行政院國家科學委員會專題研究計畫 期中進度報告

自閉症類疾患之臨床及分子基因學研究(1/3)
期中進度報告(完整版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 96-3112-B-002-033-
執 行 期 間 ：96 年 05 月 01 日至 97 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：高淑芬
共 同 主 持 人：宋維村、陳嘉祥、吳佑佑、廖漢文、黃玉書
丘彥南、蔡文哲

報 告 附 件 ：國外研究心得報告

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 97 年 03 月 13 日

行政院國家科學委員會補助專題研究計畫□ 成 果 報 告 ☑期中進度報告

自閉症類疾患之臨床及分子基因學研究(第一年)

計畫類別：☑ 個別型計畫　　□ 整合型計畫
計畫編號：NSC96-3112-B-002-033
執行期間：　96　年　5 月　1 日至　97　年　4 月　30 日

計畫主持人：高淑芬 副教授
共同主持人：宋維村 醫師、陳嘉祥 教授、吳佑佑 醫師、廖漢文 副教授、
黃玉書 醫師、丘彥南 醫師、蔡文哲 醫師
計畫參與人員： 林郁秀、徐玉容、梁郁芊、王齡萱

成果報告類型(依經費核定清單規定繳交)：□精簡報告　☑完整報告

本成果報告包括以下應繳交之附件：
☑赴國外出差或研習心得報告二份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：國立台灣大學醫學院精神科

中　華　民　國　九十七　年　三　月　十一　日

摘要

前言：自閉症是以人際社會情緒和溝通互動障礙和反覆狹窄的行為和興趣為主要表現的神經發展障礙，ICD-10 和 DSM-IV 以其症狀的數目和嚴重程度再次分為自閉症、艾斯柏格症、和非典型自閉症等，合稱自閉症類疾患（Autism Spectrum Disorder, ASD）。其盛行率約為千分之一，男女比率約為 4 比 1，ASD 有顯著家庭聚集現象，其遺傳率大於 90%, 顯示基因突變為其重要病因，因此發現自閉症之致病基因不僅有助於自閉症的診斷、病理研究與治療，且對自閉症的預防與遺傳諮詢十分重要。本研究將是國內第一個結合臨床、腦神經心理學、基因學多中心合作的自閉症研究。

目的：建立我國自閉症病患臨床症狀、腦神經心理學資料、家族樹及 DNA 資料庫，以分子遺傳學的方法找出自閉症的致病基因及其突變，並建立其臨床症狀的相關性。本研究計確切目標(specific aims)如下：

1. 準備多種中文版自閉症診斷量表及訪談工具;
2. 以結構化診斷工具(ADI-R, SCQ, SRS)建立 ASD 病患與其家屬臨床症狀與診斷資料庫、人口學資料、發育史及心理測驗資料;
3. 進行高功能 ASD 病患神經心理學功能的先導研究;
4. 建立我國 ASD 病患家族樹及 DNA 資料庫，以作為自閉症致病基因分子遺傳研究之基礎;
5. 以 family-based （parental control, trios） study 和 population-based case-control 的方式檢測一些與自閉症有關的候選基因，例如 Neuroligin gene family、Methyl-CpG-binding protein 2 (MeCP2) gene、FOXP2 gene 是否與我國自閉症病患者有相關性。

6.嘗試建立臨床症狀、神經心理學功能與基因型的相關性。

結果：本研究執行至今 (10 個月)，已完成多種自閉症診斷量表及訪談工具的翻譯，包括 ABC、AQ、CAST、SRS、SCQ、ADI-R 及 ADOS。本研究收案迄今已收集 100 個 ASD 家庭，所收集到的資料包括血液樣本、臨床心理以及腦神經心理學。目前血液樣本已完成細胞株培植。在血液分析方面，我們用高解析定序方法以第二外顯子序列來判定 HLA-DRB1 基因型。初步結果顯示，在 74 位自閉症患者與 97 個正常對照組的樣本中，HLA-DRB1 基因的頻率在 DR4、DR13、DR15 與正常對照組有差異，唯目前所顯示之差異形式與過去在高加索人種的研究不同。另外我們將 ACCN3 基因的十一個外顯子片段定序，已掃描是否有基因變異。目前在 103 位自閉症患者中發現有三個基因變異，包括在第一外顯子上第 229 核苷酸位置 A 到 T 的變異(c.229A>T)，第三外顯子上 G 到 T 的變異(c. 719 G>T)，以及第十個插入子上 T 到 C 的變異(IVS10+60T>C)。

討論：計畫第一年不但完成了預訂目標，更額外完成了 60 個個案家庭的資料收集。計畫第二年我們將以穩定的收案速度，繼續收集基因及臨床相關資料。當資料收集、整理完畢後，我們將會陸續發表臨床方面或基因分析的相關論文，希望透過我們的努力能夠找到與自閉症有關的致病基因。

關鍵詞：自閉症類疾患、家族遺傳研究、基因、表現型、內在性表現型

Abstract

Background: Autism is a pervasive neurodevelopmental disorder with prominent reciprocal social and communication impairment and restricted repetitive behavior or interest. Based on the number of symptoms and functional impairment, autistic disorder, Asperger disorder, and atypical autism are conceptualized as the autism spectrum disorder (ASD). Most recent survey estimated the prevalence of narrow diagnosis of autistic disorder to be around 0.1% to 0.2%, and 0.59 % to 0.63% for ASD, with a four-fold male predominance. Due to high heritability (> 0.9), high family recurrence risk ($\lambda = 60$), and severe impairment without effective prevention and treatment available for ASD, this disastrous disease has been prioritized for molecular genetic study from public health perspective. The proposed research is the first systematic approach combining clinical and molecular genetic study of ASD.

Objectives:

1. to establish the psychometric properties of three Chinese versions of rating scales for ASD: SCQ, SRS, and ABC;
2. to collect clinical, neuropsychological, and genetic data of ASD probands and their family; and
3. to identify the genetic variants close to etiological genes of ASD in a Taiwanese sample using candidate gene case-control association study design (e.g., Neuroligin gene family, MeCP2 gene, and FOXP2 gene, parent trio and population-based studies) and whole genome linkage analysis for multiplex families.

Results: We have translated ABC, AQ, CAST, SRS, SCQ, ADI-R and ADOS into Chinese and have successfully collected clinical, neuropsychological, and genetic data from 100 families. We also have done two preliminary genetic analyses: (1) Our association study on the HLA-DRB1 genotyping in 74 probands and 97 controls revealed that DR4, DR13, and DR15 had significantly different frequency between probands and controls. (2) Our mutation screening showed 3 single nucleotide polymorphisms in 11 exons of ACCN3 gene, including a A-to-T substitution at exon 1 (c.229A>T), a G-to-T substitution at exon 3 (c.719G>T), and a T-to-C substitution at intron10 (IVS10+60T>C).

Discussions: We have advanced data collection of additional 60 families. We will continue to work hard to assess more ASD families, to add ADOS into our standard assessment of ASD, and to conduct genetic analysis on the available sample.

Keywords：autism spectrum disorder, family genetic study, gene, phenotype, endophenotype

目錄

Introduction (前言)

Autism is a pervasive neurodevelopmental disorder with prominent reciprocal social and communication impairment and restricted repetitive behavior or interest. Based on the number of symptoms and functional impairment, autistic disorder, Asperger disorder, and atypical autism (or PDDNOS) are conceptualized as the autism spectrum disorder (ASD). Most recent survey estimated the prevalence of narrow diagnosis of autistic disorder to be around 0.1% to 0.2%, and 0.59 % to 0.63% for ASD, with a four-fold male predominance. Due to high heritability (> 0.9), high family recurrence risk (λ = 60), and severe impairment without effective prevention and treatment available for ASD, this disastrous disease has been prioritized for molecular genetic study from public health perspective.

The ultimate goals of this study are to prepare several key internationally recognized measures on studying autism, to establish the clinical data among a wide age range of children and adolescents with ASD, to validate the phenotypes and endophenotypes that are closer to the biological expression of genes underlying ASD, and to identify the genetic variants close to etiological genes of ASD in a Taiwanese sample. We propose to replicate the analysis of the candidate genes identified by previous genetic studies on ASD using the candidate gene association study design (family-based case control study using parental controls, and population-based case control study) to validate the findings from other research groups. With the accomplishment of these goals, this study will resolve controversies over inconsistent findings in previous genetic studies and contribute to the literature on the validity of ASD using clinical and genetic data. The proposed research is the first systematic approach combining clinical and molecular genetic study of ASD involving multi-sites and three research cores: assessment core (by Gau SS and Wu YY), molecular genetics core (by Chen CH), and data/statistics core (by Gau SS).

Objective

The **specific aims** of this study are:

1. Perform Psychometric Studies of three Chinese versions of rating scales for ASD: To prepare the Chinese versions of the Autism Diagnostic Interview-Revised (ADI-R), Autism Diagnostic Observation Schedule (ADOS), Social Communication Questionnaire (SCQ), Social Reciprocity Scale (SRS),and Childhood Asperger Syndrome Test (CAST) following the standard process of psychometric studies to establish the reliability and validity of the three measures.
2. Phenotype and Endophenotype Research:
   a. To ascertain a cohort of children and adolescents (n = 300) aged 3-17 with ASD;
   b. To explore the potential peri-natal and developmental factors on the risk of ASD;
   c. To collect information about the symptomatology, language development, social reciprocity, verbal and non-verbal communication, intelligence, neuropsychological functioning, parental autistic feature, and parental psychopathology among individuals with ASD to establish the first clinical data of ASD in Taiwan;
   d. To validate the subgroup of ASD using psychopathological, neuropsychological, developmental, behavioral, familial and social correlates; and
   e. To conduct a pilot study on brain imaging studies combined with a wealthy of measures of neuropsychological functioning to test whether neuropsychological and brain imaging findings can be the intermediate phenotype of ASD for genetic studies.
3. Genetic Analysis: The collected DNA samples will be subjected to molecular genetic studies including (1) replication study of the positive genetic findings from literature; (2) performance of candidate gene analysis such as FRAXA gene, neuroligin family genes, Mecp2 gene, FOXP2 gene, etc; (3) using high throughput SNP and microsatellite genotyping to perform family and population-based association study; and (3) whole genome linkage analysis for multiplex families.

The **Objectives** of the first year are:

1. Psychometric Studies of three Chinese versions of rating scales for ASD:

To prepare the Chinese versions of the ADI-R, ADOS, SCQ, CAST, and SRS following the standard process of psychometric studies to establish the reliability and validity of these measures.

2. ADI-R interview training:

To train key investigators and research assistants using ADI-R interview.

3. Phenotype and Endophenotype Research:

To ascertain a cohort of children and adolescents (n = 40) aged 3-17 with ASD and have a complete clinical and neuropsychological assessments and blood sample collection.

Background (文獻探討)

Autism is a pervasive, multi-factorial, highly heritable, clinically heterogeneous neuro-developmental disorder with prominent reciprocal social and communication impairment and restricted repetitive behavior or interest.[1] Infantile autism and autistic psychopathy were first described by Kanner in 1943[2] and Asperger in 1944,[3] respectively. The diagnosis of pervasive developmental disorder (PDD) including autistic disorder, Asperger's disorder, Rett's disorder, disintegrative disorder, and atypical autism or PDD Not Otherwise Specified (PDDNOS),[1, 4] has reached consensus in the two main psychiatric diagnosis systems, DSM-IV[1] and ICD-10.[4] Since the autistic social impairment has genetic origin[5] and a unimodal distribution in the twin population,[6] and Rett's disorder has been known to have a clear association with mutation of MECP2 gene,[7] the PDDs include in this proposal, autistic disorder, Asperger's disorder, and atypical autism, are called autism spectrum disorder (ASD).

Children with ASD may also manifest symptoms of impaired adaptive and cognitive functioning, unusual mood and sensory responses, repetitive behaviors, and attention problems; and tend to have sleep problems, and medical conditions.[8] In addition to the typical autistic features, children with autism have been reported to have more general emotional or behavioral symptoms such as thought problems,[9, 10] social problems,[11] attention/hyperactivity problem,[10] disruptive behaviors,[12] and maladaptive behaviors.[13, 14]

A systemic review of the prevalence of autism shows the median prevalence estimate of 5.2 per 10,000 children[15] with increased prevalence of ASD around 60 per 10,000 children in recent decade.[16, 17] Most recent survey estimated the prevalence of narrow diagnosis of autistic disorder to be around 0.1% to 0.2%, and 0.59 % to 0.63% for ASD,[18, 19] with a four-fold male predominance.[15, 17] The symptoms often occur and are diagnosed in early childhood and continue throughout life.[16]

A. Genetic Studies

Evidence from twin and family studies suggests that genetic factor plays an important role in the etiology of autism without evident underlying mechanism.[20-23] Familial segregation studies revealed the recurrence risk to siblings, estimated from multiple studies at 1%-3%, is between 50 and 150 times greater than that in the general population.[24, 25] Twin studies revealed a 60% to 90% concordance rate in monozygotic twins, and 0% to 10% in dizygotic twins.[26-28] These epidemiological evidences suggest a non-Mendelian mode of inheritance involving 2-10 genes,[29] or up to 15 loci[30] which interact with environmental factors to produce different presentations of ASD. The presence of genetic heterogeneity is very likely to exist. Moreover, the association of ASD with several genetic disorders, including tuberous sclerosis, fragile X syndrome, neurofibromatosis, Angelman syndrome, Prader-Willi syndrome, and untreated phenylketonuria, also suggested high importance of genetic component to autistic pathology.

There were three broad research domains in genetic research for autism: positional cloning, candidate disease gene studies, and the delineation of chromosomal abnormalities.[31]

First of all, linkage and association studies were used complementarily to identify chromosomal regions closely linked to ASD, so these regions might harbor related disease genes. To date, there are 13 published genome-wide scans of ASD.[32-44] Findings of linkage are inconsistent, and regions on 18 out of the 23 pairs of chromosomes have been reported. However, several "hot regions" have been reported across studies, among them 2q, 3q, 7q, 15q11-q13, and 17q to be the most often reported ones. However, by investigating around 50-200 affected sibling pairs (ASP) or affected relative pairs (ARP), the LOD

scores yielded were often around 2 to 3; on dividing sample according to intermediate phenotype, or using more strict criteria on defining autistic disorder, the LOD scores may approach 3 to 4.[31, 45, 46] These convergent data do suggest several regions containing candidate genes contributing to ASD. However, the strategies used to yield better results, such as grouping of samples, expanding of samples, and further stratification of samples, implies that phenotypic and genetic heterogeneity contributes much to the inconsistent finding across studies, which should be dealt with properly in our future study designs.

Second, numerous candidate gene studies were done in past 5 to 6 years, soon after the reports of several linkage and association studies. Candidate genes were selected by its proximity to the "hot region" detected, and its possible involvement in the biological pathoplastic process of ASD. Near a hundred candidate genes were reported for its association with ASD.[31, 46, 47] Yet similarly, there were still no proved consistent findings across studies. Nevertheless, some possible "hot candidates" were identified, and most of them showed biological relevance to the process of neurotransmission, neurodevelopment and CNS patterning, and synaptic assembly and dendritic development.[47]

I. Those related to neurotransmitter systems:

A. GABAergic systems: GABA-A receptor subunits (GABRB3, GABRG3, and GABRA5)[48-50]
B. Serotonergic systems: Serotonin transporter (SLC6A4)[51-63]
C. Glutamatergic systems: Glutamate receptor subunit CluR6 (GRIK2)[64]
D. Catecholamines: The maternal modifier effect at dopamine beta-hydroxylase (DBH) and MAO-A loci[65]
E. Acetylcholine: cholinergic receptor (CHRNA1)[66, 67]

II. Those related to central nervous system development:

A. Genes patterning the central nervous system: Engrailed-2 (EN2),[68] Wingless-Int-2 (Wnt2)[69]
B. Neuromigration: Reelin (RELN)[70-77]
C. Synaptic assembly and dendritic development: Neuroligin-3 and 4 (NLGN3, NLGN4),[78-82] brain-derived neurotrophic factor (BDNF), and methyl-CpG-binding protein 2 (MeCP2)[83, 84]

III. Other candidate genes: Hox genes (HOXA1, HOXB1),[85, 86] the mitochondrial aspartate/glutamate carrier (SLC25A12/AGC1),[87, 88] AVP receptor 1a (AVPR1A),[89] and FOXP2.[90]

The evidence for each gene is weakened by the contradictory internal consistencies and by the lack of support from independent studies.[31] For future studies, focus should not be put solely on the mutations in the coding regions, but to be shifted to the non-coding sequences (which may serves as regulatory components). Furthermore, investigating several genes at a time, as well as their gene-gene interactions, should also be considered.

Lastly, in 3-5% of cases, autism is attributable to gross chromosomal abnormalities, based on karyotyping examinations.[91, 92] Overlap between chromosomal abnormalities and autism has mainly be found over the chromosome region 15q11-q13,[93] 22q11.2,[93] and 7q.[94] Currently, more advanced molecular techniques, including fluorescent in situ hybridization, microarray-based comparative genomic hybridization, representational oligonucleotide microarray analysis, and SNP-based arrays were applied to the detection of chromosomal abnormalities in ASD. Such ways of investigation complements positional

cloning in its assistance of identifying disease genes (which might escape from the detection of linkage studies or are obscured by epigenetic phenomena), and in further elucidation of possible underlying molecular mechanisms.

In conclusion, although abundant evidence has shown significant association of genetic factors in the generation of ASD, no definite conclusion regarding associated genetic mechanisms can be reached. Several further points not addressed above include:

I. The major problem arises from phenotypic and genetic heterogeneity. To deal with this, the use of intermediate phenotype/endophenotype, including language function, social impairment, restricted and repetitive behaviors, serum serotonin level, head circumference, level of functioning (IQ, adaptive behaviors), savant ability, and the presence of seizure, is proposed by several recent experienced researchers.[31, 95] This strategy helps to stratify the sample into more homogenous groups, and to detect less severe pathologies in the relatives of probands. Treating phenotype as quantitative traits is another effective strategy to achieve the above goal.[96]
II. Ethnic difference should be carefully considered. Previous findings sometimes failed to be replicated in another sample of different ethnic background. Moreover, only few findings were reported from the Hans population until recently.[61, 97-99]
III. Statistical strategies to deal with heterogeneity should be employed, such as posterior probability of linkage, and ordered subset analysis.[31]

B. Neurocognitive Functioning

B.1 Cognitive profiles in autism

ASD is often characterized by unevenly developed cognitive skills in terms of IQ profiles. Although it has been noted that nonverbal, particularly visuospatial abilities, superior to verbal is strongly associated with autism,[100] this profile is not universal among individuals with autism, and is not even necessarily the cognitive model in autism.[101] Moreover, higher-functioning individuals with autism often evidence verbal abilities that are superior to their visuospatial skills in IQ testing.[102, 103]

Children with typical autism demonstrate high rate of verbal-nonverbal discrepancies,[104, 105] which is related to severity of symptoms.[105, 106] The imbalance in cognitive abilities presented by verbal-nonverbal discrepancies, particularly when verbal better than nonverbal, may reflect a particularly severe disturbance in brain development and organization and may provide a marker for an etiologically significant subtype of autism.[104] It has been argued that preserved visuospatial skills is an outcome of fundamental differences in neurocognitive development and organization, rather than a selective sparing of normal cognitive capacities and their neurobiological substrates.[107-109] For example, failure of the normal propensity for "central coherence"[110, 111] or the abnormal development of lower-level perceptual process.[112-114] Moreover, evidence also links the poorer verbal than nonverbal profile in autism to enlarged brain volume in addition to enlarged head circumference.[105]

B. 2 Executive function in autism

Three dimensions of executive functions (EF) are: (i) inhibition, (ii) flexibility, and (iii) working memory (or updating).[115, 116] Over the past 2 decades several studies have shown the impaired EFs among individuals with ASD assessed by the Wisconsin Card Sorting Test (WCST),[117] Tower of Hanoi,[118] and Trail Making Test.[119-126] Individuals with ASD demonstrated impairment in **planning** using the Tower of London as compared to patients with dyslexia, ADHD, and Tourette syndrome[120, 122, 127-130] as well as to age-matched normal developing individuals[122] and using the Stockings of Cambridge.[131] Regarding **mental flexibility** as illustrated by perseverative responses and difficulties in shifting to a different thought or action according to situational changes, individuals with ASD demonstrated poor mental flexibility in the WCST in Western samples[128, 131] and non-Western sample as well.[132] The deficit on the WCST tends to maintain over time. [129] Moreover, autistic individuals also have worse performance in the final stage of Intradimensional-Extradimensional shift (ID/ED shift) task.[131] Concerning **inhibition**, individuals with ASD also demonstrate greater deficits in the Stroop task,[122, 128, 133] Go/No-Go task,[129] and Windows and Detour-Reaching tasks.[134-137] **Semantic fluency** is assumed as an area of strength for individuals with ASD, who generate as many words from a given category as typically developing children,[103, 138, 139] although they may include a higher number of uncommon category members than expecte.[139] However, this finding was not supported by Turner,[140] who reported impaired on category fluency among individuals with high functioning autism.

In contrast, Pascualvaca et al. (1998)[141] did not find that individuals with ASD showed deficits in the tests of the CPT, a digit cancellation task, WCST, or other two tests of shifting attention. A most recent study examining several aspects of EF showed that individuals with ASD had below average performance in EF and deficits in complex verbal tasks that required cognitive switching and initiation of efficient lexical retrieval strategies but intact cognitive inhibition.[142]

C. Neuroimaging studies on autism

C. 1 Brain volume: Studies on the structural magnetic resonance imaging (MRI) have demonstrated inconsistent findings in the total and regional brain structure and volumes of ASD. Some studies support the neuropathologic findings in limbic[143-145] and cerebellar regions;[146-149] however, others have not replicated these results.[150-154] MRI studies of young autistic children show that by 2 to 4 years of age mean brain volume is increased and megalencephaly is present in some of the children,[155, 156] while about one-third of children with autism who are macrocephalic at birth or during infancy are not macrocephalic later in childhood.[157-161] From later childhood to adulthood, the difference in mean total brain volume progressively decreases;[162]. Most studies of total brain volume in adults find no significant difference. Few studies have examined the meaning of increased total brain volume showing an association of increased rate of head growth during infancy with more impairment later in childhood[163] but in contrast with higher levels of adaptive functioning in another study.[164]

Several studies revealed increased cerebellar volume, decreased size of posterior corpus callosum, enlargement of amygdale,[165] increased cerebral gray and white matter volumes at very early childhood,[155] particularly in white matter of the frontal lobe,[166] and an increase in white matter but decrease in cerebral cortical gray matter at later childhood.[167] Despite no longer increased global white matter after childhood, the early increase in gray matter seems to persist until mid-adulthood. Furthermore, a recent study revealed extensive regions of white matter deficit, mainly in left frontal-temporal and frontal-occipital regions.[168]

C. 2 Magnetic resonance spectroscopy (MRS):

Additional evidence from magnetic resonance spectroscopy (MRS) is beginning to illuminate metabolic aspects of this picture. Both ^{31}P-MRS,[169] and ^{1}H-MRS studies have shown abnormalities in frontal, [169, 170] lateral temporal[171] and medial temporal lobes,[172] and cerebellum[170, 172] in children with autism. Reduced NAA, creatine, and myo-inositol (mI) have been found in young children, aged 3-4, with ASD.[173] Older children (5-16 years old) showed a more mixed pattern, a decrease in choline in left cingulate, and an increase in right caudate.[174] Moreover, two studies have demonstrated an association between symptom severity and abnormal ^{1}H-MRS metabolite ratios in the temporal lobes of autistic subjects[175] and in the prefrontal region of Asperger's subjects.[176] Friedman and his colleague (2006)[177] reported reduced gray matter choline, creatine, NAA, and mI, prolonged transverse relaxation of choline in gray matter, and reduced choline, mI and trend-level NAA in gray matter in individuals with ASD. However, children with ASD and children with developmental delay showed a similar pattern of decreased NAA and mI levels in white matter.

C. 3 Diffusion tensor imaging:

Diffusion tensor imaging is a non-invasive tool to study white matter (the main conduit of connectivity), using the bio-physical properties of water diffusion in the brain, to assess aspects of the micro-anatomic integrity of white matter and to examine the macroscopic anatomy of white matter fiber bundles and tracts.[178] It has been applied in studying neuropsychiatric disease recently,[179-181] and there was few reports in autism.[182] In children with autism, reduced fractional anisotropy (FA) values were observed in white matter adjacent to the ventromedial prefrontal cortices and in the anterior cingulated gyri as well as in the temporoparietal junctions. Additional clusters of reduced FA values were seen in adjacent to the superior temporal sulcus bilaterally, in the temporal lobes approaching the amygdale bilaterally, in occipitotemporal tracts, and in the corpus callosum. Extensive, widely distributed abnormalities of white matter in 7 individuals with autism suggest a disruption of the micro-integrity of white matter, particularly in prefrontal and temporal-parietal regions.[182]

Methods

I. Participants

We have completed the clinical assessment (ADOS excluded) and blood collection of 100 probands (9 females; mean age±SD, 8.98±4.5) and their parents and siblings from Chung Gung Memorial Hospital (CGMH) and Children's Mental Health Center (CMHC) in Nation Taiwan University Hospital (NTUH).

I.1 Inclusion Criteria

The inclusion criteria for the proband subjects are (1) that subjects have a clinical diagnosis of autistic disorder, Asperger disorder, or atypical autism defined by the DSM-IV and ICD-10, which was made by a full-time board-certificated child psychiatrist at the first visit and following visits; (2) their ages range from 3 to 18 when we conduct the study; (3) subjects have at least one biological parent; (4) both parents are Han Chinese; and (5) subjects and their biological parents (and siblings if any) consent to participate in this study for complete phenotype assessments and blood withdraw for genetic study.

I.2 Exclusion Criteria

The proband subjects will be excluded from the study if they currently meet criteria or have a history of the following condition as defined by DSM-IV: Shizophrenia, Schizoaffective Disorder, or Organic Psychosis. Moreover, the subjects will also be excluded from the study if they completely cannot cooperate with blood withdrawal, collection of saliva, or buccal swabs.

II. Interviews for Diagnosis and Measures

II.1 Interviews for Diagnosis of ASD

II.1.1 Autism-specific developmental history and current presentation:

The Autism Diagnostic Interview-Revised (ADI-R):[183] The ADI-R, a recently modified version of the Autism Diagnostic Interview.[184] The ADI-R is a standardized, comprehensive, semi-structured, investigator-based interview covering most developmental and behavioral aspects of ASD. It is administered to the child's caregiver. Diagnostic assignment is made following a diagnostic algorithm for the DSM-IV[1] and the ICD-10 definition of autism.[4] The ADI-R is appropriate for interviewing caregivers of children with a mental age from about 18 months into adulthood. The ADI-R requires approximately 2 to 3 hours to complete with the caregiver and typically is videotaped for later scoring. The scoring yields summary scores of qualitative impairments in reciprocal social interaction, communication, and repetitive behaviors and stereotyped patterns. Published values for inter-rater reliability are good, with Kappas ranging from 0.62 to 0.89. Cut-off scores are available for making the diagnosis of autism versus individuals without a diagnosis of autism. Dr. Lord and her colleagues at the University of Chicago are conducting further research to examine ADI-R comparisons between well-defined autistic samples and other groups with pervasive developmental disorders.[185] The ADI-R also provides a dimensional measure of severity of autistic symptomatology, a quantified measure of social disability. Six clusters of variables are generated: spoken language, social intent, compulsions, developmental milestones, savant skills, and sensory aversions.[186] The co-PI (Wu YY), trained by Dr. Lord and certified to use the ADI-R, is an experienced ADI-R interviewer. The PI (Gau SS) and two co-PI (Liu SK and Lin LY) will get training by Dr. Lord and be certified to use the ADI-R before the

implementation of this study. The four investigators will be in charge of training of ADI-R in Taiwan. This instrument has been translating to Chinese and will be tested for the reliability and validity by the child psychiatrist investigators of this study.

II.2 Diagnostic Interview for Other Psychiatric Diagnosis than ASD:

II.2.1 Chinese version of the Kiddie-Schedule for Affective Disorders and Schizophrenia - epidemiology version (K-SADS-E). The K-SADS-E interview scale will be used to interview parents or caregivers by investigators or well-trained research assistants with extensive clinical experience in child psychiatry. Development of the Chinese K-SADS-E was completed by the Child Psychiatry Research Group in Taiwan.[189] This included a two-stage translation and modification of several items with psycholinguistic equivalents relevant to the Taiwanese culture. Previous studies have shown that the Chinese K-SADS-E is a reliable and valid instrument to assess child psychiatric disorders in Taiwan and has been used extensively in a variety of studies regarding childhood mental disorders in Taiwan.[189, 190]

II.3 Self-administered Questionnaire for Assisting Diagnostic Characterization of ASD

II.3.1 Social Reciprocity Scale (SRS).[5] The SRS is a 65-item questionnaire that inquires about the child's social interactions with others. Thirty-five items are directly related to reciprocal social behavior (criterion "a" for autistic disorder), 6 items are related to language deficits (criterion "b"), 20 items represent criterion "c", and 4 items inquire about miscellaneous symptoms. All items are rated on a scale from 0 (not true) to 3 (almost always true), based on the frequency of the behavior. The SRS can be completed by a parent or other adult (e.g., teachers) who routinely observes the child's social interactions with peers and adults in 15-20 minutes. The SRS is highly correlated with ADI-R algorithm scores for DSM-IV criterion and exhibited good inter-rater reliability.[191] The SRS has been shown to provide a continuous measure of social disability that has important implications for genetic research.[192]

II.3.2 Social Communication Questionnaire (**SCQ**):[193] The SCQ formerly know as the Autism Screening Questionnaire[193] is a 40 item questionnaire that is based on the (required) probes from the first version of the Autism Diagnostic Interview.[184] The SCQ measures the symptomatology associated with ASD focusing on the behaviors that are rare in non-affected individuals: reciprocal social interaction, communication, repetitive and stereotyped behaviors. It has been used as a screening tool and been shown to have good criterion reliability with the ADI-R.[193] The SCQ can be used to measure the progress of children with ASD using parents as informants.[194]

II.4 Measurement of Other Behavioral Symptoms:

Child Behavior Checklist (CBCL): Achenbach's questionnaires of the CBCL is a parental report concerning their children aged 4-18.[198] Eight narrow-band behavioral problems are derived from the 118 emotional and behavioral items of the CBCL,[198] including the symptoms of the anxious/depressed, attention problems, aggressive behaviors, delinquent behavior, social problems, thought problems, somatic complaints, and withdrawn. Two broad-band dimensions are termed internalizing and

externalizing syndromes. Each item is scored as 0 if not true, 1 if somewhat or sometimes true, and 2 if very true or often true. The Chinese version of the CBCL, a reliable and valid instrument, has been widely used in child and adolescent research in Taiwan[199-201] The CBCL has been used to measure the behavioral problems,[9, 10] or to validate the measures about social behaviors,[11, 14] or to distinguish the children with autism from those with ADHD or those with normal development[9, 10].

II.5 Intelligence:

II.5.1 Raven's Progressive Matrices.[208] The Raven's is a nonverbal test of reasoning ability based on figural test stimuli. The test measures the ability to form comparisons, to reason by analogy, and to organize spatial perceptions into systematically related wholes. The Raven's was chosen as a measure of nonverbal IQ for the molecular genetics project because of the availability of 3 sets of stimuli – the Colored Progressive Matrices (CPM), and the Standard Progressive Matrices (SPM) – provide an equivalent measure from ages 5 to adolescence. Validity coefficients with intelligence tests are between .50 and .80.[209] The norms of CPM and SPM have been established in Taiwan.

II.5.2 Wechsler Intelligence Scale for Children-3rd edition (WISC-III). The WISC-III[212] has been widely used to assess full-scale intelligence levels of children aged 6 years to 16 years, 11 months. The WISC-III is composed of 13 subtests to test children's cognitive ability of different dimensions, which are grouped into two scores: performance IQ score (7 subtests: Picture Completion, Block Design, Object Assembly, Picture Arrangement, Coding, Symbol Search and Mazes subtests) and verbal IQ score (Information, Comprehension, Arithmetic, Similarities, Digit Span and Vocabulary subtests). Four composite subscales are generated: (1) Verbal Comprehension: Information, Similarities, Vocabulary, and Comprehension; (2) Perceptual Organization: Picture Completion, Picture Arrangement, Block Design, and Object Assembly; (3) Freedom From Distractibility: Arithmetic and Digit span; and (4) Process Speed: Coding and Symbol Search.[213]

II.5.3 Wechsler Primary and Preschool Scale of Intelligence-Revised (WPPSI-R). The Chinese WPPSI-R is the most recent standardized intelligence test for young children age from 3 to 7 1/2 years old in Taiwan. The Chinese WPPSI-R demonstrates the same subtests as the English version, except the maze subtest is replaced by the matrix subtest. The correlations between the performance subtests and performance scale range from 0.44~0.58, and between verbal subtests and verbal scale ranged from .059~0.63. The test-retest reliability of the PIQ, VIQ, FSIQ are high (γ =0.89, γ = 0.88 and r = 0.91, respectively).

II.6 Neuropsychological Assessment:

The neuropsychological assessment views to cover (a) constructs that are essential for neuropsychological interpretation of other tests (e.g., handedness), and (b) clusters of constructs organized in terms of verbal and nonverbal functioning according to hypotheses regarding the neuropsychological correlates of high-functioning autism and Asperger's syndrome. Except the handedness, only selected subjects aged older than 10 will receive the neuropsychological tests (n = 40 subjects). Many of the neuropsychological constructs and measures are sometimes not applicable to younger and/or lower functioning subjects.

II.6.1 Continuous Performance Test (CPT).

The CPT computerized task which required tabbing on the space key when any character besides *X* shown on the screen.[215] The CPT has been widely used in the studies on ADHD[216] and schizophrenia and rarely used in the studies on autism.[141] The PI has done extensive research on the neurocognitive function among adolescents with ADHD.

II.6.2 Wisconsin Card Sorting Test (WCST).

The WCST is used to assess the ability to form abstract concepts, and shift and maintain the set. Examples of such indices include the number of perseverance responses, the number of perseverative errors, the failure to maintain the set, and the number of categories achieved. The WCST will be used to assess the mental flexibility in child subjects.[119, 132, 141]

II.7 Assessment of Parents

Fathers (n = 300) and mothers (n = 300) of the probands of ASD will reports on the following two questions on themselves.

II.7.1 Chinese version of the Adult Self-Report Inventory-4 (ASRI). The ASRI, a 135-item self report or interview scale, is derived from Youth Self-Report Inventory[221] for the purpose of making the DSM-IV referenced psychiatric diagnosis in adults. The reliability and validity of the Chinese ASRI was examined in a sample of 2731 young adults. The sample for test-retest reliability at a 4-week interval was 78. The test-retest reliability of subscales revealed that ICC ranged from 0.64 (CD) to 0.98 (ADHD). The internal consistency of subscales ranged from 0.60 (bulimia) to 0.85 (borderline personality disorder). The kappa value for binary diagnosis ranged from 0.32 (major depression) to 1.0 (post-traumatic stress disorder). The validity of the ASRI will be further examined by the PI.

II.7.2 Autism-Spectrum Quotient (AQ). The AQ, a 50-item self-administered questionnaire, was developed by Baron-Cohen et al. for screening normal intelligent adolescents and adults with high functioning pervasive developmental disorder (HPDD).[222] The AQ consists of five subscales: social skill, attention switching, attention to detail, communication, and imagination. AQ score ranges from 0 to 50, with a higher AQ score indicating higher autistic tendency. Dr. Chang HL at CCCH obtained written permission from Dr. Baron-Cohen in April 2005 to translate the AQ into Chinese. The test-retest reliability with a 2-week interval in 20 undergraduate students was satisfactory (Pearson correlation coefficients = 0.78).[222]

III. Procedure

A face-to-face explanation of the purpose and procedure of this study and reassurance of confidentiality was performed and the written informed consent from parents and child assent (if appropriate) must be obtained before the recruitment of subjects.

Then, the probands and their siblings were assessed using the WISC-III (or WPPSI-R), WCST, CPT, RPM, and self-reported measures. Their parents were interviewed using the ADI-R and K-SADS-E to reports on their children and completed self-reported measures about themselves and their children. The mother report child questionnaire including 12 measures which are basic information about children, pregnant history, labor history, the SCQ, SRS, CBCL, SNAP-IV and sleep questionnaire. If the age of their

children is between 1.5 to 5 years old, they have to complete CBCL for 1.5 to 5 years old. Parents also need to complete the questionnaire about their psychopathology (ASRI-4) and degree of autistic tendency (AQ). After these assessments are completed, each ADI-R and K-SADS-E interview was reviewed by another interviewer independently. The doctor in charge also made the best estimate of each psychiatric diagnosis for children. The reports of clinical and neuropsychological assessments were provided to the participants after doubly checked by the investigators. An explanation of the assessment results was provided at clinic by attending investigators.

IV. DNA Preparation

Genomic DNA was prepared from peripheral blood cells, transformed lymphocyte cell line, or saliva. Up to now, there are totally 312 subjects with transformed cell line; among them, 48 families have cell lines of probands and their parents (trios sample).

IV.1. HLA-DRB1 Genotyping:

HLA-DRB1 gene is located in 6p21.3 within a highly polymorphic region. It has 6 exons, and the second exon probably has most variants. Genotyping of HLA DRB1 exon 2 was determined by high-resolution sequencing-based typing (AlleleSEQR HLA DRB1 kit, Atria Genetics) and was compared with that of healthy controls. We have conducted a case-control association study regarding HLA-DRB1 in 100 autistic cases and healthy controls.)

IV.2. Mutation Screening of ACCN3:

We systematically screened the protein-coding region of the ACCN3 gene in a sample of 103 autistic patients using direct sequencing. Each exon was PCR amplified and subjected to autosequecing

Results

I. Translation, Preparation of Instruments:

I.1. We have translated the ADI-R (88 pages) into Chinese. The Chinese version of the ADI-R has been approved and permitted by the Western Psychological Services (WPS) to be used in our study. The PI (Gau SS) and the co-PI of the other three sites (Wu YY, Liu SK and Lin LY) got the ADI-R training by Dr. Lord's team.

I.2. We have translated the ADOS (60 Pages) into Chinese and got permission from the WPS to continue working on the back-translation, which will be completed in February. We should be able to start the ADOS training of the investigators in May and for research assistants in summer 2008.

I.3. We have prepared the Chinese version of the Autism Behavior History Questionnaire (ABC), Autism-Spectrum Quotient (AQ), Childhood Asperger Syndrome Test (CAST), Social Responsiveness Scale (SRS) and Questionnaire on Social Communication (SCQ) and conducted the pilot studies of these instruments in clinical subjects.

I.4. Interview and neuropsychological tests training:

Dr. Gau and Dr. Wu have conducted intensive interview training and inter-rater reliability for psychiatrists, psychologists and three research assistants (RAs) on ADI-R and K-SADS-E (only attention deficit/ hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), and conduct disorder (CD)). The interview training included basic reading about the phenotypes of ASD, the development of ADI-R and ADOS, and the detailed content of the two standard research instruments. The training process followed the standard procedure of interview training conducted by Dr. Lord at University of Michigan. After the training, each trainee needed to provide at least three videotaped interviews to the trainer to ensure accuracy of interview procedure and to establish the inter-rater reliability with other investigators and the trainers. The inter-rater reliability maintains within and across the four study sites. At least 20 interviews will be videotaped (audiotaped if necessary), after obtaining the informed consent from parents, for the inter-rater reliability prior to the phenotype assessment. The three RAs have reach satisfactory agreement (>90%) with Drs. Gau and Wu for more than 12 interviews.

The RAs completed the training of administrating the Wisconsin card sorting test (WCST), Conners' continuous performance test (CPT), Raven's progressive matrices (RPM), Wechsler Intelligence Scale for Children-3rd edition (WISC-III), and Wechsler Primary and Preschool Scale of Intelligence-Revised (WPPSI-R).

I.5. Interview quality control

The regular research meeting on interview training is hold every 3 months to make sure that the standard interview procedure is followed and to discuss any issue emerged from instruments and diagnosis or recruitment. As data are collected, one in 15 of each interview is randomly identified for a reliability check to decrease the interviewer's bias.

I.6. Dr. Wu has been certified as a trainer for using the ADOS in clinical and research settings. The training will be held after the Chinese version of the ADOS is ready.

II. Preliminary Results of Clinic Data:

II.1. Sample Description (Table 1)

There were 100 probands (92 males) with average age of 9.2 (S.D=4.3). More than half of their parents were college degree and above. More than half of mothers were unemployed.

II.2. Autistic symptoms based on ADI-R interview (Table 2)

The average mean score of the Qualitative Abnormalities in Reciprocal Social Interaction, Communication were 21.86 (5.96), and 15.91 (3.95), respectively. The mean score of the Restrictive, Repetitive, and Stereotyped Pattern of Behavior was 6.50 (2.50). The majority of the probands were noted to have autistic symptoms before 36 months old. Their symptoms assessed by the ADI-R all reached the cut-off for autism diagnosis.

II.3. Results from parental reports on the probands

Table 3 presents the mean scores of the three dimensions of the SCQ (reciprocal social interaction, 6.65; communication, 6.92; restricted repetitive stereotyped behavior, 5.02).

Table 4 presents that 52% of the probands had diagnosis of attention-deficit/ hyperactivity disorder, mainly combined type. Moreover, 10% of the probands were also diagnosed with oppositional defiant disorders based on the Kiddie Schedule for Affective Disorders and Schizophrenia.

Table 5 summarizes the mean scores of the SNAP-IV for measuring the ADHD-related symptoms. The results showed that autism probands had higher scores of inattention and hyperactivity/impulsivity than the normal children at the same age (*T*-score > 65) but there was no increased oppositional score.

Table 5 also presents the mean T-score of the 8 behavioral syndrome measured by the CBCL. We found that children with autism had significantly more severe symptoms of attention problems, social problems, and thought problems than general population.

Table 6 shows that 48% of probands with autism have allergic history mainly nasal allergy, 20% of them suffered from eczema, and 9% of them suffered from asthma.

II.4. Preliminary Neuropsychological Results

The results show that probands with autism did not have more impaired attention based on the CPT assessments **(Table 7)** but they demonstrated more errors, higher percent perseverative responses, and more nonperseverative errors than same-age children **(Table 8)**.

II.5. Preliminary Results about Parents

Table 9 presents parents' autism symptom. There was no difference in autistic scores between the mothers and the fathers.

Table 10 presents the DSM-IV psychiatric syndromes of parents. The rates of anxiety disorders, obsessive-compulsive disorder, tic disorders, sleep disorders and substance use disorders were 60.7%, 36.0%, 31.5%, 15.7%, and 43.8% for fathers; and 71.9%, 46.9%, 16.7%, 26.0%, and 8.3% for mothers, respectively.

III. Preliminary Results of genetic analysis:

III.1. HLA-DRB1 Genotyping:

HLA-DRB1 genotyping was confirmed in 74 subjects. In genotyping of HLA-DRB1 gene, the preliminary results based on 74 autistic cases and 97 controls revealed that DR4 (9.5% vs 15.6%), DR13 (0.7% vs 11.6%), and DR15 (14.2% vs 9.8%) had significantly different frequency in children with autism

compared with normal controls. (details of allele frequency were shown in Table 11.)

III.2. Mutation Screening of ACCN3:

We have found that there were three single nucleotide polymorphisms in 11 exons of ACCN3 gene, including a A-to-T substitution at exon 1 (c.229A>T), a G-to-T substitution at exon 3 (c.719G>T), a T-to-C substitution at intron10 (IVS10+60T>C).

Discussion

We have successfully prepared the Chinese version of several instruments for this clinical and genetic study of autism spectrum disorders to measure the phenotypes of autism. Among these instruments, only ADOS has not been used in our assessments of the first 100 probands because it has taken us two years to complete the translation and back-translation. The Chinese version of ADOS is under the process of the final review and approval by the WPS. We have completed the inter-rater reliability of the ADI-R for the three research assistants and the use of ADI-R to make diagnosis of autism in addition to clinical diagnosis have been demonstrated as a reliable method to earn the trust from the international research field of autism.

Our preliminary findings suggest that autism probands have typical autistic features measured by parental interview using the ADI-R and parental self-administered reports. Moreover, autistic probands also have more attention problems, social problems, and thought problems than the same-age children. They also have high prevalence of allergy and asthma. These findings are consistent with western studies. Regarding parental symptomatology, their parents also showed increased autistic personality characteristics, anxiety symptoms, obsessive-compulsive characteristics, tic symptoms, use of substances, and sleep disorders.

Regarding the HLA-DRB1 Genotyping, the preliminary result in our case-control association study revealed that DR4, DR13 and DR15 had significantly different frequency in children with autism compared with normal controls. HLA-DRB1 might be associated with autism in our Taiwanese autistic sample. However, the pattern of DRB1 genotyping in autism was different from that in Caucasian sample, which might have higher frequency of DR4, yet lower frequency of DR13, 14.[183-185] Further replication by a larger sample and a family sample is indicated.

Regarding the mutation Screening of ACCN3, there are several single nucleotide polymorphisms found in our preliminary study. Further validation in a larger sample, case-control association study of these polymorphism, and functional study are warranted.

We have worked hard to reach the first-year goal of this project. We have to take about four hours to assess one family (three research assistants simultaneously), therefore we can only assess 1 family in one day. When assessing these families, the most difficult challenge is to persuade those probands to have blood withdrawal because most autism and Asperger children are terribly afraid of needles. Some probands may take hours to calm them down to have blood withdrawal and some may need to come back again.

Although it was very difficult to recruit proband families to have a whole day assessment of whole family within one day, we still tried very hard to obtain their consents to participate in our study. We have successfully collected partial data from 100 families of 300 families which is our goal and the ADOS assessment and other neuropsychological assessments will be performed in the coming year. Most parents of probands are willing to join this study to find out the cause of this illness and wish to look for the effective treatment and prevention of this illness. We believe a well-designed study procedure and a report of assessment results with face-to-face explanation by the experienced research psychiatrists (PI, co-PI and other child psychiatrists) is very helpful to earn the study subject's trust and pave the way for future longitudinal study.

Reference

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed ed. Washington DC: American Psychiatric Association; 1994.
2. Kanner L. Autistic disturbances of affective contact. *Nervous Child.* 1943;2:217-243.
3. Firth U. *Autism and Asperger syndrome.* Cambridge, UK: Cambridge University Press; 1991.
4. World Health Organization. *International Statistical Classification of Diseases and Related Health Problems, 10th Revision.* Geneva: World Health Organization; 1992.
5. Constantino JN, Todd RD. Genetic structure of reciprocal social behavior. *American Journal of Psychiatry.* 2000;157(12):2043-2045.
6. Constantino JN, Todd RD. Autistic traits in the general population: a twin study. *Archives of General Psychiatry.* May 2003;60(5):524-530.
7. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2.[see comment]. *Nature Genetics.* 1999;23(2):185-188.
8. Beglinger LJ, Smith TH. A review of subtyping in autism and proposed dimensional classification model. *Journal of Autism & Developmental Disorders.* Aug 2001;31(4):411-422.
9. Duarte CS, Bordin IA, de Oliveira A, Bird H. The CBCL and the identification of children with autism and related conditions in Brazil: pilot findings. *Journal of Autism & Developmental Disorders.* Dec 2003;33(6):703-707.
10. Bolte S, Dickhut H, Poustka F. Patterns of parent-reported problems indicative in autism. *Psychopathology.* Mar-Apr 1999;32(2):93-97.
11. Constantino JN, Hudziak JJ, Todd RD. Deficits in reciprocal social behavior in male twins: evidence for a genetically independent domain of psychopathology. *Journal of the American Academy of Child & Adolescent Psychiatry.* Apr 2003;42(4):458-467.
12. Scattone D, Wilczynski SM, Edwards RP, Rabian B. Decreasing disruptive behaviors of children with autism using social stories. *Journal of Autism & Developmental Disorders.* Dec 2002;32(6):535-543.
13. Gilotty L, Kenworthy L, Sirian L, Black DO, Wagner AE. Adaptive skills and executive function in autism spectrum disorders. *Child Neuropsychology.* Dec 2002;8(4):241-248.
14. Luteijn E, Luteijn F, Jackson S, Volkmar F, Minderaa R. The children's Social Behavior Questionnaire for milder variants of PDD problems: evaluation of the psychometric characteristics. *Journal of Autism & Developmental Disorders.* Aug 2000;30(4):317-330.
15. Fombonne E. The epidemiology of autism: a review. *Psychological Medicine.* Jul 1999;29(4):769-786.
16. Chakrabarti S, Fombonne E. Pervasive developmental disorders in preschool children: confirmation of high prevalence. *American Journal of Psychiatry.* Jun 2005;162(6):1133-1141.
17. Fombonne E. The prevalence of autism. *JAMA.* Jan 1 2003;289(1):87-89.
18. Chakrabarti S, Fombonne E. Pervasive developmental disorders in preschool children. *Jama.* Jun 27 2001;285(24):3093-3099.
19. Chakrabarti S, Fombonne E. Pervasive developmental disorders in preschool children: confirmation of high prevalence. *Am J Psychiatry.* Jun 2005;162(6):1133-1141.
20. Folstein SE, Bisson E, Santangelo SL, Piven J. Finding specific genes that cause autism: a combination of approaches will be needed to maximize power. *Journal of Autism & Developmental Disorders.* Oct 1998;28(5):439-445.
21. Gillberg C. Chromosomal disorders and autism. *Journal of Autism & Developmental Disorders.* Oct 1998;28(5):415-425.
22. Lauritsen M, Mors O, Mortensen PB, Ewald H. Infantile autism and associated autosomal chromosome abnormalities: a register-based study and a literature survey. *Journal of Child Psychology & Psychiatry & Allied Disciplines.* Mar 1999;40(3):335-345.
23. Spiker D, Lotspeich L, Kraemer HC, et al. Genetics of autism: characteristics of affected and unaffected children from 37 multiplex families. *American Journal of Medical Genetics.* Mar 15 1994;54(1):27-35.
24. Szatmari P, Jones MB, Zwaigenbaum L, MacLean JE. Genetics of autism: overview and new directions. *Journal of Autism & Developmental Disorders.* Oct 1998;28(5):351-368.
25. Newschaffer CJ, Fallin D, Lee NL. Heritable and nonheritable risk factors for autism spectrum disorders. *Epidemiologic Reviews.* 2002;24(2):137-153.
26. Bailey A, Le Couteur A, Gottesman I, et al. Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med.* Jan 1995;25(1):63-77.
27. Folstein S, Rutter M. Infantile autism: a genetic study of 21 twin pairs. *J Child Psychol Psychiatry.* Sep 1977;18(4):297-321.
28. Steffenburg S, Gillberg C, Hellgren L, et al. A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden.

J Child Psychol Psychiatry. May 1989;30(3):405-416.
29. Pickles A, Bolton P, Macdonald H, et al. Latent-class analysis of recurrence risks for complex phenotypes with selection and measurement error: a twin and family history study of autism. *Am J Hum Genet.* Sep 1995;57(3):717-726.
30. Risch N, Spiker D, Lotspeich L, et al. A genomic screen of autism: evidence for a multilocus etiology. *Am J Hum Genet.* Aug 1999;65(2):493-507.
31. Wassink TH, Brzustowicz LM, Bartlett CW, Szatmari P. The search for autism disease genes. *Ment Retard Dev Disabil Res Rev.* 2004;10(4):272-283.
32. IMGSAC. A full genome screen for autism with evidence for linkage to a region on chromosome 7q. International Molecular Genetic Study of Autism Consortium. *Hum Mol Genet.* Mar 1998;7(3):571-578.
33. Barrett S, Beck JC, Bernier R, et al. An autosomal genomic screen for autism. Collaborative linkage study of autism. *Am J Med Genet.* Dec 15 1999;88(6):609-615.
34. Philippe A, Martinez M, Guilloud-Bataille M, et al. Genome-wide scan for autism susceptibility genes. Paris Autism Research International Sibpair Study. *Hum Mol Genet.* May 1999;8(5):805-812.
35. Buxbaum JD, Silverman JM, Smith CJ, et al. Evidence for a susceptibility gene for autism on chromosome 2 and for genetic heterogeneity. *Am J Hum Genet.* Jun 2001;68(6):1514-1520.
36. IMGSAC. A genomewide screen for autism: strong evidence for linkage to chromosomes 2q, 7q, and 16p. *Am J Hum Genet.* Sep 2001;69(3):570-581.
37. Auranen M, Vanhala R, Varilo T, et al. A genomewide screen for autism-spectrum disorders: evidence for a major susceptibility locus on chromosome 3q25-27. *Am J Hum Genet.* Oct 2002;71(4):777-790.
38. Shao Y, Wolpert CM, Raiford KL, et al. Genomic screen and follow-up analysis for autistic disorder. *Am J Med Genet.* Jan 8 2002;114(1):99-105.
39. Yonan AL, Alarcon M, Cheng R, et al. A genomewide screen of 345 families for autism-susceptibility loci. *Am J Hum Genet.* Oct 2003;73(4):886-897.
40. Buxbaum JD, Silverman J, Keddache M, et al. Linkage analysis for autism in a subset families with obsessive-compulsive behaviors: evidence for an autism susceptibility gene on chromosome 1 and further support for susceptibility genes on chromosome 6 and 19. *Mol Psychiatry.* Feb 2004;9(2):144-150.
41. Ylisaukko-oja T, Nieminen-von Wendt T, Kempas E, et al. Genome-wide scan for loci of Asperger syndrome. *Mol Psychiatry.* Feb 2004;9(2):161-168.
42. McCauley JL, Li C, Jiang L, et al. Genome-wide and Ordered-Subset linkage analyses provide support for autism loci on 17q and 19p with evidence of phenotypic and interlocus genetic correlates. *BMC Med Genet.* Jan 12 2005;6:1.
43. Liu J, Nyholt DR, Magnussen P, et al. A genomewide screen for autism susceptibility loci. *Am J Hum Genet.* Aug 2001;69(2):327-340.
44. Lauritsen MB, Als TD, Dahl HA, et al. A genome-wide search for alleles and haplotypes associated with autism and related pervasive developmental disorders on the Faroe Islands. *Mol Psychiatry.* Jan 2006;11(1):37-46.
45. Bacchelli E, Maestrini E. Autism spectrum disorders: molecular genetic advances. *Am J Med Genet C Semin Med Genet.* Feb 15 2006;142(1):13-23.
46. Santangelo SL, Tsatsanis K. What is known about autism: genes, brain, and behavior. *Am J Pharmacogenomics.* 2005;5(2):71-92.
47. Polleux F, Lauder JM. Toward a developmental neurobiology of autism. *Ment Retard Dev Disabil Res Rev.* 2004;10(4):303-317.
48. Bolton PF, Dennis NR, Browne CE, et al. The phenotypic manifestations of interstitial duplications of proximal 15q with special reference to the autistic spectrum disorders. *Am J Med Genet.* Dec 8 2001;105(8):675-685.
49. Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, Realmuto GR. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. *Biol Psychiatry.* Oct 15 2002;52(8):805-810.
50. Dykens EM, Sutcliffe JS, Levitt P. Autism and 15q11-q13 disorders: behavioral, genetic, and pathophysiological issues. *Ment Retard Dev Disabil Res Rev.* 2004;10(4):284-291.
51. Mulder EJ, Anderson GM, Kema IP, et al. Serotonin transporter intron 2 polymorphism associated with rigid-compulsive behaviors in Dutch individuals with pervasive developmental disorder. *Am J Med Genet B Neuropsychiatr Genet.* Feb 5 2005;133(1):93-96.
52. Maestrini E, Lai C, Marlow A, et al. Serotonin transporter (5-HTT) and gamma-aminobutyric acid receptor subunit beta3 (GABRB3) gene polymorphisms are not associated with autism in the IMGSA families. The International Molecular Genetic Study of Autism Consortium. *Am J Med Genet.* Oct 15 1999;88(5):492-496.
53. Anderson GM, Horne WC, Chatterjee D, Cohen DJ. The hyperserotonemia of autism. *Ann N Y Acad Sci.* 1990;600:331-340; discussion 341-332.
54. Cook EH, Jr., Courchesne R, Lord C, et al. Evidence of linkage between the serotonin transporter and autistic disorder. *Mol Psychiatry.* May 1997;2(3):247-250.

55. Klauck SM, Poustka F, Benner A, Lesch KP, Poustka A. Serotonin transporter (5-HTT) gene variants associated with autism? *Hum Mol Genet.* Dec 1997;6(13):2233-2238.
56. Yirmiya N, Pilowsky T, Nemanov L, et al. Evidence for an association with the serotonin transporter promoter region polymorphism and autism. *Am J Med Genet.* May 8 2001;105(4):381-386.
57. Persico AM, Pascucci T, Puglisi-Allegra S, et al. Serotonin transporter gene promoter variants do not explain the hyperserotoninemia in autistic children. *Mol Psychiatry.* 2002;7(7):795-800.
58. McCauley JL, Olson LM, Dowd M, et al. Linkage and association analysis at the serotonin transporter (SLC6A4) locus in a rigid-compulsive subset of autism. *Am J Med Genet B Neuropsychiatr Genet.* May 15 2004;127(1):104-112.
59. Coutinho AM, Oliveira G, Morgadinho T, et al. Variants of the serotonin transporter gene (SLC6A4) significantly contribute to hyperserotonemia in autism. *Mol Psychiatry.* Mar 2004;9(3):264-271.
60. Devlin B, Cook EH, Jr., Coon H, et al. Autism and the serotonin transporter: the long and short of it. *Mol Psychiatry.* Dec 2005;10(12):1110-1116.
61. Wu S, Guo Y, Jia M, et al. Lack of evidence for association between the serotonin transporter gene (SLC6A4) polymorphisms and autism in the Chinese trios. *Neurosci Lett.* Jun 10-17 2005;381(1-2):1-5.
62. Sutcliffe JS, Delahanty RJ, Prasad HC, et al. Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. *Am J Hum Genet.* Aug 2005;77(2):265-279.
63. Koishi S, Yamamoto K, Matsumoto H, et al. Serotonin transporter gene promoter polymorphism and autism: a family-based genetic association study in Japanese population. *Brain Dev.* May 2006;28(4):257-260.
64. Jamain S, Betancur C, Quach H, et al. Linkage and association of the glutamate receptor 6 gene with autism. *Mol Psychiatry.* 2002;7(3):302-310.
65. Jones MB, Palmour RM, Zwaigenbaum L, Szatmari P. Modifier effects in autism at the MAO-A and DBH loci. *Am J Med Genet B Neuropsychiatr Genet.* Apr 1 2004;126(1):58-65.
66. Agulhon C, Abitbol M, Bertrand D, Malafosse A. Localization of mRNA for CHRNA7 in human fetal brain. *Neuroreport.* Aug 2 1999;10(11):2223-2227.
67. Martin-Ruiz CM, Lee M, Perry RH, Baumann M, Court JA, Perry EK. Molecular analysis of nicotinic receptor expression in autism. *Brain Res Mol Brain Res.* Apr 7 2004;123(1-2):81-90.
68. Gharani N, Benayed R, Mancuso V, Brzustowicz LM, Millonig JH. Association of the homeobox transcription factor, ENGRAILED 2, 3, with autism spectrum disorder. *Mol Psychiatry.* May 2004;9(5):474-484.
69. Wassink TH, Piven J, Vieland VJ, et al. Evidence supporting WNT2 as an autism susceptibility gene. *Am J Med Genet.* Jul 8 2001;105(5):406-413.
70. Persico AM, D'Agruma L, Maiorano N, et al. Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. *Mol Psychiatry.* Mar 2001;6(2):150-159.
71. Persico AM, Levitt P, Pimenta AF. Polymorphic GGC repeat differentially regulates human reelin gene expression levels. *J Neural Transm.* Apr 11 2006.
72. Zhang H, Liu X, Zhang C, et al. Reelin gene alleles and susceptibility to autism spectrum disorders. *Mol Psychiatry.* 2002;7(9):1012-1017.
73. Bonora E, Beyer KS, Lamb JA, et al. Analysis of reelin as a candidate gene for autism. *Mol Psychiatry.* Oct 2003;8(10):885-892.
74. Skaar DA, Shao Y, Haines JL, et al. Analysis of the RELN gene as a genetic risk factor for autism. *Mol Psychiatry.* Jun 2005;10(6):563-571.
75. Devlin B, Bennett P, Dawson G, et al. Alleles of a reelin CGG repeat do not convey liability to autism in a sample from the CPEA network. *Am J Med Genet B Neuropsychiatr Genet.* Apr 1 2004;126(1):46-50.
76. Li J, Nguyen L, Gleason C, et al. Lack of evidence for an association between WNT2 and RELN polymorphisms and autism. *Am J Med Genet B Neuropsychiatr Genet.* Apr 1 2004;126(1):51-57.
77. Serajee FJ, Zhong H, Mahbubul Huq AH. Association of Reelin gene polymorphisms with autism. *Genomics.* Jan 2006;87(1):75-83.
78. Jamain S, Quach H, Betancur C, et al. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat Genet.* May 2003;34(1):27-29.
79. Laumonnier F, Bonnet-Brilhault F, Gomot M, et al. X-linked mental retardation and autism are associated with a mutation in the NLGN4 gene, a member of the neuroligin family. *Am J Hum Genet.* Mar 2004;74(3):552-557.
80. Vincent JB, Kolozsvari D, Roberts WS, Bolton PF, Gurling HM, Scherer SW. Mutation screening of X-chromosomal neuroligin genes: no mutations in 196 autism probands. *Am J Med Genet B Neuropsychiatr Genet.* Aug 15 2004;129(1):82-84.
81. Gauthier J, Bonnel A, St-Onge J, et al. NLGN3/NLGN4 gene mutations are not responsible for autism in the Quebec population. *Am J Med Genet B Neuropsychiatr Genet.* Jan 5 2005;132(1):74-75.
82. Yan J, Oliveira G, Coutinho A, et al. Analysis of the neuroligin 3 and 4 genes in autism and other neuropsychiatric

patients. *Mol Psychiatry.* Apr 2005;10(4):329-332.
83. Muhle R, Trentacoste SV, Rapin I. The genetics of autism. *Pediatrics.* May 2004;113(5):e472-486.
84. Longo I, Russo L, Meloni I, et al. Three Rett patients with both MECP2 mutation and 15q11-13 rearrangements. *Eur J Hum Genet.* Aug 2004;12(8):682-685.
85. Ingram JL, Stodgell CJ, Hyman SL, Figlewicz DA, Weitkamp LR, Rodier PM. Discovery of allelic variants of HOXA1 and HOXB1: genetic susceptibility to autism spectrum disorders. *Teratology.* Dec 2000;62(6):393-405.
86. Conciatori M, Stodgell CJ, Hyman SL, et al. Association between the HOXA1 A218G polymorphism and increased head circumference in patients with autism. *Biol Psychiatry.* Feb 15 2004;55(4):413-419.
87. Ramoz N, Reichert JG, Smith CJ, et al. Linkage and association of the mitochondrial aspartate/glutamate carrier SLC25A12 gene with autism. *Am J Psychiatry.* Apr 2004;161(4):662-669.
88. Segurado R, Conroy J, Meally E, Fitzgerald M, Gill M, Gallagher L. Confirmation of association between autism and the mitochondrial aspartate/glutamate carrier SLC25A12 gene on chromosome 2q31. *Am J Psychiatry.* Nov 2005;162(11):2182-2184.
89. Wassink TH, Piven J, Vieland VJ, et al. Examination of AVPR1a as an autism susceptibility gene. *Mol Psychiatry.* Oct 2004;9(10):968-972.
90. Wassink TH, Piven J, Vieland VJ, et al. Evaluation of FOXP2 as an autism susceptibility gene. *Am J Med Genet.* Jul 8 2002;114(5):566-569.
91. Lauritsen M, Mors O, Mortensen PB, Ewald H. Infantile autism and associated autosomal chromosome abnormalities: a register-based study and a literature survey. *J Child Psychol Psychiatry.* Mar 1999;40(3):335-345.
92. Wassink TH, Piven J, Patil SR. Chromosomal abnormalities in a clinic sample of individuals with autistic disorder. *Psychiatr Genet.* Jun 2001;11(2):57-63.
93. Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L. Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol Psychiatry.* Jan 2006;11(1):1, 18-28.
94. Scherer SW, Cheung J, MacDonald JR, et al. Human chromosome 7: DNA sequence and biology. *Science.* May 2 2003;300(5620):767-772.
95. Coon H. Current perspectives on the genetic analysis of autism. *Am J Med Genet C Semin Med Genet.* Feb 15 2006;142(1):24-32.
96. Chen GK, Kono N, Geschwind DH, Cantor RM. Quantitative trait locus analysis of nonverbal communication in autism spectrum disorder. *Mol Psychiatry.* Feb 2006;11(2):214-220.
97. Gong X, Jia M, Ruan Y, et al. Association between the FOXP2 gene and autistic disorder in Chinese population. *Am J Med Genet B Neuropsychiatr Genet.* May 15 2004;127(1):113-116.
98. Wu S, Jia M, Ruan Y, et al. Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. *Biol Psychiatry.* Jul 1 2005;58(1):74-77.
99. Shuang M, Liu J, Jia MX, et al. Family-based association study between autism and glutamate receptor 6 gene in Chinese Han trios. *Am J Med Genet B Neuropsychiatr Genet.* Nov 15 2004;131(1):48-50.
100. Lincoln AJ, Courchesne E, Kilman BA, Elmasian R, Allen M. A study of intellectual abilities in high-functioning people with autism. *Journal of Autism & Developmental Disorders.* Dec 1988;18(4):505-524.
101. Siegel DJ, Minshew NJ, Goldstein G. Wechsler IQ profiles in diagnosis of high-functioning autism.[see comment]. *Journal of Autism & Developmental Disorders.* Aug 1996;26(4):389-406.
102. Ozonoff S, Williams BJ, Rauch AM, Opitz JO. Behavior phenotype of FG syndrome: cognition, personality, and behavior in eleven affected boys. *American Journal of Medical Genetics.* 2000;97(2):112-118.
103. Manjiviona J, Prior M. Neuropsychological profiles of children with Asperger syndrome and autism. *Autism.* 1999;3:327-356.
104. Joseph RM, Tager-Flusberg H, Lord C. Cognitive profiles and social-communicative functioning in children with autism spectrum disorder. *Journal of Child Psychology & Psychiatry & Allied Disciplines.* Sep 2002;43(6):807-821.
105. Tager-Flusberg H, Joseph RM. Identifying neurocognitive phenotypes in autism. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences.* Feb 28 2003;358(1430):303-314.
106. Bailey A, Phillips W, Rutter M. Autism: towards an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. *Journal of Child Psychology & Psychiatry & Allied Disciplines.* Jan 1996;37(1):89-126.
107. Karmiloff-Smith A, Tyler LK, Voice K, et al. Linguistic dissociations in Williams syndrome: evaluating receptive syntax in on-line and off-line tasks. *Neuropsychologia.* 1998;36(4):343-351.
108. Karmiloff-Smith A, Grant J, Berthoud I, Davies M, Howlin P, Udwin O. Language and Williams syndrome: how intact is "intact"? *Child Development.* 1997;68(2):246-262.
109. Happe F. Autism: Cognitive deficit or cognitive style? *Trends in Cognitive Sciences.* 1999;3:216-222.
110. Frith U, Happe F. Autism: beyond "theory of mind". *Cognition.* 1994;50(1-3):115-132.

111. Happe FG. Studying weak central coherence at low levels: children with autism do not succumb to visual illusions. A research note. *Journal of Child Psychology & Psychiatry & Allied Disciplines.* Oct 1996;37(7):873-877.
112. Plaisted K, O'Riordan M, Baron-Cohen S. Enhanced visual search for a conjunctive target in autism: a research note. *Journal of Child Psychology & Psychiatry & Allied Disciplines.* Jul 1998;39(5):777-783.
113. Plaisted KC. Aspects of autism that theory of mind cannot explain. In: S. Baron-Cohen HT-F, & D. Cohen ed. *Understanding otherminds: Perspectives from autism and developmentalcognitive neuroscience (2nd ed.).* Oxford: Oxford University Press; 2000:222-250.
114. Elgar K, Campbell R. Annotation: the cognitive neuroscience of face recognition: implications for developmental disorders. *Journal of Child Psychology & Psychiatry & Allied Disciplines.* Sep 2001;42(6):705-717.
115. Miyake A, Friedman NP, Emerson MJ, Witzki AH, Howerter A, Wager TD. The unity and diversity of executive functions and their contributions to complex "Frontal Lobe" tasks: a latent variable analysis. *Cognitive Psychology.* 2000;41(1):49-100.
116. Lehto JE, Juujäarvi P, Kooistra L, Pulkkinen L. Dimensions of executive functioning: Evidence from children. . *British Journal of Developmental Psychology.* 2003;21:59-80.
117. Berg EA. A simple, objective technique for measuring flexibility in thinking .
. *Journal of General Psychology.* 1948;39:15-22.
118. Borys SV, Spitz HH, Dorans BA. Tower of Hanoi performance of retarded young adults and nonretarded children as a function of solution length and goal state. *Journal of Experimental Child Psychology.* 1982;33(1):87-110.
119. Liss M, Fein D, Allen D, et al. Executive functioning in high-functioning children with autism. *Journal of Child Psychology & Psychiatry & Allied Disciplines.* Feb 2001;42(2):261-270.
120. Bennetto L, Pennington BF, Rogers SJ. Intact and impaired memory functions in autism. *Child Development.* 1996;67:1816-1835.
121. Minshew NJ, Meyer J, Goldstein G. Abstract reasoning in autism: a dissociation between concept formation and concept identification. *Neuropsychology.* 2002;16(3):327-334.
122. Ozonoff S, Jensen J. Brief report: specific executive function profiles in three neurodevelopmental disorders.[see comment]. *Journal of Autism & Developmental Disorders.* 1999;29(2):171-177.
123. Ozonoff S, McEvoy RE. A longitudinal study of executive function and theory of mind development in autism. . *Development and Psychopathology.* 1994;6(3):415-431.
124. Rumsey JM, Hamburger SD. Neuropsychological divergence of high-level autism and severe dyslexia. *Journal of Autism & Developmental Disorders.* Jun 1990;20(2):155-168.
125. Rumsey JM, Hamburger SD. Neuropsychological findings in high-functioning men with infantile autism, residual state. *Journal of Clinical & Experimental Neuropsychology: Official Journal of the International Neuropsychological Society.* Mar 1988;10(2):201-221.
126. Battery AIT. *Manual of directions and scoring.* Washington, DC: War Department, Adjutant General's Office; 1944.
127. Ozonoff S, Rogers SJ, Pennington BF. Asperger's syndrome: evidence of an empirical distinction from high-functioning autism. *Journal of Child Psychology & Psychiatry & Allied Disciplines.* Nov 1991;32(7):1107-1122.
128. Ozonoff S, Strayer DL. Inhibitory function in nonretarded children with autism. *Journal of Autism & Developmental Disorders.* Feb 1997;27(1):59-77.
129. Ozonoff S, Strayer DL, McMahon WM, Filloux F. Executive function abilities in autism and Tourette syndrome: an information processing approach. *Journal of Child Psychology & Psychiatry & Allied Disciplines.* Sep 1994;35(6):1015-1032.
130. Sergeant JA, Geurts H, Oosterlaan J. How specific is a deficit of executive functioning for attention-deficit/hyperactivity disorder? *Behavioural Brain Research.* 2002;130(1-2):3-28.
131. Hughes C, Russell J, Robbins TW. Evidence for executive dysfunction in autism. *Neuropsychologia.* Apr 1994;32(4):477-492.
132. Shu BC, Lung FW, Tien AY, Chen BC. Executive function deficits in non-retarded autistic children. *Autism.* Jun 2001;5(2):165-174.
133. Russell J, Jarrold C, Hood B. Two intact executive capacities in children with autism: implications for the core executive dysfunctions in the disorder.[see comment]. *Journal of Autism & Developmental Disorders.* Apr 1999;29(2):103-112.
134. Russell J, Mauthner N, Sharpe S, Tidswell T. The 'windows task' as a measure of strategicdeception in preschoolers and autistic subjects. . *British Journal of Developmental Psychology.* 1991;9:101-119.
135. Russell J, Hala S, Hill EL. Mechanising an executive task: the performance of preschool children, children with autism and with moderate learning difficulties in the automated Windows Task. *Cognitive Development.* 2003;18:111-137.
136. Hughes C, Russell J. Autistic children's mental difficulty with disengagement from an object: Its implication for theories of autism. *Developmental Psychology.* 2003;29:498-510.

137. Biro S, Russell J. The execution of arbitrary procedures by children with autism. *Development & Psychopathology.* 2001;13(1):97-110.
138. Boucher J, Lewis V, Collis G. Familiar face and voice matching and recognition in children with autism. *Journal of Child Psychology & Psychiatry & Allied Disciplines.* Feb 1998;39(2):171-181.
139. Dunn M, Gomes H, Sebastian M. Prototypicality of responses of autistic and language disordered children in a word fluency task. . *Child Neuropsychology.* 1996;2:99-108.
140. Turner MA. Generating novel ideas: fluency performance in high-functioning and learning disabled individuals with autism. *Journal of Child Psychology & Psychiatry & Allied Disciplines.* Feb 1999;40(2):189-201.
141. Pascualvaca DM, Fantie BD, Papageorgiou M, Mirsky AF. Attentional capacities in children with autism: is there a general deficit in shifting focus? *Journal of Autism & Developmental Disorders.* Dec 1998;28(6):467-478.
142. Kleinhans N, Akshoomoff N, Delis DC. Executive functions in autism and Asperger's disorder: flexibility, fluency, and inhibition. *Dev Neuropsychol.* 2005;27(3):379-401.
143. Abell F, Krams M, Ashburner J, et al. The neuroanatomy of autism: a voxel-based whole brain analysis of structural scans. *Neuroreport.* 1999;10(8):1647-1651.
144. Aylward EH, Minshew NJ, Goldstein G, et al. MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. *Neurology.* Dec 10 1999;53(9):2145-2150.
145. Mountz JM, Tolbert LC, Lill DW, Katholi CR, Liu HG. Functional deficits in autistic disorder: characterization by technetium-99m-HMPAO and SPECT. *Journal of Nuclear Medicine.* Jul 1995;36(7):1156-1162.
146. Carper RA, Courchesne E. Inverse correlation between frontal lobe and cerebellum sizes in children with autism. *Brain.* Apr 2000;123(Pt 4):836-844.
147. Courchesne E, Saitoh O, Yeung-Courchesne R, et al. Abnormality of cerebellar vermian lobules VI and VII in patients with infantile autism: identification of hypoplastic and hyperplastic subgroups with MR imaging. *AJR. American Journal of Roentgenology.* 1994;162(1):123-130.
148. Courchesne E, Yeung-Courchesne R, Press GA, Hesselink JR, Jernigan TL. Hypoplasia of cerebellar vermal lobules VI and VII in autism. *New England Journal of Medicine.* 1988;318(21):1349-1354.
149. Kates WR, Mostofsky SH, Zimmerman AW, et al. Neuroanatomical and neurocognitive differences in a pair of monozygous twins discordant for strictly defined autism. *Annals of Neurology.* Jun 1998;43(6):782-791.
150. Holttum JR, Minshew NJ, Sanders RS, Phillips NE. Magnetic resonance imaging of the posterior fossa in autism. *Biological Psychiatry.* Dec 15 1992;32(12):1091-1101.
151. Kleiman MD, Neff S, Rosman NP. The brain in infantile autism: are posterior fossa structures abnormal? *Neurology.* Apr 1992;42(4):753-760.
152. Piven J, Bailey J, Ranson BJ, Arndt S. No difference in hippocampus volume detected on magnetic resonance imaging in autistic individuals.[erratum appears in J Autism Dev Disord 1998 Jun;28(3):271]. *Journal of Autism & Developmental Disorders.* Apr 1998;28(2):105-110.
153. Piven J, Nehme E, Simon J, Barta P, Pearlson G, Folstein SE. Magnetic resonance imaging in autism: measurement of the cerebellum, pons, and fourth ventricle. *Biological Psychiatry.* Mar 1 1992;31(5):491-504.
154. Saitoh O, Courchesne E, Egaas B, Lincoln AJ, Schreibman L. Cross-sectional area of the posterior hippocampus in autistic patients with cerebellar and corpus callosum abnormalities. *Neurology.* 1995;45(2):317-324.
155. Courchesne E, Karns CM, Davis HR, et al. Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology.* 2001;57(2):245-254.
156. Sparks BF, Friedman SD, Shaw DW, et al. Brain structural abnormalities in young children with autism spectrum disorder.[see comment]. *Neurology.* 2002;59(2):184-192.
157. Lainhart JE, Piven J, Wzorek M, et al. Macrocephaly in children and adults with autism. *Journal of the American Academy of Child & Adolescent Psychiatry.* 1997;36(2):282-290.
158. Bolton PF, Roobol M, Allsopp L, Pickles A. Association between idiopathic infantile macrocephaly and autism spectrum disorders. *Lancet.* Sep 1 2001;358(9283):726-727.
159. Stevenson RE, Schroer RJ, Skinner C, Fender D, Simensen RJ. Autism and macrocephaly. *Lancet.* 1997;349(9067):1744-1745.
160. Gillberg C, de Souza L. Head circumference in autism, Asperger syndrome, and ADHD: a comparative study. *Developmental Medicine & Child Neurology.* May 2002;44(5):296-300.
161. Torrey EF, Dhavale D, Lawlor JP, Yolken RH. Autism and head circumference in the first year of life. *Biological Psychiatry.* 2004;56(11):892-894.
162. Aylward EH, Minshew NJ, Field K, Sparks BF, Singh N. Effects of age on brain volume and head circumference in autism.[see comment]. *Neurology.* 2002;59(2):175-183.
163. Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism.[see comment]. *JAMA.* Jul 16 2003;290(3):337-344.

164. Dementieva YA, Vance DD, Donnelly SL, et al. Accelerated head growth in early development of individuals with autism. *Pediatric Neurology.* Feb 2005;32(2):102-108.
165. Palmen SJ, van Engeland H. Review on structural neuroimaging findings in autism. *J Neural Transm.* Jul 2004;111(7):903-929.
166. Carper RA, Moses P, Tigue ZD, Courchesne E. Cerebral lobes in autism: early hyperplasia and abnormal age effects. *Neuroimage.* Aug 2002;16(4):1038-1051.
167. Herbert MR, Ziegler DA, Deutsch CK, et al. Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. *Brain.* 2003;126(Pt 5):1182-1192.
168. Lainhart JE. Advances in autism neuroimaging research for the clinician and geneticist. *American Journal of Medical Genetics Part C, Seminars in Medical Genetics.* Feb 15 2006;142(1):33-39.
169. Minshew NJ, Goldstein G, Dombrowski SM, Panchalingam K, Pettegrew JW. A preliminary 31P MRS study of autism: evidence for undersynthesis and increased degradation of brain membranes. *Biological Psychiatry.* Jun 1-15 1993;33(11-12):762-773.
170. Chugani DC, Sundram BS, Behen M, Lee ML, Moore GJ. Evidence of altered energy metabolism in autistic children. *Prog Neuropsychopharmacol Biol Psychiatry.* May 1999;23(4):635-641.
171. Hisaoka S, Harada M, Nishitani H, Mori K. Regional magnetic resonance spectroscopy of the brain in autistic individuals. *Neuroradiology.* 2001;43(6):496-498.
172. Otsuka H, Harada M, Mori K, Hisaoka S, NishitaniH H. Brain metabolites in the hippocampus-amygdala region and cerebellum in autism: an 1H-MR spectroscopy study. . *Neuroradiology.* 1999;41:517-519.
173. Friedman SD, Shaw DW, Artru AA, et al. Regional brain chemical alterations in young children with autism spectrum disorder. *Neurology.* 2003;60(1):100-107.
174. Levitt JG, O'Neill J, Blanton RE, et al. Proton magnetic resonance spectroscopic imaging of the brain in childhood autism. *Biological Psychiatry.* 2003;54(12):1355-1366.
175. Sokol DK, Dunn DW, Edwards-Brown M, Feinberg J. Hydrogen proton magnetic resonance spectroscopy in autism: preliminary evidence of elevated choline/creatine ratio. *Journal of Child Neurology.* Apr 2002;17(4):245-249.
176. Murphy DG, Critchley HD, Schmitz N, et al. Asperger syndrome: a proton magnetic resonance spectroscopy study of brain. *Archives of General Psychiatry.* 2002;59(10):885-891.
177. Friedman SD, Shaw DW, Artru AA, Dawson G, Petropoulos H, Dager SR. Gray and white matter brain chemistry in young children with autism. *Archives of General Psychiatry.* 2006;63(7):786-794.
178. Lazar M, Weinstein DM, Tsuruda JS, et al. White matter tractography using diffusion tensor deflection. *Human Brain Mapping.* 2003;18(4):306-321.
179. Gulani V, Sundgren PC. Diffusion tensor magnetic resonance imaging. *Journal of Neuro Ophthalmology.* 2006;26(1):51-60.
180. Filippi M. MRI-clinical correlations in the primary progressive course of MS: new insights into the disease pathophysiology from the application of magnetization transfer, diffusion tensor, and functional MRI. *Journal of the Neurological Sciences.* 2003;206(2):157-164.
181. Ashtari M, Kumra S, Bhaskar SL, et al. Attention-deficit/hyperactivity disorder: a preliminary diffusion tensor imaging study. *Biological Psychiatry.* 2005;57(5):448-455.
182. Barnea-Goraly N, Kwon H, Menon V, Eliez S, Lotspeich L, Reiss AL. White matter structure in autism: preliminary evidence from diffusion tensor imaging. *Biological Psychiatry.* 2004;55(3):323-326.
183. Lee, L. C., A. A. Zachary, M. S. Leffell, C. J. Newschaffer, K. J. Matteson, J. D. Tyler, and A. W. Zimmerman. 2006. HLA-DR4 in families with autism. *Pediatr Neurol* 35 (5):303-7.
184. Torres, A. R., A. Maciulis, E. G. Stubbs, A. Cutler, and D. Odell. 2002. The transmission disequilibrium test suggests that HLA-DR4 and DR13 are linked to autism spectrum disorder. *Hum Immunol* 63 (4):311-6.
185. Warren, R. P., J. D. Odell, W. L. Warren, R. A. Burger, A. Maciulis, W. W. Daniels, and A. R. Torres. 1996. Strong association of the third hypervariable region of HLA-DR beta 1 with autism. *J Neuroimmunol* 67 (2):97-102.

Table 1 Sample Description: Demographic characteristics

N (%)	Case (N=100)
Child	
Age (Mean ± SD)	9.19±4.25
Sex	
Male	92(92)
Female	8(8)
Male/Female ratio	11.5
Father	
Age (Mean ± SD)	42.62±6.22
Education	
College / University	71(71)
Senior High	21(21)
Junior High & Below	4(4)
Job	
Professional	13(13)
Skilled work	76(76)
Others	7(7)
Developmental difficulties	2(2)
Mother	
Age (Mean ± SD)	39.41±5.78
Education	
College / University	58(58)
Senior High	32(32)
Junior High & Below	8(8)
Job	
Professional	10(10)
Skilled work	27(27)
Others	59(59)
Developmental difficulties	4(4)

Table 2 Autism symptoms based on ADI-R interview

	Mean (N=100)	**S.D.** (N=100)
A. Qualitative Abnormalities in Reciprocal Social Interaction	21.86	5.96
A1: Failure to use nonverbal behaviors to regulate social interaction	4.08	1.52
A2: Failure to develop peer relationships	6.14	2.10
A3: Lack of shared enjoyment	4.90	1.49
A4: Lack of socioemotional reciprocity	6.74	2.46
B. Qualitative Abnormalities in Communication (Verbal Total)	15.91	3.95
Qualitative Abnormalities in Communication (Nonverbal Total, only B1+B4)	8.46	3.31
B1: Lack of, or delay in, spoken language and failure to compensate through gesture	3.64	2.46
B2: Relative failure to initiate or sustain conversational interchange	3.57	1.08
B3: Stereotyped, repetitive, or idiosyncratic speech	3.63	2.21
B4: Lack of varied spontaneous make-believe or social imitative play	4.82	1.43
C. Restricted, Repetitive, and Stereotyped Patterns of Behavior	6.50	2.50
C1: Encompassing preoccupation or circumscribed pattern of interest	1.41	1.07
C2: Apparently compulsive adherence to nonfunctional routines or rituals	2.09	1.39
C3: Stereotyped and repetitive motor mannerisms	1.32	0.88
C4: Preoccupations with part of objects or nonfunctional elements of material	1.67	0.60
D. Abnormality of Development Evident at or Before 36 Months	3.80	1.32

Table 3 Autism symptoms based on SCQ

	Case (N=81)	
	Mean	**S.D.**
SCQ		
Reciprocal Social Interaction	6.65	3.86
Communication	6.92	2.84
Restricted Repetitive Stereotyped Behavior	5.02	2.46
Total	19.94	6.60

Table 4 Psychopathology based on K-SADS-E interview (DSM-IV diagnosis)

Psychiatric diagnoses	Case (N=100)	
	N	(%)
Past		
Attention-Deficit/Hyperactivity Disorder	52	(52)
Combined Type	28	(28)
Inattentive Type	18	(18)
Hyperactive-Impulsive Type	6	(6)
Oppositional Defiant Disorder	10	(10)
Conduct Disorder	3	(3)
Current		
Attention-Deficit/Hyperactivity Disorder	51	(51)
Combined Type	19	(19)
Inattentive Type	24	(24)
Hyperactive-Impulsive Type	8	(8)
Oppositional Defiant Disorder	10	(10)
Conduct Disorder	2	(2)

Table 5 Parental reports on emotional/behavioral problems

	Case (N=81)			
	Raw Score		T Score	
	Mean	S.D.	Mean	S.D.
SNAP-IV				
Inattentive	15.12	6.59	73.74	17.60
Hyperactive-impulsive	10.40	5.77	69.43	17.36
Oppositional	7.13	4.98	55.65	13.26
CBCL				
Aggressive Behavior	8.97	6.12	60.83	13.79
Anxious/Depressed	5.74	4.88	57.68	13.72
Attention Problems	10.48	3.95	75.23	13.14
Delinquent Behavior	2.35	2.44	54.69	13.14
Social Problems	6.67	2.81	74.68	14.18
Somatic Complaints	1.59	2.78	50.80	11.50
Thought Problems	3.75	2.46	70.88	17.64
Withdrawn	5.46	3.21	61.30	11.50
Internalizing problems	12.79	9.04	57.88	11.86
Externalizing problems	11.32	8.07	59.32	12.96

Table 6 Physical problems

	Case (N=100)	
	N	**(%)**
呼吸道疾病(如氣喘)	9	(9)
過敏體質(如過敏性鼻炎)	48	(48)
皮膚過敏(如濕疹)	20	(20)
B 型肝炎帶菌者	1	(1)
胃腸病	1	(1)
癲癇	5	(5)
心臟病	2	(2)
車禍或意外傷害	4	(4)

Table 7 Neuropsychological test outcome: CPT

	Case (N=52)	
	Mean	**S.D.**
Omissions	52.30	14.57
Commissions	51.03	12.04
Hit RT	51.89	16.87
Hit RT Std. Error	53.32	11.57
Variability	52.19	10.64
Detectability (d')	51.47	10.09
Response Style (B)	54.13	14.24
Perseverations	58.03	19.39
Hit RT Block Change	48.14	12.82
Hit SE Block Change	49.99	9.34
Hit RT ISI Change	51.38	12.46
Hit SE ISI Change	51.23	9.22

Table 8 Neuropsychological test outcome: WSCT

	Case (N=52)			
	T score		Percentile score	
	Mean	S.D.	Mean	S.D.
Total Number of Errors	93.96	18.67	92.10	17.46
Percent Errors	45.98	12.51	44.71	11.69
Perseverative Responses	40.19	32.21	34.12	31.05
Percent Perseverative Responses	93.13	18.96	92.92	17.85
Perseverative Errors	45.44	12.68	45.23	11.91
Percent Perseverative Errors	37.56	31.73	35.75	30.52
Nonperseverative Errors	93.54	18.65	91.73	19.21
Percent Nonperseverative Errors	45.69	12.53	44.56	12.79
Percent Conceptual Level Responses	39.35	32.02	34.35	32.09

Table 9 Parents' Autism symptoms based on AQ

Variable	Father (N=89)		Mother (N=96)	
	Mean	**S.D.**	**Mean**	**S.D.**
AQ				
Social skill	3.69	2.30	3.32	2.37
Attention switching	3.89	1.82	3.64	1.89
Attention to detail	3.73	2.20	3.71	2.06
Communication	2.42	1.86	1.93	1.88
Imagination	3.81	1.93	3.70	1.72
Total score	17.54	5.59	16.12	6.17

Table 10 Psychopathology at parents based on ASRI

Variable	Father (N=89)		Mother (N=96)	
	N	%	N	%
Anxiety Disorders	54	60.67	69	71.88
Generalized Anxiety Disorder	2	2.25	13	13.54
Specific Phobia	43	48.31	53	55.21
Panic	29	32.58	46	47.92
Social phobia	4	4.49	5	5.21
Agoraphobia	10	11.24	13	13.54
OCD	32	35.96	45	46.88
Obsessions	20	22.47	31	32.29
Compulsions	25	28.09	31	32.29
TIC	28	31.46	16	16.67
Motor Tics	17	19.10	10	10.42
Vocal Tics	21	23.60	12	12.50
Somatoform Disorder	4	4.49	10	10.42
Somatization	0	0.00	0	0.00
Hypochondria	2	2.25	5	5.21
Body dysmorph	3	3.37	7	7.29
Eating Disorder	0	0.00	3	3.13
Anorexia	0	0.00	3	3.13
Bulimia	0	0.00	2	2.08
Depressive Disorders	2	2.25	6	6.25
Major Depressive disorder	1	1.12	6	6.25
Dysthymic Disorder	1	1.12	6	6.25
ADHD	3	3.37	2	2.08
AD/HD Inatten	2	2.25	2	2.08
AD/HD Hyper-Imp	1	1.12	1	1.04
AD/HD Comb	0	0.00	1	1.04
Sleep Disorders	14	15.73	25	26.04
Insomnia	9	10.11	15	15.63
Hypersomnia	2	2.25	3	3.13
Narcolepsy	4	4.49	8	8.33
Nightmare	4	4.49	10	10.42
Conduct Problems	2	2.25	2	2.08
Conduct Disorder	2	2.25	2	2.08
Antisocial	0	0.00	2	2.08
Impulsive Disorder	6	6.74	12	12.50

Inter. Explosive	3	3.37	12	12.50
Kleptomania	3	3.37	0	0.00
Gambling	0	0.00	0	0.00
Trichotillo	0	0.00	0	0.00
Pyromania	0	0.00	0	0.00
Trauma-related	3	3.37	6	6.25
PTSD	0	0.00	2	2.08
Dissociative disorder	1	1.12	2	2.08
Borderline Personality	2	2.27	4	4.17
Schizoid	4	4.49	7	7.29
Gender Identity	0	0.00	3	3.16
Adjustment	15	17.05	22	23.16
Bipolar Disorder	0	0.00	2	2.08
ODD	3	3.37	5	5.21
Schizophrenia	1	1.12	5	5.21
Substance Use	39	43.82	8	8.33

Table 11 HLA-DR Alleles Case-control comparison

HLA allele	Autism (No.)	Autism (%)	Control (No.)	Control (%)	Control Male	Control Female
DR1	1	0.68	0	0	0	0
DR2	0	0	0	0	0	0
DR3	23	15.54	20	10.31	11	9
DR4	14	9.46	38	19.59	17	21
DR5	0	0	0	0	0	0
DR6	0	0	0	0	0	0
DR7	5	3.38	3	1.55	2	1
DR8	17	11.49	15	7.74	8	7
DR9	15	10.14	27	13.92	16	11
DR10	1	0.68	0	0	0	0
DR11	11	7.43	17	8.77	13	4
DR12	18	12.16	29	14.95	14	15
DR13	1	0.68	8	4.12	6	2
DR14	11	7.43	11	5.67	8	3
DR15	21	14.19	19	9.79	7	12
DR16	10	6.76	7	3.61	2	5
Total	148	100	194	100	104	90
Hard to identify	28		24		10	14

「基因體醫學國家型科技計畫」成果評估報告(至97年3月1日止)

計畫名稱	自閉症類疾患之臨床及分子基因學研究 (第一年)		
計畫期間	自 96 年 5 月起 至 97 年 4 月止		
計畫主持人	高淑芬副教授	電話	（02）23123456 ext.6798
執行單位	國立台灣大學醫學院精神科	E-MAIL	gaushufe@ntu.edu.tw
一、計畫摘要及目標（請以一張 A4 為限，整合型計畫不在此限）			

Background: Autism is a pervasive neurodevelopmental disorder with prominent reciprocal social and communication impairment and restricted repetitive behavior or interest. Based on the number of symptoms and functional impairment, autistic disorder, Asperger disorder, and atypical autism (or PDDNOS) are conceptualized as the autism spectrum disorder (ASD). Most recent survey estimated the prevalence of narrow diagnosis of autistic disorder to be around 0.1% to 0.2%, and 0.59 % to 0.63% for ASD, with a four-fold male predominance. Due to high heritability (> 0.9), high family recurrence risk ($\lambda = 60$), and severe impairment without effective prevention and treatment available for ASD, this disastrous disease has been prioritized for molecular genetic study from public health perspective. The proposed research is the first systematic approach combining clinical and molecular genetic study of ASD involving multi-sites and three research cores: assessment core (by Gau SS and Wu YY), molecular genetics core (by Chen CH), and data/statistics core (by Gau SS).

Objectives: The long-term objective of this study is to establish clinical and genetic database of autism and their family for etiology study, exploration of pathogenesis, and developing new treatment. The specific aims are:

1. to establish the psychometric properties of three Chinese versions of rating scales for ASD: ADI-R, ADOS, SCQ, SRS, and ABC;
2. to collect demographic, symptoms, developmental, and clinical data of ASD probands and their siblings;
3. to conduct the pilot study of neuropsychological studies on probands with high-functioning ASD;
4. to collect genetic data of families of probands with ASD;
5. to identify the genetic variants close to etiological genes of ASD in a Taiwanese sample using candidate gene case-control association study design (e.g., Neuroligin gene family, MeCP2 gene, and FOXP2 gene, parent trio and population-based studies) and whole genome linkage analysis for multiplex families.

Results: For past 10 months, we have translated several key autism instruments into Chinese, including ABC, AQ, CAST, SRS, SCQ, ADI-R and ADOS. Up to now, we have successfully collected partial data (clinical, neuropsychological, and genetic data) from 100 families out of the expected total sample size of 300 families in this 3-year grant. Cell lines have been developed from all the blood samples. We also have done two preliminary genetic analyses. Our association study on the HLA-DRB1 genotyping in 74 probands and 97 controls revealed that DR4 (9.5% vs 15.6%), DR13 (0.7% vs 11.6%), and DR15 (14.2% vs 9.8%) had significantly different frequency in probands with autism compared with normal controls. Our mutation screening showed three single nucleotide polymorphisms in 11 exons of ACCN3 gene, including a A-to-T substitution at exon 1 (c.229A>T), a G-to-T substitution at exon 3 (c.719G>T), a T-to-C substitution at intron10 (IVS10+60T>C).

Discussions: Our research team not only reach the first year goal within the 10 months but have collected the clinical and genetic data of additional 60 families. We will continue to work hard to collect more ASD families, to add ADOS into our standard assessment of ASD, and to conduct genetic analysis on the available sample.

二、執行進度差距（請依照計畫執行內容及階段為評估標準）

實際執行進度/計畫預定進度=___100___%

三、人力運用情形

參與計畫人員姓名	職稱	專長	學歷	參與年資
高淑芬	副教授	兒童精神醫學、流行病學、生物統計學	M.D., Ph.D.	10 月
宋維村	副教授	兒童少年精神醫學、家庭精神醫學、一般精神醫學、心理衛生	M.D.	10 月
丘彥南	主治醫師	兒童少年精神醫學	M.D.	10 月
蔡文哲	主治醫師	兒童少年精神醫學	M.D.	10 月
廖漢文	副教授	神經系統放射線診斷學、神經系統放射線介入性治療	M.D.	10 月
陳嘉祥	教授	人類遺傳學、精神遺傳學	M.D.	10 月
吳佑佑	主治醫師	兒童精神醫學、青少年精神醫學、自閉症、心理治療	M.D.	10 月
黃玉書	助理教授	兒童精神醫學	M.D.	10 月
林郁秀	研究助理	心理衡鑑	B.S.	8 月
梁郁苹	研究助理	精神科護理	B.N.	8 月
徐玉容	研究助理	心理衡鑑	B.S.	8 月
王齡萱	研究助理	人類遺傳學	M.M.	7 月

四、經費運用情形

1.全程經費___12550___仟元

2.本年執行進度：目前支用___2798___元/本年經費___4415___仟元=___63.36___%

五、成果差距

預期獲得(篇/項數)				實際獲得(篇/項數)			
論文	專利權	技術移轉	著作權	論文	專利權	技術移轉	著作權
0	0	0	0	0	0	0	0

六、成果評估與展望

（請就工作項目/研發成果/預期產出各方面差距做自我評估，並著眼展望於對學術、社會、經濟面的成果效益）　（請以一張A4為限，整合型計畫不在此限）

本研究自九十六年五月至九十七年二月底止已進行 10 個月，在這 10 個月中我們所完成的進度包括：(1)研究自填問卷的翻譯及前測的部分、(2)完成兩種診斷工具的分析及嚴謹的翻譯，包括會談診斷工具 Autism Diagnostic Interview – Revised (ADI-R)，及觀察診斷工具 Autism Diagnosis and Observation Scale(ADOS)、(3)已收集 100 個家庭的完整的血液及臨床資料，並開始進行初步的分析。目前的進度是遠超過第一年預期的進度。

在這 10 個月的研究進行過程中，最困難的部分在於研究工具的準備。由於在研究開始進行之前，國內完全沒有任何中文版的工具得以用來評估自閉症的症狀嚴重度、共病和情緒行為問題及其發展的功能程度、日常生活能力，故在研究開始前整整兩年的時間，在沒有任何經費支持的情況下，研究團隊中所有參與人員，包括 12 位兒童精神科醫師及 1 位心理師，已取得國外相關機構 Western Psychological Service(WPS)的同意，開始在週末及平常下班之餘，著手進行嚴謹的翻譯、討論及 back-translation。由於 ADI-R 和 ADOS 各是將近 80 頁的診斷工具，翻譯十分困難，不僅所有研究人員花了許多心力，再加上這些工具是由 WPS 所出版，翻譯版本需獲得該機構的許可及評估，是研究主持人從事研究 20 年來從未經歷過的重大困難。但是我們憑著專業能力及過去從事研究的經驗，表現出對工具準備的嚴謹及專業，終於在眾多競爭者中，獲得 WPS 對我們 ADI-R 中文版本的認可，亦即我們在此研究收集的臨床個案，可使用 ADI-R 進行會談評估，並輔以兒童精神科醫師的臨床診斷，以取得最完整的臨床症狀的診斷；至於 ADOS 的部分，目前也已完成 back-translation，正送交 WPS 評估中，進行最後確認。

目前所收的 100 位個案中，尚未完成 ADOS 的評估，但我們預計在取得 ADOS 中文版權後，由研究主持人及共同主持人(吳佑佑醫師)於暑假中進行研究助理及其他參與醫師的 ADOS 訓練。此訓練在國外的收費標準為為每位受訓人員 1500 元美金，故在暑假所舉行的訓練預計能省下不少經費，但其相關版權的取得費用以及購買工具，仍需支出相當多的經費，故此工具準備及翻譯的部分為這 10 個月中耗費最大心力的部分。我們預期在不久之後，ADOS 將可在本研究中可使用，而這些工具未來將可開放作為國內其他相關研究人員及臨床工作者的使用。

除了翻譯測驗工具及量表外，我們亦完成了 100 個家庭的資料收集。此研究需完整收集個案、手足及其父母之血液及臨床資料，以及多項神經心理學測驗結果，因此一個家庭的評估需耗時至少 5 個小時以上。為配合個案家庭時間，評估大多必須在下班後或假日完成，除了花費時間外，也需大量人力進行。由於我們的努力，並給予家屬完整評估報告及臨床的整合性服務，獲得家屬及個案的信賴，除了幼兒的血液不易抽取，須花相當長的時間與小孩建立關係之外，完整的家族血液和臨床資料的蒐集，以及個案的轉介，目前並未遇到太大困難。在此過程中，研究助理以及年輕的醫師們獲得許多重要的研究經驗：包括建立臨床可信的評估、與家屬建立良好的信賴關係及互動。至於其血液樣本，目前皆已送達實驗室進行細胞之培養，亦對所收集到的血液樣本進行初步的基因分析，結果部分如報告其他部分所呈現，此為臨床評估及實驗室分析同時並行且良好合作及努力的展現，始能在此短時間之內有此初步結果。

計畫第二年將會接續第一年的進度，繼續進行個案家庭的資料收集，並且將針對研究工具的信效度資料，著手撰寫相關論文，以便後續有關基因與臨床資料的發表。同時希望 ADOS 能在近期內能獲得 WPS 的許可，使研究主持人及共同主持人能在暑假中進行參與醫師及研究助理的訓練。

目前的研究成果是超越原先預期的進度，我們對此相當滿意。研究團隊展現相當緊密的合作，所有參與研究人員皆投入相當多的心力，積極地進行此國內第一個有關自閉症的基因研究，我們可預期未來能以穩定的收案進度，繼續收集更多基因及臨床相關資料。當資料收集、整理完畢後，我們將會陸續有臨床方面或基因分析的相關論文發表在國際或國內的學術研討會，並希望在不久的將來能很快地發表在國際期刊，亦希望透過我們的努力能夠找到與自閉症有關的致病基因

七、對基因體醫學國家型科技計畫之具體建議

非常感謝行政院國家科學委員會基因體國家型科技計畫能夠支持這個研究，因為自閉症類疾患是非常值得進行遺傳基因方面研究的疾患，而本研究是國內第一個整合了自閉症兒童臨床和基因體的研究，其重要性已經在成果報告中清楚的描述。

研究團隊中有許多從事兒童精神科的臨床醫師們，都已經累積數十年臨床工作的經驗，但過去國內一直沒有將這些臨床經驗轉化成研究的機會。我們十分感謝國家科學委員會今年在研究資源方面給予的支持，讓我們可以完成所有診斷工具的準備，並且能更深入的進行基因方面的分析。診斷工具和量表不僅是貴重的研究資源，在自閉症類疾患的臨床評估診斷或是評估治療效果，都是非常必要的，這部分也是目前國內相當缺乏的部分。

相對於國內其他研究，我們的經費算是充裕，但與國外進行相同評估測驗的自閉症研究經費差距相當大。我們在有限的經費之下，珍惜每一分辛苦得來的經費，努力以最經濟、最有效率的方式完成研究的多項目的。希望未來國家科學委員會基因體醫學國家型科技計畫能夠繼續給予支持，讓我們建立起研究的基礎後，能擴展到更深入的部份，以本土的族群，找到與自閉症相關的基因和染色體上的發現。並讓我們已經遠遠落後國際社會的研究可以急起直追，使國內自閉症類疾患方面的研究達到國際學術的水準。

赴國外研究心得報告

計畫編號	NSC 96-3112-B-002-033
計畫名稱	自閉症類疾患之臨床及分子基因學研究
出國人員姓名 服務機關及職稱	吳佑佑 (共同主持人) 長庚醫院兒童心智科　主治醫師
出國時間地點	自 2007 年 6 月 20 日至 2007 年 6 月 22 日
國外研究機構	University of Michigan

工作記要：

這次研修是到美國密西根大學接受自閉症診斷觀察量表(Autism Diagnostic Observation Schedule，以下簡稱 ADOS)的訓練，並取得訓練者之訓練資格認證，期間是 2007 年 6 月 20 日至 6 月 22 日。ADOS 在經過本院兒童心理衛生中心醫師一年的準備和翻譯，目前正準備將中文版之翻譯結果送回 WPS(Western Psychological Services)作最後確認，並希望取得中文版之授權及認可，能夠將此診斷工具之中文版使用在此研究上。

ADOS 的訓練過程乃一嚴謹之訓練：除了在研修之前，須先熟悉所有 ADOS 的診斷內容，並熟讀手冊裡所詳述之如何做 ADOS 診斷及執行步驟，並先須達成認可使用此工具之 training workshop，在獲得認證後，才可進一步前往參加為更高階訓練者準備之 trainer-in-training workshop。在訓練過程中，除須精確診斷達評估者間信度 0.90 以上，還須學習如何在臨床及研究上完整講述 ADOS 執行步驟與注意事項。

在工作坊的最後，會有實際三個個案的考試，回國後更須錄製一份英文會談錄影，將診斷結果送回國外，確認與其他訓練者之間的信度達 0.9 以上，即可取得國際認證。

在此次訓練工作坊結束之後，已完成將所有資料送達 University of Michigan，並取得其認證，獲得訓練其他醫師使用此診斷工具之訓練者資格。

赴國外研究心得報告

計畫編號	NSC 96-3112-B-002-033
計畫名稱	自閉症類疾患之臨床及分子基因學研究
出國人員姓名 服務機關及職稱	高淑芬 副教授 台灣大學醫學系精神科
出國時間地點	自 2007 年 7 月 4 日至 2007 年 7 月 7 日
國外研究機構	Monash University Medical Center

工作記要：

這次研修是到澳洲 Monash medical center 接受自閉症臨床及研究診斷工具(Autism Diagnostic Interview Schedule-Revised，以下簡稱 ADI-R)的訓練，並獲得增加認證，期間是 2007 年 7 月 4 日至 7 月 6 日。ADI-R 在經過本院兒童心理衛生中心醫師一年的準備和翻譯，並取得 WPS(Western Psychological Services)的許可，將此診斷工具之中文版使用在此研究上，目前已在研究中著手進行使用此診斷工具進行診斷會談。

此次研修主要的訓練過程，包括事前在出國前必須熟悉所有 ADI-R 的診斷內容，並熟讀手冊裡所詳述之如何做 ADI-R 診斷及執行步驟，並先須以台灣個案錄製錄影帶，之後才開始為期三天的訓練。訓練過程中除了解有關使用此診斷工具的發展，及過去研究中此診斷工具之信效度，並學習如何和臨床上診斷做連結；在大略對此診斷工具有初步了解後，即進行示範會談，由我們在旁做會談者間的信度紀錄，之後再針對每個個案做詳細討論。

第二天則實際對個案進行診斷會談，彼此交換不同個案討論，並檢測會談結果及所做診斷的精確度是否符合；此外，負責訓練的 Dr. Laura Crilley 會就之前所送達之會談 DVD，確定各人在台灣所做的會談技巧及方式如何。

第三天有實際三個個案的考試，請個案到場做 interview 或以錄影帶練習，每人須達到評估者間信度 0.9 以上，才能繼續進行下一步。三天的訓練結束後會有多次的評估者間信度及學習模擬，在過程中皆以英文進行。回國後更須進一步接受錄影帶評估，並將結果送回，確認信度達 0.9 以上，即可在台灣錄製一份英文會談錄影，將診斷結果送回國外，確認和訓練者之間的信度達 0.9 以上，即可取得國際認證。

前後五天的訓練結束之後，已完成將所有資料送達 MONACH Dr. Laura，並取得其認證，獲得許可使用此診斷工具。

赴國外研究心得報告

計畫編號	NSC 96-3112-B-002-033
計畫名稱	自閉症類疾患之臨床及分子基因學研究
出國人員姓名 服務機關及職稱	吳佑佑 (共同主持人) 長庚醫院兒童心智科 主治醫師
出國時間地點	自 2007 年 6 月 20 日至 2007 年 6 月 22 日
國外研究機構	University of Michigan

工作記要：

這次研修是到美國密西根大學接受自閉症診斷觀察量表(Autism Diagnostic Observation Schedule，以下簡稱ADOS)的訓練，並取得訓練者之訓練資格認證，期間是2007年6月20日至6月22日。ADOS在經過本院兒童心理衛生中心醫師一年的準備和翻譯，目前正準備將中文版之翻譯結果送回WPS(Western Psychological Services)作最後確認，並希望取得中文版之授權及認可，能夠將此診斷工具之中文版使用在此研究上。

ADOS的訓練過程乃一嚴謹之訓練：除了在研修之前，須先熟悉所有ADOS的診斷內容，並熟讀手冊裡所詳述之如何做ADOS診斷及執行步驟，並先須達成認可使用此工具之training workshop，在獲得認證後，才可進一步前往參加為更高階訓練者準備之trainer-in-training workshop。在訓練過程中，除須精確診斷達評估者間信度0.90以上，還須學習如何在臨床及研究上完整講述ADOS執行步驟與注意事項。

在工作坊的最後，會有實際三個個案的考試，回國後更須錄製一份英文會談錄影，將診斷結果送回國外，確認與其他訓練者之間的信度達0.9以上，即可取得國際認證。

在此次訓練工作坊結束之後，已完成將所有資料送達University of Michigan，並取得其認證，獲得訓練其他醫師使用此診斷工具之訓練者資格。

赴國外研究心得報告

計畫編號	NSC 96-3112-B-002-033
計畫名稱	自閉症類疾患之臨床及分子基因學研究
出國人員姓名 服務機關及職稱	高淑芬 副教授 台灣大學醫學系精神科
出國時間地點	自 2007 年 7 月 4 日至 2007 年 7 月 7 日
國外研究機構	Monash University Medical Center

工作記要：

這次研修是到澳洲Monash medical center接受自閉症臨床及研究診斷工具(Autism Diagnostic Interview Schedule-Revised，以下簡稱ADI-R)的訓練，並獲得增加認證，期間是2007年7月4日至7月6日。ADI-R在經過本院兒童心理衛生中心醫師一年的準備和翻譯，並取得WPS(Western Psychological Services)的許可，將此診斷工具之中文版使用在此研究上，目前已在研究中著手進行使用此診斷工具進行診斷會談。

此次研修主要的訓練過程，包括事前在出國前必須熟悉所有ADI-R的診斷內容，並熟讀手冊裡所詳述之如何做ADI-R診斷及執行步驟，並先須以台灣個案錄製錄影帶，之後才開始為期三天的訓練。訓練過程中除了解有關使用此診斷工具的發展，及過去研究中此診斷工具之信效度，並學習如何和臨床上診斷做連結；在大略對此診斷工具有初步了解後，即進行示範會談，由我們在旁做會談者間的信度紀錄，之後再針對每個個案做詳細討論。

第二天則實際對個案進行診斷會談，彼此交換不同個案討論，並檢測會談結果及所做診斷的精確度是否符合；此外，負責訓練的Dr. Laura Crilley會就之前所送達之會談DVD，確定各人在台灣所做的會談技巧及方式如何。

第三天有實際三個個案的考試，請個案到場做interview或以錄影帶練習，每人須達到評估者間信度0.9以上，才能繼續進行下一步。三天的訓練結束後會有多次的評估者間信度及學習模擬，在過程中皆以英文進行。回國後更須進一步接受錄影帶評估，並將結果送回，確認信度達0.9以上，即可在台灣錄製一份英文會談錄影，將診斷結果送回國外，確認和訓練者之間的信度達0.9以上，即可取得國際認證。

前後五天的訓練結束之後，已完成將所有資料送達MONACH Dr. Laura，並取得其認證，獲得許可使用此診斷工具。