Poster of Distinction

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THE ROLE OF A HOMOZYGOUS PROTEIN CODING VARIANT OF EPS8 IN THE PATHOGENESIS OF PEDIATRIC IBD

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Background: The rate of onset for Inflammatory Bowel Disease (IBD) is rising worldwide—most significantly in the pediatric population. The pathogenesis of the disease involves a complicated interaction between the environment and genetics. Recent findings suggest that there is a broad range of rare, single-gene mutations that correlate with the IBD phenotype in children. Some of these single-gene defects can disrupt the epithelial barrier, which alters intestinal immune homeostasis. Epidermal Growth Factor Receptor Kinase Substrate 8 (EPS8) is crucial for the stabilization of F-actin by capping and bundling the barbed end. Previous literature shows that loss of expression of EPS8 disrupts the polymerization of F-actin in intestinal microvilli resulting in shortened brush borders. We identified a pediatric IBD patient with a homozygous missense *EPS8* mutation(c.2099C>T, I700T) in the actin-binding domain. The patient presented with pancolitis, colonic strictures and other IBD-like symptoms at 6 weeks of age. Currently, the functional role of EPS8 in the pathogenesis of very early-onset IBD remains elusive.
Aims: 1)To investigate if the patient with EPS8 mutation has microvilli disorganization.
2)To determine EPS8 localization in patient samples and cell models.
3)To clarify the mechanism of how the EPS8 mutation affects F-actin *in vitro.*

Ultimately, we want to clarify how the mutation of EPS8 might contribute to the onset of pediatric IBD.
Methods: A rare, damaging mutation in *EPS8* was identified in a very early-onset IBD patient by Whole Exome Sequencing. From patient-derived colon biopsy samples, the localization of EPS8 and actin was visualized by immunofluorescence (IF) microscopy. The morphology of the patient's intestinal microvilli was assessed

using transmission electron microscopy (TEM). EPS8 expression was measured using western blot. An F-actin polymerization assay was performed comparing purified WT and mutant EPS8 protein to assess the rate of polymerization from monomeric G-actin to F-actin.
Results: IF microscopy of the colonic sections revealed lower co-localization of EPS8 and actin on the apical surfaces, compared to IBD and normal controls. Closer examination of the cell structure using TEM showed disruption of the microvilli in the EPS8 mutant, but not in IBD or normal controls. Western blots showed no differences in protein expression between wildtype and mutant EPS8 in HEK293T cells. F-actin polymerization assay revealed differences in the rate of G-actin to F-actin polymerization between wildtype and mutant EPS8.
Conclusions: The patient with EPS8 mutation had microvilli disorganization. EPS8 localized differently in the patient colon tissues compared to normal and IBD control samples. The EPS8 mutation may affect F-actin polymerization, which may lead to disruption of the microvilli.

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