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ANKKI / Taq A1 Genotyping from Dried Blood Stored on Filter Paper Among Alcohol & Nicotine Dependant Subjects

Rizwana Quraishi* and Raka Jain

National Drug Dependence Treatment Centre, Department of Psychiatry, All India Institute of Medical Sciences, New Delhi, India

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Corresponding author: Department of
Psychiatry, Room No.4080, 4th floor
All India Institute of Medical
Sciences, Ansari Nagar, New Delhi -
110029, India.

E-mail address:
rizwanaguraishi@gmail.com

ABSTRACT

This paper describes the use of blood spotted filter paper to study the genotyping of ANKKI / Taq A gene. For the study hundred patients visiting National Drug Dependence Treatment Centre (NDDTC) for the treatment of co-morbid alcohol and nicotine dependence were selected at random. Blood was spotted on to Whatman 903 filter paper, dried and stored at room temperature. Genomic DNA was extracted and genotyping was carried out from dried blood and corresponding whole blood samples. All the samples collected on to filter paper showed exact match of the genotyping when compared to fresh blood. At the end of 6 months of storage the genotyping was carried out successfully from all the samples. The present study shows that dried blood is suitable for ANKKI/ Taq A1 genotyping up to a period of 6 months at room temperatures.

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Introduction

The centre where the study was carried out is a nodal centre involved in substance abuse treatment activities in North India¹⁻³. The association of ANKKI with alcohol and nicotine co-dependence was one of the studies carried out in the centre. During this study it was observed that the study of dependence genetics among patients belonging to remote areas is fraught with many logistic problems. The study of dependence genetics among patients belonging to remote areas is fraught with many logistic problems. Transportation of blood samples to a centralized laboratory often involves problems like requirement of trained staff, sample spillage, breakage, and cross contamination. Dried blood (DB) spotted on filter paper has reported to be used for epidemiological studies, analytical measurements and genetic screening^{4,5}. The current study evaluated the feasibility of using dried blood spotted on to filter paper (Whatman international Ltd, England) for genotyping study using ANKKI as reference genes involved in alcohol & nicotine dependence.

Materials and Methods

The study was carried out for a period of eight months at National Drug Dependence Treatment Centre. The study protocol was approved by the institutional ethical committee. A written informed consent was obtained prior to the recruitment of the subjects. Blood samples from each subject were collected by venipuncture into tubes with anticoagulant. Blood spots were prepared by pipetting 200 μ L (~1.5 inch circle) of the blood onto Whatman 903 filter paper (Whatman international Ltd, England) kept on a nonabsorbent surface (thermacol) and left at room temperature for drying⁶. The room temperature was 20-30°C for the duration of

the study. After drying, the filter discs were kept in sealed plastic bags to protect them from dust and moisture, and stored at room temperature (20-30°C)⁷. Remaining blood samples were kept at -20 °C till processing.

Genomic DNA extraction was carried out from dried blood samples using the Mini blood DNA extraction kit from Qiagen (Valencia, CA). TaqI A (rs1800497) genotyping was done using previously published polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP)⁸. The digested PCR products were resolved on 2-3% agarose gel stained with ethidium bromide. Genotype profiles obtained from this was then compared with the corresponding whole blood profiling from each subject. To assess the effect of stability the blood spots were processed for DNA extraction and genotyping study for ANKKI / Taq1A gene was carried out at the end of six month.

Results and Discussion

The blood samples were collected from 100 subjects with alcohol and nicotine co-dependence. Genomic DNA isolation was carried out from dried blood and corresponding whole blood sample. After DNA extraction amplification for ANKKI / Taq1A gene was carried out and thereafter the samples were subjected to restriction digestion for genotyping (Fig 1 & 2). All the 100 samples showed exact match of number and size of alleles for ANKKI / Taq A1 in DNA extracted from both whole blood and dried blood samples.

The dried blood filter discs stored at room temperature (20-30°C) at the end of 6 month are processed for DNA extraction to study the effect of storage. After extraction genotyping for ANKKI / Taq A1 was carried out in all the samples. All the samples showed successful amplification

and genotyping and compared well with the corresponding whole blood results.

In the present study the extraction and genotyping of Taq A1 / ANKKI was done in samples from dried blood and corresponding whole blood samples. The genotyping from dried blood was in accordance with the whole blood, as also reported earlier^{6,9}. The storage of these discs at room temperature for 6 months showed no effect in the genotyping study. However, storage for longer durations may result in unsuccessful amplification in a few samples as reported earlier^{6,9}. The storage time studied is sufficient for transportation of samples from remote areas. This method appears to be suitable for countries like India with varied temperature conditions. Further such studies are required in various research areas to study the feasibility of use of filter paper.

Conclusion

The genotyping of ANKKI/Taq A1 among alcohol & nicotine dependant subjects from remote areas is feasible from dried blood stored at room temperatures for upto a period of six months.

Abbreviations

ANKKI / Taq1A: Ankyrin repeat and kinase domain containing 1 (ANKKI) gene having Taq1A polymorphism.

DNA: Deoxyribonucleic Acid.

RFLP: Restriction fragment length polymorphism.

Authors Contributions

RQ: contributes to conception, design, data collection, analysis & drafting of the manuscript.

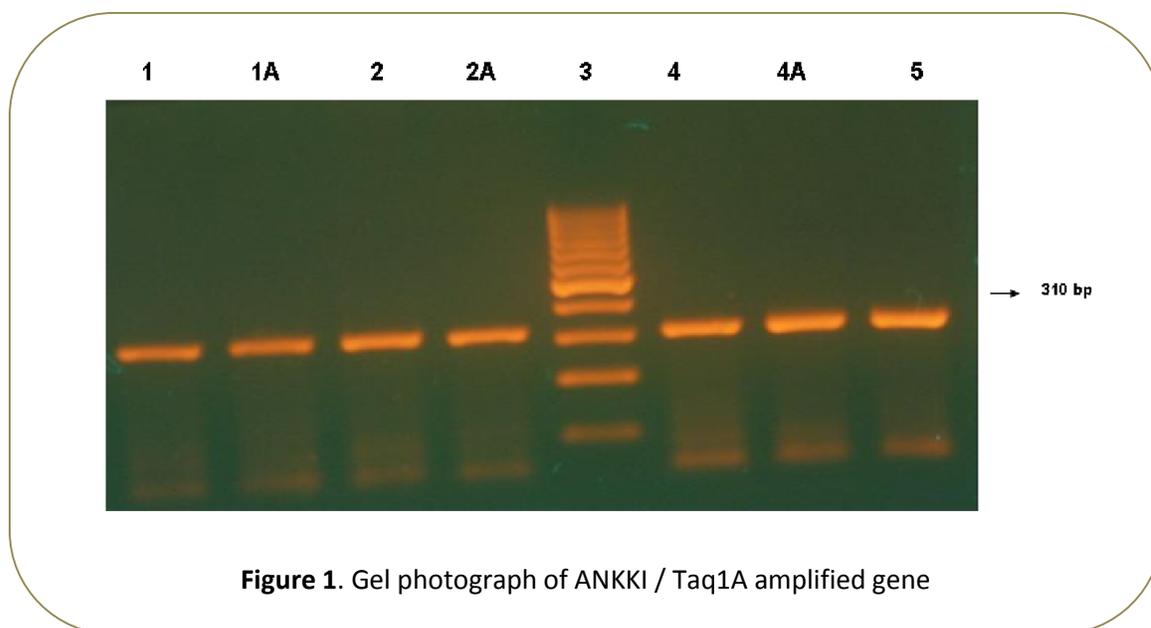
RJ: contributes to conception, and critical review of the manuscript.

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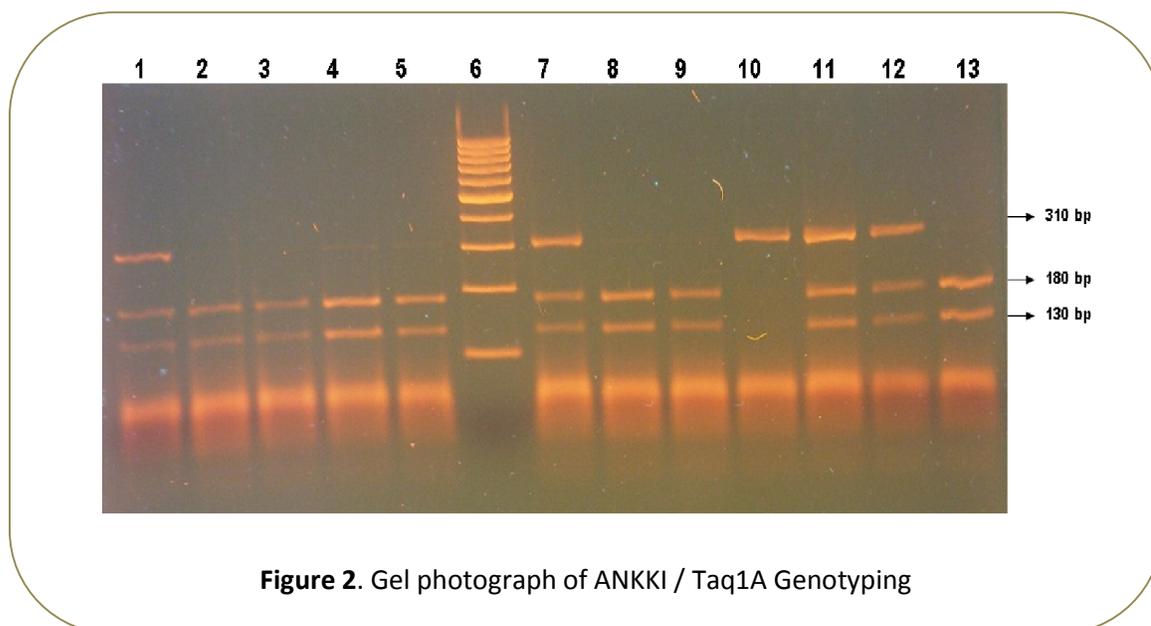
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Agarose gel (2%) showing a 310 bp amplified product of ANKKI / Taq A1 gene.
 Lane 1, 2, 4 & 5: amplified gene from direct blood extracted DNA samples.
 Lane 1A, 2A & 4A: amplified gene from respective dried blood extracted DNA samples.
 Lane 3: 100 bp DNA ladder.



Agarose gel (3 %) showing genotyping by restriction fragment length polymorphism using Taq A1 enzyme.
 Lane 1, 7, 11, 12: A1A2 Genotypes.
 Lane 10: A1A1 Genotype.
 Lane 2, 3, 4, 5, 8, 9, 13: A2A2 Genotypes.
 Lane 6: 100 bp DNA ladder.