QUANTIFICATION OF SPLICE VARIANTS USING REAL-TIME PCR AND ITS APPLICATION IN THE STUDY OF DISEASES

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INTRODUCTION

Alternative splicing is a complex post-transcriptional mechanism which generates different mRNA isoforms allowing large diversity of proteins synthesised from a small number of genes.

Alteration of splicing events can cause or modify human disease, so a reliable method to measure the expression levels of splice variants is essential. Real-time PCR is the most sensitive method for RNA quantification developed to date.

Our aim is to apply Real-Time PCR technology to quantify the different splice variants generated by Major Histocompatibility Complex (MHC) class III region genes, potentially implicated in diseases.

We are now focusing in studying *NFKBIL1 (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1)* gene, which is a susceptibility gene for rheumatoid arthritis.

THE HUMAN MHC CLASS III REGION

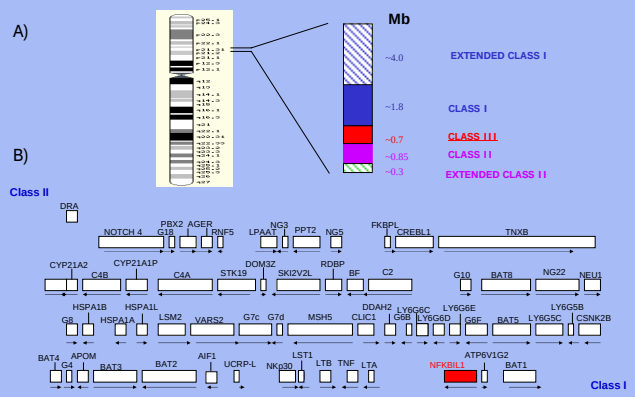

FIGURE 1. A) The human MHC located on chromosome 6p21.3. B) Schematic representation of the MHC class III region genes. In red is shown the gene under study (*NFKBIL1*).

DETECTION OF THE TWO *NFKBIL1* ISOFORMS BY REAL-TIME PCR

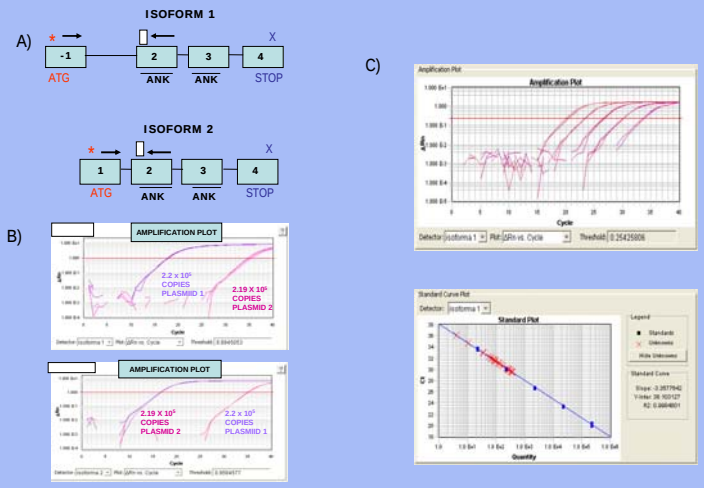

FIGURE 2. A) Location of TaqMan probe (white rectangle) and primers (arrows) for assay developed to differentiate *NFKBIL1* isoforms. B) Primer pairs were tested for cross-amplification with cloned cDNAS. (C) Standard dilutions were performed with the cloned cDNAs for absolute quantifications.

EXPRESSION OF *NFKBIL1* IN HUMAN CELL LINES AND TISSUES

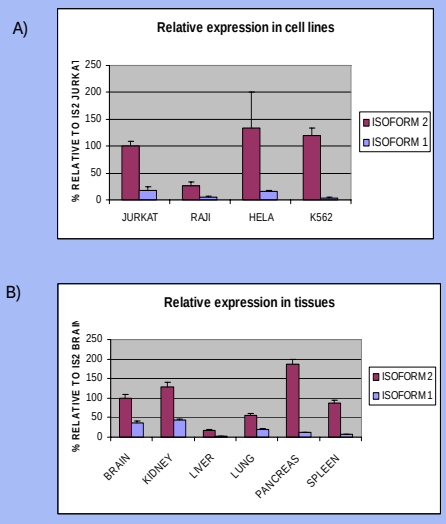

FIGURE 3. Relative expression of *NFKBIL1* isoforms in different A) cell lines and B) tissues.

RNA ANALYSIS FROM BLOOD SAMPLES

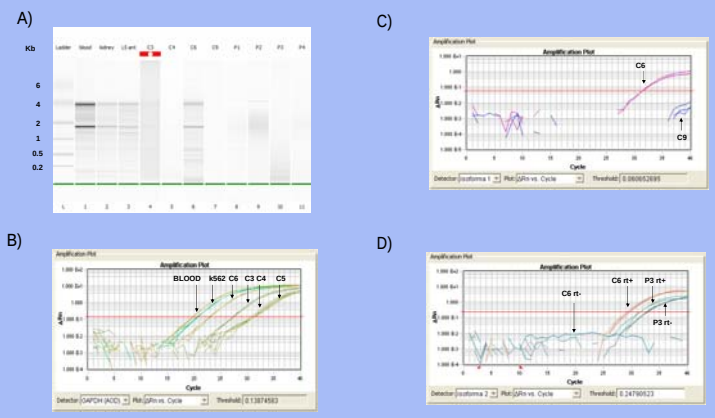

FIGURE 4. A) Results from Bioanalyzer indicating degradation of some RNAs. B) Heterogeneity of GAPDH amplification in different blood RNA samples. (C) NFKBIL1 isoform1 assay showing lack of amplification of some blood samples due to RNA degradation. (D) NFKBIL1 isoform 2 Real-Time PCR observing similar amplifications in RT+ and RT- from RNA of sample P3, but not from C6. C: blood RNA from control individuals, P: blood RNA from patiens with rheumatoid arthritis, LS: synovial fluid from patients, BLOOD, KIDNEY and K562: comercial RNAs.

CONCLUSIONS

•We have designed a Real-Time PCR assay which differentiates the two NFKBIL1 mRNA isoforms.

• The NFKBIL1 isoform 2 is the most abundantly expressed in all the samples studied.

•The RNA Bioanalyzer analysis demonstrates degradation of RNA from some blood samples and the amplification of GAPDH heterogeneity, so the quantifications of *NFKBIL1* isoforms are not reliable in those RNA samples.

REFERENCES

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