Signal joint T-cell Receptor Rearrangement Excision Circle as a Potential Marker for Age Estimation in a Sample of Egyptians

Thesis

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Omnia Saad

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List of Abbreviations

Abb.	Full term

ΔC_T ..... delta Cycle Threshold

AE Buffer ..... Animal Elution Buffer

AGEs..... Advanced Glycation End products

AL Buffer ..... Animal Lysis Buffer

ATL Buffer ..... Animal Tissue Lysis Buffer

AUC ..... Area Under Curve

AW Buffer ..... Animal Wash Buffer

CD..... Cluster of Differentiation

CI ..... Confidence Interval

DNA..... Deoxyribonucleic acid

GC..... Gluco Corticoids

HSCT..... Hematopoietic stem cell transplantation

HVR.....Hypervariable region

MHC ..... Major histocompatibility complex

NPV..... Negative predictive value

PBMCs..... Peripheral blood mononuclear cells

PPV ..... Positive predictive value

qPCR ..... quantitative polymerase chain reaction

R^2..... R-Squared

List of Abbreviations cont…

Abb.	Full term
ROC	*Receiver operating characteristic curve*
ROS	*Reactive oxygen species*
rpm	*revolutions per minute*
SCID	*Severe combined immunodeficiency*
SDS	*Safety Data Sheet*
SjTREC	*Signal joint T-cell receptor Rearrangement Excision Circle*
TBP	*TATA box binding protein*
TCR	*T-cell receptor*
TREC	*T-Cell Receptor Excision Rearrangement Circles*

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Abstract

Age can be determined very accurately in childhood using developmental methods like dental eruption, dimensions of bones, presence of ossification centers and epiphyseal fusion. However, in adults this methodology is less accurate. For that reason, new biochemical and molecular methodologies have been developed.

Biochemical methods are based on the natural process of aging, which including different biochemical changes that lead to age related alterations in cells and tissues. Some methods that could be used in age estimation, such as aspartic acid racemization, collagen content in human teeth, bone and cartilage and lead accumulation in teeth.

Molecular biology approaches analyzed DNA alterations that occur with aging such as age-dependent accumulation of deletions that occur in mitochondrial DNA, telomere shortening and DNA methylation.

Keywords: *Reactive oxygen species- revolutions per minute - Severe combined immunodeficiency- Safety Data Sheet- TATA box binding protein - T-cell receptor*

INTRODUCTION

Individual age is one of the major factors determining human appearance. Establishing the age of unknown person may provide important leads in police investigations, disaster victim identification, identity fraud cases… etc. ***(Meissner and Ritz-Timme, 2010).***

Currently used methods of age determination rely mostly on physical examination, teeth or skeleton maturation. The prerequisite for using these techniques is the availability of human remains such as teeth, bones or even whole body ***(Zubakov et al., 2010)***.

The development of the molecular methods for age estimation using biological samples that possess no morphological information as blood stains, would be extremely practically valuable as this type of samples commonly seen at the crime scene ***(Ou et al., 2012).***

However, previously proposed genetic indicators for human age estimation, including the mitochondrial deoxyribonucleic acid (mt DNA) 4977 deletion accumulations or telomere shortening, have suffered from low accuracy and

technical problems due to the interference of the environmental, genetic and disease effects ***(Ren et al., 2009)***.

It could be imagined that individual age is too complex to allow only a simple molecular indicator in age estimation from biological materials. Thus, complementary studies on newer age-related indicators are expected to improve the age predicting accuracy with the assistance of methods based on molecular biology ***(Ou et al., 2012).***

The critical role of the thymus in the generation of a diversified population of peripheral T lymphocytes is well-established. Signal joint T-cell receptor Rearrangement Excision Circle (sjTREC) becomes one of the new tools for measuring thymic export ***(Politikos and Boussiotis, 2014).***

The sjTREC is extra-chromosomal DNA by-products of the rearrangements of gene segments encoding the variable parts of T-cell receptor (TCR) chains during intra-thymic development. In this process, the intervening DNA segments in the TCR loci are deleted and the circularized DNA molecules are formed, so-called sjTREC ***(Ringhoffer et al., 2013).***

The content of sjTRECs has been reported to be lower in older people. This may be related to thymic involution (i.e., the

shrinking of the thymus) and consequent thymic function loss, which begins shortly after birth and increases with age. Therefore, the sjTREC content per total T cell content or with respect to the level of a constant gene is expected to decrease with age (***Cho et al., 2014).***

AIM OF THE WORK

The aim of this study is to investigate the levels of T lymphocytes' (sjTREC) in fresh and stored blood stains obtained from peripheral blood in Egyptians' sample of different age groups, in order to asses the role of sjTREC as a potential molecular marker for age determination in forensic practice.