Association of *AKT1* Gene Polymorphisms With Risk of Schizophrenia and With Response to Antipsychotics in the Chinese Population

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Background: A number of studies have pointed to the involvement of *AKT* signaling pathways in the etiology of schizophrenia. The purpose of this study was to determine whether the *AKT1* gene is involved in the etiology of schizophrenia and whether it affects therapeutic outcomes in the Chinese population.

Method: Five single nucleotide polymorphisms (SNPs) were genotyped among 384 schizophrenic patients (DSM-IV criteria) and 384 healthy controls from the Chinese population. We systematically analyzed the association of the *AKT1* gene with schizophrenia on the basis of sex, age at onset, therapeutic response to typical antipsychotics and atypical antipsychotics, and presence or absence of extrapyramidal syndrome. The study was conducted from May 2004 to June 2006.

Results: We found a positive association of the G allele of the SNP marker rs3803300 with schizophrenia (p = .003), both in early-onset and late-onset subjects, and that a haplotype A-G-C-G-A constructed by the 5 SNPs showed significant association (p = .00004886). However, we found no relationship between any of the 5 SNP markers and therapeutic response to typical and atypical antipsychotics and chlorpromazine-induced extrapyramidal syndrome.

Conclusions: Our study suggests that *AKT1* is a susceptibility gene for schizophrenia in the Chinese population and that the *AKT1* gene may play no major role in the therapeutic response to antipsychotics or in chlorpromazine-induced extrapyramidal syndrome.

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Schizophrenia is a severe and chronic disabling brain disorder involving genetic, biological, and environmental factors. Although schizophrenia afflicts approximately 1% of the population throughout the world,[1] the ultimate biological cause of the disorder remains elusive. Lines of evidence suggest that neurodevelopmental abnormalities of specific brain areas, including disturbances of neuron migration, alteration in neural plasticity, and changes in synaptic connection, are important factors in the pathogenesis of this disease.[2–4]

AKT1, an isoform of serine/threonine protein kinase *AKT* isolated from an *AKR* mouse thymoma cell line transforming with murine retrovirus *AKT8*[5] (also known as protein kinase B), is involved in the regulation of intracellular signaling pathways,[6] including some that might be important for neurodevelopment, neural plasticity,[7] and working memory formation.[8] The *AKT1* gene is located on human chromosome 14q32.32, and chromosome 14q has been reported in a genome-wide scan as a susceptible locus for schizophrenia.[9]

AKT1 was first reported as having a role in the development of schizophrenia by Emamian et al.[10] on the basis of convergent evidence for impairment of *AKT1-GSK3β* signaling, and several other lines of evidence have

pointed to the involvement of *AKT1* in the pathophysiology of the disease, the most notable of which are as follows.

1. *AKT1* protein levels and *GSK3β* phosphorylation have been found to be decreased in peripheral lymphocytes, as well as in the hippocampus and frontal cortex of postmortem brains of schizophrenic patients.[10]
2. Haloperidol (a typical antipsychotic) treatment of mice has been shown to induce increased phosphorylation of *AKT1* and *GSK3β*.[10,11]
3. *AKT1*-deficient mice have shown a greater sensitivity to the sensorimotor-gating disruptive effect of amphetamine, as measured by prepulse inhibition of a startle response.[10]
4. DNA variants located in the gene encoding for *AKT1* on chromosome 14q32.32 have provided further evidence for association with schizophrenia. Nominally significant distortion of transmission has been detected for 1 of the 5 tested single nucleotide polymorphisms (SNPs), as well as for a 3-locus haplotype in a sample of 58 extended families and 210 proband parent trios.[10]
5. The core 2-SNP haplotype has been found to be associated with schizophrenia and with impaired *AKT1* expression in lymphoblast cell lines from individuals with schizophrenia.[10]

Even though strong evidence for association was presented by Emamian et al.[10] and subsequently was further confirmed by Schwab et al.,[12] 2 independent studies of these 5 SNPs of *AKT1* in the Japanese population revealed contradictory results.[13,14] In order to determine whether the *AKT1* gene is involved in the etiology of schizophrenia and affects therapeutic outcomes in the Chinese population, we used a case-control study to research the effects of these 5 SNPs of the *AKT1* gene on the risk of schizophrenia, on the response to clozapine and chlorpromazine, and on the chlorpromazine-induced extrapyramidal syndrome.

MATERIAL AND METHOD

Subjects and Psychiatric Assessment

A total of 384 unrelated schizophrenia patients (214 men and 170 women; mean ± SD age = 43.22 ± 11.85 years) were recruited from the Shanghai Mental Health Center. All of these subjects were inpatients and had been diagnosed according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, Text Revision. Diagnosis and review of psychiatric case records were independently checked and verified by 2 senior psychiatrists.

In accordance with the procedures reported in previous, related publications,[15–18] age at onset was determined as the patient's age during first hospitalization at which the diagnosis of schizophrenia was used (mean ± SD = 26.0 ± 11.7 years), and early or late onset of schizophrenia was defined on the basis of a first diagnosis at or before 25 years of age (early) or after 25 years of age (late). This cutoff of 25 years was chosen because, in most cases, the first symptoms (whether specific or not) occur at or before the age of 25 years.[16,17,19] It fitted approximately to the mean ± 1 SD of our sample. Early-onset, late-onset, and patients with no known age at onset corresponded to 41% (N = 156), 38% (N = 144), and 22% (N = 84), respectively, of the subjects under study.

Of the 384 patients, 141 were treated with an atypical antipsychotic only, i.e., clozapine, and another 138 with a typical antipsychotic only, i.e., chlorpromazine. Patients underwent a washout period of 2 to 4 weeks during which, unless clinically necessary, they received no medications before this study.[20–22] (Two patients in the prospective clozapine treatment group and 3 in the prospective chlorpromazine treatment group were allowed medication deemed clinically necessary during the washout period.) The applied dosage was in the range of 300 to 600 mg per day for both clozapine and chlorpromazine (the dosage was variable in case there was intolerance to the maximum dosage).[20,21] Clozapine and chlorpromazine blood levels were monitored throughout the course of treatment to ascertain compliance. The Brief Psychiatric Rating Scale (BPRS)[23,24] scores were evaluated before the 2 types of antipsychotics were prescribed followed by at least 8 weeks of antipsychotic treatment. To evaluate therapeutic response, the overall BPRS score was used for analysis, as described by previous publications.[16,20,24] To compare clinical response to clozapine and to chlorpromazine for the 3 *AKT1* genotypes, therapeutic response was evaluated by comparing the percentage score reduction for total BPRS score: [(baseline score – posttreatment score) × 100/baseline score]. Responders were defined as showing a minimum 40% decrease in the BPRS general score after antipsychotic treatment.[16,20,24] Of the 141 patients treated with clozapine only, 95 were responders and 46 were nonresponders; of another 138 patients treated with chlorpromazine only, 55 were responders and 83 were nonresponders.

The extrapyramidal syndrome (tardive dyskinesia symptoms and parkinsonism) status of each patient was assessed by at least 2 independent psychiatrists after the patients had taken chlorpromazine for 8 weeks. As in previous studies,[25–28] tardive dyskinesia symptoms were assessed with the Abnormal Involuntary Movement Scale (AIMS),[29] akathisia with the Barnes Akathisia Scale[30] and parkinsonism with the Simpson-Angus Scale.[31] Tardive dyskinesia was diagnosed if at least moderate abnormal involuntary movements in 1 or more of the 7 body areas or at least mild movements in 2 or

Table 1. The Primers Used in the Study of *AKT1* in the Etiology of Schizophrenia

SNP ID	Distance, Base Pair	Location	Genotyping Method	Primer Sequence	Annealing Temperature, °C	Product Size, Base Pair
rs3803300 (G > A)[a]	0	5′ UTR	Sequencing	5′-GTGGCATCATTGTCACTCGG-3′ 5′-CCATTAGGCAGGCAAAGCA-3′	58	586
rs1130214 (G > T)	10045	5′ UTR	Sequencing	5′-CCCCTTTGACTTCTTTGACCC-3′ 5′-TGCTCCTCACTGACGGACTTG-3′	58	406
rs3730358 (C > T)	23372	Intron 3	Sequencing	5′-GCCAGTGCTTGTTGCTTGC-3′ 5′-GGCGAGGGTCTGACGGGTA-3′	60	314
rs2498799 (G > A)	29885	Intron 11	Sequencing	5′-CTGTGATCTTAATGTGCCCGTC-3′ 5′-GGCTGGTCCCTTCCCTGT-3′	59	446
rs2494732 (A > G)	30587	Intron 12	Sequencing	5′-GCAGGGTTGGGGACAGAGG-3′ 5′-GTGCTGGAGGACAATGACTACG-3′	59	356

[a]G > A = a single nucleotide polymorphism (SNP) between alleles G and A in which allele G is a wild type and allele A is a mutated type. The expressions G > T, C > T, and A > G represent SNPs with the same type of relationship between alleles.
Abbreviation: SNP ID = single nucleotide polymorphism identifier.

more areas were present. Akathisia was diagnosed if a patient scored mild or worse on the global clinical assessment on the Barnes Akathisia Scale. If a patient had a total score of 0.2 or more on the Simpson-Angus Scale,[28] parkinsonism was diagnosed. Among the 138 patients treated with chlorpromazine, 53 experienced an extrapyramidal syndrome.

Three hundred eighty-four unrelated healthy individuals (202 males and 182 females; mean ± SD age = 43.75 ± 12.92 years), screened for absence of major mental disorders and family history, were recruited locally as control subjects. Patients did not differ significantly from controls with regard to gender distribution ($p = .17$) or age ($p = .36$).

All subjects (384 schizophrenic patients and 384 healthy controls) were Han Chinese in origin. The psychiatric assessment was completed before genetic analysis, and the choice for analysis factors was done blind to the genotyping results. The study, which was conducted from May 2004 to June 2006, received the approval of the Shanghai Ethical Committee of Human Genetic Resource. All subjects gave their written informed consent prior to the study.

Genotyping

Peripheral blood samples were obtained from the subjects for DNA extraction using the phenol-chloroform method. The 5 SNPs (National Center for Biotechnology Information [NCBI] dbSNP ID: rs3803300, rs1130214, rs3730358, rs2498799, and rs2494732) studied by Emamian et al.[10] were genotyped by direct DNA sequencing. Polymerase chain reaction (PCR) amplification was carried out in a final volume of 15 μL, containing 10 ng genomic DNA, 0.2 μm of each primer, 2.5 mm manganese chloride, 2 mm deoxyribonucleoside triphosphate (dNTP), 1.5 μL of 10 × buffer (Qiagen, Basel, Switzerland), and 1 U HotStar Taq DNA polymerase (Qiagen, Basel, Switzerland). Thermocycling was performed on a Gene Amp PCR system 9700 (Applied Biosystems, Foster City, Calif.) according to a modified touchdown protocol, with an initial denaturation at 95°C for 10 minutes, followed by 60 cycles of denaturation at 95°C for 30 seconds and extension at 72°C for 1 minute. The annealing temperature was decreased from 64°C by 0.5°C per cycle for a total of 20 cycles, followed by 30 cycles at the final annealing temperature (the detailed annealing temperature for each of the 5 SNPs is given in Table 1), and final extension of 10 minutes at 72°C. Preparation of DNA for sequencing included incubation of PCR products with 0.1 U shrimp alkaline phosphatase (Roche, Basel, Switzerland) at 37°C for 45 minutes, followed by heat inactivation at 85°C for 20 minutes. The PCR products were sequenced using an ABI Prism BigDye Terminator Cycle Sequencing Kit, version 3.1 (Applied Biosystems, Foster City, Calif.) on an ABI Prism 3100 sequencer (Applied Biosystems, Foster City, Calif.). In order to ensure that the genotypes obtained were valid, reverse-direction regenotyping was performed on 100 randomly selected DNA samples for each of the 5 SNPs. All the results of regenotyping were identical to those obtained from the first round of genotyping. Details of the 5 markers and the primer sequences are listed in Table 1.

Statistical Analysis

Allele frequencies were calculated using the software SPSS 11.0 for Windows (SPSS Inc., Chicago, Ill.). Deviations from Hardy-Weinberg equilibrium were tested on an online calculator at http://www.kursus.kvl.dk/shares/vetgen/_Popgen/genetik/applets/kitest.htm. SPSS 11.0 was also used to compare the discrepancies of allele, genotype, and haplotype frequencies between patient and control subjects based on the χ^2 test and to calculate the odds ratios (ORs) with 95% confidence intervals (95% CIs). The pairwise linkage disequilibrium (LD), measured by standardized D′, was estimated using the 2LD software.[32] Haplotypes were inferred by the Bayesian method[33] and implemented on the software PHASE 1.0, which is available at http://www.stat.washington.edu/stephens/software.html. The statistical power of the sample size was estimated using the G*Power program.[34]

Table 2. Genotype and Allele Distributions of the 5 Single Nucleotide Polymorphisms Within *AKT1* Gene

A. rs3803300 (G > A)[a]

	Genotype Distribution				Allele Distribution			Test for HWE	
Subjects	N	GG	GA	AA	No.	G	A	Frequency of G Allele	χ^2	p Value
Controls, all	355	33	127	195	710	193	517	0.272	3.294	.070
Male	186	20	60	106	372	100	272	0.269	5.987	.014
Female	165	12	65	88	330	89	241	0.270	0.000	1.000
Patients, all	349	45	150	154	698	240	458	0.344	0.787	.375
Male	179	21	83	75	358	125	233	0.349	0.073	.787
Female	136	17	58	61	272	92	180	0.338	0.305	.581
Early-onset	148	18	68	62	296	104	192	0.351	0.010	.922
Late-onset	128	17	54	57	256	88	168	0.344	0.540	.463
Responders to chlorpromazine	48	8	19	21	96	35	61	0.365	1.019	.313
Nonresponders to chlorpromazine	70	8	30	32	140	46	94	0.329	0.058	.810
Responders to clozapine	93	10	45	38	186	65	121	0.349	0.383	.536
Nonresponders to clozapine	44	4	18	22	88	26	62	0.295	0.013	.908
EPS for chlorpromazine	46	8	20	18	92	36	56	0.391	0.351	.554
No EPS for chlorpromazine	62	6	26	30	124	38	86	0.306	0.011	.916

B. rs1130214 (G > T)

	Genotype Distribution				Allele Distribution			Test for HWE	
Subjects	N	GG	GT	TT	No.	G	T	Frequency of G Allele	χ^2	p Value
Controls, all	375	298	71	6	750	667	83	0.889	0.545	.460
Male	194	157	33	4	388	347	41	0.894	1.941	.164
Female	178	138	38	2	356	314	42	0.882	0.118	.731
Patients, all	286	213	67	6	572	493	79	0.862	0.073	.787
Male	150	113	35	2	300	261	39	0.870	0.149	.699
Female	100	75	22	3	200	172	28	0.860	0.746	.388
Early-onset	112	89	19	4	224	197	27	0.879	4.473	.034
Late-onset	111	80	30	1	222	190	32	0.856	1.010	.315
Responders to chlorpromazine	48	36	8	4	96	80	16	0.833	7.680	.006
Nonresponders to chlorpromazine	73	59	14	0	146	132	14	0.904	0.821	.365
Responders to clozapine	61	41	20	0	122	102	20	0.836	2.345	.126
Nonresponders to clozapine	27	20	7	0	54	47	7	0.870	0.599	.439
EPS for chlorpromazine	50	42	7	1	100	91	9	0.910	1.056	.304
No EPS for chlorpromazine	63	48	13	2	126	109	17	0.865	0.848	.357

C. rs3730358 (C > T)

	Genotype Distribution				Allele Distribution			Test for HWE	
Subjects	N	CC	CT	TT	No.	C	T	Frequency of C Allele	χ^2	p Value
Controls, all	337	294	41	2	674	629	45	0.933	0.189	.663
Male	173	147	25	1	346	319	27	0.922	0.003	.955
Female	161	144	16	1	322	304	18	0.944	0.551	.458
Patients, all	345	306	38	1	690	650	40	0.942	0.025	.875
Male	172	150	21	1	344	321	23	0.933	0.080	.778
Female	132	118	14	0	264	250	14	0.947	0.414	.520
Early-onset	136	120	15	1	272	255	17	0.938	0.471	.493
Late-onset	130	116	14	0	260	246	14	0.946	0.421	.516
Responders to chlorpromazine	39	32	7	0	78	71	7	0.910	0.379	.538
Nonresponders to chlorpromazine	74	61	12	1	148	134	14	0.905	0.210	.647
Responders to clozapine	88	78	10	0	176	166	10	0.943	0.319	.572
Nonresponders to clozapine	42	40	2	0	84	82	2	0.976	0.025	.874
EPS for chlorpromazine	43	38	4	1	86	80	6	0.930	3.452	.063
No EPS for chlorpromazine	67	54	13	0	134	121	13	0.903	0.773	.379

(continued)

In this study, the p values were 2 tailed and significance was set at $p < .05$.

RESULTS

Table 2 shows the genotype and allele frequencies of all 5 SNPs among patients and control subjects, according to sex, age at onset, therapeutic efficacy, and presence or absence of extrapyramidal adverse effects. The genotype distributions of all 5 SNPs among all control subjects showed no significant deviations from Hardy-Weinberg equilibrium.

Individual SNP Analysis

According to sex, age at onset, therapeutic response, and extrapyramidal adverse effects, we stratified and compared the discrepancies of alleles and genotypes among cases and controls for each of the 5 SNPs using the χ^2-based tests. The allele- and genotype-based analytic results for the individual SNPs are presented in Table 3.

Table 2 (continued). Genotype and Allele Distributions of the 5 Single Nucleotide Polymorphisms Within *AKT1* Gene

D. rs2498799 (G > A)

	Genotype Distribution				Allele Distribution		Frequency	Test for HWE	
Subjects	N	GG	GA	AA	No.	G	A	of G Allele	χ^2	p Value
Controls, all	361	59	162	140	722	280	442	0.388	1.089	.297
Male	184	33	84	67	368	150	218	0.408	0.550	.458
Female	174	25	78	71	348	128	220	0.368	0.227	.634
Patients, all	320	56	134	130	640	246	394	0.384	4.245	.039
Male	160	27	77	56	320	131	189	0.409	0.004	.952
Female	122	24	44	54	244	92	152	0.377	6.582	.010
Early-onset	131	24	56	51	262	104	158	0.397	1.503	.220
Late-onset	121	24	51	46	242	99	143	0.409	1.989	.158
Responders to chlorpromazine	43	10	16	17	86	36	50	0.419	2.386	.122
Nonresponders to chlorpromazine	66	14	24	28	132	52	80	0.394	3.753	.053
Responders to clozapine	76	14	35	27	152	63	89	0.414	0.199	.655
Nonresponders to clozapine	44	4	20	20	88	28	60	0.318	0.100	.752
EPS for chlorpromazine	44	10	17	17	88	37	51	0.420	1.889	.169
No EPS for chlorpromazine	61	13	23	25	122	49	73	0.402	2.834	.092

E. rs2494732 (A > G)

	Genotype Distribution				Allele Distribution		Frequency	Test for HWE	
Subjects	N	AA	AG	GG	No.	A	G	of A Allele	χ^2	p Value
Controls, all	369	27	144	198	738	198	540	0.268	0.014	.907
Male	191	19	75	97	382	113	269	0.296	0.631	.427
Female	175	8	67	100	350	83	267	0.237	0.592	.442
Patients, all	362	8	154	200	724	170	554	0.235	12.237	.000
Male	179	4	83	92	358	91	267	0.254	8.897	.003
Female	141	3	55	83	282	61	221	0.216	3.194	.074
Early-onset	147	4	60	83	294	68	226	0.231	3.213	.073
Late-onset	135	3	62	70	270	68	202	0.252	6.457	.011
Responders to chlorpromazine	53	1	22	30	106	24	82	0.226	1.813	.178
Nonresponders to chlorpromazine	76	1	32	43	152	34	118	0.224	3.427	.064
Responders to clozapine	90	1	43	46	180	45	135	0.250	6.760	.009
Nonresponders to clozapine	41	1	15	25	82	17	65	0.207	0.525	.469
EPS for chlorpromazine	48	0	19	29	96	19	77	0.198	2.923	.087
No EPS for chlorpromazine	71	3	30	38	142	36	106	0.254	0.961	.327

[a]G > A = a single nucleotide polymorphism (SNP) between alleles G and A in which allele G is a wild type and allele A is a mutated type.
The expressions G > T, C > T, and A > G represent SNPs with the same type of relationship between alleles.
Abbreviations: EPS = extrapyramidal syndrome, HWE = Hardy-Weinberg equilibrium.

The SNP marker rs3803300 (between alleles G and A [G > A]) showed great statistical significance for allele and genotype frequencies among all 384 patients and 384 healthy control subjects (for allele G vs. allele A: p = .003, OR = 1.404, 95% CI = 1.118 to 1.762; for genotypes GG and GA vs. genotype AA: p = .004, OR = 1.543, 95% CI = 1.146 to 2.077). After stratified analysis based on sex and age at onset, significant discrepancies of allele and genotype frequencies appeared between male patients and male healthy control subjects (for allele G vs. allele A: p = .019, OR = 1.459, 95% CI = 1.064 to 2.001; for genotypes GG and GA vs. genotype AA: p = .004, OR = 1.837, 95% CI = 1.213 to 2.783). The frequency of allele G in female patients (34%) was greater than that in female control subjects (27%). Both in early- and late-onset subjects, we found significant discrepancies of allele and genotype frequencies among patients and all 384 healthy controls: for early-onset subjects, (1) allele G versus allele A: p = .012, OR = 1.451, 95% CI = 1.085 to 1.940 and (2) genotypes GG and GA versus genotype AA: p = .008, OR = 1.691, 95% CI = 1.147 to 2.491; and for late-onset subjects, (1) allele G versus allele A: p = .030, OR = 1.403, 95% CI = 1.033 to 1.906 and (2) genotypes GG and GA versus genotype AA: p = .043, OR = 1.518, 95% CI = 1.011 to 2.279; however, we found no significant discrepancies of allele and genotype frequencies between early-onset patients and late-onset patients, between patients with response to chlorpromazine and those without response to chlorpromazine, between patients with response to and those without response to clozapine, or between patients with chlorpromazine-induced extrapyramidal syndrome and those without the syndrome.

For the other 4 SNP markers, we found almost no significant discrepancies of allele or genotype frequencies either from global statistical analyses or from stratified analyses.

All the results from individual SNP analyses showed that the allele G of the SNP marker rs3803300 was a risk allele, especially in male subjects, and that the other 4 SNP markers appeared to have no role in schizophrenia, which are inconsistent with the findings of Emamian et al.[10] and Schwab et al.[12]: a global transmission distortion on the SNP marker rs3730358 (p = .005) was identified in the Emamian et al.[10] study, and a statistical significance

Table 3. Allele- and Genotype-Based Statistical Analysis for the 5 Polymorphisms of *AKT1* Gene[a]

	Patients vs Controls		Male Patients vs Male Controls		Female Patients vs Female Controls		Early-Onset vs Controls	
Comparison	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)
rs3803300 (G > A)[b]								
Allele G vs allele A	.003**	1.404 (1.118 to 1.762)	.019*	1.459 (1.064 to 2.001)	.068	1.384 (0.976 to 1.963)	.012*	1.451 (1.085 to 1.940)
Genotype GG vs GA + AA	.128	1.444 (0.898 to 2.324)	.767	1.103 (0.576 to 2.113)	.126	1.821 (0.838 to 3.961)	.332	1.351 (0.735 to 2.485)
Genotype GG + GA vs AA	.004**	1.543 (1.146 to 2.077)	.004**	1.837 (1.213 to 2.783)	.143	1.405 (0.891 to 2.216)	.008**	1.691 (1.147 to 2.491)
Genotype GG vs AA	.030*	1.727 (1.051 to 2.837)	.254	1.484 (0.752 to 2.929)	.079	2.044 (0.911 to 4.584)	.097	1.716 (0.903 to 3.258)
rs1130214 (G > T)								
Allele G vs allele T	.132	0.777 (0.559 to 1.079)	.324	0.791 (0.496 to 1.261)	.453	0.822 (0.492 to 1.372)	.682	0.908 (0.572 to 1.442)
Genotype GG vs GT + TT	.129	0.754 (0.523 to 1.086)	.210	0.720 (0.430 to 1.206)	.633	0.870 (0.490 to 1.543)	1	1.000 (0.593 to 1.686)
Genotype GG + GT vs TT	.635	0.759 (0.242 to 2.378)	NA		NA		NA	
Genotype GG vs TT	.564	0.715 (0.227 to 2.246)	NA		NA		NA	
rs3730358 (C > T)								
Allele C vs allele T	.502	1.163 (0.749 to 1.805)	.571	1.181 (0.663 to 2.104)	.879	1.057 (0.516 to 2.168)	.810	1.073 (0.603 to 1.910)
Genotype CC vs CT + TT	.559	1.148 (0.723 to 1.821)	.548	1.206 (0.654 to 2.223)	.990	0.995 (0.471 to 2.102)	.767	1.097 (0.595 to 2.023)
Genotype CC + CT vs TT	NA		NA		NA		NA	
Genotype CC vs TT	NA		NA		NA		NA	
rs2498799 (G > A)								
Allele G vs allele A	.897	0.986 (0.792 to 1.226)	.963	1.007 (0.839 to 1.203)	.819	1.040 (0.742 to 1.459)	.795	1.039 (0.778 to 1.388)
Genotype GG vs GA + AA	.688	1.086 (0.727 to 1.622)	.796	0.929 (0.531 to 1.625)	.227	1.460 (0.789 to 2.701)	.605	1.148 (0.680 to 1.937)
Genotype GG + GA vs AA	.623	0.926 (0.681 to 1.259)	.785	1.063 (0.683 to 1.655)	.553	0.868 (0.544 to 1.386)	.976	0.994 (0.659 to 1.497)
Genotype GG vs AA	.922	1.022 (0.660 to 1.582)	.946	0.979 (0.526 to 1.820)	.491	1.262 (0.651 to 2.448)	.706	1.117 (0.630 to 1.980)
rs2494732 (A > G)								
Allele A vs allele G	.140	0.837 (0.660 to 1.060)	.205	0.811 (0.587 to 1.122)	.535	0.888 (0.610 to 1.293)	.220	0.821 (0.598 to 1.126)
Genotype AA vs AG + GG	.001**	0.286 (0.128 to 0.639)	NA		NA		NA	
Genotype AA + AG vs GG	.666	0.938 (0.701 to 1.255)	.906	0.976 (0.649 to 1.467)	.758	0.932 (0.594 to 1.461)	.564	0.893 (0.608 to 1.312)
Genotype AA vs GG	.002**	0.293 (0.130 to 0.661)	NA		NA		NA	

[a]NA means that the corresponding analysis based on the genetic model was ignored due to limited rare homozygotes.
(In a 2-by-2 contingency table, if the expected value for each of the 4 cells is less than 5, the χ^2 test may present problems.)
[b]G > A = a single nucleotide polymorphism (SNP) between alleles G and A in which allele G is a wild type and allele A is a mutated type.
The expressions G > T, C > T, and A > G represent SNPs with the same type of relationship between alleles.
*p < .05.
**p < .01.
Abbreviation: EPS = extrapyramidal syndrome.

was also observed in both SNP marker rs1130214 and rs3730358 in the Schwab et al.[12] study. We found no relationship between any of the 5 SNP markers and therapeutic response to typical or atypical antipsychotics or chlorpromazine-induced extrapyramidal syndrome. The *AKT1* gene may therefore play no major role in therapeutic response to these antipsychotics or in chlorpromazine-induced extrapyramidal adverse effects.

Because of limited sample size, individual SNP-based stratified analyses were not done for sex, age at onset, therapeutic response, or extrapyramidal adverse effects.

Haplotype Analysis

The results of linkage disequilibrium tests between each pair of all 5 SNP markers among all patients and control subjects are presented in Table 4. All D′ were greater than 0.5; therefore, we analyzed those common haplotypes that consisted of all 5 markers (at least 1% frequency in either case or control group).

All the χ^2-based statistical p values corresponding to haplotypes are shown in Table 5. Haplotypes with probabilities greater than 1% accounted for the majority of haplotype diversity (> 95%). The overall haplotype frequency showed significant differences between patients and control subjects (p < .000001). Haplotype G-T-C-G-G, which was more frequent in patients (6.647%) than in healthy control subjects (3.934%), was observed to be significantly associated with schizophrenia as a risk haplotype (p = .016, OR = 1.755, 95% CI = 1.105 to 2.787), while the frequency of haplotype A-G-C-G-A in the total set of 384 patients (6.984%) was less than that in the total set of 384 controls (13.102%) indicating that the haplotype A-G-C-G-A was statistically related to schizophrenia as a protective haplotype (p = .00004886, OR = 0.491, 95% CI = 0.346 to 0.696). These findings are consistent with those based on the SNP rs3803300 because the protective haplotype A-G-C-G-A includes the protective allele of the SNP rs3803300, while the risk haplotype G-T-C-G-G includes the risk allele of the SNP rs3803300.

The haplotype composed of rs1130214-rs3730358-rs2498799 (T-C-G) showed highly significant association (p = .0006) in the study of Emamian et al.[10] and marginal

Late-Onset vs Controls		Early-Onset vs Late-Onset		Responders to Clozapine vs Nonresponders to Clozapine		Responders to Chlorpromazine vs Nonresponders to Chlorpromazine		EPS vs No EPS	
p Value	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)
.030*	1.403 (1.033 to 1.906)	.852	1.034 (0.728 to 1.469)	.375	0.781 (0.451 to 1.351)	.567	1.172 (0.680 to 2.022)	.194	1.455 (0.826 to 2.564)
.204	1.494 (0.801 to 2.788)	.781	0.904 (0.445 to 1.838)	.414	1.550 (0.538 to 4.463)	.764	0.830 (0.245 to 2.810)	.238	1.965 (0.631 to 6.118)
.043*	1.518 (1.011 to 2.279)	.659	1.114 (0.691 to 1.795)	.975	0.989 (0.477 to 2.048)	.314	0.691 (0.336 to 1.421)	.338	1.458 (0.673 to 3.162
.087	1.762 (0.915 to 3.394)	.944	0.973 (0.458 to 2.069)	NA		.461	1.524 (0.459 to 4.689)	.190	2.222 (0.663 to 7.445)
.175	0.739 (0.477 to 1.146)	.462	1.229 (0.709 to 2.129)	.560	1.317 (0.521 to 3.328)	.102	0.530 (0.246 to 1.144)	.293	1.577 (0.671 to 3.706)
.100	0.667 (0.411 to 1.082)	.198	1.499 (0.808 to 2.782)	.446	0.712 (0.297 to 1.708)	.520	1.394 (0.506 to 3.839)	.306	1.641 (0.633 to 4.254)
NA		NA		NA		NA		NA	
NA		NA		NA		NA		NA	
.467	1.257 (0.678 to 2.331)	.670	0.854 (0.412 to 1.769)	.236	2.470 (0.529 to 11.533)	.905	1.060 (0.409 to 2.745)	.483	1.433 (0.523 to 3.924)
.556	1.212 (0.639 to 2.299)	.798	0.905 (0.423 to 1.938)	.960	0.974 (0.354 to 2.684)	.224	2.564 (0.536 to 12.267)	.282	1.830 (0.602 to 4.462)
NA		NA		NA		NA		NA	
NA		NA		NA		NA		NA	
.558	1.093 (0.812 to 1.470)	.781	0.951 (0.666 to 1.358)	.138	0.659 (0.379 to 1.146)	.717	1.108 (0.637 to 1.925)	.784	1.081 (0.619 to 1.886)
.379	1.266 (0.748 to 2.145)	.760	0.907 (0.483 to 1.700)	.801	1.126 (0.448 to 2.828)	.168	0.443 (0.136 to 1.441)	.863	1.086 (0.427 to 2.764)
.881	1.033 (0.676 to 1.578)	.881	0.962 (0.579 to1.599)	.283	1.512 (0.710 to 3.224)	.765	1.127 (0.515 to 2.464)	.809	1.103 (0.499 to 2.437)
.706	1.117 (0.630 to 1.980)	.470	1.238 (0.693 to 2.211)	NA		.753	1.176 (0.428 to 3.233)	.814	1.131 (0.404 to 3.166)
.600	0.918 (0.667 to 1.264)	.569	0.894 (0.608 to 1.315)	.451	0.785 (0.417 to 1.476)	.959	1.016 (0.561 to 1.839)	.318	0.727 (0.388 to 1.362)
NA		NA		NA		NA		NA	
.719	1.075 (0.724 to 1.596)	.438	0.830 (0.519 to 1.328)	.998	0.999 (0.492 to 2.027)	.293	0.669 (0.316 to 1.418)	.457	0.754 (0.359 to 1.586)
NA		NA		NA		NA		NA	

significance (p = .02) in the study of Schwab et al.,[12] but was not significant in our study (p = .818).

Due to limited sample size, haplotype-based stratified analyses were not done for sex, age at onset, therapeutic response, or extrapyramidal adverse effects.

Statistic Power Estimation

By performing power calculations, we found that the sample size recruited in this work had > 85% power in detecting a significant association ($\alpha < .05$) for both allele and haplotype-based analysis when an effect size index of 0.1 (corresponding to "weak" gene effect) was used.

DISCUSSION

Our study using a relatively large Chinese sample supports the finding of a positive association between *AKT1* and schizophrenia. In our study, the 5-SNP–constructed haplotype A-G-C-G-A showed a great significance (p = .00004886, OR = 0.491, 95% CI = 0.346 to 0.696). Given that individual haplotypic analysis represents a spin-off from multi-SNP haplotype systems and therefore needs no further correction in individual haplotypic analysis,[13,35] and although we applied Bonferroni correction independently from global haplotypic analyses, the lowest p value (the 5-SNP–constructed haplotype A-G-C-G-A) could withstand 1023 corrections. Therefore, we have enough reason to assume the positive association of the *AKT1* gene with the etiology of schizophrenia.

Through individual SNP and individual haplotypic analyses, we observed different positively and negatively associated SNP alleles and haplotypes than those observed by Emamian et al.[10] and Schwab et al.,[12] which might be derived from the variations in human genetic structure. Different ethnic populations exhibit significant discrepancies, including SNP allele frequency, recombination hotspots, haplotype block, and haplotypic frequency.[36,37] Even though the studies of both Emamian et al.[10] and Schwab et al.[12] were derived from Caucasian populations by using family-based study design, their results were not completely the same. In our study, for the 5-marker–constructed haplotype structure in the general

Table 4. Pairwise Linkage Disequilibrium in *AKT1*[a]

Marker	rs3803300	rs1130214	rs3730358	rs2498799	rs2494732
rs3803300	…	**0.865218**	0.576453	0.492994	0.369166
rs1130214	**0.622795**	…	**0.666978**	0.503080	0.861323
rs3730358	0.032780	**0.845911**	…	0.467383	**0.929153**
rs2498799	0.387741	0.420607	**0.709765**	…	**0.728612**
rs2494732	0.214218	0.491293	0.260090	**0.814312**	…

[a]Numbers of upper diagonal are D′ of schizophrenia subjects; numbers of lower diagonal are D′ of control subjects. If D′ > 0.6, the 2 SNP markers are considered to be in strong linkage disequilibrium.
Symbol: … = not applicable.

Table 5. Estimated Haplotype Frequencies and Association Significance

Haplotype	rs3803300 (G > A)[a]	rs1130214 (G > T)	rs3730358 (C > T)	rs2498799 (G > A)	rs2494732 (A > G)	Frequency, % Patients, N = 384	Frequency, % Controls, N = 384	χ^2	p Value	Odds Ratio (95% CI)
1	A	G	C	A	A	2.6759	1.7630	1.099	.295	1.444 (0.724 to 2.880)
2	A	G	C	A	G	42.2751	44.1849	0.531	.466	0.928 (0.758 to 1.135)
3	A	G	C	G	A	6.9842	13.1023	16.492	.00004886	0.491 (0.346 to 0.696)
4	A	G	C	G	G	8.4697	7.0578	1.134	.287	1.266 (0.842 to 1.785)
5	A	G	T	A	G	4.1092	3.6661	0.167	.683	1.115 (0.662 to 1.877)
6	G	G	C	A	A	1.1077	1.0207	0.0009	.996	1.003 (0.374 to 2.685)
7	G	G	C	A	G	5.9656	4.4977	1.608	.205	1.338 (0.852 to 2.102)
8	G	G	C	G	A	10.9354	8.5111	2.739	.098	1.332 (0.948 to 1.872)
9	G	G	C	G	G	3.3605	2.9848	0.198	.656	1.138 (0.643 to 2.013)
10	G	T	C	A	G	3.3221	3.4812	0.074	.785	0.926 (0.532 to 1.610)
11	G	T	C	G	G	6.6470	3.9343	5.806	.016	1.755 (1.105 to 2.787)
12	…	G	C	A	…	52.7411	51.3998	0.263	.608	1.054 (0.863 to 1.288)
13	…	G	C	G	…	31.0734	32.0598	0.164	.686	0.956 (0.771 to 1.186)
14	…	G	T	A	…	3.7970	4.7476	0.768	.381	0.800 (0.485 to 1.319)
15	…	T	C	A	…	4.8239	4.7777	0.000	.991	1.003 (0.629 to 1.600)
16	…	T	C	G	…	6.0048	5.6688	0.053	.818	1.051 (0.687 to 1.610)
17	A	G	…	…	…	64.4810	70.1830	5.648	.017	0.772 (0.623 to 0.956)
18	A	T	…	…	…	1.4495	2.2076	1.294	.255	0.644 (0.299 to 1.383)
19	G	G	…	…	…	22.1740	18.8000	2.793	.095	1.236 (0.964 to 1.585)
20	G	T	…	…	…	11.8950	8.8090	3.779	.052	1.388 (0.996 to 1.933)
21	…	G	C	…	…	82.0706	83.0000	0.760	.383	0.888 (0.680 to 1.160)
22	…	G	T	…	…	5.5065	6.2000	0.285	.594	0.890 (0.580 to 1.366)
23	…	T	C	…	…	12.1342	10.0000	1.140	.286	1.188 (0.865 to 1.632)
24	…	…	C	A	…	57.4882	56.0586	0.273	.601	1.055 (0.862 to 1.292)
25	…	…	C	G	…	37.0910	37.7676	0.077	.782	0.971 (0.709 to 1.194)
26	…	…	T	A	…	4.0550	5.1459	1.172	.279	0.768 (0.475 to 1.241)
27	…	…	T	G	…	1.3657	1.0279	0.230	.631	1.257 (0.493 to 3.201)
28	…	…	…	A	A	3.6821	3.0908	0.329	.566	1.176 (0.675 to 2.048)
29	…	…	…	A	G	57.1440	58.1229	0.125	.723	0.964 (0.787 to 1.181)
30	…	…	…	G	A	20.1250	23.6737	2.895	.089	0.810 (0.636 to 1.033)
31	…	…	…	G	G	19.0490	15.1125	4.238	.04	1.324 (1.013 to 1.729)

[a]G > A = a single nucleotide polymorphism (SNP) between alleles G and A in which allele G is a wild type and allele A is a mutated type. The expressions G > T, C > T, and A > G represent SNPs with the same type of relationship between alleles.
Symbol: … = not applicable.

population, the D′, a measure of intermarker linkage disequilibrium, was smaller between rs1130214 and rs3803300 compared with others. This might indicate increased recombination and suggest that the causal variant of the disorder is located in the region distal to SNP marker rs3803300.

This study has several limitations. First, although we found no significant heterozygosis after sequencing all nonsynonymous SNP markers on the exons and 2 SNP markers on the 3′ untranslated region of the *AKT1* gene presented in the updated human SNP database at the NCBI Web site for 50 schizophrenic patients and 50 healthy individuals (NCBI dbSNP ID: rs11555436, rs11555433, rs12881616, and rs11555432, rs1130245, rs3803305), we did not perform a systematic mutation search in the untranslated regions and did not analyze additional SNP markers at the junction of extron and intron by using a large Chinese schizophrenia sample. Second, considering the genetic heterogeneity and gene-gene interaction involved in the etiology of schizophrenia, the other candidate genes of the *AKT-GSK3β* signaling pathway, such as *AKT2*, *AKT3*, and *GSK3β*, need to be analyzed together. Third, we did not analyze the relationship between the 5 SNP markers and each subtype of schizo-

phrenia and did not produce haplotype-based analytic results for the therapeutic response to antipsychotics or the chlorpromazine-induced extrapyramidal syndrome due to limited sample size after stratified analysis.

As regards the association of the 5 polymorphisms of the *AKT1* gene with the therapeutic response to typical (chlorpromazine) and atypical (clozapine) antipsychotics and the extrapyramidal syndrome, no meaningful results were observed. In this respect, our findings were inconsistent with those of Emamian et al.[10] and Ukai et al.,[11] namely that haloperidol (a typical antipsychotic) treatment could increase phosphorylation of the *AKT1* gene and that the *AKT1* gene is involved in the typical antipsychotic target. We did not get any implication of functional mechanisms for antipsychotic medication response from the 5 SNP markers. But the mechanism of antipsychotic action is very complex, and antipsychotic targets are mainly focused on the dopaminergic and serotonin systems. To date, no studies have dealt with the interactions between the *AKT1-GSK3β* signaling pathway and the dopaminergic or serotonin pathways. Therefore, the relationship between *AKT1* gene polymorphisms and therapeutic response to antipsychotics needs further study.

In conclusion, our study suggests that, in the Chinese population, the *AKT1* gene has a significant effect on the risk of schizophrenia, especially in male subjects, and the effect exists both in early-onset subjects and late-onset subjects, but that the *AKT1* gene might play no major role in the therapeutic response to antipsychotics or to chlorpromazine-induced extrapyramidal adverse effects. Our overall conclusion on the relationship between the polymorphisms of the *AKT1* gene and therapeutic response to antipsychotics and chlorpromazine-induced extrapyramidal adverse effects is that further well-designed, large, sample-based studies are required.

Drug names: chlorpromazine (Thorazine, Sonazine, and others), clozapine (FazaClo, Clozaril, and others), haloperidol (Haldol and others).

REFERENCES

1. Gottesman II. Schizophrenia Genesis: The Origins of Madness. New York, NY: WH Freeman & Co; 1999
2. Arnold SE, Franz BR, Gur RC, et al. Smaller neuron size in schizophrenia in hippocampal subfields that mediate cortical-hippocampal interactions. Am J Psychiatry 1995;152:738–748
3. Arnold SE, Hyman BT, van Hoesen GW, et al. Some cytoarchitectural abnormalities of the entorhinal cortex in schizophrenia. Arch Gen Psychiatry 1991;48:625–632
4. Lauer M, Beckmann H, Senitz D. Increased frequency of dentate granule cells with basal dendrites in the hippocampal formation of schizophrenics. Psychiatry Res 2003;122:89–97
5. Staal SP. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. Proc Natl Acad Sci USA 1987;84:5034–5037
6. Nicholson KM, Anderson NG. The protein kinase B/Akt signaling pathway in human malignancy. Cell Signal 2002;14:381–395
7. Wang Q, Liu L, Pei L, et al. Control of synaptic strength, a novel function of Akt. Neuron 2003;38:915–928
8. Mizuno M, Yamada K, Takei N, et al. Phosphatidylinositol 3-kinase A molecule mediating BDNF-dependent spatial memory formation. Mol Psychiatry 2003;8:217–224
9. Bailer U, Leisch F, Meszaros K, et al. Genome scan for susceptibility loci for schizophrenia. Neuropsychobiology 2000;42:175–182
10. Emamian ES, Hall D, Birnbaum MJ, et al. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. Nat Genet 2004;36: 131–137
11. Ukai W, Ozawa H, Tateno M, et al. Neurotoxic potential of haloperidol in comparison with risperidone: implication of Akt-mediated signal changes by haloperidol. J Neural Transm 2004;111:667–681
12. Schwab SG, Hoefgen B, Hanses C, et al. Further evidence for association of variants in the AKT1 gene with schizophrenia in a sample of European sib-pair families. Biol Psychiatry 2005;58:446–450
13. Ikeda M, Iwata N, Suzuki T, et al. Association of AKT1 with schizophrenia confirmed in a Japanese population. Biol Psychiatry 2004;56: 698–700
14. Ohtsuki T, Inada T, Arinami T. Failure to confirm association between AKT1 haplotype and schizophrenia in a Japanese case-control population. Mol Psychiatry 2004;9:981–983
15. Kampman O, Anttila S, Illi A, et al. Interaction of tumor necrosis alpha-G308A and epidermal growth factor gene polymorphisms in early-onset schizophrenia. Eur Arch Psychiatry Clin Neurosci 2005;255:279–283
16. Krebs MO, Guillin O, Bourdell MC, et al. Brain derived neurotrophic factor (BDNF) gene variants association with age at onset and therapeutic response in schizophrenia. Mol Psychiatry 2000;5:558–562
17. Sato T, Bottlender R, Schroter A, et al. Psychopathology of early-onset versus late-onset schizophrenia revisited: an observation of 473 neuroleptic-naive patients before and after first-admission treatments. Schizophr Res 2004;67:175–183
18. Jeste DV, Harris MJ, Krull A, et al. Clinical and neuropsychological characteristics of patients with late-onset schizophrenia. Am J Psychiatry 1995;152:722–730
19. Hafner H, Maurer K, Loffler W, et al. Onset and early course of schizophrenia. In: Hafner H, Gattaz WF, eds. Search for the Causes of Schizophrenia, vol 3. Berlin, Germany: Springer Verlag; 1995:43–66
20. Wu S, Xing Q, Gao R, et al. Response to chlorpromazine treatment may be associated with polymorphisms of the DRD2 gene in Chinese schizophrenic patients. Neurosci Lett 2005;376:1–4
21. Kane J, Honigfeld G, Singer J, et al. Clozapine for the treatment-resistant schizophrenic: a double-blind comparison with chlorpromazine. Arch Gen Psychiatry 1988;45:789–796
22. Hwang R, Shinkai T, De Luca V, et al. Association study of 12 polymorphisms spanning the dopamine D(2) receptor gene and clozapine treatment response in two treatment refractory/intolerant populations. Psychopharmacology (Berl) 2005;181:179–187
23. Overall JE, Gorham DR. The Brief Psychiatric Rating Scale. Psychol Rep 1962;10:799–812
24. Lachar D, Bailley SE, Rhoades HM, et al. Use of BPRS-A percent change scores to identify significant clinical improvement: accuracy of treatment response classification in acute psychiatric inpatients. Psychiatry Res 1999;89:259–268
25. Kaiser R, Tremblay PB, Klufmoller F, et al. Relationship between adverse effects of antipsychotic treatment and dopamine D(2) receptor polymorphisms in patients with schizophrenia. Mol Psychiatry 2002;7: 695–705
26. Plesnicar BK, Zalar B, Breskvar K, et al. The influence of the CYP2D6 polymorphism on psychopathological and extrapyramidal symptoms in the patients on long-term antipsychotic treatment. J Psychopharmacol 2006;20:829–833
27. Gerlach J, Lublin H, Peacock L. Extrapyramidal symptoms during long-term treatment with antipsychotics: special focus on clozapine and D1 and D2 dopamine antagonists. Neuropsychopharmacology 1996;14 (suppl 3):35S–39S
28. Srinivasan TN, Thara R, Padmavathi R, et al. Relationship of extrapyramidal symptoms to age at onset and drug treatment in middle-aged and elderly schizophrenic patients. Schizophr Res 2001;47:69–75
29. Psychopharmacology Research Branch, National Institute of Mental Health. Abnormal Involuntary Movement Scale (AIMS). In Guy W, ed. ECDEU Assessment Manual for Psychopharmacology. Revised. US Dept Health, Education, and Welfare publication (ADM) 76-338. Rockville, Md: National Institute of Mental Health; 1976:534–537
30. Barnes TRE. A rating scale for drug-induced akathisia. Br J Psychiatry 1989;154:672–676

31. Simpson GM, Angus JWS. A rating scale for extrapyramidal side effects. Acta Psychiatr Scand Suppl 1970;45(suppl 212):11–19
32. Zhao JH. 2LD, GENECOUNTING and HAP: computer programs for linkage disequilibrium analysis. Bioinformatics 2004;20:1325–1326
33. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–989
34. Erdfelder E, Faul F, Buchner A. G*Power: a general power analysis program. Behav Res Methods Instrum Comput 1996;28:1–11
35. van Den Bogaert A, Schumacher J, Schulze TG, et al. The DTNBP1 (dysbindin) gene contributes to schizophrenia, depending on family history of the disease. Am J Hum Genet 2003;73:1438–1443
36. Schork NJ, Fallin D, Thiel B, et al. The future of genetic case-control studies. Adv Genet 2001;42:191–212
37. Altshuler D, Brooks LD, Chakravarti A, et al. International HapMap Consortium: a haplotype map of the human genome. Nature 2005;437: 1299–1320