Summary
Schizophrenia is a devastating neuropsychiatric disorder affecting 1% of the world population and ranks as one of the disorders providing the most severe burden for society. Schizophrenia etiology remains obscure involving multi-risk factors, such as genetic, environmental, nutritional, and developmental factors. In Chapter 1 of the thesis, a general introduction of schizophrenia is presented including its historical and recent concept, diagnosis and symptoms, treatment and prognosis, and genetics. The main aim of the research presented in this thesis is to evaluate several schizophrenia candidate genes implied by newly accepted hypotheses or by GWAS. So, Chapter 2 to Chapter 5 present candidate gene-based association studies of schizophrenia, investigating four genes, STON2, MSI2, NAT2 and SOX11, respectively. In each association study, we have chosen SNP markers covering most of the gene-encoding region or a region related to the gene function.

To investigate the potential involvement of the STON2 gene (an endocytosic protein for synaptic vesicle protein recognition) in schizophrenia (Chapter 2), we examined this gene in a sample of 768 Chinese Han schizophrenia cases and 1347 Chinese Han controls. 11 SNPs were genotyped and statistically analyzed. The results showed that three SNPs were significantly associated with schizophrenia, of which two were located in exons (rs2241621: allelic p=0.0005; rs3813535: allelic p=0.0078) and one was intronic (rs9323698: allelic p=0.0019). When haplotype analysis was performed, two LD (linkage disequilibrium) blocks showed significant differences in frequency between cases and controls. Remarkably, we discovered an over-transmitted functional haplotype C-C (Pro307-Ala851) in schizophrenia cases. Our data imply that STON2 may be a schizophrenia susceptibility gene justifying further exploration of the role of STON2 in schizophrenia.

In Chapter 3 we investigated the association of N-acetyltransferase 2 (NAT2), an enzyme with a crucial role in xenobiotic metabolism, and schizophrenia, and genotyped six selected NAT2 exonic functional SNPs in a sample of 761 Chinese Han schizophrenia cases and 976 Chinese Han healthy controls. Functional NAT2 polymorphisms result in various enzyme acetylation phenotypes. Three SNPs (rs1801280T/341C, rs1799930/G590A and rs1208/A803G), one protective fast-acetylation haplotype (NAT2*4) and two risk slow-acetylation haplotypes (NAT2*5B and NAT2*6A) showed significant differences between case and control groups, indicating that NAT2 may be a susceptibility gene for schizophrenia in the Chinese Han population, and the risk haplotypes might cause NAT2 functional impairment in metabolizing neurotoxic substances.

A previous GWAS implied an association between the MSI2 gene and schizophrenia. MSI2 is a member of the Musashi family, playing a substantial role in neural stem-cell maintenance, asymmetric division and differentiation during neurogenesis. To further verify this association, in chapter 4, three MSI2 SNPs with the highest significance were selected for a replication study in an independent sample of 921 Chinese Han schizophrenia cases and 1244 Chinese Han healthy controls. Two SNPs (rs9892791 and rs11657292) showed remarkable differences in allele and genotype distribution frequencies between two groups. When combing GWAS and replication samples, three SNPs were all strongly associated with schizophrenia (rs9892791: allelic P = 1.07E-5; rs11657292: allelic P = 1.95E-12; rs1822381: allelic P = 1.44E-4). By downloading genotyping data from the 100 Genome Project, we identified linkage SNPs for the three SNPs. Several of the linkage SNPs have a significant cis-eQTL effect for MSI2 gene, suggesting that different variants of the schizophrenia-associated SNPs may affect the MSI2 expression level. Our data indicate MSI2 as a susceptibility gene for schizophrenia and encourage future research on the functional relationship between MSI2 and schizophrenia.
The human SOX11 gene has been indicated as a candidate gene for schizophrenia in our previous GWAS. SOX11 is a member of the developmentally essential SOX (Sry-related HMG box) transcription factor gene family and mapped to chromosome 2p, a potential candidate region for schizophrenia. To further investigate the association between SOX11 and schizophrenia (Chapter 5), we selected 15 SNPs along the SOX11 gene region for an independent replication study in a sample of 786 Chinese Han schizophrenia cases and 1348 Chinese Han healthy controls. Four SNPs showed a significant association with schizophrenia (rs16864067, allelic p=0.0022; rs12478711, allelic p=0.0009; rs2564045, allelic p=0.0027; and rs2252087, allelic p=0.0025). Two LD blocks were strongly associated with schizophrenia between two groups. These data pointed to SOX11 as a susceptibility gene for schizophrenia. We used a luciferase reporter gene assay in the mouse Neuro2A neuroblastoma cell line to assess the functionality of the 6 SNPs and found that the schizophrenia-associated SNPs located in SOX11 3’ near gene indeed do influence the level of expression of SOX11. SOX11 has been suggested as an regulatory transcription factor in the development of the CNS and the PNS. By in vitro cell culture and in utero electroporation, we further defined the role of SOX11 in neurodevelopment in mice (Chapter 6). Manipulating the expression of Sox11 in neural stem cells (NSCs) isolated from E14 C57BL/6J mice, demonstrated that Sox11 inhibits the proliferation and promotes the differentiation of the NSCs. Over-expressing and down-regulating Sox11 in mouse primary cortical neurons disturbed their neurite growth and cell maturation. Knockdown experiments in E14 mouse embryos using in utero electroporation showed that reduction of Sox11 resulted in delayed cortical radial neuronal migration and severely impaired neuronal leading process. These results indicated that the susceptibility gene for schizophrenia, SOX11, may affect the formation of schizophrenia associated neuronal circuitry during neurodevelopment. In the final Chapter 7, we focus on the transcription factor SOX2 which is critical for regulating self-renewal and homeostasis of NSCs during neurodevelopment. In mouse NSC cultures and in utero injection approaches we aimed to identify the downstream targets of SOX2 to explain underlying molecular mechanism. In this somewhat separated chapter, we reported that Sox2 directly up-regulates survivin, an inhibitor in mitochondria-dependent apoptotic pathway of neural stem cells, and found a novel Sox2/survivin pathway that regulates NSC survival and homoeostasis, thus revealing a new mechanism of brain development, neurological degeneration and such aging-related disorders.

Limitations and Future Studies

Although GWAS accomplish remarkable and exciting successes in discovering candidate genes and regions, guiding researchers into new biological insights into certain disorders, considerable limitations inherently exist in psychiatric genetic studies and hinder a more conclusive interpretation of these studies performed herein. For GWAS, there are several primary limitations: 1) genotype-phenotype association strategies may easily lead to false positive results due to the multiple comparisons performed; therefore, a quite large sample size is needed to reach an optimal statistical power; 2) GWAS is hypothesis-free and association does not equal causality; 3) Significant SNPs shown in GWAS usually have only moderate or weak effects with relatively low odds ratio compared to certain environmental factors and might be easily overlooked; 4) Costs for conducting GWAS are very high with limited potential of immediate clinical applicability. All these limitations require replication of the loci with the most significance or convinced hypothesis-based relativity in independent samples. Follow-up deep-going functional studies are necessary for interpreting the biological mechanisms.
For each individual association study, heterogeneous phenotype and individual risk variants of apparently small effect are the two primary limitations. Due to the combination of these two limitations, the ultimate goals of the genetic studies (rigorous evaluation and statistical interpretation) may be substantially affected in inadequate sample sizes. Typically, the sample collected in an association study is reasonably powered to detect an odds ratio of 1.5 under ideal circumstances, such as genotyping the actual risk variant. If considering detection of a realistic association, thousands of cases may be required for identifying statistical significance. For an individual test, the threshold is usually an alpha of 0.05, however, after correction; it is certainly inflated for a large scale study to extremely stringent. A majority of the associated SNPs detected were located in introns with unknown function, and may be not the causal SNPs but in linkage disequilibrium with the functional SNPs.

Our four genetic studies all revealed significant association of the relevant genes with schizophrenia, and represented a reasonable investigation of the common genetic risk to the disorder. However, these studies were all applied in the Chinese Han population, and further prospective replicated studies in other ethnic populations are required to confirm our positive results. If the variants associated with schizophrenia were to be evaluated further, sequencing for novel mutations of certain genes might provide the most beneficial prediction in discovering the causal loci and functional mechanisms. Future studies will take this approach to attentively evaluate the \textit{MSI2} and \textit{SOX11} genes. We have already constructed a conditional Sox11-deficient mouse model, and we will focus on three research directions: 1) observe the core events in neurodevelopment in the transgenic Sox11 deficient mouse model; 2) investigate the transcriptional profile of Sox11 in neurodevelopment based on Sox11 RNAi cDNA array analysis; 3) explore the related molecular mechanism of Sox11 regulation in neurodevelopment and neuroplasticity. We expect to provide important data revealing the regulatory function of the schizophrenia susceptibility gene \textit{SOX11} and its potential role in the etiology of schizophrenia.