Association of Cholinergic Muscarinic 2 Receptor Gene Polymorphisms with Learning Aptitude among Medical and Fine Arts Students

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Background: *Cholinergic muscarinic 2 receptor (CHRM2) is believed to be involved in neuronal excitability, synaptic plasticity and feedback regulation of acetylcholine release. Polymorphism in the CHRM2 gene had been shown a significant association with intelligence.*
Objective: *To investigate single nucleotide polymorphisms (SNPs) in CHRM2 gene with the learning aptitude among medical and fine arts students at Srinakharinwirot university, Thailand.*
Material and Method: *A total of two hundred blood samples were withdrawn from medical (100) and fine arts (100) students. DNAs were extracted using DNA extraction kit. SNP primers were designed and screened. Genotyping was performed by real-time PCR and high-resolution melting (HRM) analysis and confirmed by sequencing. The difference in genotypic distribution was analyzed using Pearson's Chi-square test implemented in SPSS program version 11.5. Significant level was set at $p<0.05$.*
Results: *Two SNPs in CHRM2 gene, rs2061174 and rs6948054, showed significant difference in genotype distribution between medical and fine arts students ($p<0.05$). The rs2061174 showed significant at $p = 0.001$, OR and 95% CI were 3.78 (2.00-7.14), whereas the rs6948054 was significant at $p = 0.012$, OR and 95% CI were 2.50 (1.32-4.77).*
Conclusion: *Two SNPs in CHRM2 gene, rs2061174 and rs6948054, may be used as biomarker to distinguish the learning aptitude among Thai individual.*

Keywords: *Cholinergic muscarinic 2 receptor, Polymorphisms, Biomarker, CHRM2 gene, SNPs*

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Differences in individual human intelligence (IQ) are thought to represent an inborn potential that correlates with future academic success, occupational status, and health[1-3]. Intelligence is a quantitative trait with substantial heritability that increase with age estimate about 30% to 80% in very young child to adulthood[4,5]. The genetic variability of several genes were observed to be associated with intelligence. The Val158Met polymorphism of the catechol-O-methyltransferase (COMT) gene is supposed to exert its effects on cognition by modifying dopamine signaling in the frontal lobes[6]. Single-nucleotide polymorphism (SNP) has been identified in the human BDNF gene (Val66Met) that leads to the decrease in BDNF secretion and impairments in specific forms of learning in human or effects on cognitive processes[7]. The BDNF gene encodes a secretory protein that has an important function in the CNS, from glutamate signaling to cell survival and plasticity. The BDNF protein was significant for both intelligence and memory constructs[8]. A genome-wide association study (GWAS) was conducted to identify common genetic variants that are associated with human intelligence or general cognitive ability[9] and this identified the SNPs in the genes which associated with intelligence such as *ApoE, COMT, DRD2, DTNBP1, BDNF* and *CHRM2*[10].

The cholinergic muscarinic receptor (CHRM2) family belongs to the superfamily of G-protein coupled receptors. *CHRM2* gene is thought to be involved in neuronal excitability, synaptic plasticity and feedback

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regulation of acetylcholine (ACh) release and has been associated in higher cognitive processing, including learning and memory[11]. The gene is located on chromosome 7q which contains numerous SNPs. This receptor is primarily a presynaptic auto-receptor that modulates release of acetylcholine[12]. However, several publications have reported GWAS as well as related cognitive traits and the polymorphism at the *CHRM2* gene showed a significant associated with intelligence[13]. A GWAS performed in the Netherlands showed that a SNP (rs2061174) in intron region of *CHRM2* gene was association with IQ[14].

This study aims to investigate the presence of the association between single nucleotide polymorphisms (SNPs) of *CHRM2* gene and learning aptitude among medical and fine arts students.

Material and Method

Subjects

Two hundred volunteered, medical (100) and fine arts (100) students were participated in this study, and all signed an informed consent. This research project was approved by the ethical committees of the Faculty of Medicine, Srinakharinwirot University, Thailand.

Genomic DNA extraction

Five ml of blood were collected in heparinized-tube for DNA extraction using Flexigene DNA kit (QIAGEN, German). Briefly, 12.5 ml lysis buffer were added to the whole blood and mixed by inverting the tube, then centrifuged for 15 min at 5,300 xg. Supernatant was discarded and the pellets was partially dried for 2 min. Add 2.5 ml of denaturation buffer and 25 µl of protease into the tube, vortex immediately until the pellets completely dissolved and incubated at 65°C for 10 min. After that, 2.5 ml of isopropanol (100%) were added, mix thoroughly by inversion, then centrifuged for 15 min at 5,300 xg and discarded the supernatant. DNA pellet was washed with 70% ethanol (v/v) and centrifuged for 15 min at 5,300 xg. DNA pellet was dried at room temperature and dissolved with FG3 buffer. The DNA concentration was measured using Nanodrop 2000 (Scientific™). Then, the DNA concentration was adjusted to 25 ng/µl for PCR amplification.

SNPs primers

The primers for detecting the SNPs in the *CHRM2* gene by real-time PCR were designed using the program from website; http://www.ncbi.nlm.nih.gov/projects/SNP/. All primer sequences are shown in Table 1. The position of two selected SNPs (rs2061174, and rs6948054) in *CHRM2* gene are shown in Fig. 1.

Real-time PCR, high-resolution melting analysis and sequencing

PCR was carried out in a 20 µl mixture contains 2 µl of DNA (25 ng), 10 µl of supermix, 0.6 µl of 10 µM forward primer, 0.6 µl of 10 µM reverse primer and 6.8 µl of sterile-distilled water. PCR was performed for 40 cycles; pre-denaturing at 98°C for 3 min, denaturation at 98°C for 10 s, annealing at 60°C for 15 s, and extension at 72°C for 20 s. PCR products were further genotyped by HRM analysis at temperature between 65°C to 95°C using a rotor gene 6000 (Corbett, Australia). Genotype was confirmed by sequencing using ABI 3730xl (Applied Biosystems).

Statistical analysis

The difference in genotype distribution between SNPs of the two groups (medical and fine arts students) were analyzed using Pearson's Chi-square test implemented in SPSS program version 11.5 for windows. Significant level was set at $p<0.05$.

Results

SNPs located in intronic region of *CHRM2* genes were investigated from DNA of medical and fine arts students. The results are summarized in Table 2. Two SNPs, rs2061174 and rs6948054, showed significant differences in genotype distribution ($p<0.05$). The SNPs, rs2061174 was significant at $p =$

Table 1. Two selected SNP primers of *CHRM2* gene used in real-time PCR

db SNPs ID	Location	Primer sequence 5' to 3'	Size of PCR product (bp)
rs2061174	Intron	F1: CCTGTTGTTGCTAATGTCTAGTCC R1: AAAGGGAAAACCTGCTGAGA	90
rs6948054	Intron	F2: CAGAAACAGAACTCAGTGTATGGAA R2: TCCAGAAACATTTATCAGGCAGT	98

A

B

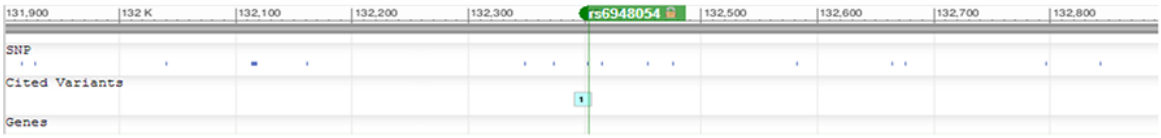

Fig. 1 The positions of selected SNPs, rs2061174 (A) and rs6948054 (B) in *CHRM 2* gene.

Table 2. Distribution of allele and genotype frequencies of *CHRM* 2 gene in medical and fine arts students

SNP	Allele	Medical student		Fine arts student		*p*-value	OR	95% CI
		n = 100		n = 100				
rs2061174	C/T	n	%	n	%			
	CC	31	31	29	29			
	CT	22	22	52	52	0.001	3.78	2.00-7.14
	TT	47	47	19	19			
	Allele C	84	42	110	55			
	Allele T	116	58	90	45			
rs6948054	A/G	n	%	n	%			
	AA	24	24	37	37			
	AG	39	39	44	44	0.012	2.50	1.32-4.77
	GG	37	37	19	19			
	Allele A	87	44	118	59			
	Allele G	113	56	82	41			

0.001, with OR and 95% CI of 3.78 (2.00-7.14), whereas rs6948054 was significant at $p = 0.012$, with OR and 95% CI of 2.50 (1.32-4.77).

Discussion

We found two SNPs, rs2061174 and rs6948054, located in intron region of mRNA of *CHRM2* gene at position 113,002, and 132,403 bp, respectively, significantly associated with learning skill among medical and fine arts students. The *CHRM2* gene was selected to study because this gene was reported to involve in learning and memory although there are several genes that involved in intelligence. The *CHRM2* gene activate a multitude of signaling pathway important for neuronal excitability, synaptic plasticity and feedback regulation of acetylcholine (ACh) release and has previously been implicated in higher cognitive processing, including learning and memory[15,16]. The medical and fine arts students were chosen to represent the skill in learning sciences and arts, because both groups showed evidence of obtaining high scores in sciences and arts subjects, respectively. The results revealed that two SNPs in *CHRM2* gene, rs2061174 and rs6948054, appeared to be significant associated

with intelligence and can be used to distinguish the learning skill among medical and fine arts students in Thai ethnic. The rs2061174 showed significant at p = 0.001, odd ratio value and 95% CI [3.78 (2.00-7.14)], whereas the rs6948054 showed significant at p = 0.012, odd ratio value and 95% CI [2.50 (1.32-4.77)]. These are useful information to guide the parent to support their children to study in subject or faculty corresponding to the predicted learning aptitude from SNPs of this gene.

Conclusion

Two SNP in intronic regions of *CHRM2* gene, rs2061174 and rs6948054, were significant associated with learning skill among medical and fine arts students. It may be used as biomarker to distinguish the learning aptitude of individual in Thai ethnic.

What is already known on this topic?

Cholinergic muscarinic 2 receptor (*CHRM2*) gene has previously been implicated in higher cognitive processing, including learning and memory.

What this study adds?

Two SNPs in *CHRM2* gene, rs2061174 and rs6948054, can be distinguished the learning aptitude among medical and fine arts students of Thai ethnic.

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Potential conflicts of interest

None.

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ความสัมพันธ์ระหว่างความแปรผันของจีนตัวรับโคลิเนอร์จิคมัสคาร์รินิก 2 กับความถนัดในการเรียนรู้ของนิสิตแพทย์และนิสิตศิลปะ

พลเพชร ทองเกตุ, ชิดพล เพลินศิริ, วันเพ็ญ ธุรกิตต์วัณณการ, วาสนา สุขุมศิริชาติ

ภูมิหลัง: *จีนตัวรับโคลิเนอร์จิคมัสคาร์รินิก 2 (Cholinergic muscarinic 2: CHRM2) เชื่อกันว่ามีความเกี่ยวข้องกับการกระตุ้นสมอง และการควบคุมแบบป้อนกลับของการหลั่งแอซิติลโคลีน (acetylcholine) การแปรผันของจีน CHRM2 มีรายงานว่าเกี่ยวข้องกับความฉลาดในการเรียนรู้*

วัตถุประสงค์: *เพื่อหาความสัมพันธ์ของการแปรผันหนึ่งเบส (สนิปส์) ในจีนตัวรับ CHRM2 กับความถนัดในการเรียนรู้ระหว่างนิสิตแพทย์และนิสิตศิลปะมหาวิทยาลัยศรีนครินทรวิโรฒ ประเทศไทย*

วัสดุและวิธีการ: *จำนวนตัวอย่างเลือดทั้งหมด 200 ตัวอย่าง ได้จากนิสิตคณะแพทยศาสตร์ (100 คน) และนิสิตคณะศิลปกรรมศาสตร์ (100 คน) ทำการสกัดดีเอ็นเอโดยใช้ชุดสกัดดีเอ็นเอ ออกแบบและคัดเลือกไพรเมอร์เพื่อตรวจสนิปส์ ทำการหาจีโนไทด์ของดีเอ็นเอจากตัวอย่างทั้งหมดโดยเทคนิคเรียลไทม์พีซีอาร์ (real-time PCR) และไฮเรสโซลูชัน เมลติ้ง อะนาไลซิส (high-resolution melting analysis) และหาลำดับเบสเพื่อยืนยันจีโนไทด์ ความแตกต่างของการกระจายตัวของจีโนไทด์วิเคราะห์โดยใช้ Pearson's Chi-square test ของโปรแกรม SPSS รุ่น 11.5 โดยกำหนดค่าความแตกต่างอย่างมีนัยสำคัญทางสถิติเมื่อค่า p น้อยกว่า 0.05*

ผลการศึกษา: *สนิปส์ 2 ตำแหน่งในจีน CHRM2 คือ rs2061174 และ rs6948054 มีความแตกต่างอย่างมีนัยสำคัญทางสถิติของการกระจายตัวของจีโนไทด์ ระหว่างนิสิตคณะแพทยศาสตร์และนิสิตคณะศิลปกรรมศาสตร์ ($p<0.05$) สนิปส์ตำแหน่ง rs2061174 มีความแตกต่างอย่างมีนัยสำคัญทางสถิติที่ค่า $p = 0.001$, OR และ 95% CI เท่ากับ 3.78 (2.00-7.14) ขณะที่สนิปส์ตำแหน่ง rs6948054 มีความแตกต่างอย่างมีนัยสำคัญทางสถิติที่ ค่า $p = 0.012$, OR และ 95% CI เท่ากับ 2.50 (1.32-4.77)*

สรุป: *สนิปส์ 2 ตำแหน่งในจีน CHRM2 คือ rs2061174 และ rs6948054 อาจใช้เป็นตัวบ่งชี้ความถนัดในการเรียนรู้ของแต่ละบุคคลในคนไทยได้*