行政院國家科學委員會專題研究計畫　期中進度報告

精神分裂症致病基因之定位研究（2/3）

計畫類別：個別型計畫
計畫編號：NSC92-3112-B-002-019-
執行期間：92 年 05 月 01 日至 93 年 04 月 30 日
執行單位：國立臺灣大學醫學院精神科

計畫主持人：胡海國

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報告類型：完整報告
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中　華　民　國 93 年 4 月 29 日

基因體醫學國家型科技計畫

National Research Program for Genomic Medicine
National Science Council, the Executive Yuan, ROC.

計畫名稱-精神分裂症致病基因之定位研究
A Positional Cloning Study of Schizophrenia

報告類別：☐ 新進研究計畫 (New Proposal) ☐ 修正後計畫書 (Revised Proposal) ☑ 年度成果報告 (Progress Report)

計畫類別：☑ 個別型計畫 (Individual Project) ☐ 整合型計畫 (Program Project)

計畫編號：**91-GMP029-1**

計畫主持人 (Principle Investigator)：胡海國 Hai-Gwo Hwu

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Department of Psychiatry, Medical College, National Taiwan University

中華民國 93 年 4 月 30 日

National Research Program for Genomic Medicine
National Science Council, the Executive Yuan, ROC.
Progress Report—Research Project

基因體醫學國家型科技計畫

國科會延續性計畫進度報告

個別型計畫 (Individual Project)

Program Classification:

☑ **Genomic Medicine** ☐ **Bioinformatics**

☐ **Proteomics & Structural Genomics** ☐ **ELSI**

Project Number: **91-GMP029-1**
NSC Funding Number: NSC 92-3112-B-002-019 (93 年度國科會**預核**編號)

Title of Project 計畫名稱	(in Chinese) 中文 精神分裂症致病基因之定位研究
	(in English) 英文 A Positional Cloning Study of Schizophrenia
Institution 研究（執行）單位	(in Chinese) 中文 國立台灣大學醫學院精神科
	(in English) 英文 Department of Psychiatry, Medical College, National Taiwan University
Principle Investigator 計畫主持人	(in Chinese) 中文 胡海國
	(in English) 英文 Hai Gwo Hwu

FY	2002	2003	2004	Total
Budget	4,778,900 **(NGC:2000,000)**	4,791,800 **(NGC:7,109,760)**	**4,835,400** **(NGC:9,878,400)**	

(in NT dollars: 1USD = 34 NTD)

Signature of the PI：_____Date：_____

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Progress Report

1. Response to previous reviewers' critiques

The reviewers' comments (A):

This project is of fine mapping study, and only uses a candidate region as narrow as 2cM for SNP genotyping study. The reviewers suggest that traditional linkage approach is unlikely and suggest to use linkage disequilibrium approach using trios.

Answer:

We share the same concerns as the reviewer's over the resolution of conventional linkage analysis; therefore the transmission disequilibrium tests (TDT) were conducted in addition to traditional linkage analyses. To extract as much information as possible from the data that have been collected, we performed TDT that uses pedigree data such as PDT, TDT/S-TDT and TRANSMIT program. Quantitative trait outcomes were also considered by using QTDT program. Multipoint transmission tests were also carried out using the TRANSMIT program. In addition, haplotype analysis using pedigree data were carried out using the FBAT program.

The reviewers' comments (B):

The reviewers also stated that a carefully through out analytical plan is needed to laid out as to why around 100 SNPs is required can be extracted. Specifically, it would seem wise to carry out this fine mapping approach in two stages.

Answer:

As a matter of fact, the two-stage strategy has been adopted in this project. For the first screening step, we selected 31 trios at random plus 2 normal controls; these DNA samples were placed into a 96-well plate for SNP genotyping. Allele frequencies, Hardy Weinburg equilibrium (HWE), Mendelian inheritance (MI), inter-marker LD coefficients, missing rate etc, were estimated. Markers that failed HWE and MI, data were rechecked for quality control concern. SNP markers were considered in the second stage genotyping for all samples if the three conditions were met; 1) no violation of MI, 2) missing rate less than 30%, 3) minor allele frequency larger than 10%. For example, 120 SNPs located at 1q42.1 were identified using bioinformatic tools, after our first stage screening; only 45 were retained for the second stage typing. Although, TDT and haplotype analysis (haplotype were grouped by LD information) were conducted based on the 45 SNPs as preliminary results, the information were not used as one of our criterion to enter second stage typing due to small sample size.

The two-stage strategy will be used through out the rest of the project.

Ideally, in a cluster of SNPs with strong LD, only a few needs to be selected (preferably CSNP) for the second stage genotyping, in the example of 1q42.1, this information was not used due to low retention rate (45/120 = 37.5%). LD information will be used as one of the criterion if more SNPs are available in other regions.

The reviewers' comments (C):

My recommendation is that the investigators are asked to prepare for a sensible analytical plan….

Answer:

The analytical plan is as the following:

1) Although the project is for fine mapping, the range of the candidate region may span more than a few centi-Morgans, probability of IBD sharing from multiple STRP markers using affected sibpair will be first checked to confirm the likelihood of the region.
2) If the region does show a positive signal of linkage from the results of probability of IBD sharing using STRP markers, two- stage SNP genotyping will be launched. The first stage is for the screening using subset of samples. SNP markers that met certain criterion will be genotyped in the second stage using all samples.
3) The analyses for the SNP markers consist of two parts.
   (1) For single SNP: the following programs will be carried out: Transmission Disequilibrium Test and Sib Transmission Disequilibrium Test, Pedigree Transmission Test, Quantitative Transmission Test, TRANSMIT program, Family Based Association Test, etc.
   (2) For multipoint SNP: inter-marker LD coefficient will first be determined (for example using the GOLD program), the TRANSMIT program will be performed for haplotype analysis. Haplotype Family Based Association Test (HaploFBAT) will also be carries out. In additional, haplotypes will be estimated by using the SIMWALK2 program then the information will be used in the analysis of haplotype transmission test.
4) As for the available quantitative outcomes, heritability for each of them will be calculated to decide the possible genetic component. QTDT will be performed for those with higher value of heritability.
5) The interpretation of positive association will depend on a few factors:
   (1) p-values after multiple comparison adjustment
   (2) Available sample size under the assumption for each of the test
   (3) Consistent trend across different tests.

The above analytical plan will be revised according to the development of new methodology.

Reference:

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- TDT/S-TDT program
  — Spielman, et al. Am J Hum Genet, 52:506-516 (1993).
  — Spielman, Ewens, Am J Hum Genet, 62:450-458 (1998).
- TRANSMIT program
  — David Clayton, Am. J. Hum. Genetics, 65:1170-7 (1999).
- QTDT program
  — Abecasis, Cardon and Cookson, Am J Hum Genet, 66:279-292 (2000).
- FBAT program
  — Laird, Horvath, Xu, Genetic Epi, supp 1, 19:36-42 (2000).
  — Rabinowitz, Laird , Hum. Heredity, 50:211-223 (2000).
  — Horvath, Xu, Laird ,European J Hum Genet. 9:301-306 (2001).
- HaploFBAT program
  — Laird NM ,Genet Epidemiol, 26(1):61-69 (2004).
- GOLD program
  — Abecasis, Cookson , Bioinformatics, 16(2):182-183 (2000).
- SIMWALK2 program
  — Weeks, Sobel, O'Connell, Lange, Am J. Hum Genet, 56, 1506-1507 (1995).
  — Sobel, Lange, O'Connell, Weeks, in *Genetic Mapping and DNA Sequencing* , Vol 81 in

The IMA Volumes in Mathematics and its Applications, eds TP Speed, MS Waterman (Springer-Verlag, 1996), pages 49-63.

— Sobel, Lange, Am. J. Hum. Genet. 58, 1323-1337 (1996).

The reviewers' comments (D):

In addition, the investigators should provide a rationale to prioritize the four remaining candidate regions for genotyping…

Answer:

We, the investigators, reconsider the genotyping plan. We finally had the following conclusions:

a. To do a STRP genotyping with 6 marker around each of the initial dinucleotide repeat markers of D6S296 (6p22), D8S1222(8p14), D15S976 (15q14), and D22S278 (22q12), which was found to have suggestive evidence of linkage with schizophrenia in our previous study. This work intended to replicate the original finding, and to reduce the number of candidate regions, and to narrow down the chromosome region for further SNP genotyping.
b. To do a set of SNP genotyping study on possible candidate genes, which have expression in CNS and have possible pathophysiological significance related to schizophrenia theoretically, reported in literature located in these 4 other candidate chromosome regions. This work intends to catch up the frontier work of world schizophrenia genetic studies. Those candidate genes reported in literature near those candidate regions found by our previous study might be meaningful to us POCOS project.
c. To do serial SNP genotyping studies on the other four candidate regions with the sequence of (1) D22S278 (22q12), (2) D6S296 (6p22), (3) D15S976(15q14), (4)D8S1222(8p14).

The rationale for this sequence is that: The reason for choosing D22S278 (22q12) as the first candidate region to do fine mapping is that there are many functional genes in this region. There is no report of candidate genes, except in the region of 22q11, in this region. It is worthwhile to do it as quick as possible. The D6S296 (6p22) was as the second candidate region to do fine mapping. The reason is similar to the first one. Actually, there is rare report of candidate genes in this region yet, other than TNFA, NOTCH4 and MRDS1 (6p24.3).

The 3rd and the 4th candidate regions are D15S976 (15q14) and D8S1222 (8p14). The reason is that there already some promising candidate genes found in these two regions.

However, we intend to speed up all fine genotyping studies in all these 4 candidate regions as soon as possible in this 3-year study period. After all, all research centers were not definitely sure that the candidate genes are really true vulnerability genes. It deserves us to find the candidate genes by ourselves for further neurobiological studies. This is the only was to be in the frontier line to do schizophrenia molecular genetic study.

2. Specific Aims

This positional cloning study of schizophrenia (POCOS) has three specific aims: (1) To locate and identify the vulnerability genes of the phenomenological phenotype as well as the endophenotypes of schizophrenia (SCH) at specific chromosome regions; (2) To find specific polymorphism of candidate genes associated with endophenotypes and/or phenomenological phenotypes of schizophrenia; (3) To collect more data of continuous performance test (CPT) and Wisconsin Card Sorting Test (WCST) in the families with DNA samples.

3. Progress Results Summary

We focused the following seven works in this period (4/1/2003~4/30/2004):

1. Collecting more data of Continuous Performance Test (CPT) and Wisconsin Card Sorting Test (WCST) of the TSLS family with DNA sample for endophenotype definition.
2. Preparation of the DNA samples of Taiwan Schizophrenia Linkage Study (TSLS), totally 586 coaffected sibpair families, 2389 DNA samples.
3. Completion of the fine mapping study, including analysis, of chromosome 1q42.1 in 102 coaffected sibpair families of schizophrenia.
4. Replication of the SNPs of significant results in the fine mapping studies of chromosome 1q42.1 in the randomly selected 203 families of TSLS samples.
5. Replication of linkage evidences of five chromosome regions (1q42.1, 6p24-22, 8p21, 15q13-14, 22q12) with 30 simple tandem repeat polymorphisms (STRPs) markers in all the family samples to guide further fine-mapping studies.
6. Searching for polymorphism in the POCOS located vulnerability gene for neurobiological and/or clinical studies.
7. Phenotype refinement according to the clinical phenotype and neuropsychological endophenotypes of CPT and WCST for further genetic association study.
8. We prepare to genotype the polymorphisms (SNPs) in 10 positional candidate genes, including RGS4 on chromosome 1q21, MRDS1 on chromosome 6p24, DTNBP1 on 6p22, TNF-alpha and NOTCH4 on 6p21, PPP3CC and NRG1 on 8p12-21, G72 on 13q and DAAO interacting with G72, and CHRNA7 on 15q14.
9. We have also selected 99 SNPs markers for SNP fine mapping on the candidate region of 22q12, around the STRP marker D22S278. Three step methods will be adopted too in this genotyping work. We hypothesize that we may obtain one or two significant candidate genes in this specific candidate region of 22q12. Currently, the first stage work of SNP validation has been completed.
10. We prepare to genotype 3 SNPs in NRG1 gene in 905 adolescent assessed by schizotypal personality questionnaire (SPQ) and CPT to test the hypothesis NRG1 is a candidate gene for schizotypal personality features. The genotyping work has been completed and the preliminary result will be presented as the following section.

Collection of CPT and WCST data

The work of collecting more data of CPT and WCST of study families with DNA sample has been preceded smoothly.

Up to now, we have collected 573 and 570 families with CPT and WCST data, respectively of 600 TSLS families with DNA samples.

We also collected blood samples for genetic study: (1) collecting fresh blood of 20subjects for FISH study on the validity study of a novel positive probe for identifying possible balanced translocation of t(1:11) (q42.1;q14.3) (attached manuscript submitted); (2) collecting serum of the 11 sample patients with C/C and C/T genotypes of the novel SNP polymorphism of DISC1gene.

Preparation of the DNA samples of TSLS data bank

This is a laborious work for genotyping study in this year. Up to the present time, The DNA sample have been well prepared.

Fine mapping using SNP genotyping on the 1q42.1 candidate region near marker D1S251 using 102 NTUH samples (3rd work)

Please see the attached manuscript, **'**A SNP Fine Mapping Study on Chromosome 1q42.1 Reveals Vulnerability Genes of GNPAT and DISC1 in Schizophrenia: Association with Impairment of Sustained Attention'

Replication study of haplotype association analysis between GNPAT gene and schizophrenia (Work No. 4)

We randomly selected 203 sibpair families from our TSLS sample (total available family number is 388 families) in order to replicate the previous of significant association between GNPAT gene polymorphisms and schizophrenia in our MPSS family sample (3rd work). Five SNPs (SNP 482, 485, 479, 488, 489) from the 3rd work with the total distance of 32 kbp were selected for the replication study.

We found the five SNPs were in a high LD block using GOLD program (please see Fig 1). Single point association analysis didn't reveal significant association with any of the five SNPs using either Transmit v2.5.4 or FBAT v1.4.1 (see Table 1). Haplotype association analysis revealed not significant association with the risk haplotype (2.2.2.2.2) reported in the 3rd work (Table 2). We failed to replicate the significant association between schizophrenia and GNPAT gene in a larger family sample. However, the risk haplotype was reported to be associated with the sustained attention deficit assessed by CPT in the previous study. We prepare to do further analysis to stratify the larger sample according to the CPT data to see if the risk haplotype of GNPAT gene is associated with specific subtype of schizophrenia assessed by CPT.

Replication of linkage evidences of five chromosome regions [1q42.1(DIS251), 22q12(D22S278), 6p22(D6S296), 15q14(D15S976), 8p14(D8S1222)](work No.5).

Partly following the suggestion of the reviewers' comment, we (investigators) intended to replicate our previous linkage results of these 5 candidate chromosome regions using STRP genotyping.

We designed 6 STRP markers with high polymorphism (50%-90%) in each chromosome candidate region. A total of 30 STRP markers were chosen for study. The markers for 1q41-42 were DIS2847, DIS2833, DIS1656, DIS251, D1S2709, D1S459; for 6p24-22 were D6S1640, D6S277, D6S296, D6S940, D6S470, D6S1034; for 8p21 were D8S1223, D8S339, D8S1222, D8S1810, D8S1477, and D8S283; for 15q13-14 were D15S1019, D15S1048, D15S165, D15S976, D15S1031; and for 22q12-13 were D22S1162, D22S691, D22S424, D22S278, D22S683, and D22S1177. In average, the marker-marker distance of each region was about 1 cM. Of the 30 STRP selected, 7 markers failed to be genotyped using high throughput method. Therefore, 23 STRP markers were included into the linkage analysis. (Table 3).

After validation analysis and checking up for Mendelian compatibility, we have available STRP genotyping data on 480 families, including 92 families of MPSS sample and 388 families of TSLS sample.

Single point analysis using SAGE 3.1 SIBPAL revealed significant linkage evidence with D8S283 (p=0.026) in narrow model, with D22S1162 (p=0.0378) in broad model, and with

D22S1177 (p=0.0304 in narrow model; p=0.0122 in broad model) in our MPSS families. In TSLS families, significant linkage evidence with D1S251 (p=0.0287 in narrow model; p=0.0283 in broad model) were observed. Borderline significant linkage was observed in D8S1447 (p=0.06) and D22S1177 (p=0.056) in the TSLS sample. (Table 4). Single point analysis using GeneHunter 2.1 didn't reveal any significant result with these markers either in MPSS or TSLS sample. (Table 5). Multipoint analysis didn't reveal significant result in MPSS family, but reveal significant linkage with D15S976 (p=0.04 in narrow model, p=0.03 in broad model) in TSLS sample. (Table 6). The fine-mapping study revealed suggestive linkage evidence to marker D15S976 on chromosome 15q14 from the multipoint analysis in our TSLS family sample. In current stage, we failed to show linkage evidence to other 4 linked regions (1q42, 6p24, 8p21, 22q12), previously reported in Taiwanese families, in the large family sample. However, the linkage evidence to chromosome 15q14 may guide further fine-mapping work.

Since the phenotype definition in the MPSS and TSLS sample is the same (DSM-IV schizophrenia) despite different recruitment period and procedure, we will pool the two family set to perform further linkage analysis. Increase heterogeneity in the large sample may contribute the failure to replicate the previous linkage results. Therefore, we will try to use the CPT performance as an endophenotype to stratify the large sample to see if different type of sub sample linked to different chromosome regions.

Searching for polymorphism in the vulnerability candidate gene located in this fine mapping study

We have located 2 candidate vulnerability genes in 1q42.1 region: (1) GNPAT gene and (2) DISC1 gene. We have identified one C→T SNP polymorphism site in DISC1 near promoter region (TATA box).

We found that this C→T polymorphism site is binding to IL-6 transcription factor. We hypothesized that this C→T SNP polymorphism may play a role in the pathophysiological process of a proportion of schizophrenia, if not all of the schizophrenic population, We also found that the C/C genotype has worse impairment in CPT, which is found to be a genetic trait marker of schizophrenia. We'll continue to study the functional expression and the possible clinical significance of this SNP of the DISC1 gene.

Regarding the GNPAT gene having 16 exons, we have found 3 haplotype-specific risk SNPs in exon 1, exon7 and exon11. These polymorphism are T→C, A→G and A→A, respectively. Functional and clinical pathological studies are going on now to explore the possible biological significance.

Phenotype refinement according to the clinical phenotype and neuropsychological endophenotype of CPT and WCST data

We have initially analyzed the potential variable of age of onset, negative symptom, positive symptoms, d' and lß of CPT indicators, and 9 parameters of WCST, using the QTDT of pedigree tests of transmission disequilibrium.

In this analysis we used 7 risk SNP markers. These 7 markers were S482, S485, S475, S488, S 489, S517, and S518 with distance (bp) of 8989,13648,6701,3154,470229,14268 respectively. The family number of study was 102, and study individuals of 478 (204 founders, 274 non founders). The family size was 4-7, and generation was of 2. The estimated heritability (h^2) of possibly significant variables of age of onset (P=0.1029), negative symptom (p=0.0198), adjusted d' Z value (P=8e-07) of undegraded CPT, adjusted d'Z value (p= 2e-07) of degraded CPT and

adjusted lß Z value (p=0.0019)of degraded CPT were0.220, 0.357, 0.381, 0.751, and 0.474, respectively.

Across these 7 markers , the mean additive genetic factors (σa) of these variables of age of onset, negative sympton , d'Z of undegraded CPT, d'Z of degraded and lβZ of degraded CPT were 0.219 (P=0.0941), 0.205(p=0.0891), 0.525(P=2e-05), 0.626(P=9e-07)and 0.305(p=0.0162), respectively. Apparently, these significant variables could be used as the indicators to sub-classify the study subjects or families for further association and/ or linkage analysis. We are now using these variables to refine our 301 families for further analyses on SNP genotyping data.

Ten candidate genes association study (Work No.8)

The 10 potential candidate genes illustrated in literature are DTNBP1(6p22.3), G72(13q33.2), DAAO (12q), NRG1(8P21), RGS4(1q23.3), PPP3CC(8P21.3), TNFA(6P21), NOTCH4(6p21), MRDS1(6P24.3), and CHRNA7(15q13.2). Those SNPs located in the exons, promotor region or exon-intron junction region were picked up for fine mapping study. A total of 141 SNPs were selected for fine mapping study. Through the first stage of SNP validation with the criteria of minor allele frequency above 0.1 in a sub sample, 82 SNPs were validated and listed in Table 7. Most of the SNPs were compatible with the Hardy Weinberg equilibrium. However, the 14 SNPs from CHRNA7 were all incompatible to Hardy-Weinberg equilibrium. We are working on this issue with National Genotyping Center. Therefore, the genotyping data of CHRNA7 will not be presented here. Considering the budget limitation, a total of 218 families, 86 from MPSS sample and 132 from TSLS sample, were included into the study.

The result were listed as following:

1. RGS4

   Single point analysis revealed significant association with the SNP 6554 (p=0.004~0.008) and SNP 6557 (p=0.04) in both TSLS and MPSS families using Transmit v2.5.4. (Table 8). Haplotype association analysis revealed significant transmission distortion of haplotype 2.1.2.1 composed of SNP 6554, 6557, 6568, 6556 (p=0.048) in TSLS families (Table 9).
2. MRDS1

   Single point analysis revealed significant association with the SNP 6623 (p=0.0365), SNP 6598 (p=0.008~0.0232) and SNP 6595 (p=0.02) in MPSS families and with SNP 6594 (p=0.02) in TSLS families using Transmit v2.5.4. (Table 8). Haplotype association analysis revealed significant transmission distortion of haplotype 2.2.2.2.2 composed of SNP 6624, 6596, 6597, 6598, 6595 (p=0.0264~0.0442) in MPSS sample (Table 10).
3. DTNBP1

   Single point analysis revealed significant association with the SNP 6496 (p=0.0004~0.0008), SNP 6497 (p=0.0078~0.0197), SNP 6498 (p=0.0031~0.0066), and SNP 6495 (p=0.0046-0.0123) in MPSS families and with SNP 6498 (p=0.005) in TSLS families using Transmit v2.5.4. (Table 8). Haplotype association analysis revealed significant transmission distortion of haplotype 1.1.1 (p=0.01) and of haplotype 1.1.2 (p=0.0002~0.0003) composed of SNP 6504, 6494, 6496 in MPSS sample and significant transmission distortion of haplotype 2.1.2.2 (p=0.0062) composed of SNP 6497, 6513, 6498, 6495 in TSLS sample (Table 11).
4. TNF-alpha

   Single point analysis didn't reveal significant association with the two SNPs selected from this gene in both MPSS families and TSLS families using Transmit v2.5.4. (Table 8). Haplotype association analysis revealed significant transmission distortion of haplotype 1.1

(p=0.04) composed of SNP 6563, 6584 in MPSS sample (Table 12).

5. NOTCH4

   Single point analysis didn't reveal significant association with the SNPs in MPSS families but revealed significant association with SNP 6581 (p=0.0064~0.0082) and SNP 6575 (P=0.0477) in TSLS families using Transmit v2.5.4. (Table 8). Haplotype association analysis revealed significant transmission distortion of haplotype 2.1.2 (p=0.0393) composed of SNP 6581, 6575, 6582, in TSLS sample (Table 13).

6. PPP3CC

   Single point analysis didn't reveal significant association with the SNPs in MPSS families but revealed significant association with SNP 6577 (p=0.003) in TSLS families using Transmit v2.5.4. (Table 8). Haplotype association analysis revealed significant transmission distortion of haplotype 2.1.1.2.1 (p<0.0001) composed of SNP 6555, 6560, 6569, 6561, 6562 in MPSS sample (Table 14). But the frequency of the haplotype 2.1.1.2.1 is very low (0.0016).

7. NRG1

   Single point analysis didn't reveal significant association with the SNPs in MPSS families but revealed significant association with with SNP 6539 (p=0.02) in TSLS families using Transmit v2.5.4. (Table 8). Haplotype association analysis revealed significant transmission distortion of haplotype 2.2.1 (p=0.0337) composed of SNP 6524, 6529, 6530 in MPSS sample (Table 15).

8. DAAO

   Single point analysis didn't reveal significant association with the SNPs in both MPSS and TSLS families using Transmit v2.5.4. (Table 8). Haplotype association analysis revealed significant transmission distortion of haplotype 1.2.1 (p=0.02), of haplotype 2.1.2 (p=0.003) and of haplotype 2.2.2 (p=0.004) composed of SNP 6521, 6522, 6519 in TSLS sample (Table 16).

9. G72

   Single point analysis revealed significant association with the SNP 6511 (p=0.0057~0.01), SNP 6517 (p=0.0112) in MPSS families but didn't reveal significant association result in TSLS families using Transmit v2.5.4. (Table 8). Haplotype association analysis didn't reveal significant association in both MPSS and TSLS sample (Table 17).

In conclusion, we found significant association between schizophrenia and the candidate genes of RGS4, MRDS1, DTNBP1, NOTCH4, NRG1 either through single point or haplotype association analysis. We will pool the MPSS and TSLS family sample for further analysis. In order to refine the phenotype, we will integrate the clinical and endophenotype data as CPT into further analysis. We will compare our results with the literature to see whether the associated region and the direction of association is consistent with the study of other ethnic sample.

22q12 Regions of SNP Selection Strategies and Validation (Work no. 9)

The D22S278 marker region of SNP selection was combining the consideration of candidate schizophrenia marker locus and the candidate genes in this area. A total of 2 Mb region covering

the upstream and downstream 1 Mb of marker D22S278 was searched according to the following strategies; first, the chromosomal location of the marker, second, the genes (in the order of exon, 5'-untranslated region, 3'-untranslated region, and intron) and the promoter region of each gene. The center locus of marker D22S278 is located in chromosome position of 34678466 bp of chromosome 22q from NCBI UniSTS website (http://www.ncbi.nlm.nih.gov/genome/sts/sts.cgi?uid=37090). The upstream chromosome position (33678466 bp) and the downstream chromosome position (35678466 bp) of marker D22S278 were filled into the position of UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgTracks) on Human (Fig.1). The 2 Mb region covers twenty-three genes, HMG2L1, TOM1, HMOX1, MCM5, RASD2, MB, APOL6, APOL5, RBM9, APOL3, APOL4, APOL2, APOL1, MYH9, TXN2, EIF3S7, CACNG2, RABL4, PVALB, NCF4, CSF2RB, TST, and MPST. The name of each gene or promoter region was linked to GeneCards website (http://bioinfo.weizmann.ac.il/cards/index.html), which leads to the recognition of whether a gene expressed in brain. The promoter and the exon SNPs of each gene were further selected from Biochip website (http://snpper.chip.org/bio/snpper-enter-gene).

There were 97 SNPs selected and validated from the D22S278 marker region on 94 independent individuals. The average distance between two SNPs is bps. There were 35 SNPs with allele frequencies less than 10%. Therefore, there should have only 62 SNPs qualified for further large samples (around 250 families) of genotyping.

Neuregulin 1 as a Candidate Gene Influencing Schizotypal Personality Features or Sustained Attention in Adolescents (Work No.10)

Please see attached draft, 'Neuregulin 1 as a Candidate Gene Influencing Schizotypal Personality Features or Sustained Attention in Adolescents'

Fig.1 The LD of SNP 482, 485, 479, 488, 489 in the GNPAT gene

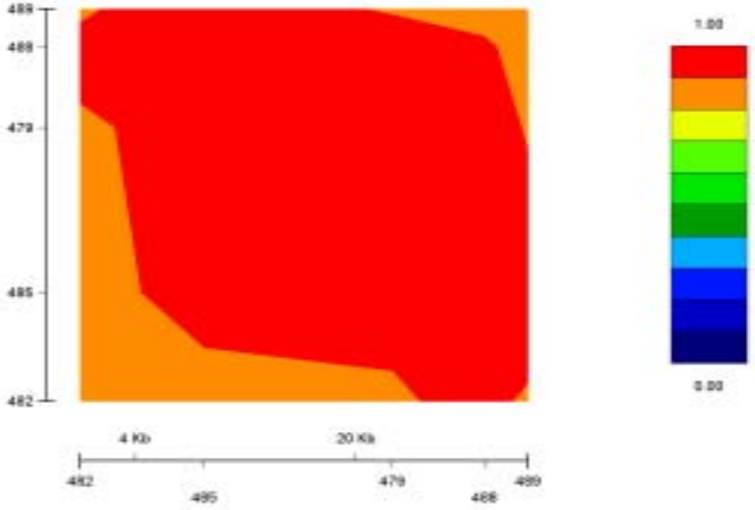

Table 1. Single point analysis of the 5 SNPs of GNPAT using Transmit v2.4.5 and FBAT v1.4.1

(a) Transmit version 2.5.4 (10000 bootstrap tests)

SNP	Narrow Model			Broad Model		
	N	Chi-square	P-value	N	Chi-square	P-value
482	194	0.9397	0.9267	194	1.1555	0.2873
485	193	0.0030	0.9615	193	0.0034	0.9583
479	191	0.0005	0.9797	191	0.0187	0.8919
488	196	0.1451	0.7221	196	0.2452	0.6240
489	199	0.1550	0.7113	199	0.2223	0.6204

N: Number of families with transmissions to affected offspring

(b) FBAT version 1.4.1 (empirical variance)

SNP	Allele	Narrow Model			Broad Model		
		N	Z	P-value	N	Z	P-value
482	1	71	-0.807	0.4194	70	-0.854	0.3928
485	1	74	0.070	0.9445	73	0.023	0.9815
479	1	72	0.048	0.9617	71	0.000	1.0000
488	1	73	-0.092	0.9270	72	-0.138	0.8905
489	1	70	0.250	0.8025	71	0.175	0.8614

N: Number of informative families

Table 2. Haplotype association analysis of 5 SNPs of GNPAT gene using Transmit v2.5.4 and FBAT v1.4.1

(a) Transmit version 2.5.4 (10000 bootstrap tests)

Haplotype	Frequency	Narrow Model (N:195)				Broad Model (N:195)			
		obs	Exp.	Chi-square	P-value	obs	Exp.	Chi-square	P-value
1.1.1.1.1	0.5891	466.7	463.90	0.1064	0.7540	466.7	464.54	0.0635	0.8059
2.2.2.2.2	0.3779	302.6	297.85	0.3041	0.5920	306.6	300.70	0.4667	0.4971
Global test				6.3887	0.0245			7.1157	0.0162

Degree of freedom Global test is 2

(b) Haplotype FBAT version 1.4.1 (additive model; empirical variance)

Haplotype	Frequency	Narrow Model			Broad Model		
		N	Z	P-value	N	Z	P-value
11111	0.584	52	1.067	0.2858	52	0.994	0.3202
22222	0.370	52	-0.319	0.7497	52	-0.216	0.8292
Global test			4.059	0.1314		4.164	0.1247

N: Number of informative families

Table 3. Descriptive data of 23 STRP markers used in the linkage analysis

Table I. MPSS data

	STRP	Allele no	Fam. no	Indiv. no	Founder missing		Nonfounder missing	Hetero-zygosity	HW test	
					DNA	All			Chi-s.	Exact
1	D1S2847	8	89	326	0.0935	0.4728	0.0615	0.5670	**<.0001**	**0.0266**
2	D1S2833	11	88	327	0.0561	0.4511	0.0738	0.7624	0.5085	0.1997
3	D1S1656	10	89	332	0.0654	0.4565	0.0492	0.7900	0.4980	0.1246
4	D1S251	11	89	332	0.0748	0.4620	0.0451	0.7677	0.9565	0.4976
5	D1S2709	4	83	306	0.1869	0.5272	0.1025	0.6322	0.6963	0.6678
6	D1S459	7	89	332	0.0654	0.4565	0.0492	0.7400	0.2596	0.2882
7	D6S277	11	84	280	0.1869	0.5272	0.2090	0.8506	0.9858	0.6767
8	D6S940	7	90	333	0.0654	0.4565	0.0451	0.7100	0.7449	0.7081
9	D8S1223	9	83	301	0.1682	0.5163	0.1311	0.6854	0.1218	0.0801
10	D8S1222	11	90	320	0.1121	0.4837	0.0779	0.7895	**<.0001**	**0.0210**
11	D8S1477	7	75	247	0.2991	0.5924	0.2951	0.7467	0.0595	0.2745
12	D8S283	9	89	334	0.0654	0.4565	0.0410	0.7000	0.8965	0.4844
13	D15S1019	12	82	297	0.1869	0.5272	0.1393	0.6782	0.0048	0.0859
14	D15S1048	9	89	321	0.0841	0.4674	0.0861	0.6837	0.7221	0.6448
15	D15S165	11	86	321	0.1215	0.4891	0.0697	0.2979	0.1257	0.3040
16	D15S976	4	84	295	0.1776	0.5217	0.1516	0.5909	0.2043	0.1468
17	D15S1031	12	82	270	0.2804	0.5815	0.2090	0.7922	0.7524	0.4854
18	D22S1162	6	89	326	0.0935	0.4728	0.0615	0.7320	0.0459	0.5667
19	D22S691	6	85	303	0.1869	0.5272	0.1148	0.8161	0.5289	0.5902
20	D22S424	3	88	327	0.0841	0.4674	0.0615	0.6429	**0.0398**	**0.0257**
21	D22S278	8	86	312	0.1682	0.5163	0.0861	0.7303	0.3739	0.3251
22	D22S683	14	71	178	0.5607	0.7446	0.4631	0.7872	0.7929	**0.0353**
23	D22S1177	9	80	287	0.2150	0.5435	0.1680	0.7738	**0.0347**	**0.0078**

data after check Mendelian

Table II. TSLS data

	STRP	Allele no	Fam. no	Indiv. no	Founder missing		Nonfounder missing	Hetero-zygosity	HW test	
					DNA	All			Chi-sq.	Exact
1	D1S2847	7	378	1488	0.0499	0.3131	0.0507	0.5760	0.9596	0.7249
2	D1S2833	13	373	1477	0.0606	0.3209	0.0557	0.8083	0.2998	**0.0205**
3	D1S1656	11	381	1520	0.0267	0.2964	0.0318	0.8333	0.5770	0.4923
4	D1S251	12	373	1464	0.0588	0.3196	0.0696	0.7841	<.0001	0.8384
5	D1S2709	6	353	1417	0.1141	0.3595	0.0855	0.6901	**0.0010**	**<.0001**
6	D1S459	11	384	1515	0.0250	0.2951	0.0378	0.7331	0.9988	0.7354
7	D6S277	15	377	1506	0.0339	0.3015	0.0417	0.7952	0.9183	0.2162
8	D6S940	9	381	1519	0.0285	0.2977	0.0318	0.7505	0.4985	0.4315
9	D8S1223	11	386	1540	0.0143	0.2874	0.0189	0.5841	0.8352	0.2971
10	D8S1222	15	372	1486	0.0446	0.3093	0.0557	0.8153	0.1569	0.0742
11	D8S1477	12	371	1478	0.0642	0.3235	0.0527	0.7886	0.9800	0.2461
12	D8S283	11	385	1538	0.0214	0.2925	0.0169	0.6576	0.9996	0.9547
13	D15S1019	14	371	1440	0.0713	0.3286	0.0865	0.6948	<.0001	0.1832
14	D15S1048	12	373	1473	0.0535	0.3157	0.0636	0.6949	0.9382	0.7394
15	D15S165	11	376	1480	0.0553	0.3170	0.0557	0.3094	0.9116	0.4790
16	D15S976	8	338	1334	0.1586	0.3918	0.1431	0.5911	0.2667	**0.0106**
17	D15S1031	16	306	1116	0.2816	0.4807	0.2913	0.7891	0.6631	0.0481
18	D22S1162	8	383	1514	0.0339	0.3015	0.0338	0.7362	0.3215	0.2935
19	D22S691	10	354	1381	0.1087	0.3557	0.1243	0.7320	0.9974	0.9054
20	D22S424	4	386	1539	0.0160	0.2887	0.0189	0.5054	0.9645	0.8701
21	D22S278	11	344	1333	0.1426	0.3802	0.1531	0.7796	**<.0001**	**0.0010**
22	D22S683	26	354	1384	0.1052	0.3531	0.1233	0.8167	0.0861	0.2266
23	D22S1177	10	365	1433	0.0677	0.3260	0.0954	0.7094	0.5217	0.7060

data after check Mendelian

Table 4-1. Two-Point Nonparametric Linkage Analysis of 23 STRP markers in 5 chromosome regions

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MPSS data		Narrow Model			Broad Model		
		N[1]	Proportion[2]	P-value	N[1]	Proportion[2]	P-value
	Marker						
1	D1S2847	80	.5006	.4918	97	.4976	.5362
2	D1S2833	83	.5120	.3655	97	.5065	4218
3	D1S1656	84	.5018	.4780	101	.5009	.4883
4	D1S251	85	.4687	.8292	101	.4685	.8468
	D1S2709	81	.4950	.5835	96	.5004	4921
6	D1S459	85	.4972	.5384	102	.4995	.5072
7	D6S277	62	.5255	.2463	76	.5302	.1749
8	D6S940	84	.5109	.3637	98	.5122	.3281
9	D8S1223	77	.5183	.2593	93	.5360	.0849
10	D8S1222	80	.5337	.1445	95	.5256	.1996
11	D8S1477	60	.5423	.1346	67	.5504	.0843
12	D8S283	87	.5538	**.0260**	103	.5380	.0633
13	D15S1019	74	.4567	.9093	89	.4668	.8720
14	D15S1048	79	.4920	.5967	95	.5049	4333
15	D15S165	81	.5087	.3522	98	.5084	3414
16	D15S976	69	.5027	.4635	83	.4984	.5239
17	D15S1031	68	.4452	.9445	77	.4618	.8853
18	D22S1162	82	.5429	.0629	97	.5457	**.0378**
19	D22S691	75	.5332	.1745	92	.5224	.2323
20	D22S424	83	.5158	.2262	99	.5111	.2889
21	D22S278	78	.5154	.3153	94	.5123	.3358
22	D22S683	37	.5835	**.0304**	43	.5890	**.0122**
23	D22S1177	75	.4984	.5200	84	.5045	.4410

[1]: Number of affected sibpair. [2]: Estimated proportion of marker alleles shared i. b. d.

Table 4-2. Two-Point Nonparametric Linkage Analysis of 23 STRP markers in 5 chromosome regions

TSLS data		Narrow Model			Broad Model		
		N[1]	Proportion[2]	P-value	N[1]	Proportion[2]	P-value
	Marker						
1	D1S2847	398	.5075	.2809	404	.5089	.2436
2	D1S2833	396	.5200	.1007	402	.5202	.0955
3	D1S1656	408	.5211	.0837	414	.5223	.0711
4	D1S251	389	.5289	**.0287**	395	.5286	**.0283**
5	D1S2709	390	.5180	.0778	394	.5188	.0676
6	D1S459	401	.5010	.4717	407	.5009	.4739
7	D6S277	403	.5113	.2291	409	.5092	.2717
8	D6S940	406	.5036	.4023	412	.5014	.4619
9	D8S1223	414	.5138	.1287	420	.5085	.2451
10	D8S1222	398	.5139	.1908	404	.5106	.2512
11	D8S1477	400	.5231	.0674	406	.5214	.0804
12	D8S283	416	.5135	.1609	422	.5113	.2022
13	D15S1019	382	.5064	.3210	388	.5057	.3390
14	D15S1048	394	.5203	.0720	400	.5196	.0771
15	D15S165	396	.4998	.5091	402	.4998	.5077
16	D15S976	350	.5143	.1539	356	.5155	.1341
17	D15S1031	289	.5017	.4626	293	.5005	.4892
18	D22S1162	404	.5002	.4935	410	.4938	.6687
19	D22S691	363	.4841	.8631	368	.4838	.8709
20	D22S424	412	.4973	.6055	418	.4978	.5841
21	D22S278	349	.5223	.0747	354	.5172	.1387
22	D22S683	361	.5154	.1351	367	.5000	.4996
23	D22S1177	375	.5181	.0854	381	.5223	.0568

Table 5-1. Two-point nonparametric analysis of 23 STRP markers in 5 chromosome regions using GeneHunter 2.1

MPSS data		Narrow Model		Broad Model	
		NPL_score	p-value	NPL_score	p-value
	Marker				
1	D1S2847	0.1130	0.4515	0.0444	0.4804
2	D1S2833	0.2162	0.4094	0.2548	0.3959
3	D1S1656	-0.0251	0.5108	0.1682	0.4304
4	D1S251	-0.6174	0.7449	-0.5904	0.7261
5	D1S2709	-0.1125	0.5476	-0.0685	0.5270
6	D1S459	-0.0836	0.5348	0.0612	0.4739
7	D6S277	0.5212	0.2892	0.6640	0.2482
8	D6S940	0.2147	0.4079	0.3165	0.3723
9	D8S1223	0.4252	0.3261	0.9800	0.1592
10	D8S1222	0.6583	0.2426	0.7442	0.2242
11	D8S1477	0.7252	0.2197	0.8847	0.1839
12	D8S283	1.3438	0.0771	1.1708	0.1171
13	D15S1019	-0.9947	0.8555	-1.0298	0.8536
14	D15S1048	-0.2055	0.5869	-0.0781	0.5309
15	D15S165	0.0440	0.4812	-0.0233	0.5087
16	D15S976	-0.0198	0.5079	-0.2269	0.5907
17	D15S1031	-1.0433	0.8665	-1.0009	0.8462
18	D22S1162	0.9891	0.1458	1.1151	0.1272
19	D22S691	0.6015	0.2598	0.3977	0.3416
20	D22S424	0.4548	0.3135	0.4423	0.3245
21	D22S278	0.2554	0.3934	0.3265	0.3684
22	D22S683	0.7746	0.2042	0.9309	0.1704
23	D22S1177	-0.0955	0.5411	-0.0015	0.4993

Table 5-2. Two-point nonparametric analysis of 23 STRP markers in 5 chromosome regions using GeneHunter 2.1

TSLS data		Narrow Model		Broad Model	
		NPL_score	p-value	NPL_score	p-value
	Marker				
1	D1S2847	0.5022	0.2960	0.5759	0.2715
2	D1S2833	1.3657	0.0797	1.3781	0.0784
3	D1S1656	1.1729	0.1126	1.1942	0.1092
4	D1S251	1.4723	0.0649	1.3932	0.0762
5	D1S2709	0.8663	0.1832	0.9623	0.1593
6	D1S459	0.0947	0.4505	0.0663	0.4622
7	D6S277	0.6446	0.2482	0.4985	0.2978
8	D6S940	0.2652	0.3833	0.1590	0.4251
9	D8S1223	0.8859	0.1777	0.5930	0.2657
10	D8S1222	0.7873	0.2048	0.6498	0.2472
11	D8S1477	1.1222	0.1224	1.0873	0.1304
12	D8S283	0.9697	0.1565	0.8728	0.1819
13	D15S1019	0.6093	0.2597	0.6020	0.2629
14	D15S1048	1.0931	0.1287	1.1060	0.1267
15	D15S165	0.0209	0.4801	0.0479	0.4694
16	D15S976	0.9455	0.1628	1.0246	0.1446
17	D15S1031	0.3373	0.3559	0.3299	0.3592
18	D22S1162	-0.2180	0.5759	-0.3366	0.6220
19	D22S691	-0.7383	0.7633	-0.8066	0.7837
20	D22S424	-0.0374	0.5035	-0.0050	0.4907
21	D22S278	0.8821	0.1789	0.9214	0.1694
22	D22S683	0.4511	0.3142	0.4031	0.3319
23	D22S1177	1.3534	0.0815	1.2814	0.0937

Table 6-1. MultiPoint Nonparametric Linkage Analysis of 23 STRP markers in 5 chromosome regions using GeneHunter 2.1

MPSS data			Narrow Model		Broad Model	
			NPL_score	p-value	NPL_score	p-value
	Marker	Position				
1	D1S2847	233.15	-0.3069	0.6283	-0.2938	0.6170
2	D1S2833	233.97	-0.2754	0.6152	-0.1110	0.5438
3	D1S1656	234.69	-0.6378	0.7510	-0.4686	0.6832
4	D1S251	235.23	-0.5809	0.7322	-0.6802	0.7556
5	D1S2709	235.37	-0.6174	0.7449	-0.8029	0.7938
6	D1S459	237.28	-0.5411	0.7183	-0.5104	0.6978
7	D6S277	20.86	0.6633	0.2393	0.8432	0.1938
8	D6S940	22.65	0.4373	0.3188	0.6618	0.2488
9	D8S1223	51.25	0.1353	0.4421	0.5924	0.2731
10	D8S1222	52.66	0.2542	0.3942	0.4905	0.3084
11	D8S1477	53.64	0.7609	0.2102	0.9109	0.1768
12	D8S283	54.23	0.7574	0.2102	0.7569	0.2207
13	D15S1019	20.59	-1.1944	0.8981	-1.1765	0.8855
14	D15S1048	21.07	-1.3008	0.9169	-1.1022	0.8696
15	D15S165	22.64	-1.2097	0.9005	-1.0078	0.8481
16	D15S976	22.65	-1.1585	0.8918	-0.9556	0.8351
17	D15S1031	24.63	-1.3997	0.9326	-1.1295	0.8758
18	D22S1162	38.55	0.1979	0.4161	-0.1013	0.5404
19	D22S691	39.06	0.0167	0.4911	-0.2838	0.6126
20	D22S424	39.85	0.2049	0.4123	-0.2248	0.5901
21	D22S278	40.73	-0.2192	0.5907	-0.3907	0.6542
22	D22S683	40.84	-0.2797	0.6154	-0.3791	0.6497
23	D22S1177	43.51	-0.6020	0.7383	-0.6207	0.7362

Table 6-2. MultiPoint Nonparametric Linkage Analysis of 23 STRP markers in 5 chromosome regions using GeneHunter 2.1

TSLS data			Narrow Model		Broad Model	
			NPL_score	p-value	NPL_score	p-value
	Marker	Position				
1	D1S2847	233.15	0.8523	0.1869	0.8199	0.1966
2	D1S2833	233.97	0.9409	0.1639	0.8903	0.1776
3	D1S1656	234.69	0.8149	0.1973	0.7504	0.2165
4	D1S251	235.23	0.9075	0.1723	0.8836	0.1793
5	D1S2709	235.37	0.5598	0.2762	0.5585	0.2773
6	D1S459	237.28	0.5430	0.2819	0.5130	0.2928
7	D6S277	20.86	0.2708	0.3811	0.1098	0.4447
8	D6S940	22.65	0.3620	0.3466	0.2286	0.3980
9	D8S1223	51.25	1.2431	0.0994	1.0281	0.1434
10	D8S1222	52.66	0.7088	0.2281	0.5710	0.2730
11	D8S1477	53.64	1.2405	0.0998	1.1485	0.1179
12	D8S283	54.23	1.1878	0.1095	1.0593	0.1366
13	D15S1019	20.59	0.6883	0.2347	0.6817	0.2374
14	D15S1048	21.07	1.1462	0.1178	1.1579	0.1162
15	D15S165	22.64	1.5729	0.0530	1.6388	0.0466
16	D15S976	22.65	1.6484	**0.0453**	1.7151	**0.0396**
17	D15S1031	24.63	0.7739	0.2090	0.7799	0.2079
18	D22S1162	38.55	-0.1577	0.5518	-0.2262	0.5790
19	D22S691	39.06	-0.6912	0.7484	-0.7577	0.7689
20	D22S424	39.85	-0.1398	0.5447	-0.1830	0.5620
21	D22S278	40.73	-0.0869	0.5235	-0.1689	0.5563
22	D22S683	40.84	0.3187	0.3629	0.1830	0.4157
23	D22S1177	43.51	0.4939	0.2990	0.3643	0.3463

Table 7-1. The descriptive data of validated 82 SNPs from 10 candidate genes

	Genes	Chromosome	Primer	SNP ID	Location	Type	MPSS data			TSLS data		
							Minor Allele Freq	HW Test		Minor Allele Freq	HW Test	
								P-value	Exact		P-value	Exact
1	RGS4	1	6554	SNP 1	159728876	G=1 A=2	0.1691	**<.0001**	**<.0001**	0.0459	**<.0001**	**<.0001**
2			6557	rs951436	159729374	A=1 C=2	0.5054	0.5831	0.6649	0.4727	0.8138	0.8753
3			6568	SNP7	159729725	G=1 A=2	0.4388	0.7250	0.8191	0.4453	0.5788	0.6486
4			6556	SNP18	159735811	A=1 G=2	0.4674	0.4121	0.5105	0.4408	0.8934	1.0000
5	MRDS1	6	6594	rs1925767	9969042	T=1 C=2	0.1613	0.1996	0.2300	0.1953	0.1411	0.1423
6			6601	rs1925773	9984452	T=1 C=2	0.3454	0.0555	0.0905	0.2835	0.6277	0.7269
7			6623	rs2327219	10017872	C=1 A=2	0.4595	**0.0002**	**0.0005**	0.4217	**<.0001**	**<.0001**
8			6624	rs201264	10044086	T=1 A=2	0.4121	0.1112	0.1738	0.4461	0.7368	0.7425
9			6596	rs201260	10056935	A=1 C=2	0.3776	0.7762	0.8187	0.3549	0.7256	0.8722
10			6597	rs201252	10073630	C=1 T=2	0.2283	0.0993	0.1157	0.2432	0.8594	0.8423
11			6598	rs1206988	10087353	T=1 C=2	0.1789	0.1073	0.1354	0.2042	0.4491	0.4832
12			6595	rs855407	10102400	G=1 A=2	0.1868	0.0955	0.1286	0.1995	0.9554	1.0000
13			6608	rs1407621	10128531	T=1 C=2	0.1823	0.5436	0.4896	0.2173	0.8433	1.0000
14			6609	rs1407625	10131336	G=1 A=2	0.1768	0.4193	0.4656	0.2120	0.9921	1.0000
15	DTNBP1	6	6492	rs742106	15587019	A=1 G=2	0.4570	0.5592	0.6667	0.4172	0.1350	0.1885
16			6504	rs3829893	15678176	G=1 A=2	0.2903	0.6468	0.7943	0.3185	0.4335	0.5736
17			6494	rs2619539	15683394	C=1 G=2	0.4066	0.3257	0.3550	0.3810	0.1229	0.1768
18			6496	rs1011313	15695971	C=1 T=2	0.2055	0.6568	0.6818	0.1845	0.7556	1.0000
19			6497	rs2619528	15712368	C=1 T=2	0.1099	0.2318	0.5945	0.0909	0.7878	1.0000
20			6513	rs2005976	15713341	C=1 T=2	0.1250	0.1870	0.3476	0.0625	0.4197	1.0000
21			6498	rs760761	15713671	G=1 A=2	0.1111	0.2281	0.5814	0.0559	0.1771	0.2447
22			6495	rs2619522	15716188	A=1 C=2	0.1087	0.2354	0.5891	0.0909	0.7878	1.0000
23			6500	rs1018381	15719609	G=1 A=2	0.1087	0.2354	0.5936	0.0592	0.4477	1.0000
24			6501	rs909706	15723410	T=1 C=2	0.4000	0.5762	0.6357	0.3876	0.0625	0.0921
25			6499	rs441539	15827177	T=1 C=2	0.1739	0.2760	0.2536	0.2012	0.0481	0.0727
26	TNFa	6	6563	rs2009658	31597109	C=1 G=2	0.1173	0.2852	0.5915	0.1528	0.1704	0.2603
27			6584	rs2256974	31614256	C=1 A=2	0.3889	0.6334	0.8108	0.4785	**0.0055**	**0.0062**

Table 7-2. The descriptive data of validated 82 SNPs from 10 candidate genes

	Genes	Chromosome	Primer	SNP ID	Location	Type	MPSS data			TSLS data		
							Minor Allele Freq	HW Test		Minor Allele Freq	HW Test	
								P-value	Exact		P-value	Exact
28	NOTCH4	6	6581	rs204993	32181580	A=1 G=2	0.4141	0.4337	0.5219	0.3750	0.3745	0.4248
29			6575	rs2071285	32206436	A=1 T=2	0.1868	0.5668	0.7254	0.1716	0.7303	1.0000
30			6582	rs3131290	32209207	G=1 A=2	0.1173	0.0158	0.0434	0.1425	0.8424	0.7648
31			6583	rs415929	32215059	T=1 C=2	0.1753	0.0520	0.0676	0.1632	0.5044	0.7721
32			6587	rs915895	32216244	C=1 T=2	0.5300	0.7140	0.8286	0.4923	**0.0020**	**0.0015**
33			6592	rs915894	32216417	T=1 G=2	0.4176	0.3647	0.3670	0.4673	**0.0236**	**0.0272**
34			6593	rs397081	32218641	T=1 C=2	0.1739	0.2354	0.4431	0.1627	0.1873	0.2470
35	PPP3CC	8	6577	rs2272080	22119295	T=1 G=2	0.1059	0.3765	1.0000	0.2018	0.5391	0.6039
36			6555	rs2461491	22181821	G=1 A=2	0.3778	0.8042	1.0000	0.4371	0.4631	0.5048
37			6560	rs2469758	22183601	T=1 C=2	0.3737	0.4883	0.6531	0.4308	0.5059	0.5484
38			6569	rs2461489	22190822	A=1 G=2	0.4286	0.4958	0.6643	0.4895	0.4582	0.4587
39			6561	rs2469770	22200425	A=1 G=2	0.3814	0.7225	0.8189	0.4356	0.7576	0.7616
40			6562	rs2449340	22210469	G=1 T=2	0.3150	0.1824	0.2277	0.3135	0.8689	1.0000
41			6559	rs1482337	22216078	A=1 G=2	0.4000	0.7108	0.8178	0.4921	0.8255	0.8861
42			6566	rs2252471	22225797	C=1 G=2	0.3788	0.3930	0.4893	0.4450	0.9404	1.0000
43	NRG1	8	6524	SNP8NRG221	31330342	C=1 T=2	0.4540	0.0951	0.1088	0.4042	0.7744	0.8654
44			6529	rs4452759	31343650	C=1 T=2	0.4271	0.2182	0.2615	0.3763	0.8810	0.8798
45			6530	rs4476964	31348868	C=1 T=2	0.3316	0.0867	0.0866	0.3177	0.3486	0.3966
46			6526	SNP8NRG243	31351791	T=1 C=2	0.4261	**0.0130**	**0.0196**	0.3935	0.8534	1.0000
47			6544	rs1481736	31836842	G=1 A=2	0.5114	0.6516	0.6499	0.4556	0.0471	0.0582
48			6539	rs4733331	32134140	T=1 A=2	0.1927	0.3235	0.3136	0.1718	0.4693	0.6085
49			6543	rs4489283	32255862	C=1 T=2	0.2363	0.5258	0.5490	0.2440	0.3903	0.5145
50			6523	rs3924999	32309558	A=1 G=2	0.2021	0.0641	0.1078	0.2367	0.8817	0.8227
51			6549	rs3924999	32309558	A=1 G=2	0.2050	0.0452	0.0594	0.2371	0.7125	0.6810
52			6550	rs2439325	32373968	A=1 G=2	0.2371	0.3958	0.3721	0.2333	0.2094	0.3088
53			6540	rs2954041	32378826	G=1 T=2	0.3696	0.2528	0.3604	0.3667	0.7576	0.7493
54			6547	rs3735775	32441623	G=1 A=2	0.3594	0.2419	0.3347	0.3910	0.8708	0.8814
55			6552	rs3735776	32441634	C=1 A=2	0.3636	0.1444	0.1723	0.3846	0.8935	0.8778
56	DAAO	12	6521	rs2111902	109211693	G=1 T=2	0.4550	0.7140	0.8335	0.4922	0.8769	1.0000
57			6522	rs3918346	109214830	A=1 G=2	0.3778	**0.0109**	**0.0215**	0.3883	**<.0001**	**<.0001**
58			6519	rs3741775	109216549	A=1 C=2	0.3878	0.9436	1.0000	0.3995	0.9841	1.0000

Table 7-3. The descriptive data of validated 82 SNPs from 10 candidate genes

	Genes	Chromosome	Primer	SNP ID	Location	Type	MPSS data			TSLS data		
							Minor Allele Freq	HW Test		Minor Allele Freq	HW Test	
								P-value	Exact		P-value	Exact
59	G72	13	6506	rs3916965	104939998	T=1 C=2	0.3596	0.3071	0.3535	0.3735	0.6243	0.7237
60			6503	rs3916966	104947533	C=1 A=2	0.3830	0.2462	0.3657	0.3817	0.6336	0.7345
61			6510	rs3916967	104953986	C=1 T=2	0.3626	0.2193	0.2522	0.3817	0.6336	0.7236
62			6507	rs2391191	104956084	A=1 G=2	0.3626	0.2193	0.2535	0.3817	0.6336	0.7377
63			6511	rs3916968	104957057	C=1 T=2	0.1966	0.7061	0.7298	0.1607	0.6146	0.5584
64			6516	rs3916970	104976300	T=1 C=2	0.3989	0.1077	0.1754	0.3680	**0.0275**	**0.0387**
65			6517	rs3916971	104978873	T=1 C=2	0.4286	0.4245	0.5220	0.4275	0.2062	0.2297
66			6518	rs947267	104998639	T=1 G=2	0.4316	0.7380	0.8270	0.4948	0.0527	0.0504
67			6515	rs778293	105005837	T=1 C=2	0.3454	0.9829	1.0000	0.3151	0.8023	0.8658
68			6520	rs3918342	105022387	T=1 C=2	0.4898	0.0881	0.0894	0.4510	0.2032	0.2254
69	CHRNA7	15	6610	rs2924987	28229586	G=1 A=2	0.3163	**<.0001**	**<.0001**	0.3212	**<.0001**	**<.0001**
70			6599	rs286103	28233774	G=1 A=2	0.5000	**<.0001**	**<.0001**	0.4949	**<.0001**	**<.0001**
71			6605	rs2253967	28234754	G=1 A=2	0.2500	**0.0010**	**0.0006**	0.2605	**<.0001**	**<.0001**
72			6606	rs1042724	28234820	G=1 A=2	0.3093	**<.0001**	**<.0001**	0.3316	**<.0001**	**<.0001**
73			6607	rs2564605	28239618	G=1 A=2	0.3053	**<.0001**	**<.0001**	0.3226	**<.0001**	**<.0001**
74			6612	rs2564606	28239668	C=1 G=2	0.3032	**<.0001**	**<.0001**	0.3221	**<.0001**	**<.0001**
75			6618	rs384470	28239762	C=1 T=2	0.3167	**<.0001**	**<.0001**	0.3244	**<.0001**	**<.0001**
76			6620	rs2651439	28243491	C=1 T=2	0.3407	**<.0001**	**<.0001**	0.3225	**<.0001**	**<.0001**
77			6621	rs2611608	28244413	G=1 A=2	0.3495	**<.0001**	**<.0001**	0.3225	**<.0001**	**<.0001**
78			6615	rs1042722	28244537	T=1 C=2	0.4889	**<.0001**	**<.0001**	0.5000	**<.0001**	**<.0001**
79			6626	rs386715	28244573	G=1 A=2	0.4731	**<.0001**	**<.0001**	0.5000	**<.0001**	**<.0001**
80			6627	rs408234	28245254	T=1 C=2	0.4457	**<.0001**	**<.0001**	0.4671	**<.0001**	**<.0001**
81			6628	rs2653242	28266172	C=1 G=2	0.4947	**<.0001**	**<.0001**	0.4948	**<.0001**	**<.0001**
82			6629	rs3901746	28275516	A=1 G=2	0.5000	**<.0001**	**<.0001**	0.5000	**<.0001**	**<.0001**

Table 8-1. Single Point Association Analysis of 10 candidate genes using Transmit v2.5.4

	Candidate genes	Primer	MPSS data						TSLS data					
			Narrow Model			Broad Model			Narrow Model			Broad Model		
			N	Chi	P	N	Chi	P	N	Chi	P	N	Chi	P
1	RGS4	6554	64	7.31	**0.0068**	65	6.90	**0.0086**	84	8.31	**0.0039**	84	8.31	**0.0039**
2		6557	79	3.47	0.0627	81	4.13	**0.0421**	115	0.60	0.4403	115	0.50	0.4805
3		6568	84	1.48	0.2245	86	1.77	0.1828	132	0.09	0.7579	132	0.10	0.7498
4		6556	79	1.00	0.3182	81	1.20	0.2732	115	1.01	0.3161	115	0.89	0.3463
5	MRDS1	6594	80	0.02	0.8803	82	0.15	0.7014	115	5.16	**0.0231**	115	4.84	**0.0279**
6		6601	84	3.17	0.0750	86	3.37	0.0663	132	0.08	0.7819	132	0.05	0.8216
7		6623	69	4.37	**0.0365**	71	1.97	0.1606	86	2.57	0.1088	86	2.57	0.1088
8		6624	80	0.07	0.7944	82	0.00	0.9583	115	0.02	0.8761	115	0.06	0.8040
9		6596	84	0.59	0.4410	86	0.82	0.3652	132	0.02	0.8896	132	0.05	0.8205
10		6597	83	0.91	0.3413	85	1.34	0.2477	132	0.32	0.5687	132	0.22	0.6417
11		6598	81	5.15	**0.0232**	83	7.02	**0.0080**	132	1.90	0.1686	132	1.60	0.2064
12		6595	81	5.27	**0.0217**	84	5.41	**0.0200**	132	1.12	0.2898	132	0.91	0.3395
13		6608	82	0.68	0.4095	85	0.53	0.4674	132	0.41	0.5210	132	0.42	0.5175
14		6609	83	0.46	0.4965	86	0.37	0.5429	132	0.39	0.5306	132	0.40	0.5272
15	DTNBP1	6492	80	0.00	0.9886	82	0.03	0.8596	115	0.50	0.4815	115	0.56	0.4542
16		6504	80	1.53	0.2157	82	1.07	0.3019	115	0.00	0.9438	115	0.00	0.9516
17		6494	80	1.13	0.2878	82	0.73	0.3933	115	0.24	0.6209	115	0.23	0.6285
18		6496	70	11.27	**0.0008**	72	12.66	**0.0004**	129	1.55	0.2125	129	1.40	0.2370
19		6497	80	5.44	**0.0197**	82	7.08	**0.0078**	128	2.46	0.1169	128	2.13	0.1441
20		6513	80	0.03	0.8699	82	0.04	0.8349	115	0.26	0.6079	115	0.40	0.5260
21		6498	79	7.39	**0.0066**	82	8.78	**0.0031**	120	7.89	**0.0050**	120	7.89	**0.0050**
22		6495	81	6.27	**0.0123**	83	8.01	**0.0046**	128	2.46	0.1169	128	2.13	0.1441
23		6500	80	0.02	0.8912	82	0.01	0.9310	115	1.03	0.3099	115	1.03	0.3110
24		6501	80	0.83	0.3621	82	0.40	0.5265	115	0.08	0.7839	115	0.12	0.7284
25		6499	80	0.27	0.6052	82	0.41	0.5231	115	0.52	0.4708	115	0.51	0.4743

Table 8-2. Single Point Association Analysis of 10 candidate genes using Transmit v2.5.4

	Candidate genes	Primer	MPSS data						TSLS data					
			Narrow Model			Broad Model			Narrow Model			Broad Model		
			N	Chi	P	N	Chi	P	N	Chi	P	N	Chi	P
26	TNF-alpha	6563	84	1.64	0.2003	86	0.60	0.4395	132	0.01	0.9293	132	0.00	0.9984
27		6584	81	0.96	0.3276	83	1.84	0.1752	129	0.85	0.3573	129	1.10	0.2938
28	NOTCH4	6581	84	0.52	0.4728	86	0.11	0.7369	132	6.99	**0.0082**	132	7.42	**0.0064**
29		6575	80	0.06	0.8129	82	0.01	0.9123	115	3.92	**0.0477**	115	3.47	0.0626
30		6582	84	3.12	0.0774	86	1.93	0.1653	132	0.00	0.9773	132	0.00	0.9842
31		6583	83	0.07	0.7973	85	0.04	0.8463	132	1.02	0.3115	132	0.87	0.3498
32		6587	84	0.11	0.7356	86	0.17	0.6764	132	0.26	0.6097	132	0.18	0.6672
33		6592	80	0.19	0.6605	82	0.10	0.7495	115	0.54	0.4643	115	0.38	0.5371
34		6593	79	0.02	0.8820	82	0.05	0.8172	115	2.01	0.1560	115	2.00	0.1573
35	PPP3CC	6577	78	0.02	0.9006	80	0.11	0.7390	115	8.58	**0.0034**	115	8.61	**0.0033**
36		6555	79	1.39	0.2387	81	1.12	0.2897	115	0.22	0.6425	115	0.20	0.6572
37		6560	84	0.71	0.4010	86	0.55	0.4580	132	0.06	0.8137	132	0.05	0.8286
38		6569	84	1.41	0.2355	86	1.02	0.3130	132	0.20	0.6524	132	0.22	0.6422
39		6561	84	1.02	0.3129	86	0.82	0.3647	132	0.00	0.9965	132	0.00	0.9818
40		6562	84	1.20	0.2732	86	1.19	0.2762	131	0.65	0.4208	131	0.69	0.4064
41		6559	82	0.98	0.3218	84	0.83	0.3615	128	0.08	0.7813	128	0.14	0.7034
42		6566	84	0.83	0.3622	86	0.55	0.4575	132	0.00	0.9811	132	0.02	0.8937
43	NRG1	6524	79	2.97	0.0846	81	1.62	0.2038	115	0.00	0.9948	115	0.00	0.9582
44		6529	83	0.72	0.3965	85	0.20	0.6512	132	0.01	0.9416	132	0.00	0.9986
45		6530	82	0.29	0.5886	84	1.10	0.2936	132	1.35	0.2451	132	1.16	0.2814
46		6526	78	0.09	0.7683	80	0.48	0.4889	115	0.18	0.6756	115	0.22	0.6408
47		6544	79	0.02	0.8748	81	0.31	0.5759	115	0.30	0.5865	115	0.37	0.5426
48		6539	84	0.67	0.4119	86	0.33	0.5663	132	5.08	**0.0242**	132	5.06	**0.0245**
49		6543	80	0.52	0.4714	82	1.27	0.2601	115	0.03	0.8665	115	0.03	0.8720
50		6523	80	0.04	0.8362	82	0.01	0.9218	115	1.64	0.2006	115	1.65	0.1985
51		6549	83	0.28	0.5977	85	0.18	0.6713	132	0.29	0.5891	132	0.30	0.5855
52		6550	84	1.70	0.1928	86	1.41	0.2355	132	0.13	0.7222	132	0.08	0.7720
53		6540	84	0.04	0.8426	86	0.15	0.7027	132	2.88	0.0897	132	2.79	0.0948
54		6547	83	2.44	0.1186	85	2.46	0.1167	128	1.09	0.2955	128	1.24	0.2654
55		6552	84	2.49	0.1143	86	3.02	0.0823	132	1.91	0.1670	132	2.10	0.1475

Table 8-3. Single Point Association Analysis of 10 candidate genes using Transmit v2.5.4

	Candidate genes	Primer	MPSS data						TSLS data					
			Narrow Model			Broad Model			Narrow Model			Broad Model		
			N	Chi	P	N	Chi	P	N	Chi	P	N	Chi	P
56	DAAO	6521	84	0.34	0.5617	86	0.03	0.8701	132	0.20	0.6528	132	0.19	0.6617
57		6522	81	0.25	0.6158	83	0.88	0.3493	132	1.40	0.2369	132	1.43	0.2311
58		6519	83	0.05	0.8300	85	0.00	0.9441	132	0.79	0.3754	132	0.77	0.3811
59	G72	6506	80	1.53	0.2169	81	0.98	0.3231	115	0.20	0.6524	115	0.16	0.6918
60		6503	80	1.56	0.2110	82	1.12	0.2893	115	0.14	0.7124	115	0.10	0.7502
61		6510	78	1.80	0.1803	80	1.53	0.2167	115	0.10	0.7461	115	0.07	0.7843
62		6507	78	1.96	0.1616	80	1.49	0.2225	115	0.08	0.7751	115	0.06	0.8136
63		6511	79	6.63	**0.0100**	81	7.65	**0.0057**	115	0.93	0.3344	115	0.94	0.3319
64		6516	80	1.56	0.2117	82	1.36	0.2436	131	0.25	0.6190	131	0.20	0.6508
65		6517	84	6.43	**0.0112**	86	2.49	0.1146	132	0.48	0.4901	132	0.42	0.5160
66		6518	83	2.46	0.1167	85	1.99	0.1583	132	0.15	0.7026	132	0.17	0.6763
67		6515	84	2.82	0.0931	86	2.90	0.0888	132	0.86	0.3547	132	0.84	0.3591
68		6520	84	2.12	0.1457	86	2.20	0.1381	132	0.63	0.4256	132	0.71	0.4007

Table 9. Haplotype Association Analysis of SNPs 6554,6557,6568,6556 in RGS4 gene using Transmit v2.5.4

Haplotype	MPSS data					Haplotype	TSLS data				
	HF	Narrow Model(N=78)		Broad Model (N=80)			HF	Narrow Model(N=131)		Broad Model(N=131)	
		Chi	P	Chi	P			Chi	P	Chi	P
1.1.1.1	0.0456	0.00	0.9962	0.00	0.9746	1.1.1.1	0.0623	0.07	0.7849	0.18	0.6693
1.2.1.1	0.0605	3.15	0.0762	1.75	0.1854	1.2.1.1	0.0575	1.84	0.1749	1.84	0.1749
1.1.2.1	0.2794	1.44	0.2298	1.42	0.2342	1.1.2.1	0.3091	1.14	0.2854	1.14	0.2854
2.1.2.1	0.1506	1.21	0.2710	0.95	0.3286	2.1.2.1	0.1178	3.90	**0.0484+**	3.90	**0.0484+**
1.2.1.2	0.4463	0.03	0.8552	0.12	0.7308	1.2.1.2	0.4378	1.01	0.3156	0.89	0.3447
2.1.2.2	0.0175	0.65	0.4203	0.32	0.5694	1.1.2.2	0.0156	0.30	0.5821	0.30	0.5821

HF: Haplotype Frequency +: risk -: protect

Table 10. Haplotype Association Analysis of SNPs 6624,6596,6597,6598,6595 in MRDS1 gene using Transmit v2.5.4

Haplotype	MPSS data					Haplotype	TSLS data				
	HF	Narrow Model(N=84)		Broad Model (N=86)			HF	Narrow Model(N=132)		Broad Model(N=132)	
		Chi	P	Chi	P			Chi	P	Chi	P
1.1.1.1.1	0.5423	0.13	0.7222	0.25	0.6143	1.1.1.1.1	0.5111	0.15	0.7019	0.22	0.6396
2.1.1.1.1	0.0993	0.38	0.5402	0.20	0.6576	2.1.1.1.1	0.1126	0.52	0.4728	0.52	0.4701
1.2.1.1.1	0.0033	1.00	0.3180	1.00	0.3179	1.2.1.1.1	0.0068	3.08	0.0792	3.08	0.0792
2.2.1.1.1	0.1256	0.10	0.7565	0.13	0.7170	2.2.1.1.1	0.1157	0.10	0.7482	0.11	0.7448
1.1.2.1.1	0.0052	0.58	0.4446	0.58	0.4461	1.1.2.1.1	0.0040	0.94	0.3325	0.94	0.3327
2.1.2.1.1	0.0032	1.00	0.3165	1.00	0.3165	2.2.2.1.1	0.0401	1.24	0.2656	1.24	0.2647
2.2.2.1.1	0.0493	2.55	0.1105	1.56	0.2122	2.2.2.2.1	0.0059	0.26	0.6086	0.26	0.6091
2.2.2.2.1	0.0034	1.00	0.3166	1.00	0.3166	1.1.1.2.2	0.0020	1.00	0.3176	1.00	0.3176
1.1.1.1.2	0.0032	1.01	0.3159	0.99	0.3196	2.1.1.2.2	0.0020	0.99	0.3195	0.99	0.3195
1.2.1.1.2	0.0094	1.47	0.2252	1.49	0.2223	1.1.2.2.2	0.0080	0.22	0.6403	0.22	0.6409
2.1.1.2.2	0.0031	1.01	0.3160	1.01	0.3157	1.2.2.2.2	0.0025	0.14	0.7085	0.15	0.7029
1.1.2.2.2	0.0047	0.86	0.3543	0.86	0.3527	2.2.2.2.2	0.1893	0.85	0.3555	0.68	0.4098
2.2.2.2.2	0.1480	4.05	**0.0442-**	4.93	**0.0264-**						

HF: Haplotype Frequency +: risk -: protect

Table 11. Haplotype Association Analysis of SNPs in DTNBP1 gene using Transmit v2.5.4

Haplotype	MPSS data					Haplotype	TSLS data				
	HF	Narrow Model(N=78)		Broad Model (N=79)			HF	Narrow Model(N=126)		Broad Model(N=126)	
		Chi	P	Chi	P			Chi	P	Chi	P
					(6504, 6494, 6496)						
1.1.1	0.4617	6.52	**0.0106+**	5.45	**0.0196+**	1.1.1	0.4410	0.20	0.6581	0.24	0.6263
1.2.1	0.0844	0.13	0.7223	0.07	0.7876	1.2.1	0.0721	1.52	0.2176	1.51	0.2188
2.2.1	0.2381	0.03	0.8641	0.16	0.6861	2.2.1	0.3063	0.10	0.7514	0.09	0.7591
1.1.2	0.1710	13.38	**0.0003-**	13.55	**0.0002-**	1.1.2	0.1755	0.12	0.7263	0.08	0.7745
1.2.2	0.0059	0.08	0.7772	0.01	0.9052	2.2.2	0.0051	1.13	0.2872	1.13	0.2871
2.2.2	0.0390	2.49	0.1146	2.47	0.1164						
					(6497, 6513, 6498, 6495)						
1.1.1.1	0.8088	1.72	0.1895	2.29	0.1302	1.1.1.1	0.8975	1.30	0.2541	1.08	0.2988
1.2.1.1	0.0698	0.70	0.4013	0.42	0.5152	1.2.1.1	0.0094	0.00	0.9693	0.00	0.9693
1.1.2.1	0.0036	1.01	0.3159	1.01	0.3158	2.1.2.2	0.0232	7.48	**0.0062-**	7.48	**0.0062-**
2.1.2.2	0.1008	1.47	0.2250	2.01	0.1563	2.2.2.2	0.0698	0.15	0.6972	0.26	0.6114
2.2.2.2	0.0170	0.09	0.7596	0.08	0.7712						

HF: Haplotype Frequency +: risk -: protect

Table 12. Haplotype Association Analysis of SNPs 6563, 6584 in TNF-alpha gene using Transmit v2.5.4

Haplotype	MPSS data					Haplotype	TSLS data				
	HF	Narrow Model(N=163)		Broad Model (N=177)			HF	Narrow Model(N=131)		Broad Model(N=131)	
		Chi	P	Chi	P			Chi	P	Chi	P
1.1	0.4718	3.97	0.0463-	4.18	0.0410-	1.1	0.3841	0.64	0.4247	0.64	0.4247
2.1	0.1200	3.03	0.0816	1.72	0.1901	2.1	0.1590	0.01	0.9146	0.01	0.9146
1.2	0.4006	0.56	0.4539	1.26	0.2613	1.2	0.4570	0.46	0.4970	0.46	0.4970
2.2	0.0076	0.98	0.3232	1.76	0.1842						

HF: Haplotype Frequency +: risk -: protect

Table 13. Haplotype Association Analysis of SNPs 6581,6575,6582 in NOTCH4 gene using Transmit v2.5.4

Haplotype	MPSS data					Haplotype	TSLS data				
	HF	Narrow Model(N=84)		Broad Model (N=86)			HF	Narrow Model(N=132)		Broad Model(N=132)	
		Chi	P	Chi	P			Chi	P	Chi	P
1.1.1	0.4720	0.34	0.5610	0.71	0.4001	1.1.1	0.4827	3.03	0.0817	3.27	0.0707
2.1.1	0.2201	0.85	0.3552	0.11	0.7409	2.1.1	0.2034	0.11	0.7380	0.25	0.6181
1.2.1	0.0036	0.88	0.3482	0.89	0.3455	2.2.1	0.1721	3.68	0.0550	3.31	0.0687
2.2.1	0.1706	0.00	0.9825	0.02	0.8969	1.1.2	0.1176	0.47	0.4921	0.47	0.4921
1.1.2	0.1131	3.35	0.0673	2.44	0.1182	2.1.2	0.0243	4.25	**0.0393+**	4.25	**0.0393+**
2.1.2	0.0205	0.08	0.7818	0.14	0.7041						

HF: Haplotype Frequency +: risk -: protect

Table 14. Haplotype Association Analysis of SNPs 6555,6560,6569,6561,6562 in PPP3CC gene using Transmit v2.5.4

Haplotype	MPSS data					Haplotype	TSLS data				
	HF	Narrow Model(N=80)		Broad Model (N=82)			HF	Narrow Model(N=132)		Broad Model(N=132)	
		Chi	P	Chi	P			Chi	P	Chi	P
1.1.1.1.1	0.5521	1.00	0.3179	0.83	0.3637	1.1.1.1.1	0.5283	0.45	0.5021	0.47	0.4910
1.1.2.1.1	0.0293	0.00	0.9543	0.01	0.9368	2.1.1.1.1	0.0023	0.95	0.3287	0.96	0.3284
1.1.1.2.1	0.0017	0.26	0.6068	0.26	0.6068	1.2.1.1.1	0.0011	0.02	0.8966	0.02	0.8966
2.1.1.2.1	0.0016	42.96	0.0000+	42.60	0.0000+	2.2.1.1.1	0.0009	0.02	0.8855	0.02	0.8855
1.1.2.2.1	0.0029	0.74	0.3882	0.75	0.3872	1.1.2.1.1	0.0518	1.03	0.3094	1.03	0.3111
2.1.2.2.1	0.0033	1.00	0.3167	1.00	0.3166	2.2.2.1.1	0.0020	0.95	0.3300	0.95	0.3299
2.2.2.2.1	0.0804	0.17	0.6765	0.26	0.6117	2.1.2.2.1	0.0039	0.15	0.7025	0.15	0.7031
1.1.2.2.2	0.0036	1.02	0.3129	1.02	0.3125	2.2.2.2.1	0.1079	0.75	0.3868	0.76	0.3836
2.2.2.2.2	0.3251	1.39	0.2378	1.37	0.2414	1.1.2.2.2	0.0020	1.00	0.3176	1.00	0.3176
						2.2.2.2.2	0.2999	0.55	0.4589	0.59	0.4438

HF: Haplotype Frequency +: risk -: protect

Table 15. Haplotype Association Analysis of SNPs 6524,6529,6530 in NRG1 gene using Transmit v2.5.4

Haplotype	MPSS data					Haplotype	TSLS data				
	HF	Narrow Model(N=83)		Broad Model (N=85)			HF	Narrow Model(N=132)		Broad Model(N=132)	
		Chi	P	Chi	P			Chi	P	Chi	P
1.1.1	0.5455	1.96	0.1613	0.84	0.3591	1.1.1	0.5744	0.00	0.9906	0.00	0.9906
2.1.1	0.0207	0.53	0.4683	0.55	0.4597	2.1.1	0.0253	0.00	0.9724	0.06	0.8118
2.2.1	0.0926	4.51	**0.0337-**	3.44	0.0638	2.2.1	0.0721	2.22	0.1367	2.22	0.1367
1.1.2	0.0034	1.01	0.3156	1.01	0.3152	1.1.2	0.0080	0.46	0.4993	0.46	0.4993
2.2.2	0.3378	0.47	0.4908	1.28	0.2574	2.2.2	0.3202	0.81	0.3674	0.67	0.4131

HF: Haplotype Frequency +: risk -: protect

Table 16. Haplotype Association Analysis of SNPs 6521,6522,6519 in DAAO gene using Transmit v2.5.4

Haplotype	MPSS data					Haplotype	TSLS data				
	HF	Narrow Model(N=78)		Broad Model (N=79)			HF	Narrow Model(N=120)		Broad Model(N=120)	
		Chi	P	Chi	P			Chi	P	Chi	P
1.1.1	0.5831	0.07	0.7856	0.20	0.6571	1.1.1	0.4982	0.53	0.4650	0.55	0.4575
2.1.1	0.0042	1.01	0.3158	1.02	0.3120	2.1.1	0.0326	3.53	0.0603	3.54	0.0600
1.2.1	0.0025	0.49	0.4856	0.49	0.4856	1.2.1	0.0141	5.05	**0.0246-**	5.05	**0.0247-**
2.2.1	0.0391	0.26	0.6073	0.22	0.6393	2.2.1	0.0692	0.00	0.9741	0.00	0.9766
2.1.2	0.0567	0.93	0.3344	0.78	0.3761	1.1.2	0.0044	0.90	0.3426	0.90	0.3431
1.2.2	0.0078	1.78	0.1817	1.79	0.1814	2.1.2	0.0989	8.34	**0.0039-**	8.52	**0.0035-**
2.2.2	0.3067	0.00	0.9762	0.01	0.9026	1.2.2	0.0065	0.04	0.8364	0.04	0.8370
						2.2.2	0.2761	8.06	**0.0045+**	8.29	**0.0040+**

HF: Haplotype Frequency +: risk -: protect

Table 17. Haplotype Association Analysis of SNPs 6506,6503,6510,6507 in G72 gene using Transmit v2.5.4

Haplotype	MPSS data					Haplotype	TSLS data				
	HF	Narrow Model(N=80)		Broad Model (N=82)			HF	Narrow Model(N=115)		Broad Model(N=115)	
		Chi	P	Chi	P			Chi	P	Chi	P
1.1.1.1	0.6269	1.81	0.1782	1.20	0.2725	1.1.1.1	0.6247	0.15	0.6976	0.11	0.7349
1.2.1.1	0.0033	1.00	0.3165	1.00	0.3165	2.1.1.1	0.0023	0.88	0.3490	0.88	0.3488
2.2.1.1	0.0033	1.00	0.3184	1.00	0.3185	1.1.1.2	0.0023	1.01	0.3160	1.01	0.3160
2.1.1.2	0.0033	1.00	0.3184	0.99	0.3198	2.2.2.2	0.3708	0.14	0.7075	0.11	0.7450
1.2.2.2	0.0068	1.99	0.1584	1.99	0.1579						
2.2.2.2	0.3564	1.79	0.1810	1.32	0.2514						

HF: Haplotype Frequency +: risk -: protect

Projected Timeline & Brief Summary of Plans for Next Year

Next year is the third year of this POCOS, we'll continue to work on the 7working domains as that of the second year in general. We'll continue to do the fine mapping study using SNP genotyping on 3 other candidate chromosome regions of 8p21(D8S1222), 6P24-22(D6S 296)and 15q13-14(D15S976), using 3-step genotyping method developed by this POCOS project. We'll do replication study of the initially located vulnerability genes by using big TSLS sample of 600 families. We'll also search for polymorphism of identify candidate vulnerability genes for neurobiological and clinical study. Sub-classification of schizophrenia using clinical as well as endophenotype variables will be done to help genetic analyses. Finally, we'll write manuscripts for submission to the appropriate journal. In summary, we plan to complete the fine SNP mapping on all 5 candidate chromosome regions proposed in the POCOS study. We may be able to reach the following conclusions: (1) We may be able to identify about 5 vulnerability candidate genes. (2) Multiple vulnerability genes are responsible for schizophrenic; (3) There are interactions among these vulnerability genes; (4) Schizophrenia is heterogeneous in clinical phenotype and it can be sub-classified using meaningful clinical variables of age of onset, negative symptom, and indicators of CPT. (5) There may have specific vulnerability genes for each subtype of schizophrenia. Those results will be of value for further molecular genetic, neurobiological and clinical studies on schizophrenia.

The propose Time line of the next year work will be as the following:

(1) May 1,2004~ April 30, 2005. Continuing data analysis on genotyping data, and continue paper writing for publication.
(2) May1,2004-Oct31, 2005; SNP genotyping work on 3 other candidate chromosome regions of 8P21(D8S1222), 6P24-22(D6S296), and 15q13-14(D15S976). Management of DNA bank for DNA sample retrieval for laboratory work.
(3) May 1, 2004-April 30, 2005: Polymorphism detection study on all positive candidate vulnerability genes obtained in this POCOS.
(4) Oct.1, 2004-April 30, 2005: Analysis on gene-gene interaction in association study. We'll proceed to study, the multiple gene-gene interaltion after identifying multiple vulnerability genes from POCOS. The interaction effect will be examined at the level of significance level of association , transmission effect, and at the level of clinical and endophenotype variables, especially at the level of sub-classification of sample individual, and families , as well as the subtypes of schizophrenia .
(5) May1,2004-April,2005: Planning for further genetic studies on schizophrenia in the era of post-POCOS stage
(6) May 1, 2004-April, 2005: Continuing sample collection of serum, plasma, DNA, CPT, WCST if needed for laboratory work, especially for functional and/or clinical studies.

4. Personnel

Name		Position Title	Education Degree	% of personal effort on this project	Job Description or Responsibilities
In Chinese	In English				
胡海國	Hai-Gwo Hwu	主持人(PI)	M.D.	30%	Design and management of the whole project; supplementary data collection; data management and paper writing; business management
劉智民	Chih-Min Liu	共同主持人 (Co-PI)	M.D.	30%	Supplementary data collection; management of DNA, cell-line and family data bank

5. Publications and/or Patents

6a. Publications

Appendix

1. Manuscripts submitted /prepared

(1) Liu YL , Liu CM, Fann CS-J , Wu J-Y, Hung SY, Chen WJ, Chen JH, Jon YS, Hwu HG ;Linkage of chromosome 1q42.1 with schizophrenia containing vulnerability genes of GNPAT and DISC1using SNP fine mapping.

(2) Liu YL, CM Liu, Hwu HG: An IL-6 Transcription factor recognition single nucleotide polymorphism located around the TATA box region of disrupted-in schizophrenia gene.

(3) Liu YL, CM Liu, Hwu HG: Construction of a positive probe for screening balanced translocation t(1;11)(q42.1;q14.3)causing disruption in a candidate gene for schizophrenia

(4) Hwu HG, Tsuang MT, Liu CM, Faraone S, Tsuang MM, Chen WJ, Liu SK, Shieh MH, Huang TC, and TSLS study group: Taiwan schizophrenia linkage study: Field Study.

2. Conference proceeding paper

(1) Hwu HG, Liu CM , Liu YL, Fann CSJ, Wu JY, Lin CY, Ou-Yang WC, Jann HY, and Chen JJ; Genetic variation in the 1q42.1 locus and susceptibility to schizophrenia (Presented in XI ICOPS, Quebec, Canada, Oct, 2003; Neuropsychiatric Genetics, 122B;131-132,2003)

(2)Liu YL, Liu Cm, and Hwu HG; dhplc Analysis of the TATA BOX region of Disc1 Gene (Presented in XI ICOPS, Quebec, Canada, Oct, 2003; Neuropsychiatric Genetics, 122B;86-87,2003)

(3)Liu CM, Liu YL, Hwu HG, Fann CSJ, and Lin CY; Significant Association Evidences Between Polymorphisms of Prodh and Schizophrenia (Presented in XI ICOPS, Quebec, Canada, Oct, 2003; Neuropsychiatric Genetics, 122B;116-117,2003)

(4)Huang LC, Liu CM, and Hwu HG; Polymorphism of G308a Tumor Necrosis Factor Alpha Gene in Schizophrenia. (Presented in XI ICOPS, Quebec, Canada, Oct, 2003; Neuropsychiatric Genetics, 122B;116-117,2003)

(5)Liu YL, Liu CM, and Hwu HG; Construction of A Positive Probe For Screening Balanced Translocation Candidate Gene for Schizophrenia.(Presented in XI ICOPS, Quebec, Canada, Oct, 2003; Neuropsychiatric Genetics, 122B; 81,2003)

(6)Hwu HG, Tsuang MT, Liu CM, Faraone S, Tsuang MM, Chen WJ, Liu SK, Shieh MH, Huang TC, and TSLS study group ; Taiwan schizophrenia Linkage Study; Field Study.(Presented in XI ICOPS, Quebec, Canada, Oct, 2003; Neuropsychiatric Genetics, 122B;84,2003)

6b. Patents

Appendix

Neuregulin 1 as a Candidate Gene Influencing Schizotypal Personality Features or Sustained Attention in Adolescents

Method

Subject

The subjects in this study are 905 (459 girls and 446 boys) junior high school students in Taipei City. The sampling of the participants has been described in detail elsewhere (Lin et al 2000). Briefly, we stratified the 73 public junior high schools in Taipei City in 1996 by educational levels of the residents into the three groups, and randomly selected one school from each group. Then, three classes were randomly selected from each grade in each of the three selected schools. In total, there were 971 students eligible for this study. After informed written consent was obtained from the students and their parents, 905 (93%) students completed the self-report questionnaire, and 878 did a mouth rinse with 4% sucrose. One-sixth of participants (n=161) were randomly selected to take the Continuous Performance Test (CPT).

Measurement instruments

The participants were asked to complete a questionnaire inquiring demographic features and schizotypal personality features, including the PAS (Chapman et al 1978) and SPQ (Raine 1991). The answers to the 35 items of the PAS were either *yes* or *no*. So are those to 74 items of the SPQ. The two parts were intermingled to minimize the potential offensiveness of some questions regarding aberrations in bodily perception, as suggested Chapman et al. (Chapman et al 1978). It took about ten to fifteen minuets to fill out the questionnaire.

The internal consistency alphas were 0.84 for the PAS and 0.95 for the SPQ, whereas the ICCR for the 1-week test-retest reliability was 0.80 for the PAS and 0.86 for the SPQ (Chen et al 1998b).

CPT performance

We used a CPT machine from Sunrise System, Version 2.20 (Pembroke, MA). Briefly, numbers from 0 to 9 were randomly presented for 50ms each, at a rate of one per second. The probability of critical stimulus was set at 10%. Each student undertook two CPT sessions: the undegraded 1-9 task and the 25% degraded 1-9 task. During the undegraded task, students responded to the target stimulus (the number 9 proceeded by the number 1) by pressing a button. A total of 331 trials, 31 of them targets, were presented over 5 minutes. During the degraded task, a pattern of snow was used to toggle background and foreground dots so that the image was not distinct. The further stimulus encoding and analysis processes of the degraded CPT demand a substantial allocation of attentional capacity. Standardized instructions were given before the test to make sure the students understood the rules. The undegraded session was administered first and then degraded session. Once the test began, no rewards or cues were given to the student.

The signal-detection indices of CPT performance were derived from the hit rate (h; rate of response to target trials) and false alarm (f; rate of response to nontarget trials; (Nuechterlein 1991)). The sensitivity index (d'), calculated as z(h)-z(f), reflects the degree to which the frequency the distribution of the internal perceptual evidence generated by signal trials is separated from that generated by nonsignal or noise trials, whereas the response criterion index (Inβ), calculated as $\ln\{y[z(h)]/y[z(f)]\}$, assesses the amount of perceptual evidence an individual requires prior to making a decision to response to a stimulus as a signal. Variables extraneous to the intended comparison (d'),

such as differences in motivation or in cooperativeness between the groups, would yield differences in the response criterion rather than in d'.

The ICCRs of the performance indices for the undegraded and degraded CPT were, respectively, 0.89 and 0.81 for the hit rate, 0.37 and 0.52 for the false alarm rate, 0.83 and 0.82 for d', amd 0.49 and 0.72 for lnβ ((Chen et al 1998a)).

Statistical analysis

We conducted the confirmatory factor analysis using SAS 8.2. The three factor model (Raine et al 1994), was constructed on the basis of factor analytic studies of schizophrenic symptoms and contains the following three factors: (a) Cognitive-Perceptual (ideas of reference, magical thinking, unusual perceptual experience and paranoid ideation); (b) Interpersonal (social anxiety, no close friends, constricted affect, and paranoid ideation); (c) Disorganization (odd behavior and odd speech). Several indices have been developed to assess the fit for a model. We judged the fitness by using the following indices: the goodness of fit (GFI), the adjusted GFI (AGFI), the normed fit index (NFI), the Akaike information criterion (AIC). Values of GFI and NFI greater than 0.9, and a AGFI value greater than 0.8 indicate a good fit, whereas smaller values of AIC indicate a better fit.

Then, we performed one-way ANOVA (or two-way ANOVA, if needed to adjusted sex effect) to test whether there's a significant difference in schizotypal scores or CPT indices between genotype in each marker. And, to avoid omitting the possible dose-response effect of allele, the trend test was further used to detect such relationship. Schizotypal scores were regressed on genotype in multiple linear regressions with sex as a covariate. All those multiple comparisons were dealt with Bonferroni correction.

Result

According as GFI was 0.9210, AGFI was 0.8455, NFI was 0.8861 and AIC was 302.007, the three-factor model provided the quite fit to the data. Same structure was found in adolescent sample in Chinshan Township (Chen et al 1997). The mean age (SD) of 905 students was 13.97 (0.89). The measurement of PAS, SPQ and CPT performance indices were outlined in table 1. Most measurements didn't show significant difference between girls and boy, except SPQ total score, cognitive-perceptual deficits score and d' in 25% degraded session of CPT. Girls expressed higher schizotypal personality score than boys, especially in cognitive-perceptual aspect. Meanwhile, boys tended to perform better than girls in degraded CPT session. This was also coincident with Chinshan Study(Chen et al 1998a; Chen et al 1997) .

The success rates of genotyping each of the three SNP markers from 878 students' buccal cells were above 99%. The genotype distributions and allele frequencies were counted (Table 2). The distributions of the three markers all fit Hardy-Weinberg Equilibrium. We used the ANOVA to test the personality score difference in each genotype group, only the marker on the second in position 12, rs3924999, revealed significant relationship with PAS total score ($F_{2, 858; 0.05} = 3.93$, p=0.02), SPQ total score (two-way ANOVA with sex adjustment; $F_{2, 857; 0.05} = 3.05$, p = 0.048) and disorganization score ($F_{2, 858; 0.017} = 4.17$, p=0.016) (Table 3). When it comes to trend test, we had only found significant trend effect in PAS and SPQ total scores. PAS score increased with number of allele A in rs3924999. Regressing PAS score or SPQ scores on genotype, we found the regression coefficients were significant positive. It evidenced their positive correlation with the number of allele A (table 4). However, we didn't get any significant finding when comparing four CPT indices in each genotype

group (Table 5) by using either ANOVA or the trend test.

Whether linkage disequilibrium of the three SNPs shows more information on schizotypal personality festures or sustained attention, we need to do haplotype analysis further.

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The CALIS Procedure

Covariance Structure Analysis: Maximum Likelihood Estimation

Fit Function	0.3850
Goodness of Fit Index (GFI) >0.9	0.9210
GFI Adjusted for Degrees of Freedom (AGFI) >0.8	0.8455
Root Mean Square Residual (RMR) <0.05	0.2953

Fit the three-factor structure of SPQ as before.

Parsimonious GFI (Mulaik, 1989)	0.5884
Chi-Square	348.0070
Chi-Square DF	23
Pr > Chi-Square	<.0001
Independence Model Chi-Square	3054.8
Independence Model Chi-Square DF	36
RMSEA Estimate	0.1250
RMSEA 90% Lower Confidence Limit	0.1136
RMSEA 90% Upper Confidence Limit	0.1368
ECVI Estimate	0.4342
ECVI 90% Lower Confidence Limit	0.3712
ECVI 90% Upper Confidence Limit	0.5055
Probability of Close Fit	0.0000

Bentler's Comparative Fit Index	0.8923
Normal Theory Reweighted LS Chi-Square	348.7233
Akaike's Information Criterion (smaller is better)	302.0070
Bozdogan's (1987) CAIC	168.4245
Schwarz's Bayesian Criterion	191.4245
McDonald's (1989) Centrality	0.8356
Bentler & Bonett's (1980) Non-normed Index	0.8315
Bentler & Bonett's (1980) NFI >0.9	0.8861
James, Mulaik, & Brett (1982) Parsimonious NFI	0.5661
Z-Test of Wilson & Hilferty (1931)	15.0875
Bollen (1986) Normed Index Rho1	0.8217
Bollen (1988) Non-normed Index Delta2	0.8928
Hoelter's (1983) Critical N	93

Table 1. Demographic characteristics and measurements of PAS, SPQ and CPT performance of students

	Boy		Girl		Total	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Age	445	13.95 (0.89)	458	14.00 (0.90)	903	13.97 (0.89)
PAS	446	5.42 (4.44)	459	5.33 (4.33)	905	5.37 (4.38)
SPQ*		22.55 (12.38)		24.90 (11.33)		23.75 (11.91)
Cognitive-perceptual deficits†		10.46 (6.15)		12.44 (6.04)		11.46 (6.17)
Interpersonal deficits		10.07 (6.52)		10.66 (6.20)		10.37 (6.37)
Disorganization		4.80 (3.57)		5.00 (3.45)		4.90 (3.51)
CPT indices						
Undegraded	80		81		161	
Hit rate (%)		94.31 (8.64)		94.98 (6.12)		94.65 (7.46)
False alarm rate (%)		0.69 (0.88)		0.47 (0.70)		0.58 (0.80)
d'		4.23 (0.64)		4.33 (0.53)		4.28 (0.58)
Lnβ		1.81 (0.73)		2.04 (0.67)		1.92 (0.70)
Degraded, 25%	79		81		160	
Hit rate (%)		91.14 (0.12)		87.26 (11.70)		89.17 (11.91)
False alarm rate (%)		0.95 (1.29)		1.19 (1.45)		1.07 (1.37)
d' ‡		4.00 (0.82)		3.69 (0.76)		3.84 (0.80)
Lnβ		1.86 (0.75)		2.04 (0.83)		1.95 (0.79)

*: significant sex difference for the t-test ($p < 0.05$)

†: significant sex difference, t-test with Bonferroni correction, $p \leqq 0.05/3 = 0.017$

‡: significant sex difference, t-test with Bonferroni correction, $p \leqq 0.05/4 = 0.125$

Table 2. Genotype distribution and allele frequency of the SNPs

Genotype	Genotype distributions (%)	Allele frequencies	
Rs3924999 (n=870)			
AA	538 (61.84)	A	G
AG	295 (33.91)	0.79	0.21
GG	37 (4.25)		
SNP8NRG221533 (n=867)			
CC	254 (29.30)	C	T
CT	455 (52.48)	0.56	0.44
TT	158 (18.22)		
Rs2954041 (n=871)			
GG	298 (34.21)	G	T
GT	419 (48.11)	0.58	0.42
TT	154 (17.68)		

NOTE: The three markers all fit Hardy-Weinberg Equilibrium

Table 3. Means and standard deviations of PAS and SPQ total score and its three factor scores in each genotype group

Genotype	N	PAS	SPQ	Cognitive-perceptual deficits	Interpersonal deficits	Disorganization
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Rs3924999						
AA	533	5.62 (4.53)†	24.48 (12.27)‡	11.71 (6.36)	10.75 (6.47)	5.12 (3.62)*
AG	291	4.84 (3.90)†	22.40 (11.45)‡	11.02 (5.98)	9.78 (6.21)	4.39 (3.24)*
GG	37	4.41 (4.41)†	23.13 (10.26)‡	10.70 (5.63)	10.16 (5.93)	5.00 (3.61)*
Trend test		*P = 0.0056*	*P = 0.0300*	*P = 0.0809*	*P = 0.0674*	*P = 0.0279*
SNP8NRG221533						
CC	250	5.20 (4.00)	24.88 (12.16)	11.97 (6.04)	11.00 (6.60)	5.04 (3.52)
CT	451	5.33 (4.35)	23.22 (11.85)	11.09 (6.29)	10.25 (6.29)	4.74 (3.51)
TT	157	5.48 (4.96)	23.11 (11.72)	11.50 (6.16)	9.74 (6.10)	4.91 (3.50)
Trend test		*P = 0.5384*	*P = 0.1149*	*P = 0.3574*	*P = 0.0432*	*P = 0.6057*
Rs2954041						
GG	293	5.35 (4.33)	23.36 (11.66)	11.31 (6.08)	10.08 (6.11)	4.86 (3.52)
GT	417	5.26 (4.09)	24.14 (12.00)	11.50 (6.17)	10.88 (6.56)	4.86 (3.51)
TT	153	5.58 (5.10)	23.78 (12.26)	11.75 (6.44)	9.86 (6.30)	5.05 (3.51)
Trend test		*P = 0.6887*	*P = 0.5205*	*P = 0.3563*	*P = 0.9149*	*P = 0.6475*

†: p = 0.02 for the one-way ANOVA ($F_{2, 858; 0.05} = 3.93$)

‡: p = 0.048 for the two-way ANOVA with sex adjustment ($F_{2, 857; 0.05} = 3.05$)

*: significant genotype difference for the one-way ANOVA with Bonferroni correction, $p \leqq 0.05/3 = 0.017$

Table 4. Regression coefficient estimate for the regressions of schizotypal scores on genotype

Genotype	PAS	SPQ§	Cognitive-perceptual deficits§	Interpersonal deficits	Disorganization
Rs3924999	0.71†	1.53†	0.63	0.69	0.46
SNP8NRG221533	-0.14	0.94	0.28	0.65	0.09
Rs2954041	-0.09	-0.37	-0.27	-0.03	-0.08

§: with sex adjustment

†: significant for the t-test, p = 0.0056

Table 5. Means and standard deviations of CPT indices in each genotype group*

Genotype	Undegraded					Degraded, 25%				
	N	Hit rate (%)	False alarm rate (%)	d'	Lnβ	N	Hit rate (%)	False alarm rate (%)	d'§	Lnβ
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Rs3924999										
AA	91	94.26 (7.99)	0.57 (0.82)	4.27 (0.61)	1.96 (0.69)	90	87.35 (13.24)	0.99 (1.14)	3.77 (0.82)	2.05 (0.77)
AG	55	95.54 (7.12)	0.59 (0.83)	4.32 (0.56)	1.82 (0.75)	55	91.73 (8.44)	0.99 (1.24)	3.97 (0.73)	1.92 (0.78)
GG	7	91.70 (5.85)	0.65 (0.72)	4.01 (0.61)	2.20 (0.67)	7	93.09 (6.30)	1.71 (1.99)	3.84 (0.59)	1.45 (1.16)
Trend test		*P = 0.8398*	*P = 0.8040*	*P = 0.7780*	*P = 0.7203*		*P = 0.0169*	*P = 0.3791*	*P = 0.1974*	*P = 0.0655*
SNP8NRG221533										
CC	54	95.10 (7.77)	0.47 (0.63)	4.36 (0.52)	1.96 (0.75)	54	88.89 (11.24)	0.85 (0.91)	3.86 (0.75)	2.07 (0.75)
CT	72	94.44 (6.75)	0.65 (0.94)	4.23 (0.61)	1.92 (0.74)	71	88.96 (12.51)	1.02 (1.33)	3.84 (0.82)	2.02 (0.74)
TT	27	93.67 (9.45)	0.67 (0.76)	4.20 (0.68)	1.86 (0.61)	27	90.80 (10.07)	1.31 (1.44)	3.84 (0.72)	1.66 (0.98)
Trend test		*P = 0.4188*	*P = 0.2193*	*P = 0.1984*	*P = 0.6621*		*P = 0.6621*	*P = 0.1113*	*P= 0.7193*	*P = 0.0483*
Rs2954041										
GG	57	95.13 (6.54)	0.70 (0.97)	4.26 (0.57)	1.83 (0.83)	57	89.02 (11.58)	1.18 (1.36)	3.78 (0.77)	1.87 (0.78)
GT	69	93.69 (8.70)	0.55 (0.78)	4.24 (0.64)	2.02 (0.64)	68	88.09 (12.98)	0.95 (1.26)	3.84 (0.85)	2.12 (0.78)
TT	28	95.62 (6.58)	0.44 (0.39)	4.38 (0.47)	1.90 (0.64)	28	90.55 (10.20)	1.05 (1.06)	3.86 (0.70)	1.80 (0.86)
Trend test		*P = 0.9708*	*P = 0.1354*	*p = 0.4803*	*P = 0.4211*		*P = 0.7510*	*P = 0.5227*	*P = 0.6318*	*P = 0.9024*

§: with sex adjustment

*: multiple comparison with Bonferroni correction, $p \leqq 0.05/4 = 0.0125$

A SNP Fine Mapping Study on Chromosome 1q42.1 Reveals Vulnerability Genes of GNPAT and DISC1 in Schizophrenia: Association with Impairment of Sustained Attention

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ABSTRACT
Linkage evidence of schizophrenia to chromosome 1q41-42 has been reported from our previous work at the marker D1S251. In further fine mapping study, two stages were approached to pinpoint the candidate genes of schizophrenia. First, we validated 120 single nucleotide polymorphisms (SNPs) in 1 Mb around D1S251 from a subset of sample. A total of 47 SNPs with average intermarker distance of 32.5 kbp met the validation criterion of minor allele frequency above 0.1 was selected from the 120 SNPs. Second, the 47 validated SNPs in 102 families with at least 2 siblings affected with schizophrenia were genotyped. In single locus association and haplotype association analyses, we found two genetic SNP blocks significantly associated with schizophrenia. A five SNPs block located within the introns of GNPAT gene showed significant association in either single locus ($p<0.05$) or GATTT haplotype association analyses ($p=0.0461$). A two SNPs block located within the introns of DISC1 gene also showed significant result in the single locus ($p<0.05$) and AG or GA haplotypes association analyses ($p=0.0091$ or $p=0.0008$, respectively). In further symptomatic phenotype of sustained attention function assessed by Continuous Performance Test (CPT) with the SNP haplotypes of the two genes, we found that the d' indices of undegraded CPT was significantly associated with the DISC1 gene, and the degraded CPT was associated with the GNPAT gene. This study suggested two potential candidate genes, GNPAT and DISC1, might involve in the pathophysiological process of the sustained attention impairment in schizophrenia.

KEY WORDS: schizophrenia, sustained attention, DISC1, GNPAT, haplotype association, quantitative TDT

INTRODUCTION

Schizophrenia is a devastating and stigmatized psychiatric disorder with brain pathology and high genetic loading. Genetic epidemiological studies revealed that schizophrenia is a familial disease and the risk to first-degree relatives is approximately ten times the risk to relatives of controls (1-4). Monozygous twin pairs had concordance rates of 46%~53% and 14%~15% for dizygotic twin pairs (5-7). The heritability was around 0.7. However, the concordance rate in MZ twins is far from 100%, the environmental factors should also be considered. The evidence of genetic contribution to the etiology of schizophrenia was further supported by adoption study (8-10). Segregation analyses indicate that the model of multiple genes better fit the observed patterns of schizophrenia in family studies than do single major locus model (11-13). It was suggested that several genes (3 to 5 in number) in epistasis might responsible for genetic etiology of schizophrenia (14).

In linkage analysis, there is no priori hypothesis to focus on any given chromosomal region except the sex chromosomes (15, 16). There are a few genome-wide scans of schizophrenia with suggestive evidence for linkage, which include chromosome 1q21-22, 1q31-42, 2p22-21, 4q24-32, 6p24-22, 6q16-23, 8p24-21, 10p14-13, 13q14-32, 15q13-14, 22q11-13 (17-26). One of the promising chromosome region is 1q 42, where a balance translocation (1; 11)(q42.1; q14.3) has been found associating with major mental illness including schizophrenia in a large Scottish family pedigree (27, 28). Two novel genes named DISC1 (Disrupted-in-Schizophrenia 1) and DISC2 (Disrupted-in-Schizophrenia 2) at chromosome 1q42.1 were disrupted at the breakpoint (29, 30). This region of linkage evidence has also been confirmed in a Finnish family sample (25) and in Taiwan family samples (31, 32). In our previous study, we found that the marker D1S251 located near the breaking point of DISC1 gene had significant linkage with schizophrenia (32). In this study, we continued to pursue the possible significant association of SNP markers located near by the D1S251 region using the SNP fine mapping. The hypothesis is that there are some SNP markers near by the D1S251 marker significantly associated with schizophrenia. These SNP markers would belong to a significant haplotype located in some functional genes expressed in the brain.

The neuropsychological functions of sustained attention, as assessed by the continuous performance test (CPT) (33), and executive functions, as assessed using the Wisconsin card sort test (WCST) (34, 35), were obtained from the study subjects. These executive functions are known to be impaired in schizophrenic patients (36, 37) and their first degree relatives (38). Among schizophrenic patients, impaired executive functioning has been related to hypofrontality (39), and to a mutation of the gene regulating the catecholamine catabolizing enzyme catechol-O-methyl transferase (40). Impaired sustained attention, as assessed by the CPT, was found to be impaired in schizophrenic patients (41), and in the first degree relatives (42, 43) of schizophrenic patients. Patients with schizotypal disorder were also found to have impaired sustained attention (43). The CPT and WCST data could be used to define endophenotypes for

schizophrenia. As such they should be useful for addressing the heterogeneity and variable expression of schizophrenia in linkage analyses. In this study, we intend to use quantitative TDT (44-46) to analyze the significance of associations of these phenotypic status and the significant SNPs in genes. The hypothesis is that the significant SNP markers would have significant association with these phenotypic variables.

MATERIALS AND METHODS

Subjects and CPT assessment

Schizophrenic probands who had at least two affected siblings were identified from the Department of Psychiatry, National Taiwan University Hospital and the University-affiliated Taoyuan Psychiatric Center. Data collection was initiated after informed consents were obtained from the identified study subjects and their families. All of the family members were personally interviewed by the research psychiatrists using the Psychiatrist Diagnostic Assessment (PDA) (47). The final diagnostic assessment was formulated by integrating the PDA data and clinical information of medical chart records. The final diagnosis was done following DSM-IV criteria for schizophrenia, schizoaffective disorder, and other non-affective psychoses. Clinical data of age at onset of initial symptoms, negative symptoms, and positive symptoms were collected. The negative and positive symptoms were assessed using schedule for assessment of negative symptoms (SANS)(47) and schedule for assessment of positive symptoms (SAPS)(48) with satisfactory reliability. Negative symptom score was the sum of all global scores of five negative symptom dimensions, including affective flattening, alogia, avolition/apathy, anhedonia/asociality, and impaired attention. Positive symptom score was the sum of all global scores of 4 positive symptom dimensions, including hallucination, delusion, excitements, and thought derailment.

One hundred and two schizophrenic nuclear families with at least two affected siblings were recruited in this study. A total of 399 subjects were recruited for this genotype study, of which about 231 individuals received undegraded CPT test and 225 individuals received degraded CPT test. The d' value represents the sensitivity of CPT performance. The higher the d' value means the better the CPT performance. The 1β value represent the motivation factor of CPT performance. The higher the 1β value means the better motivation of CPT performance. These indicators are influenced by the demographic variables of age, sex, and education. The adjusted Z values of d' and 1β were used for statistical analyses. A 164 out of 399 subjects received WCST assessment and had available WCST data for analyses. The indicators of WCST used in analyses were total error, non-perseverative error, perseverative error, perseverative response, categories achieved, learn to learn response, failure to maintain set.

SNP selection criteria and validation

Fine mapping studies were using SNP dense markers spreading upstream and down stream of the dinucleotide marker D1S251 located at 1q42.1. In the defined region of 2cM around DIS251, a total of 120 SNPs were selected. The inter SNP marker distance ranged from 5 kb to 60 kb, with an average of 32.5 kb. According to the location of these SNPs relative to the position of the functional genes, the SNPs were selected based upon the following priority of exon, 5'-untranslated region, 3-untranslated region, introns, promoter (CpG island), and gaps between functional genes, which expressed in the central nervous system. We used 31 trios and 2 independent individuals of a totally 95 individuals to validate the 120 SNPs from public

database (http://www.ensembl.org/Homo_sapiens/martview).

SNP genotyping

All SNPs were typing by the method of matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS). A DNA fragment (100-300 bp) encompassing the SNP site of 2.5 ng/ul concentration was amplified using the polymerase chain reaction (PCR) according to the manufacturer's instruction.

After PCR amplification, the un-incorporated deoxynunleotide triphosphate (dNTP), and the primer pair of the PCR reaction mixture were neutralized by treating with SAP at 37℃ for 20 minutes. The reaction mixture were incubated at 85℃ for 5 minutes to inactivate the SAP activity. A primer which sits just next to the SNP site, Thermo Sequenase (Amersham) and appropriate dideoxynucleotide triphosphate (ddNTP) mixture were added to the above reaction mixture and 40 cycles of denaturing at 94℃ for 5 sec, annealing at 52℃ for 5 sec, and extension at 72℃ for 5 sec, were carried out in the GeneAmp 9700 thermocycler (ABI, U.S.A.). Different extension products were differentiated by mass through MALDI-TOF in later step.

Resin was added to the above reaction mixture to get rid of salt, which would interfere with the mass reading in the MALDI-TOF analysis. After desalting, the reaction mixture was spotted onto the SpectroCHIP (using the SpoectroPOINT) where they were co-crystallized with the matrix. After introduced to the SpectroREADER, a nitrogen laser with nanosecond-wide pulses interrogated the samples on the SpectroCHIP in the high-vacuum environment of the time-of-flight mass spectrometer. Upon laser irradiation, the matrix crystals absorbed the laser's energy and the extension products were converted into gas phase ions, which upon acceleration undergo mass-dependent separation over approximately 1-meter flight path. Molecular masses were determined with 0.01 to 0.02% estimated accuracy. Acquired spectra were transferred to the MassARRAY Server (Sequenom, San Diego, CA) where they were automatically interpreted and corrected for their genotypes.

Statistics analysis

In order to verify the sample accuracy included family relationship and genotype, PEDCHECK version 1.1 (O'Connell and Weeks 1998) and UNKNOWN version 5.23 (Terwilliger and Ott 1994) were employed to check Mendelian inheritance and Procedure ALLELE in SAS/GENETICS release 8.2 (SAS Institute 2002) was used to test for Hardy-Weinberg equilibrium. Linkage disequilibrium of inter-markers was measured using coefficient D' (Hedrick 1987) which was also used to define haplotype blocks. A graphic presentation of block pattern was completed using GOLD software (Abecasis and Cookson 2000).

Family-based transmission disequilibrium tests were applied to test linkage disequilibrium. Both of single-locus and haplotype-based association analyses were carried out simultaneously using two popular programs for the nuclear family data, haplotype FBAT version 1.4.1 (Horvath et al 2001; Laird et al 2000; Horvath et al 2004) and TRANSMIT version 2.5.4 (Clayton 1999). Moreover, GEE method (Liang and Zeger 1986) was applied to test the interaction between haplotype blocks.

Besides the analysis of qualitative trait, quantitative analysis using highly heritable quantitative trait was also considered. The analysis of heritability and

quantitative-type transmission disequilibrium test based on variance component approach was applied using QTDT version 2.4.3 (Abecasis et al 2000a,b).

RESULTS

SNP Validation

A SNP was considered valid if the frequency of minor allele was larger than 0.1 and genotyping missing rate was smaller than 30%. Forty-seven out of 120 SNPs met the validity criteria. The 47 SNPs span across 1591 kb around D1S251 marker (Table I) and cover 11 known functional genes of COG2, AGT, CAPN9, FLJ14525, FLJ 22584, ARV1, GNPAT, DKFZP547NO43, EGLN1, TSNAX, and DISC1. Except five SNPs (SNP 495, 506, 513, 527, 581), the other 42 SNP markers are compatible with the Hardy-Weinberg equilibrium.

Construction of SNP block

In order to perform haplotype analysis haplotype block was evaluated by inter-marker linkage disequilibrium coefficient. . Two SNP blocks were identified by using 2 criteria: 1) inter-marker association test based on chi-squared test was significant and 2) Coefficient D' was higher than 0.8. The locations of these two SNP blocks are shown Figure 1. The first block covers the SNP markers of 482 (intron 2), 485 (intron 2), 479 (intron 5), 488 (intron 11) and 489 (intron 13) in the GNPAT gene region; the second block covers the markers of 517 (intron 4) and 518 (intron 5) in the DISC1 gene region as shown in Figure 2.

Single-locus association analysis

The preliminary analysis investigates the effect of single locus under phenotype models and provides information for the later multi-loci analysis. From the result of single-locus association analyses using FBAT program version 1.4.1 (Horvath et al 2001; Laird et al 2000), we found some SNP variants exhibited significant consequences in the broad and narrow models of schizophrenia phenotypes, respectively. In the broad model, the significant SNP marker on GNPAT gene is 485 (rs508908) (p=0.038), and on DISC1 gene are 517 (rs2793092) (p=0.005) and SNP518 (rs2793091) (p=0.008). In the narrow model, SNP marker 485 (rs508908) on GNPAT gene shows a modest effect p=0.070, and the highly significant results on DISC1 gene are 517 (rs2793092) (p=0.001) and 518 (rs2793091) (p=0.007). The details are summarized in Table 2. The results based on TRANSMIT program version 2.5.4 (Clayton 1999) yielded more significant results with similar pattern, especially for SNPs on GNPAT gene under narrow model, 485 (rs508908) (p=0.019) and 479 (rs538643) (p=0.049).

Haplotype-based association analysis

In order to evaluate the relationship between the haplotypes that were constructed and schizophrenia, haplotype-based association analysis was applied. The results from haplotype FBAT program version 1.4.1 (Horvath et al 2004) showed that GATTT haplotype in the GNPAT gene SNP block (block 1) is only slightly significant for the broad model of schizophrenia phenotype (p=0.0461). The AG and the GA haplotypes in the DISC1 gene SNP block (block 2) reveal significant association for both of the narrow model and the broad model of schizophrenia. It seems that haplotype GA is a risk haplotype and AG possesses protection effect. The corresponding p-values are p=0.0091 and p=0.0008 for the narrow model, and p=0.03 and p=0.002 for the broad model. The results are summarized in Table 2. Analyses carried out by TRANSMIT

program version 2.5.4 (Clayton 1999) yielded similar pattern and the results are not shown here. The interaction of two haplotype blocks was investigated by using the GEE method (Liang and Zeger 1986). However, no significance was found.

Quantitative TDT for phenotypic indicators

When correlating the SNP genotypes with the pathological symptoms of schizophrenia, we analyzed the symptomatic variables of age of onset of the initial symptom, global score of negative symptom, global score of positive symptoms, adjusted Z value of undegraded and degraded CPT, adjusted 1 β value of undegraded and degraded CPT, and WCST indicators of total errors, perseverative errors, perseverative response, categories achieved, learn to learn response, and failure to maintain set.

The analysis of heritability using QTDT version 2.4.3 (Abecasis et al 2000a,b) was conducted to screen the important quantitative phenotypes and the results are shown in Table III. Table III lists the variance components due to environment (σ_E^2) and genetic effect (σ_G^2). Heritability h^2 is defined as the explained proportion due to genetic component. Two highly heritable phenotypes, d' indices of undegraded CPT and degraded CPT, were identified by likelihood ratio tests. The former yields a very significant p-value $8*10^{-7}$ and heritability h^2 is 0.831, the latter yields p-value $2*10^{-7}$ and h^2 is 0.751.

Quantitative haplotype analysis based on variance component analysis was investigated using these two highly heritable traits. Based on the Akaike information criterion (AIC) of model selection, the optimum variance-component models for two traits and haplotypes in two blocks were selected respectively. Indices YLN and MYLN are regarded as the adjusted covariates in the model fitting. The results show that significant association between the first haplotype block and degraded CPT index. The second haplotype block is associated to undegraded CPT index.

DISCUSSION

DISC1 gene has been known involved in major psychiatric illness, including schizophrenia (50-52). In our previous study, we have found that the D1S251 marker upstream the DISC1 gene has the maximum NPL score of 1.73 (p=0.03) in narrow phenotype model, and the highest peak NPL score of 2.18 (p=0.01) in the broad phenotype model (32). In exactly the same marker, the populations of British and Iceland have shown LOD scores of 2.5 (p=0.002) (31). These results suggest that there are positional candidate genes in this region susceptible to schizophrenia.

In further fine mapping study, the Finnish schizophrenia samples have shown a two SNP haplotype located between the intron 1 and exon 2 of the DISC1 gene significantly under-transmitted to affected individuals (p=0.0031) with gender differences (51). In this study, the result has suggested a two SNP haplotype between intron 4 and intron 5 of DISC1 gene significantly associated with schizophrenia (p<0.01). The exon1 and 2 encode the putative globular domains, and the exon3-13 encode the putative helical tail (coiled-coil motif) of DISC1 gene (30). The DISC1 181-357 domain (within exon 2) binds strongly with the cytoplasmic microtubules of α-tubulin, and the C-terminal coiled-coil motif is essential for interaction with neurodevelopmental protein Nudel (53). Therefore, we suspect that in different ethnic groups may have different

pathogenic mechanisms associated with DISC1 gene. There is no difference in our haplotype genetic transmission between the male and the female. Consistently in both studies agreed that the DISC1 gene is not associated with the onset of schizophrenia. Since we have rule out the possible existence of the balanced translocation in our schizophrenia DNA samples (submitted manuscript), we would confirm that the participation of DISC1 gene in our schizophrenia patients may be exerted through other mechanisms besides genetic disruption in the pathological process of schizophrenia.

Most documents have been noticed the 1q42.1 regions are important for the pathological cause of schizophrenia (51, 54-57). Here is the first report showing another gene located within this region may have also involved in the pathological process of schizophrenia, the dihydroxyacetone phosphate acyltransferase (DHAPAT or GNPAT). This enzyme has been shown deficiency of varying degrees than normal in the congenital peroxisomal disorders (58). In recessive peroxisomal disorder of cerebrohepatorenal syndrome (ZS), the patients do not survive beyond the first year, and their DHAPAT activity is found only 10% of normal control (59). In mental status of phenotype, a case report has shown severe mental retardation, developmental delay and growth failure in a DHAPAT deficient girl (60). Our result suggests that the GNPAT may not be a schizophrenia specific etiological factor, but may play a role in the broad definition of schizophrenia. Further works in our future study, we will examine the SNP markers located within the exons of GNPAT gene and analyze their association degree to schizophrenia.

The continuous performance test (CPT) has been used as a measure of sustained attention and demonstrated highly sensitive to brain damage or dysfunction (61). These brain dysfunction areas measured by CPT have demonstrated involving in the interaction of cortical (frontal, temporal, parietal), subcortical (limbic, basal ganglia), and functional systems including the pathways between the basal ganglia, thalamus, right hemisphere and frontal lobes (61). These brain area matches the DISC1 gene expression regions found in primates (62). In our previous study, we have found a heritability of performance on the CPT ranged from 0.48 to 0.62 in the schizophrenic patients of Taiwan (63). We have further identified the CPT as a marker of genetic susceptibility to schizophrenia due to reasons of the existence of CPT deficit in probands' CPT performance, and not amenable to neuroleptic treatment (64). In this study, we further demonstrate that the DISC1 gene haplotype associated with undegraded CPT and the GNPAT gene haplotype associated with degraded CPT. This suggests that the two genes linking to schizophrenia may have different pathological process of attention deficit. This result along with our another finding in the promoter region of DISC1 gene which significantly associated with attention deficit (submitted manuscript) suggest that the 1q42.1 region may be important in regulating the attention process in the pathophysiological dysfunction of schizophrenia.

The Wisconsin Card Sorting Test (WCST) is a test paradigm to detect working memory as an indicator for the function of human frontal lobe in schizophrenia (65, 66). In this study, the candidate genes of DISC1 and GNPAT have no significant association with the pathological process of working memory as tested by WCST. This result is different from the Finnish twin study which demonstrated significant association between the 1q D1S2833 marker and the spatial working memory performance measured by the Visual Span subtest (67). The D1S2833 (deCODE map position at 233.97 cM) and the D1S251 (deCODE map position at 235.23 cM) have a chromosomal distance over 1.26

cM beyond the screening region of this study. This study did not stratified our data to examine the association between the spatial working memory, we suspect other gene As the gene declared significantly associating with working memory is the muscarinic m1 receptor genetic polymorphism located at 11q13 (68). The 1q42.1 region may have less contribution on the pathological process of working memory in schizophrenia.

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FIGURE LEGENDS

Fig. 1. Linkage disequilibrium of all SNP markers showed two significant SNP blocks located within a block of 482, 485, 479, 488, and 489, and a block of 517 and 518.

Fig. 2. Blocks of SNPs located within genes of GNPAT and DISC1 showed significant linkage disequilibrium and associations among 9 other functional genes in the 2 cM ranges around D1S251 marker.

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