) SDF1-3'A with alleles frequencies or with the frequencies of 9 different combined genotypes of CCR2 and SDF1 in Table1 between all pairs of 36 populations from sampled counties, the genetic distance measure, (D_N)[34], was selected to calculate the genetic distograms[27-28]. Genetic distograms representing graphs where mean genetic distances between all pairs of population belonging to a spatial distance class (s_q) were plotted against the spatial distance classes. The construction of genetic distograms had two advantages. Firstly, they could describe spatial patterns for multiple variables simultaneously (not only the frequency of one allele). Secondly, they could use established concepts of genetic distance to measure dissimilarities. For data sets of CCR2-64I and SDF1-3'A with genotypes (wt/wt, wt/mt, mt/mt) between all pairs of individuals from each sampled county or from all 36 sampled counties, the genetic distance measure, (D_N)[35] was selected to calculate the genetic distograms[27- 28].

For the analysis of spatial genetic structure on the scale of both populations and individuals above, a permutation procedure using Monte-Carlo simulations was used to test significant deviation from spatial random distribution of each calculated measure[36]. Each permutation consisted of a random redistribution of genetic or phenotypic data over the spatial co-ordinates of the sampled points. For each of the spatial distance classes, observed values were compared with the distribution obtained after *N* permutations. Then an *X%* confidence interval for the parameters was constructed as the interval from the *CI* 1. to the *CI* 2. ordered permutation estima- tes[37]. Where *N* is the number of permutations, which should be made at least 500 permutations, *X%* is the definition of the confidence interval, and the 95% confidence interval is often used, *CI* 1 is the lower limit of the confidence interval, and *CI* 2 is the upper limit of the confidence interval.

The Spatial Genetic Software (SGS) (http//kourou.criad.fr/genetique/software.html) was used to calculate the genetic distograms, and their permutation test.

Predicting and estimating the spatial distribution of frequency SDF1-3'A or CCR2-64I The kriging technique, a geostatistics method for spatial data analysis[29], was used to predict and estimate the spatial distribution of frequency SDF1-3'A or CCR2-64I. The software GS+ (a geostatistics for the environmental science software, download from http://www.gammadesign.com) was used for the step-by-step procedure of kriging.

Predicting and estimating the spatial distribution of RH on different AIDS definitions In order to predict and estimate the spatial distribution of *RH* on SDF1-3'A and CCR2-64I, the *RH* value on the combination of different genotypes was evaluated for each population on the basis of the two-locus genotype of each individual. The *RH* value for each of the nine possible two-locus genotypes was adapted on the basis of the published data of the cohort studies[13]. Three AIDS definitions were considered in the *RH* evaluations: AIDS-1993, AIDS-1987, and Related Deaths[7], The *RH* indices for each two-locus conserved genotype used in this report are shown in Table 1. The *RH* of a population was estimated by the

equation $RH=\Sigma W_i P_i$, where W_i and P_i are the genotype-specific RH and frequencies (grouped by distinct *RH* values in Table 1), and the summation was over all groups of combined genotypes. Then, the inverse distance-weighted method[30] was used for predicting and estimating the spatial distribution of *RH* values on there different AIDS definitions (AIDS-1993, AIDS-1987, and related deaths).

TABLE 1

RH of Three AIDS Definitions of 9 Different Combined Genotypes of CCR2 and SDF1

No.	Combined Genotypes of CCR2 and SDF1	AIDS-1993 (*RH*1)	AIDS-1987 (*RH*2)	Death (*RH*3)
A	(CCR2-64V/ CCR2-64V) and (SDF1-3'G / SDF1-3'G)	1	1	1
B	(CCR2-64V/ CCR2-64V) and (SDF1-3'G / SDF1-3'A)	1	1	1
C	(CCR2-64V/ CCR2-64I) and (SDF1-3'G / SDF1-3'A)	0.55	0.31	0.00
D	(CCR2-64I/ CCR2-64I) and (SDF1-3'G / SDF1-3'A)	0.55	0.31	0.00
E	(CCR2-64V/ CCR2-64V) and (SDF1-3'G / SDF1-3'G)	0.63	0.35	0.23
F	(CCR2-64V/ CCR2-64I) and (SDF1-3'G / SDF1-3'G)	0.65	0.66	0.60
G	(CCR2-64V/ CCR2-64I) and (SDF1-3'G / SDF1-3'A)	0.65	0.66	0.60
H	(CCR2-64I/ CCR2-64I) and (SDF1-3'A / SDF1-3'A)	0.65	0.66	0.60
I	(CCR2-64I/ CCR2-64I) and (SDF1-3'G / SDF1-3'A)	0.65	0.66	0.60

RESULTS

Frequencies of the Genotypes and Alleles of CCR2-64I and SDF1-3'A

The frequencies of the genotypes and alleles of CCR2-64I and SDF1-3'A were surveyed in 3147 and 3172 individuals from 36 sampled counties respectively (Fig.1, Table 2). Of the 3 147 individuals tested for CCR2-64I, 1023 (38.23%) who were carriers of the CCR2-64I alleles, 79 (2.51%) were homozygous with CCR2-64I/ CCR2-64I, and 1124 (35.72%) were heterozygous with CCR2-64V/ CCR2-64I (Table 2). In contrast, of the 3172 individuals tested for SDF1-3'A, 1372(43.25%) who were carriers of the SDF1-3'A alleles, 98 (3.09%) were homozygous with SDF1-3'A / SDF1-3'A, and 1274 (40.16%) were heterozygous with SDF1-3'G / SDF1-3'A (Table 2). There were some differencies in the frequencies of genotypes of CCR2-64I and SDF1-3'A between the 36 sampled counties (Table 2). Our results indicated that the mean frequency of the CCR2-64I allele in the population of Shandong Province was 20.37% (N=3147), but there were some differencies in the frequencies of CCR2-64I allele between the 36 sampled counties (Table 2).The frequency of the SDF1-3'A allele also varied among the 36 sampled counties, with the mean frequency of the SDF1-3'A allele being 23.17% (N=3172) in the population of Shandong Province (Table 2). The spatial distribution of frequency SDF1-3'A or CCR2-64I were predicted and estimated using the kriging technique.

Spatial Genetic Structures Analysis on the Scale of Populations or Individuals

On the scale of populations, the distograms were calculated by the frequencies of alleles CCR2-64I and SDF1-3'A, and by the frequencies of 9 different combined genotypes of CCR2 and SDF1s (Table 1) respectively, which represented graphs where mean genetic distances between all pairs of population from 36 sampled counties belonging to a spatial distance class were plotted against the spatial distance classes (Fig. 3). The distogram Fig. 3 (a) was calculated by the frequencies of CCR2-64 allele, a stronger continuous increase of genetic distance with geographic distances was observed in this allele. Genetic distances were significantly lower than expected by chance in the first distance classes (up to 187.50 km) and significantly higher in the classes ranging from 412.50 km to 487.50 km. The distogram Fig. 3(b) was calculated by the frequencies of SDF1-3'A allele, whereas no spatial structure was found in this allele. The distogram Fig. 3 (c) was calculated by the frequencies of two alleles (CCR2-64I and SDF1-3'A), showing an increase of genetic distance over space, but it was not stronger than the CCR2-64I allele solely in Fig. 3 (a), with the distance classes 75 km showing lower , and the distance classes 412.50 km showing higher than expected genetic distances. Similarly, the distogram corresponding to the frequencies of 9 different combined genotypes of CCR2 and SDF1 shown in

Table 1, also presented an increase of genetic distance over space. It was also not stronger, with the distance classes 37.5km showing lower than expected genetic distances (Fig. 3 (d)).

TABLE 2

Distribution of CCR2-64I and SDF1-3'A Alleles in the 36 Sampled County of Shandong Province, China

Sampled County	Size	CCR2-64I				(SDF1-3'A)				Allele Frequency	
		Tested Samples	Genotypes			Tested Samples	Genotypes			CCR2-64I	SDF1-3'A
			wt/wt	wt/mt	mt/mt		wt/wt	wt/mt	mt/mt		
01:Dongming	100	95	64	30	1	93	45	44	4	0.1684	0.2796
02:Mudan	100	95	64	30	1	92	45	43	4	0.1684	0.2772
03:Chengwu	100	97	66	31	0	93	47	42	4	0.1600	0.2688
04:Jinxiang	112	92	60	29	3	92	47	41	4	0.1902	0.2663
05:Yanzhou	102	91	56	31	4	89	43	45	1	0.2143	0.2640
06:Dongping	60	58	34	23	1	57	30	25	2	0.2155	0.2544
07:Feicheng	72	53	32	20	1	53	32	21	0	0.2075	0.1981
08:Taian	81	63	39	24	0	63	31	29	3	0.1905	0.2778
09:Qihe	113	63	39	21	3	63	34	28	1	0.2143	0.2381
10:Gaotang	99	97	60	33	4	92	45	43	4	0.2113	0.2717
11:Decheng	101	91	56	32	3	92	50	40	2	0.2088	0.2391
12:Ningjin	114	83	50	29	4	87	47	36	4	0.2229	0.2529
13:Shanghe	100	100	62	36	2	100	60	39	1	0.2000	0.2050
14:Zhanhua	99	99	62	34	3	99	57	39	3	0.2020	0.2273
15:Lijin	106	90	58	28	4	96	59	37	0	0.2000	0.1927
16:Zouping	100	94	60	30	4	97	60	36	1	0.2021	0.1959
17:Huantai	101	86	54	31	1	88	54	32	2	0.1919	0.2045
18:Zhangqiu	134	100	60	39	1	100	59	39	2	0.2050	0.2150
19:Zichuan	112	91	57	32	2	93	57	30	6	0.1978	0.2258
20:Yiyuan	108	97	60	35	2	92	56	30	6	0.2010	0.2283
21:Laiwu	100	83	51	31	1	85	44	35	6	0.1988	0.2765
22:Zoucheng	100	89	56	31	2	89	46	39	4	0.1966	0.2640
23:Shanting	104	98	63	31	4	102	61	37	4	0.1990	0.2206
24:Feixian	103	89	55	34	0	92	58	30	4	0.1910	0.2065
25:Xuecheng	124	95	61	30	4	104	62	38	4	0.2000	0.2212
26:Cangshan	118	102	60	40	2	110	63	45	2	0.2157	0.2227
27:Linshu	103	93	53	40	0	94	52	41	1	0.2151	0.2287
28:Qingzhou	108	92	56	36	0	93	58	30	5	0.1957	0.2151
29:Guangrao	100	95	59	33	3	92	57	34	1	0.2053	0.1957
30:Changyi	107	95	56	38	1	93	59	29	5	0.2105	0.2097
31:Rizhao	107	82	48	34	0	84	54	30	0	0.2073	0.1786
32:Jiaonan	51	50	30	17	3	50	33	16	1	0.2300	0.1800
33:Jimo	52	50	29	19	2	50	33	16	1	0.2300	0.1800
34:Penglai	108	102	60	37	5	102	54	46	2	0.2304	0.2451
35:Muping	103	96	54	38	4	100	53	45	2	0.2396	0.2450
36:Wendeng	105	101	60	37	4	101	55	44	2	0.2228	0.2376
Total	3607	3147	1944	1124	79	3172	1800	1274	98	0.2037	0.2317

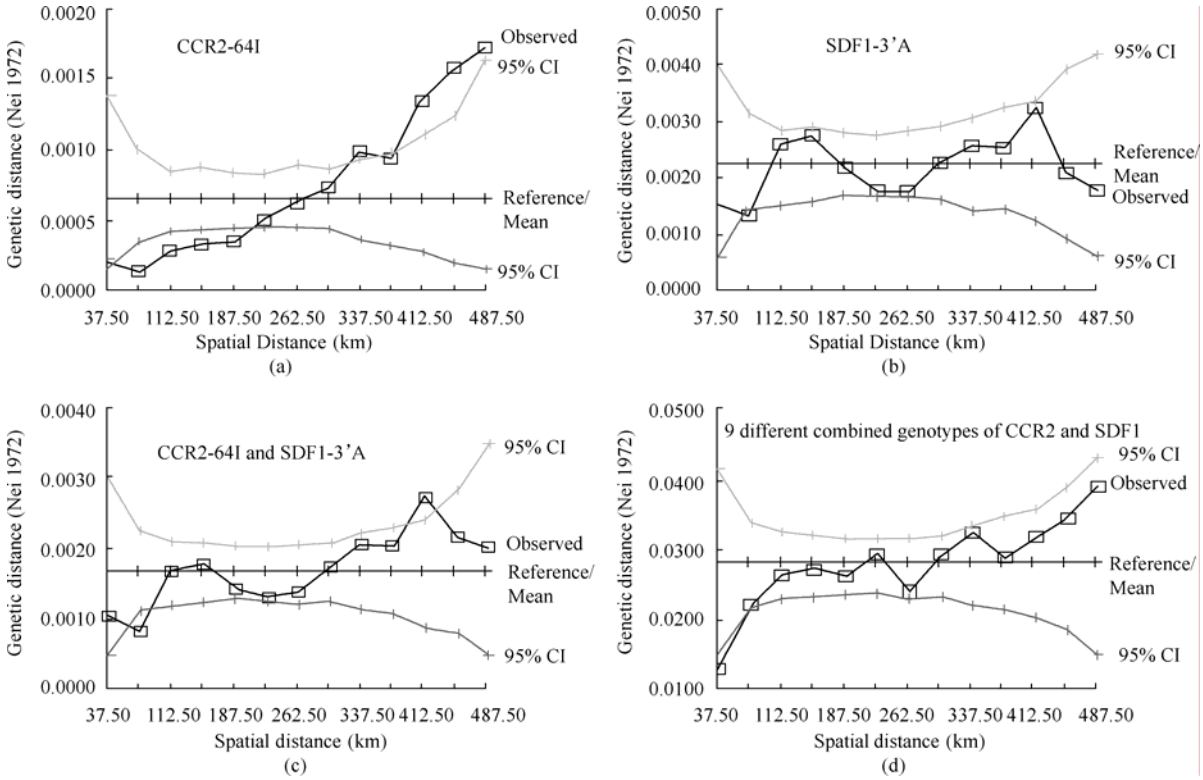

FIG. 3. Distograms of average genetic distance in 13 spatial distance classes. All of the (a), (b), (c), (d) were calculated by using genetic distance measures (Nei 1972).

On the scale of individuals, for individuals in each sampled county, and for all the sampled individuals in the whole area of Shandong Province, the average genetic distance measure (D_G)[35] in different number of spatial distance classes was calculated using individuals' genotypes (CCR2-64V/ CCR2-64V, CCR2-64V/ CCR2-64I, CCR2-64I/ CCR2-64I, SDF1-3'G / SDF1-3'G, SDF1-3'A / SDF1-3'A, SDF1-3'A / SDF1-3'A) (Table 3). However, the results in Table 3 do not present significant value at most spatial distance classes for individuals in each sampled county. There were only slightly spatial genetic structures with several distance classes in several sampled counties (25: Xuecheng, 30: Changyi, 33: Jimo, 34: Penglai, 36: Wendeng). Similarly, for all the sampled individuals in the whole area of Shandong Province, no spatial structure was found in individuals' genotypes. This result was different from that on the scale of populations in Fig. 3.

Predicting and Estimating the Spatial Distribution of Frequency SDF1-3'A or CCR2-64I and the RH Based on Different AIDS Definitions

To smooth the frequency surfaces of CCR2-64I or SDF1-3'A allele and produce their informative visual representations, the Kriging technique was used for interpolation using the GS+ software. Based on the isotropy of semivariogram in the distance range shorter than 496.55 km for the CCR2-64I allele, and 441.55 km for SDF1-3'A allele, the parameters for isotropy spherical model of semivariogram were applied to ordinary block Kriging (Fig. 4). For Kriging, the area of Shandong Province was divided into 5 km × 5 km square blocks and the integral calculation for the blocks was replaced by 2 × 2 discrete points. The contour maps presented obvious geographic diversities in the two alleles. However, their changing directions were inverse. The frequency of CCR2-64I allele gradually increased from the southwest to the northeast, while the frequency of SDF1-3'A allele gradually increased from the northeast to the southwest with a lower area in east of the Province (Fig. 4 (b) and (e)).

In order to predict and estimate the spatial distribution of *RH* on SDF1-3'A and CCR2-64I, the RH value for the 9 combined types of different genotypes (Table 1) was evaluated for each population on the basis of the two-locus genotype of each individual. The three types of *RH* according to AIDS' three definitions are shown in Table 4 for each population from 36 sampled counties, their corresponding contour maps are shown in Fig. 5. The

results in Table 4 and Fig. 5 do not represent obvious geographic diversity on the whole area of the Province. The range of three types of *RH* was all significantly narrow, with *RH*1 being 0.8396 to 0.8770 calculated by definition of AIDS-1993, *RH*2 being 0.8312 to 0.8749 calculated by definition of AIDS-1987, and *RH*3 being 0.7921 to 0.8529 calculated by definition of AIDS- Death[7].

TABLE 3

The Mean Genetic Distances (Gregorius 1978) Between All Pairs Genotypes Belonging to CCR2 and SDF1 of Individuals From Each 36 Sampled Counties or From the Whole Area of Shandong Province

Sampled	Spatial Distance (km)									Reference/
County	5.625	11.250	16.875	22.500	28.125	33.750	39.375	45.000	50.625	Mean
01:Dongming	0.3425	0.3434	0.3378	0.3466	0.3288	0.3610	0.3379	0.3632		0.3427
02:Mudan	0.3391	0.3443	0.3448	0.3399	0.3462	0.3624	0.3773	0.4081	0.3810	0.3473
03:Chengwu	0.3256	0.3177	0.3238	0.3298	0.3151	0.3439	0.3200			0.3234
04:Jinxiang	0.3542	0.3492	0.3536	0.3560	0.3381	0.3481	0.3500			0.3512
05:Yanzhou	0.3026	0.2940	0.3131	0.3185	0.3600	0.4444				0.3111
06:Dongping	0.3311	0.3262	0.3304	0.3401	0.3021	0.3245	0.2658	0.2705	0.2727	0.3199
07:Feicheng	0.3228	0.3168	0.3065	0.3210	0.3420	0.3695	0.3173	0.2500		0.3258
08:Taian	0.3503	0.3346	0.3554	0.3713	0.3896					0.3522
09:Qihe	0.3438	0.3642	0.3594	0.3686	0.3295	0.3365	0.3537	0.3854	0.3318	0.3523
10:Gaotang	0.3526	0.3595	0.3713	0.3685	0.3470	0.3562	0.4500			0.3623
11:Decheng	0.3515	0.3494	0.3365	0.3268	0.3609	0.3236	0.2500			0.3427
12:Ningjin	0.3416	0.3589	0.3548	0.3268	0.3401	0.3717	0.5000			0.3471
13:Shanghe	0.3296	0.3375	0.3140	0.3279	0.3256	0.3267	0.2465	0.1944		0.3243
14:Zhanhua	0.3454	0.3244	0.3368	0.3300	0.3269	0.3438	0.3367	0.3110	0.2500	0.3322
15:Lijin	0.3113	0.3147	0.3050	0.3028	0.2758	0.3197	0.3260	0.3316	0.3626	0.3138
16:Zouping	0.3279	0.3150	0.3124	0.3224	0.3415	0.3256	0.3328	0.3500	0.2868	0.3247
17:Huantai	0.2919	0.2911	0.3118	0.3457*	0.3415					0.3040
18:Zhangqiu	0.3314	0.3295	0.3359	0.3395	0.3147	0.3299	0.3158	0.3304	0.3136	0.3303
19:Zichuan	0.3073	0.3138	0.3213	0.3503	0.3579	0.3440	0.4167	0.3571	0.3750	0.3316
20:Yiyuan	0.2855	0.3042	0.3057	0.3326	0.3509	0.3636	0.3475	0.3958	0.3166	0.3243
21:Laiwu	0.3732	0.3582	0.3570	0.3676	0.3628	0.3367	0.3109	0.3325	0.3125	0.3581
22:Zoucheng	0.3642	0.3444	0.3291	0.3579	0.3469	0.3431	0.3584	0.4187	0.4500	0.3514
23:Shanting	0.3251	0.3382	0.3375	0.3333	0.3274	0.3337	0.3395	0.3636	0.4047	0.3352
24:Feixian	0.3214	0.3209	0.3149	0.3292	0.3265	0.3111	0.3049	0.3144	0.3103	0.3195
25:Xuecheng	0.3310	0.3309	0.3230	0.3227	0.3006	0.2245	0.1667	0.0938*		0.3231
26:Cangshan	0.3455	0.3561	0.3455	0.3417	0.3343	0.3464	0.3237	0.2500	0.2500	0.3411
27:Linshu	0.3457	0.3541	0.3501	0.3388	0.3577	0.3486	0.2805			0.3483
28:Qingzhou	0.3448	0.3297	0.3409	0.3418	0.3191	0.3188	0.3162	0.2654		0.3318
29:Guangrao	0.3077	0.2867	0.3115	0.3087	0.3333	0.3067	0.2759			0.3067
30:Changyi	0.3039*	0.3184	0.3211	0.3238	0.3412	0.3510	0.3327	0.3950*	0.3445	0.3331
31:Rizhao	0.2943	0.2768	0.3147	0.3158	0.3076	0.3083	0.3019	0.3109	0.2738	0.3040
32:Jiaonan	0.3405	0.4375	0.3396	0.3677	0.3648	0.3514	0.3914	0.3178	0.5000	0.3629
33:Jimo	0.3486	0.3173*	0.3515	0.3881	0.3742	0.3886	0.3234	0.3659	0.2984	0.3573
34:Penglai	0.3836*	0.3320*	0.3578	0.3590	0.3780	0.3851	0.4958*			0.3639
35:Muping	0.3710	0.3743	0.3655	0.3888	0.3761	0.3811	0.3518	0.4025	0.3702	0.3739
36:Wendeng	0.3578	0.3581	0.3526	0.3565	0.3426	0.3524	0.4056*	0.4107	0.2500	0.3558
Spatial Distance (km)		67.50	135.00	202.50	270.00	337.50	405.00	472.50	540.00	
Shandong Province		0.3333	0.3328	0.3356	0.3346	0.3364	0.3430	0.3501	0.3535	0.3368

Note. (1) Reference/Mean: value indicating absence of spatial autocorrelation; (2)*: values outside the 95% confidence interval of 500 permutations.

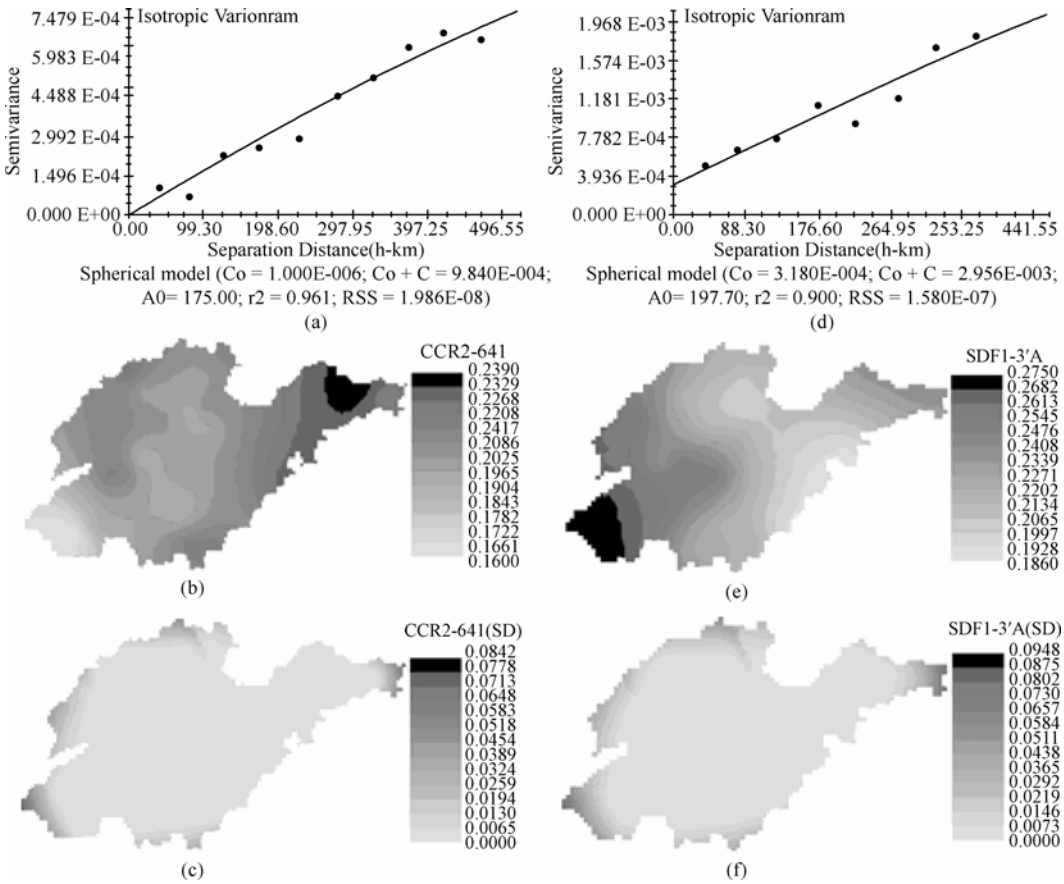

FIG. 4. Spatial Prediction and Estimation of Frequency of Allele (CCR2-64I or SDF1-3'A). (a) is the graph of the observed semivariogram values and the theoretical semivariogram of the curve calculated by frequency of CCR2-64I allele; (b) is the map of Kriging estimation of the frequency of allele CCR2-64I; (c) is the map of Kriging estimation standard deviation of the frequency of allele CCR2-64I, it indicates predicting error of the Kriging estimation; (d) is the graph of the observed semivariogram values and the theoretical semivariogram of the curve calculated by frequency of SDF1-3'A allele; (e) is the map of Kriging estimation of the frequency of allele SDF1-3'A; (f) is the map of Kriging estimation standard deviation of the frequency of allele SDF1-3'A.

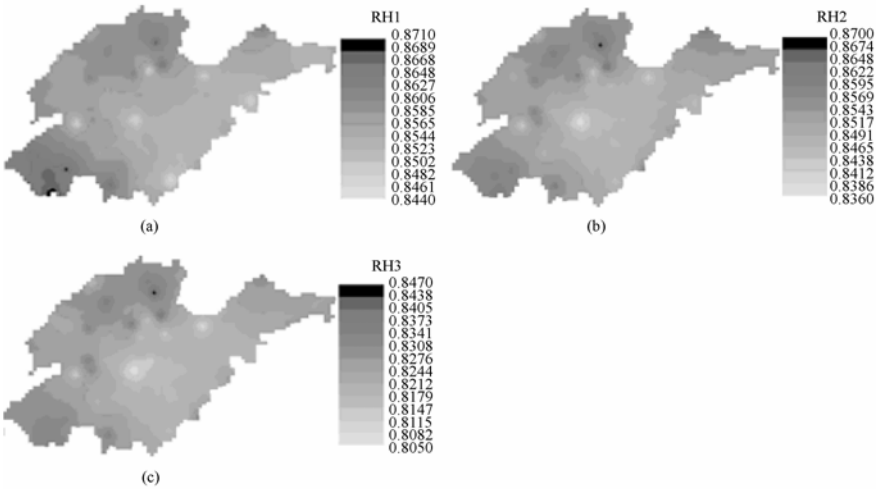

FIG. 5. Spatial Prediction and Estimatiion of RH of Three AIDS Definitions on 9 Different Genotypes of CCR2 and SDF1. (a) is the map of inverse distance-weighted estimation of RH1 calculated by definition of AIDS-1993; (b) is the map of inverse distance-weighted estimation of RH2 calculated by definition of AIDS-1987; (c) is the map of inverse distance-weighted estimation of RH3 calculated by definition of AIDS- Death[7].

TABLE 4

Frequency of 9 Different Combined Genotypes of CCR2 and SDF1 and Their Relative Hazard (*RH*) Based on the Three AIDS Definitions in Table 1

Sampled	Combined Genotypes of CCR2 and SDF1									Relative Hazard (*RH*)		
County	A	B	C	D	E	F	G	H	I	*RH*1	*RH*2	*RH*3
01:Dongming	0.3226	0.3333	0.0215	0.0000	0.0215	0.1505	0.1398	0.0000	0.0108	0.8770	0.8688	0.8415
02:Mudan	0.3146	0.3034	0.0000	0.0000	0.0449	0.1573	0.1685	0.0000	0.0112	0.8679	0.8576	0.8315
03:Chengwu	0.3407	0.2967	0.0110	0.0000	0.0330	0.1648	0.1538	0.0000	0.0000	0.8713	0.8626	0.8362
04:Jinxiang	0.3297	0.3077	0.0220	0.0000	0.0220	0.1758	0.1099	0.0110	0.0220	0.8705	0.8623	0.8337
05:Yanzhou	0.3810	0.2262	0.0000	0.0000	0.0119	0.2381	0.0952	0.0357	0.0119	0.8623	0.8628	0.8385
06:Dongping	0.3684	0.1754	0.0000	0.0000	0.0351	0.2807	0.1228	0.0175	0.0000	0.8396	0.8339	0.8045
07:Feicheng	0.4151	0.1887	0.0000	0.0000	0.0000	0.1698	0.2075	0.0189	0.0000	0.8613	0.8653	0.8415
08:Taian	0.3333	0.2540	0.0000	0.0000	0.0476	0.1587	0.2063	0.0000	0.0000	0.8545	0.8449	0.8172
09:Qihe	0.3492	0.2698	0.0159	0.0000	0.0000	0.1587	0.1587	0.0317	0.0159	0.8650	0.8648	0.8380
10:Gaotang	0.3370	0.2609	0.0109	0.0000	0.0326	0.1413	0.1739	0.0217	0.0217	0.8575	0.8494	0.8206
11:Decheng	0.3333	0.2644	0.0000	0.0000	0.0230	0.1954	0.1494	0.0115	0.0230	0.8587	0.8561	0.8306
12:Ningjin	0.3378	0.2568	0.0270	0.0000	0.0270	0.1486	0.1622	0.0405	0.0000	0.8548	0.8443	0.8116
13:Shanghe	0.4200	0.1900	0.0000	0.0000	0.0100	0.1700	0.1900	0.0100	0.0100	0.8633	0.8343	0.8403
14:Zhanhua	0.3265	0.2857	0.0000	0.0000	0.0204	0.2347	0.1020	0.0204	0.0102	0.8638	0.8618	0.8373
15:Lijin	0.4023	0.2299	0.0000	0.0000	0.0000	0.2184	0.1034	0.0345	0.0115	0.8713	0.8749	0.8529
16:Zouping	0.3474	0.2737	0.0000	0.0000	0.0105	0.2211	0.1053	0.0421	0.0000	0.8672	0.8680	0.8446
17:Huantai	0.3378	0.2568	0.0135	0.0000	0.0270	0.1622	0.1757	0.0000	0.0000	0.8387	0.8312	0.8036
18:Zhangqiu	0.3400	0.2500	0.0100	0.0000	0.0100	0.2500	0.1300	0.0100	0.0000	0.8553	0.8540	0.8263
19:Zichuan	0.3478	0.2500	0.0326	0.0000	0.0326	0.2609	0.0543	0.0109	0.0109	0.8553	0.8417	0.8075
20:Yiyuan	0.4000	0.2000	0.0222	0.0000	0.0444	0.2111	0.1111	0.0111	0.0000	0.8568	0.8424	0.8102
21:Laiwu	0.3373	0.2169	0.0120	0.0000	0.0602	0.1566	0.2048	0.0000	0.0120	0.8414	0.8254	0.7921
22:Zoucheng	0.2921	0.2921	0.0000	0.0000	0.0449	0.2135	0.1348	0.0112	0.0112	0.8534	0.8446	0.8169
23:Shanting	0.3776	0.2347	0.0102	0.0000	0.0306	0.1939	0.1122	0.0204	0.0204	0.8627	0.8551	0.8275
24:Feixian	0.4045	0.1910	0.0225	0.0000	0.0225	0.2247	0.1348	0.0000	0.0000	0.8557	0.8476	0.8164
25:Xuecheng	0.3871	0.2473	0.0000	0.0108	0.0215	0.1828	0.1183	0.0215	0.0108	0.8706	0.8653	0.8394
26:Cangshan	0.3469	0.2347	0.0102	0.0000	0.0102	0.2347	0.1429	0.0000	0.0204	0.8523	0.8510	0.8227
27:Linshu	0.2727	0.2727	0.0000	0.0000	0.0114	0.2955	0.1477	0.0000	0.0000	0.8407	0.8419	0.8139
28:Qingzhou	0.3587	0.2283	0.0326	0.0000	0.0217	0.2609	0.0978	0.0000	0.0000	0.8518	0.8414	0.8072
29:Guangrao	0.3626	0.2747	0.0000	0.0000	0.0110	0.2418	0.0989	0.0110	0.0000	0.8728	0.8733	0.8509
30:Changyi	0.3548	0.2151	0.0323	0.0000	0.0215	0.2688	0.0968	0.0108	0.0000	0.8459	0.8359	0.8007
31:Rizhao	0.3951	0.1975	0.0000	0.0000	0.0000	0.2593	0.1481	0.0000	0.0000	0.8574	0.8615	0.8370
32:Jiaonan	0.3000	0.3000	0.0200	0.0000	0.0000	0.2000	0.1200	0.0400	0.0200	0.8580	0.8570	0.8280
33:Jimo	0.3000	0.2600	0.0000	0.0000	0.0200	0.2200	0.1600	0.0400	0.0000	0.8456	0.8442	0.8166
34:Penglai	0.3663	0.2178	0.0198	0.0000	0.0000	0.1683	0.1980	0.0000	0.0495	0.8653	0.8347	0.8336
35:Muping	0.2826	0.2727	0.0000	0.0000	0.0000	0.1739	0.2391	0.0217	0.0217	0.8510	0.8555	0.8281
36:Wendeng	0.3267	0.2574	0.0099	0.0000	0.0099	0.1980	0.1584	0.0198	0.0198	0.8532	0.8520	0.8240
Total	0.3491	0.2502	0.0101	0.0003	0.0206	0.2051	0.1411	0.0137	0.0101	0.8585	0.8539	0.8260

DISCUSSION

In the Chinese mainland, higher frequencies of mutation CCR2-64I (19.15%-28.79%) and SDF1-3'A (19.10%-29.86%) alleles have been found in subjects of 8 ethnic groups[20]. The present study indicated that the frequencies of mutation CCR2-64I and SDF1-3'A alleles in Han population of Shanding Province, China, were 20.37% and 23.17% respectively. Though Shandong is a small area compared with the whole Chinese mainland, the different prevalence of the CCR2-64I and SDF1-3'A allele frequencies in this province reflects the trend observed in the population of the whole Chinese mainland population[17-18]. In the Chinese mainland, there is an obvious geographic diversity–gradual increase from the south to the north for the frequency of CCR2-64I allele. On the contrary, for the frequency of SDF1-3'A allele, there is an obvious geographic diversity–gradual increase from north to the south[18]. In the present study, similar geographic diversities were found in Shandong Province. Also, there is a gradual increase from the southwest to the northeast for the frequency of CCR2-64I allele and a gradual increase from the northeast to the southwest for the frequency of SDF1-3'A allele.

Geographic patterns of genetic diversity allow us to make inferences about population histories and the evolution of inherited disease[24]. In the present paper, the genetic distance measures[34-35] were combined with the spatial statistical technique to analyze the spatial genetic structure of two CCR2-64I (or SDF1-3'A) loci on the scales of populations and individuals. The results indicate that there are significant spatial genetic structures at different spatial distance classes on the scale of populations, but on the scale of individuals, no spatial structure is found in either the whole area of Shandong Province or the area of each sampled county. This situation indicates that the spatial genetic structure is different between the scales of populations and individuals, and that the frequency of allele usually has some spatial genetic structures or spatial autocorrelation at different spatial distance classes, but the genotypes of individuals have random distributions in the same geographic area.

We observed that the spatial pattern of the frequencies of CCR2-64I and SDF1-3'A alleles did not reflect the spatial pattern of their *RH* to AIDS. The frequencies of CCR2-64I alleles increased gradually from the southwest to the northeast, and the frequency of SDF1-3'A allele increased gradually from the northwest to the southeast. However, the *RH* of three AIDS definitions of each population from 36 sampled counties, evaluated by using the 9 combined types of different genotypes on the basis of the two-locus genotype of each individual, did not represent obvious geographic diversity on the whole area of the province. This indicates that evaluating spatial distribution of the genetic susceptibility to HIV (AIDS) based on CCR2-64I and SDF1-3'A alleles, should focus on the frequencies of combined genotypes of CCR2 and SDF1 based on the two-locus genotype of each individual rather than the frequencies of CCR2-64I and SDF1-3'A alleles.

According to the research objective, several statistics can be used to quantify the spatial genetic structure: (a) Spatial autocorrelation analysis of genetic structure: Moran's index and Geary's index are among the most frequently used measures[38-39]. Other approaches can also be used for detecting the spatial autocorrelation of genetic structure. In this paper, in order to combine the genetic distance measures with the spatial statistics, the genetic distograms[27-28] representing graphs where mean genetic distances between all pairs of population (or individuals) belonging to a spatial distance class (s_q) were plotted against the spatial distance classes, were used for analyzing the spatial genetic structure of CCR2-64I and SDF1-3'A alleles at both populations and individuals scale. (b) Spatial interpolation and prediction of genes or genetic structure: a number of approaches can be used for modeling or predicting the spatial distribution of genes or genetic structure at a given geographical location using data from the surrounding regions: Cavalli-Sforza method in genography–a technique currently used in mapping geographic distribution of genes[40], inverse distance-weighted methods[30], trends surface analysis[41] and splines[42]. For example, using the method of Cavalli-Sforza, Barbujani G has mapped the frequency of the CCR5-Δ32 mutation in various countries of Europe[24] from the data of Stephens[43] and displayed the geographic patterns of the frequency of the CCR5-Δ32 in Europe. However, this mapping only used the frequency of CCR5-Δ32 of different population in an large region rather than a specific small area. On the other hand, the method of Cavalli-Sforza, has two limitations. Firstly, they fix tuning constants or make prior assumptions that do not take advantage of the spatial genetic structure of the variable. Secondly, they do not allow for estimation of the error of prediction. So the Kriging technique[29], a geostatistics method which can overcome the limitations of Cavalli-Sforza above, was used for predicting and estimating the spatial distribution of frequency of SDF1-3'A and CCR2-64I in the present study. However, when the genetic variables were distributed randomly in the geographic

area, the Kriging technique could not predict or estimate their spatial distribution accurately. As a result, the inverse distance-weighted methods[30] was used to predict and estimate the spatial distribution of RH values on there different AIDS definitions (AIDS-1993, AIDS-1987, and Related Deaths) in this study.

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