ENDOGENOUS RETROVIRAL DYSREGULATED EXPRESSION IN PEDIATRIC CROHN'S DISEASE

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BACKGROUND

Human Endogenous Retroviruses (HERVs) comprise 8% of the modern human genome and their dysregulated expression is linked to inflammation (1). Aberrant HERV expression was recognized in adult patients with Crohn's Disease (CD) (2,3). We aimed to investigate the expression of HERVs in the ileal mucosa of children with CD compared to healthy children and its correlation to the expression of genes involved in mucosal inflammation.

METHODS

We used online available high throughput RNA-sequencing data of ileal biopsies of 26 children with CD and of 8 age-matched healthy controls. We used Bowtie2 (4) for the alignment against the hg19 human genome assembly; we used Samtools (5) and Bedtools (6) to retrieve read counts corresponding to 13 HERV families (HERV-H, HERV-W, HERV-L, HERV-E, HERV-I, HERV-9, HERV-FRD, HML-1/2/3/4/5/6) and a panel of 20 mucosal immunity genes related to chemotaxis (CXCL10, CXCL11, CXCL13, CXCL16, CCL21, CXCR3, TLR4, SPAK) and cellular adhesion (PECAM1, ICAM1, ICAM2, ICAM3, VCAM1, ESAM, MADCAM1, SELP, SELL, SELE, ADAM10, ADAM17). We evaluated HERV expression at a family level by summing the reads corresponding to each HERV family. HERV and immunity gene expression was normalized by means of four housekeeping genes (SDHA, HPRT1, RBX1, RRAGA). Statistical Analysis: Normalized expressions were ln-transformed and we used independent sample t-tests, to compare the expression of HERV families and immunity genes in the ileal biopsies of children with CD and controls. Spearman's rho correlation coefficient was used to identify correlations between the expression of differentially expressed HERVs and immunity genes.

Dysregulated expression of *HERVs* in children with *CD*, correlated to the expression levels of genes involved in the *perpetuation of mucosal inflammation*.

RESULTS

We recognized upregulated expression of HERV-FRD (1.54-fold, p=0.031) and downregulated expression of HERV-9 (0.52-fold, p=0.013) in children with CD (**Figure 1**). Of the examined immunity genes, we found significant upregulation in the expression of CXCL-10 (5.9-fold, p<0.001), CXCL-11 (4.3-fold, p=0.001), CXCL-16 (1.64-fold, p=0.002), SPAK (1.4-fold, p=0.014), ADAM-10 (1.5-fold, p=0.047), TLR-4 (2.1-fold, p=0.036) in CD. HERV-FRD expression correlated to the expression of SPAK (Rho=0.68, p<0.001) and ADAM-10 (Rho=0.411, p=0.037) **(Figure 2)**, while HERV-9 expression correlated to the expression of CXCL-10 (Rho=-0.496, p=0.001), CXCL-11 (Rho=-0.5, p=0.009), TLR-4 (Rho=-0.5, p=0.008) and SELE ((Rho=-0.596, p<0.001) **(Figure 3).**

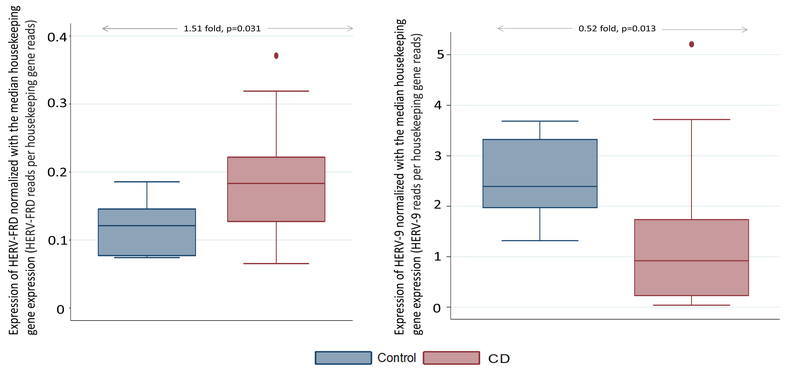


Figure 1: Differentially expressed HERVs in the terminal ileum of children with CD compared to healthy controls.

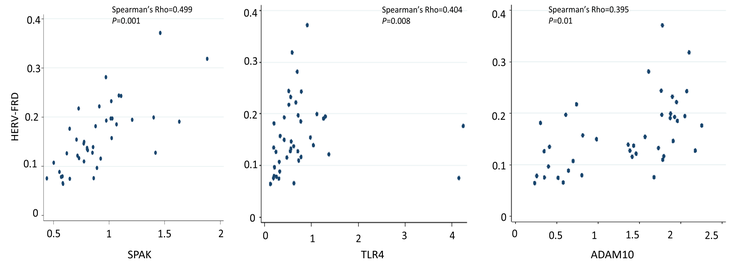


Figure 2: Positive correlation between the expression of HERV-FRD and SPAK, TLR4 and ADAM10 in the terminal ileum. All expressions are normalized by means of four housekeeping genes (reads per housekeeping genes reads).

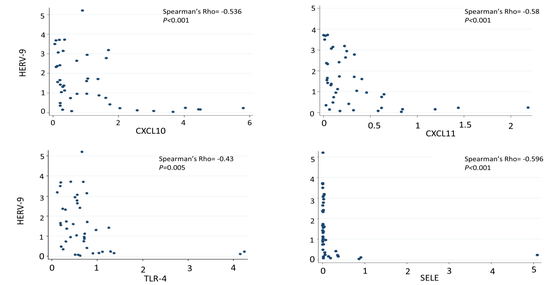


Figure 3: Negative correlation between the expression of HERV-9 and CCL10, CCL11, TLR4 and SELE in the terminal ileum. All expressions are normalized by means of four housekeeping genes (reads per housekeeping genes reads).

CONCLUSIONS

We recognized dysregulated expression of HERV-FRD and HERV-9 in children with CD, correlated to the expression of genes involved in proinflammatory processes in ileal mucosa and the perpetuation of mucosal inflammation. These findings merit further research on the HERV role in intestinal mucosa fitness to confirm the HERV involvement in pediatric IBD.

ADDITIONAL KEY INFORMATION

Data Availability: The data used in this study are available at the NCBI Gene Expression Omnibus (Edgar, Domrachev and Lash, 2002) under the accession number GSE57945 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57945). (Haberman et al., 2014, 2018).

References: 1. Hancks and Kazazian Curr Opin Genet Dev 2012, 2. Klag et al Inflamm Intest Dis 2019, 3. Kakuta e al Sci Rep 2020, 4. Langmead and Salzberg Nat Methods 2012, 5. Li Bioinformatics 2009, 6. Quinlan and Hall Bioinformatics 2010.

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Funding: This study was supported through a donation of SYN-ENOSIS, the Greek Shipowners' Social Welfare Company.