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# HUMORAL BETA-CELL AUTOIMMUNITY, AGER GENE POLYMORPHISM, AND CIRCULATING SOLUBLE RAGE IN PRE-CLINICAL AND CLINICAL TYPE 1 DIABETES

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ACADEMIC DISSERTATION

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# ABSTRACT

Type 1 diabetes is a disease characterized by a preclinical phase with autoimmunity against beta cells of the pancreas and eventually hyperglycemia and dependence on external insulin treatment. Finland has the highest annual incidence of the disease worldwide. Incidence has been increasing over the decades at an accelerated rate, although the incidence rates from the past few years suggest that a peak may have been reached. The reason for the increase is unknown. Children and adolescents with significantly increased risk for type 1 diabetes can be recognized from the general population based on HLA-genotypes associated with increased risk for type 1 diabetes and using a panel of beta-cell autoantigen-specific antibody assays. However, the initial trigger for the autoimmune process remains obscure. Recently, a novel autoantibody specificity was discovered, the zinc transporter 8 antibodies (ZnT8A).

The advanced glycation end products (AGEs) are produced by a non-enzymatic glycation reaction between reducing sugars and proteins or lipids. AGEs and their interaction with the receptor molecule, receptor for advanced glycation end products (RAGE), have been suggested to play a role in the pathogenesis and complications of both type 1 and type 2 diabetes. The soluble form of RAGE (sRAGE) counteracts the effects of the proinflammatory membrane-bound RAGE. The circulating concentration of sRAGE is associated with various disease states. An initial low sRAGE might be a risk factor for inflammatory and metabolic disease, and a decrease in sRAGE has been observed in various studies on acute inflammation, both autoimmune and caused by other factors. In contrast, higher than normal sRAGE concentrations have also been observed in pathological states, especially in diabetic populations and subjects with compromised renal function. Polymorphisms of the gene encoding RAGE, *AGER*, are associated with the risk for diabetes and the complications of diabetes according to numerous studies.

The first study of this thesis focused on the role of ZnT8A positivity in the phenotype and the prevalence of ZnT8A in newly diagnosed patients with type 1 diabetes. In concordance with previous studies, 63% of newly diagnosed Finnish children and adolescents with type 1 diabetes had ZnT8A. Positivity for ZnT8A was related to age and metabolic state at diagnosis as well as to HLA genotype. ZnT8A-assay did not significantly improve the detection rate of beta-cell autoimmunity in the current study population when used in addition to previously available autoantibody analyses since only 0.3% of the subjects had ZnT8A as their single autoantibody.

The objective of Studies II, III, and IV was to identify possible roles for RAGE and AGEs in the clinical phenotype of newly diagnosed patients with type 1 diabetes and the emergence of humoral autoimmunity against islet cell antigens during the preclinical phase. In the second publication, we defined the relationship between humoral beta-cell autoimmunity, HLA-genotype, state of metabolic compensation, polymorphisms of the AGER gene, and concentrations of sRAGE in 2115 children with newly diagnosed type 1 diabetes. Children who did not have type 1 diabetes and tested negative for beta-cell autoantibodies were used as controls. In the third part of the thesis, we analyzed serum concentrations and sRAGE from 114 children who progressed to type 1 diabetes during prospective observation in the Diabetes Prediction and Prevention study (DIPP-study). Concentrations of an abundant AGE, carboxymethyllysine (CML), were analyzed as well. We assessed the dynamics of sRAGE during the disease process leading to type 1 diabetes also in the fourth publication by measuring sRAGE before and after the appearance of beta-cell-specific autoantibodies and annually after the seroconversion, until the diagnosis or end of follow-up. In Study IV, the study population included 211 children with permanent positivity for at least two autoantibodies.

The results of this thesis suggest that AGEs and RAGE seem to have a role in the pathogenesis of type 1 diabetes. Concentrations of soluble RAGE are positively associated with age in children with newly diagnosed type 1 diabetes but not in the control population. The aggressiveness of humoral beta-cell autoimmunity does not correlate with sRAGE concentrations, but the state of metabolic decompensation at diagnosis and the HLA-genotype associated with the highest risk of type 1 diabetes, the DR3/DR4 heterozygosity, were related to lower sRAGE concentrations. Interestingly, two polymorphic variants of the *AGER* gene associated with increased risk for type 1 diabetes were associated with lower levels of sRAGE as well.

Prediabetic children seem to have higher circulating concentrations of sRAGE than autoantibody-negative controls. A reduction in the circulating sRAGE concentrations coincides with the appearance of diabetes-predictive autoantibodies in children progressing to overt type 1 diabetes, but not in healthy controls. We could not see a similar drop in sRAGE in children seroconverting to autoantibody positivity later in childhood. After the seroconversion, the sRAGE concentrations remained stable in both groups. The CML concentrations were similar between cases and controls in the samples taken before the appearance of autoantibodies against beta cells in the prediabetic children, but the RAGE/AGE ratio was higher in the cases than in the controls.

To conclude, sRAGE, which has been considered cytoprotective in previous studies, is positively associated with older age at disease onset,

protection from metabolic decompensation at diagnosis, and *AGER* genotypes with a lower risk for type 1 diabetes. Children who seroconvert to humoral islet cell autoimmunity early in childhood experience a drop in sRAGE concentration coinciding with the appearance of the first autoantibodies. They have higher sRAGE concentrations and sRAGE/AGE ratio than the controls before seroconversion. These observed associations might be a result of an intrinsic protective mechanism that fails at seroconversion.

Keywords: Type 1 diabetes, prediabetes, AGEs, RAGE, ZnT8A