The association between transcriptional regulation of macrophage differentiation and activation and genetic susceptibility to inflammatory bowel disease

Claire L O'Brien*[1,2], Kim M Summers*[3], Natalia M Martin[2], Dylan Carter-Cusack[3], Rasel Barua[2], Ojas VA Dixit[1], David A Hume*[3], Paul Pavli*[2,4]

[1]Centre for Research in Therapeutics Solutions, Faculty of Science and Technology, University of Canberra, Canberra, Australian Capital Territory, Australia.

[2]Inflammatory Bowel Disease Research group, Canberra Hospital, Canberra, Australian Capital Territory, Australia.

[3]Mater Research Institute-University of Queensland, Translational Research Institute, Brisbane, Australia.

[4]Gastroenterology Unit, Canberra Hospital, Canberra, Australian Capital Territory, Australia.

* These authors contributed equally

Correspondence to:

Paul.Pavli@anu.edu.au

David.Hume@uq.edu.au

Abstract

The abundant macrophage population of the intestinal lamina propria turns over rapidly and is replaced by blood monocytes. The differentiation and survival of resident intestinal macrophages depends upon signals from the macrophage colony-stimulating factor receptor (CSF1R). The response of human monocyte-derived macrophages (MDM) grown in macrophage colony-stimulating factor (CSF1) to bacterial lipopolysaccharide (LPS) has been proposed as a model for the differentiation and adaptation of monocytes entering the intestinal lamina propria. We hypothesized that dysregulation of this response leads to susceptibility to chronic inflammatory bowel disease (IBD). To address this hypothesis we analyzed transcriptomic variation in MDM from affected and unaffected sib pairs/trios from 22 IBD families and 6 healthy controls. There was no overall or inter-sib distinction between affected and unaffected individuals in basal gene expression or the stereotypical time course of the response to LPS. However, the basal or LPS-inducible expression of individual genes including inflammatory cytokines and many associated with IBD susceptibility in genome-wide association studies (GWAS) varied by as much as 100-fold between subjects. Extreme independent variation in the expression of pairs of HLA-associated transcripts (*HLA-B/C, HLA-A/F* and *HLA-DRB1/DRB5*) was associated with HLA genotype providing a novel explanation for the HLA association with disease susceptibility. The relationship between single nucleotide variant (SNV) genotype and gene expression at other loci was weaker and inconsistent suggesting that much of the variation arises from the integration of multiple *trans*-acting effects. For example, expression of *IL1B* at 2 hrs of LPS treatment was significantly associated with local SNV genotype and with peak expression of IL23A at 7 hrs. By contrast, there was no evidence of association between peak *IL6* mRNA at 7hrs, *IL6*-associated SNV genotype or *IL1B* at 2 hrs. Our results support the view that gene-specific dysregulation in macrophage adaptation to the intestinal milieu provides a plausible explanation for genetic

susceptibility to IBD. The analysis also suggests that the molecular basis of susceptibility is unique to each individual which may contribution to variation in the precise environmental trigger, the consequent pathology and response to treatment.

Author summary

Cells of the innate immune system called macrophages are abundant in the wall of the gut, providing a first line of defense against potential pathogens. These cells must also avoid an inappropriate or excessive response to the abundant microbial population (the microbiome) of the intestine. We have previously proposed that genetic differences between individuals in macrophage adaptation to the unique environment of the intestine underlie genetic susceptibility to inflammatory bowel disease (IBD). In this study we developed a model of the adaptation of macrophages and used that model to identify surprisingly extreme variation in the response amongst a cohort of affected and unaffected siblings in families with IBD. The response did not distinguish affected individuals from their unaffected siblings. Our results support the view that each individual within IBD-susceptible families carries a unique set of genetic variants of large effect that together predispose to uncontrolled gut inflammation in the face of an environmental trigger.

63 **Introduction**

64 The major chronic inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative
65 colitis, clearly have significant heritability. The strongest associations are with the human
66 major histocompatibility gene complex (HLA), contributing up to 30% of shared risk amongst
67 siblings for CD and even greater for ulcerative colitis [1, 2]. In terms of mechanism, there is a
68 clear consensus that IBD arises from an aberrant interaction between cells of the immune
69 system in the intestinal mucosa and the luminal microbiota [3]. Several rare monogenic early-
70 onset forms of IBD have provided some mechanistic insights [4, 5]. Modern population
71 genome-wide association studies (GWAS) have identified hundreds of additional non-HLA
72 variants associated with significantly increased risk [3, 6-8]. Collectively, this genetic variation
73 still explains only 20-30% of the heritability. The missing heritability has been attributed in
74 part to limitations in sample size and other constraints on GWAS and to complex interactions
75 between genes and environment. The genomic intervals identified by GWAS contain multiple
76 genes and few SNVs are likely to be functional. To extend association analysis, whole genome
77 sequencing has been applied to identify rare and/or causal variants within expression
78 quantitative trait loci (eQTL) [9-11]. After sequencing 100 candidate genes in 6600 CD patients
79 and 5500 controls, Momozawa *et al.* [10] concluded that > 10-fold greater sample sizes would
80 be required to demonstrate causality. Accordingly, Sazonovs *et al.* [11] analyzed whole exome
81 or whole genome sequences of >30,000 CD patients and 80,000 controls to implicate coding
82 variation in an additional 10 genes in susceptibility.

83 The strength and weakness of GWAS is the lack of a hypothesis that can focus analysis on a
84 smaller set of genomic loci to obviate the tyranny of appropriate correction for multiple testing.
85 There have been numerous hypothesis-driven candidate gene analyses providing evidence of
86 associations between disease and SNVs associated with inflammatory mediators. For example,
87 Richard *et al.* [12] identified a disease-associated functional regulatory variant linked to

88 reduced expression of *TNFSF15* (TL1A) which has been implicated in gut inflammation.
89 Recently, Klunk *et al.* [13] proposed that the medieval plague epidemic led to selection for
90 variants affecting immune response gene expression. They highlighted a SNV linked to
91 expression of the *ERAP2* gene in macrophages. The same SNV was previously associated with
92 IBD risk [14]
93 The intestinal lamina propria contains an abundant population of resident tissue macrophages
94 which turn over constantly and are replaced by recruitment of blood monocytes. Following
95 their entry to the mucosal niche, monocytes must rapidly modulate their response to the
96 products of microorganisms to avoid inflammatory activation [15, 16]. The differentiation of
97 lamina propria macrophages depends upon macrophage colony-stimulating factor (CSF1) and
98 these cells are rapidly depleted by administration of anti-CSF1R antibody [17]. Accordingly,
99 the differentiation of human monocytes *in vitro* in response to CSF1 and their subsequent
100 response to bacterial agonists such as lipopolysaccharide (LPS) provides a model of
101 differentiation of monocyte-derived macrophages in the ileum and colon [18].
102 Based upon extensive gene expression analysis in monocytes and macrophages produced with
103 the FANTOM5 consortium, loci associated with IBD were strongly and specifically enriched
104 for promoters that were regulated during monocyte differentiation or activation [18].
105 Furthermore, amongst known IBD susceptibility loci, the vast majority contained promoters
106 that were regulated during the CSF1-dependent monocyte-macrophage transition and/or in
107 response to LPS [18, 19]. These analyses also indicated that even amongst the most supported
108 IBD susceptibility loci, genes in the same genomic region as the highlighted candidate are
109 either co-regulated in monocytes (e.g. *SNX20* and *CYLD* with *NOD2*) or provide more
110 plausible candidates (e.g. *INPP5D* within the *ATG16L1* locus) [18].
111 Siblings presumably share some components of both genotype and environment. It has been
112 known for many years that the relative risk of developing CD is greater amongst the siblings

113 of affected individuals. However, for monozygotic twins shared risk is estimated at only 15-
114 30% and concordance even amongst dizygotic twins (who may share a more similar
115 environment compared to siblings) may be <5% [20, 21]. In the current study we have
116 performed gene expression analysis of monocyte-derived macrophages (MDM) and their
117 response to LPS in a cohort of sib-pairs discordant for IBD disease. The extended cultivation
118 of monocytes in CSF1 *in vitro* to produce MDM provides a common environment to reduce
119 possible direct effects of disease status on monocyte function and enrich for detection of effects
120 of genotype. Previous studies have identified allele-associated variation in regulated gene
121 expression in monocytes and monocyte-derived macrophages amongst healthy individuals and
122 associated with autoimmune disease susceptibility [22-26] Network analysis of the monocyte
123 data [22] identified *trans*-acting impacts of variation in expression of *IFNB1* and downstream
124 transcription factors IRF7 and IRF9 [27].
125 The genome of each affected individual with IBD must contain only a subset of the hundreds
126 of disease-associated genetic variants that have been identified in population-based analyses
127 and may also contain private variants of larger effect. If disease onset occurs purely in response
128 to an environmental trigger that unaffected siblings have avoided (which is strongly implicated
129 by the low level of concordance amongst siblings) then the entire cohort of affected individuals
130 and first degree relatives will be strongly enriched for disease-associated variants regardless of
131 disease status. Alternatively, unaffected siblings may be discordant for risk-associated variants
132 and consequently at lower genetic risk of disease. In this study we demonstrate that MDM
133 from both affected and unaffected individuals within families affected by IBD display extreme
134 variation in expression of individual genes and classes of genes that may predispose to
135 dysregulation of the response to microbial challenge and hence affect risk of developing IBD
136 in response to an environmental trigger.
137

138 **Results**

139 Monocyte-derived macrophages were prepared from a total of 56 individuals, including
140 affected and unaffected sib pairs/trios from 22 IBD families and a set of 6 healthy individuals
141 with no known family history of IBD, including one sib-pair. The response of human MDM to
142 LPS is a sequential cascade of transient induction and repression of sets of transcripts [18].
143 Based upon the previous dense time course analysis using Cap analysis of gene expression
144 (CAGE) tag sequencing [18], MDM populations were sampled at zero time, and 2, 7 and 21
145 hours after addition of a maximal effective concentration (100ng/ml) of *Salmonella Minnesota*
146 Re595 LPS, a pure TLR4 agonist [28, 29]. The chosen time points represent the peaks of
147 expression of early, mid and late response genes, also applied in previous comparative analysis
148 of mouse and human responses [28] and multiple other mammalian species [29]. RNA-seq was
149 performed on mRNA extracted at each time point and initially quantified using Kallisto as
150 described previously [29]. The patient metadata is provided in **Table S1A** and the complete
151 dataset organized by individual and family is provided in **Table S1B.**

152 To overview the data, we initially performed a network analysis using Biolayout [30]. A
153 sample-to-sample network shown in **Figure 1A** (left panel) demonstrates that samples from
154 each of the time points tend to group together, with a transition across the network from pre-
155 LPS to 21 hours post-LPS reflecting the progressive temporal profile of the response. This is
156 consistent with the global changes in gene expression elicited in response to LPS [18] but there
157 was no class separation between affected and unaffected individuals (**Figure 1A** (right panel)).
158 **Figure 1B** shows the main element of the gene-to-gene network graph based on the entire
159 dataset, and **Table S2** shows the cluster gene lists alongside the average gene expression
160 profiles of each cluster. **Cluster 1,** the largest, contains 1903 transcripts that were expressed
161 constitutively and relatively invariant across the entire dataset with a small decline with time
162 in response to LPS. This cluster comprises mainly house-keeping genes, but also includes

163 macrophage-enriched transcription factors (*SPI1, CEBPA/B*) and surface markers (*ITGAM,*
164 *ITGAX*). The consistent detection of these transcripts provides a data quality control and a
165 reference point for much greater variation amongst other transcripts. A second large cluster,
166 **Cluster 4** (401 transcripts) is even less variable between individuals and highly-enriched for
167 mitochondria-related transcripts and ribosomal protein genes. **Cluster 12** (146 transcripts) was
168 highly variable between individuals at 0 time and down-regulated by LPS. This cluster
169 includes transcription factors *ETV5, MAFB*, *MEF2C, NFXL1*, *RB1* and multiple macrophage-
170 enriched differentiation markers and cell surface receptors (*AIF1, CD163, CD28, CD302, CD4,*
171 *CLEC10A, CSF1R, FOLR2, GAS6/7, GPR34, GSDMA, INPP5D, MERTK, MPEG1, MRC1,*
172 *TLR7, TYROBP, STAB1, TNFRSF11A, VSIG4*). Their correlated expression indicates that there
173 is significant variation in CSF1-induced differentiation amongst individuals. ETV5 [31],
174 MAFB [32] and MEF2C [33] have each been identified as regulators of monocyte-macrophage
175 differentiation, and variation in their expression (in each case >10-fold in the 0 time samples)
176 likely contributes to the co-regulation of this cluster of genes. Many of these genes (including
177 *MAFB* and *CSF1R*) form part of what was classified as a gut resident macrophage signature in
178 single cell-RNA-seq analysis of IBD lesions [34].
179 There are several large clusters that reinforce previous analysis of the progressive temporal
180 profile of the response of human MDM to LPS [18]. Transcripts in **Cluster 11** (161 transcripts)
181 peaked at 2 hours and then declined rapidly. They include genes encoding the key feed forward
182 activators, *TNF, IFNB1* and *IFNL1*, transcriptional regulators (e.g. *ATF3, FOSL1, KLF6,*
183 *KLF7, MYC, NFATC1, NFE2L2, NFIL3, NFKBIZ*, *NR4A3*) and many negative feedback
184 regulators (e.g. *DUSP1, GPR183, NFKBIA, OTUD1, TNFAIP3* and *ZFP36L2*) that serve to
185 constrain the response. Transcripts within **Cluster 9** (209 transcripts) were also induced by 2
186 hours, but the expression declined less rapidly and was retained at 7 hours. This cluster includes

187 additional transcription factors *IRF1, IRF8*, several interferon target genes (*IFIT2*), inducible
188 cytokines (*IL1B, IL12A*) and chemokines (*CXCL1, CCL3, CCL4*).
189 The large **Cluster 2** (819 transcripts) groups the set of transcripts that was maximally induced
190 at 7 hours post-stimulation and declined by 21 hours. It includes inducible transcription factors
191 (*BATF, BATF2, BATF3, IRF9, NFE2L3, STAT2,3,4*) and further interferon target genes (e.g.
192 *IFIT1, ISG15, MNDA, MX1, OAS1*). Similarly, **Cluster 3** (445 transcripts) contains transcripts
193 that were inducible by 7 hours but continued to increase, peaking at 21 hours in the response.
194 This cluster also contains transcripts encoding transcriptional regulators (*CEBPB, IRF6, RELB,*
195 *STAT1*) and a distinct cohort of chemokines, surface receptors (e.g *CLEC4E*) and interferon-
196 inducible transcripts including the receptor *IFNAR2* and *IFI6, IFI27, IFITM1/2/3.* It is notable
197 that *IRF7,* which also formed part of an IFN-inducible regulon in monocytes responding to
198 LPS [27, 35] was not found in any cluster. Indeed, by contrast to the induction reported in
199 monocytes *IRF7* was induced by LPS in MDM derived from only 9/56 individuals. On the
200 other hand, *IRF1* (which may be affected by an autoimmune disease risk variant in monocytes
201 [24]) was strongly induced by LPS to a similar extent in MDM in all individuals.
202 Although blood monocytes are predominantly post-mitotic, CSF1 is able to promote
203 proliferation in a subset of cells and this response is inhibited by LPS [36]. Accordingly,
204 **Cluster 13** (104 transcripts) contains the key cell cycle transcription factors
205 (*E2F1/E2F2/E2F8, FOXM1, MYBL2*) and numerous structural and regulatory genes that are
206 induced specifically in S phase and mitosis [37]. The expression of transcripts in this cluster
207 was relatively low, and down-regulated by LPS, but clustering arises because of the substantial
208 variation between individuals at the earlier time points.
209 **Cluster 70** (17 transcripts) contains all of the mitochondrial encoded transcripts, clustered
210 because of the extensive and correlated variation between individuals. This observation is
211 consistent with evidence for high levels of variation in expression of these transcripts in human

212 populations [38]. No corresponding variation was detected in nuclear-encoded mitochondrial
213 genes.

214 **Variation in the temporal profile of expression of key cytokine genes**

215 Many pro-inflammatory cytokines produced by macrophages are targets for successful
216 therapeutic intervention in IBD and/or are affected by regulatory variants that are in turn
217 associated with susceptibility to inflammatory disease. Our focus in this study was based on
218 the idea that affected individuals and their siblings provide a discovery cohort to identify
219 extreme regulatory variation. As noted above, the majority of transcripts varied by less than 2-
220 fold amongst the 50 individuals from IBD families at each of the 4 time points. To identify the
221 most highly variable transcripts, in **Tables S1C-F** the genes have been ranked in order of
222 maximum/minimum each time point. At each of the three time points post LPS treatment (2hrs,
223 7hrs, 21hrs) there were around 100 transcripts that varied more than 100-fold in expression
224 between individuals (**Table S1D-F**). These include each of the cytokines mentioned above,
225 and numerous chemokine genes.

226 In some cases, the variation reflects temporal differences. **Figure 2** shows the time courses of
227 regulation of selected cytokines (*IL1A, IL1B, IL10, IL12B, IL23A, IL6, TNF* and *TNFSF15*)
228 across the whole dataset. The transient induction of *TNF* with a peak at 2hrs and of *IL12B* at
229 7hrs was relatively consistent between individuals apart from a small number of outliers with
230 low or sustained expression (**Figure 2, Table S1B**). For other transcripts, the pattern of
231 regulation varied. For example, in 9 individuals, *IL6* mRNA peaked at a relatively low level at
232 2 hrs then declined, where in the majority, peak expression at a much higher level occurred at
233 7 hrs. In the majority (35/56), *IL1B* peaked at 2 hrs then declined rapidly, but in 21/56 *IL1B*
234 mRNA was sustained or increased further at 7 and 21 hrs.

235 IL23, a dimeric protein comprised of IL12B and IL23A, is a therapeutic target in IBD [39, 40].
236 **Figure 2** shows that *IL23A* and *IL12B* were each strongly induced by LPS but with distinct

237 temporal profiles. In the cluster analysis, *IL23A* forms part of the small hypervariable **Cluster**
238 **118** (10 transcripts) whereas *IL12B* was not correlated with any other transcript at the threshold
239 of r = 0.7. This observation raises the possibility that some individuals are IL23-deficient
240 through a failure to coordinate the expression of the two subunits. **Figure 3A** shows that the
241 expression of the genes encoding the two subunits is significantly correlated across the whole
242 dataset. However, there are some individuals where one gene is very highly expressed while
243 the other is low (**Figure 3B**). In addition, the peak expression of *IL23A* is an order of magnitude
244 higher than expression of *IL12B.* IL23A may also form dimers with EBI3 to produce an
245 alternative effector of the IL23R, although there is some doubt as to whether this occurs in
246 humans [41]. Unlike *IL12B* and *IL23A*, *EBI3* forms part of the late response **Cluster 3** and was
247 amongst the most highly-induced transcripts with relatively little variation amongst
248 individuals. EBI3 is also a dimer partner with IL27A to form IL27. *IL27* mRNA was also LPS
249 inducible and varied greatly between individuals, but unlike the dimer partner, it is part of
250 **Cluster 2** (peaking at 7hrs) and is a known interferon target gene [42].
251 In humans, *CSF1* mRNA is not detectable in freshly-isolated monocytes but is induced during
252 monocyte-macrophage differentiation. MDM become autocrine for this growth factor [18].
253 *CSF1* was indeed highly-expressed in unstimulated cells in most individuals and further-
254 induced around 3-fold by LPS at 2hrs, but expression varied independently of other cytokines.
255 A relatively common SNV variant at the *CSF1* locus is linked to Paget's disease [43] but we
256 found no association between SNVs at the *CSF1* locus and the level of *CSF1* mRNA in MDM
257 (not shown). One feature of the LPS response in human MDM that has not previously been
258 reported is the induction of the chemokine receptor, *CCR7* and its ligand, *CCL19.* Both were
259 variable between individuals and not correlated with each other (**Table S1B**).
260 Transcripts encoding the three subunits of complement component C1q (*C1QA, CIQB, C1QC*)
261 are also undetectable in monocytes and highly-expressed in MDM. C1q has multiple functions

262 in autoimmunity and in resistance to infection [44]. Each *C1Q* transcript varied more than 10-
263 fold between individuals. *C1QA* and *C1QC* were correlated with each other (Cluster 443) at
264 r>0.75. *C1QB* was correlated at r=0.41 (with *C1QC*) and r=0.54 (with *C1QA*). Potter *et al.*
265 [45] provided evidence of increased synthesis and catabolism of C1q in IBD patients. An IBD
266 susceptibility locus, defined by rs12568930, is located at Chromosome 1:22,375,738. The
267 nearest gene is *ZTBT40,* but the three C1Q genes lie within the genomic interval [18]. Our data
268 suggest C1q provides stronger candidates than others in the interval.

269 **eQTL analysis.**

270 The large majority of genes expressed in human monocytes show evidence of heritable
271 variation in expression, either in the basal state or following stimulation with LPS or interferon-
272 gamma [22, 35, 46]. The relatively small population analyzed herein, and the obvious and
273 deliberate relatedness, is not optimal for any genome-wide association analysis, including
274 eQTL [47]. However, at least some of the variation in gene expression we detected suggested
275 the presence of null expression alleles that might be linked to common SNVs. To test this
276 possibility, we genotyped each of the individuals using a focused immune chip and performed
277 eQTL analysis. **Figure 4** shows the Manhattan plots for each of the 4 time points and **Table**
278 **S3** contains all associations with a p value of $<10^{-6}$. A major peak of *cis*-acting variation was
279 detected over the HLA region of chromosome 6 at every time point, but otherwise the weaker
280 candidate eQTL associations in **Table S3** are predominantly *trans*-acting.

281 **Association of the regulation of MHC gene expression with HLA haplotypes**

282 Because of the extensive polymorphism of HLA and known variation associated with specific
283 haplotypes, it is possible to determine the HLA genotype based upon expressed SNVs [48] and
284 in turn to assess the relationship between HLA type and expression of specific HLA transcripts.
285 We also used the published analytical pipeline RNA2HLA to establish the HLA types of each
286 of the individuals in this cohort to confirm family relationships and sample identity and to link

287 variation to specific haplotypes. In the cluster analysis, the class II MHC regulator *CIITA*, and
288 chaperone, *CD74*, were not correlated with class II MHC gene expression. *CD74* was highly
289 and relatively uniformly expressed and clustered only with *HLA-DPA1 (***Cluster 870**). The
290 only co-regulated HLA genes were pairs located adjacent in the genome, *HLA-B/HLA-C, HLA-*
291 *A/HLA-F* and *HLA-DRB1/HLA-DRB5*, correlated with each other because of extreme variation
292 between individuals. The expression of these gene pairs across the whole data set is shown
293 **Figure 5,** sorted by family and separately by disease status. In each case, there are siblings
294 with >100-fold differences in expression, with examples of concordant and discordant sib-pairs
295 that are not related to disease status. However, in overview, all but 18 of the 56 tested
296 individuals over-expressed at least one of these HLA gene pairs in MDM. **Table 1** relates the
297 HLA genotypes of each individual to the level of expression of four of these HLA genes.
298 Expression of each of the correlated pairs was not strictly-related to HLA genotype across the
299 cohort, but was correlated with HLA genotype in sib-pairs.

300 **Association of expression with genotype at selected candidate gene loci**

301 Aside from the HLA region, the genome-scale analysis of eQTL does not highlight any clear
302 *cis* SNV associations with the highly-variable expression of individual cytokine genes in **Table**
303 **S1**. To test the relationship between genotype and expression we examined SNV associations
304 at selected candidate gene loci. *ERAP2* encodes an aminopeptidase involved in antigen
305 processing and lies within a genomic interval associated with IBD susceptibility [18]. There
306 are two major haplotypes with almost equal frequency in human populations [13] one of which
307 encodes a splice isoform that introduces premature stop codons leading to nonsense-mediated
308 decay (NMD). Klunk *et al.* [13] provided evidence that the functional allele was favoured by
309 pathogen selection in survivors of the black death pandemic, caused by the bacterium *Yersinia*
310 *pestis*, and suggested a consequential increase in susceptibility to inflammatory disease
311 including IBD. **Figure 6A** shows that a subset (13/56) of our cohort has very low expression

312 *ERAP2* in MDM, approximately the proportion of homozygotes anticipated from the allele
313 frequency. In those individuals that expressed *ERAP2* mRNA, expression was not highly
314 variable and was marginally induced by 21hrs. There was no association with disease status
315 within the IBD families. Analysis of expressed SNVs revealed that these individuals were
316 indeed homozygous for the rs1056893 variant (**Figure 6B**). The neighbouring *ERAP1* gene
317 was more highly-expressed than *ERAP2* in MDM and also relatively consistent amongst
318 individuals (**Table S1B-F**). ERAP1 is also involved in peptide processing for presentation on
319 MHC1, and extensive protein-coding variation has been associated with susceptibility to
320 several inflammatory diseases [49]. The calpastatin (*CAST*) locus overlaps *ERAP1* on the
321 opposite strand and has also been implicated in regulation of inflammation in colitis models
322 [50]. *CAST* is highly-expressed by MDM in our data and further induced by LPS. In summary,
323 *ERAP2* is not the only candidate gene within the genomic interval associated with IBD
324 susceptibility.
325 *IL1B* is of particular interest because of its implication in IBD pathology [51] and because LPS-
326 inducible expression is at least 10-fold lower in MDM compared to freshly-isolated monocytes.
327 Hence, extreme high expression of *IL1B* in response to LPS is an indication of failure of CSF1-
328 induced differentiation. SNV rs1143634 encodes a synonymous substitution with a minor
329 allele frequency (MAF) around 0.2 in most ethnicities (gnomad.broadinstitute.org). That allele
330 frequency is not significantly different in our cohort. **Figure 6B** shows level of expression of
331 *IL1B* mRNA at each time point for each genotype. The distribution of peak expression values
332 is highly skewed; there is a significant correlation between the presence of the minor allele and
333 low expression. Aschenbrenner *et al.* [51] proposed that IL1B provides an autocrine signal
334 regulating expression of another therapeutic target *IL23A* [39, 52] by stimulated monocytes.
335 Consistent with that view, *IL23A* was induced later in the LPS response (peak at 7-21 hrs;
336 **Figure 2**) and expression of *IL23A* at 7 hrs was significantly correlated with *IL1B* at 2 hrs

337 (**Figure 6C**). In contrast there was no correlation between *IL1B* and *IL23A* at 2 hours (**Figure**
338 **6C**).
339 IL6 is another therapeutic target in many inflammatory diseases [53]. *IL6* mRNA at 7hrs varied
340 >100-fold between individuals (**Figure 2**). The *IL6* locus contains an expressed SNV
341 (rs42534390) with an allele frequency of around 50% and a common IL6 promoter variant (-
342 174G/C, rs1800795) has been associated with circulating IL6, variation in responsiveness to
343 LPS and other stimuli [53]. **Figure 6D** shows the relationship between SNV genotype and *IL6*
344 mRNA expression level in our cohort. There was no correlation between *IL6* mRNA and *IL6*
345 SNV genotype, nor with the level of *IL1B* at 2 hrs.
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347
348

349 **Discussion**
350
351 This project was based upon three hypotheses: (1) within human populations there are
352 uncommon, non-coding variants of large effect that influence transcription of key genes in
353 macrophages; (2) differentiation of monocytes in response to CSF1 and a regulated response
354 to microbial stimulus is a key event in adaptation to the pro-inflammatory environment of the
355 lower GI tract; (3) if they exist, regulatory variants affecting basal or inducible gene expression
356 in monocytes differentiated in CSF1 would be prevalent in IBD families. These hypotheses
357 were based upon substantial enrichment identified in large-scale GWAS studies for IBD
358 association with SNVs in the vicinity of the promoters of regulated macrophage-expressed
359 genes [18]. The analysis herein provides further strong support for the view that transcriptional
360 dysregulation in macrophages has a causal relationship to IBD susceptibility. Based upon
361 inducible expression of an NF-κB-regulated luciferase in patient macrophages in a cohort of
362 similar size to ours, Papoutsopoulou *et al*. [54] claimed that CD patients were hyper-responsive
363 to TLR4 signalling. In our data, the overall patterns of gene expression in stimulated
364 macrophages clustered based upon time, reflecting the major changes induced by LPS, but
365 there was no relationship with disease status (**Figure 1B**). Hence, the data provide no support
366 for the idea that IBD arises from a global hyper-responsiveness to TLR4 signals.
367 The available evidence of coding variation in IBD also supports macrophages as the main focus
368 of IBD susceptibility variants. Based upon mass scale genome and exome sequencing in
369 thousands of individuals, Sazonovs *et al.* [11] confirmed known and identified a number of
370 new coding variants apparently enriched in IBD patients. The pattern of expression and
371 function of transcripts containing these coding variants in monocytes and macrophages is
372 summarized in **Table 2.** In overview, the large majority of these coding mutations are likely to
373 impact macrophage function. With the exceptions of *FUT2, IL32R, HGFAC* and *IRGM,* all
374 IBD-associated genes with coding variation identified through genome sequencing are

375 expressed by monocytes and/or MDM and in many cases share the pattern of down-regulation
376 in MDM with *NOD2*, and/or regulation by LPS. In the case of *FUT2* and *IL23R,* variation may
377 impact downstream of macrophage secreted products, IL1B and IL23A. The discussion of roles
378 of *HGFAC* variants by Sazonovs *et al.* [11] focusses on known biology of hepatocyte growth
379 factor (HGF), but *HGFAC* is expressed exclusively in hepatocytes [55] so the mechanistic
380 connection to IBD susceptibility is not obvious. The FANTOM5 analysis implied strongly that
381 *IRGM* in humans is a pseudogene. There is no evidence of expression of *IRGM* mRNA in either
382 the massive CAGE dataset [55] or in the RNA-seq data generated herein. However, active
383 monocyte-macrophage expressed enhancers were detected throughout the *IRGM* locus and
384 flanking DNA. As previously discussed [18] the neighbouring *TNIP1* gene (aka *ABIN3*)
385 encodes a more obvious regulator of inflammation located in this genomic region and
386 regulatory variation in this gene has been detected in patients with systemic lupus
387 erythematosus[56]. *TNIP1* mRNA was highly-expressed in MDM, further induced > 10-fold
388 by LPS and highly variable amongst individuals (**Table S1C-F**).
389 The top 100 hypervariable transcripts at 2, 7 and 21 hrs (**Table S1D-F**) include numerous
390 chemokines (*CCL2, CCL4, CCL5, CCL19, CCL20, CCL3L3, CCL4L2, CCL8, CXCL1,*
391 *CXCL5, CXCL8, CXCL9, CXCL10, CCL15, CXCL3, CCL22, CXCL11*) and cytokines (*CSF2,*
392 *CSF3, EBI3, IFNB1, TNF, TNFSF10, IL1A, IL1B, IL6, IL12B, IL23A, IL27, IL32*) many of
393 which are considered targets for therapeutic intervention. This cocktail of inducible
394 macrophage-derived effectors likely contributes to the formation of tertiary lymphoid tissue
395 that is a feature of IBD [57]. In each case, a simple NCBI search on gene name AND
396 polymorphism identified evidence of an association with disease susceptibility and many of
397 these genes lie within genomic intervals associated with IBD in GWAS analysis (e.g. CXCL
398 chemokines on Chr4 with rs2472649 and CCL chemokines on Chr17 with rs3091316). For
399 each of the cytokines in **Figure 2,** we have also performed pairwise comparison of affected

400 and unaffected siblings as well as global comparisons of affected versus unaffected and healthy
401 donors (not shown). Consistent with the lack of detection of *cis*-acting variants in the eQTL
402 analysis in **Table S3** we found no evidence for any significant association between magnitude
403 or pattern of expression of any gene and disease**.** In the specific cases of *IL1B* and *IL6,* the
404 analysis in **Figure 6** supports the view that the extensive variation is only partly influenced by
405 *cis*-acting variants. There has been one report of an association between IBD and a common
406 *IL6* promoter polymorphism [58] but we found no evidence of SNV association with the peak
407 level of *IL6* mRNA. The *IL6* locus has at least nine LPS-responsive inducible enhancers [18]
408 so there is considerable complexity in transcriptional regulation and it somewhat unlikely that
409 a single variant is causal. Given the extensive network of feedforward and feedback regulation
410 analysed previously [18] expression of each transcript is essentially a complex trait.
411 Identification of *cis* or *trans*-acting regulatory variants of large effect would be even more
412 challenging than the search for coding variants. Functional annotation of non-coding SNVs
413 would be even more challenging. The published analysis of HLA sequences identified
414 thousands of non-coding variants within putative regulatory elements [56].
415 Aside from cytokines and chemokines, each hypervariable regulated transcript expressed in
416 MDM can form the basis for a hypothetical link to IBD susceptibility. For example, *IFITM1,*
417 *IFITM2* and *IFITM3* contribute to endocytic targeting of incoming virus particles, restricting
418 virus replication [59]. Each of these transcripts is highly-expressed by monocytes and down-
419 regulated by CSF1, enabling entry of influenza virus, expression of viral proteins and induction
420 of pro-inflammatory genes [60]. *IFITM3* variants are associated with severe disease in
421 influenza infection and an *Ifitm3* knockout mouse is susceptible to low pathogenicity infections
422 [61]. Mice lacking the entire IFITM locus develop spontaneous colitis [62]. In unstimulated
423 MDM, *IFITM1* was barely detectable but there was residual expression of *IFITM2* and
424 *IFITM3.* Each transcript was induced by LPS with differences in time course and magnitude,

425 and large variations between individuals, but they were sufficiently correlated to form part of
426 the interferon-response **Cluster 3** in the current data set**.** Could the extreme variation in
427 expression of these and other LPS-inducible interferon-responsive genes (*CXCL10, IDO1,*
428 *IFIT2, ISG15, ACOD1, RSAD2, OSAL, IFIH, ISG20*) predispose to hyper- or hypo-
429 responsiveness to enteric viruses as the initial environmental trigger of chronic inflammation?
430 The relative abundance and function of inducible negative feedback regulators of macrophage
431 activation highlighted previously [18] also varied between individuals. For example, the set of
432 highly-expressed and hypervariable transcripts in **Table S1D-F** includes *ACOD1, DUSP1,*
433 *GPR183, OTUD1, PMAIP1, PTGS2 (aka COX2), SERPINE1, SOCS3, TNFAIP3, TNFAIP6*
434 and *ZFP36*. These have been described as inflammation suppressor genes, in that even
435 heterozygous mutations in any one of them can lead to spontaneous inflammation [63, 64].
436 Variation in their expression is likely to contribute to the variation in temporal profiles of
437 cytokine induction, for example the transient or sustained expression of IL1B (**Figure 2**). In
438 mice, transcripts encoding anti-inflammatory IL10 (*Il10* gene) and IL10 receptor (*Il10ra* gene)
439 appear to be required for homeostatic maintenance and regulation of IL23 production but the
440 available evidence implicates T cells as the main source of IL10. Conditional deletion of either
441 ligand or receptor in macrophages did not lead to spontaneous inflammation but influenced the
442 response to pathogenic bacterial challenge [65]. *IL10* mRNA was induced in MDM by LPS in
443 all individuals but the level of expression was relatively low and both the time course and the
444 peak expression were highly variable (see **Table S1D-F, Figure 2**). The receptor gene,
445 *IL10RA,* was expressed in unstimulated MDM and induced 6-7 fold further by 7 hrs following
446 LPS addition (**Table S1E**). This suggests that main axis of control is the regulated response of
447 macrophages to IL10 produced by other cells. SNVs in both *IL10* and *IL10RA* have been linked
448 to IBD susceptibility in GWAS and loss-function mutations are associated with very early
449 onset disease [66].

450 The profound induction by LPS of transcripts encoding the chemokine receptor, CCR7, and its
451 major ligand, CCL19, has not been reported previously. At each of the time points, *CCR7*
452 expression varied between individuals (**Table S1C-F**). The literature on CCR7 biology
453 focusses exclusively on its expression by dendritic cells (DC) and the function in guiding these
454 cells to draining lymph nodes (e.g. [67]). Transcripts encoding inducible co-
455 stimulatory/inhibitory molecules detected on active human DC [68], CD83, CD80, CD40 and
456 CD274 (PDL-1) were inducible >20 fold by LPS in MDM and highly variable between
457 individuals. The macrophages of the lamina propria of mice, conflated with dendritic cells
458 because of their expression of CD11c (*Itgax*) (see [69]) express CCR7 and migrate in response
459 to CCL21 [70]. As expected, the human monocytes differentiated in CSF1 expressed *ITGAX*
460 (CD11c) and *ITGAM* (CD11b) mRNA. They also express *ITGAE* (CD103), commonly used
461 as a marker of mouse and rat DC [69], but lack detectable FLT3. Studies in the rat have
462 demonstrated that migrating "dendritic cells" in lymphatics draining the gut are derived from
463 blood monocytes [71]. We conclude that monocytes differentiated in CSF1 can be primed to
464 respond to microbial agonists by becoming migratory antigen-presenting cells (APC).
465 Of course, the primary determinant of APC activity is the expression of MHC molecules to
466 enable presentation of antigenic peptides to T cell receptors. If monocyte differentiation in
467 CSF1 provides a model for adaptation to the gut mucosa [18], then the differential expression
468 of both Class I and Class II MHC genes, independent of their antigen binding specificity, may
469 well underlie the selective response to classes of mucosal antigens leading to inflammation. As
470 noted in the introduction, shared haplotypes in the HLA region provide the greatest prediction
471 of shared risk of IBD amongst members of the same family [1, 72]. High-density SNV typing
472 of the HLA region in >32,000 individuals with IBD implicated several HLA alleles, with the
473 greatest effect size associated with HLA-DRB1*01:03 in both CD and ulcerative colitis [73].
474 Seven of the individuals in our cohort (five affected, two unaffected) have this allele, all as

475 heterozygotes. This included three members of one family (MN11), with one unaffected
476 individual, one with CD and one with ulcerative colitis. The discussion of these specific HLA
477 associations has focused on variation in the antigen-binding sites, with the presumption that
478 these variants predispose in some way to response to an environmental trigger. There is also
479 evidence of associations of class I MHC (specifically HLA-C) alleles with IBD [74] and of
480 regulatory polymorphism affecting HLA-C expression [75]. High HLA-C expression on
481 peripheral blood leukocytes was associated with increased risk of CD [76]. 11/50 individuals
482 in our cohort expressed high levels of HLA-C (>400TPM) in MDM (compared to an average
483 of <2TPM in non-expressors) but there was no correlation with disease status (**Figure 6**). By
484 contrast to mouse, where class II MHC genes are almost undetectable in macrophages grown
485 in CSF1 (see BioGPS.org), Class II MHC genes are expressed to varying degrees in human
486 blood monocytes and retained or increased in MDM grown in CSF1. The relationships
487 between HLA alleles and expression in relevant cell types has not been explored in the context
488 of IBD. Raj *et al* [56] reported the existence of numerous regulatory polymorphisms affecting
489 expression of Class II MHC genes in monocytes cultured in CSF2 (GM-CSF) (so-called
490 monocyte-derived DC, moDC), as related to susceptibility to systemic lupus erythematosus.
491 However, the effect sizes were small. By contrast, our analysis of MDM grown in CSF1
492 revealed apparent null expression of class I (HLA-A, HLA-B, HLA-C, HLA-E, HLA-F) and
493 class II (HLA-DRA, HLA-DRB1, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DMB, HLA-
494 DPA1) in certain individuals, linked to HLA haplotypes (**Table 1**). HLA-C is of particular
495 interest as the major determinant of natural killer (NK) cell recognition [77, 78]. The small
496 number of individual monocytes and MDM profiles in the FANTOM5 project also show >10-
497 fold variation in expression of each of these genes among individuals. These large differences
498 could regulate acquired immune responses to broad classes of microflora that might in turn
499 initiate a chronic inflammatory state.

500 **Conclusions**

501 This study has not identified a common global pattern of dysregulated variation in macrophage
502 activation that distinguishes affected and unaffected individuals or discordant sib-pairs. What
503 it has done is to confirm that in macrophages differentiated in CSF1 there are very large inter-
504 individual differences in basal and LPS-inducible expression of specific pro-inflammatory or
505 anti-inflammatory genes. Whereas analysis of common SNVs by GWAS reveals variants of
506 small effect on disease susceptibility at a population level, we suggest that within individual
507 families, there are smaller numbers of regulatory variants of much larger effect that impact
508 gene expression in macrophages and could only be associated with disease through
509 conventional linkage analysis in large pedigrees. The concept that each family is unique has
510 been termed the Anna Karenina hypothesis for the genetics of complex disease [79]. The
511 corollary of the view is that IBD may, in fact, be many different diseases with unique
512 underlying genetic basis and corresponding variation in treatment efficacy.

513

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516

517 **Materials and methods**

518 All patients provided consent and the study was approved by the ACT Health Human Research
519 Ethics Committee (ETH.5.07.464).

520 *Blood monocyte isolation*. Approximately 65 mL of blood was drawn from each subject into
521 ACD-B Vacuette® tubes, transferred to sterile 50 mL tubes, and centrifuged for 30 min at
522 12,000 x g with no brake. A 200 uL aliquot of blood was kept for DNA extraction. Plasma was
523 removed from the tubes so that only a 1 cm layer remained. Approximately 9 mL of buffy coat
524 was aspirated from each sample and diluted 1/1.5 in RPMI with no additives. The buffy coat
525 dilution was gently layered on to Lymphoprep™ to a total volume of 50ml in a 50mL tube,
526 then spun for 45 min at 200 x g at room temperature with no brake. The clear top layer was
527 removed and the mononuclear cells (peripheral blood mononuclear cells; PBMC) was
528 recovered, diluted in 30ml RPMI1640 and pelleted by centrifugation. Cells were counted,
529 washed once with phosphate buffered saline. CD14-positive cells were enriched by using the
530 Classical Monocyte Isolation Kit (Miltenyi 130-117-337) according to the Manufacturer'
531 instructions. A 10 uL aliquot of the magnetic column eluate was removed for cell counts and
532 purity check. The remainder of the elution was centrifuged for 10 min at 300 x g at 4°C.

533 *Cell culture*. $CD14^+$ monocyte pellet was resuspended in complete culture medium; RPMI1640
534 (Sigma-Aldrich) containing 2mM Glutamax (Gibco), 20ug/ml penicillin/streptomycin (Sigma-
535 Aldrich), and 10% v/v human AB serum (Sigma-Aldrich). Recombinant human macrophage
536 colony-stimulating factor (CSF1, Gibco-Thermo Fisher, PHC9501) was then added at a final
537 concentration of 100 ng/ml. The cells were then plated at 10^6 cells per well, to a final volume
538 of 2 mL. The 6-well plates were coated with 1ug/cm^2 human plasma fibronectin (Sigma-
539 Aldrich) as per the manufacturer's instructions to create a more physiologically relevant
540 substratum. The 6-well plates were placed in the incubator at 37°C with 5% CO_2. At day four

541 of incubation 1 mL of fresh complete medium with 3 X CSF1 concentration was added to each
542 well. On day six, the medium was replaced with 2 mL complete medium with 1X concentration
543 of CSF1. On day seven of incubation, the time course experiments commenced (Day 1), LPS
544 10 ng/ml lipopolysaccharide *Salmonella enterica* serotype Minnesota Re 595 (Sigma-Aldrich)
545 was added to each well. Time point 0 hr was collected before LPS was added. At each time
546 point (2 hr, 7 hr, 21 hr), cells were lysed using 0.5 mL TRIzol (Invitrogen), after which 100 uL
547 chloroform was added. The plates were rocked back and forth for 15 sec and incubated at 37°C
548 with 5% CO_2 for 2 min. The cells were then placed in sterile microcentrifuge tubes and placed
549 at -80°C.

550 *Transcriptome sequencing*. A total of 224 samples underwent whole genome transcriptome
551 sequencing. Lysed cell samples were thawed and RNA extracted using a Qiagen RNeasy
552 miniprep kit, including DNase digestion (Qiagen). RNA concentration and purity was assessed
553 on a Tapestation (Agilent). Library preparation was performed using Illumina TruSeq Stranded
554 mRNA kits. Sequencing was performed on a NovaSeq 6000 instrument using S1 flowcells
555 (three in total) in a 100 bp paired-end format. Library preparation and sequencing were
556 performed by the Biomolecular Resource Facility of the Australian National University,
557 Canberra, Australia, according to manufacturer's instructions.

558 *SNV genotyping.* DNA was extracted from a 200 uL blood sample for each subject using
559 QIAamp DNA Blood Mini kits with RNaseA digestion (Qiagen). SNV genotyping was
560 performed using Illumina Infinium ImmunoArray-24 v2.0 BeadChips
561 (InfiniumImmunoArray-24v2-0; Illumina, Inc., San Diego, CA, USA). A total of 4 uL DNA
562 was loaded onto the array, as per the manufacturer's guidelines. The BeadChips were scanned
563 using an Illumina iScan. Samples were genotyped using Illumina's GenomeStudio 2.0.4 with
564 Genotyping module 2.0.4 software, using default settings. The Illumina
565 InfiniumImmunoArray-24v2-0 manifest file and project specific cluster file were used for the

566 analysis (InfiniumImmunoArray-24v2- 0_A_ClusterFile.egt). SNV genotyping was performed
567 by Australian Genome Research Facility, Melbourne, Australia.
568 *Bioinformatics.* FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was
569 used to assess the quality of RNA-seq results. Assignment of reads to transcripts was then
570 conducted using the pseudoaligner Kallisto v0.44 [80]. Ensembl release 103 of the GRCh38
571 genome was used for all kmer matching and subsequent annotation. The R package tximeta
572 [81] was used to calculate gene-level expression, corrected for effective gene length, resulting
573 in a file for abundance, in counts per million (CPM). Ensembl gene and primary transcript IDs,
574 NCBI stable gene IDs, Ensembl gene symbols and HGNC symbols were included for each
575 gene. This preparation of the data for analysis was carried out by the ANU Bioinformatics
576 Consultancy, Australian National University, Canberra, Australia.
577 Network analysis was then performed on the abundance file. Lowly expressed genes
578 (CPM<1.0) and genes expressed in <2 sample records (N records = 222; N samples = 56) were
579 removed. The resultant dataset consisted of 19,972 genes. The network analysis tool BioLayout
580 (http://biolayout.org, formerly BioLayout *Express*^{3D} [30]) was used to visualize the
581 transcriptomic data and explore relationships between samples (sample-to-sample analysis)
582 and genes (gene-to-gene analysis). For the sample-to-sample analysis, a threshold Pearson
583 correlation coefficient of 0.87 was used; this was the maximum value that included all samples.
584 For gene-to-gene analysis, a Pearson correlation coefficient of 0.7 was chosen to maximise the
585 number of genes included while minimizing the computational complexity due to the number
586 of connections between them [82].
587 Analysis of the SNV genotyping data was performed by Australian Genome Research Facility,
588 Melbourne, Australia. The hg38 build of the human reference genome was used for analysis.
589 Final reports were merged and converted to PLINK PED/MAP format using gcta v1.91.2
590 (Yang et al., 2011). Alleles were flipped to the positive strand using the ImmunoArray v2.0

591 strand designations (N=102,173). Quality control was carried out using PLINK [83]. Filtered
592 SNVs were pruned by pairwise genetic correlation to create a set of independent variants
593 appropriate for principle components analysis (PCA). LD pruning was calculated using a
594 window size of 1000 bp and 50 bp step with r^2 threshold of 0.5. Annotated PCA plots were
595 generated using the ggplot2 package [84] in the R statistical environment. PCA plots were
596 annotated for phenotype, family, age, and sex. Expression QTL (eQTL) analysis was
597 performed for 56 samples using Matrix-eQTL version 2.3 [85] in the R statistical environment
598 version 4.1.2. Four timepoints were modelled in linear mode (least squares), with sex applied
599 as a covariate. A total of 1,194,937,309 eQTL were modelled for each timepoint. A stringent
600 minor allele frequency threshold of 0.2 was applied to reduce inflation generated by rare alleles
601 [86] (approximately 100,000 SNVs remaining in the analysis). To reduce the resultant file
602 sizes, only sites with a significance score of $P<1x10^{-5}$ were exported. Quantile-quantile plots
603 and p-value histograms were generated to assess the effectiveness of the model and data QC.
604 Following initial QC of SNV results, assignment of the time point samples to individuals was
605 verified and HLA-A, HLA-B, HLA-C and HLA-DRB1 typing was obtained using RNA2HLA
606 [48]. Assignment of sex was validated using expression of Y chromosome genes.

607 **Data availability**

608 All of the processed RNA-seq data is presented in Table S1. The raw RNA-seq sequence files
609 that support the findings of this study are available on reasonable request from the
610 corresponding authors. The data are not publicly available due to privacy and ethical
611 restrictions.

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617 Additional bioinformatic analysis was performed by Dr Lesley Gray at the Australian Genome
618 Research Facility.
619

620 **Legends to Figures**

621 **Figure 1. Network analysis of gene expression in macrophages stimulated with LPS.**

622 Gene expression profiles from RNA-seq analysis of the time course of the LPS response of
623 MDM from 56 individuals (primary data in **Table S1**) were subjected to network analysis using
624 Biolayout as described in Materials and Methods.

625 *Figure 1A. Sample-to-sample network.*

626 Each sphere represents a sample (individual time point). Edges between them (Pearson
627 correlations of ≥ 0.87) have been omitted for clarity. Note the clear separation of the samples
628 based upon time of incubation (left) but not on disease status (right).

629 *Figure 1B. Gene-to-gene network.*

630 Each sphere represents a gene. Edges between them (Pearson correlations of ≥ 0.7) gave been
631 omitted for clarity and only the main element of the network is shown. Spheres are coloured
632 by their membership of a cluster (MCL inflation value 2.2), Graphs show the averaged
633 expression of genes in key clusters; colours of the columns match those in the network graph.
634 Y axis – gene expression in CPM. X axis – the samples are ordered by time point and disease
635 status; each column represents a single sample. Upper bar – disease status (blue: affected
636 (Crohn's disease or ulcerative colitis); green: unaffected sibling of an affected individual;
637 yellow: healthy donor with no family history of inflammatory bowel disease). Lower bar –
638 time point (red: 0 hrs; pink: 2 hrs; turquoise: 7 hrs; salmon: 21 hrs). The profiles for all clusters
639 are provide in **Table S2.** Arrows indicate the position of the cluster in the network. The position
640 of Cluster 70 is not indicated as it forms a small separate element not linked to the largest
641 element shown here.

642 **Figure 2. Individual variation in patterns of transcriptional regulation of key cytokine**
643 **genes in LPS-stimulated MDM.**

644 Gene expression profiles for the indicated cytokine genes were extracted from the RNA-seq
645 analysis of the time course of the LPS response of MDM from 56 individuals (primary data in
646 **Table S1B-F**). Each line joins the 4 time points from a single individual.

647 **Figure 3. Expression of *IL23A* and *IL12B*.**

648 *IL23A* and *IL12B* encode the two subunits of the heterodimeric cytokine, IL23. Expression of
649 the two genes was extracted from RNA-seq analysis of the time course of the LPS response of
650 MDM from 56 individuals (primary data in **Table S1**)

651 *A. Correlation between IL23A and IL12B expression.*

652 Graph shows the relationship between the two transcripts across the full dataset (all 4 time
653 points) including the line of best fit. Spearman $r = 0.8766$, $p < 0.0001$. Zero time values are
654 clustered at left. Note the numbers of outliers above (high *IL23A*) and below (high *IL12B*) the
655 line.

656 *B. Patterns of IL23A and IL12B expression across the time course***.**

657 Y axis – gene expression in CPM. X axis – samples ordered by time point and disease status;
658 each column represents a single sample. Upper bar – disease status (blue: affected (Crohn's
659 disease or ulcerative colitis); green: unaffected sibling of an affected individual; yellow:
660 healthy donor with no family history of inflammatory bowel disease). Lower bar – time point
661 (red: 0 hrs; pink: 2 hrs; turquoise: 7 hrs; salmon: 21 hrs).

662 **Figure 4. Expression QTL analysis of the response to MDM to LPS**

663 Gene expression profiles from RNA-seq analysis of the time course of the LPS response of
664 MDM from 56 individuals (primary data in **Table S1**) were correlated with SNV genotype as
665 described in Materials and Methods. Y axis – p value (-log10) of the correlation co-efficient
666 for the SNV genotype. Red line shows the p value considered significant after correction for
667 multiple testing. X axis – chromosome number and position for each analysed SNV. Colours

668 alternate with odd numbered chromosomes in blue and even numbered chromosomes in
669 yellow.

670 **Figure 5. Expression of MHC genes in IBD patients and families**

671 Expression of the Class I and Class II MHC genes was extracted from RNA-seq analysis of the
672 time course of the LPS response of MDM from 56 individuals (primary data in **Table S1**)

673 *A. Expression of MHC genes.*

674 Histograms on the left show the levels of expression for each individual across the four time
675 points. Y axis – expression in CPM. X axis – individuals and time points. Each column
676 represents one sample and the four time points for each individual are adjacent to each other
677 and next to other family members. Expression of *HLA-A* and *HLA-F* (red), *HLA-B* and *HLA-C*
678 (black) and *HLA-DRB1* and *HLA-DRB5* (blue). Panels on the right show the distribution of
679 expression values for the four time points. Y axis – expression (TPM, $\log_{10}$ scale). X-axis –
680 time point. Upper bar – family (arranged as in Table S1). Lower bar – disease status (red:
681 affected with Crohn's disease or ulcerative colitis; green: unaffected sibling; dark green –
682 healthy donor with no family history of IBD.

683 *B. Coexpression of Class I MHC genes.*

684 Histogram shows expression of *HLA-A* (red) and *HLA-B* (black) in each individual. Y axis –
685 expression in CPM. X axis – individuals and time points. Each column represents one sample
686 and the four time points for each individual are adjacent to each other and next to other family
687 members. Upper bar – family (arranged as in Table S1). Lower bar – disease status (red:
688 affected with Crohn's disease or ulcerative colitis; green: unaffected sibling; dark green –
689 healthy donor with no family history of IBD. Some individuals express one or other pair of
690 genes, others express both and some express neither. In several families, members show
691 different patterns (boxes).

692 **Figure 6. Relationship between SNV genotype and expression of key genes.**

693 Expression of the genes indicated was extracted from RNA-seq analysis of the time course of
694 the LPS response of MDM from 56 individuals (primary data in **Table S1**) and SNV genotype
695 was determined as described in Materials and Methods.
696 *A. Expression of ERAP2.*
697 Histogram in the upper panel shows the levels of expression of *ERAP2* for each individual
698 across the four time points. Y axis – expression in CPM. X axis – individuals and time points.
699 Each column represents one sample and the four time points for each individual are adjacent
700 to each other and to other family members. Y axis – expression (CPM). X-axis – genotype.
701 Upper bar – family (arranged as in **Table S1**). Lower bar – disease status (red: affected with
702 Crohn's disease or ulcerative colitis; green: unaffected sibling; dark green – healthy donor with
703 no family history of IBD). Graphs in the lower panel show the expression values for each
704 genotype at SNV rs1056893 for the four time points. Kruskal-Wallis test showed a highly
705 significant difference among genotypes for all time points ($p < 0.0001$), with the AA genotype
706 showing lower expression than the GG genotype and the heterozygous genotype being
707 intermediate.
708 *B. Expression of IL1B.*
709 Graphs show the expression values for each genotype at SNV rs1143634 for the four time
710 points. Y axis – expression (CPM). X-axis – genotype. Kruskal-Wallis test showed no
711 difference among genotypes for 0 hrs ($p = 0.17$) and 21 hrs ($p = 39$). There was a small
712 difference among genotypes at 2 hrs ($p = 0.03$) and 7 hrs ($p = 0.02$), with the TT genotype
713 showing lower expression than the CC genotype.
714 *C. Correlation between IL1B and IL23A expression.*
715 Left panel shows the relationship between *IL1B* and *IL23A* expression at 2 hrs. Spearman $r =$
716 0.05577, $p = 0.68$. Right panel shows the relationship between expression of *IL1B* at 2 hrs and
717 *IL23A* at 7 hrs. Spearman $r = 0.5573$, $p < 0.0001$.

718 *D. Correlation between IL1B and IL6 expression.*

719 Left panel shows the relationship between *IL1B* and *IL6* expression at 2 hrs. Spearman r =

720 0.08593, p = 0.53. Right panel shows the relationship between expression of *IL1B* at 2 hrs and

721 *IL6* at 7 hrs. Spearman r = -0.119, p = 0.39.

722

723 **Supplementary Tables**

724

725 **Table S1. Gene expression data.**

726 S1A contains the metadata contains metadata describing the individuals and their disease

727 status. Table S1B contains the full set of gene expression based upon quantification using

728 Kallisto as described in Materials and Methods, sorted based upon individual, family and gene

729 name. Tables S1C, S1D, S1E and S1F contain the data for individual time points, 0, 2, 7 and

730 21 hrs respectively, sorted based upon maximum/minimum expression.

731 **Table S2. Co-expression clusters**

732 Table shows the complete set of co-expression clusters generated by network analysis using

733 Biolayout. For each cluster with at least 10 transcripts the Tables shows the average expression

734 profile across the dataset in the same format as in Figure 1B.

735 **Table S3. Expression QTL analysis**

736 The Table shows the relationship between SNV genotype and level of expression of specific

737 transcripts at each of the 4 time points for all associations with p value$<10^{-6}$.

738

739 **Table 1:**

740 **HLA genotypes and level of expression of HLA genes.**

741 HLA genotypes derived from RNA2HLA (see Methods). Expression levels are given as

742 indicated for each gene.

743 For *HLA-A*, <250 CPM – 0, 250 CPM≤x<1000 CPM – +, 1000 CPM≤x<3000 CPM – ++,

744 ≥3000 CPM – +++. The maximum value was 8227 CPM and the time point for maximum

745 expression was 21 hrs.

746 For *HLA-B*, <250 CPM – 0, 250 CPM≤x<1000 CPM – +, 1000 CPM≤x<3000 CPM – ++,

747 ≥3000 CPM – +++. The maximum value was 6948 CPM and the time point for maximum

748 expression was 21 hrs.

749 For *HLA-C*, <100 CPM – 0, 100 CPM≤x<500 CPM – +, 500 CPM≤x<1500 CPM – ++,

750 ≥1500 CPM – +++. The maximum value was 3132 CPM and the time point for maximum

751 expression was 21 hrs.

752 For *HLA-DRB1*, <25 CPM – 0, 25 CPM≤x<100 CPM – +, 100 CPM≤x<300 CPM – ++, ≥300

753 CPM – +++. The maximum value was 704 CPM and the time point for maximum expression

754 was 0 hrs for most individuals.

Individual ID	Status	HLA-A allele1	HLA-A allele2	Max HLA-A expression	HLA-B allele1	HLA-B allele2	Max HLA-B expression	HLA-C allele1	HLA-C allele2	Max HLA-C expression	HLA-DRB1 allele1	HLA-DRB1 allele2	Max DRB1 Expression
MN1AF	AF	A*01:01	A*02:01	0	B*08:01	B*40:01	++	C*03:04	C*07:01	0	DRB1*15:01	DRB1*13:02	+++
MN1UN	UN	A*01:01	A*68:01	0	B*08:01	B*44:02	0	C*07:04	C*07:04	+	DRB1*15:01	DRB1*11:01	++
MN5AF	AF	A*26:01	A*02:01	0	B*13:02	B*52:01	0	C*06:02	C*12:02	0	DRB1*15:02	DRB1*07:01	++
MN5UN	UN	A*02:03	A*01:01	0	B*13:02	B*38:02	0	C*06:02	C*07:02	+++	DRB1*16:02	DRB1*07:01	++
MN6AF	AF	A*02:05	A*02:05	0	B*50:01	B*15:01	+	C*18:01	C*06:02	0	DRB1*13:01	DRB1*07:01	0
MN6UN	UN	A*24:02	A*02:01	0	B*15:01	B*15:01	0	C*04:01	C*03:03	0	DRB1*13:01	DRB1*03:17	0
MN8AF	AF	A*25:01	A*11:01	+	B*39:01	B*35:01	+	C*04:01	C*12:03	0	DRB1*01:03	DRB1*07:01	+
MN8UN	UN	A*29:02	A*02:01	0	B*44:02	B*44:02	0	C*05:01	C*16:01	0	DRB1*04:01	DRB1*07:01	0
MN9AF	AF	A*03:01	A*29:02	+++	B*44:03	B*15:01	0	C*03:04	C*16:01	0	DRB1*15:01	DRB1*07:01	+++
MN9UN	UN	A*03:01	A*29:02	+++	B*44:02	B*40:01	0	C*05:01	C*16:01	0	DRB1*01:03	DRB1*07:01	+
MN11AFC	AF	A*31:01	A*02:01	0	B*39:01	B*07:02	+++	C*12:03	C*07:02	+++	DRB1*01:03	DRB1*04:04	+
MN11AFU	AF	A*31:01	A*02:01	0	B*39:01	B*07:02	+++	C*12:03	C*07:02	+++	DRB1*01:03	DRB1*04:04	+
MN11UN	UN	A*31:01	A*02:01	0	B*39:01	B*07:02	+++	C*12:03	C*07:02	+++	DRB1*01:03	DRB1*04:04	+
MN12AF	AF	A*01:01	A*01:01	0	B*08:01	B*08:01	0	C*07:01	C*07:01	0	DRB1*03:01	DRB1*03:01	0
MN12UN	UN	A*01:01	A*01:01	0	B*08:01	B*08:01	0	C*07:01	C*07:01	0	DRB1*03:01	DRB1*03:01	0
MN13AF	AF	A*03:01	A*02:01	+++	B*44:02	B*07:02	+++	C*07:02	C*05:01	+++	DRB1*01:03	DRB1*04:01	++
MN13UN	UN	A*11:01	A*11:01	++	B*44:02	B*35:01	0	C*04:01	C*05:01	0	DRB1*04:08	DRB1*14:103	0
MN14AF	AF	A*01:01	A*01:01	0	B*08:01	B*08:01	0	C*07:01	C*07:01	0	DRB1*03:01	DRB1*03:01	0
MN14UN	UN	A*01:01	A*03:01	+++	B*08:01	B*35:03	0	C*07:01	C*04:01	0	DRB1*03:01	DRB1*10:01	0
MN15AF	AF	A*03:01	A*02:01	+++	B*18:01	B*57:01	0	C*06:02	C*07:01	0	DRB1*11:04	DRB1*07:01	0
MN15UN	UN	A*32:01	A*02:01	0	B*44:02	B*15:01	0	C*05:01	C*03:03	0	DRB1*12:01	DRB1*04:01	0
MN16AF	AF	A*30:02	A*03:01	+++	B*18:01	B*53:01	0	C*04:01	C*05:01	0	DRB1*11:01	DRB1*03:17	0
MN16UN	UN	A*30:02	A*03:01	+++	B*18:01	B*53:01	0	C*04:01	C*05:01	0	DRB1*11:01	DRB1*03:17	0
MN17AF	AF	A*02:01	A*03:01	0	B*44:02	B*15:01	0	C*05:01	C*03:03	0	DRB1*01:03	DRB1*04:01	++
MN17UN1	UN	A*01:01	A*01:01	0	B*08:01	B*52:01	0	C*07:01	C*12:02	0	DRB1*03:01	DRB1*15:02	+++
MN17UN2	UN	A*01:01	A*01:01	0	B*08:01	B*52:01	0	C*07:01	C*12:02	0	DRB1*03:01	DRB1*15:02	+++
MN18AFC	AF	A*02:01	A*01:01	0	B*57:01	B*35:01	0	C*06:02	C*18:01	0	DRB1*11:01	DRB1*07:01	0

MN18AFU	AF	A*02:01	A*31:01	0	B*57:01	B*40:01	++	C*06:02	C*03:04	0	DRB1*11:01	DRB1*07:01	0
MN19AF	AF	A*24:02	A*02:01	0	B*49:01	B*15:01	+	C*07:01	C*03:04	0	DRB1*11:02	DRB1*01:01	+
MN19UN	UN	A*25:01	A*02:01	0	B*18:01	B*15:01	0	C*12:03	C*03:04	0	DRB1*07:01	DRB1*01:01	+
MN20AF	AF	A*30:04	A*03:01	+++	B*51:08	B*35:01	0	C*04:01	C*16:02	0	DRB1*01:01	DRB1*13:02	+
MN20UN	UN	A*31:01	A*02:01	0	B*51:01	B*44:02	0	C*15:02	C*16:01	0	DRB1*04:07	DRB1*07:01	0
MN21AF	AF	A*68:01	A*02:01	0	B*51:01	B*40:01	++	C*16:02	C*03:04	0	DRB1*08:01	DRB1*04:07	0
MN21UNa	UN	A*68:01	A*02:01	0	B*51:01	B*40:01	++	C*16:02	C*03:04	0	DRB1*08:01	DRB1*04:07	0
MN21UNb	UN	A*68:01	A*02:01	0	B*51:01	B*40:01	++	C*16:02	C*03:04	0	DRB1*08:01	DRB1*04:07	0
MN22AF	AF	A*01:01	A*03:01	+++	B*37:01	B*07:02	+++	C*07:02	C*06:02	+++	DRB1*15:01	DRB1*14:01	++
MN22UN	UN	A*01:01	A*11:01	+	B*08:01	B*35:01	0	C*07:01	C*04:01	0	DRB1*03:01	DRB1*14:01	0
MN23AF	AF	A*24:02	A*02:01	0	B*44:02	B*18:01	0	C*16:04	C*07:01	0	DRB1*11:04	DRB1*13:01	0
MN23UNa	UN	A*24:02	A*02:01	0	B*44:02	B*18:01	0	C*16:04	C*07:01	0	DRB1*11:04	DRB1*13:01	0
MN23UNb	UN	A*24:02	A*24:02	0	B*44:02	B*51:01	0	C*16:04	C*01:02	0	DRB1*11:04	DRB1*03:01	0
MN24AF	AF	A*24:02	A*03:01	+++	B*57:01	B*07:02	+++	C*06:02	C*07:02	+++	DRB1*15:01	DRB1*07:01	+++
MN24UN	UN	A*32:01	A*02:01	0	B*38:01	B*40:02	+	C*02:02	C*12:03	0	DRB1*10:01	DRB1*07:01	0
MN25AF1	AF	A*03:01	A*03:01	+++	B*07:02	B*47:01	+++	C*07:02	C*06:02	+++	DRB1*15:01	DRB1*04:05	+++
MN25AF2	AF	A*11:01	A*03:01	+++	B*18:01	B*47:01	0	C*07:01	C*06:02	0	DRB1*15:01	DRB1*04:05	+++
MN25UN	UN	A*03:01	A*03:01	+++	B*07:02	B*35:01	++	C*07:02	C*04:01	++	DRB1*15:01	DRB1*01:01	+++
MN26AF	AF	A*01:01	A*02:01	0	B*44:02	B*57:01	0	C*06:02	C*05:01	0	DRB1*04:01	DRB1*07:01	0
MN26UN	UN	A*30:02	A*31:01	+	B*35:01	B*18:01	0	C*04:01	C*05:01	0	DRB1*04:07	DRB1*03:01	0
MN27AF	AF	A*03:01	A*24:02	+++	B*08:01	B*47:01	0	C*06:02	C*07:06	0	DRB1*03:01	DRB1*03:01	0
MN27UN1	UN	A*03:01	A*24:02	++	B*08:01	B*47:01	0	C*06:02	C*07:06	0	DRB1*03:01	DRB1*03:01	0
MN27UN2	UN	A*03:01	A*24:02	++	B*08:01	B*47:01	0	C*06:02	C*07:01	0	DRB1*03:01	DRB1*03:01	0
MN2HD	HD	A*01:02	A*02:01	0	B*08:01	B*44:02	0	C*05:01	C*07:01	0	DRB1*15:01	DRB1*03:01	+++
MN5HD	HD	A*03:01	A*24:02	++	B*38:01	B*35:03	0	C*04:01	C*12:03	0	DRB1*15:02	DRB1*13:01	++
MN7HD	HD	A*68:01	A*24:02	0	B*51:01	B*07:02	+++	C*14:02	C*07:02	+++	DRB1*15:01	DRB1*10:01	+++
MN8HD	HD	A*03:01	A*02:01	++	B*08:01	B*44:02	0	C*05:01	C*07:01	0	DRB1*04:01	DRB1*07:01	0
MN9HDa	HD	A*32:01	A*02:05	0	B*35:03	B*57:01	0	C*18:02	C*06:02	0	DRB1*11:01	DRB1*07:01	0
MN9HDb	HD	A*32:01	A*02:01	0	B*35:03	B*08:01	0	C*04:01	C*07:01	0	DRB1*11:01	DRB1*03:01	0

Table 2:

Macrophage-related function of candidate IBD susceptibility genes

Genes below were shown to have coding sequence variants significantly linked to IBD susceptibility in large-scale whole genome sequencing analysis of affected individuals and controls [11]. Comments relate to expression in monocytes and macrophages in the current dataset and/or FANTOM5 CAGE data analyzed previously [18]. Selected references highlight known functions of gene of interest and/or neighbouring genes in macrophages or inflammation.

Gene name	**Function/pathway**	**Comments**	References
NOD2	CARD15. Pathogen pattern recognition	Several coding variants. *NOD2* mRNA high in monocytes, down-regulated by CSF1. Co-regulated with *SNX20* and *CYLD*	[87, 88]
ATG16L1	Autophagy	T300A coding variant. Mechanism/function still unclear. Widely-expressed. Neighbouring *INPP5D* (SHIP1) gene high in monocytes, down-regulated by CSF1.	[89, 90]
SLC39A8	Zinc transporter (ZIP8)	Monocyte/macrophage specific. Highly-inducible by LPS Note adjacent *NFKB1*, also inducible by LPS. Highly variable expression	[91, 92]
TYK2	Tyrosine kinase	Broad expression. Neighbouring gene *ICAM3* high in monocytes, down-regulated by CSF1, implicated in IBD	[93]
IFIH1	MDA5. Anti-viral defense	Strongly induced by LPS in monocytes and MDM. Variable expression. Link to viral triggers?	[94, 95]
LRRK2	Multifunctional kinase. PARK8/Dardarin/RIPK7	High in monocytes and granulocytes. Down-regulated by CSF1. Regulator of phagosome-lysosome homeostasis	[96-98]
SLAMF8	Cell surface receptor (CD353)	Undetectable in monocytes, induced by CSF1. Inhibitor of reactive oxygen production & inflammation	[99, 100]
PLCG2	Phospholipase/eicosanoid regulation	Coding mutants in autoinflammatory diseases. Constitutive in monocytes and MDM. Downstream regulator of CSF1 signals	[101]
CARD9	Signalling adaptor protein	High in monocytes. Repressed by CSF1 and by LPS. Regulation of *IL1B* and inflammasome activation	[102]
ADCY7	Adenylate cyclase	Monocyte-macrophage specific promoter. Regulates LPS responsiveness. Coding variants associated with ulcerative colitis	[103, 104]

GPR65	pH-sensing receptor	High in monocytes and granulocytes, Down-regulated by CSF1. Regulator of inflammation	[105]
SMAD3	Transcription factor	High in monocytes, down-regulated by CSF1. Regulator of inflammation	[106]
IL10RA	IL10 receptor	High in monocytes, down-regulated by CSF1 but highly-induced by LPS and variable. Mutation in early-onset IBD	[107]
PTAFR	Platelet activating factor receptor	High in monocytes, down-regulated by CSF1.	[108]
TAGAP	RhoGTPase activating protein	High in monocytes, down-regulated by CSF1, induced LPS, highly variable. Shared risk with celiac disease.	[109]
IRGM	Putative regulator of autophagy.	No detectable mRNA in any tissue Neighbouring *TNIP1* (ABIN3) highly-expressed in MDM, induced further by LPS. Binding partner of TNFAIP3	[110]
RELA	NFkB transcription factor	Major transducer of LPS signaling. High in monocytes and MDM, further induced by LPS	[54]
DOK2	Adaptor protein, regulator of tyrosine kinase signals	High in monocytes, down-regulated by CSF1. Further repressed by LPS	[111]
CCR7	Chemokine receptor	Induced around 500-fold by LPS by 21 hrs. Very variable between individuals. Major ligand, CCL19, also induced >1000-fold. Generation of tertiary lymphoid structures	[112]

References

1. Ahmad T, Marshall SE, Jewell D. Genetics of inflammatory bowel disease: the role of the HLA complex. World J Gastroenterol. 2006;12(23):3628-35. doi: 10.3748/wjg.v12.i23.3628. PubMed PMID: 16773677;
2. Ashton JJ, Latham K, Beattie RM, Ennis S. Review article: the genetics of the human leucocyte antigen region in inflammatory bowel disease. Aliment Pharmacol Ther. 2019;50(8):885-900. doi: 10.1111/apt.15485. PubMed PMID: 31518029;
3. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012;491(7422):119-24. doi: 10.1038/nature11582. PubMed PMID: 23128233;
4. Nambu R, Warner N, Mulder DJ, Kotlarz D, McGovern DPB, Cho J, et al. A Systematic Review of Monogenic Inflammatory Bowel Disease. Clin Gastroenterol Hepatol. 2022;20(4):e653-e63. doi: 10.1016/j.cgh.2021.03.021. PubMed PMID: 33746097;
5. Loddo I, Romano C. Inflammatory Bowel Disease: Genetics, Epigenetics, and Pathogenesis. Front Immunol. 2015;6:551. doi: 10.3389/fimmu.2015.00551. PubMed PMID: 26579126;
6. Liu JZ, van Sommeren S, Huang H, Ng SC, Alberts R, Takahashi A, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet. 2015;47(9):979-86. doi: 10.1038/ng.3359. PubMed PMID: 26192919;
7. Lee JC, Biasci D, Roberts R, Gearry RB, Mansfield JC, Ahmad T, et al. Genome-wide association study identifies distinct genetic contributions to prognosis and susceptibility in Crohn's disease. Nat Genet. 2017;49(2):262-8. doi: 10.1038/ng.3755. PubMed PMID: 28067912;

8. Huang H, Fang M, Jostins L, Umicevic Mirkov M, Boucher G, Anderson CA, et al. Fine-mapping inflammatory bowel disease loci to single-variant resolution. Nature. 2017;547(7662):173-8. doi: 10.1038/nature22969. PubMed PMID: 28658209;
9. Somineni HK, Nagpal S, Venkateswaran S, Cutler DJ, Okou DT, Haritunians T, et al. Whole-genome sequencing of African Americans implicates differential genetic architecture in inflammatory bowel disease. Am J Hum Genet. 2021;108(3):431-45. doi: 10.1016/j.ajhg.2021.02.001. PubMed PMID: 33600772;
10. Momozawa Y, Dmitrieva J, Theatre E, Deffontaine V, Rahmouni S, Charloteaux B, et al. IBD risk loci are enriched in multigenic regulatory modules encompassing putative causative genes. Nat Commun. 2018;9(1):2427. doi: 10.1038/s41467-018-04365-8. PubMed PMID: 29930244;
11. Sazonovs A, Stevens CR, Venkataraman GR, Yuan K, Avila B, Abreu MT, et al. Large-scale sequencing identifies multiple genes and rare variants associated with Crohn's disease susceptibility. Nat Genet. 2022;54(9):1275-83. doi: 10.1038/s41588-022-01156-2. PubMed PMID: 36038634;
12. Richard AC, Peters JE, Savinykh N, Lee JC, Hawley ET, Meylan F, et al. Reduced monocyte and macrophage TNFSF15/TL1A expression is associated with susceptibility to inflammatory bowel disease. PLoS Genet. 2018;14(9):e1007458. doi: 10.1371/journal.pgen.1007458. PubMed PMID: 30199539;
13. Klunk J, Vilgalys TP, Demeure CE, Cheng X, Shiratori M, Madej J, et al. Evolution of immune genes is associated with the Black Death. Nature. 2022;611(7935):312-9. doi: 10.1038/s41586-022-05349-x. PubMed PMID: 36261521;
14. Di Narzo AF, Peters LA, Argmann C, Stojmirovic A, Perrigoue J, Li K, et al. Blood and Intestine eQTLs from an Anti-TNF-Resistant Crohn's Disease Cohort Inform IBD Genetic

Association Loci. Clin Transl Gastroenterol. 2016;7(6):e177. doi: 10.1038/ctg.2016.34. PubMed PMID: 27336838;

15. Mowat AM, Scott CL, Bain CC. Barrier-tissue macrophages: functional adaptation to environmental challenges. Nat Med. 2017;23(11):1258-70. doi: 10.1038/nm.4430. PubMed PMID: 29117177;
16. Joeris T, Muller-Luda K, Agace WW, Mowat AM. Diversity and functions of intestinal mononuclear phagocytes. Mucosal Immunol. 2017;10(4):845-64. doi: 10.1038/mi.2017.22. PubMed PMID: 28378807;
17. MacDonald KP, Palmer JS, Cronau S, Seppanen E, Olver S, Raffelt NC, et al. An antibody against the colony-stimulating factor 1 receptor depletes the resident subset of monocytes and tissue- and tumor-associated macrophages but does not inhibit inflammation. Blood. 2010;116(19):3955-63. doi: 10.1182/blood-2010-02-266296. PubMed PMID: 20682855;
18. Baillie JK, Arner E, Daub C, De Hoon M, Itoh M, Kawaji H, et al. Analysis of the human monocyte-derived macrophage transcriptome and response to lipopolysaccharide provides new insights into genetic aetiology of inflammatory bowel disease. PLoS Genet. 2017;13(3):e1006641. doi: 10.1371/journal.pgen.1006641. PubMed PMID: 28263993;
19. Baillie JK, Bretherick A, Haley CS, Clohisey S, Gray A, Neyton LPA, et al. Shared activity patterns arising at genetic susceptibility loci reveal underlying genomic and cellular architecture of human disease. PLoS Comput Biol. 2018;14(3):e1005934. doi: 10.1371/journal.pcbi.1005934. PubMed PMID: 29494619;
20. Halme L, Paavola-Sakki P, Turunen U, Lappalainen M, Farkkila M, Kontula K. Family and twin studies in inflammatory bowel disease. World J Gastroenterol. 2006;12(23):3668-72. doi: 10.3748/wjg.v12.i23.3668. PubMed PMID: 16773682;

21. Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. Gastroenterology. 2011;140(6):1704-12. doi: 10.1053/j.gastro.2011.02.046. PubMed PMID: 21530736;
22. Fairfax BP, Humburg P, Makino S, Naranbhai V, Wong D, Lau E, et al. Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. Science. 2014;343(6175):1246949. doi: 10.1126/science.1246949. PubMed PMID: 24604202;
23. Fan J, Hu J, Xue C, Zhang H, Susztak K, Reilly MP, et al. ASEP: Gene-based detection of allele-specific expression across individuals in a population by RNA sequencing. PLoS Genet. 2020;16(5):e1008786. doi: 10.1371/journal.pgen.1008786. PubMed PMID: 32392242;
24. Brandt M, Kim-Hellmuth S, Ziosi M, Gokden A, Wolman A, Lam N, et al. An autoimmune disease risk variant: A trans master regulatory effect mediated by IRF1 under immune stimulation? PLoS Genet. 2021;17(7):e1009684. doi: 10.1371/journal.pgen.1009684. PubMed PMID: 34314424;
25. Kim-Hellmuth S, Aguet F, Oliva M, Munoz-Aguirre M, Kasela S, Wucher V, et al. Cell type-specific genetic regulation of gene expression across human tissues. Science. 2020;369(6509). doi: 10.1126/science.aaz8528. PubMed PMID: 32913075;
26. Kim-Hellmuth S, Bechheim M, Putz B, Mohammadi P, Nedelec Y, Giangreco N, et al. Genetic regulatory effects modified by immune activation contribute to autoimmune disease associations. Nat Commun. 2017;8(1):266. doi: 10.1038/s41467-017-00366-1. PubMed PMID: 28814792;
27. Hume DA, Freeman TC. Transcriptomic analysis of mononuclear phagocyte differentiation and activation. Immunol Rev. 2014;262(1):74-84. doi: 10.1111/imr.12211. PubMed PMID: 25319328;

28. Schroder K, Irvine KM, Taylor MS, Bokil NJ, Le Cao KA, Masterman KA, et al. Conservation and divergence in Toll-like receptor 4-regulated gene expression in primary human versus mouse macrophages. Proc Natl Acad Sci U S A. 2012;109(16):E944-53. doi: 10.1073/pnas.1110156109. PubMed PMID: 22451944;
29. Bush SJ, McCulloch MEB, Lisowski ZM, Muriuki C, Clark EL, Young R, et al. Species-Specificity of Transcriptional Regulation and the Response to Lipopolysaccharide in Mammalian Macrophages. Front Cell Dev Biol. 2020;8:661. doi: 10.3389/fcell.2020.00661. PubMed PMID: 32793601;
30. Theocharidis A, van Dongen S, Enright AJ, Freeman TC. Network visualization and analysis of gene expression data using BioLayout Express(3D). Nat Protoc. 2009;4(10):1535-50. doi: 10.1038/nprot.2009.177. PubMed PMID: 19798086;
31. Gazova I, Lefevre L, Bush SJ, Clohisey S, Arner E, de Hoon M, et al. The Transcriptional Network That Controls Growth Arrest and Macrophage Differentiation in the Human Myeloid Leukemia Cell Line THP-1. Front Cell Dev Biol. 2020;8:498. doi: 10.3389/fcell.2020.00498. PubMed PMID: 32719792;
32. Sarrazin S, Mossadegh-Keller N, Fukao T, Aziz A, Mourcin F, Vanhille L, et al. MafB restricts M-CSF-dependent myeloid commitment divisions of hematopoietic stem cells. Cell. 2009;138(2):300-13. doi: 10.1016/j.cell.2009.04.057. PubMed PMID: 19632180;
33. Schuler A, Schwieger M, Engelmann A, Weber K, Horn S, Muller U, et al. The MADS transcription factor Mef2c is a pivotal modulator of myeloid cell fate. Blood. 2008;111(9):4532-41. doi: 10.1182/blood-2007-10-116343. PubMed PMID: 18326819;
34. Martin JC, Chang C, Boschetti G, Ungaro R, Giri M, Grout JA, et al. Single-Cell Analysis of Crohn's Disease Lesions Identifies a Pathogenic Cellular Module Associated with Resistance to Anti-TNF Therapy. Cell. 2019;178(6):1493-508 e20. doi: 10.1016/j.cell.2019.08.008. PubMed PMID: 31474370;

35. Knight JC. Genomic modulators of the immune response. Trends Genet. 2013;29(2):74-83. doi: 10.1016/j.tig.2012.10.006. PubMed PMID: 23122694;
36. Clanchy FI, Holloway AC, Lari R, Cameron PU, Hamilton JA. Detection and properties of the human proliferative monocyte subpopulation. J Leukoc Biol. 2006;79(4):757-66. doi: 10.1189/jlb.0905522. PubMed PMID: 16434691;
37. Giotti B, Chen SH, Barnett MW, Regan T, Ly T, Wiemann S, et al. Assembly of a parts list of the human mitotic cell cycle machinery. J Mol Cell Biol. 2019;11(8):703-18. doi: 10.1093/jmcb/mjy063. PubMed PMID: 30452682;
38. Wang G, Yang E, Mandhan I, Brinkmeyer-Langford CL, Cai JJ. Population-level expression variability of mitochondrial DNA-encoded genes in humans. Eur J Hum Genet. 2014;22(9):1093-9. doi: 10.1038/ejhg.2013.293. PubMed PMID: 24398800;
39. Sewell GW, Kaser A. Interleukin-23 in the Pathogenesis of Inflammatory Bowel Disease and Implications for Therapeutic Intervention. J Crohns Colitis. 2022;16(Supplement_2):ii3-ii19. doi: 10.1093/ecco-jcc/jjac034. PubMed PMID: 35553667;
40. Parigi TL, Iacucci M, Ghosh S. Blockade of IL-23: What is in the Pipeline? J Crohns Colitis. 2022;16(Supplement_2):ii64-ii72. doi: 10.1093/ecco-jcc/jjab185. PubMed PMID: 35553666;
41. Ecoeur F, Weiss J, Schleeger S, Guntermann C. Lack of evidence for expression and function of IL-39 in human immune cells. PLoS One. 2020;15(12):e0242329. doi: 10.1371/journal.pone.0242329. PubMed PMID: 33259477;
42. de Marcken M, Dhaliwal K, Danielsen AC, Gautron AS, Dominguez-Villar M. TLR7 and TLR8 activate distinct pathways in monocytes during RNA virus infection. Sci Signal. 2019;12(605). doi: 10.1126/scisignal.aaw1347. PubMed PMID: 31662487;

43. Albagha OM, Visconti MR, Alonso N, Langston AL, Cundy T, Dargie R, et al. Genome-wide association study identifies variants at CSF1, OPTN and TNFRSF11A as genetic risk factors for Paget's disease of bone. Nat Genet. 2010;42(6):520-4. doi: 10.1038/ng.562. PubMed PMID: 20436471;
44. Lu JH, Teh BK, Wang L, Wang YN, Tan YS, Lai MC, et al. The classical and regulatory functions of C1q in immunity and autoimmunity. Cell Mol Immunol. 2008;5(1):9-21. doi: 10.1038/cmi.2008.2. PubMed PMID: 18318990;
45. Potter BJ, Hodgson HJ, Mee AS, Jewell DP. Clq metabolism in ulcerative colitis and Crohn's disease. Gut. 1979;20(11):1012-9. doi: 10.1136/gut.20.11.1012. PubMed PMID: 527870;
46. Fairfax BP, Makino S, Radhakrishnan J, Plant K, Leslie S, Dilthey A, et al. Genetics of gene expression in primary immune cells identifies cell type-specific master regulators and roles of HLA alleles. Nat Genet. 2012;44(5):502-10. doi: 10.1038/ng.2205. PubMed PMID: 22446964;
47. Sul JH, Martin LS, Eskin E. Population structure in genetic studies: Confounding factors and mixed models. PLoS Genet. 2018;14(12):e1007309. doi: 10.1371/journal.pgen.1007309. PubMed PMID: 30589851;
48. Chelysheva I, Pollard AJ, O'Connor D. RNA2HLA: HLA-based quality control of RNA-seq datasets. Brief Bioinform. 2021;22(5). doi: 10.1093/bib/bbab055. PubMed PMID: 33758920;
49. Lopez de Castro JA. How ERAP1 and ERAP2 Shape the Peptidomes of Disease-Associated MHC-I Proteins. Front Immunol. 2018;9:2463. doi: 10.3389/fimmu.2018.02463. PubMed PMID: 30425713;
50. Huang Z, Rose AH, Hoffmann FW, Hashimoto AS, Bertino P, Denk T, et al. Calpastatin prevents NF-kappaB-mediated hyperactivation of macrophages and attenuates colitis. J

Immunol. 2013;191(7):3778-88. doi: 10.4049/jimmunol.1300972. PubMed PMID: 23986533;

51. Aschenbrenner D, Quaranta M, Banerjee S, Ilott N, Jansen J, Steere B, et al. Deconvolution of monocyte responses in inflammatory bowel disease reveals an IL-1 cytokine network that regulates IL-23 in genetic and acquired IL-10 resistance. Gut. 2021;70(6):1023-36. doi: 10.1136/gutjnl-2020-321731. PubMed PMID: 33037057;
52. Schmitt H, Neurath MF, Atreya R. Role of the IL23/IL17 Pathway in Crohn's Disease. Front Immunol. 2021;12:622934. doi: 10.3389/fimmu.2021.622934. PubMed PMID: 33859636;
53. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014;6(10):a016295. doi: 10.1101/cshperspect.a016295. PubMed PMID: 25190079;
54. Papoutsopoulou S, Burkitt MD, Bergey F, England H, Hough R, Schmidt L, et al. Macrophage-Specific NF-kappaB Activation Dynamics Can Segregate Inflammatory Bowel Disease Patients. Front Immunol. 2019;10:2168. doi: 10.3389/fimmu.2019.02168. PubMed PMID: 31572379;
55. Consortium F, the RP, Clst, Forrest AR, Kawaji H, Rehli M, et al. A promoter-level mammalian expression atlas. Nature. 2014;507(7493):462-70. doi: 10.1038/nature13182. PubMed PMID: 24670764;
56. Raj P, Rai E, Song R, Khan S, Wakeland BE, Viswanathan K, et al. Regulatory polymorphisms modulate the expression of HLA class II molecules and promote autoimmunity. Elife. 2016;5. doi: 10.7554/eLife.12089. PubMed PMID: 26880555;
57. McNamee EN, Rivera-Nieves J. Ectopic Tertiary Lymphoid Tissue in Inflammatory Bowel Disease: Protective or Provocateur? Front Immunol. 2016;7:308. doi: 10.3389/fimmu.2016.00308. PubMed PMID: 27579025;

58. Cantor MJ, Nickerson P, Bernstein CN. The role of cytokine gene polymorphisms in determining disease susceptibility and phenotype in inflammatory bowel disease. Am J Gastroenterol. 2005;100(5):1134-42. doi: 10.1111/j.1572-0241.2005.40979.x. PubMed PMID: 15842590;
59. Spence JS, He R, Hoffmann HH, Das T, Thinon E, Rice CM, et al. IFITM3 directly engages and shuttles incoming virus particles to lysosomes. Nat Chem Biol. 2019;15(3):259-68. doi: 10.1038/s41589-018-0213-2. PubMed PMID: 30643282;
60. Clohisey S, Parkinson N, Wang B, Bertin N, Wise H, Tomoiu A, et al. Comprehensive Characterization of Transcriptional Activity during Influenza A Virus Infection Reveals Biases in Cap-Snatching of Host RNA Sequences. J Virol. 2020;94(10). doi: 10.1128/JVI.01720-19. PubMed PMID: 32161175;
61. Everitt AR, Clare S, Pertel T, John SP, Wash RS, Smith SE, et al. IFITM3 restricts the morbidity and mortality associated with influenza. Nature. 2012;484(7395):519-23. doi: 10.1038/nature10921. PubMed PMID: 22446628;
62. Alteber Z, Sharbi-Yunger A, Pevsner-Fischer M, Blat D, Roitman L, Tzehoval E, et al. The anti-inflammatory IFITM genes ameliorate colitis and partially protect from tumorigenesis by changing immunity and microbiota. Immunol Cell Biol. 2018;96(3):284-97. doi: 10.1111/imcb.12000. PubMed PMID: 29356071;
63. Wells CA, Ravasi T, Hume DA. Inflammation suppressor genes: please switch out all the lights. J Leukoc Biol. 2005;78(1):9-13. doi: 10.1189/jlb.1204710. PubMed PMID: 15774547;
64. Kondo T, Kawai T, Akira S. Dissecting negative regulation of Toll-like receptor signaling. Trends Immunol. 2012;33(9):449-58. doi: 10.1016/j.it.2012.05.002. PubMed PMID: 22721918;

65. Krause P, Morris V, Greenbaum JA, Park Y, Bjoerheden U, Mikulski Z, et al. IL-10-producing intestinal macrophages prevent excessive antibacterial innate immunity by limiting IL-23 synthesis. Nat Commun. 2015;6:7055. doi: 10.1038/ncomms8055. PubMed PMID: 25959063;
66. Moran CJ, Walters TD, Guo CH, Kugathasan S, Klein C, Turner D, et al. IL-10R polymorphisms are associated with very-early-onset ulcerative colitis. Inflamm Bowel Dis. 2013;19(1):115-23. doi: 10.1002/ibd.22974. PubMed PMID: 22550014;
67. Alrumaihi F. The Multi-Functional Roles of CCR7 in Human Immunology and as a Promising Therapeutic Target for Cancer Therapeutics. Front Mol Biosci. 2022;9:834149. doi: 10.3389/fmolb.2022.834149. PubMed PMID: 35874608;
68. Hubo M, Trinschek B, Kryczanowsky F, Tuettenberg A, Steinbrink K, Jonuleit H. Costimulatory molecules on immunogenic versus tolerogenic human dendritic cells. Front Immunol. 2013;4:82. doi: 10.3389/fimmu.2013.00082. PubMed PMID: 23565116;
69. Summers KM, Bush SJ, Hume DA. Network analysis of transcriptomic diversity amongst resident tissue macrophages and dendritic cells in the mouse mononuclear phagocyte system. PLoS Biol. 2020;18(10):e3000859. doi: 10.1371/journal.pbio.3000859. PubMed PMID: 33031383;
70. Jang MH, Sougawa N, Tanaka T, Hirata T, Hiroi T, Tohya K, et al. CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes. J Immunol. 2006;176(2):803-10. doi: 10.4049/jimmunol.176.2.803. PubMed PMID: 16393963;
71. Yrlid U, Jenkins CD, MacPherson GG. Relationships between distinct blood monocyte subsets and migrating intestinal lymph dendritic cells in vivo under steady-state conditions. J Immunol. 2006;176(7):4155-62. doi: 10.4049/jimmunol.176.7.4155. PubMed PMID: 16547252;

72. Satsangi J, Welsh KI, Bunce M, Julier C, Farrant JM, Bell JI, et al. Contribution of genes of the major histocompatibility complex to susceptibility and disease phenotype in inflammatory bowel disease. Lancet. 1996;347(9010):1212-7. doi: 10.1016/s0140-6736(96)90734-5. PubMed PMID: 8622450;
73. Goyette P, Boucher G, Mallon D, Ellinghaus E, Jostins L, Huang H, et al. High-density mapping of the MHC identifies a shared role for HLA-DRB1*01:03 in inflammatory bowel diseases and heterozygous advantage in ulcerative colitis. Nat Genet. 2015;47(2):172-9. doi: 10.1038/ng.3176. PubMed PMID: 25559196;
74. Jung ES, Cheon JH, Lee JH, Park SJ, Jang HW, Chung SH, et al. HLA-C*01 is a Risk Factor for Crohn's Disease. Inflamm Bowel Dis. 2016;22(4):796-806. doi: 10.1097/MIB.0000000000000693. PubMed PMID: 26891255;
75. Vince N, Li H, Ramsuran V, Naranbhai V, Duh FM, Fairfax BP, et al. HLA-C Level Is Regulated by a Polymorphic Oct1 Binding Site in the HLA-C Promoter Region. Am J Hum Genet. 2016;99(6):1353-8. doi: 10.1016/j.ajhg.2016.09.023. PubMed PMID: 27817866;
76. Apps R, Qi Y, Carlson JM, Chen H, Gao X, Thomas R, et al. Influence of HLA-C expression level on HIV control. Science. 2013;340(6128):87-91. doi: 10.1126/science.1232685. PubMed PMID: 23559252;
77. Parham P. The genetic and evolutionary balances in human NK cell receptor diversity. Semin Immunol. 2008;20(6):311-6. doi: 10.1016/j.smim.2008.10.002. PubMed PMID: 19036608;
78. Papuchova H, Meissner TB, Li Q, Strominger JL, Tilburgs T. The Dual Role of HLA-C in Tolerance and Immunity at the Maternal-Fetal Interface. Front Immunol. 2019;10:2730. doi: 10.3389/fimmu.2019.02730. PubMed PMID: 31921098;

79. McClellan J, King MC. Genetic heterogeneity in human disease. Cell. 2010;141(2):210-7. doi: 10.1016/j.cell.2010.03.032. PubMed PMID: 20403315;

80. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. Nat Biotechnol. 2016;34(5):525-7. doi: 10.1038/nbt.3519. PubMed PMID: 27043002;

81. Love MI, Soneson C, Hickey PF, Johnson LK, Pierce NT, Shepherd L, et al. Tximeta: Reference sequence checksums for provenance identification in RNA-seq. PLoS Comput Biol. 2020;16(2):e1007664. doi: 10.1371/journal.pcbi.1007664. PubMed PMID: 32097405;

82. Bush SJ, Freem L, MacCallum AJ, O'Dell J, Wu C, Afrasiabi C, et al. Combination of novel and public RNA-seq datasets to generate an mRNA expression atlas for the domestic chicken. BMC Genomics. 2018;19(1):594. doi: 10.1186/s12864-018-4972-7. PubMed PMID: 30086717;

83. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81(3):559-75. doi: 10.1086/519795. PubMed PMID: 17701901;

84. Ginestet-C. ggplot2: Elegant Graphics for Data Analysis. JRoyal StatSoc Series A. 2011;164:245-6.

85. Shabalin AA. Matrix eQTL: ultra fast eQTL analysis via large matrix operations. Bioinformatics. 2012;28(10):1353-8. doi: 10.1093/bioinformatics/bts163. PubMed PMID: 22492648;

86. Wang T, Liu Y, Ruan J, Dong X, Wang Y, Peng J. A pipeline for RNA-seq based eQTL analysis with automated quality control procedures. BMC Bioinformatics. 2021;22(Suppl 9):403. doi: 10.1186/s12859-021-04307-0. PubMed PMID: 34433407;

87. Keestra-Gounder AM, Tsolis RM. NOD1 and NOD2: Beyond Peptidoglycan Sensing. Trends Immunol. 2017;38(10):758-67. doi: 10.1016/j.it.2017.07.004. PubMed PMID: 28823510;

88. Tang Y, Reissig S, Glasmacher E, Regen T, Wanke F, Nikolaev A, et al. Alternative Splice Forms of CYLD Mediate Ubiquitination of SMAD7 to Prevent TGFB Signaling and Promote Colitis. Gastroenterology. 2019;156(3):692-707 e7. doi: 10.1053/j.gastro.2018.10.023. PubMed PMID: 30315770;

89. Chesney KL, Men H, Hankins MA, Bryda EC. The Atg16l1 gene: characterization of wild type, knock-in, and knock-out phenotypes in rats. Physiol Genomics. 2021;53(6):269-81. doi: 10.1152/physiolgenomics.00114.2020. PubMed PMID: 33818130;

90. Fernandes S, Srivastava N, Sudan R, Middleton FA, Shergill AK, Ryan JC, et al. SHIP1 Deficiency in Inflammatory Bowel Disease Is Associated With Severe Crohn's Disease and Peripheral T Cell Reduction. Front Immunol. 2018;9:1100. doi: 10.3389/fimmu.2018.01100. PubMed PMID: 29872435;

91. Hall SC, Smith DR, Dyavar SR, Wyatt TA, Samuelson DR, Bailey KL, et al. Critical Role of Zinc Transporter (ZIP8) in Myeloid Innate Immune Cell Function and the Host Response against Bacterial Pneumonia. J Immunol. 2021;207(5):1357-70. doi: 10.4049/jimmunol.2001395. PubMed PMID: 34380651;

92. Kalita CA, Brown CD, Freiman A, Isherwood J, Wen X, Pique-Regi R, et al. High-throughput characterization of genetic effects on DNA-protein binding and gene transcription. Genome Res. 2018;28(11):1701-8. doi: 10.1101/gr.237354.118. PubMed PMID: 30254052;

93. Bernstein CN, Sargent M, Rawsthorne P, Rector E. Peripheral blood lymphocyte beta 2 integrin and ICAM expression in inflammatory bowel disease. Dig Dis Sci. 1997;42(11):2338-49. doi: 10.1023/a:1018887222296. PubMed PMID: 9398815;

94. Adiliaghdam F, Amatullah H, Digumarthi S, Saunders TL, Rahman RU, Wong LP, et al. Human enteric viruses autonomously shape inflammatory bowel disease phenotype through divergent innate immunomodulation. Sci Immunol. 2022;7(70):eabn6660. doi: 10.1126/sciimmunol.abn6660. PubMed PMID: 35394816;
95. Zervou MI, Andreou AC, Eliopoulos EE, Goulielmos GN. Functional significance of the rare rs35667974 IFIH1 gene polymorphism, associated with multiple autoimmune diseases, using a structural biological approach. Autoimmunity. 2022;55(7):455-61. doi: 10.1080/08916934.2022.2103799. PubMed PMID: 35918839;
96. de Guilhem de Lataillade A, Caillaud M, Oullier T, Naveilhan P, Pellegrini C, Tolosa E, et al. LRRK2 expression in normal and pathologic human gut and in rodent enteric neural cell lines. J Neurochem. 2022. doi: 10.1111/jnc.15704. PubMed PMID: 36219522;
97. Eguchi T, Kuwahara T, Sakurai M, Komori T, Fujimoto T, Ito G, et al. LRRK2 and its substrate Rab GTPases are sequentially targeted onto stressed lysosomes and maintain their homeostasis. Proc Natl Acad Sci U S A. 2018;115(39):E9115-E24. doi: 10.1073/pnas.1812196115. PubMed PMID: 30209220;
98. Lee H, Flynn R, Sharma I, Haberman E, Carling PJ, Nicholls FJ, et al. LRRK2 Is Recruited to Phagosomes and Co-recruits RAB8 and RAB10 in Human Pluripotent Stem Cell-Derived Macrophages. Stem Cell Reports. 2020;14(5):940-55. doi: 10.1016/j.stemcr.2020.04.001. PubMed PMID: 32359446;
99. Romero-Pinedo S, Barros DIR, Ruiz-Magana MJ, Maganto-Garcia E, Moreno de Lara L, Abadia-Molina F, et al. SLAMF8 Downregulates Mouse Macrophage Microbicidal Mechanisms via PI3K Pathways. Front Immunol. 2022;13:910112. doi: 10.3389/fimmu.2022.910112. PubMed PMID: 35837407;
100. Zeng X, Liu G, Peng W, He J, Cai C, Xiong W, et al. Combined deficiency of SLAMF8 and SLAMF9 prevents endotoxin-induced liver inflammation by downregulating TLR4

expression on macrophages. Cell Mol Immunol. 2020;17(2):153-62. doi: 10.1038/s41423-018-0191-z. PubMed PMID: 30552382;

101. Jing H, Reed A, Ulanovskaya OA, Grigoleit JS, Herbst DM, Henry CL, et al. Phospholipase Cgamma2 regulates endocannabinoid and eicosanoid networks in innate immune cells. Proc Natl Acad Sci U S A. 2021;118(41). doi: 10.1073/pnas.2112971118. PubMed PMID: 34607960;

102. Drummond RA, Desai JV, Hsu AP, Oikonomou V, Vinh DC, Acklin JA, et al. Human Dectin-1 deficiency impairs macrophage-mediated defense against phaeohyphomycosis. J Clin Invest. 2022;132(22). doi: 10.1172/JCI159348. PubMed PMID: 36377664;

103. Duan B, Davis R, Sadat EL, Collins J, Sternweis PC, Yuan D, et al. Distinct roles of adenylyl cyclase VII in regulating the immune responses in mice. J Immunol. 2010;185(1):335-44. doi: 10.4049/jimmunol.0903474. PubMed PMID: 20505140;

104. Luo Y, de Lange KM, Jostins L, Moutsianas L, Randall J, Kennedy NA, et al. Exploring the genetic architecture of inflammatory bowel disease by whole-genome sequencing identifies association at ADCY7. Nat Genet. 2017;49(2):186-92. doi: 10.1038/ng.3761. PubMed PMID: 28067910;

105. Tcymbarevich I, Richards SM, Russo G, Kuhn-Georgijevic J, Cosin-Roger J, Baebler K, et al. Lack of the pH-sensing Receptor TDAG8 [GPR65] in Macrophages Plays a Detrimental Role in Murine Models of Inflammatory Bowel Disease. J Crohns Colitis. 2019;13(2):245-58. doi: 10.1093/ecco-jcc/jjy152. PubMed PMID: 30535144;

106. Fowler SA, Ananthakrishnan AN, Gardet A, Stevens CR, Korzenik JR, Sands BE, et al. SMAD3 gene variant is a risk factor for recurrent surgery in patients with Crohn's disease. J Crohns Colitis. 2014;8(8):845-51. doi: 10.1016/j.crohns.2014.01.003. PubMed PMID: 24461721;

107. Kelsen JR, Dawany N, Moran CJ, Petersen BS, Sarmady M, Sasson A, et al. Exome sequencing analysis reveals variants in primary immunodeficiency genes in patients with very early onset inflammatory bowel disease. Gastroenterology. 2015;149(6):1415-24. doi: 10.1053/j.gastro.2015.07.006. PubMed PMID: 26193622;

108. Liu G, Baird AW, Parsons MJ, Fan K, Skerrett-Byrne DA, Nair PM, et al. Platelet activating factor receptor acts to limit colitis-induced liver inflammation. FASEB J. 2020;34(6):7718-32. doi: 10.1096/fj.201901779R. PubMed PMID: 32293760;

109. Festen EA, Goyette P, Green T, Boucher G, Beauchamp C, Trynka G, et al. A meta-analysis of genome-wide association scans identifies IL18RAP, PTPN2, TAGAP, and PUS10 as shared risk loci for Crohn's disease and celiac disease. PLoS Genet. 2011;7(1):e1001283. doi: 10.1371/journal.pgen.1001283. PubMed PMID: 21298027;

110. Zhou M, He J, Shi Y, Liu X, Luo S, Cheng C, et al. ABIN3 Negatively Regulates Necroptosis-induced Intestinal Inflammation Through Recruiting A20 and Restricting the Ubiquitination of RIPK3 in Inflammatory Bowel Disease. J Crohns Colitis. 2021;15(1):99-114. doi: 10.1093/ecco-jcc/jjaa131. PubMed PMID: 32599618;

111. Shinohara H, Inoue A, Toyama-Sorimachi N, Nagai Y, Yasuda T, Suzuki H, et al. Dok-1 and Dok-2 are negative regulators of lipopolysaccharide-induced signaling. J Exp Med. 2005;201(3):333-9. doi: 10.1084/jem.20041817. PubMed PMID: 15699069;

112. Koscso B, Kurapati S, Rodrigues RR, Nedjic J, Gowda K, Shin C, et al. Gut-resident CX3CR1(hi) macrophages induce tertiary lymphoid structures and IgA response in situ. Sci Immunol. 2020;5(46). doi: 10.1126/sciimmunol.aax0062. PubMed PMID: 32276965;