Research Paper
Article de recherche

Prefrontal executive function and D_1, D_3, 5-HT_{2A} and 5-HT_6 receptor gene variations in healthy adults

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Objective: The Val158Met polymorphism of the catechol-*O*-methyltransferase gene has been demonstrated to be associated with prefrontal executive function explaining 4% of variance in perseverative errors on the Wisconsin Card Sorting Test (WCST). Studies suggest that dopamine D_1 and D_3 and serotonin 5-HT_{2A} and 5-HT_6 receptors may also be involved in prefrontal cognitive function and that genetic polymorphisms (D_1 A-48G, D_3 Ser9Gly, 5-HT_{2A} T102C, and 5-HT_6 T267C) of these receptors may be associated with brain glucose metabolism or neurophysiological function. The current study's objective was to investigate whether executive function varies with these genetic variations. **Methods:** A sample of 216 healthy Han Chinese adults were measured with the WCST and genotyped for the 4 genetic polymorphisms. **Results:** Kruskal–Wallis tests showed a significant difference in WCST perseverative errors among the genotypes D_3 Ser9Gly (p = 0.009), 5-HT_{2A} T102C (p = 0.038) and 5-HT_6 T267C (p = 0.010), but not in the genotype D_1 A-48G. Multiple regression analysis for the WCST natural logarithm values (i.e., for fulfilling the normal distribution requirement) showed that subjects' perseverative errors were significantly influenced by D_1 A-48G, D_3 Ser9Gly, 5-HT_{2A} T102C and 5-HT_6 T267C polymorphisms after adjustment of other variables. **Conclusion:** The preliminary data suggest that D_1, D_3, 5-HT_{2A} and 5-HT_6 genetic mutations may influence prefrontal executive cognition in healthy adults. Further studies in larger samples with other ethnicities or in mentally ill patients are warranted.

Objectif : On a démontré qu'il y avait, entre le polymorphisme Val158Met du gène de la catéchol-*O*-méthyltransférase et la fonction d'exécution préfrontale, un lien qui explique 4 % de la variation des erreurs de persévérance révélées par le test Wisconsin de classification catégorielle de cartes (WCST). Des études indiquent que les récepteurs de la dopamine D_1 et D_3 et de la sérotonine 5-HT_{2A} et 5-HT_6 peuvent aussi jouer un rôle dans la fonction cognitive préfrontale et qu'il peut y avoir un lien entre les polymorphismes génétiques (D_1 A-48G, D_3 Ser9Gly, 5-HT_{2A} T102C et 5-HT_6 T267C) de ces récepteurs et le métabolisme du glucose dans le cerveau ou la fonction neurophysiologique. L'étude en cours visait à déterminer si la fonction d'exécution varie selon ces variations génétiques. **Méthodes :** On a mesuré un échantillon de 216 adultes chinois Han en bonne santé au moyen du questionnaire WCST et l'on a établi leur génotype pour les 4 polymorphismes génétiques. **Résultats :** Des tests de Kruskal–Wallis ont montré une différence importante au niveau des erreurs de persévérance révélées par le test WCST chez les génotypes D_3 Ser9Gly (p = 0,009), 5-HT_{2A} T102C (p = 0,038) et 5-HT_6 T267C (p = 0,010), mais non chez le génotype D_1 A-48G. Une analyse de régression multiple portant sur les valeurs logarithmiques naturelles WCST (c.-à-d. pour la satisfaction de l'exigence relative à la distribution normale) a montré que les polymorphismes D_1 A-48G, D_3 Ser9Gly, 5-HT_{2A} T102C et 5-HT_6 T267C exerçaient une influence importante sur les erreurs de persévérance des sujets, après rajustement en fonction d'autres variables. **Conclusion :** Les données préliminaires indiquent que les mutations génétiques D_1, D_3, 5-HT_{2A} et 5-HT_6 peuvent avoir un effet sur la cognition exécutive préfrontale chez les adultes en bonne santé. D'autres études portant sur des échantillons plus importants d'autres origines ethniques ou de patients atteints de maladie mentale sont justifiées.

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Medical subject headings: receptor, dopamine, D_1; receptor, dopamine, D_3; receptor, serotonin, 5-HT_{2A}; receptor, serotonin, 5-HT_6; prefrontal; cognition.

J Psychiatry Neurosci 2008;33(1):47-53.

Submitted Mar. 20, 2007; Revised July 15, 2007; Accepted Aug. 2, 2007

Introduction

Genetic factors can influence frontal structures and related test performances.[1,2] Converging evidence suggests that dopaminergic neurotransmission affects prefrontal functioning[3] and other cognitive domains.[4–6] Catechol-*O*-methyltransferase (COMT) is the key enzyme regulating dopamine metabolism within the prefrontal cortex.[7] It has been demonstrated that Val158Met, a functional polymorphism in the *COMT* gene, is associated with prefrontal cognitive function, explaining 4% of variance in perseverative errors on the Wisconsin Card Sorting Test (WCST) in schizophrenia patients, unaffected siblings and healthy individuals.[8] The WCST, a widely used measure of prefrontal cognitive functions (including executive function, abstraction and working memory), is sensitive to a person's ability to generate hypotheses, establish response sets and fluently shift sets.[9,10] A recent meta-analysis[11] suggests that there is a small but significant relation between Val158Met genotype and WCST performance in healthy people but not in those with schizophrenia.

Because the *COMT* Val158Met polymorphism accounts for a small portion of the variability in prefrontal cognition, it is warranted to explore whether other genetic mutations have potential effects. Of dopamine receptors, D_1 and D_3 may deserve foremost attention. D_1 receptors play a critical role in the activation of prefrontal cognition such as working memory.[12] In the the rat medial prefrontal cortex, the D_1 receptor is also essential for selecting response in skilled nonautomatic tasks.[3] In addition, D_1 activation modulates rodents' social cognition,[13] which depends substantially on prefrontal function.[14] Among genetic variants of D_1 receptors, the A-48G polymorphism is associated with glucose metabolic rates in human brain regions, including the prefrontal cortex.[15]

The D_3 receptor, although structurally highly homologous to other D_2-like dopamine receptors, differs from them in regulating neuropsychological performance.[16,17] In healthy people, D_2 receptor occupancy is correlated with motor tasks rather than with domains involving prefrontal cognitive functions.[6] D_2 may also regulate spatial working memory, but the latter is principally attributed to D_1 receptor–mediated mechanisms.[16] In comparison, D_3 receptors mediate dopamine-related prefrontal neurocognition[18] independently with D_1 activation.[16] Of D_3 genetic variances, a functional polymorphism, Ser9Gly, determines eye movement disturbances,[19] which are related to deficient working memory[20,21] and attention.[22]

By interacting with the dopaminergic system, serotonergic transmission also plays a significant role in prefrontal cognitive function.[23] Its action is mediated via specific receptors, possibly serotonin 5-HT_{2A} and 5-HT_6, located in crucial brain structures, primarily the nucleus basalis magnocellularis–frontal cortex. In rats, 5-HT_{2A} receptors regulate prefrontal cortex–related execution of primed responses.[3] In healthy volunteers, 5-HT_{2A} agonists impair the continuous performance task,[24] which is mainly determined by prefrontal function.[25] Among 5-HT_{2A} receptor polymorphisms, T102C has received considerable attention. For instance, this genetic variance affects treatment response to antipsychotics.[26] It also affects N100 amplitude of event-related potentials,[27] which are mediated by both frontal and temporal networks.[28] Although the T102C substitution does not alter amino acid sequence, recent evidence[29] indicates that total levels of 5-HT_{2A} receptor messenger ribonucleic acid (mRNA) and protein in healthy people with the C/C genotype are lower than those in healthy people with the T/T genotype.

The 5-HT_6 receptor is responsible for endogenous 5-HT–mediated facilitation of dopamine release in the rat prefrontal cortex[30] and regulates brain cholinergic neurotransmission.[31] This receptor is thus involved in rodents' learning and memory function.[31,32] It has been indicated that, among 5-HT_6 receptor polymorphisms,[33] T267C is associated with Alzheimer disease.[34] Whether this polymorphism also contributes to variances in prefrontal function requires investigation. Although this is a silent mutation, it has been suggested that this variant may affect translation through the secondary structure and stability of mRNA or that it may be in linkage disequilibrium with a functional polymorphism.[35]

The current study aims to explore the effects of the aforementioned genetic variances of D_1, D_3, 5-HT_{2A} and 5-HT_6 on WCST performance, which reflects prefrontal executive function, in a healthy population.

Methods

Participants

This study was approved by the institutional review board of China Medical University Hospital and carried out in accordance with the Declaration of Helsinki (www.wma.net/e/policy/b3.htm). The participants were 216 unrelated healthy volunteers (81 men and 135 women) with a mean age of 48.6 (standard deviation [SD] 9.4) years (range 20–65 y) and a mean education level of 10.9 (SD 3.7) years (range 0–20 y). All were Han Chinese living in Taiwan. The subjects in this study gave their consent to participate after procedures were explained to them. All subjects were free of any axis I or II psychiatric disorders, as determined by a research psychiatrist using the Structured Clinical Interview for DSM-IV.[36] All participants were also in good physical health, as determined by physical examination, electrocardiogram and laboratory tests including liver, renal and thyroid function tests and urinalysis.

WCST assessments

An experienced psychologist (Y-LC) administered the WCST.[9,10] In this test, subjects are required to sort stimulus cards on the basis of perceptual attributes (colour, form, number). The only feedback provided by the administrator is to indicate whether each response is correct or incorrect. The sorting rule is changed after 10 consecutive correct responses. Testing is discontinued when the subject has reached 128 trials.[37] The a priori primary outcome measure was the percentage of perseverative errors because it is sensitive to a person's ability to fluently shift cognitive sets and is thought to best reflect the prefrontal cognitive function.[8] The accessory

outcome variable was the number of categories completed, as used by other investigators.[34] To minimize multiple comparisons, we determined a priori not to analyze other WCST parameters.

Genotyping

Genomic DNA was extracted from the subjects' white blood cells. The 4 polymorphisms were genotyped according to the polymerase chain reaction-restriction fragment length polymorphism technique.

Forward and reverse primers and restriction enzymes for each polymorphism were as follows: 5′-ACT GAC CCC TAT TCC CTG CT-3′, 5′-AGC ACA GAC CAG CGT GTT C-3′, DdeI for A-48G polymorphisms in the promoter region of the dopamine D_1 receptor gene;[38] 5′-GCT CTA TCT CCA ACT CTC ACA-3′, 5′-AAG TCT ACT CAC CTC CAG GTA-3′, MscI for the D_3 Ser9Gly polymorphism;[39,40] 5′-TCT GCT ACA AGT TCT GGC TT-3′, 5′-CTG CAG CTT TTT CTC TAG GG-3′, MspI for 5-HT_{2A} T102C;[41,42] and 5′-AAC TTC TTC CTG GTG TCG CTC TTC-3′, 5′-ATG AGC AGG TAG CGG TCC AGG-3′, RsaI for T267C polymorphisms in the 5-HT_6 receptor gene.[37,43]

DNA fragments were visualized with 2% agarose gel electrophoresis and staining with ethidium bromide. Duplicate genotyping was conducted for all 4 polymorphisms, with 100% concordance. All assays were performed blind to the subjects' cognitive measurements.

Data analyses

We used a 2-tailed Mann–Whitney *U* test for WCST performance comparisons between male and female subjects. Kruskal–Wallis tests were carried out with each genetic polymorphism as the independent variable and percentage of perseverative errors and number of categories as the dependent measures. These nonparametric statistical methods were used because the distributions of perseverative errors and categories numbers were skewed to the right (data not shown). We also compared the subjects' demographic characteristics among genotypes of each genetic polymorphism, using Kruskal–Wallis tests for continuous variables and χ^2 tests for categorical variables. Statistical significance was defined as $p < 0.05$ or corrected for multiple comparisons as needed ($p < 0.0125 = 0.05/4$ [genetic polymorphisms]).

To control for the effects of other confounding factors, we used multiple linear regression in a single analysis to evaluate impacts of sex, age, sex × age interaction, education level and the 4 genetic polymorphisms simultaneously on WCST performance. Because all these comparisons were performed in a single multiple regression analysis, no correction for multiple comparisons was required. However, the distributions of the WCST data were skewed to the right and unsuitable for regression analyses. To convert the skewed distribution to a normal one, we measured each perseverative error value in its natural logarithmic scale before the regression analysis.[44,45]

Results

In all 216 subjects, the mean percentage of WCST perseverative errors was 20.1% (SD 13.1%) and the mean number of WCST categories completed was 4.18 (SD 3.08). Men and women had comparable mean perseverative errors at 18.7 (SD 12.0) and 20.9 (SD 13.8) ($p = 0.46$), respectively, and comparable mean numbers of categories completed at 4.4 (SD 3.2) and 4.1 (SD 3.0) ($p = 0.55$).

Allele frequencies of the genetic polymorphisms in our Han Chinese sample are similar to those in other Han Chinese populations but may be different from those in white populations (Table 1). The genotype distribution of each of the 4 genetic polymorphisms did not deviate significantly from the Hardy–Weinberg equilibrium: D_1 A-48G ($\chi^2 = 0.839$, $p = 0.657$), D_3 S er9G1y ($\chi^2 = 1.873$, $p = 0.392$), 5-HT_{2A} T102C ($\chi^2 = 0.76$, $p = 0.683$) and 5-HT_6 T267C ($\chi^2 = 1.17$, $p = 0.558$).

WCST perseverative errors and genotypes

Table 2 shows differences in WCST perseverative errors among genotypes of each of the 4 genetic variances. D_3 Ser9Gly, 5-HT_{2A} T102C and 5-HT_6 T267C, but not D_1 A-48G, significantly influenced perseverative errors. If corrected for multiple comparisons of the 4 genetic polymorphisms, D_3 Ser9Gly and 5-HT_6 T267C remained significant in modulating the functional outcome measures ($\alpha = 0.05/4 = 0.013$). Sex, age and education level did not significantly differ among genotypes of each genetic polymorphism (all *p* values > 0.05, data not shown).

Table 1: Allele frequencies of various genetic polymorphisms in different populations

Gene	Polymorphism	Allele	Population	Frequency, %	Reference
D_1	A-48G	G	Han Chinese	87	This study
			White	63	Ni et al[38]
D_3	Ser9Gly	Gly	Han Chinese	31	This study
			White	27	Segman et al[40]
5-HT_{2A}	T102C	C	Han Chinese	37	This study
			Han Chinese	38	Chen et al[41]
			White	59	Arranz et al[26]
5-HT_6	267T/C	C	Han Chinese	79	This study
			Han Chinese	73	Yu et al[27]
			White	85	Masellis et al[43]

Because various genetic factors may simultaneously determine executive function, we then conducted multiple linear regression analysis to evaluate effects of the 4 genetic polymorphisms on WCST perseverative errors after controlling for sex, age, gender × age interaction and education level. Prior to multiple regression, each WCST value was transformed to its natural logarithm to obtain normal distributions (data not shown). As revealed in Table 3, D_1 A-48G, D_3 Ser9Gly, 5-HT_{2A} T102C and 5-HT_6 T267C polymorphisms significantly influence perseverative errors after adjustment for sex, age, sex × age interaction, education level and other genetic variants. Compared with subjects having the D_1 A-48G A/A genotype, those with A/G had a 74% more perseverative errors ($e^{0.5561}$ = 1.7438, 1.7438 – 1 = 0.7438), and those with G/G had a 101% more perseverative errors ($e^{0.6968}$ = 1.9447, 2.0073 – 1 = 1.0073). Compared with subjects with the D_3 Ser9Gly Ser/Ser genotype, those with Ser/Gly had 23% more perseverative errors ($e^{0.2103}$ = 1.2340). However, the difference in perseverative errors between those with Gly/Gly and those with Ser/Ser did not achieve statistical significance. Compared with subjects having the 5-HT_{2A} T102C T/T genotype, those with T/C committed 25% more perseverative errors ($e^{0.2257}$ = 1.2532). The 2 homozygote groups, however, did not have significant differences in perseverative errors. Compared with subjects with the 5-HT_6 T267C T/T genotype, those with T/C had 42% fewer perseverative errors ($e^{-0.5468}$ = 0.5788, 1 – 0.5788 = 0.4212) and those with C/C had 32% fewer errors ($e^{-0.3920}$ = 0.6757, 1 – 0.6757 = 0.3243). Because all these comparisons were performed in a single multiple regression analysis (Table 3), no correction for multiple comparisons was needed. There was a sex × age interaction effect on perseverative errors. Compared with men, perseverative errors made by women increased less rapidly with age. Education level did not significantly influence the cognitive measures.

Table 2: Percentage of perseverative errors in Wisconsin Card Sorting Test in healthy individuals with various genotypes

Polymorphisms and genotypes	% Perseverative errors; mean (and SD)	*p* value*
D_1 A-48G		
A/A (*n* = 5)	10.8 (6.9)	0.13
A/G (*n* = 45)	17.8 (9.7)	
G/G (*n* = 166)	20.9 (13.9)	
D_3 Ser9Gly		
Ser/Ser (*n* =107)	18.2 (12.5)	0.01
Ser/Gly (*n* = 83)	22.8 (13.2)	
Gly/Gly (*n* = 26)	19.3 (14.4)	
5-HT_{2A} T102C		
T/T (*n* = 88)	18.0 (12.2)	0.04
T/C (*n* = 95)	22.6 (14.0)	
C/C (*n* = 33)	18.3 (12.1)	
5-HT_6 T267C		
T/T (*n* = 12)	29.5 (19.0)	0.01
T/C (*n* = 66)	17.6 (12.5)	
C/C (*n* = 138)	20.4 (12.5)	

SD = standard deviation.
*Difference among genotypes as analyzed by Kruskal–Wallis test. If corrected for multiple comparisons of the 4 genetic polymorphisms, the results for D_3 Ser9Gly and 5-HT_6 T267C remain significant ($p < 0.013 = 0.05/4$).

Genotypes and WCST categories completed

Regarding the number of categories completed, we found that only the D_3 Ser9Gly polymorphism had significant influence. The mean number of categories completed for the subjects with the Ser/Ser genotype was 4.7 (SD 3.1); for those with

Table 3: Effects of genetic polymorphisms on natural logarithms of percentage of perseverative errors in Wisconsin Card Sorting Test by multiple linear regression analysis*†

Variable	Estimated coefficient	SE	95% CI	*p* value‡
Women compared with men	0.6683	0.2797	0.1168 to 1.2200	0.018
Age, 1-y increments	0.0133	0.0055	0.0025 to 0.0241	0.016
Sex × age	–0.0127	0.0055	–0.0236 to –0.0018	0.023
Education level, 1-y increments	–0.0002	0.0004	–0.0010 to 0.0006	0.683
D_1 A-48G polymorphism				
A/G compared with A/A	0.5561	0.2712	0.0214 to 1.0908	0.042
G/G compared with A/A	0.6968	0.2588	0.1865 to 1.2071	0.008
D_3 Ser9Gly polymorphism				
Ser/Gly compared with Ser/Ser	0.2103	0.0838	0.0451 to 0.3755	0.013
Gly/Gly compared with Ser/Ser	0.0271	0.1273	–0.2240 to 0.2781	0.832
5-HT_{2A} T102C polymorphism				
T/C compared with T/T	0.2257	0.0853	0.0575 to 0.3940	0.009
C/C compared with T/T	0.0621	0.1181	–0.1707 to 0.2948	0.600
5-HT_6 T267C polymorphism				
T/C compared with T/T	–0.5468	0.1803	–0.9022 to –0.1914	0.003
C/C compared with T/T	–0.3920	0.1726	–0.7323 to –0.0517	0.024
Constant	–2.8962	0.4182	–3.7207 to –2.0717	NA

SE = standard error; CI = confidence interval; NA = not applicable.
*The amount of variance (R^2) in perseverative errors explained by the genetic polymorphisms was 0.1698.
†$F_{12, 203} = 3.46$.
‡Because all the comparisons are conducted in a single regression analysis, no correction for multiple comparisons is needed.

Ser/Gly, it was 3.7 (SD 3.1); and for those with Gly/Gly, it was 3.5 (SD 2.9) (p = 0.029). However, if corrected for the 4 multiple comparisons (see the above section), the difference did not achieve statistical significance. Multiple linear regression analysis also did not reveal statistical significance for any genetic variance. These findings support the notion that the percentage of perseverative errors is the most sensitive of the WCST parameters in terms of reflecting prefrontal function.[8]

Discussion

The main finding is that D_1 A-48G, D_3 Ser9Gly, 5-HT_{2A} T102C and 5-HT_6 T267C variants can significantly influence executive function in healthy adults, supporting the hypothesis that D_1, D_3, 5-HT_{2A} and 5-HT_6 receptors are involved in prefrontal executive cognition. Certainly, chance false-positive findings might have happened. Although we included a total of 216 subjects, cell sizes for some genotypes that were used as the basis of between-genotype comparisons were small (n = 5 and n = 12 for the D_1 A-48G and 5-HT_6 T267C polymorphisms, respectively). Larger samples are needed to substantiate the results.

Interestingly, while subjects homozygous for the D_3 Ser/Ser genotype performed better in terms of perseverative errors than those heterozygous for Ser/Gly, subjects homozygous for Gly/Gly tended to have intermediate performances (although this was nonsignificant). This phenomenon is not unique for functional studies of D_3 Ser/Gly polymorphisms. For instance, people homozygous for Gly/Gly demonstrate intermediate levels of eye movement disturbances whether they are schizophrenia patients or healthy subjects.[19] Ser/Gly heterozygotic subjects have lower 5-hydroxyindoleacetic acid levels in their cerebral spinal fluid than Ser/Ser and Gly/Gly homozygotic subjects.[46] Excess homozygosity (both the wild type and the variant) can also be a feature of certain subgroups of schizophrenia patients, such as those who respond to neuroleptic treatment.[47] The heterozygote of the 5-HT_{2A} T102C polymorphism also displayed unusual functional consequences. Whereas subjects with the 5-HT_{2A} T/T genotype had the fewest perseverative errors, those with T/C (rather than those with C/C) had the poorest performances (Table 2, Table 3). In accordance, heterozygous patients with T/C have the lowest N100 amplitude when compared with the other 2 Han Chinese genetic groups.[27] Last, 5-HT_6 T267C polymorphisms may have similar effects. The T/T genotype was associated with the highest perecentage of perseverative errors, and the C/C genotype tended to have intermediate performance (Table 2, Table 3). Likewise, schizophrenia patients with the 5-HT_6 C/C genotype show intermediate treatment response to clozapine, compared with those with T/T or T/C.[35] Such findings on "positive or negative heterosis (the highest or lowest phenotypic expression in heterozygotes)"[48] have been reported in the neuroscience[49] and other[50,51] literature.

An advantage of this study is the apparent ethnic homogeneity of Han Chinese people. In general, the Han Chinese population is genetically homogeneous. However, given the large population and the wide distribution of the Han Chinese, we cannot exclude the potential population admixture effect on the study results. Of note, cognitive capacity, as measured by performance on neuropsychological tests, is related to many aspects of function in schizophrenia patients.[52,53] Whether our findings could be extrapolated to other ethnic populations or to patients with psychiatric disorders remains unclear. In another study,[54] which recruited antipsychotics-treated schizophrenia patients, D_1 A-48G, but not D_3 Ser9Gly or D_4 C-521T, influenced WCST performance. In female patients, a relation was also found between D_2 -141C Ins/Del polymorphism and perseverative errors. Patients with the G/G genotype of the D_1 receptor gene tended to obtain poorer WCST results,[54] similar to our finding in healthy people. The findings for the D_3 variances differ from ours. Because both first- and second-generation antipsychotics act on D_3 receptors,[17,39] the potential confounding influences from drugs need consideration. Differences in subject ethnicity may also contribute to the discrepancy.

The WCST perseverative error performance in this study is similar to that in another healthy Chinese population[55] but inferior to that in a healthy white population.[56] Further, the percentage of perseverative errors and the number of completed categories in the healthy population in the current study are similar to the results obtained in schizophrenia patients in studies performed in the United States and Europe.[8,11] In conclusion, although our results are of interest, the definitive answers and the possible applications to other ethnic groups or mentally ill patients require future, larger studies. Besides, the question of whether polymorphisms of other serotonin or dopamine receptors or serotonin or norepinephrine transporters (the latter of which is responsible for dopamine reuptake in the prefrontal cortex) are also associated with WCST performance deserves more study. Finally, glutamate neurotransmission[57–59] and other pathways[2] are also crucial for high cortical function in the prefrontal areas. Therefore, possible effects of other genetic variances than dopamine- or serotonin-related ones on executive function or other neuroncognitions[60] also require elucidation.

Acknowledgements: This study was supported by the National Science Council (Taiwan) NSC-94-2314-B-039-026, NSC-95-2314-B-006-119, NSC-95-2314-B-006-118-MY3; the National Health Research Institutes (Taiwan) NHRI-EX-96-9405PI, the National Research Program for Genomic Medicine (Taiwan) 94DOH004, the Committee on Chinese Medicine and Pharmacy (Taiwan) CCMP94-RD-041-1, the Department of Health (Taiwan), China Medical University Hospital (Taiwan) DMR-94-064, and National Cheng Kung University Project of Promoting Academic Excellence and Developing World Class Research Centers (Taiwan).

Competing interests: None declared.

Contributors: Drs. Lane and Liu designed the study. Drs. Lane, Huang, Hsieh, Mr. Y-L Chang and Ms. L. Chang acquired the data, which Drs. Lane, Liu, Y-C Chang and W-H Chang analyzed. Dr. Lane wrote the article, and all authors revised it. All authors gave final approval for the article to be published.

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