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Association of SNPs and Haplotypes in APOL1, 2 and 4 with schizophrenia

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Abstract

Prior work found the APOL1, 2 and 4 genes, located on chromosome 22q12.3-q13.1, to be upregulated in brains of schizophrenic patients. We performed a family-based association study using 130 SNPs tagging the APOL gene family (APOL1-6). The subjects were 112 African-American (AA), 114 European-American (EA), 109 Chinese (Ch) and 42 Japanese (Jp) families

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Contributors

Authors Sakae Takahashi, Jesen A. Fagerness, Takuya Kojima, Stephen V. Faraone, Ming T. Tsuang, and staffs of Department of Psychiatry, Harvard Medical School, and Psychiatric and Neurodevelopmental Genetics Unit and MIT/Whitehead Institute Center Biomedical Research designed the study and wrote the protocol. Authors Sakae Takahashi, Jesen A. Fagerness, Stephen V. Faraone, and staffs of Department of Psychiatry, Harvard Medical School, and Psychiatric and Neurodevelopmental Genetics Unit and MIT/Whitehead Institute Center Biomedical Research managed the literature searches and analyses, and undertook the statistical analysis. Authors Sakae Takahashi wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest

All authors declare that they have no conflicts of interest.

with schizophrenia (377 families, 1171 genotyped members and 647 genotyped affecteds in total). Seven SNPs had p -values < 0.05 in the APOL1, 2 and 4 regions for the AA, EA and combined (AA and EA) samples. In the AA sample, two SNPs, rs9610449 and rs6000200 showed low p -values; and a haplotype which comprised these two SNPs yielded a p -value of 0.00029 using the global test (GT) and the allele specific test (AST). The two SNPs and the haplotype were associated with risk for schizophrenia in African-Americans. In the combined (AA and EA) sample, two SNPs, rs2003813 and rs2157249 showed low p -values; and a three SNP haplotype including these two SNPs was significant using the GT ($p = 0.0013$) and the AST ($p = 0.000090$). The association of this haplotype with schizophrenia was significant for the entire (AA, EA, Ch and Jp) sample using the GT ($p = 0.00054$) and the AST ($p = 0.00011$). Although our study is not definitive, it suggests that the APOL genes should be more extensively studied in schizophrenia.

Keywords

schizophrenia; chromosome 22; APOL gene family cluster; APOL1; APOL2; APOL4

1. Introduction

Family, twin, and adoption studies suggest that the etiology of schizophrenia is influenced by genetic factors (Tsuang and Faraone 1995; Tsuang 2000). Moreover, recent molecular genetic studies have pointed to a variety of chromosomal regions that could genetically influence schizophrenia (chromosomes 1q, 5p, 6p, 8p, 10p, 13q and 22q) (Kirov et al. 2005). Several researchers suggested that loci at chromosome 22q12.3-q13.1 might be linked to susceptibility to schizophrenia (Lasseter et al. 1995; Vallada et al. 1995a; Vallada et al. 1995b; Gill et al. 1996; Blouin et al. 1998; Shaw et al. 1998; Schizophrenia Collaborative Linkage Group for Chromosome22 1998; DeLisi et al. 2002). We also found significant associations between this region and schizophrenia in not only the European-American sample from the National Institute of Mental Health (NIMH) genetics initiative for schizophrenia but also in a combined sample of European-American and Chinese samples (Takahashi et al. 2003). These findings suggest that a gene associated with the etiology of schizophrenia may exist on chromosome 22q12.3-q13.1, which harbors the apolipoprotein L (APOL) gene family cluster. Mimmack et al. (2002) found that APOL1, 2 and 4 of this gene family were significantly upregulated in the prefrontal cortex of schizophrenic patients. McGhee et al. (2005) performed a case-control association study of the APOL gene family using a three-stage DNA pooling strategy. They did not find evidence for an association between APOL genes and schizophrenia.

To test the hypothesis that APOL genes are associated with schizophrenia, we performed two experiments. First, we performed haplotype mapping across the 697kb region of this gene family cluster using a dense map of single nucleotide polymorphisms (SNPs). Family-based association analyses were performed for individual SNPs and then for haplotypes. Samples of African-American and European-American origin were obtained from the NIMH Genetics Initiative on Schizophrenia and the Department of Veteran Affairs (DVA) Cooperative Studies Program (CSP). Second, we performed family-based association analyses in the Chinese and Japanese samples using SNPs and haplotypes that were significant in the African-American and European-American samples.

2. Materials and methods

2.1. Subjects

We constructed the haplotype block map of the APOL gene family region using DNA samples from the Centre d'Etude du Polymorphisme Humain (CEPH) pedigrees stored at the

Coriell Institute for Medical Research (12 pedigrees containing 93 family members). These samples were also used for assessing the quality of the assays designed for each SNP. We performed two experiments. The first experiment used the African-American (AA) and European-American (EA) schizophrenia family samples from the NIMH Genetics Initiative on Schizophrenia and the DVA CSP schizophrenia family samples. Detailed descriptions of the ascertainment and extension rules, diagnostic assessment, and informed consent have been presented in previous publications (Cloninger et al. 1998; Faraone et al. 1998; Kaufmann et al. 1998; Tsuang et al. 2000; Faraone et al. 2005). A description of the AA and EA samples is shown in Table 1. For the second experiment, Chinese (Ch) and Japanese (Jp) schizophrenic families were recruited through 1. Peking University, Beijing, China, and 2. Nihon University Hospital, Tokyo, Japan and five affiliated hospitals in Tokyo and its environs. After the purpose and procedures of the project were fully explained, written informed consent was obtained from the probands and participating relatives. This project was approved by the Ethics Committees of Peking University and Nihon University. A description of the samples from Ch and Jp is given in Table 1. For all of the samples, each affected family member met DSM-III-R criteria for schizophrenia or schizoaffective disorder, depressive type.

2.2. SNP selection, genotyping and statistical analysis

All SNPs were selected from the National Center for Biotechnology Information (NCBI)/dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/>; dbSNP, build 118) and the Celera (<http://www.appliedbiosystems.com/>) database (in this paper, we changed Celera SNP numbers to rs numbers). These SNPs were validated in the CEPH samples to assess assay quality and to build our initial haplotype block map of the region. For the AA, EA and CEPH samples, genotyping was performed using the Sequenom system which uses Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). For the Ch and Jp samples, genotyping used Sequence-Specific Primer Cycle Elongation (SSPCE) and Fluorescence Correction Spectrometry (FCS): SSPCE-FCS (Hori et al. 2003). To test associations between our SNPs of interest and schizophrenia, we used the Family Based Association Test (FBAT) program version 1.7.3 (<http://www.biostat.harvard.edu/~fbat/fbat.htm>). For haplotype mapping and visualization, we used HaploView (<http://www.broad.mit.edu/personal/jcbarret/haploview/>) and LocusView (<http://www.broad.mit.edu/mpg/locusview/>).

3. Results

3.1. SNP selection

The APOL gene family cluster is located on chromosome 22q at position 34,316kb-34,936kb on the physical map (NCBI, build 34). The order of the genes is as follows: centromeric-APOL6-APOL5-APOL3-APOL4-APOL2-APOL1-telomeric. SNPs were selected across the entire 697 kb region (Figure 1).

Two hundred thirty nine SNPs were selected at an average spacing of 3kb. We removed 49 SNPs that failed quality control tests after genotyping was performed on the CEPH families. We removed an additional 59 SNPs that failed quality control tests after genotyping the NIMH and DVA samples. A total of 130 SNPs met our quality control criteria. The average distance between these SNPs was 5,397 bp. The SNPs in the APOL1, 2, 3 and 4 regions are shown in Figure 2.

3.2. Family-based association study and definition of haplotype blocks

Since there are only two alleles for each SNP, the results for the minor and major alleles are identical apart from the signs of the Z statistics in single-marker analyses of the FBAT

program. A minor allele with a positive Z statistic is referred to as a high risk variant; and a minor allele with negative value is referred as a protective variant (Table 2, 5 and 7) (FBAT Manual, Feng et al. 2004; Bosse et al. 2005).

SNPs with $p < 0.05$ for the combined (AA and EA) sample are summarized in Table 2. Ten SNPs yielded p -values < 0.05 , and seven of those were in the APOL4-1 region. The SNPs which showed the smallest p -value, rs2157249 ($Z = -3.01$, $p = 0.0027$; the variant C allele is the protective allele), and the second smallest p -value, rs2003813 ($Z = -2.83$, $p = 0.0046$; the variant C allele is the protective allele) were in the APOL2 gene. Only rs2157249 yielded a p -value < 0.05 in both the AA and EA samples. Since APOL1, 2 and 4 were all located nearby, this region in the APOL2 gene was +20kb from APOL4 and -20kb from APOL1 (Figure 2, b). In the AA sample, five of the above seven SNPs in the APOL4-1 region had $p < 0.05$ (see Table 2). In the EA sample, only the above two SNPs with the smallest and second smallest p -values had $p < 0.05$ (see Table 2). In the AA and EA samples, rs9610449 and rs6000200 variants were associated with a risk for schizophrenia whereas rs2016988, rs2003813 and rs2157249 variants were protective effect against schizophrenia.

Figure 3 shows the haplotype block structure using the AA or EA full family trios in the APOL4-1 region (rs132664 - rs2071733; distance: 117,176bp). We found two blocks for the EA sample: blocks A and B, and four blocks for the AA sample: blocks C, D, E and F. We performed haplotype analyses utilizing the above block structures. The global test (GT) and the allele specific test (AST) for the EA and AA samples are summarized in Tables 3 and 4. For the EA sample, only one haplotype for block B showed $p < 0.05$ (Table 3 - block B SNPs - haplotype: A-C-T-C-T - AST). However, the GT for this block did not yield $p < 0.05$ (Table 3 - block B -GT P-value). As shown in Table 4, the haplotype that showed the smallest p -value for the AA sample was in block E ($Z = 3.36$, $p = 0.00029$; the C-G variant is the high risk haplotype). The GT p -value was 0.00029.

We performed the haplotype analyses using SNPs within the blocks defined by AA or EA in each ethnic sample. Although the analyses based on race are suitable for block-based analyses, these also have a problem of reducing sample sizes. Moreover, if we use a large block with many SNPs, the sample sizes also decrease. The FBAT analysis for haplotype is able to adjust for population admixture. Furthermore, the blocks for the AA and EA samples almost overlapped (Figure 3). Hence, in order to maximize the statistical power of our analysis, we performed the haplotype test with a small number of SNPs in the combined (AA and EA) sample as well. The above two SNPs with the smallest and second smallest p -values in the single SNP analyses using the combined samples (rs2157249 and rs2003813) were in the block A defined by EA (Figure 3). These two SNPs also showed the smallest and second smallest p -values in the EA sample (Table 2). Therefore we paid attention to these two SNPs and block A, and performed the haplotype analysis using three SNP haplotype composed of only SNPs with $p < 0.05$ (rs2016988 -rs2003813 - rs2157249) within the block A. We found our second lowest GT p -value, 0.0013 (chi-square = 15.6) and the lowest AST p -value, 0.000090 ($Z = 3.92$) in the combined samples (see Haplotype rs2016988 - rs2003813 - rs2157249 within the Block A of the combined sample (AA and EA) in Table 4). The haplotype with the lowest p -value consisted of major alleles (G, C and T). The other haplotypes bearing the variant alleles (C, T or C) showed negative Z statistics. Hence, the haplotypes with variant alleles, C, T or C may be protective against schizophrenia. However, because the significance levels of the haplotypes with variant alleles were marginal, these findings should be interpreted cautiously.

We also performed family-based association tests using five SNPs with $p < 0.05$ (rs9610449, rs6000200, rs2016988, rs2003813 and rs2157249; see Table 2) within the interesting blocks

(block A and E; see Table 3 and 4, and figure 3) in the Ch and Jp samples. Replicated SNPs for the Ch and Jp samples are summarized in Table 5. The number of informative Ch and Jp families was small and allele frequencies for each SNP were similar in the two groups. Thus, we performed the association analyses combining Ch and Jp. There were no significant associations in the Asian sample (Ch and Jp in Table 5). Moreover, the markers rs2003813 and rs2157249 in the Asian sample were not examined because the numbers of informative families were less than 10. In the entire sample (AA, EA, Ch and Jp), rs9610449 and rs6000200 did not show $p < 0.05$; rs2016988, rs2003813 and rs2157249 yielded $p < 0.05$ (Table 5). However, as mentioned above, the numbers of informative families for rs2003813 and rs2157249 in the Asian sample were very small. Therefore, the results for the Asian sample did not influence the results for the entire sample. For the haplotype analyses, haplotype rs9610449 - rs6000200 of block E did not show $p < 0.05$ for either the GT or the AST; haplotype rs2016988 - rs2003813 - rs2157249 within block A yielded $p = 0.00054$ (chi-square = 17.6, GT) and $p = 0.00011$ ($Z = 3.87$, AST of G-C-T haplotype) in the entire sample (Table 6 and figure 3). In the entire sample, the G-C-T haplotype with the lowest p -value was not the major haplotype (haplotype frequency = 0.32, see Table 6), and bore the variant G allele of rs2016988 (Table 5). The Z statistic for this haplotype was positive. Thus, this haplotype may be a risk variant for schizophrenia. However, these results were not consistent with the findings of the combined AA and EA sample. In the AA and EA sample, the G allele of rs2016988 was the major allele; and the G-C-T haplotype was also the major haplotype. Therefore, we should interpret this result cautiously.

Because we performed 432 tests in this study, the Bonferroni corrected alpha level was 0.00012. The GT and AST results for block E in the AA sample were close to the Bonferroni corrected threshold ($p = 0.00029$, Table 4). The AST results for the G-C-T haplotype within block A in the combined (AA and EA) sample ($p = 0.00009$, Table 3) and the entire (AA, EA, Ch and Jp) sample ($p = 0.00011$, Table 6) were still significant after the Bonferroni correction.

4. Discussion

The results of this study are summarized in Table 7 and 8. We performed a family-based association study with 130 SNPs covering all members of the APOL gene family for the AA, EA and combined (AA and EA) samples. Moreover, we examined these genes in the Asian (Ch and Jp) and combined (AA, EA, Ch and Jp) samples. In Table 7, rs9610449 and rs6000200 of the APOL4 and APOL2 regions showed low p -values in the AA sample. These SNPs did not reach nominal significance in the entire sample. The SNPs, rs2003813 and rs2157249 of the APOL2 and APOL1 regions yielded low p -values for the combined (AA and EA) and entire samples. However, the allele frequencies of the two SNPs in the Asian sample were quite different from those of the AA and EA samples. As a result, although 151 Asian families were genotyped, there were only two informative families. Therefore, the Asian sample is not useful for association studies of these two SNPs.

In the APOL4, 2 and 1 regions, we found two blocks for the EA sample: blocks A and B, and four for the AA sample: blocks C, D, E and F (Figure 3). As seen in Table 8, haplotype rs2016988 - rs2003813 - rs2157249 within block A yielded a significant p -value using the GT and AST in the combined (AA and EA) sample. Moreover, these results were replicated in the entire sample when we added the Asian sample. In the AST for the combined sample, the haplotype with the lowest p -value comprised major alleles (G, C and T); and the frequency of this haplotype was the highest. Z statistics for other haplotypes bearing the variant alleles were negative but their significance levels were marginal. On the other hand, the frequency of the G-C-T haplotype with the lowest p -value was second, and it bore the G variant for the entire sample. Moreover, the Z statistic for this haplotype was positive.

Hence, the results from the combined sample were not consistent with those for the entire sample. These results may be influenced by the low frequency of G-C-T haplotype of Asian sample. Thus, it is premature to conclude that this haplotype is associated with schizophrenia.

McGhee et al. (2005) reported a case-control study of the APOL gene family using a novel DNA pooling strategy. They found no association between APOL genes and schizophrenia. However, they performed single SNP analyses only. They stated that the APOL gene family would warrant more investigation assessing the haplotype structure across this gene family region with the use of a larger sample to increase statistical power. In this study, we did the block-based analysis and our sample was relatively large.

In summary, our results were as follows: 1) rs9610449, rs6000200 and block E comprised of these two SNPs yielded a low p -value in the AA sample. These SNPs and block E were associated with a risk for schizophrenia. 2) rs2003813, rs2157249 and a three SNP haplotype including these two SNPs within the block A showed a low p -value in the combined (AA and EA) sample. Similar results for this haplotype were seen in the entire (AA, EA, Ch and Jp) sample. The results of the haplotype for the combined sample ($p = 0.00009$, Table 3) and the entire sample ($p = 0.00011$, Table 6) remained significant after the Bonferroni correction. Variant alleles of these two SNPs had a protective effect against schizophrenia in the combined sample. As for the haplotype, we could not determine if was a risk or protective haplotype for schizophrenia. 4) The above SNPs and blocks were in the APOL4-1 region (Figure 2 and 3). Mimmack reported APOL1, 2 and 4 were upregulated in the schizophrenia brain. Additionally, several researchers have found evidence for linkage and association in the region that includes APOL1, 2 and 4. Therefore, we conclude that APOL1, 2 and/or 4 may play a risk or protective role in the etiology of schizophrenia. However, we were not able to decide whether the APOL gene had risk or protective effect. Nevertheless, our results suggest that the APOL genes should be more extensively studied in schizophrenia. We also found that the Asian sample was not suitable for association studies using SNPs from the APOL4-1 region. Hence, in order to confirm the present results, more studies for APOL genes using a larger single race, AA or EA samples will be needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Genes in the APOL family cluster region
The genes were based the NCBI/RefSeq.

Figure 2. Positions of SNPs with $P < 0.05$ in the entire sample

The genes were based the NCBI/RefSeq. The direction of arrows is a direction of transcription. The end of Figure a and the first of Figure b are same position. The “hCV” means SNPs from the Celera database and the “rs” means SNPs from the dbSNP database. SNPs highlighted by a red font had $P < 0.05$

Figure 3. Four Gamete Rule (FGR) blocks detected by the European-American or African-American full family trio in the APOL1, 2 and 4 regions
The genes were based the NCBI/RefSeq. The direction of arrows is a direction of transcription. SNPs highlighted by a red font had $P < 0.05$

Table 1
Description of the African-American, European-American Chinese, Japanese and entire samples

	Ped	Nuclear families	Genotyped members	Genotyped affecteds	Ped	Nuclear families	Genotyped members	Genotyped affecteds	Ped	Nuclear families	Genotyped members	Genotyped affecteds
	AA				EA				AA and EA			
NIMH	46	61	172	121	56	63	204	124	102	124	376	245
DVA	51	51	163	110	50	51	167	112	101	102	330	222
Total	97	112	335	231	106	114	371	236	203	226	706	467
	Ch				Jp				Ch and Jp			
	109	109	322	118	42	42	133	62	151	151	455	180
									Entire sample			
									354	377	1161	647

Ped: pedigrees; AA: African-American; EA: European-American; NIMH: National Institute of Mental Health; DVA: Department of Veteran Affairs; Total: NIMH and DVA; Ch: Chinese; Jp: Japanese; Entire sample: African-American, European-American, Chinese and Japanese

Table 2

SNPs with $P < 0.05$ in the African-American and European-American samples

SNPs	Gene (see Figure 2 and 3)	Allele	Allele frequency				Informative family				p -value (Z)	
			AA	EA	AA and EA	AA and EA	AA	EA	AA and EA	AA and EA		
rs2092786	RBM9	A	0.51	0.85	0.70	31	18	49	0.076 (-1.77)	0.14 (-1.48)	0.021 (-2.31)	
		T	0.49	0.15	0.30				0.076 (1.77)	0.14 (1.48)	0.021 (2.31)	
rs16996261	out of gene region	G	0.51	0.14	0.30	30	17	47	0.23 (1.21)	0.058 (1.90)	0.037 (2.09)	
		T	0.49	0.86	0.70				0.23 (-1.21)	0.058 (-1.90)	0.037 (-2.09)	
rs5995197	out of gene region	A	0.63	0.27	0.43	27	31	58	0.19 (1.31)	0.088 (1.71)	0.031 (2.15)	
		T	0.34	0.73	0.57				0.19 (-1.31)	0.088 (-1.71)	0.031 (-2.15)	
rs132736	APOL4	C	0.60	0.41	0.49	27	36	63	0.012 (-2.51)	0.63 (0.49)	0.049 (-1.97)	
		T	0.40	0.59	0.51				0.012 (2.51)	0.63 (0.49)	0.049 (1.97)	
rs9610449	APOL4/APOL2	C	0.22	0.34	0.29	22	31	53	0.0029 (2.98)	0.33 (0.98)	0.011 (2.56)	
		T	0.78	0.66	0.71				0.0029 (-2.98)	0.33 (-0.98)	0.011 (-2.56)	
rs6000200	APOL4/APOL2	C	0.71	0.66	0.71	21	31	52	0.00060 (-3.43)	0.33 (-0.98)	0.0052 (-2.79)	
		G	0.29	0.34	0.29				0.00060 (3.43)	0.33 (0.98)	0.0052 (2.79)	
rs2016988	APOL4/APOL2	C	0.38	0.07	0.21	31	12	43	0.016 (-2.40)	0.83 (-0.22)	0.033 (-2.14)	
		G	0.62	0.93	0.79				0.016 (2.40)	0.83 (0.22)	0.033 (2.14)	
rs2003813	APOL2/APOL1	C	0.81	0.67	0.74	18	31	49	0.068 (1.83)	0.029 (2.18)	0.0046 (2.83)	
		T	0.19	0.33	0.26				0.068 (-1.83)	0.029 (-2.18)	0.0046 (-2.83)	
rs2157249	APOL2/APOL1	C	0.19	0.27	0.27	17	32	49	0.041 (-2.04)	0.024 (-2.26)	0.0027 (-3.01)	
		T	0.81	0.73	0.73				0.041 (2.04)	0.024 (2.26)	0.0027 (3.01)	
rs2413394	APOL2/APOL1	A	0.01	0.19	0.11	2	23	25	*	0.076 (1.77)	0.043 (-2.03)	
		G	0.99	0.81	0.89				*	0.076 (1.77)	0.043 (2.03)	

AA: African-American sample; EA: European-American sample. The "rs" means SNPs from the dbSNP database.

* A default value of useful minimum number of family in FBAT is 10. The marker rs2413394 in the African-American sample was not calculated because the number of family was under 10.

Table 3

Haplotype analyses of blocks including SNPs with $P < 0.05$ in European-American

Block A SNPs				Size (bp)	Haplotype frequency	Informative family	Global test (GT) p -value (df , chi- square)	Allele specific test (AST) p -value (Z)
rs1075803	rs2016988 *	rs2269597	hCV11521267					
			rs2003813 *					
			rs2157249 *					
C	G	A	C	T	C	C	0.16 (5, 7.9)	0.097 (-1.66)
T	G	G	T	C	T	C		0.28 (1.08)
C	G	A	C	C	T	A		0.79 (-0.26)
C	G	A	C	C	T	C		0.059 (1.89)
C	C	A	C	C	T	C		0.67 (-0.42)
Haplotype rs201 6988 * - rs2003813 * - rs2157249 * within the Block A of the combined sample (AA and EA)								
	G		C	C	T	65	0.0013 (3, 15.6)	0.000090 (3.91)
	G		T	C	C	45		0.013 (-2.48)
	C		C	C	T	44		0.046 (-1.99)
Block B SNPs								
rs9610448	rs11704345	rs9610449 *	rs6000200 *	rs132753				
A	C	T	C	T	0.36	29	0.37 (5, 5.4)	0.046 (-2.0)
A	C	T	C	C	0.23	33		0.39 (0.86)
G	T	C	G	T	0.21	28		0.52 (0.64)
G	C	C	G	T	0.11	16		0.28 (1.07)
G	C	T	C	T	0.08	12		0.88 (-0.15)

The "hCV" means SNPs from the Celera database and the "rs" means SNPs from the dbSNP database. Haplotypes with the IF > 10 are not shown for each block because a default value of useful minimum number of family in FBAT is 10.

* SNPs with $p < 0.05$ in the single SNP association test in the African-American and European-American samples. AA: African-American sample; EA: European-American sample.

Table 4
Haplotype analyses of blocks including SNPs with $P < 0.05$ in African-American

Block C SNPs		Size (bp)	haplotype frequency	Informative family	Global test (GT) <i>p</i> -value (<i>df</i> , chi-square)	Allele specific test (AST) <i>p</i> -value (<i>Z</i>)
rs2003813 *	rs2157249 *	4,789			0.10 (2, 4.5)	
	rs916264					
C	T		0.79	21		0.054 (1.93)
T	C		0.16	25		0.037 (-2.09)
Block D SNPs						
rs132753	rs1075803	11,094			0.066 (2, 5.5)	
	rs2016988 *					
	rs2269597					
	hCV11521267					
T	C		0.48	35		0.021 (2.30)
T	C		0.38	30		0.045 (-2.00)
Block E SNPs						
rs9610449 *	rs6000200 *				0.00029 (1, 13.1)	
T	C		0.8	19		0.00029 (-3.63)
C	G		0.2	19		0.00029 (3.63)

IF: informative family; haplotypes with the IF > 10 are not shown for each block because a default value of useful minimum number of family in FBAT is 10.

* SNPs with $p < 0.05$ in the single SNP association test in the African-American and European-American samples

Table 5

Replicated SNPs in the Chinese and Japanese samples, and the entire sample

SNPs	Gene (see Figure 2 and 3)	Allele	Allele frequency			Informative family			p-value (Z)
			Ch	Jp	Ch and Jp	Entire	Ch	Jp and Ch	
rs9610449	APOL4/APOL2	C	0.07	0.08	0.07	0.19	25	3	0.21 (-1.26)
		T	0.93	0.92	0.93	0.81		JP 28	0.11 (1.60)
rs600200	APOL4/APOL2	C	0.93	0.92	0.93	0.81	26	3	0.21 (1.26)
		G	0.07	0.08	0.07	0.19		29	0.11 (-1.60)
rs2016988	APOL4/APOL2	C	0.96	0.97	0.96	0.54	16	4	0.16 (1.41)
		G	0.04	0.03	0.04	0.46		20	0.090 (-1.70)
rs2003813	APOL2/APOL1	C	0.995	1.0	0.996	0.85	2	0	0.16 (-1.41)
		T	0.005	0	0.004	0.15		2	0.090 (1.70)
rs2157249	APOL2/APOL1	C	0.005	0	0.004	0.15	2	0	0.51 (-0.66)
		T	0.996	1.0	0.996	0.85		2	0.029 (-2.91)
								51	0.51 (0.66)
									*
									0.0020 (3.09)
									*
									0.0020 (-3.09)
									*
									0.0011 (-3.26)
									*
									0.0011 (3.26)

Ch: Chinese; Jp: Japanese; Entire: African-American, European-American, Chinese and Japanese.

* A default value of useful minimum number of family in FBAT is 10. The marker rs2413394 in the African-American sample was not calculated because the number of family was under 10.

Table 6

Haplotype consisted of the replicated SNPs in the entire sample

3 SNPs Haplotype within the Block A defined by EA				Haplotype frequency	Informative family	Global test (G ² T) <i>p</i> -value (<i>df</i> , chi-square)	Allele specific test (AST) <i>p</i> -value (<i>Z</i>)
rs9610449	rs6000200	rs2016988	rs2003813	rs2157249			
		C	C	T	66	0.00054 (3, 17.6)	0.082 (-1.74)
		G *	C *	T *	85		0.00011 (3.87)
		G	T	C	45		0.013 (-2.48)
Block E (2 SNPs haplotype) defined by AA							
T **	C **			0.81	77	0.13 (2, 4.05)	0.098 (-1.65)
C **	G **			0.19	76		0.081 (1.75)

AA: African-American; EA: European-American; entire sample: African-American, European-American, Chinese and Japanese. Haplotypes with the IF > 10 are not shown for each block because a default value of useful minimum number of family in FBAT is 10.

*The most significant haplotype in the combined sample (African-American and European-American).

**The most significant haplotype in the African-American sample.

Table 7

Summary of the single SNP association results in the African-American, European-American, Chinese and Japanese samples

SNPs (Gene, see Figure 2 and 3)	Allele	Allele frequency				Informative family					p-value (Z)	EA	AA and EA	Ch and JP	Entire	
		AA	EA	AA and EA	Ch and JP	Entire	AA	EA	AA and EA	Ch and JP						
rs9610449 (APOL4/APOL2)	C	0.22	0.34	0.29	0.07	0.19	22	31	53	28	81	0.0029 (2.98)	0.33 (0.98)	0.011 (2.56)	0.21 (-1.26)	0.11 (1.60)
	T	0.78	0.66	0.71	0.93	0.81						0.0029 (-2.98)	0.33 (-0.98)	0.011 (-2.56)	0.21 (1.26)	0.11 (-1.60)
rs6000200 (APOL4/APOL2)	C	0.71	0.66	0.71	0.93	0.81	21	31	52	29	81	0.00060 (-3.43)	0.33 (-0.98)	0.0052 (-2.79)	0.16 (1.41)	0.090 (-1.70)
	G	0.29	0.34	0.29	0.07	0.19						0.00060 (3.43)	0.33 (0.98)	0.0052 (2.79)	0.16 (-1.41)	0.090 (1.70)
rs2016988 (APOL4/APOL2)	C	0.38	0.07	0.21	0.96	0.54	31	12	43	20	63	0.016 (-2.40)	0.83 (-0.22)	0.033 (-2.14)	0.51 (-0.66)	0.029 (-2.91)
	G	0.62	0.93	0.79	0.04	0.46						0.016 (2.40)	0.83 (0.22)	0.033 (2.14)	0.51 (0.66)	0.029 (2.91)
rs2003813 (APOL2/APOL1)	C	0.81	0.67	0.74	0.996	0.85	18	31	49	2	51	0.068 (1.83)	0.029 (2.18)	0.0046 (2.83)	*	0.0020 (3.09)
	T	0.19	0.33	0.26	0.004	0.15						0.068 (-1.83)	0.029 (-2.18)	0.0046 (-2.83)	*	0.0020 (-3.09)
rs2157249 (APOL2/APOL1)	C	0.19	0.27	0.27	0.004	0.15	17	32	49	2	51	0.041 (-2.04)	0.024 (-2.26)	0.0027 (-3.01)	*	0.0011 (-3.26)
	T	0.81	0.73	0.73	0.996	0.85						0.041 (2.04)	0.024 (2.26)	0.0027 (3.01)	*	0.0011 (3.26)

AA: African-American; EA: European-American; Ch: Chinese; JP: Japanese; Entire: African-American, European-American, Chinese and Japanese.

Table 8

Summary of the haplotype association results in the African-American, European-American, Chinese and Japanese samples

3 SNPs Haplotype within the Block A defined by EA				Haplotype frequency			Informative family		Global test (GT) p-value (df, chi-square)		Allele specific test (AST) p-value (Z)	
rs9610449	rs6000200	rs2016988	rs2003813	rs2157249	AA	EA	AA and EA	Ch and Jp	Entire	AA and EA	Entire	Entire
					0.46	0.59	0.53	0.32	65	85	0.0013 (3, 15.6)	0.00054 (3, 17.6)
	G *	C *	T *		0.16	0.34	0.25	0	0.15	45	0.013 (-2.48)	0.00090 (3.91)
	G	T	C		0.38	0.066	0.21	0.96	0.52	44	0.046 (-1.99)	0.0011 (3.87)
	C	C	T							66		0.013 (-2.48)
												0.082 (-1.74)
Block E (2 SNPs haplotype) defined by AA												
					AA	EA	AA and EA	Ch and Jp	Entire	AA	Entire	Entire
T **	C **				0.80	0.67	0.73	0.93	0.81	19	77	0.00029 (-3.63)
										0.00029 (1, 13.1)	0.13 (2, 4.05)	0.098 (-1.65)
C **	G **				0.20	0.33	0.27	0.067	0.19	19	76	0.00029 (3.63)
												0.081 (1.75)

AA: African-American; EA: European-American; Entire: African-American, European-American, Chinese and Japanese.

* The most significant haplotype in the combined sample (African-American and European-American).

** The most significant haplotype in the African-American sample.