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Age prediction using the novel dual sjTREC probe assay

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ABSTRACT

In crime investigation, some 40% of the DNA profiles recovered from the crime scene do not match to DNA profiles in the criminal DNA database. Hence, the ability to predict age from DNA samples provides additional information that would facilitate crime investigations. The present study aimed to develop a correlation model between signal joint T-cell receptor excision circle (sjTREC) quantity and age. A dual-TaqMan® probe qPCR assay was used to quantify sjTREC numbers, normalised against the single copy gene albumin. This novel dual-probe assay showed an increased sensitivity as sjTREC could be amplified in donors up to 67 years old using only 12 ng of DNA with less than 40 PCR cycles. In comparison, literature had cited at least 50 ng of DNA with more than 40 PCR cycles. The age prediction accuracy of ±8.6 years for the Singapore Chinese population was comparable to previous studies. The results also suggested that the trend of sjTREC numbers with age could be different between the Singapore Chinese and Malay populations. However, a larger Malay sample size would be required to ascertain this observation.

1. Introduction

The usefulness of DNA profiling in crime investigation is limited by the availability of reference DNA profiles in the criminal DNA databases. The ability to predict visible characteristics such as eye and hair colour from DNA samples recovered from the crime scene could, therefore, assist the police in the identification of the suspect before he can re-offend or escape the jurisdiction [1]. Age is another important characteristic that could be predicted by quantifying sjTREC numbers via standard qPCR methods. This would make the age prediction assay relatively simple to perform and adopt.

2. Materials and methods

2.1. Primers and probes

The dual-TaqMan® probe assay consisted of one primer set [2] and two dual-quencher sjTREC probes: (1) SLP (FAM-CCT CTG GTT-ZEN TTT GTA AAG GTG CCC AC-IABkFQ) and (2) AZP (FAM-CAG GTG CCT-ZEN ATG CAT CAC CGT G-IABkFQ). Albumin was selected for normalisation of sjTREC quantitation to a single cell. Albumin primers and probe sequences were adapted from Zubakov [3].

2.2. QPCR cycling conditions

QPCR analysis of sjTREC and albumin was performed using the TaqMan® Fast Advance Master Mix on the 7500 Real-Time PCR System with the following thermal cycling conditions: (1) 50 °C for 2 min; (2) 95 °C for 20 s; and (3) 45 cycles of 95 °C for 3 s, 60 °C for 30 s.

2.3. Regression model

The sjTREC/Albumin (S/A) ratio was determined for each sample using the formula: $\left((2^{(\text{sjTREC})C_T})/(2^{(\text{albumin})C_T})\right)^{-1}$. A regression model was established using $\text{Log}_{(S/A)}$ and the age of the sample donor. Standard error of estimate (prediction accuracy) was calculated using the formula: $\sqrt{\Sigma(Y-Y')^2/N}$, where Y is the actual age, Y' is the predicted age and N is the number of donors whose age can be predicted.

3. Results and discussion

3.1. Increased sjTREC qPCR assay sensitivity

This dual-probe sjTREC assay had increased sensitivity over conventional single-probe assays. The C_T values obtained were at

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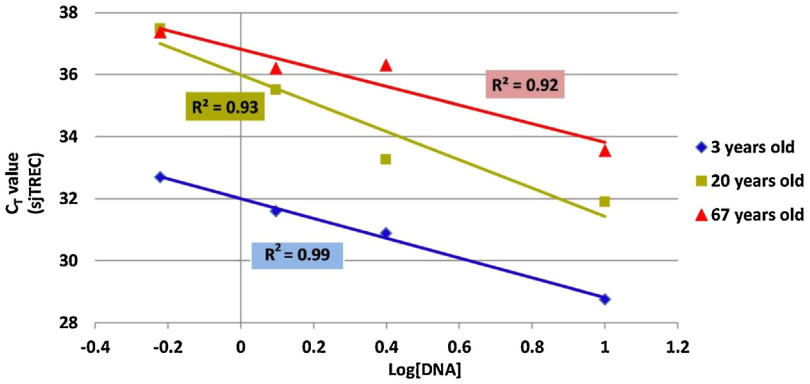

Fig. 1. Linearity study of the sjTREC with three different DNA samples. Four different quantities of DNA (log concentration) in a total reaction volume of 20 μL: (1) 12 ng (−0.22); (2) 25 ng (0.10); (3) 50 ng (0.40); and (4) 200 ng (1.00), were tested with 700 nM primers and 250 nM probes in a duplex qPCR reaction. The R^2 value was determined for each of the different DNA samples used.

least one PCR cycle lower as compared to using the single-probe assays [2–4]. This enhancement of assay sensitivity could be attributed to: (1) the additional sjTREC probe increasing the fluorescence intensity and/or (2) the dual-quencher reducing the background 'noise' level.

3.2. Dynamic range of dual-probe assay

A linearity study was conducted to determine the dynamic range of the assay (Fig. 1). As little as 12 ng of DNA from a 67 years old donor gave positive sjTREC amplification with a C_T value of less than 38 PCR cycles. This suggested that the amount of DNA could be further reduced as theoretically, 3 ng of DNA would yield a C_T value of around 39 cycles. This indicated greater sensitivity of this dual-probe sjTREC assay over previous studies [5], where the authors reported that at least 50 ng of DNA from older donors (70 years old) and no less than 40 PCR cycles were required for positive sjTREC amplification.

3.3. Good correlation between sjTREC quantity and age for the Chinese population

In the present study, regression models with R^2 value of 0.80 and 0.45, respectively, were established for the Singapore Chinese and Malay populations (Fig. 2). A non-linear regression model had a better fit for our data as a linear regression model would under-estimate the age of donors in the age group of 0–4 years old. Furthermore, sjTREC numbers appeared to plateau after 60 years old for the Chinese population. As for the Malay population, a larger cohort of donors beyond the age of 60 years old would be needed to ascertain the plateauing trend.

The prediction accuracy of the regression model for the Chinese and Malay populations, up to 60 years old, was ±8.6 years and ±12.2 years, respectively. The difference observed could be attributed to: (1) the R^2 value obtained, as the Chinese samples appeared to have less variation in S/A ratios as compared to the Malay samples; and (2) the comparatively smaller number of Malay samples. However, when the Chinese regression model was used to predict the age of all the Malay donors, the prediction accuracy was ±14.0 years. This suggested inherent differences in the trend of sjTREC numbers between the different populations in Singapore. Hence, there is a need to further explore this phenomenon using larger sample sizes.

4. Conclusion

The dual-probe sjTREC assay developed for quantifying sjTREC showed increased sensitivity over previous studies while maintaining comparable age prediction accuracy (Singapore Chinese population). The next phase of this study would involve larger cohorts from the Singapore Malay and Indian populations to evaluate the trend of their sjTREC numbers with age. Nonetheless, age prediction accuracy using sjTREC numbers appeared to be limited to around ±8 years; future work towards improvement of

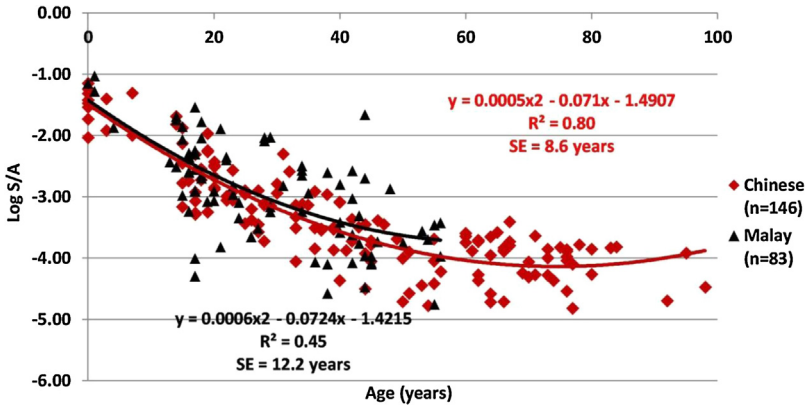

Fig. 2. Quantitative PCR analysis of sjTREC in relation to age. One hundred and forty six DNA samples from the Chinese population (red) and 83 DNA samples from the Malay population (black) were evaluated in the sjTREC and albumin duplex qPCR assay. The S/A ratio was determined for each sample, and plotted against the age of the sample donor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

this sjTREC assay would, therefore, focus on sensitivity to reduce the amount of DNA required.

Ethical standards

The authors have no financial interests to disclose regarding this work.

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