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Determination of dopamine receptor (D2) –141C Ins/Del polymorphism in whole-blood and buccal swabs by PCR-RFLP genotyping using GC-Rich® solution

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Introduction

The –141C Ins/Del polymorphism is a single nucleotide polymorphism (SNP) of the promoter region of the human dopamine D2 receptor (DRD2) gene, characterized by the deletion of one cytosine nucleotide at the position of –141 (1). It occurs in relatively high frequencies; 9% in Caucasians (2), and 39% in African–Americans (3). *In vitro* (1) and *in vivo* (4, 5) studies suggest that –141C Ins/Del SNP affects DRD2 gene expression and receptor density. Other studies have shown that this SNP might be associated with schizophrenia (1, 6, 7), personality trait detachment (8), bipolar affective disorder (9), alcoholism (10), heroin abuse (11), and stature (12). Moreover, as DRD2 plays an important role in the mechanism of action of antipsychotics, –141C Ins/Del SNP might affect the clinical response to antipsychotics. Pharmacogenetic studies have shown that –141C Ins/Del SNP might be associated with antipsychotic–induced extrapyramidal symptoms (13), antipsychotic–induced anxiolytic and antidepressive effects (14), and treatment–resistance to these agents (15). Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) genotyping for this SNP has been described earlier using whole-blood DNA isolates (1). However, amplification of the dopamine promoter region containing the SNP has been difficult to achieve, due to its high GC content (16). In this study, we describe an optimized method for the detection of the –141C Ins/Del SNP using GC-Rich® solution and *FastStart Taq*® polymerase, which shows superior effectiveness in amplifying high GC-content amplicons as compared to *SuperTaq*® or *AmpliTaq Gold*®. Furthermore, we describe for the first time the non-invasive detection of –141C Ins/Del SNP using DNA obtained from buccal swabs.

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Materials and Methods

PCR

DNA was isolated from 200 µl whole blood (Wb) using *High Pure Template Kit* (Roche) or from buccal swabs (Sw) using *BuccalAmp*™ DNA Extraction Kit (Epicentre) according to the manufacturer's instructions. DNA isolates were stored at -20 °C until analysis. For the amplification of the fragment containing the SNP, the following forward primer 5'– ACT GGC GAG CAG ACG GTG AGG ACC C –3', and reverse primer 5'–TGC GCG CGT GAG GCT GCC GGT TCG G –3' were used. Each PCR reaction of 50 µl contained the following: 2µl of patient's DNA, 200 µmol/l dNTP's, 20 pmol of each primer, Taq polymerase (either *SuperTaq*® (Sphaero Q), *AmpliTaq Gold*® (Applied Biosystems), or *FastStart*® (Roche) at 2 Units/reaction), 1X buffer containing appropriate $MgCl_2$ and KCl concentrations, and additives such as 5% v/v DMSO as a final concentration (when using *SuperTaq*® or *AmpliTaq Gold*®) or 1X GC-Rich® solution (Roche) when using *FastStart*® Taq polymerase. PCR was performed on a *Perkin Elmer 2400* thermal cycler (Applied Biosystems). Cycling conditions were as follows; 4 minutes (or 10 minutes when using *AmpliTaq Gold*®) at 95 °C for DNA denaturation/activation of the polymerase, 35 cycles (30 sec at 95 °C (denaturation), 30 sec at 68 °C (annealing), and 30 sec at 72 °C (extension)), and finally 10 minutes at 72 °C. Using the aforementioned primer combination, an amplicon size of 304 bp is expected (1).

RFLP

After amplification, 10 µl of amplified product was digested with 2.5 Units of *Bst*NI (New England Biolabs) in a total volume of 15 µl containing 1X BSA, 1X NE Buffer II (New England Biolabs), and H_2O for 2 hours at 60 °C. Fragments were separated on 3% Proranose LM-3 agarose gel (Sphaero Q) and visualized using ethidium bromide. Wild type alleles are cut at the –141 position in the DRD2 gene generating two fragments (160 bp and 144 bp), while mutated alleles loose this restriction site.

Results

Amplification of the region containing the –141C Ins/Del polymorphism was carried out using previously established protocols (1). The use of *Amplitaq*

Gold® or *SuperTaq*® in our setting yielded poor results with faint amplicons (results not shown). Since the –141C Ins/Del region has a high GC-content, new PCR amplifications were conducted in the presence of 5% v/v DMSO, which has been previously shown to facilitate DNA denaturation and amplification of high GC-content amplicons (17, 18). This did not improve the yield of the PCR reaction (results not shown). *FastStart*® Taq DNA polymerase has been previously used to amplify GC rich (65%) fragments in the serotonin receptor 2C gene (19). Figure 1A shows the PCR results when *FastStart*® Taq polymerase was used in the presence (+) or absence (-) of 1X GC-Rich® solution. Using *FastStart*®Taq polymerase, clear amplicons were obtained with the expected size (approximately 304 bp). Addition of GC-Rich® solution greatly enhanced the PCR yield. DNA sequence analysis (BaseClear, Leiden, The Netherlands) of the amplicons confirmed the validity of the PCR reactions (results not shown). Comparison of amplicons obtained from Wb - or Sw DNA (paired samples from the same patient) showed complete concordance (figure 1B), indicating that Sw is an appropriate alternative for obtaining DNA samples.

Discussion and Conclusion

The determination of functional polymorphisms in the promoter region of the DRD2 can be a useful tool in identifying risk factors that predispose individuals to particular disorders or that may affect clinical response to antipsychotics. In this paper, we describe an improved method for the determination of –141C Ins/Del SNP in the DRD2 promoter region using PCR-RFLP techniques.

The determination of this polymorphism has been previously described by others, but the amplification of the fragment containing the aforementioned SNP has proven to be difficult (16). Using *FastStart*®Taq DNA polymerase in combination with GC-Rich® solution, we were able to successfully amplify this fragment while obtaining high yield amplicons. *FastStart*®Taq in combination with GC-Rich® solution has been especially formulated to facilitate amplification of difficult DNA targets such as those that contain high (e.g. > 50%) GC-content. An alternative to GC-Rich® solution is Q-solutions (Qiagen). Using *FastStart*®Taq in combination with GC-Rich® solution, it was also possible to amplify the same fragment from DNA obtained from buccal swabs. The latter offers a non-invasive method for obtaining sufficient DNA amounts for genotyping purposes, and provides an attractive alternative to whole-blood DNA in psychiatric and mentally-ill patients.

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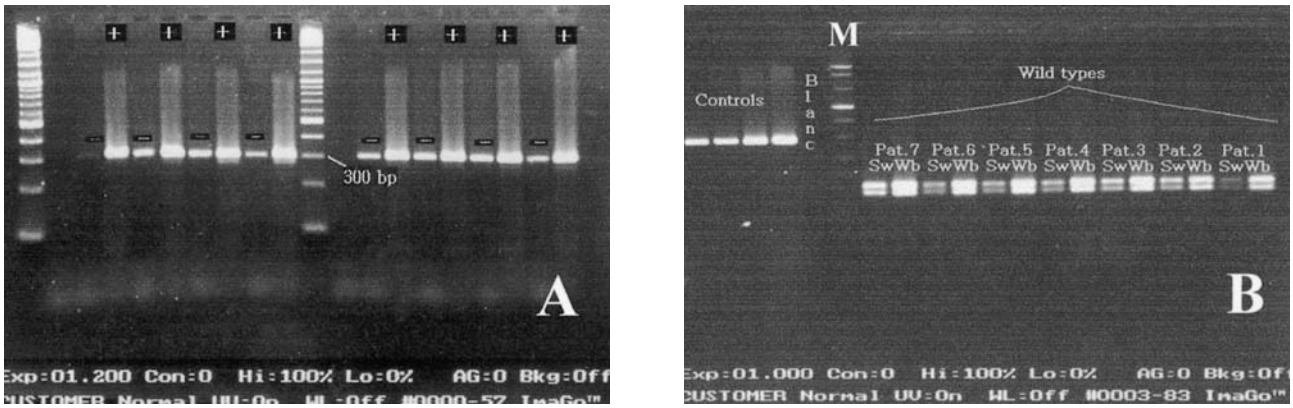

Figure 1. Results of PCR amplification of the DRD2 promoter region which contains the –141C Ins/Del polymorphism using *FastStart*® Taq DNA polymerase in the presence (+) or absence (-) of GC-Rich® solution (A), and comparison of –141C Ins/Del genotypes from whole-blood DNA (Wb) or buccal swab (Sw) obtained from the same patient (B). Blanc indicates a PCR reaction with all components except genomic DNA, while controls represent undigested amplicons. Pat. X (X= 1 to 7): paired DNA samples from patient No.X, M: molecular weight marker.

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Het belang van commuteerbare referentiematerialen en externe SKML-monsters bij enzymstandaardisatie

Studie in het kader van het SKML-project 'Kalibratie 2000'

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Inleiding

Bij het verkleinen van de tussenlaboratoriumvariatie zijn twee initiatieven belangrijk: het gebruik van gestandaardiseerde methodes en het toepassen van referentiematerialen. Het promoten van aanbevolen methodes door NVKC en IFCC in de jaren 1970-1990 resulteerde in de daling van de tussenlaboratoriumvariatie van 50% naar ca 15%. Aanvankelijke pogingen om een verdere reductie te verkrijgen door het gebruik van kalibratoren lukten maar ten dele vanwege de niet-commuteerbaarheid van de gebruikte materialen (1). Recente strategieën in dit verband zijn neergelegd in het 'Reference System'-concept (2, 3) waarin de nadruk wordt gelegd op het gebruik van (commuteerbare) referentiematerialen en het gebruik van een netwerk van referentielaboratoria. De in werking getreden EU-IVD-richtlijn benadrukt eveneens het belang van zulke referentiesystemen. Omdat de tot nu toe beschikbare referentiematerialen duur zijn en slechts geschikt bij het gebruik van primaire referentiemethoden is er behoefte aan secundaire referentiematerialen die commuteerbaar zijn en daardoor in staat zijn om de juistheid over te dragen van de primaire referentiemethoden naar de in gebruik zijnde routinemethoden. We beschrijven hier het onderzoek naar commuteerbaar referentiemateriaal en rapporteren over de resultaten die in 2003 werden behaald met het gebruik van de zo ontwikkelde Nederlandse Enzymkalibrator.

Methoden

Tien potentiële referentiematerialen (PRM's) werden op hun commuteerbaarheid onderzocht door 37 geselecteerde laboratoria in een zogenaamde Twin-studie (4) te laten participeren. Vijf PRM's waren van commerciële herkomst, de andere vijf waren zelf bereide serumvarianten. De enzymen die werden gebruikt voor het 'spiken' waren afkomstig van Asahi Chemical Co (Tokyo, Japan) en werden bereid uit humane cellijnen met recombinanttechnieken, waardoor de isoenzymsamenstelling overeenkomt met de samenstelling zoals in natief humaan serum. Het preparaat met uiteindelijk de beste eigenschappen qua commuteer-

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