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Ph.D. Thesis Title: "miRNA as disease modifier in HLA-DQ2/DQ8 matched first-degree relatives of patients with celiac disease"

Abstract

Background: Celiac disease (CD) is an autoimmune enteropathy arising in genetically susceptible individuals exposed to gluten, which activates both innate and adaptive immunity. First-degree relatives (FDRs) of patients with celiac disease (CD) have a lifetime risk of 10% for seroconversion. This study aimed to investigate the role of microRNAs (miRNAs) in the seroconversion of FDR of CD patients who have similar HLA DQ2/DQ8 haplotypes. Differentially expressed miRNAs were evaluated for its role as potential early diagnostic circulatory biomarker for seroconversion in FDRs.

Methodology: Total 247 CD patients FDRs were prospectively screened for anti-tTG antibody. Fresh frozen D2/3 biopsy samples from 5 seropositive FDRs, 3 seronegative FDRs, and 3 disease controls were subjected to deep RNA sequencing (test cohort). The findings were validated using quantitative polymerase chain reaction (qPCR) in the same Test cohort (n=11, biopsy tissue). Differentially expressed microRNAs were further validated in both tissue biopsy and in serum using quantitative real-time polymerase chain reaction (qRT-PCR). The validation cohort includes separate seropositive (n=10) FDR, seronegative (n=17) FDRs and disease controls (n=6). The target genes of these altered miRNAs were identified bioinformatically and validated in tissue biopsy by qRT-PCR. Gene ontology and pathway enrichment analyses were performed using the Database for Annotation, Visualization, and

Integrated Discovery (DAVID). Differential potential for miRNAs both in tissues and sera were evaluated using area under receiver operating characteristic (AUROC) curve analysis.

Results: Next-generation miRNA-sequencing analysis of tissue samples identified 20 miRNAs those are differentially expressed (16 up- and 4 downregulated, log fold change $\geq \pm 2$) between seropositive and seronegative FDRs with false discovery rate P values < 0.05. Furthermore, a comparison of miRNA expression profiles between seropositive FDRs and disease controls revealed differential expression patterns for 10 miRNAs, while a comparison of seronegative FDRs and disease control revealed 14 differentially expressed miRNAs. In validation cohort (n=33), differential expression of miR-16-1, miR-23a, miR-502, miR-15b, miR-125b and miR-195b, found significant in tissue biopsy. Further our qPCR serum data showed significant miRNA expression in miR-193 and miR-941.

BACH2 gene, is an important regulator of Treg and effector T cells, was significantly down-regulated ($P < 0.02$) in biopsies, while target genes from other differentially expressed miRNAs were not significantly altered in biopsies. Correlation analyses between miR15b and BACH2 expression indicated a significant inverse correlation (r value of -0.394 and P 0.041). In ROC curve analysis, two miRNAs (miR-502 and miR-15b) expression can differentiate seropositive FDRs vs seronegative FDRs, whereas six miRNAs (miR-16-1, miR-16-2, miR-23a, miR-125, miR-193, miR-941) can differentiate seropositive FDRs vs Disease Controls.

Conclusion: High-throughput small RNA sequencing identified miRNAs which have potential to differentiate anti-tTG seropositive vs seronegative FDRs. Target gene identification of these miRNAs and functional gene enrichment analysis provides valuable information on the non-HLA gene-mediated pathogenesis of CD and seroconversion in CD patients FDRs. These findings require validation in a larger multicentre cohort before these miRNAs can be used as non-invasive biomarkers for seroconversion and posttreatment response monitoring.