Identification of short-length oligonucleotides biomarker for canine species detection using mitochondrial cytochrome b gene

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ABSTRACT

Introduction: Stray dogs are still available in certain countries without any offered price and made it as a potential source for adulteration with costly meats for more benefit. Furthermore, human forensic evidences from crime scenes were often integrated with biomaterial of canine origin. Most of the DNA based assay for canine species detection used longer amplicon size (>150 bp) which are not suitable for highly degraded food or forensic sample analysis. Therefore, in this study for development of short length canine specific biomarker, mitochondrial cytochrome b (cytb) gene was targeted using simple PCR assay.

Objective: Detection of canine species using short length DNA biomarker targeting cytb gene.

Methods: The assay targeted a 100-bp fragment of cytochrome b gene using a pair of canine specific primers. The primers specificity were tested under Insilico, as well as in real PCR assay using dog and eight other species DNAs. The consensus 100 bp canine specific site along with cytb sequences of 14 species including dog and human were used for analysis of pair wise distances, construct dendogram and primers mismatch calculation. The stability of the biomarker was tested under commonly used cooking condition and extensive autoclaving state which was known for degradation of target DNA. The sensitivity of the assay was tested using binary admixture composed of dog and most consumed chicken DNA pool.

Results & Discussion: The biomarker was 100% canine specific and successfully amplified 100 bp region of canine cytb gene specific target. It was highly stable and sensitive enough to detect as low as 0.1% (0.02 ng) of canine specific target from admixed DNAs.

Conclusion: The primers provided the shortest DNA biomarker for canine species detection. The shortest amplicon length, high stability and sensitivity offered its potentiality for canine biomaterials determination from food as well as from degraded samples.

Circulating microRNA as biomarkers for young dyslipidemia men

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ABSTRACT

Introduction: Cardiovascular diseases (CVD) are the major cause of morbidity and mortality in Malaysia, and dyslipidemia is a known contributing factor to the development of CVD. MicroRNAs (miRNAs) have been identified as potent post–transcriptional regulators of lipid metabolism, making it an important area of research to be explored in lipid disorders. Until today, no published data was reported on circulating miRNA among young men with dyslipidemia.

Objective: To determine the differentially expressed miRNA between with dyslipidemia and nondyslipidemia in young men.

Methods: Fourteen young males (32 ± 0.45 years) were recruited at UKMMC after obtaining written consent and were divided into dyslipidemia and non–dyslipidemia. Mean values for TC and LDL were significantly higher in dyslipidemia men compared to non–dyslipidemia (4.20 ± 0.8 mg/dl vs. 3.24 ± 0.33 mg/dl and 5.9 ± 0.67 mg/dl vs. 4.9 ± 0.31 mg/dl) (p<0.05), respectively. TG and HDL levels were not significant between both groups. Ten ml of blood was collected for total RNA extraction which then subjected to quantitative PCR using miRCURY LNA™ Universal RT microRNA PCR Serum/Plasma Focus Panel. All data was analysed using GenEx software (reference gene: miR-423-5p) and SPSS v.16 with p<0.05 was considered significant.

Results & Discussion: Our results revealed six miRNAs were significantly upregulated and two were downregulated in dyslipidemia men compared to non–dyslipidemia (p<0.05). miR–144 is the most significant upregulated miRNA. This miRNA plays an important role in lipid metabolism as it targeted Liver X Receptor (LXR), an established key modulators of lipid metabolism. Eventually, it led to accumulation of cholesterol and attenuated cholesterol efflux by suppressing ABCA1 expression. ABCA1 is known to facilitate the efflux of cholesterol and phospholipids to lipid–poor lipoproteins.

Conclusion: The study has successfully discovered a list of miRNA in circulating blood of young men with dyslipidemia. Further validation of these candidate miRNA is required before they are used as biomarkers for the current health problem in our community.