Preclinical and clinical evidence of epigenetic regulation of cannabinoid receptor type 1 in anorexia nervosa and effects of *Lactobacillus planatrum* IMC513 probiotic treatment in the genetic animal model

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Anorexia nervosa (AN) is an eating disorder with the highest mortality rate among all psychiatric disorders, with underlying joint contribution of genetic and environmental factors. Metagenomic studies have revealed profound microbiome perturbations in AN, that appear to be associated with extreme weight loss, energy metabolism and emotional imbalance (anxiety and depression). Modulation of intestinal microbiota through probiotic treatment hence represents a promising intervention strategy for AN. The aims of this study were: (1) to investigate the epigenetic regulation of the endocannabinoid system (ECS), proven for the regulation of feeding behaviour and impaired signaling in AN, in a mice genetic model as well as in human subjects; (2) evaluate the significance of *Lactobacillus plantarum* IMC513 probiotic treatment in the animal model. The *anx/anx* mouse is a unique spontaneous genetic model for some of the core features of AN, such as elective starvation and emaciation, where animals homozygous for the *anx* mutation eat significantly less than their wild type (wt) littermates, leading to premature death. We observed that body weight loss was reduced in both *anx/anx* and wt animals upon probiotic administration, even if not enough to rescue anorectic animals from dying at 3 weeks postnatal. Among all ECS components, a selective and significant down-regulation of the gene encoding for the cannabinoid receptor type 1 (*Cnr1*) was observed in the prefrontal cortex region of *anx/anx* animals compared to the wt (Mann Whitney test: 0.59 ± 0.05, $P < 0.05$) whereas *L. plantarum* probiotic treatment restored *Cnr1* expression in the *anx/anx* mice (Mann Whitney test: 0.924 ± 0.114). Based on our previous findings on deviations in microbiota taxonomy between the wt and *anx/anx* mice, we are currently assessing bacterial diversity by 16s RNA sequencing in feces upon probiotic administration. We also hypothesize that *L. plantarum* IMC513 impose alterations in the production of short chain fatty acids (SCFAs), the major product of dietary fiber fermentation by enteric microbial community, which remains yet to be evaluated in serum using mass spectrometry. Consistent with animal studies, preliminary results in the saliva of anorectic

individuals also point out relevance of *cnr1* epigenetic regulation. In fact, we observed significant increase in the DNA methylation mark at its promoter region, at the average of all CpG sites under study combined in AN subjects when compared to healthy controls (multiple t-test, P=0.0044) more relevant in those subjects reporting mental health comorbidities (multiple t-test 4^{th} CpG site: P=0.0042; average CpG: P=0.0032) or presence of other environmental cues (multiple t-tests at 4^{th} CpG site: P=0.0004 and P=0.0087; 5^{th} CpG site: P=0.0068, average CpG: P=0.0047 and P=0.0076). In conclusion, this is the first demonstration of selective regulation of *Cnr1* in AN in both animal and human context, with suggestive beneficial effect of probiotic strain *L. plantarum* IMC513. Our data thus might open avenue to environmental strategies of intervention, also due to the reversible nature of the epigenetic hallmark at *Cnr1*, suggesting epigenetic modulation of *Cnr1* as a biomarker for AN development.