

Studies of Dopaminergic System in Tourette Syndrome Comorbid Attention
Deficit Hyperactivity Disorder

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Content

I. Preface

In our previous studies, we used single nucleotide polymorphisms (SNPs) as markers to study the association between dopamine regulators and TS in Taiwanese children. A total of 160 Taiwanese children with TS and 83 normal control subjects were included. We have identified that dopamine receptor D2 gene polymorphisms are associated in Taiwanese children with TS (10), and the dopamine receptor D1 gene polymorphism are not associated (39). This is consistent with animal and human studies showing an important role of dopamine in rewarding TS. However, we examined the noradrenergic genes (*ADRA2A*, *ADRA2C*) and found no association (40). We also reported that genetic screening in the *Slit* and *Trklike1* gene for the recently identified mutations does not appear to be of utility in the diagnosis of TS (41). Based on these experiences, we will focus on neurobiological data that link the genetic variants to biological models that reflect dysfunction in the dopamine systems. Second, TS and ADHD are highly co-morbidity, and the neurobiologic relationship between TS and ADHD is not yet clear. In our experience, most of the TS patients we have tested fall into ADHD, and since we have also examined multiple sets of controls, we feel that this wide range of individuals will allow us to examine the role of the dopaminergic candidate genes in pure TS, pure ADHD and TS comorbid ADHD. We hypothesize that TS and ADHD are variant expressions of a common set of vulnerability genes, with one subtype of ADHD being a variant expression of the underlying vulnerability genes for TS. Both conditions evolve from disturbances in a shared neural substrate, such as dysfunction of the basal ganglia. Therefore, we subclass three diagnostic groups in this study exploring the dopaminergic genes of TS only, TS with comorbid ADHD, and ADHD. This approach includes clinical symptoms, neuroimaging studies, and treatment response to methylphenidate. Results from the study will be crucial in elucidating the pathogenesis of this condition.

II. Research Aim

Tourette's Syndrome (TS) and attention deficit hyperactivity disorder (ADHD) are now recognized as a common neuropsychiatric disorder with a spectrum of neurological, behavioral, and cognitive features. We aim to characterize affected individuals in patients with ADHD and TS. The neuropsychological function and comorbid disorders and of TS will be examined. We will further use the Yale Global Tic Severity Scale to compare the severity of TS with the single nucleotide polymorphisms (SNPs) markers. Four models of alterations in the central neurotransmitters have been suggested as a cause of TS and ADHD.

III. Background and Rationale

Tourette Syndrome Comorbid Attention Deficit Hyperactivity Disorder

Tourette syndrome (TS) and attention deficit hyperactivity disorder (ADHD) are common neuropsychiatric disorders found in children, and they frequently co-occur in the same individual (1, 2). Tic disorders are characterized by bouts of brief, involuntary or semivoluntary movements and sounds. Tic disorders are classified as either transient (present for less than one year), chronic (motor or vocal tics lasting longer than one year), or as TS, in which the presence of chronic motor and vocal tics endure beyond one year. It occurs in 1-3% of school-age children (1).

Attention deficit hyperactivity disorder (ADHD) is characterized by hyperactivity, inattention, and impulsivity. The DSM-IV classification of ADHD distinguishes three subtypes: predominantly hyperactive, predominantly inattentive, and combined type, in which both hyperactive and inattentive symptoms are present. The majority of studies report prevalence rate of 3-6% within a school-aged population (2).

The diagnosis and effective treatment of ADHD in a child with TS can be critical to their academic, social and interpersonal functioning. The school performance of a child with TS may

be impaired by various combinations of contributing factor (2). In a recent study of social and emotional adjustment in 72 children with TS, tic severity and frequency was not associated with social, behavioral, or emotional functioning even after controlling for the effects of medication. However, ADHD diagnosis was found to be highly correlated with those outcomes (2).

Genetics of Tourette Syndrome and Attention Deficit Hyperactivity Disorder

In the 1970s, investigators first demonstrated that TS shows a familial concentration. Susceptibility to TS was then demonstrated to be transmitted vertically from generation to generation, and studies of twin pairs confirmed a genetic influence. In 1986, Pauls and Leckman used segregation analysis of affected families to indicate that TS is inherited in an autosomal dominant pattern, with variable expression that also includes chronic tic disorder and obsessive-compulsive disorder (OCD). This information raised hopes that the application of linkage analysis techniques to ascertained pedigree would identify a genetic marker linked to the disease. To date, the gene search in TS has been unsuccessful (4) and is illustrative of the many factors that can complicate genetic analysis of complex human traits.

ADHD is also highly hereditary, and the familial and twin studies estimate heritability at 80%–90% (2). It is estimated that 50-75% of children with TS will have comorbid ADHD (2). The high rate of comorbidity between ADHD and TS is hypothesized to involve shared pathophysiology of basal ganglia circuitry (3).

Neurobiolog Issues in Tourette Syndrome

There is general consensus from pathophysiological studies that TS represents a disorder localized to a series of parallel frontal subcortical circuits that link specific regions of the frontal cortex to subcortical structures (5). Although a variety of neurotransmitters are localized within these circuits, the possibility of a dopaminergic abnormality in TS continues to receive strong consideration. Evidence supporting the dopamine hypothesis includes the therapeutic response to dopamine antagonists and results from a variety of postmortem studies and nuclear imaging protocols (6). Postulated changes in dopamine synaptic transmission include: (A) variable increases in the number of receptors (7); (B) elevated levels of transporters (8); and (C) increased intrasynaptic release (9).

Progress in Gene Location in Tourette Syndrome and Attention Deficit Hyperactivity Disorder

Base on these evidence, candidate genes investigated to date in TS belong primarily to the dopaminergic system. In our previous study, the dopamine receptor 2 gene (DRD2) TaqI restriction site locus has shown a possible association with TS (10) Besides, the 7-repeat allele of the dopamine receptor 4 gene (DRD4) (11) have also shown an association with TS. Several investigators have examined patients with both TS and ADHD (TS+ADHD) and shown positive associations for polymorphisms within the DRD2, DRD4, dopamine beta-hydroxylase (DBH), and acid phosphatase 1 (ACP1) genes (13). For example, the TaqI polymorphism of DBH, the enzyme responsible for the conversion of dopamine to norepinephrine, has been associated with an additive effect on continuous ADHD scores in TS patients (13). In a study evaluating TS+ADHD, the frequency of non A alleles of the ACP*1A polymorphism was significantly increased in TS+ADHD cases (12). In 2007, Singer et al has identified a significant association between the presence of TS and a dopamine transporter, DAT1 *DdeI* polymorphism (14).

Involvement of the dopaminergic system has been also suggested in patients suffering from ADHD since the symptoms can be successfully treated with methylphenidate, a potent blocker of the dopamine transporter (DAT). ADHD is a very promising area for pharmacogenetic studies. Among the 15 ADHD pharmacogenetic studies in the literature (15, 16), 10 investigations addressed the dopamine transporter gene (DAT1). In a previous study, an association between homozygosity for the 10-repeat allele at DAT1 and worse response to methylphenidate (MPH) in a sample of 50 European-Brazilian patients (17), replicating previous results from Winsberg and Comings (18). Recently, Cheon et al. (19) also replicated this association. However, several others found different results (20-24). Five studies addressed the association between the 48-bp

VNTR polymorphism at the D4 dopamine receptor (DRD4) and response to MPH (19, 21, 25-27) found that patients presenting the 7-repeat allele at DRD4 (DRD4*7) required higher doses for symptom improvement. Cheon et al. (19) reported that children who were homozygous for the 4-repeat allele at the DRD4 presented a better response to MPH.

Imaging in Attention Deficit Hyperactivity Disorder and Treatment Response

In recent years, investigators have begun to focus on the possible neurobiological factors involved in ADHD by evaluating the functional activity of the brain through the use of newly available imaging techniques. These studies have reported on either brain metabolism (28) or perfusion (29, 30). Recently, animal and human studies have been published on a newly developed and one of the first technetium-labelled ligands for imaging the dopamine transporter, [^{99m}Tc]TRODAT-1 (31-37). In the previous study, [^{99m}Tc]TRODAT-1 was used to assess the status of the DAT in adults suffering from ADHD in the newly diagnosed, untreated condition and after commencement of treatment with methylphenidate (38). They found that the decrease in available DAT binding sites under treatment with methylphenidate correlates well with the improvement in clinical symptoms. [^{99m}Tc]TRODAT-1 may help to elucidate the complex dysregulation of the dopaminergic neurotransmitter system in patients suffering from ADHD and the effect of treatment with psychoactive drugs.

VI. Instruments

SNAP-IV

The Swanson, Nolan, and Pelham scale—version IV (SNAP-IV)—the primary outcome measure—is a revision of the Swanson, Nolan, and Pelham (SNAP) Questionnaire (43). Its subscales provide scores for inattention, hyperactivity, and oppositional symptoms. The scale is completed by the subjects' parents and teachers. The Taiwanese version of ADHD evaluation scale was standardized by Gau, et al (44). Secondary outcome measures include the Clinical Global Assessment (45) and the Barkley's Stimulants Side Effects Rating Scale (SERS) to assess stimulants side events (46).

YGTSS

Yale Global Tic Severity Scale (YGTSS), a semi-structured interview with multiple informants, was designed to elicit information concerning the specific character and anatomical distribution of tics observed during the course of 1-week interval before the clinical assessment (47). With number, frequency, intensity, complexity and interference, examiners rate these characteristics for motor and phonic tics independently to generate a total tic score, an overall impairment rating, a global severity score and there is also a checklist for specific types for the tics.

CY-BOCS

The Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS) is a 10 item clinician-administered scale for Obsessive-Compulsive Disorder (OCD) that represents a total scale as well as two subscales (obsessions and compulsions). Both subscales are identically structured, with five items assessing frequency, distress, interference, resistance, and control over either obsessions or compulsions. Each item is rated from 0 to 4, with higher numbers indicating severity of symptoms (49)

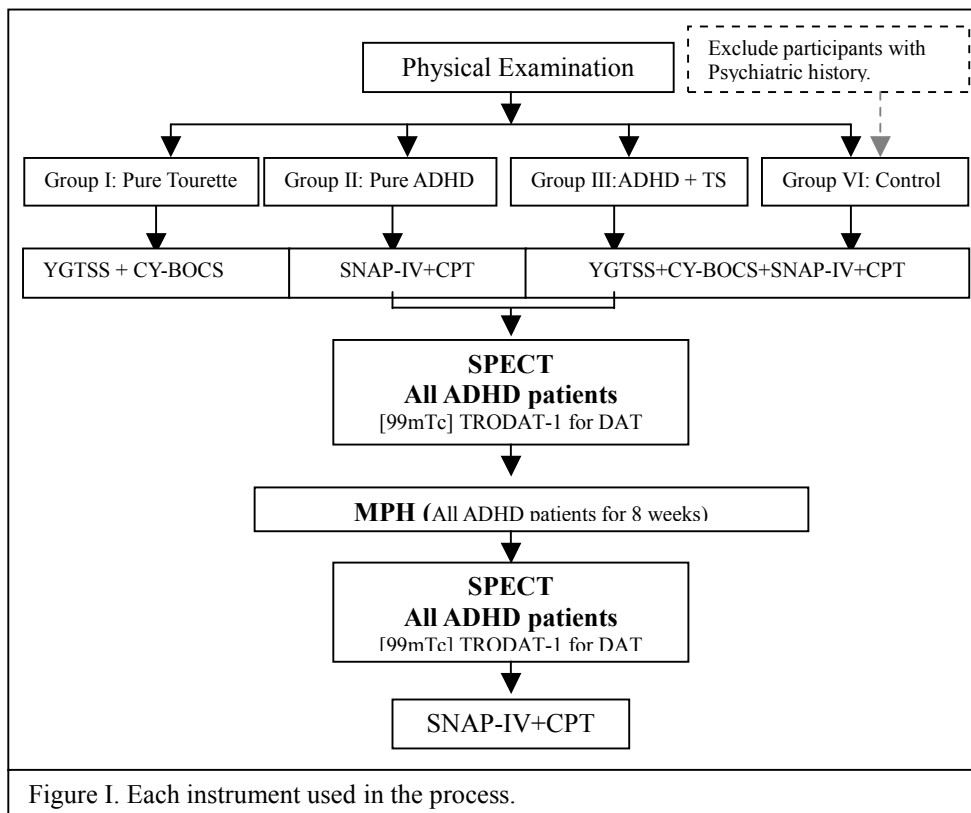
CPT II

Conner's Continuous Performance Test II (CPT II) is an attention test widely used in ADHD research and clinical testing. Examiner measures response times, errors, change in reaction time speed and consistency according to Signal Detection Theory. The standard version of the test is a non-X- type presented visually for 14 minutes. The participants need to respond by pressing the space bar every time a letter (target) appear on the computer screen, expect the letter X (non-target) appears (48).

V. Methods

Include Taiwanese children with Tourette's syndrome and ADHD patients (group 1; TS=200, ADHD=200, ADHD comorbid TS=200) and normal control subjects (group 2; n=200). This study was approved by the Ethics Committee of the China Medical College Hospital, Taichung, Taiwan. All parents signed informed consent before blood tests were performed. Cases were matched with controls according to age, sex, ethnicity and geographic location of origin. Diagnosis of TS and ADHD followed the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV). The severity of TS will be measured by the YGTSS, to compare the severity of TS with the SNP markers; the attention data of ADHD patients will be measured by CPT and SNAP-IV. All cases were from unrelated kindred. The controls were healthy volunteers with no history of psychiatric treatment (Figure I).

All children will undergo peripheral blood sampling for genotype analyses. Genomic DNA was isolated from peripheral blood by mean of a DNA extractor kit (Genomaker DNA extraction kit; Blossom, Taipei, Taiwan). A total of 50 ng of genomic DNA was mixed with 20 pmol of each polymerase chain reaction (PCR) primer in a total volume of 25 ul containing 10mM Tris-hydrochloride, pH 8.3; 50mM potassium chloride; 2.0mM magesium chloride; 0.2mM each deoxyribonucleotide triphosphate; and 1 U of DNA polymerase (Amplitaq; Perkin Elmer, Foster City, Calif., USA). PCR primers were used to amplify the correlated gene. The subjects of this research are not required to have follow-up program. Allelic frequencies were expressed as a percentage of the total number of alleles. Genotypes and allelic frequencies polymorphisms in both groups were compared.



VI. International Cooperation

In March 2009, the National Institute of Neurological Disorders and Stroke hold a “Tourette Syndrome GWAS Replication Workshop” in Bethesda, Maryland. In the conference, Prof. David Pauls mentioned a global project for collecting and genotyping the TS GWAS samples to replication. In table I, there are TS samples contributed by each site. The project that they applied to NIH is build on a long-standing program of research conducted by the Tourette Syndrome

Association International Consortium on Genetics (TSAICG) that was formed to help clarify the genetics of Gilles de la Tourette Syndrome (GTS). We attend the group to advance the genetic research and become one of a member of GTS GWAS replication steering committee.

All individuals who will be included in the current project have signed informed consent for their DNA to be used in the studies. De-identified DNA samples and phenotypic information will be sent to the coordinating site at the Psychiatric and Neurodevelopmental Genetic Unit in the Center of Human Genetic Research at Massachusetts General Hospital in Boston. This replication/extension project will incorporate a case-control design. We are looking forward to this development.

Table I: Expected number of subjects contributed by each site

Site	Ethnicity/ ancestry	# of TS samples
TSAICG	US/Canadian	328
TSAICG	French Canadian	73
TS GeneSEE (Hungary)	Hungarian	66
TS GeneSEE (Poland)	Polish	20
TS GeneSEE (Greece)	Greek	16
TS GeneSEE (Italy)	Italian	50
Utrecht	Dutch	20
Vienna, Austria	Austrian	107
Essen	German	107
Hannover	German	98
Taiwan	Han Chinese	91
Tel Aviv	Israeli Jewish	28
NYU	US	35
Cincinnati	US	92
Hopkins	US	250
Rutgers	US	30
Paris	French	127
TOTAL		1538

VII. Discussion

We've gathered 93 patients with TS or ADHD comorbid TS in the last year, and the male/female ratio is about 1:5. Still there are subjects needed to follow up, so in the 1st year of this three-year project, we've searched and developed instruments to perfect the data we want to collect and analyze from participants.

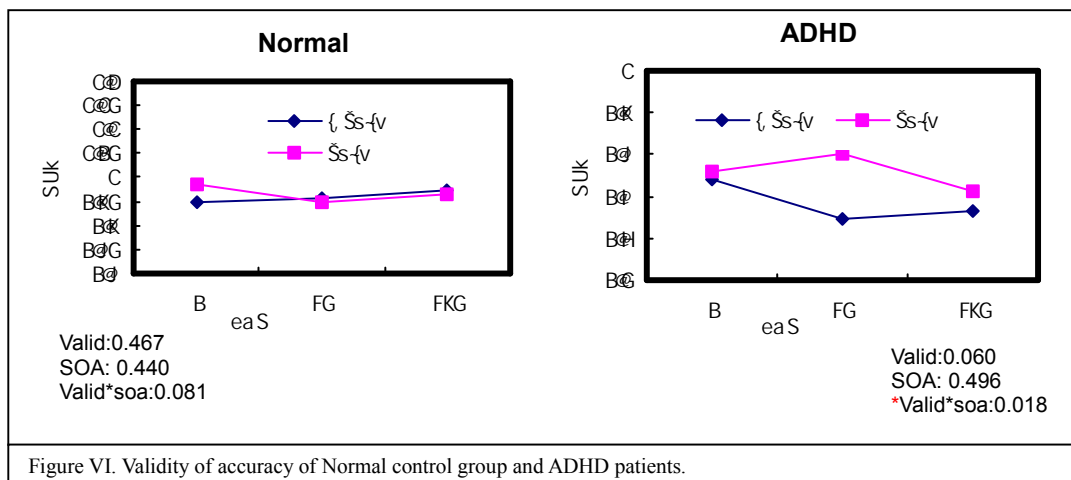
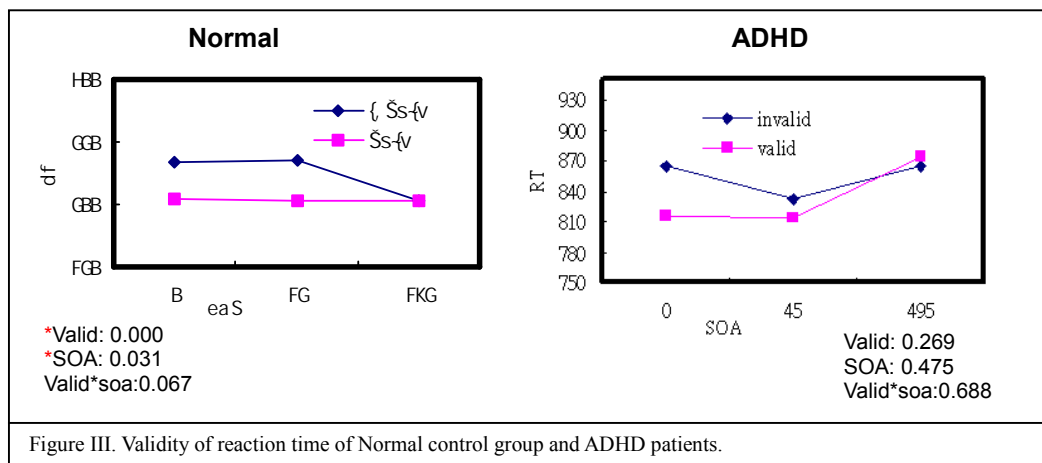
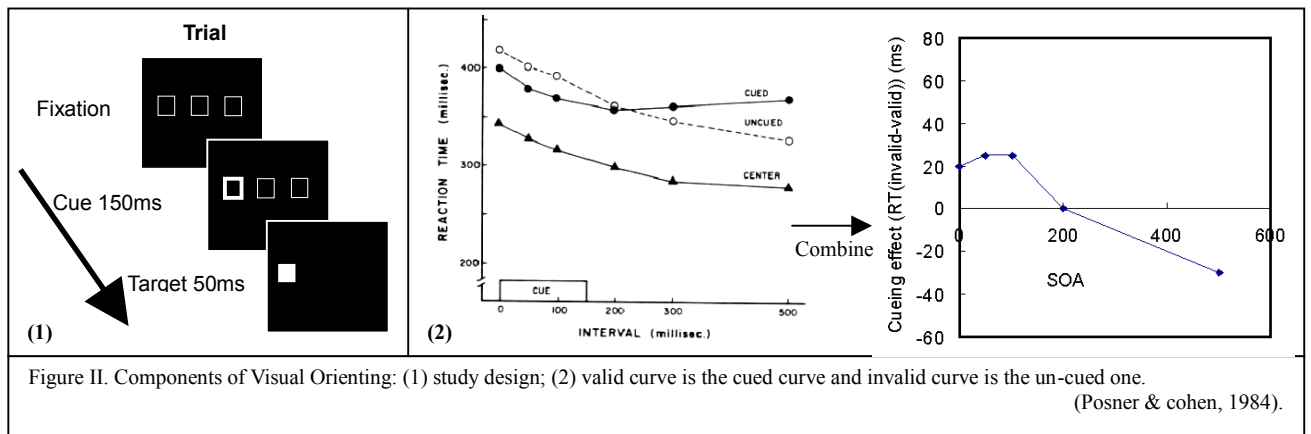
1. The cueing paradigm:

Thanks for Dr. Hsu, Li-Chuan and Dr. Tien, Yi-Min provide an incredible instrument- an idea- for ADHD patients to find out if they are different from normal people. Cohen and Posner (1984) had developed a paradigm for attention orientating (50). And other investigators (Bashinski & Bachrach, 1980; Jonides, 1981; Posner, 1980; Posner, Nissen, & Ogden, 1978) used central and peripheral cues to direct attention to various places in the visual fields and showed that even under the most austere conditions of empty visual fields and clear, bright stimuli, various dependent variables showed improved efficiency when subjects were cued to location of the event (51-54). The paradigm is (Figure II): Top panel is before a trial; subject fixates at central box; brightening of the outline of one peripheral box initiates a trial (middle panel); and a small but a bright target will appear in the center of one of the boxes to which a response is required.

According to results of Posner (1984)'s study, when SOA is 0, 50 and 100 ms, there is a Facilitation (curves of reaction time become lower) in this interval, means that when the time getting longer, participants will respond more correctly. But when SOA is between 300 to 500 ms, an Inhibition appears (the curves become higher); it means participants take more time to respond the target when SOA is getting longer in this field (Figure II).

The study is a 3*2*4 experimental design, 180 trials in total, manipulating the stimulus onset asynchrony (SOA) time (0, 50, 100 ms), cues (only appears at left or right side of the

three boxes) and targets (left, right, center and without targets). There are 15 ADHD patients and 13 control persons included. According to the results (figure III), there are two significances, “Valid” and SOA, in the normal group; for the accuracy, there is an interaction between SOA and “Valid” in ADHD group (figure VI). We may notice that in the normal group, even “valid” is significant, but the RT time seems the same at every different SOA. RT in ADHD group even shows no significant, but the “invalid” curve has the effect of Facilitation and both of “valid” and “invalid” curves shows the Inhibition. And even ADHD patients take more time to respond; there is a significant interaction for accuracy (0.62-0.80). For the research, we think probably the numbers of trails is not enough; the SOA time is not various, participants didn’t truly matched and most of all, sample is not big enough. So we’ll manipulate these variables for the further research to clarify the mechanism of attentional orienting on ADHD patients and develop the study to an effective assessment in clinic.



2. Translating scales of YGTSS and CY-BOCS: We translate the scales to make the data collected more easier for the examiner. In further, we can form a Chinese-version tic severity scale to build a Taiwanese norm. CY-BOCS is an OCD assessment; we include this scale in the process to check if the participant is co-morbidity between OCD and TS.
3. Using the CPT to quantify the attention data: We use two instruments to assess the severity of ADHD patients. Use SNAP-IV to confirm the diagnosis at first and CPT to compare the difference from ADHD patients' before and after medical therapy for ADHD.

VII. Self-assessment

First year of project				
Subject	Status			Notification
YGTSS translation	Un finished	In processing	Complete	
CY-BOCS translation	Un finished	In processing	Complete	Will be completed in June, 2009
CPT sourcing	Un finished	In processing	Complete	Will be completed in June, 2009
The evaluation of attentional orienting in patients with ADHD	Un finished	In processing	Complete	The cueing system is replaced by CPT in this project, and for its further research will keep going on by another project.
Case report form	Un finished	In processing	Complete	Will be completed in June, 2009
Participant-retrievals	Un finished	In processing	Complete	Will be completed in the 2 nd year.
International cooperation for TS	Un finished	In processing	Complete	For replicating the genetics of TS.

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