ABSTRACT

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Title of diploma thesis: Alpha-1-antitrypsin deficiency analysis using real-time PCR

This diploma thesis focuses on the validation of the method PCR in real-time (real-time PCR) to investigate the Z and S mutations in the gene SERPINA1, located at the long arm of chromosome 14 (14q32.13) and provides instructions for making a protein named alpha-1-antitrypsin (A1AT). A1AT is a serine protease inhibitor that protects tissues from degradation by neutrophil elastase. Deficiency, which is most commonly caused by these mutations, can cause children and adult´s liver and lung disease. A method of real-time PCR was applied after successful validation to a set of 46 clinical samples of DNA extracted from blood samples and 30 samples of the DNA from cells of the buccal mucosa. DNA isolation was performed by kit extraction QIAamp ® DNA Mini Kit by QIAGEN. I used a set of primers and hybridization probes according to *Snyder (2006)* for genotyping. Melting curve analysis was carried out in the thermocycler LightCycler 1.2. Results of DNA samples obtained from blood were compared with the results obtained through an accredited method based on the principle of PCR/RFLP. Both methods gave a 100% identical result. In these samples were determined frequencies 14 % of the Z allele and 86 % of the wt allele. The frequency of the S allele was zero. In samples of DNA extracted from buccal swabs were found frequencies 3 % of the Z allele and 97% of the wt allele. The frequency of the S allele in this group was zero.