Amino Acids (2006)
DOI 10.1007/s00726-005-0292-8

Amino Acids
Printed in Austria

Abnormal changes of plasma acute phase proteins in schizophrenia and the relation between schizophrenia and haptoglobin (Hp) gene

C. Wan[1,2,*], **Y. La**[1,2,*], **H. Zhu**[1,2], **Y. Yang**[1,2], **L. Jiang**[1,2], **Y. Chen**[1,2], **G. Feng**[3], **H. Li**[3], **H. Sang**[4], **X. Hao**[4], **G. Zhang**[5], and **L. He**[1,2]

[1] Bio-X Life Science Research Center, Shanghai Jiao Tong University, Shanghai, China
[2] Institutes for Nutritional Sciences, Shanghai Institute of Biological Sciences, Chinese Academy of Sciences, Shanghai, China
[3] Shanghai Institute of Mental Health, Shanghai, China
[4] Kaixuan Hospital of Changchun, Jilin Province, China
[5] Shanghai Zhabei Institute of Mental Health, Shanghai, China

Received September 15, 2005
accepted November 7, 2005
Published online August 10, 2006;

Summary. In this study we focused on detecting schizophrenia related changes of plasma proteins using proteomic technology and examining the relation between schizophrenia and haptoglobin (*Hp*) genotype. We investigated plasma proteins from schizophrenic subjects ($n = 42$) and healthy controls ($n = 46$) by two-dimensional gel electrophoresis (2-DE) in combination with mass spectrometry. To further reveal the genetic relationship between acute phase proteins (APPs) and schizophrenia disease, we tested *Hp* α1/*Hp* α2 (*Hp* 1/2) polymorphism and two single nucleotide polymorphisms (SNPs) of *Hp*, rs2070937 and rs5473, for associations with schizophrenia in the Chinese Han population. With the relatively high number of samples for 2-DE work, we found that four proteins in the family of positive APPs were all up-regulated in patients. In genetic association study, we found significant associations existing between schizophrenia and *Hp* polymorphisms, *Hp* 1/2 and rs2070937 variants. Schizophrenia is accompanied by both an altered expression of Hp protein and a different genotype distribution of *Hp* gene, demonstrating that Hp is associated with schizophrenia. The results from proteomic and genomic aspects both indicate that acute phase reaction is likely to be an aetiological agent in the pathophysiology of schizophrenia, but not just an accompanying symptom. The positive APPs are schizophrenic related proteins, with the highly concordant results on four positive APPs.

Keywords: Schizophrenia – Acute phase proteins – Proteomics – Plasma – Haptoglobin

Introduction

The etiology of schizophrenia is complex and mostly speculative, and its pathophysiology and psychopathology have a large degree of variability. Clear genetic susceptibility has been shown by twin concordance, familial risk, and studies of adopted offspring of patients with schizophrenia (Thaker and Carpenter, 2001; McGuffin et al., 1995; Kendler, 1988; Kety et al., 1994). The linkage studies to date have shown that major effect genes are not common causes of schizophrenia, although they provide information concerning the possible location of some moderate effect genes (Shaw et al., 1998). Most gene expression studies suggest that abnormalities in monoamine neurotransmission play a role in the aetiology of schizophrenia. However, the precise pathological mechanisms remain obscure. In spite of significant advances in the treatment and management of the illness over the past 50 years, there are no descriptive biological criteria as yet and it is defined only by the negative symptoms. The improved diagnosis, management and treatment of this disorder is therefore of considerable significance.

Proteomics, the large-scale analysis of proteins, will contribute greatly to our understanding of schizophrenia in the post-genomic era. Pandey et al. mention 'differential display' proteomics for comparing protein levels (Pandey and Mann, 2000). It has potential application in the research into complex diseases, using 2-DE to separate a mixture of proteins and subsequent identification by mass spectrometry. The main goal of our study has been to find some susceptible proteins relevant to schizophrenia using this high throughput proteomics analysis.

Systemic immunological and inflammatory abnormalities have been widely reported in schizophrenic patients

* These authors contributed equally.

(Rothermundt et al., 2001; Zhang et al., 2002; Maes et al., 1997). Schizophrenia is characterized by the activation of the response system with increasing plasma concentration of positive APPs, such as Hp, transferrin and albumin. As a typical APP familial member, Hp is a plasma α-sialoglycoprotein that binds free hemoglobin. Hp is a tetrameric structure joined by disulfide linkages among the 2α and 2β chains. Based on the length of α chain, there are two kinds of Hp α in the population, Hp 1 and Hp 2 (Langlois and Delanghe, 1996). These phenotypes are an expression of two common autosomal codominant alleles *HP* 1 and *HP* 2 (Smithies and Walker, 1956; Smithies, 1955). In a Northwestern Italian population the frequency of the *Hp* 2 allele was observed to be significantly higher in schizophrenic patients as compared with controls (Maes et al., 2001). Till now, there has been no published association study of *Hp* SNPs in schizophrenia or any other diseases. To explore the possible mechanism of *Hp* in the pathogenesis of schizophrenia, we also studied the genetic association between the *Hp* gene polymorphisms and schizophrenia in a Chinese Han population.

Materials and methods

Instruments used

All apparatus for proteomics technology were from GE healthcare (USA). Immobilized pH gradient (IPG) strips, IPG buffer, urea, glycerol, glycine and dithiothreitol (DTT) were purchased from GE healthcare. Acrylamide and the other reagents for the polyacrylamide gel preparation were obtained from Bio-Rad Laboratories (USA). CHAPS, iodoacetamide (IAA), ortho-phosphoric acid and ammonium sulfate, thiourea, acetonitrile, trifluoroacetic acid and α-cyano-4-hydroxycinnamic acid were from Sigma Chemical Co (St. Louis, MO). Methanol was obtained from Merck (Spain).

Subjects

Plasma samples from schizophrenic patients ($n = 42$, 26 males, 16 females, age 33.76 ± 19.65) undergoing chlorpromazine treatment and controls ($n = 46$, 22 males, 24 females, age 39.36 ± 11.97) were collected from the Shanghai Institute of Mental Health, following the guidelines of the local ethical committee. All these subjects were derived from Shanghai City. In association study of *Hp* two SNPs with schizophrenia, we prepared two sets of case-control sample panels. Panel I comprised 329 schizophrenics (203 males, 126 females; mean age, 44.54 ± 11.48 years) and 288 mentally healthy controls (159 males, 129 females; mean age, 32.02 ± 8.16 years). All samples of Panel I were derived from Shanghai City. Panel II comprised 192 schizophrenics (99 males, 93 females; mean age, 34.99 ± 10.48 years) and 192 mentally healthy controls (95 males, 97 females; mean age, 32.31 ± 9.01 years). All samples of Panel II were derived from Jilin City. All the subjects in the present study were unrelated Han Chinese. The diagnosis of schizophrenia was assigned according to CCMD-II-R (a counterpart diagnostic criterion of DSM-III-R in China) and DSM-III (Spitzer et al., 1992). Within the control group, subjects with a positive personal or familial history of major psychiatric disorders were excluded. After complete description of the study to the subjects, written informed consent was obtained.

Two-dimensional gel electrophoresis

The plasma samples were stored at −80 °C until assay by 2-DE. The protein content was determined using the Coomassie blue method. Protein concentration was approximately 60 mg/ml in the plasma from controls and patients. After thawing, 13 µl plasma was add to the 440 µl of IEF sample buffer (7 M urea, 2 M thiourea, 4% CHAPS, 40 mM Tris pH 8.8, 65 mM 1,4-dithioerythritol) with 0.5% IPG buffer, and then centrifuged at 49,000 rpm and 4 °C for 30 min. The supernatant sample for IEF was applied on immobilized pH 3–7 nonlinear gradient strips (24 cm). IEF was performed under the following conditions: 100 V (rehydration, 1,400 Vhr), 500 V (500 Vhr), 1,000 V (1,000 Vhr), 4,000 V (4,000 Vhr) and 8,000 V (80,000 Vhr). Subsequent to IEF, each strip was incubated for 15 min in an equilibration buffer (50 mM Tris-HCl pH 8.8, 6 M urea, 30% glycerol, 2% SDS) containing 2% DTT and for another 15 min in an equilibration buffer containing 2.5% IAA before loading onto SDS-PAGE gels. The second-dimensional separation was performed in 12.5% SDS polyacrylamide gels. The gels ($260 \times 200 \times 1.5$ mm) were run at 50 mA per gel for about 4.5 hours.

After the second-dimensional separation, gels were fixed with 50% methanol, containing 5% ortho-phosphoric acid for 2 h and then exposed

Table 1. The primers used for common PCR and allele-specific PCR

Marker	Primer sequence	Annealing temp. (°C)	Distance[a] kb	Polymorphism function
rs2070937	5′GGTGGGCTCAGTTTCTGGCT/C 5′CTATCTTTGCGGTGTCTGAGGG	60	1.3	A/G (Intron 2)
rs5473	5′TGCCTTTTGTTTCAGGATTA/Gb 5′CCCACCTGCTTCACATTC	62	2.7	A/G (Exon 5) Synonymous
rs3852780	5′AGGCGTCCAGCGGGGAAA/C 5′CTGCGGTTCTCCCACATCCCT	52	4.7	A/C (Intron 7)
rs12646	5′TATGGTCTTCTGAACCCACTC/Gb 5′CACCTGGTATGCGACTGG	60	6.3	C/G (Exon 8) Nonsynonymous
rs470428	5′TTATCAAAGCTTAAGATCCCCGT/C[b] 5′GCAGCCCATACTGAATGAACAC	60	6.2	A/G (Exon 8) Nonsynonymous

[a] Positions of SNPs are shown as distances from rs5472

[b] An additional mismatch was deliberately put at position-3 from the 3′-terminus of the allele-specific primer to confer the specificity of PCR amplification

overnight to staining solution (containing with 8% ammonium sulfate, 5% ortho-phosphoric acid, 0.1% Coomassie blue G-250 and 20% methanol). Excess dye was washed out from the gels with 20% ammonium sulfate until the background was completely clear. Gels were scanned in a UMAX PowerLook III scanner (resolution 300). ImageMaster 2-D Platinum software was used for the analysis of Coomassie-stained gels. The protein spots from different gels were matched and the percentage of the volume of the spots representing a certain protein was determined in comparison with the total proteins present in the 2-DE gel.

Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS)

Mass spectrometry sample preparation was performed automatically in Ettan Spot Handling Workstation on the basis of the protocol set out in the Ettan Spot Handling Workstation manual. The dried peptide fragments were resuspended with 3 µl of matrix solution, consisting of 50% acetonitrile, 0.03% trifluoroacetic acid and semi-saturated α-cyano-4-hydroxycinnamic acid. Samples were analyzed in a time-of-flight mass spectrometer (Ettan MALDI-ToF/Pro, GE healthcare). Peptide matching and protein searching were performed using the Ettan MALDI-ToF/Pro instrument's software. The peptide masses were compared with the theoretical peptide masses of all available proteins from all species. Mono isotopic masses were used and a mass tolerance of 1 Dalton was allowed.

SNPs genotyping by real-time allele-specific PCR

High molecular weight genomic DNA was extracted from venous blood using the standard phenol chloroform extraction procedure. In our study, we firstly selected five SNPs of *Hp* from the NCBI dbSNP database http://www.ncbi.nlm.gov/SNP/. We then examined their allele frequencies using the real-time allele-specific PCR procedure. Five candidate SNPs were genotyped by allele-specific PCR to specifically amplify the reference allele or its variant in separate PCR reactions in real-time allele-specific PCR on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, San Jose, CA, USA) (Greenwood et al., 2001). All the PCR primers were designed by a tetra-primer ARMS-PCR design program http://cedar.genetics.soton.ac.uk/public_html/primer1.html (Ye et al., 2001). The standard PCR reactions of 5 ml were carried out using Taqman® Universal PCR Master Mix reagent kits as in the guidelines. The assay combining kinetic (real-time quantitative) PCR with allele-specific amplification was performed as described by Germer et al. (2000). The detailed information about SNPs and PCR amplification condition is listed in Table 1. Allele calling was manually performed as in previous research in our laboratory (Tang et al., 2003).

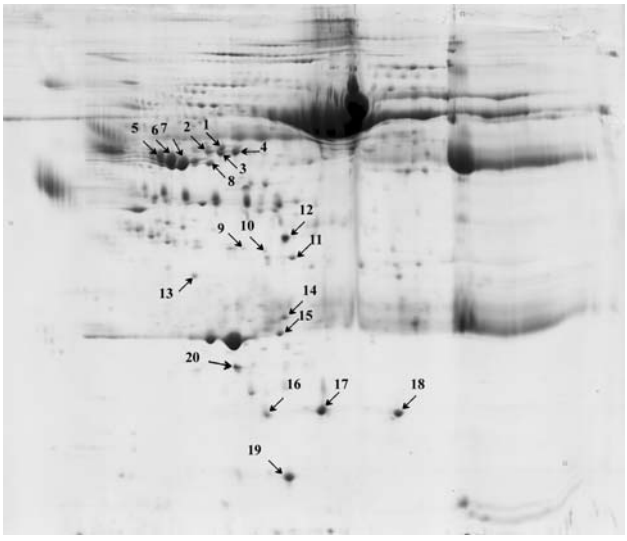

Fig. 1. Typical 2-DE picture of normal human plasma (800 µg protein sample was loaded). The gel was separated on a 26 × 20 cm plate and Coomassie blue stained. The horizontal axis represents the IEF dimension, which stretches from PH 3–7 (nonlinear). The vertical axis represents 12.5% SDS-PAGE gel

Table 2. List of proteins from human plasma analyzed by 2-DE and identified by MALDI-MS, following in-gel digestion with trypsin. The number of matching peptides and sequence coverage is listed

Protein spots	Protein ID	Swiss-Prot accession no.	Peptides matched	Sequence coverage (%)	pI	Mass (kDa)	Expectation
1	Antithrombin III	P01008	10	27.4	6.3	52.5	<0.001
2	Vitamin D binding protein	P02774	18	50.4	5.2	51.21	<0.001
3	Vitamin D binding protein	P02774	18	50.4	5.2	51.21	<0.001
4	Vitamin D binding protein	P02774	18	50.4	5.2	51.21	<0.001
5	α-1-antitrypsin	P01009	7	41.6	5.4	74.23	<0.001
6	α-1-antitrypsin	P01009	7	41.6	5.4	74.23	<0.001
7	α-1-antitrypsin	P01009	7	41.6	5.4	74.23	<0.001
8	α-1-antitrypsin	P01009	7	41.6	5.4	74.23	<0.001
9	Apolipoprotein E	P02649	19	56.8	5.6	36.14	<0.001
10	Apolipoprotein E	P02649	19	56.8	5.6	36.14	<0.001
11	Apolipoprotein E	P02649	19	56.8	5.6	36.14	<0.001
12	Transthyretin (tetramer)	P02766	5	51.3	5.3	12.83	<0.001
13	α1-microglobulin	P02760	7	28.1	6.0	38.98	0.008
14	Serum amyloid P-component	P02743	5	24.7	6.1	25.37	<0.001
15	Apolipoprotein A-I	P02647	9	36.9	5.4	28.94	<0.001
16	Haptoglobin	P00738	5	16.9	6.3	41.51	0.02
17	Haptoglobin	P00738	5	16.9	6.3	41.51	0.02
18	Haptoglobin	P00738	5	16.9	6.3	41.51	0.02
19	Transthyretin	P02766	5	51.3	5.3	12.83	<0.001
20	Retinol binding protein	P02753	8	67	5.3	20.94	<0.001

Table 3. Mean concentrations of plasma APPs in schizophrenia subjects and normal controls. Values are shown as mean ± SD

Protein (protein spot in Fig. 1)	Schizophrenic subjects (% vol)	Control subjects (% vol)	P value	Chromosomal loci
Haptoglobin α	1.25 ± 0.41	0.66 ± 0.3	<0.01	16q22.1
α1-Antitrypsin	2.54 ± 0.61	2.08 ± 0.49	<0.01	14q32.1
α1-Microglobulin	0.04 ± 0.01	0.03 ± 0.01	<0.01	9q32-q33
Serum amyloid P-component	0.12 ± 0.04	0.10 ± 0.05	0.034	1q21-q23
Retinol binding protein	0.15 ± 0.04	0.14 ± 0.09	0.513	10q24
Transthyretin (tetramer)	0.10 ± 0.07	0.10 ± 0.06	0.800	18q12.1
Transthyretin	0.37 ± 0.11	0.34 ± 0.08	0.243	18q12.1
Antithrombin III	0.17 ± 0.04	0.14 ± 0.05	<0.01	1q23-q25.1
Vitamin D binding protein	0.25 ± 0.08	0.19 ± 0.06	<0.01	4q12-q13
Apolipoprotein E	0.11 ± 0.04	0.13 ± 0.16	0.609	19q13.2
Apolipoprotein A-I	0.13 ± 0.04	0.13 ± 0.06	0.965	11q23

Statistical analysis

Differences of plasma protein levels between schizophrenia patients and normal controls were evaluated by comparing mean values of the two groups using the Student's t-test analysis (two-tailed). Statistical analysis was performed using the Statistical Package, for Social Science (SPSS) for Windows, version 10.0. Hardy-Weinberg equilibrium analysis for each *Hp* polymorphism was conducted using the online calculator, http://www.kursus.kvl.dk/shares/vetgen/_Popgen/genetik/applets/kitest.htm. Differences in genotypic and allelic distributions were estimated using the program CLUMP 1.9 (Sham and Curtis, 1995).

Results

Differences in plasma protein expression

To search for protein variation in schizophrenia, two-dimensional protein electrophoresis combined with Coomassie blue staining and computer-assisted image analysis was employed. This technique allowed screening of an average of 1000 protein spots per gel containing 13 µl plasma (800 µg protein). Twenty spots including 11 kinds of proteins were identified using the PMF (peptide-mass fingerprinting) method of MALDI-TOF MS (Fig. 1). The identification results were evaluated automatically by the instrument's software (Table 2).

We compared the differential expression of plasma proteins, easily discernable in 2-DE gels, between schizophrenic patients and normal controls (Table 3). The expression of Hp α chain, α1-antitrypsin, serum amyloid P-component, α1-microglobulin, antithrombin III and vitamin D binding protein was significantly higher in the plasma of schizophrenic patients in comparison with that of healthy controls. We also determined the levels of retinol binding protein, transthyretin, apolipoprotein E and apolipoprotein A-I, although no statistical difference was found at gene expression level. Four of the six differential expressed proteins, namely Hp α chain, α1-antitrypsin, serum amyloid P-component and α1-microglobulin, belong to positive APPs.

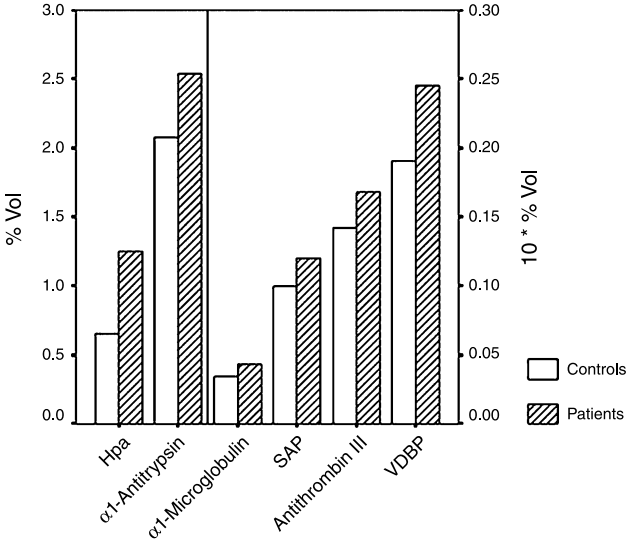

Fig. 2. Relative plasma proteins expression differences in 42 schizophrenia subjects and 46 control samples. Significant differences are indicated by P value <0.05 as determined by unpaired Student's t test (two-tailed)

We found that they were all up-regulated, while no obvious change occurred on the two negative APPs, transthyretin and retinol binding protein.

Genotyping of the Hp gene based on and 2-DE gels and RT-PCR

The genotypes for *Hp* 1/2 were assayed using 2-DE and Coomassie blue staining. Based on the length of alpha chain, there are two kinds of Hp α in the population, Hp 1 and Hp 2. Individuals can be homozygote or heterozygote for the alpha1 and alpha2 chains. Figure 3 illustrates how we genotype haptoglobin alpha1/alpha2 polymorphism with the images obtained from 2-DE gels. The frequencies for *Hp* 1/1, *Hp* 1/2 and *Hp* 2/2 were 0, 63.0%, 37.0% and 11.9%, 40.5%, 47.6% in schizophrenia patients and

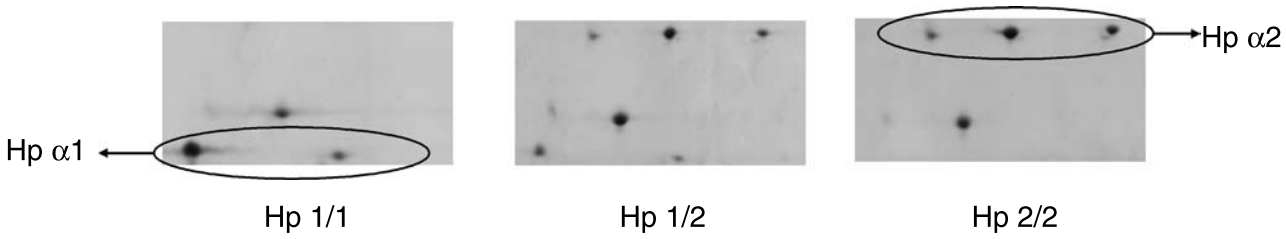

Fig. 3. Representative patterns of Hp genotypes 1/1, 1/2 and 2/2 after two-dimensional gel electrophoresis and Coomassie blue staining of human plasma

Table 4. The distributions of genotypes for three polymorphisms of the *Hp* gene

District	Group	*n*	Genotype distribution			df	χ^2	P value
	Hp 1/2[a]		11	12	22			
Shanghai	Control	42	5 (11.9%)	17 (40.5%)	20 (47.6%)	2	8.209	0.0136
	Patients	46	0	29 (63.0%)	17 (37.0%)			
	rs2070937		AA	AG	GG			
Shanghai	Control	284	133 (46.8%)	129 (45.4%)	22 (7.7%)	2	0.847	0.650
	Patients	327	141 (43.1%)	159 (48.6%)	27 (8.3%)			
Jilin	Control	192	90 (46.9%)	90 (46.9%)	12 (6.3%)	2	6.866	0.033
	Patients	191	108 (56.5%)	65 (34.0%)	18 (9.4%)			
	rs5473		GG	AG	AA			
Shanghai	Control	277	99 (35.7%)	128 (46.2%)	50 (18.1%)	2	2.992	0.233
	Patients	319	135 (42.3%)	137 (42.9%)	47 (14.7%)			
Jilin	Control	181	64 (35.4%)	84 (46.4%)	33 (18.2%)	2	0.818	0.687
	Patients	187	58 (31.0%)	94 (50.3%)	35 (18.7%)			

[a] The genotypes for *Hp* 1/2 were assayed using 2-DE and Coomassie blue staining

controls respectively (Table 4). The distribution of genotypes in controls was in Hardy-Weinberg equilibrium, while showed significant Hardy-Weinberg disequilibrium in patients ($P = 0.002$). The distribution of *Hp* 1/2 genotypes showed a strong association with schizophrenia ($\chi^2 = 8.20$, df = 2, $P = 0.0136$).

During the assay development, three SNPs (rs3852780, rs12646 and rs470428) were rejected due to their low frequency (minor allele frequencies <5%). Finally, we used 2 candidate SNPs, rs2070937 in intron 2 and rs5473 in exon 5 for genotyping. A research on Chinese population genetic structure showed that a cryptic genetic borderline exists between Shanghai and Jilin districts (Shi et al., 2004). Thus, we tested these variants for association with schizophrenia in the two districts separately. Genotype distributions were within Hardy-Weinberg equilibrium in both control and case populations from Shanghai and Jilin. Comparisons of the genotypic and allelic frequencies for rs5473 G/A displayed no significant differences between schizophrenia and controls. As for rs2070937 A/G, genotypic distribution differed significantly between schizophrenia and controls in Jilin district ($P = 0.033$) (Table 4). Bonferroni correction is very strict in the context of genetic association studies, so correction for multiple comparisons was not made here. On the other hand, in our case-control study P values were assessed using the Monte Carlo approach instead of χ^2 distribution, so that the P values have been corrected to some extent. The distribution of genotypic or allelic frequencies showed no association with schizophrenia in Shanghai district. Analysis of sample II from Jilin district showed that rs2070937 A/G was a protective genotype with an odds ratio of 0.585 (95% confidence interval, 0.387–0.883). Because we tested only two SNPs in *Hp* gene, no further measure of pairwise linkage disequilibrium (LD) was calculated.

Discussion

The study found that there were higher levels of plasma Hp α chain, α1-antitrypsin, serum amyloid P-component, α1-microglobulin, antithrombin III and vitamin D binding protein in schizophrenia patients as compared to healthy controls. Among these six proteins, four were APPs. The levels of APPs rise in response to inflammation.

These findings suggest the presence of an inflammatory process in schizophrenia. Various functions of APPs have been postulated including regulation of inflammatory mediator levels during inflammation and increase of susceptibility to bacterial phagocytosis. The up-regulated expression of all of the positive APPs investigated in the present study provides strong evidence that schizophrenia can be accompanied by an immunological response, a finding which was formerly confirmed by other researchers (Vander Putten et al., 1996; Maes et al., 1997; Meisenzahl et al., 2001; Wong et al., 1996). The convergence of results on all four positive APPs is a promising indicator that APPs are schizophrenia related proteins.

Except for APPs, VDBP and antithrombin III are significantly higher in the plasma of schizophrenic patients. VDBP, the major carrier of vitamin D and its metabolites in blood, is synthesized predominantly in the liver and secreted into the bloodstream (Song et al., 1998). Guha et al. reported IL-6 increased VDBP mRNA approximately twofold in Hep3B hepatocytes, and thus suggested that a high level of VDBP synthesis was maintained during the acute phase response (Guha et al., 1995). Antithrombin III activity has also been suggested as reflecting acute-phase reaction in cats in a stress-responsive state (Welles et al., 1993). Inflammation would lead to an increase of plasma antithrombin III. It seems that all of the six higher-level expressed plasma proteins are relevant to acute phase reaction. Thus, we concluded that APPs play an important role in schizophrenia development.

In the present study we compared the differential expression of plasma proteins, which could be easily discerned in 2-D gels, but not including all the plasma proteins. In future project we will quantify other APPs, such as C-Reactive Protein, using method of ELISA. We did not find any response in negative acute phase reactants. The possible explanation is that the deviation between individual subjects is large and due to limitations in subgroup sample size, significant changes of negative acute phase proteins may have been masked.

Genome-wide scans have shown suggested linkage to schizophrenia on many chromosome regions. The four genes of differently expressed positive APPs in this study are localized in or nearby positive linkage chromosomal regions previously identified from genome-wide linkage studies, 1q21-q23 for Serum Amyloid P-Component (Brzustowicz et al., 2002; Floyd-Smith et al., 1986; Ionasescu et al., 1987), 9q32-q33 for α1-Microglobulin (Diarra-Mehrpour et al., 1989), 14q32.1 for α1-Antitrypsin (Turleau et al., 1984) and 16q22.1 for *Hp* α (Simmers et al., 1986). Replicated linkage of schizophrenia to these chromosomal loci: 1q21-q22, 9q32-q34, 14q32.3 and 16q22 has been reported (Brzustowicz et al., 2000, 2002; Maziade et al., 2002; Kaufmann et al., 1998; Shaw et al., 1998). The overlapping of APPs gene loci with the schizophrenia candidate chromosomal regions further supports the identification of APPs as schizophrenia related proteins.

In the comparison of the plasma protein expression between schizophrenia and control subjects, Hp showed the most significant difference. The plasma levels of several Hp α components, as well as total Hp α, were found to be significantly ($P<0.01$) higher in the schizophrenic subjects. Total Hp α was highly significantly up-regulated at 1.89-fold ($P<0.01$). To explore whether the gene loci of APPs relate to schizophrenia, we selected *Hp* gene to carry out an association study in a large Chinese cohort. This analysis revealed a significant association between schizophrenia and *Hp* $1/2$ polymorphism and also an association between this disease and *Hp* gene rs2070937 variant in Jilin population, although not in Shanghai. The genotypes of *Hp* $1/2$ show significant Hardy-Weinberg disequilibrium in patients. In patients the selection criterion is based on disease-susceptibility genotypes, rather than on independently selected alleles (Nielsen et al., 1998), which leaves the possibility that genotype distribution might show a deviation from Hardy-Weinberg equilibrium, especially when the invested locus is a disease susceptibility locus itself. In our population the allele frequency of Hp 1 in normal controls is 32.1%, which is in close agreement with previously reported in Chinese population by Saha et al. (1992). In the *Hp* system, the present result is highly concordance with Rudduck's research (Rudduck et al., 1985). Rudduck et al. reported a significant departure from the Hardy-Weinberg equilibrium with an excess of heterozygotes was found among the schizophrenic patients. Similarly, it was reported that the distribution of *Hp* $1/2$ types in schizophrenic patients was different from that of controls in the study of Northwestern Italians (Maes et al., 2001).

In view of the cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), being the key mediators of the acute phase response, many previous researches on cytokines also give their explanations to our observation, acute phase reaction being involved in schizophrenia. Associations of TNF-α and IL-1 polymorphism with schizophrenia have been found in several populations (Duan et al., 2004; Hashimoto et al., 2004; Meisenzahl et al., 2001). Elevated IL-1, IL-6, and TNF-α serum levels in schizophrenic patients have also been widely reported (Lin et al., 1998; Zhang et al., 2002; Maes et al., 1995; Katila et al., 1994; Kowalski et al., 2001).

The estimation of activity of these cytokines levels and investigation of these genes polymorphisms provides more evidences on the involvements of APPs in schizophrenia.

One limitation of our study is that we used medicated schizophrenics but not neuroleptic-free schizophrenics. Generally, schizophrenic patients go to hospital after a period time of medical treatment and it is therefore very difficult to collect plasma of neuroleptic-free schizophrenics. We are now tying to collect these samples and will carry on this analysis in the further work. Despite this limitation our conclusion of acute response in schizophrenia is creditable, but not likely to be a false positive result caused by medications. Maes et al. (1997) carried out a research on acute phase reaction in psychotic diseases with a major finding of plasma APPs being significantly higher in none-medicated schizophrenic subjects than in patients medicated with antipsychotic agents. This result suggested that an acute phase response was suppressed by (sub)chronic treatment with psychotropic drugs. Hornig et al. (1998) have also show that subchronic treatment play a role in normalizing systemic immune activation associated with depression. The acute response in schizophrenia observed in our research therefore not likely to be caused by medications. Various possible causes might produce an acute phase response in schizophrenic patients. Psychological stress can lead to changes in hormonal levels, which may result in the changes in the hepatic synthesis of these proteins. Activation of the inflammatory response system found in schizophrenia could also be involved in the development of autoimmune responses in schizophrenia or a viral infection or reactivation occurring in schizophrenia (Lin et al., 1998).

In summary, we find: (i) plasma APPs express at a higher level in schizophrenic patients; (ii) there is a significant association between schizophrenia and two polymorphisms of *Hp* gene, *Hp* $1/2$ and rs2070937; and (iii) all the positive APP genes that were differentially expressed found in this study locate in or nearby positive linkage chromosomal regions of schizophrenia. These three pieces of evidences indicate that chronic systemic inflammation may be an aetiological agent of the pathophysiology of schizophrenia, but not just as an accompanying symptom. The evidence that APPs are involved in schizophrenia at both the proteomic and genetic levels suggest that mediators of the acute phase response would point to a possible underlying metabolic defect in schizophrenia sufferers. It would be of interest to study the mechanisms underlying peripheral changes in the immune system in schizophrenia.

Acknowledgements

This work was supported by grants from the national 973 and 863 Programs of China, the National Natural Science Foundation of China, the Ministry of Education, PRC and the Shanghai Municipal Commission for Science and Technology.

References

Brzustowicz LM, Hayter JE, Hodgkinson KA, Chow EW, Bassett AS (2002) Fine mapping of the schizophrenia susceptibility locus on chromosome 1q22. Hum Hered 54: 199–209

Brzustowicz LM, Hodgkinson KA, Chow EW, Honer WG, Bassett AS (2000) Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. Science 288: 678–682

Diarra-Mehrpour M, Bourguignon J, Sesboue R, Mattei MG, Passage E, Salier JP, Martin JP (1989) Human plasma inter-alpha-trypsin inhibitor is encoded by four genes on three chromosomes. Eur J Biochem 179: 147–154

Duan S, Xu Y, Chen W, Liu Z, Guo T, Gao J, Bian L, Zhen Y, Li X, Zhang X, Pan Y, Gu N, Feng G, He L (2004) No association between the promoter variants of tumor necrosis factor alpha (TNF-alpha) and schizophrenia in Chinese Han population. Neurosci Lett 366: 139–143

Floyd-Smith G, Whitehead AS, Colten HR, Francke U (1986) The human C-reactive protein gene (CRP) and serum amyloid P component gene (APCS) are located on the proximal long arm of chromosome 1. Immunogenetics 24: 171–176

Germer S, Holland MJ, Higuchi R (2000) High-throughput SNP allele-frequency determination in pooled DNA samples by kinetic PCR. Genome Res 10: 258–266

Greenwood TA, Alexander M, Keck PE, McElroy S, Sadovnick AD, Remick RA, Kelsoe JR (2001) Evidence for linkage disequilibrium between the dopamine transporter and bipolar disorder. Am J Med Genet 105: 145–151

Guha C, Osawa M, Werner PA, Galbraith RM, Paddock GV (1995) Regulation of human Gc (vitamin D-binding) protein levels: hormonal and cytokine control of gene expression in vitro. Hepatology 21: 1675–1681

Hashimoto R, Yoshida M, Ozaki N, Yamanouchi Y, Iwata N, Suzuki T, Kitajima T, Tatsumi M, Kamijima K, Kunugi H (2004) Association analysis of the $-308G>A$ promoter polymorphism of the tumor necrosis factor alpha (TNF-alpha) gene in Japanese patients with schizophrenia. J Neural Transm 111: 217–221

Hornig M, Goodman DB, Kamoun M, Amsterdam JD (1998) Positive and negative acute phase proteins in affective subtypes. J Affect Disord 49: 9–18

Ionasescu V, Burns T, Searby C, Ionasescu R (1987) Linkage between the loci for Duffy (FY) and serum amyloid P component (APCS) on human chromosome 1. Cytogenet Cell Genet 45: 240–241

Katila H, Appelberg B, Hurme M, Rimon R (1994) Plasma levels of interleukin-1 beta and interleukin-6 in schizophrenia, other psychoses, and affective disorders. Schizophr Res 12: 29–34

Kaufmann CA, Suarez B, Malaspina D, Pepple J, Svrakic D, Markel PD, Meyer J, Zambuto CT, Schmitt K, Matise TC, Harkavy Friedman JM, Hampe C, Lee H, Shore D, Wynne D, Faraone SV, Tsuang MT, Cloninger CR (1998) NIMH Genetics Initiative Millenium Schizophrenia Consortium: linkage analysis of African-American pedigrees. Am J Med Genet 81: 282–289

Kendler KS (1988) The impact of varying diagnostic thresholds on affected sib pair linkage analysis. Genet Epidemiol 5: 407–419

Kety SS, Wender PH, Jacobsen B, Ingraham LJ, Jansson L, Faber B, Kinney DK (1994) Mental illness in the biological and adoptive relatives of schizophrenic adoptees. Replication of the Copenhagen Study in the rest of Denmark. Arch Gen Psychiatry 51: 442–455

Kowalski J, Blada P, Kucia K, Madej A, Herman ZS (2001) Neuroleptics normalize increased release of interleukin-1 beta and tumor necrosis factor-alpha from monocytes in schizophrenia. Schizophr Res 50: 169–175

Langlois MR, Delanghe JR (1996) Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem 42: 1589–1600

Lin A, Kenis G, Bignotti S, Tura GJ, De Jong R, Bosmans E, Pioli R, Altamura C, Scharpe S, Maes M (1998) The inflammatory response system in treatment-resistant schizophrenia: increased serum interleukin-6. Schizophr Res 32: 9–15

Maes M, Bosmans E, Calabrese J, Smith R, Meltzer HY (1995) Interleukin-2 and interleukin-6 in schizophrenia and mania: effects of neuroleptics and mood stabilizers. J Psychiatry Res 29: 141–152

Maes M, Delange J, Ranjan R, Meltzer HY, Desnyder R, Cooremans W, Scharpe S (1997) Acute phase proteins in schizophrenia, mania and major depression: modulation by psychotropic drugs. Psychiatry Res 66: 1–11

Maes M, Delanghe J, Bocchio CL, Bignotti S, Tura GB, Pioli R, Zanardini R, Altamura CA (2001) Haptoglobin polymorphism and schizophrenia: genetic variation on chromosome 16. Psychiatry Res 104: 1–9

Maziade M, Fournier A, Phaneuf D, Cliche D, Fournier JP, Roy MA, Merette C (2002) Chromosome 1q12-q22 linkage results in eastern Quebec families affected by schizophrenia. Am J Med Genet 114: 51–55

McGuffin P, Owen MJ, Farmer AE (1995) Genetic basis of schizophrenia. Lancet 346: 678–682

Meisenzahl EM, Rujescu D, Kirner A, Giegling I, Kathmann N, Leinsinger G, Maag K, Hegerl U, Hahn K, Moller HJ (2001) Association of an interleukin-1beta genetic polymorphism with altered brain structure in patients with schizophrenia. Am J Psychiatry 158: 1316–1319

Nielsen DM, Ehm MG, Weir BS (1998) Detecting marker-disease association by testing for Hardy-Weinberg disequilibrium at a marker locus. Am J Hum Genet 63: 1531–1540

Pandey A, Mann M (2000) Proteomics to study genes and genomes. Nature 405: 837–846

Rothermundt M, Arolt V, Bayer TA (2001) Review of immunological and immunopathological findings in schizophrenia. Brain Behav Immun 15: 319–339

Rudduck C, Franzen G, Frohlander N, Lindstrom L (1985) Haptoglobin and transferrin types in schizophrenia. Hum Hered 35: 65–68

Saha N, Liu Y, Tay JS, Basair J, Ho CH (1992) Association of haptoglobin types with serum lipids and apolipoproteins in a Chinese population. Clin Genet 42: 57–61

Sham PC, Curtis D (1995) Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. Ann Hum Genet 59: 97–105

Shaw SH, Kelly M, Smith AB, Shields G, Hopkins PJ, Loftus J, Laval SH, Vita A, De Hert M, Cardon LR, Crow TJ, Sherrington R, DeLisi LE (1998) A genome-wide search for schizophrenia susceptibility genes. Am J Med Genet 81: 364–376

Shi Y, Zhao X, Yu L, Tao R, Tang J, La Y, Duan Y, Gao B, Gu N, Xu Y, Feng G, Zhu S, Liu H, Salter H, He L (2004) Genetic structure adds power to detect schizophrenia susceptibility at SLIT3 in the Chinese Han population. Genome Res 14: 1345–1349

Simmers RN, Stupans I, Sutherland GR (1986) Localization of the human haptoglobin genes distal to the fragile site at 16q22 using in situ hybridization. Cytogenet Cell Genet 41: 38–41

Smithies O (1955) Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. Biochem J 61: 629–641

Smithies O, Walker NF (1956) Notation for serum-protein groups and the genes controlling their inheritance. Nature 178: 694–695

Song YH, Ray K, Liebhaber SA, Cooke NE (1998) Vitamin D-binding protein gene transcription is regulated by the relative abundance of hepatocyte nuclear factors 1alpha and 1beta. J Biol Chem 273: 28408–28418

Spitzer RL, Williams JB, Gibbon M, First MB (1992) The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. Arch Gen Psychiatry 49: 624–629

Tang JX, Zhou J, Fan JB, Li XW, Shi YY, Gu NF, Feng GY, Xing YL, Shi JG, He L (2003) Family-based association study of DTNBP1 in 6p22.3 and schizophrenia. Mol Psychiatry 8: 717–718

Thaker GK, Carpenter WT Jr (2001) Advances in schizophrenia. Nat Med 7: 667–671

Turleau C, de Grouchy J, Chavin-Colin F, Dore F, Seger J, Dautzenberg MD, Arthuis M, Jeanson C (1984) Two patients with interstitial del (14q), one with features of Holt-Oram syndrome. Exclusion mapping of PI (alpha-1-antitrypsin). Ann Genet 27: 237–240

Vander Putten DM, Torrey EF, Larive AB, Merril CR (1996) Plasma protein variations in monozygotic twins discordant for schizophrenia. Biol Psychiatry 40: 437–442

Welles EG, Boudreaux MK, Tyler JW (1993) Platelet, antithrombin, and fibrinolytic activities in taurine-deficient and taurine-replete cats. Am J Vet Res 54: 1235–1243

Wong CT, Tsoi WF, Saha N (1996) Acute phase proteins in male Chinese schizophrenic patients in Singapore. Schizophr Res 22: 165–171

Ye S, Dhillon S, Ke X, Collins AR, Day IN (2001) An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Res 29: E88

Zhang XY, Zhou DF, Zhang PY, Wu GY, Cao LY, Shen YC (2002) Elevated interleukin-2, interleukin-6 and interleukin-8 serum levels in neuroleptic-free schizophrenia: association with psychopathology. Schizophr Res 57: 247–258

Authors' address: Dr. Lin He, Shanghai Jiao Tong University, Bio-X Life Science Research Center, Hao Ran Building, 1954 Huashan Road, Shanghai 200030, China; or Institute for Nutritional Sciences, SIBS, Chinese Academy of Sciences, 294 Taiyuan Road, Shanghai 200031, China,

Fax: +86-21-62822491, E-mail: helin@sjtu.edu.cn