

No Association between Tryptophan Hydroxylase-2 Gene G1463A Polymorphism and Unipolar Depression in a Southern Chinese Han Population (translated version)

KX Xue, CH Fan, XL Li

Abstract

Objective: To investigate the association between the tryptophan hydroxylase-2 (*TPH2*) gene (G1463A) polymorphism and unipolar depression in a southern Chinese Han population.

Participants and Methods: The allelic and genotypic frequencies of *TPH2* gene G1463A polymorphism were examined with the amplification refractory mutation system–polymerase chain reaction technique in 123 unipolar depressive patients and 122 healthy individuals. Patients and healthy controls were all from the Guangdong Han population in southern China.

Results: No subjects among the cases or controls were found to carry any *TPH2* 1463A allele.

Conclusion: There is no association identified between the *TPH2* G1463A polymorphism and unipolar depression in the southern Chinese Han population.

Key words: Genes; Mood disorders; Polymorphism, genetic; Tryptophan hydroxylase

Introduction

Depression is a common psychiatric disease which may lead to mental disability and suicide. The life-time prevalence of depression is about 5 to 17%.¹ The incidence of depression is highly modulated with environmental factors, however, there is also a strong genetic contribution to the disease pathogenesis with an estimated heritability of 40 to 70%.¹ Family studies, twin studies, and foster children studies show an association between genetic factors and depression.² Until now, there are still no confirmed genes or related DNA sequences associated with the onset of depression. Therefore, studies to identify the genetic associations of depression will be an interesting area of concern.

Results from psychopharmacology and neurobehavioural research show serotonin (5-hydroxytryptamine [5-HT]) dysfunction in the central nervous system is associated with the incidence of depression.² Decreased level of 5-HT is associated with depressed mood, reduced appetite, insomnia, circadian

rhythm disturbance, endocrine and sexual dysfunction, anxiety, and reduced activity.³ Furthermore, the central serotonin neural system is the target for most commonly prescribed antidepressants such as tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), and monoamine oxidase inhibitors.⁴ Central serotonin is synthesised at dorsal raphe nuclei of the brainstem.⁵ The biological processes related to serotonin in the body include its biosynthesis, storage, transportation, release, receptor binding, reuptake, and metabolism. Proteins and corresponding related genes which regulate these processes include tryptophan hydroxylase-1 and -2 (*TPH1* and *TPH2*), vesicular monoamine transporter 2 (*VMAT2*), serotonin transporter (*5-HTT*), monoamine oxidase A (*MAOA*), and the serotonin receptors including: 5-HT_{1A}, -2A, -2C, -3, -4, -5, -6, -7.⁶ Therefore, these serotonin-related genes are the candidates for research into the aetiology of depression.

Tryptophan hydroxylase is the rate-limiting enzyme of 5-HT biosynthesis, which includes *TPH1* and *TPH2*. The former (*TPH1*) is predominately expressed in peripheral organs such as the heart, lungs, duodenum, liver, and adrenal glands, and also in some parts of the brain. It is also the main regulator of peripheral 5-HT synthesis. The latter (*TPH2*) is neuron-specific and only expressed in the brain 5-HT neuron. In some regions of the brain (such as the hippocampus, frontal lobe, thalamus, hypothalamus, and amygdala), the expression levels of *TPH1* and *TPH2* are almost equal. However, *TPH2* is predominately expressed in the brain stem where 5-HT neurons originate.⁷ Recent studies have shown inconsistent results regarding any association between *TPH2* gene polymorphism and unipolar depression.⁸⁻¹⁶ In 2005, Zhang et al⁴ found that the human *TPH2* gene coding region contains a functional polymorphism, G1463A, leading to the replacement of the 441 arginine position with histidine (R441H). This may cause

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approximately 80% of TPH2 function loss and significantly reduce 5-HT generation. Zhang et al⁴ also analysed the TPH2 G1463A polymorphism and its relationship with the affective disorder. Results showed that among 87 patients with unipolar depression, 9 carried the 1463A mutant allele, while in 219 control subjects, only 3 carried the mutant allele. This suggested that the R441H mutation may be an important risk factor in unipolar depression. In order to verify this result in the Chinese Han population, this study used an association analysis method to study the TPH2 G1463A polymorphism in the southern Chinese Han population and its relevance to unipolar depression.

Methods

Subjects

All study subjects were of Han nationality in the Guangdong Province of southern China and gave signed informed consent prior to enrolment.

Case Group

A total of 123 patients were included in the study — 42 were male, 81 were female. They were aged 16 to 65 years with a mean of 38 (standard deviation [SD], 14) years. All were recruited from the Guangdong General Hospital Institute of Mental Health outpatient clinics and inpatient services. For all patients, the diagnosis of unipolar depression was made by 2 veteran psychiatrists, according to diagnostic criteria in the Chinese Classification and Diagnostic Criteria of Mental Disorders (3rd edition), and in the Diagnostic and Statistical Manual of Mental Disorders (4th edition). The 24-item version of the Hamilton Depression Scale was used to evaluate the severity of depression and the total scores for all patients were greater than 20. Patients who were diagnosed with neurological disorders, other serious physical illnesses, secondary depression, bipolar disorder, and other functional mental disorders were excluded.

Control Group

Another 122 healthy individuals without severe physical diseases, genetic diseases, and mental illness were recruited as controls. They were hospital staff, internship students, and volunteers from the Guangdong General Hospital Medical Center and without any blood relationships to any of the patients. Among the control were 55 males and 67 females; they aged 18 to 65 years, with a mean of 37 (SD, 14) years. No significant statistical differences were found with regard to gender and age distribution between cases and controls ($P > 0.05$).

Specimen Collection and Genomic DNA Extraction

From each patient suffering from depression and each healthy control, 5 ml of peripheral venous blood was acquired and mixed with sodium citrate anticoagulant. A column-blood genomic DNA extraction kit was used to extract genomic DNA (according to kit instructions).

TPH2 G1463A Genotype Analysis

The amplification refractory mutation system–polymerase chain reaction (ARMS-PCR) method⁴ was used to analyse TPH2 G1463A genotypes. The PCR reaction primers included 1 allele-specific primer (G or A allele) and 2 positive control primers (forward and reverse) flanking the site of the G1463A polymorphism. According to human *TPH2* gene coding DNA sequences, a forward positive control primer “F” was designed as: 5'-ATG TGT GAA AGC CTT TGA CCC AAA GAC A-3'; a reverse positive control primer “R” as: 5'-TGC GTT ATA TGA CAT TGA CTG AAC TGC T-3'; G allele-specific primer P_G as: 5'-TAG GGA TTG AAG TAT ACT GAG AAG GCA C; A allele-specific primer P_A as: 5'-TAG GGA TTG AAG TAT ACT GAG AAG GCA T. For each sample, 2 PCR reactions were conducted to detect the possible G / A alleles using primers F + R + P_G for the G allele detection and primers F + R + P_A for the A allele. The 25 µl PCR reaction volume was composed of 12.5 µl GoTaq Green Master Mix, 2 × (reaction buffer, pH > 8.5; 400 µM dNTP; 3 Mmol MgCl₂), DNA template 2 µl (≥ 100 µg), each primer 1 µl (10 µmol/l) and ddH₂O 8.5 µl. The PCR reaction conditions were: 94°C predenatured for 5 minutes (94°C for 30 sec, 63°C for 30 sec, 72°C for 30 sec) × 40 cycles, final extension at 72°C for 5 minutes. For genotyping, 5 µl PCR product samples separated via 2% agarose gel electrophoresis were stained with EB and the results observed. According to electrophoresis banding patterns, TPH2 G1463A genotype was identified: 2 bands of 492-bp and 294-bp indicated for “positive” (G allele or A allele positive, respectively, according to the type of PCR primers used) and one 492-bp band for “negative” (G allele or A allele negative, respectively). Some samples of the PCR products involved DNA sequencing.

DNA Sequencing of Polymerase Chain Reaction Products

In order to verify the accuracy of the ARMS-PCR method in TPH2 G1463A genotyping, 8 samples of PCR products were taken for DNA sequencing. DNA sequencing was conducted following the routine protocol by Shanghai Invitrogen Biotechnology Co., Ltd, Guangzhou Office. It was carried out as follows: (1) recycling of unpurified PCR product in gel; (2) examination of samples' band brightness to decide the template quantity added to reaction mixture; (3) ddH₂O, primers and template were added to the reaction mixture for heat-denaturation at 96°C; (4) after being cooled on ice and added with 1 µl BDT, the reaction mixture was taken for amplification in a thermocycler for 25 cycles; (5) after being cooled on ice, the mixture was added with EDTA and laid aside for about 5 minutes; (6) centrifugation for 30 minutes at 4°C after being added with 15 µl anhydrous ethanol; (7) after centrifugation, the upper solution was discarded, and the precipitate added with 50 µl of 70% iced ethanol and centrifuged for another 15 minutes at 4°C; (8) the precipitate was air dried after discarding the upper solution; (9) 10 µl of deionised formamide added and subjected to denaturation; (10) after being cooled, the sample was taken to the sequencing analyser 3730 to obtain a final result.

Statistical Analysis

The Statistical Package for the Social Sciences (Windows version 13.0; SPSS Inc, Chicago [IL], US) was used to carry out data management and counting of genotype and allele frequency. The allele and genotype frequency distribution differences between the two groups were analysed using the Chi-square (χ^2) test. If the theoretical value was less than 5, Fisher's exact probability analysis would be used.

Results

Agarose gel electrophoresis and DNA sequencing results showed that in all subjects the *TPH2* 1463A mutant allele was not present (Fig); all 123 cases of unipolar depression and the 122 healthy controls were homozygous for 1463G. According to the report of Zhang et al,⁴ 1463A allele frequency in patients with unipolar depression was 5.17%. In accordance with this frequency, among all patients there should have been 6 subjects carrying the 1463A mutant allele. However, in neither the cases or controls was there a single 1463A allele carrier.

Discussion

Zhang et al⁴ first reported in 2005 that the coding region of human *TPH2* gene contains a G1463A functional polymorphism. However, in subsequent studies, many research groups could not replicate Zhang's results. Garriock et al¹³ analysed *TPH2* G1463A polymorphism in a sample of 182 unipolar depression patients which was similar to

Zhang's sample in race and gender distribution, including 83 cases of treatment resistance, 8 bipolar affective disorder patients (treatment resistant), and 186 healthy controls, and found that no subjects were 1463A mutant allele carriers. For the first time, they also studied the 11th intron in the *TPH2* gene, searching for the other 2 single-nucleotide deletion mutations (C1487G and T1578G), and the sequence analysis showed no single nucleotide variations. Van Den Bogaert et al¹⁴ carried out *TPH2* G1463A genotyping on 2 independent case-control samples. One sample consisted of 135 cases of unipolar depression (mean age, 57 years) and 182 cases of bipolar affective disorder patients (mean age, 56 years) forming the case group, and 364 gender-, age-, and race-matched individuals formed the control group. The other sample consisted of 182 cases of unipolar depression patients (mean age, 47 years) and 182 cases of bipolar affective disorder patients (mean age, 56 years) forming the case group, and 364 cases of gender-, age- and race-matched individuals forming the control group. Within the 2 study samples, there was not a single carrier of 1463A. In addition, they discussed the reason for the discrepancy between Zhang's and their study — the sample of patients selected in Zhang's study⁴ had a rather high average age (>60 years), and 7 patients with unipolar depression carrying the 1463A allele were unresponsive to SSRI treatment. Glatt et al¹⁵ carried out G1463A genotype analysis on 1023 cases of unipolar depression from a wide range of ethnic representatives, and found no carriers of the 1463A allele. Zhou et al¹⁶ carried out *TPH2* G1463A genotyping on 779 cases of unrelated individuals (403

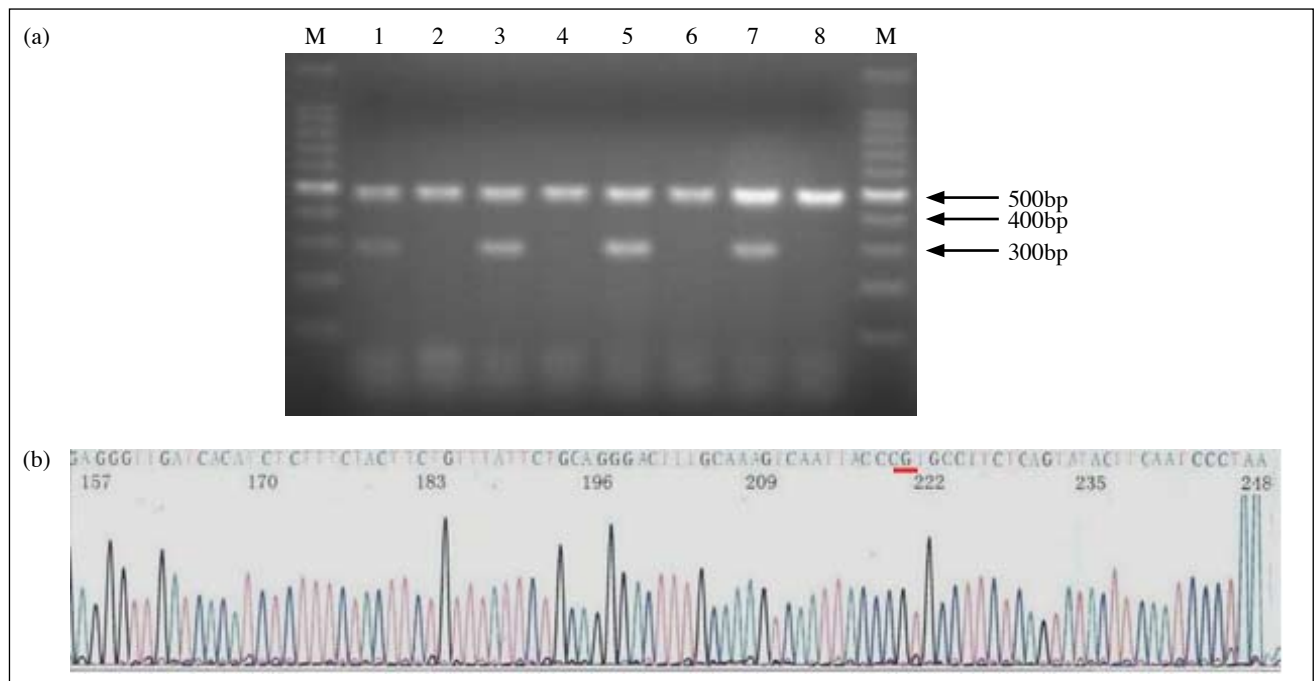


Figure . Tryptophan hydroxylase-2 (*TPH2*) G1463A genotype electrophoresis analysis of (a) and DNA sequencing (b) of polymerase chain reaction (PCR) products.

(a) M denotes DNA marker; lanes 1, 3, 5, 7: PCR products using G allele-specific primer (G allele positive); lanes 2, 4, 6, 8: PCR products using A allele-specific primer (A allele negative); (b) position 1463's G base is underlined in red.

cases of unipolar depression, with an average age of >60 years) and 1740 patients with major depression, and did not find any carrier of the 1463A allele. They speculated that the TPH2 1463A allele is a very rare mutation, and may not have any relationship with major depression or other behaviours. If there is a relationship with other behaviours, based on their own samples of old-age patients in the study, the 441H allele may be related to late onset of depression. Additionally, Delorme et al¹² used 3 different methods: ARMS-PCR, restriction fragment length polymorphism (RFLP), and direct sequencing of 1071 cases with mental disorders (265 cases of unipolar depression, 297 cases of severe depression, 84 cases of bipolar disorder, 201 cases of obsessive compulsive disorder, 224 cases of autism patients), and 246 healthy volunteers to genotype the TPH2 G1463A polymorphism. They found all subjects were G1463G homozygotes.

This study also found no TPH2 1463A allele carrier within the sample of southern Chinese Han population, and was in accordance with the findings of the above-mentioned authors but inconsistent with Zhang's earliest results.⁴ The probable underlying reasons may be: (1) with regard to depression itself, there may exist geographic and ethnic differences; (2) compared with Zhang's study sample (unipolar depression, patients' age \geq 60 years), patients in this study had a lower mean age (38; SD, 14 years) and therefore lower onset age of depression. If the age of onset is different, the genetic backgrounds may also be different; (3) the TPH2 1463A allele may be a rare mutant allele and has not been detected in this study due to its relatively small sample size; (4) the 1463A allele may be seen mainly in patients with a poor response to SSRI treatment. Zhang's study⁴ found that among the 9 cases who carried the A mutant allele, 7 exhibited a poor response to treatment with SSRI, and the other 2 required a very high dose. In this study sample however, no patients had a poor response to SSRI treatment, which may be another reason for inconsistency; (5) since this and other studies did not detect the 1463A allele, the *TPH2* gene G1463A polymorphism should not be a major genetic factor in depression. In short, the results of this study do not support a correlation between the TPH2 G1463A gene polymorphism and depression among the southern Chinese Han population.

The present study has some limitations, one of which is its relatively small sample size. Usually, rare mutant alleles are detected only in a large sample. If the TPH2 1463A allele is a rare mutant allele in Chinese Han population, a large sample would be needed to detect it. In this study, although there was no evidence of an association between *TPH2* gene G1463A polymorphism and unipolar depression in southern Chinese Han population, our findings cannot exclude a possible correlation between *TPH2* gene and unipolar depression, because there are several other polymorphisms affecting this gene.⁸⁻¹¹ To clarify the relationship between 5-HT-related genes like *TPH2* and unipolar depression, further studies with a larger sample and consideration of

gene-gene and gene-environment interaction are necessary.

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中國南方漢族人色氨酸羥化酶-2基因G1463A多態性與單相抑鬱症不存在相關

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摘要

目的：探討中國南方漢族人群中色氨酸羥化酶-2 (TPH2) 基因G1463A多態性與單相抑鬱症的相關性。

方法：採用擴增阻滯突變系統—PCR (ARMS-PCR) 對123例單相抑鬱症患者及122例正常健康人進行TPH2 G1463A基因多態性的基因分型。患者和健康對照均為廣東漢族人。

結果：病例組與對照組沒有發現一例攜帶TPH2 1463A突變等位基因。

結論：TPH2 G1463A多態性可能與中國南方漢族人單相抑鬱症不存在關聯。

關鍵詞：基因；單相抑鬱症；多態性；遺傳；色氨酸羥化酶

前 言

抑鬱症是一種常見的、可導致精神殘疾和自殺死亡的精神疾病。其終生患病率約為5至17%^[1]。雖然抑鬱症的發病與環境因素有關，但遺傳因素佔有較大的比重，其遺傳度約為40至70%^[1]。家系研究、雙生子和寄養子研究均顯示遺傳因素與抑鬱症有關^[2]。目前，與抑鬱症發病相關的具體基因或相關DNA序列還未得到確認。因此，尋找與抑鬱症相關的致病基因仍然是目前抑鬱症病因學研究的熱點之一。

精神藥理學和神經行為研究結果表明，中樞5-羥色胺 (5-HT) 功能異常與抑鬱症的發病相關^[2]。5-HT功能活動降低與抑鬱症患者的抑鬱心境、食欲減退、失眠、晝夜節律紊亂、內分泌功能紊亂、性功能障礙、焦慮不安、活動減少等密切相關^[3]。而且，中樞5-HT系統是目前大多數抗抑鬱藥如三環類、選擇性5-HT再攝取抑制劑 (SSRIs)、單胺氧化酶抑制劑等的作用靶點^[4]。中樞5-HT主要在腦幹背側縫核中合成^[5]。其在體內的生物過程包括合成、儲藏、轉運、釋放、作用於靶受體、攝取、代謝。調節和執行這些生物過程的蛋白質和相應的基因有：色氨酸羥化酶-1和-2 (TPH1及TPH2)、2型囊泡單胺轉運體 (VMAT2)、5-HT轉運體 (5-HTT)、單胺氧化酶-A (MAOA) 和5-HT系列受體包括5-HT1A、2A、2C、3、4、5、6、7等^[6]。這些5-HT相關基因由此成為抑鬱症病因學研究的候選基因。

色氨酸羥化酶 (TPH) 是5-HT生物合成的限速酶，它包括色氨酸羥化酶-1 (TPH1) 和色氨酸羥化

酶-2 (TPH2)。TPH1主要分布在外週組織如心臟、肺、十二指腸、肝臟和腎上腺中，也在部分腦區表達，主要控制外週5-HT合成。TPH2則為神經元特異性，只在腦區5-HT神經元中表達。TPH1和TPH2在部分腦區 (如海馬、額葉、丘腦、下丘腦、杏仁核) 中的表達量幾乎相等，但TPH2在腦幹中佔絕對優勢，而腦幹是5-HT神經元的產地^[7]。近年來，有關TPH2基因多態性與抑鬱症的遺傳關聯研究結果很不一致^[8-16]。其中在2005年，美國Duke大學醫學中心的Zhang及其研究小組^[4]發現，人類TPH2基因編碼區存在一個G1463A的功能多態，導致第441位精氨酸被組氨酸替代 (R441H)，從而引起TPH2喪失約80%的功能，致使5-HT生成明顯減少。Zhang等^[4]還分析了TPH2 G1463A多態性與情感障礙的關係，結果顯示，87例單相抑鬱病人中有9例攜帶1463A突變等位基因，而219例對照組中僅3例攜帶該突變等位基因。這提示R441H的變異可能是單相抑鬱的一個重要危險因子。為了在中國漢族人群中驗證這結果，本研究採用關聯分析的方法，研究中國南方漢族人群中TPH2 G1463A多態性與單相抑鬱症的相關性。

對象與方法

研究對象

所有研究對象均為中國南方廣東省的漢族人，入組前均簽訂知情同意書。

病例組

病例組123例，均來自廣東省人民醫院廣東省精神衛生研究所門診和住院部。患者中，男42例，女81例，年齡介乎16至65歲，平均年齡38歲，標準差14歲。抑鬱症的診斷由課題組中2名精神科副主任醫師或以上職稱的人員採用《中國精神障礙分類與診斷標準 (第三版)》(CCMD-3)中抑鬱發作與美國《精神障礙診斷與統計手冊 (第四版)》(DSM-IV)中重性抑鬱發作的診斷標準共同確診為單相抑鬱症。漢密爾頓抑鬱量表24項版 (HAMD-24) 評分總分均為20分以上。排除患有神經系統疾病、其他嚴重的軀體疾病、繼發性抑鬱、雙相障礙和其他功能性精神障礙者。

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對照組

對照組122例，來自廣東省人民醫院體檢中心的健康體檢者及醫院的工作人員、實習學生。122例中，男55例，女67例，年齡介乎18至65歲，平均年齡37歲，標準差14歲。排除嚴重的軀體疾病、遺傳性疾病和精神疾病，相互間無血緣關係。經統計檢驗，病例組和對照組在性別分布和年齡上無顯著性差異 ($P>0.05$)。

標本採集及基因組DNA提取

採集抑鬱症患者及正常健康對照外週靜脈血各5 ml，檸檬酸鈉抗凝，用柱式血液基因組DNA提取試劑盒提取DNA（操作按試劑盒說明書）。

TPH2 G1463A基因型分析

採用擴增阻滯突變系統-PCR (ARMS-PCR) 法^[4]分析TPH2 G1463A基因型。PCR反應引物由2條正反向的陽性對照側翼引物及1條G或A等位基因特異性引物組成。根據人TPH2基因的編碼DNA序列，分別設計正向對照引物F為：5'-ATG TGT GAA AGC CTT TGA CCC AAA GAC A-3'；反向對照引物R為：5'-TGC GTT ATA TGA CAT TGA CTG AAC TGC T-3'；G等位基因特異性引物P_G為：5'-TAG GGA TTG AAG TAT ACT GAG AAG GCA C；A等位基因特異性引物P_A為：5'-TAG GGA TTG AAG TAT ACT GAG AAG GCA T。對每個樣品進行2次PCR，分別檢測可能的G/A等位基因，用於檢測G等位基因的引物是F+R+P_G，檢測A等位基因的引物是F+R+P_A。PCR反應體積為25 μ l，12.5 μ l GoTaq Green Master Mix，2 \times (reaction buffer, pH > 8.5；400 μ M dNTP；3 Mmol MgCl₂)；DNA模板2 μ l (≥ 100 μ g)；

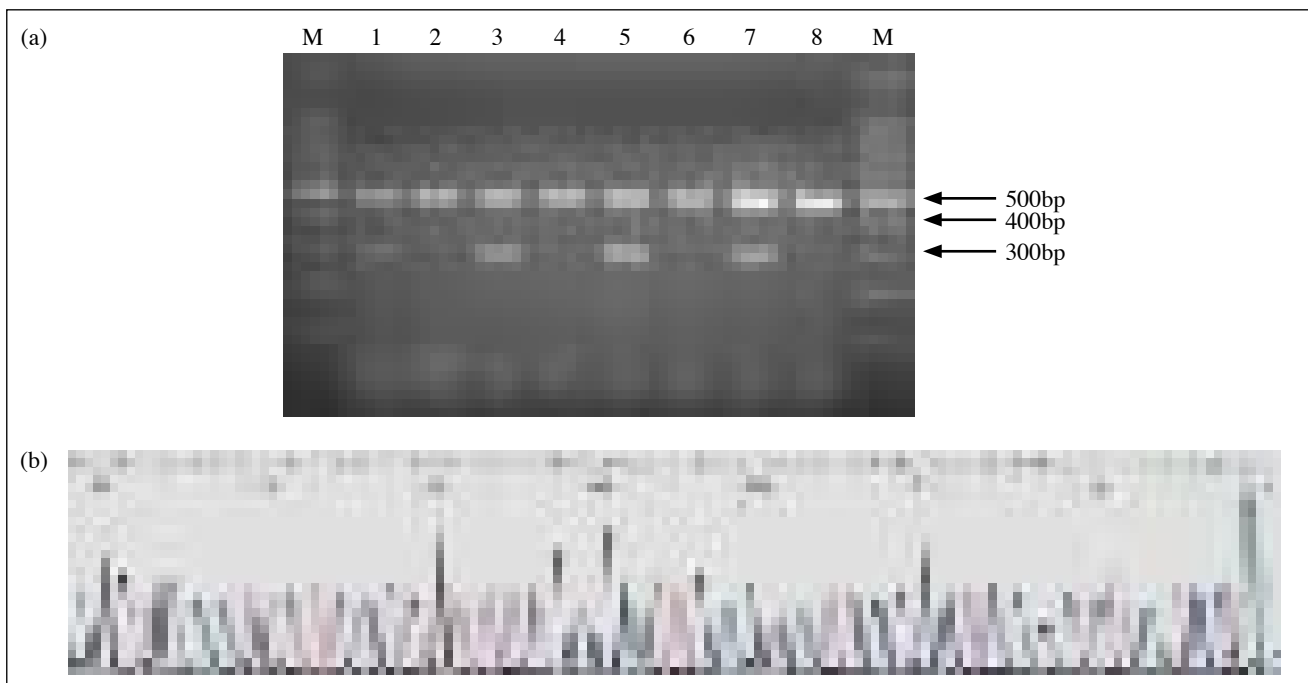
引物各1 μ l (10 μ mol/l)；ddH₂O 8.5 μ l。PCR反應條件：94°C預變性5分鐘 (94°C 30秒、63°C 30秒、72°C 30秒) \times 40循環，最後72°C延伸5分鐘。取PCR產物5 μ l，2%瓊脂糖凝膠電泳分離產物，EB染色後觀察結果，根據電泳帶型確定TPH2 G1463A基因型：即根據所用檢測G或A等位基因的引物組合，PCR產物只有492 bp條帶為陰性，有492 bp和294 bp兩個條帶為陽性。部分PCR產物牽涉DNA序列測定。

PCR產物的DNA序列測定

為驗證採用ARMS-PCR法進行TPH2 G1463A基因分型結果的準確性，對部分 (8例) 樣品DNA的PCR產物進行測序鑑定。由上海英駿生物技術有限公司廣州分公司 (Shanghai Invitrogen Biotechnology Co., Ltd, Guangzhou Office) 代理開展。具體步驟如下：(1) PCR未純化產物割膠回收；(2) 鑑定樣品的條帶亮弱，決定做反應時所加模板的量；(3) 以水、引物、模板的順序將反應體系加到96孔板，上PCR 96°C儀變性；(4) 冷卻後加1 μ l BDT，再上PCR儀，擴增25個循環；(5) 冷卻後加EDTA放置5分鐘左右；(6) 加15 μ l無水乙醇，4°C下離心30分鐘；(7) 倒離心後加50 μ l 70%冰乙醇，4°C下離心15分鐘 (重複一次)；(8) 倒離心後晾乾；(9) 加10 μ l去離子甲酰胺，上PCR儀變性；(10) 冷卻後，上3730測序儀收集信號。

統計學分析

採用SPSS13.0進行數據管理，計算基因型頻率、等位基因頻率。兩組間基因型頻率和等位基因頻率分布差異



圖：TPH2 G1463A基因型的PCR產物電泳分析 (a) 和DNA測序圖 (b)。

(a) M=DNA marker；lanes 1、3、5、7：採用G等位基因特異性引物的PCR產物 (陽性)；lanes 2、4、6、8：採用A等位基因特異性引物的PCR產物 (陰性)。(b) 對應其1463位G城基的位置用紅色下劃線標出。

採用 χ^2 檢驗，理論值少於5時採用Fisher確切概率分析。

結果

瓊脂糖凝膠电泳和DNA測序結果顯示(圖)，在所有研究對象中均未檢測到TPH2 1463A突變等位基因，123例單相抑鬱和122例健康對照均為是1463G的純合子。按照Zhang等^[4]的報道，單相抑鬱患者中1463A等位基因的頻率為5.17%，按照這個頻率，在單相抑鬱的樣本中應該觀察到6例攜帶G1463A的患者。但是，無論是病例組還是對照組，一例1463A等位基因的個體也沒有觀察到。

討論

Zhang等^[4]於2005年首先報導了人類TPH2基因編碼區存在一個G1463A的功能多態性，但在隨後的研究中，許多研究組並沒有重複出Zhang等人的發現。Garriock等^[13]通過對與Zhang等的研究在種族和性別分布方面相似的182例單相抑鬱病人(其中83例對治療抵抗，8例對治療抵抗的雙相情感障礙病人，和186例健康對照)的TPH2 G1463A序列進行分析，結果在TPH2 G1463A位點沒有發現一例攜帶1463A突變等位基因。他們還首次對TPH2基因第11外含子的其他兩個單核苷酸缺失變異(C1487G和T1578G)進行了研究，結果對C1487G和T1578G的序列分析也沒有發現單核苷酸的變異。Van Den Bogaert等^[14]對兩個獨立的病例一對照樣本進行了TPH2 G1463A基因分型。一個樣本由135例單相抑鬱病人(平均年齡57歲)和182例雙相情感障礙病人(平均年齡56歲)組成病例組，364例性別、年齡、種族相匹配的個體作為對照組。另一樣本由182例單相抑鬱病人(平均年齡47歲)，182例雙相情感障礙病人(平均年齡56歲)組成病例組，364例性別、年齡、種族相匹配的個體構成對照組。結果在兩個研究樣本中沒有觀察到一例攜帶1463A等位基因的個體。此外，他們還分析了Zhang等觀察到突變等位基因而他們則沒有觀察到的原因：Zhang等^[4]的研究樣本選擇的病人年齡偏高(60歲以上)，並且7例攜帶1463A的抑鬱症患者對SSRI治療無效。Glatt等^[15]對來自具有廣泛種族代表性的1023例單相抑鬱症患者進行了TPH2 G1463A的基因型分析，也沒有發現一例攜帶1463A等位基因的個體。Zhou等^[16]對779例無關個體(403例單相抑鬱，平均年齡60歲以上)和1740例重性抑鬱患者進行了TPH2 G1463A基因分型，結果仍然沒有發現一例攜帶1463A等位基因的個體。他們推測TPH2 1463A等位基因是非常罕見的突變基因，可能和重性抑鬱或其他行為沒有相關性。如果和其他的行為有相關性，對比他們自己所研究的老年群體，認為441H等位基因可能和抑鬱的遲發有關。另外，Delorme等^[12]使用3種不同的方法：ARMS-PCR、限制性片段長度多態性(RFLP)和直接測序對1071例精神障礙患者(265例單相抑鬱、297例重性抑鬱、84例雙相障礙、201例強迫衝動障礙、224例孤獨症患者)和246例健康志願者進行了TPH2 G1463A基因分型，結果亦全部是1463G純合子。

本研究在中國南方漢族人群中亦沒有發現TPH2 1463A等位基因，和上述多位作者的研究結果相

似，但與Zhang^[4]最早的報道結果不一致。究其原因有：(1)抑鬱症本身可能存在著地域和種族的差異。(2)和Zhang的研究樣本(單相抑鬱病人年齡60歲或以上)相比，本研究的病人平均年齡為(38±14)歲，發病年齡偏小。因為發病年齡不同，可能遺傳背景也不同。(3)TPH2 1463A等位基因可能是一種罕見的突變基因，由於本研究的樣本量不是很大，從而影響了TPH2 1463A等位基因的檢出。(4)1463A等位基因可能主要見於SSRI(5-羥色胺再攝取抑制劑)治療反應差的抑鬱症個體，Zhang的研究發現，9例攜帶A突變等位基因者中，有7例對SSRI治療無效，另2例則需要很高劑量。本研究樣本中未見明顯SSRI治療無效者，可能導致未測出該等位基因。(5)由於本研究和其他研究均未檢出1463A等位基因，該基因多態性和抑鬱症可能沒有關係。總之，本研究結果不支持在中國南方漢族人群中TPH2 G1463A基因多態性和抑鬱症存在相關性。

本研究的不足之處在於其樣本量還不是足夠大。另外，本研究結果雖然未發現在中國南方漢族人群中TPH2 G1463A基因多態性和抑鬱症存在遺傳關聯，但由於TPH2基因存在多個不同多態位點^[8-11]，因此本文結果並不能排除TPH2基因其他多態位點與抑鬱症的遺傳相關性。為了充分揭示TPH2基因多態性與抑鬱症的關係，在今後進一步的研究中，除應該提高研究樣本量外，還應該分析TPH2基因不同多態性間的交互作用以及環境因素對基因多態性表達的影響。

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