

Background

163 genetic variances (SNPs) have been found to be associated with IBD. Jostins *et al.* concluded that the majority of significant IBD associated loci are involved in 'regulation of cytokine production' including IFN- γ , TNF α and IL-10. The effect of DNA variants in these regulatory loci can best be studied in stimulated cells, determining interactions between genetic predisposition and exposure to environmental factors. We aimed to assess functional implication of IBD-related SNPs by analyzing cytokine expression in peripheral blood of IBD patients.

Methods

In 40 CD patients visiting the out-patient IBD clinic, genotyping was performed by a customized GWAS chip (ImmunoChip) including all IBD-related SNPs. Whole blood was stimulated with α CD3/ α CD28 antibodies and LPS to stimulate naïve CD4 cell- and monocyte fractions, respectively. Twenty-eight cytokines were measured in supernatant by Luminex assay. The clinical phenotype was documented. For statistics, the ANOVA test was performed on cytokine induction fold-changes (FC).

Results

Cytokine levels are differently expressed when stratifying IBD patients for their IBD-associated risk alleles (RA).

We focused on analyzing SNPs associated with TNFSF members, hereby providing the TNFSF15/TL1A SNP related to expression of IL-10, TNF α and IFN- γ .

29 patients carried one or more RA ('G') for this SNP, and showed significantly less IL-10 release following a 24-hour LPS stimulation (Fig 1). Also, upregulation of pro-inflammatory TNF α was suppressed in these RA carriers, both at 4 hours and at 24 hours (Fig 2). In this graph, all patients being treated with anti-TNF agents are depicted as red dots. Both with and without α CD3/ α CD28 stimulation there was a trend of higher IFN- γ production when carrying more RA, albeit not significant (Fig 3).

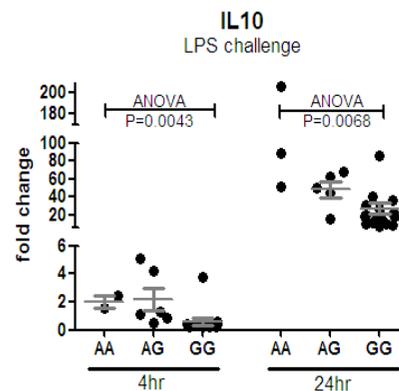


Fig 1. FC \pm s.d.

	after 4hr:	after 24hr:
AA	2.0 \pm 0.6	115.2 \pm 81.2
AG	2.2 \pm 2.0	48.0 \pm 20.6
GG	0.6 \pm 0.9	26.9 \pm 31.5

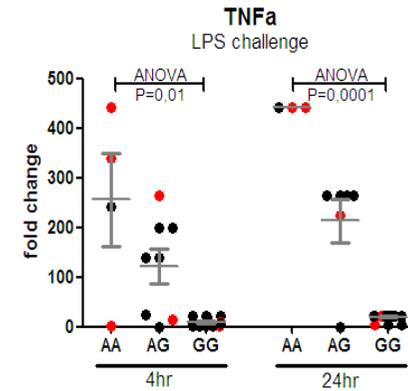


Fig 2. FC \pm s.d.

	after 4hrs:	after 24hrs:
AA	255.5 \pm 188.3	441.4 \pm 0
AG	122.3 \pm 99.1	213.1 \pm 105.6
GG	9.0 \pm 7.5	20.1 \pm 6.1

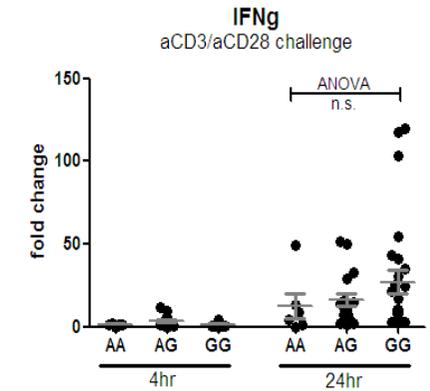


Fig 3.

Discussion

Following the proposed concept of genetic variance relating to cytokine regulation, we were able to stratify patients for presence of RA and report differently expressed cytokines after mimicking the human immune response by performing whole blood stimulation assays *in vitro*. Despite reported inter-individual variation in the innate immune reactivity, we found significant patterns of genetic variance correlating to a modified immunological response. TL1A is important in Th1-

regulation, and thus a gene of interest to study in relation to CD and cytokine expression. By performing further analyses on this data by including more cytokines as well as validating results by qPCR, we hope to find what immunological pathways are affected by such genetic variation. Further research should also focus on single cell studies, to assess their role in the specific immunological processes underlying the complex nature of IBD.