DNA Extraction from Buccal Cells for Determination of Nucleotide Polymorphism at rs 1800497 Position in Dopamine D2 Receptor Gene

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ABSTRACT

Substance use disorder is influenced by complex genetic and environmental factors. Many studies found that genetics may play an important role in the development of alcohol dependence. Twin and family history studies revealed that genetics affect a heritability of alcoholism approximately 50-60%. The neurotransmitter in brain especially dopamine may associate with rewarding system that might increase risk in alcohol dependence. The dopamine D2 receptor gene (*DRD2*) allelic status may influence on development of alcohol dependence due to the change in number of receptor. This study aimed to demonstrate that buccal cells could be used as an alternative source of DNA for PCR-RFLP based method to determine the allelic status of *DRD2*. The DNA samples were extracted from buccal cells using proteinase K and 5% chelex solution, then amplified by PCR method with some modification of the primer sequence. The optimization for annealing temperature was performed. Our PCR system using an annealing temperature at 56 °C yielded the most specific amplified DNA of approximately 200 ng/µl. The PCR product was digested with TaqI restriction enzyme at 65 °C overnight. The digested product was separated in 8.5% polyacrylamide gel elctrophoresis for genotyping.

Keywords: Alcohol dependence, PCR-RFLP, *DRD2*, buccal cells.

INTRODUCTION

Many studies found that addictive behaviour are influenced by genetics and environmental factors. Twin and family history studies revealed that genes may play a role in substance dependence including 50% to 60% of alcohol dependence linked to genetic factor.[1,2] Genetic effects may contribute to the alcohol susceptibility in individuals. It is suggested that neurotransmitter in brain may associate to the alcohol dependence development. In the family with positive history of alcoholics, the biological offspring have 3-5 folds risk to develop alcohol dependence in adolescence than the negative family.[1,3] The individual difference in alcohol susceptibility may be a result of DNA variation called DNA polymorphism that might affect protein structure or function. The difference region and pattern of DNA polymorphism can effects protein synthesis and its quality.[4]

The mesocorticolimbic dopamine system in ventral tegmental area (VTA) of the brain may plays important role on brain reward mechanism[5,6] and

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associate with addictive behaviour. The addictive drugs including alcohol can overstimulation dopamine release in nucleus accumbens (NAC) reinforce the user to repeat drug administration. The chronic overstimulation of dopamine causes drug tolerance, followed by addiction.[7,8]

The studies of 5 dopamine receptor gene (*DRD1-DRD5*) found that dopamine D2 receptor gene (*DRD2*) located on chromosome 11 q22-23 is associated with alcohol dependence.[9] The polymorphism at TaqI A site (rs1800497 in dbSNP; C/T) A1 alleleic status (TT or TC genotype) prevalence is significantly higher in alcoholic patients than control group.[10] Many studies were performed in different ethnic group show the similar result. For the Asian population[11] also found more prevalence of A1 allele in Japanese alcoholic patients than the control group. Besides that the prevalence of A1 allele was also correlated with the severity of the disease.

Most studies for A1 allele determination in *DRD2* were conducted by using DNA extraction from blood.[11,12] Since buccal cell is more simple to collected with non-invasive method, it should be used as a source of DNA for the test. We demonstrated the technique for determination of polymorphism at TaqI site of *DRD2* using buccal cells as a sample of choice.

MATERIALS AND METHODS

Buccal cell collection

The buccal cells samples were collected from the volunteers by scraping (about 10 times) the oral mucosa with the blunt end of a toothpick. The sample was soaked with 1 ml sterile distilled water in the 1.5 ml centrifuge tube and stored in -20°C until extraction.

DNA extraction from buccal cells

The samples were thawed at room temperature then centrifuged for 1 min at 14,000 xg. After the supernatant was discarded, the pellet was washed with 1 ml of sterile distilled water for 3 times and then 290 µl of sterile distilled, 5 µl of proteinase K solution (1 mg/ml; Sigma, USA) and appropriate amount of chelex resin (Sigma, USA) were added. The sample tubes were incubated at 55°C for 60 min. After the buccal cells were completely digested, samples tubes were reincubated at 100 °C for 8 min. The sample were centrifuged at 14,000 xg for 1 min then stored at 4 °C until amplification.

DNA amplification

The extracted DNA was used as a template for the polymerase chain reaction method (PCR) by the procedure previously described[12,13] with some modification of primer sequence as followed (Invitrogen USA): forward primer: 5'ACG GCT GGC CAA GTT GTC TA 3', reverse primer: 5' CCT TCC TGA GTG TCA TCA AC 3'. PCR was performed in a final volume of 20 µl and carried out in a mastercycler ep (Eppendorf Germany). About 2 µl of extracted DNA were used as a template in a final volume of 20 µl containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.25 mM of each dNTPs, 0.01% BSA, 0.05% Tween 20, 0.5 Unit of of Taq DNA polymerase and 0.25 µM of each primer (Invitrogen USA). To optimize the reaction, we have performed PCR using different annealing temperature ranging from 50-57°C. The PCR products were checked for 310 bp product by 2% agarose gel electrophoresis.

Genotyping

The amplicon was digested with TaqI restriction enzyme (Invitrogen USA) at 65°C overnight and then separated by 8.5% polyacylamide gel electrophoresis. The 3 different genotypes of the polymorphic site were revealed as: A1A1 (TT) genotype will be separated as a single undigested fragment, for the heterozygous genotype (CT) will

show 3 fragments of 130, 180 and 310 bp and the A2A2 (CC) genotype will revealed as 2 fragments of 130 and 180 bp.

RESULTS AND DISCUSSION

Optimal annealing temperature

The PCR was conducted with different annealing temperatures according to the following protocol: 94°C 2 min, 94 °C 30 sec, annealing temperature ranging from 50-57°Cfor 30 sec and 72 °C 1 min 30 sec. The optimal annealing temperature was around 56°C as shown in Figure 1. The brightest ban was found in lane 10 with the lowest signal of background.

DNA quantitation

The PCRs were performed using extracted DNA from 6 volunteers. The amplicons were quantitated using UV spectrophotometry. 250 µl mixture containing 10 µl of amplified DNA and 240 µl of ultrapure water (1:25 dilution) was filled in cuvette and measured the absorption at 260 nm (A260). Approximately 200 ng/µl of product was yielded (Table 1 and Figure 2). The amplified DNA concentration was calculated using the formula:

DNA concentration (ng/µl)

= A260 x dilution factor x 50

*Absorbance at 260 nm

= 1.0 equals to 50 ng/ µl of DNA concentration

Genotyping

The A2A2 (CC) genotype was used as the pilot sample to test for completeness of restriction enzyme digestion. Approximately 1,600 ng of amplicon was digested in 4 different amount of TaqI restriction enzyme (Invitrogen, USA). To the target DNA 8, 4, 2 and 1 Unit of restriction enzyme and 1x react 2 buffer were added to make a final volume of 30 µl. The reaction tubes were incubated at 65° C overnight. Digested product was separated by

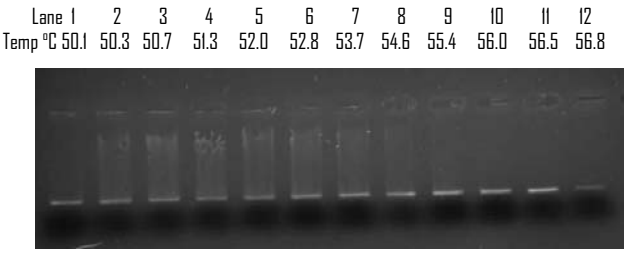
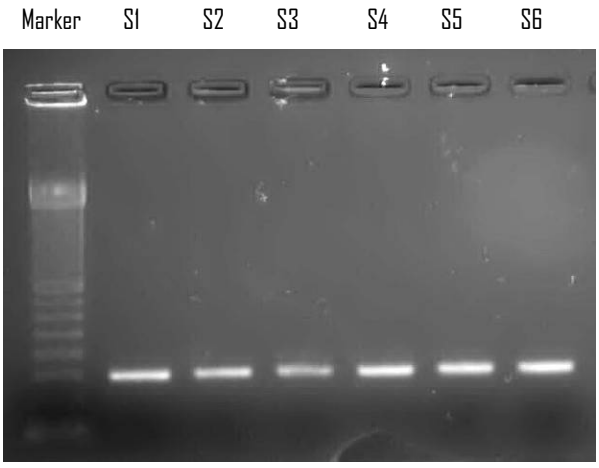


Figure1 PCR products with different annealing temperature

Table 1 Concentration of amplified DNA from 6 volunteers.

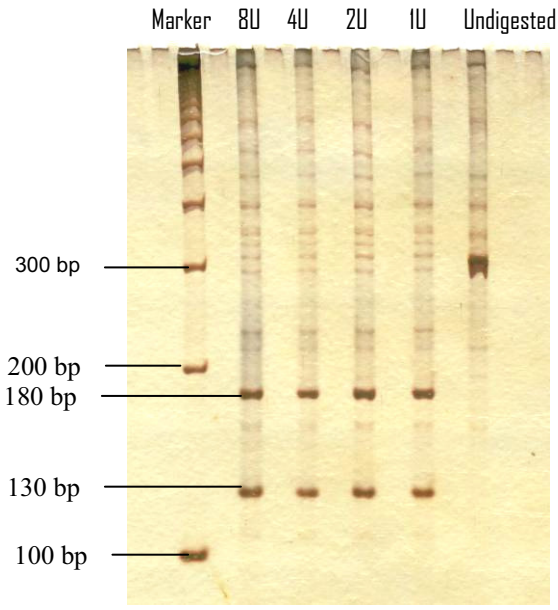
Sample	Absorbance at 260 nm (A260)	Concentration (ng/µl)
S1	0.166A	207.5
S2	0.153A	191.25
S3	0.149A	186.25
S4	0.162A	202.5
S5	0.168A	210.0
S6	0.160A	200.0

Figure 2 The 310 bp amplicon from 6 volunteers in 2% agarose gel electrophoresis



8.5% polyacrylamide gel electrophoresis. It revealed 2 bans of 130 and 180 bp compared to 310 bp of undigested product. We found the minimal amount of TaqI restriction enzyme that

Figure 3 PCR-RFLP analysis of A2A2 genotype



could digest 1,600 ng of DNA completely was about 1 Unit as shown in Figure 3.

The study shows effectiveness of using buccal cell samples to determine TaqI A1 allele of *DRD2*. Since the way to collect buccal cell is simpler and inexpensive. DNA from buccal cell yields enough quantity suitable for PCR. Therefore, we encourage using buccal cells as a sample of choice in detection of alcohol related gene.

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การสกัดสารพันธุกรรมจากเซลล์เยื่อบุกระพุ้งแก้มเพื่อใช้ตรวจสอบภาวะพหุสัณฐานของนิวคลีโอไทด์ที่ตำแหน่ง rs 1800497 ในยีนตัวรับโดปามีนชนิดที่ 2

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บทคัดย่อ

เป็นที่ยอมรับกันว่าการติดสารเสพติดส่วนหนึ่งเป็นผลมาจากพันธุกรรมที่ซับซ้อนและผลจากสิ่งแวดล้อม มีการศึกษาพบว่าพันธุกรรมอาจเกี่ยวข้องกับการติดสุราเรื้อรัง จากการศึกษาเปรียบเทียบในฝาแฝดและประวัติครอบครัวพบว่าพันธุกรรมมีผลถึงร้อยละ 50-60 ที่จะเพิ่มโอกาสเสี่ยงต่อการติดสุราเรื้อรังในอนาคต สารสื่อประสาทในสมองที่เรียกว่าโดปามีน ซึ่งเกี่ยวข้องกับความรู้สึกพึงพอใจหรือ rewarding system อาจมีผลต่อความเสี่ยงของการติดสุราเรื้อรัง สาเหตุอาจเกิดจากความผันแปรของลำดับนิวคลีโอไทด์หรือการเกิดภาวะพหุสัณฐานบนยีนตัวรับโดปามีนชนิดที่ 2 ที่อาจมีผลต่อจำนวนตัวรับในสมองทำให้แต่ละบุคคลมีความเสี่ยงต่อการติดสุราเรื้อรังได้ต่างกัน การศึกษานี้เสนอทางเลือกในการใช้เซลล์เยื่อบุกระพุ้งแก้มเพื่อศึกษาภาวะพหุสัณฐานของยีนบนตำแหน่งดังกล่าวด้วยวิธี PCR-RFLP โดยทำการสกัดสารพันธุกรรมจากเซลล์เยื่อบุกระพุ้งแก้มด้วยเอนไซม์ proteinase K และสารละลาย 5% chelex จากนั้นเพิ่มปริมาณสารพันธุกรรมด้วยเทคนิค PCR จากการทดสอบอุณหภูมิที่เหมาะสมสำหรับกระบวนการ annealing พบว่าที่ 56 องศาเซลเซียสจะให้ PCR product ที่เหมาะสมที่มีความเข้มข้น 200 ng/µl นำ PCR products ไปแช่อบที่ 65 องศาเซลเซียสพร้อมกับ *Taq*I restriction enzyme ข้ามคืน จากนั้นจึงตรวจสอบลักษณะของจีโนไทป์ด้วย 8.5% polyacrylamide gel electrophoresis.

คำสำคัญ: การติดสุราเรื้อรัง, PCR-RFLP, *DRD2*, เซลล์เยื่อบุกระพุ้งแก้ม

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