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REVIEWS

The Relationship Between CSF1R Signaling, Monocyte-Macrophage Differentiation, and Susceptibility to Inflammatory Bowel Disease

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SUMMARY

Here we argue that susceptibility to inflammatory bowel disease arises from dysregulation of monocyte adaptation in the intestinal milieu. Therapeutic targeting of ETS2, a myeloid-specific transcription factor, has the potential to prevent macrophage colony–stimulating factor-1-dependent monocyte differentiation into resident macrophages that are unresponsive to bacterial stimuli, and consequently may exacerbate intestinal inflammation.

More than 300 genomic loci have been associated with increased susceptibility to inflammatory bowel disease (IBD) through genome-wide association studies. A major challenge in the translation of genome-wide association studies to mechanistic insights lies in connecting non-coding variants to function. For example, single-nucleotide variants (SNVs) in the vicinity of the gene encoding the transcription factor ETS2 on human chromosome 21 are associated with the risk of developing IBD in Europeans. The peak of SNV association lies within a distal enhancer that may regulate *ETS2* transcription. The interpretation of this and many other SNV associations with IBD depends on a model linking variation in transcriptional regulation to the likelihood of developing chronic intestinal inflammation. One model for the *ETS2* locus is that overexpression in monocytes is causally associated with the risk allele, which in turn leads to a hyperinflammatory state. Here we summarize evidence for an alternative mechanism focused on negative regulators of monocyte-macrophage activation. We argue that IBD susceptibility arises from dysregulation of monocyte adaptation in the intestinal milieu to form resident intestinal macrophages that are anergic to inflammatory stimuli. This process depends on signals initiated by macrophage colony–stimulating factor (CSF1) binding to its receptor (CSF1R). Within this framework, ETS2 is a myeloid-specific transcription factor, expressed in pluripotent and committed progenitors and monocytes, and is down-regulated by CSF1, in common with many genes associated with IBD susceptibility, including *NOD2*. ETS2 is also both a downstream target and a mediator of the CSF1/CSF1R signaling pathway. Therapeutic targeting of ETS2 and its upstream regulators has the potential to prevent CSF1-dependent monocyte differentiation toward a prorepair resident macrophage phenotype and consequently exacerbate intestinal inflammation. *(Cell Mol Gastroenterol Hepatol 2025;19:101510; https://doi.org/10.1016/j.jcmgh.2025.101510)*

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Genome-wide association studies (GWAS) of inflammatory bowel disease (IBD) susceptibility have identified hundreds of genomic loci linked to increased risk.[1] In considering the relationship between genotype and IBD susceptibility it is important to recognize that known susceptibility loci account for only a fraction of the estimated heritability and that polygenic risk scores have limited predictive value.[2] At least part of the explanation lies in gene-by-environment interaction,[3,4] the idea that a genetically susceptible individual only develops IBD if exposed to an environmental trigger. Dissection of the mechanism whereby individual single-nucleotide variants (SNV) contribute to increased IBD susceptibility has been challenging because most variants do not alter protein-coding sequences. The obvious exception is *NOD2*, where 3 major loss of function mutations are associated with the greatest increased risk of developing IBD. *NOD2* encodes an intracellular receptor for muramyl dipeptide, derived from microbial peptidoglycans. Even among individuals with homozygous mutations, most do not develop IBD.[5] Analysis of a mouse model of NOD2 deficiency highlighted the gene-by-environment interaction and gene-gene interaction. Mice with a combined deficiency of NOD2 and another IBD susceptibility gene, NADPH oxidase (*CYBB*), developed colitis that was dependent on

Abbreviations used in this paper: eQTL, expression quantitative trait locus; GWAS, genome-wide association studies; IBD, inflammatory bowel disease; IL, interleukin; LPS, lipopolysaccharide; MDM, monocyte-derived macrophages; SNV, single-nucleotide variants.

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differences in microbiota between mice obtained from different commercial suppliers.[6]

Genomic intervals identified by GWAS commonly contain multiple coding genes and even peaks of association identified by statistical fine mapping contain multiple SNVs in linkage disequilibrium.[1] Increasingly, genome-wide analysis of transcriptional regulatory elements provides a link between risk variants and candidate target genes, which may not be the most proximal to the variant.[7,8] The hypothesis that risk variants in a particular locus act by altering transcriptional regulation leads to multiple questions: (1) Which SNVs are causal? (2) Which genes are regulated? (3) What signals control transcriptional regulation and how is the response altered by risk variants? (4) How does a difference in transcription increase the likelihood of developing chronic disease? (5) How can the inferred mechanism be translated into therapeutic interventions? These questions are a significant component of the grand challenges for IBD genetics and genomics identified in a recent overview by leaders in the field.[1]

One recent study highlights those challenges. This study examined the molecular basis for an association between genetic variation on Chr21 and an increase in the risk of developing IBD, through an effect on the *ETS2* gene.[9] In this brief review we propose an alternative interpretation of that association and a framework for understanding the relationship between monocyte-macrophage differentiation in the intestinal mucosa and IBD susceptibility. The difference in interpretation has important implications for potential therapies.

ETS2 Transcription is Controlled by a Super-enhancer

Like most loci linked to IBD susceptibility,[1] the peak of genetic association on Chr21 with IBD covers multiple linked SNVs (a block of linkage disequilibrium or haplotype) within noncoding DNA. Stankey et al[9] presented evidence that a single variant within the risk haplotype identified by statistical fine mapping, the common G allele at rs2836882, is associated with the activity of an enhancer element that controls monocyte transcription of the *ETS2* gene, some 200 kb distant in the genome. Evidence for a monocyte-specific physical interaction between the *ETS2* promoter and the upstream enhancer was extracted from published large-scale promoter chromatin capture (HiC) analysis of 17 primary human cell populations.[7] The proposed mechanism was based in part on a correlation between the SNV genotype and the level of *ETS2* mRNA in monocytes (an expression quantitative trait locus [eQTL]). The proposed model is that the risk allele leads to overexpression of *ETS2*, which encodes a transcriptional regulator, in blood monocytes, which in turn has a proinflammatory effect in the intestine.

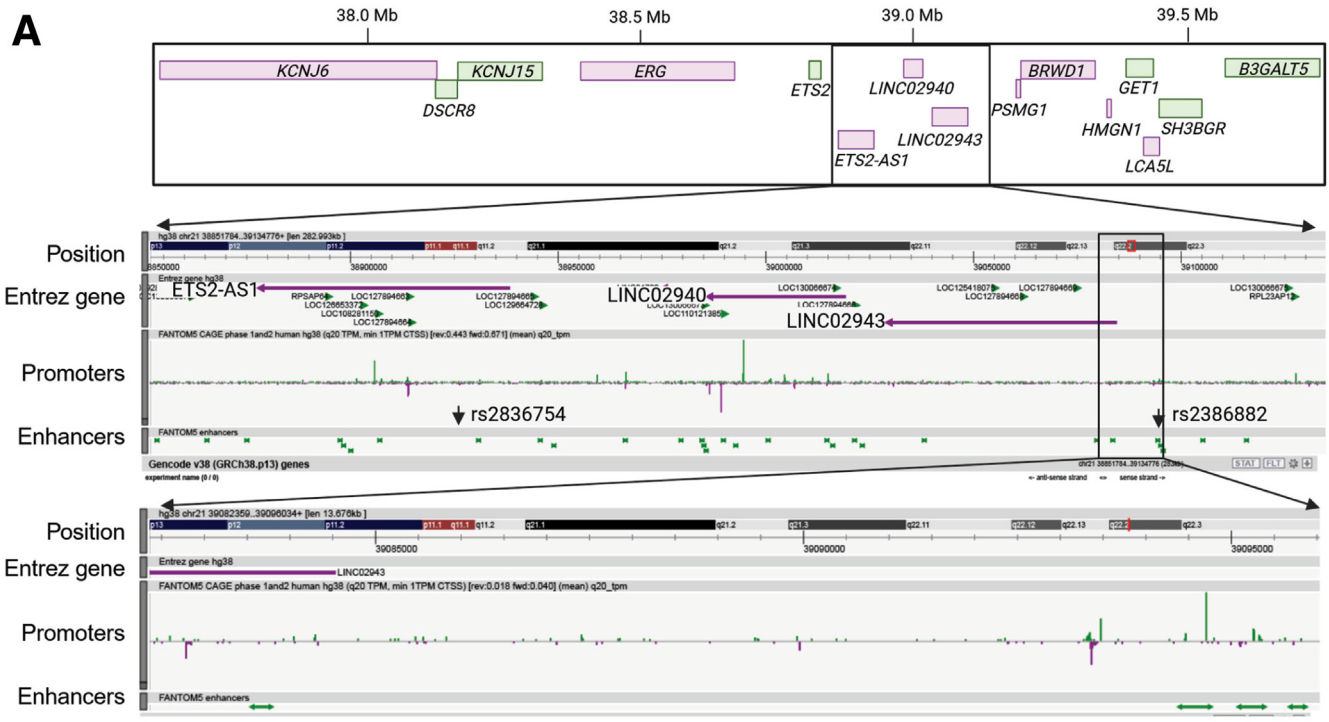
Genome-scale transcriptomic analysis by the FANTOM5 consortium[10] revealed regulated myeloid-specific bidirectional promoter activity, a feature of active enhancers,[11] in the vicinity of the identified risk variant rs2836882 but also throughout the interval between *ETS2* and *PSMG1* (Figure 1*A*). The entire interval has the characteristics of a so-called super-enhancer[12] including the regulated transcription of at least 3 long noncoding RNAs (*ETS2-AS1*, *LINC02940*, *LINC02943*) (Figure 1*A*). The FANTOM5 data were consistent with previous analysis in monocytes that revealed the epigenetic signatures of active enhancers (open chromatin, histone acetylation) throughout this interval.[13,14] Chen et al[15] analyzed a second enhancer in the interval, more proximal to *ETS2.* They linked variation in expression of *ETS2* in colorectal tumor cells to SNV rs2836754 (Figure 1*A*). The minor allele at this SNV has also been associated with IBD risk[16] and with variation in *ETS2* expression in unstimulated monocytes.[17,18] The published monocyte HiC data[7] linked the *ETS2* promoter to the region surrounding rs2836754. Thus, there is more than 1 well-established colocation of IBD GWAS hits with active monocyte enhancers that could contribute to *ETS2* transcriptional regulation.

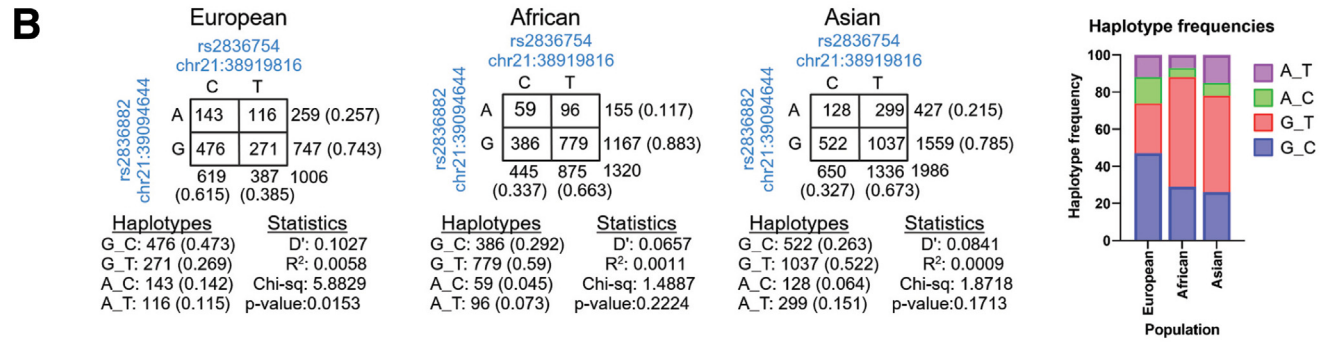
ETS2 is a Regulator of Myeloid Differentiation

The FANTOM5 data also indicates that *ETS2* mRNA expression is not associated with inflammatory activation; it is constitutively expressed in blood monocytes but also in hematopoietic stem cells, committed myeloid progenitors, granulocytes, and leukemia cell lines.[10] *ETS2* is also commonly overexpressed in acute myeloid leukemia.[19] Myeloid-specific promoters including the *ETS2* promoter share a unique architecture, lacking a TATA box and instead initiating transcription in the vicinity of multimerized purine-rich elements.[10] The archetype of this class of promoters drives expression of the monocyte-macrophage specific *CSF1R* gene, encoding the receptor for the lineage-specific growth factors, CSF1 and interleukin (IL)34.[20] Purine-rich promoter elements are bound by the lineage-specific ETS family transcription factor, PU.1 (*SPI1* gene), which acts as a pioneer to establish the open chromatin architecture required for active transcription.[21] However, PU.1 alone is not sufficient to support transcription initiation. The binding of PU.1 enables recruitment of additional transcription factors including ETS2 and other ETS family members that bind related purine-rich motifs[21] leading to cooperative activation of transcription. The ETS family contains numerous members expressed by human macrophages,[22] and dependent on genetic background, neither PU.1 nor ETS2 is absolutely required for macrophage differentiation in mice.[20,23,24] Nevertheless, the myeloid-specific pattern of expression combined with demonstrated activity as a regulator of myeloid promoters, indicates that *ETS2* is involved in myeloid differentiation rather than inflammation. Indeed, ETS2 variation may be associated with heritable differences in myeloid cell numbers in blood. In a recent study, the *ETS2*-associated SNV rs2836882 was associated with circulating neutrophil counts in a GWAS of blood cell traits in UK Biobank samples.[25]

The Cellular Basis of Genetic Susceptibility to IBD: Failure of Feedback Regulation

Any model for genetic susceptibility to IBD needs to explain why inflammation is chronic and why it is specific to





B

European

rs2836754
chr21:38919816

rs2836882 chr21:39094644	C	T	
A	143	116	259 (0.257)
G	476	271	747 (0.743)
	619 (0.615)	387 (0.385)	1006

Haplotypes	Statistics
G_C: 476 (0.473)	D': 0.1027
G_T: 271 (0.269)	R^2: 0.0058
A_C: 143 (0.142)	Chi-sq: 5.8829
A_T: 116 (0.115)	p-value:0.0153

African

rs2836754
chr21:38919816

rs2836882 chr21:39094644	C	T	
A	59	96	155 (0.117)
G	386	779	1167 (0.883)
	445 (0.337)	875 (0.663)	1320

Haplotypes	Statistics
G_C: 386 (0.292)	D': 0.0657
G_T: 779 (0.59)	R^2: 0.0011
A_C: 59 (0.045)	Chi-sq: 1.4887
A_T: 96 (0.073)	p-value:0.2224

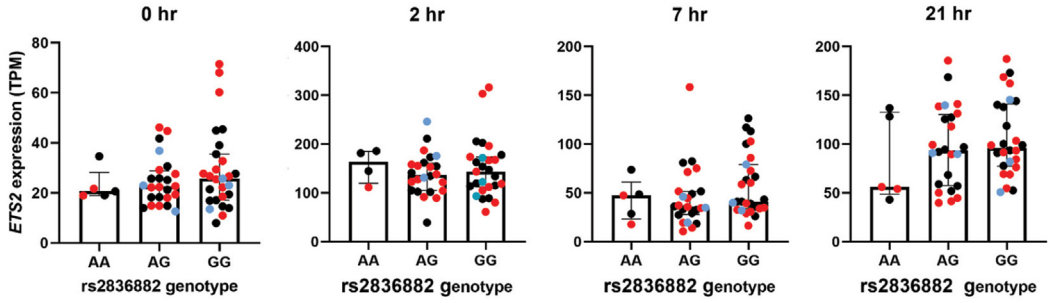
Asian

rs2836754
chr21:38919816

rs2836882 chr21:39094644	C	T	
A	128	299	427 (0.215)
G	522	1037	1559 (0.785)
	650 (0.327)	1336 (0.673)	1986

Haplotypes	Statistics
G_C: 522 (0.263)	D': 0.0841
G_T: 1037 (0.522)	R^2: 0.0009
A_C: 128 (0.064)	Chi-sq: 1.8718
A_T: 299 (0.151)	p-value:0.1713

C



D

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Mouse  AAAGCAGATTGACCTCGGGCACACGCCTTATTTCCCTAGGTTGTGTCTGCTTTTTTCTTGAATGGAATTGTTAGGTT-GT
Human  AAGGCTGGCTAACTGTGGGTGCAAGTC--ATTTCTCAAAGCAGTGTCTGTTCTTTTCTTTAATGGAATTGTTTTGTTCAT
       ** ** *  * **   ***  ** * *  ***** * * *  ******* * ******* ***********  ***  *

Mouse  TTGTTTCGGTTCCTCTTTGCGGATACCAAGGAAGCCGTGTTCCCCTTTTCCTGGTACTAGAAGTGCGTCAGTTTGAGTAA
Human  TTGTTTCACTTCCTCTTTGGGGTTCCCAAGGAACATGAGTTCCTTTTTTCCTGGTATTAGAAGTGCGTCAGTTCAAGTAT
       *******  ********** ** * ********  * *****  ********** *********.******  ****

Mouse  TAGCAAGTTAAAATAGTGAGGACTCAGGGGC-GGCTTTGCGGCTGGACTGTCGGGCATCTGA---GGGAGGTACCTCTCA
Human  GAGTACATGAAAA--GTGAAGAATAAGAGGCTGGCTCCATTAGGTGA-TGACATGTACCTGCTATGAGATTTGGGAAGCC
        ** *  * ****  **** ** * ** *** ****        ** ** *  * * ***   * **  *      *
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the lower gastrointestinal tract. Potential mechanisms involving autophagy, antimicrobial function, Th17 lymphocyte development, and inflammatory cytokine production have been inferred from numerous published analyses of disease tissue, most recently involving single-cell technologies.[26] Arguably, such analyses reveal the consequences rather than the causes of susceptibility to chronic inflammation. The focus of the analysis of *ETS2* function in IBD[9] is on its putative function in promoting excessive acute inflammatory gene expression in individual monocytes. However, excessive inflammatory activation does not provide a clear explanation for chronicity. We and others have focused instead on negative regulators of inflammatory activation; taking the view that chronicity arises primarily from an ongoing failure to resolve the initial inflammation. There are hundreds of feedback regulators of inflammatory activation, which need to be activated to suppress inflammation caused by exposure to inflammatory stimuli. Loss of function mutations in many individual "inflammation suppressor genes" are sufficient to generate spontaneous colitis in mouse models,[22,27–30] suggesting that dysregulation of any of these genes could predispose to chronic inflammation.

To understand the specific localization of chronic inflammation to the intestine we propose the following alternative mechanistic framework. The intestinal lamina propria contains an abundant resident macrophage population that, unlike other tissue resident macrophage populations, turns over rapidly in the normal steady state and is constantly replaced by blood monocytes.[31] During adaptation to the intestinal environment, monocytes must rapidly down-regulate proinflammatory pathways to avoid inappropriate responses to gut flora. This process of adaptation and maintenance of intestinal macrophages depends on signals from CSF1R,[32] the receptor for macrophage colony–stimulating factor (CSF1) and presumably involves an initial encounter with products of the intestinal microbiota. Binding of CSF1 to monocytes triggers terminal differentiation to noninflammatory resident macrophages. The same process of down-regulation occurs during the resolution of inflammation, as recruited inflammatory monocytes transition to a prorepair phenotype. Accordingly, CSF1 administration promotes tissue repair in a diverse range of inflammation models.[33–35]

To model the process of adaptation, Baillie et al[22] cultivated monocytes in CSF1 to generate monocyte-derived macrophages (MDM) and analyzed a dense time course of stimulation by the TLR4 agonist, lipopolysaccharide (LPS [endotoxin]), to simulate encounter with microbial products. The LPS time course revealed a complex sequential cascade of inducible feed forward and feedback regulation (including transient or sustained induction of numerous inflammation suppressor genes) ultimately producing an LPS-unresponsive state, analogous to the state of resident intestinal macrophages.[31] Importantly, the induction of inflammatory cytokines, such as *IL1B*, in response to LPS in MDM was short-lived and repressed around 100-fold compared with monocytes. Conversely, the anti-inflammatory cytokine, IL10, was highly-inducible in MDM but not in monocytes.[22] Genetic deficiencies in IL10 signaling are associated with very early onset IBD[36] and 2 cases associated with neutralizing anti-IL10 antibodies have been reported.[37] Interestingly, the *CSF2RB* gene, encoding a subunit of the receptor for granulocyte-macrophage colony–stimulating factor (CSF2) shows the same pattern as *IL10* in the FANTOM5 data, being profoundly LPS-inducible in MDM.[22] Rare frame-shift mutations in *CSF2RB* are associated with Crohn's disease in Ashkenazi Jews[38] and autoantibodies against CSF2 have been associated with disease severity and progression.[39,40] These data suggest that CSF2, like CSF1, contributes to disease resolution in the gut. Indeed, CSF2 administration was shown to have some therapeutic efficacy in experimental models and clinical trials in patients with IBD (reviewed in[41]).

Rather than viewing macrophages generated by cultivation of monocytes for 6 days in CSF1 as a resting or M0 activation state[9] we suggest their transcriptomic profile and dampened response to inflammatory activation provides a model for the adaptation of monocytes to the intestinal milieu via terminal differentiation to macrophages. Based on this model, genes associated with IBD susceptibility are likely to be highly expressed or inducible in monocytes and down-regulated during differentiation to resident intestinal macrophages in response to CSF1.[22] The extensive promoter-based transcriptomic data for monocytes and macrophages generated by the FANTOM5 consortium identified 2413 promoters with this expression pattern.[22] That set included *ETS2* and the archetypal IBD susceptibility gene *NOD2* and *PPIF*, highlighted in a recent analysis of enhancer association with disease susceptibility loci that also used the FANTOM5 data.[8] Both *ETS2* and *NOD2* were

Figure 1. (***See previous page***). **Gene expression and variation at chr21q22.** (*A*) Screen shots of selected tracks from the FANTOM5 Human hg38 promoterome (https://fantom.gsc.riken.jp/zenbu/) for the *ETS2-PSMG1* interval. The upper track highlights the three spliced LINC RNAs transcribed in this interval. The promoter tags show peaks of capped 5' end mRNA tags (CAGE) with green indicating forward and purple reverse orientation. Selecting the interval on the FANTOM5 Zenbu browser (https://fantom.gsc.riken.jp/zenbu/) reveals an expression table showing highest expression in monocytes (not shown). The zoomed interval highlights the close association between rs2836882 (Chr21:39094644) and enhancer-associated CAGE tags. (*B*) Haplotype variation associated with ethnicity in the *ETS2-PSMG1* interval. Relationships between rs2836882 and rs2836754 in Europeans, Africans and South and East Asians were analysed using LDpair on https://ldlink.nih.gov. (*C*) The relationship between rs2836882 SNV genotype and ETS2 expression in MDM derived from member of IBD families (black; unaffected individual, n = 25; red affected individual, n = 25; blue healthy controls with no family history of IBD, n = 6). No differences are significant. Expression was similarly unrelated to rs2836754 genotype (not shown). (*D*) Alignment of mouse and human genomic DNA sequences in the vicinity of rs2836882. The red asterisk highlights the SNV (G in the major allele, A in the minor allele). Purple highlights the extended AP1-like element that is identical to the X2 box of HLA-DRA. Green highlights predicted STAT1,2 binding sites. Blue highlights conserved motif including a PU.1 consensus site (GAGGAA).

down-regulated in MDM compared with monocytes and reinduced to lower levels in response to LPS. The low expression of *ETS2* in resident intestinal macrophages compared with monocytes was confirmed in published single-cell transcriptomic data.[42] Down-regulation of *ETS2* in monocytes in response to CSF1 is associated with almost complete loss of the transcriptional and epigenetic signatures of active enhancers in the *ETS2-PSMG1* interval.[11,13,14] The same pattern of chromatin remodeling is evident in the *NOD2-SNX20* interval, the *PPIF* locus, and many other inflammation suppressor genes.[22] Indeed, detailed curation of chromosomal intervals associated with IBD by GWAS revealed that most contain genes that fit our proposed criteria.[22] Furthermore, additional variants associated with Crohn's disease by GWAS at a lower genome-wide threshold were strongly enriched in the regions surrounding the set of CSF1-regulated promoters, whereas no significant enrichment was observed for control sets of loci/SNVs associated with other traits or diseases, notably including rheumatoid arthritis.[22]

Implications of Allele Frequencies of rs2836882 Variants

The association between rs2836882 and IBD has only been reported in Europeans. The risk allele associated with the *ETS2* locus is the ancestral human allele and is very common; the allele frequency is 0.75 in Europeans but >0.9 in those of African or Asian ethnicity (https://gnomad.broadinstitute.org/). The high prevalence of the risk allele was proposed to be a product of balancing selection; inferring that the reduced *ETS2* expression associated with the minor allele would decrease the risk of chronic inflammatory disease but also compromise innate immunity.[9] This proposal depends on the risk allele being sufficiently deleterious to affect transmission to progeny. An alternative interpretation, more consistent with population data, is that the minor allele, associated with reduced disease risk, has been subjected to recent positive selection in Europeans, analogous to the selection against loss-of-function *ERAP2* variants by plague.[43] Indeed, tuberculosis is 1 candidate agent of selection because there is a substantial overlap between susceptibility loci for IBD and for mycobacterial infection.[44] Regardless, reference to *ETS2* as a causal gene that directs macrophage inflammation[9] is difficult to reconcile with the fact that most individuals who are homozygous for the risk allele (>50% of Europeans), apparently leading to high expression of *ETS2*, do not develop IBD.

We consider 3 further caveats to the interpretation of the association between rs2836882 and IBD. First, the major haplotype defined by the 2 enhancer-associated SNVs, rs2836882 and rs2836754, differs in frequency between Europeans and African, East and South Asian populations (Figure 1*B*), providing further evidence of positive selection in Europeans and raising the possibility of interactions between SNVs located in the super-enhancer in *ETS2* regulation. In other words, Europeans with a particular combination of variants may have a lesser relative risk of disease. Second, there are 3 long noncoding mRNAs generated from this region. An inducible long noncoding mRNA generated from *ETS2-PSMG1* interval is among those detected in an analysis of LPS-stimulated monocytes.[45] These long noncoding mRNAs could be targets of the enhancer and potentially act as *cis*-acting or *trans*-acting regulators independent of ETS2. Third, the region of regulated chromatin structure in MDM compared with monocytes[13] extends into the nearby *KCNJ15* locus, which is also regulated in monocytes of individuals with tuberculosis.[46] Hence, *ETS2* may not be the only candidate gene in this region. By analogy, protein coding variation in *NOD2* (on Chr16) is clearly associated with susceptibility to IBD, but the possible function of nearby gene *SNX20*, which shares an enhancer with *NOD2*, has not been explored. Similarly, identification of a loss-of-function coding variant in the *ATG16L1* gene (T300A) focused attention on dysregulation of autophagy in IBD susceptibility. There is recent evidence that ATG16L1 is a negative feedback regulator of inflammatory cytokine expression.[47] However, the *ATG16L1* gene contains an enhancer for the neighboring *INPP5D* gene (*SHIP1*),[22] confirmed in monocyte HiC data,[7] which is linked in turn to altered expression of *INPP5D* in patients with IBD and to spontaneous colitis associated with homozygous loss of function mutations in mice.[48,49] Arguably, *INPP5D* dysregulation provides a more plausible explanation for the SNV association with IBD susceptibility at this locus.

Mechanisms for the Association Between rs2836882 and *ETS2* Expression

The proposed link between the major allele at rs2836882, *ETS2* transcription and inflammation is not supported by direct evidence of variation in ETS2 protein abundance or function and that remains a challenge. The mechanistic analysis[9] focused on monocyte-specific *ETS2* eQTL also associated with rs2836882.[17] The eQTL effect size was relatively small and accounts for a minor fraction of the population variance in monocyte *ETS2* mRNA. Hence, most variance is probably a consequence of *trans*-acting variation at other loci and environment. Chun et al[50] have critically analyzed the limited evidence for shared effects of individual SNVs on disease risk and gene expression.

The mechanistic link between the variation in expression detected by the eQTL and inflammation was investigated by analyzing the effect of deletion of the putative enhancer, deletion of *ETS2*, or overexpression of *ETS2* in MDM.[9] The deletion experiments used a nontargeting control that does not recognize any sequence in the human genome and hence would not cause any DNA damage. A nontargeting control does not control for the known impact of double-stranded DNA breaks (introduced by targeted CRISPR-Cas9 editing) on differentiation and activation in macrophages.[51] The analysis of overexpression of *ETS2* involved mRNA transfection of MDM and analysis of their subsequent response to a complex proinflammatory cocktail including CSF2, tumor necrosis factor, prostaglandin E_2, and a toll-like receptor 2 agonist. The contribution of the individual agonists in this

mixture to ETS2 regulation is difficult to evaluate, because CSF2 as the sole stimulus also down-regulates *ETS2* mRNA in monocytes and *TLR2* is strongly down-regulated in MDM compared with monocytes.[9] The level of overexpression (35–60 fold) and the target cell (MDM not monocytes) are both unrelated to the small variation in *ETS2* mRNA detected in monocytes by eQTL analysis. Given the pattern of expression of *ETS2*, we argue that deletion mimics, and overexpression reverses the differentiation response of monocytes to CSF1, rather than demonstrating the inflammatory effects of ETS2.

Regulation of *ETS2* Expression in Macrophages in IBD Families

We recently generated RNA-seq data from control and LPS-stimulated MDM cultured from a cohort of affected and unaffected sib-pairs/trios from 22 IBD families to analyze transcriptomic variation in MDM as a model for understanding IBD susceptibility.[52] Regardless of disease status, MDM from all individuals responded to LPS with transient induction of the key inflammatory cytokine, *TNF*. Hence, there is no support for the idea that IBD arises from a global hyperresponsiveness to TLR4 signals that distinguishes affected and unaffected individuals.[53] Instead, the analysis revealed extreme interindividual variation that was gene-specific, notably including all-or-nothing expression of specific HLA genes that are also subject to regulation by CSF1.[22] The magnitude of the interindividual variation in specific genes detected in MDM grown in CSF1 is much greater than reported in published eQTL for monocytes.[17,54,55] Further analysis of these results showed that *ETS2* mRNA varied >10-fold between individuals in both basal and LPS-stimulated cells, but we could not demonstrate an association with either rs2836882 or rs2836754 SNV genotype at any timepoint (Figure 1*C*). As an alternative way of dissecting the function of ETS2, we considered whether natural interindividual variation in *ETS2* in our study cohort was correlated with other known regulators. The most correlated transcriptional regulator, the repressor *NFIL3*, also lies within an IBD susceptibility locus identified by GWAS and like *ETS2* is strongly down-regulated by CSF1.[22] *Nfil3*$^{-/-}$ mice develop spontaneous colitis.[56] Most importantly, variation in *ETS2* expression between individuals was not correlated with altered expression of any proinflammatory target genes.

The Relationship Between rs2836882 and Transcription Factor Binding

The rs2836882 minor allele was associated with reduced chromatin H3K27 acetylation and binding of the macrophage transcription factor, PU.1.[9] PU.1 is also bound to the *ETS2* promoter, to an intronic enhancer within the *ETS2* gene, and to multiple sites in the intergenic interval.[13] A missing link in understanding the functional impact of rs2836882 is the identity of transcriptional regulators that bind directly to the segment of DNA containing this SNV. In the analysis of regulatory elements, cross-species sequence comparison can highlight conserved binding sites for transcriptional regulators. Active bidirectional transcription throughout the *ETS2-PSMG1* interval is also detected in macrophages in mice.[11] In mouse monocytes, *Ets2* is highly expressed and the region corresponding to the human enhancer is in open chromatin.[57] Indeed, the sequence around rs2836882 is conserved across mammalian species (Figure 1*D*) and the SNV lies within a perfectly conserved 17 bp element containing an AP1-like motif (core sequence TGCGTCA). The proposed risk variant G allele at rs2836882 is not simply the ancestral human allele, it is the ancestral mammalian allele. The conserved AP1 motif is identical to the X2 box of the human HLA-DRA genes, required for induction by interferon-γ and by phorbol esters, and shown to bind multiple members of the ATF/CREB family ([58]and references therein). We speculate that the protective A variant at rs2836882 favors binding of a repressor. One candidate is BATF, a member of the ATF family, which is selectively and strongly induced by LPS in MDM but not in

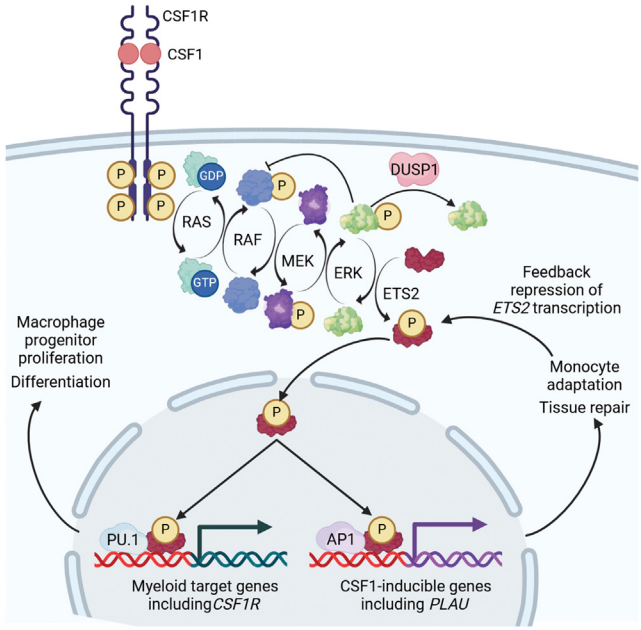

Figure 2. CSF1 signaling leading to ETS2 activation. A simplified view of CSF1 signal transduction. For a more detailed review see Stanley & Chitu.[60] CSF1 binding leads to receptor (CSF1R) leads to dimerization and autophosphorylation which initiates a phosphorylation cascade through RAS (*HRAS, KRAS, NRAS* genes), RAF (*RAF1, BRAF, ARAF*), MEK (*MAP2K1, MAP2K2*) and ERK1,2 (*MAPK3/MAPK1*) to phosphorylate ETS2 (P = phosphate group). ETS2 moves to the nucleus where it interacts with PU.1 (*SPI1*) or AP1 complexes to activate transcription of target genes including *CSF1R* and *PLAU*. The signaling cascade is subject to feedback at every level. The CSF1R-CSF1 complex is dephosphorylated, internalised and degraded.[60] The diagram shows two examples highlighted in the text. MEK phosphorylates ERK on threonine and tyrosine. Activated ERK acts in a feedback loop to inhibit RAF phosphorylation. The inducible dual specificity phosphatase DUSP1 dephosphorylates and inactivates ERK.

monocytes and lies adjacent to an IBD GWAS peak on Chr14.[22] A motif near rs2836882 was suggested as the PU.1 binding site that may be influenced in some way by the SNV.[9] This site is annotated by JASPAR on the UCSC genome browser (https://genome.ucsc.edu/) as a binding site for STAT1 and STAT2. There is a perfect conserved PU.1 motif around 40 bp away (Figure 1*D*). In any case, chromatin structure at this locus can be dissociated from PU.1 binding. The loss of the enhancer chromatin signature in monocytes in response to CSF1 occurs without the loss of bound PU.1.[13] We suggest that the observed allele-associated variation in bound PU.1[9] is more likely a consequence rather than a cause of altered chromatin structure. The conservation of the *ETS2* enhancer between mouse and human opens up the possibility of testing function by deletion, mutation, or replacement in the mouse genome.

Therapeutic Implications of ETS2 Involvement in IBD

The focus on ETS2 as a proinflammatory transcriptional regulator leads logically to discussion of therapeutic strategies for IBD via inhibition of the upstream regulator mitogen-activated protein kinase, MAP2K1 (MEK1).[9] An MEK inhibitor was found to inhibit inflammatory cytokine expression in macrophages in vitro and in explants of inflamed tissue ex vivo.[9] The effect of MEK inhibition on inducible cytokine expression in macrophages was reported previously, but the inferred mechanism involved phosphorylation of the ELK1 transcription factor and induction of *EGR1*[59] rather than ETS2. Any discussion of MAP2K1 as a target should consider the possible consequences of inhibiting CSF1R signaling. The tyrosine kinase signaling pathway activated by CSF1 binding to CSF1R involves the classical RAS-RAF-MAP kinase pathway shared by many growth factor receptors, leading to the activation of MAP2K1.[60] This pathway is shown in Figure 2. The major transcriptional effectors of this pathway in different cell types, including tumor cells, are the closely related ETS1 and ETS2 proteins.[61,62] In macrophages, phosphorylation and activation of the targets of MAP2K1, MAPK1 (ERK2), and MAPK3 (ERK1) leads in turn to phosphorylation of the pointed domain of ETS2, which is required for transcriptional activation.[63] The sustained activation of this pathway by CSF1 leads to induced expression of ETS2 target genes including urokinase plasminogen activator (*PLAU*) through interaction with inducible transcription factors in the AP1 family.[64,65] Like all signaling pathways, the RAS-RAF-MAP kinase pathway is limited by inducible feedback control at multiple levels, including the down-regulation of *ETS2*. A key inducible feedback regulator, *DUSP1* encodes the major dual specificity phosphatase that inactivates MAP kinases. Monocyte differentiation to MDM in response to CSF1 involves and likely requires complete down-regulation of *DUSP1*, which is expressed constitutively in monocytes.[22] Interestingly, *DUSP1* is also within an IBD susceptibility locus, transiently reinduced in MDM by LPS and coregulated with *ETS2*. Based on the idea that ETS2 is a proinflammatory mediator, MAP2K1 (MEK1) was proposed as a potential therapeutic target.[9] If, as we suggest, CSF1 provides a differentiation signal to drive a resident macrophage phenotype and resolution of inflammation, any therapeutic intervention to prevent ETS2 activation is likely to be counterproductive because it will block this necessary process. The same argument applies to CSF1R and the 2 ligands, CSF1 and IL34. Expression of both ligands is increased in experimental and human IBD and IL34 was proposed as target for therapy.[66] We argue that neutralizing either ligand or blocking the receptor is more likely to exacerbate disease.

The potential for ERK as a target is further compromised by the negative feedback that attenuates RAF protein kinase activity (Figure 2). Blocking ERK activation through MEK inhibition relieves this feedback inhibition of RAF and promotes flow through the pathway, activating ERK and hence ETS2. For this reason, MEK inhibitors have limited clinical use.[62] In any case, *DUSP1*, which inactivates ERK, is profoundly inducible in macrophages by glucocorticoids in both humans and mice.[67] Although now commonly replaced by immunotherapies, glucocorticoids were previously a frontline therapy in acute IBD exacerbation, producing clinical remission but with limited evidence of mucosal healing.[68,69]

Conclusions

In overview, we propose that IBD susceptibility and chronic inflammation arise from dysregulation of the CSF1/CSF1R-dependent adaptation of monocytes (via terminal differentiation to macrophages) to the environment of the lower intestine in which down-regulation of ETS2 is essential. Indeed, the proresolution, anti-inflammatory actions may provide a rationale for application of CSF1 as a therapy in inflammatory disease. That framework gives rise to alternative explanations for the reported association between rs2836882, transcriptional regulation of *ETS2*, and IBD risk[9] that applies equally to many other susceptibility loci identified by GWAS. Our critical analysis illustrates the many challenges in linking noncoding SNVs identified by GWAS to mechanism. The corollary of the recent finding that each individual has a unique macrophage transcriptome[52] is that the power of GWAS analysis may be compromised by viewing IBD as a single entity. In essence, the genetic contribution to IBD risk may be unique to each individual or family. Such variation may contribute to the known IBD disease heterogeneity and variation in treatment efficacy[70] and to the nature of the environmental trigger.

References

1. Gibson G, Rioux JD, Cho JH, et al. Eleven grand challenges for inflammatory bowel disease genetics and genomics. Inflamm Bowel Dis 2025;31:272–284.
2. Sun Y, Yuan S, Chen X, et al. The contribution of genetic risk and lifestyle factors in the development of adult-onset inflammatory bowel disease: a prospective cohort study. Am J Gastroenterol 2023;118:511–522.
3. Noble AJ, Nowak JK, Adams AT, et al. Defining interactions between the genome, epigenome, and the

environment in inflammatory bowel disease: progress and prospects. Gastroenterology 2023;165:44–60.e2.
4. Caruso R, Lo BC, Chen GY, et al. Host-pathobiont interactions in Crohn's disease. Nat Rev Gastroenterol Hepatol. Published online October 24, 2024. https://doi.org/10.1038/s41575-024-00997-y
5. Brant SR, Wang MH, Rawsthorne P, et al. A population-based case-control study of CARD15 and other risk factors in Crohn's disease and ulcerative colitis. Am J Gastroenterol 2007;102:313–323.
6. Caruso R, Mathes T, Martens EC, et al. A specific gene-microbe interaction drives the development of Crohn's disease-like colitis in mice. Sci Immunol 2019;4:eaaw4341.
7. Javierre BM, Burren OS, Wilder SP, et al. Lineage-specific genome architecture links enhancers and non-coding disease variants to target gene promoters. Cell 2016;167:1369–1384.e19.
8. Nasser J, Bergman DT, Fulco CP, et al. Genome-wide enhancer maps link risk variants to disease genes. Nature 2021;593:238–243.
9. Stankey CT, Bourges C, Haag LM, et al. A disease-associated gene desert directs macrophage inflammation through ETS2. Nature 2024;630:447–456.
10. Forrest AR, Kawaji H, Rehli M, et al. A promoter-level mammalian expression atlas. Nature 2014;507:462–470.
11. Andersson R, Gebhard C, Miguel-Escalada I, et al. An atlas of active enhancers across human cell types and tissues. Nature 2014;507:455–461.
12. Lam MT, Li W, Rosenfeld MG, et al. Enhancer RNAs and regulated transcriptional programs. Trends Biochem Sci 2014;39:170–182.
13. Pham TH, Benner C, Lichtinger M, et al. Dynamic epigenetic enhancer signatures reveal key transcription factors associated with monocytic differentiation states. Blood 2012;119:e161–e171.
14. Schmidl C, Renner K, Peter K, et al. Transcription and enhancer profiling in human monocyte subsets. Blood 2014;123:e90–e99.
15. Chen Y, Ying Y, Wang M, et al. A distal super-enhancer activates oncogenic ETS2 via recruiting MECOM in inflammatory bowel disease and colorectal cancer. Cell Death Dis 2023;14:8.
16. Parkes M, Barrett JC, Prescott NJ, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. Nat Genet 2007;39:830–832.
17. Fairfax BP, Humburg P, Makino S, et al. Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. Science 2014;343:1246949.
18. Farh KK, Marson A, Zhu J, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. Nature 2015;518:337–343.
19. Baldus CD, Liyanarachchi S, Mrozek K, et al. Acute myeloid leukemia with complex karyotypes and abnormal chromosome 21: amplification discloses overexpression of APP, ETS2, and ERG genes. Proc Natl Acad Sci U S A 2004;101:3915–3920.
20. Rojo R, Pridans C, Langlais D, et al. Transcriptional mechanisms that control expression of the macrophage colony-stimulating factor receptor locus. Clin Sci (Lond) 2017;131:2161–2182.
21. Minderjahn J, Schmidt A, Fuchs A, et al. Mechanisms governing the pioneering and redistribution capabilities of the non-classical pioneer PU.1. Nat Commun 2020;11:402.
22. Baillie JK, Arner E, Daub C, 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:e1006641.
23. Henkel GW, McKercher SR, Yamamoto H, et al. PU.1 but not ets-2 is essential for macrophage development from embryonic stem cells. Blood 1996;88:2917–2926.
24. Lichanska AM, Browne CM, Henkel GW, et al. Differentiation of the mononuclear phagocyte system during mouse embryogenesis: the role of transcription factor PU.1. Blood 1999;94:127–138.
25. Ulirsch JC, Lareau CA, Bao EL, et al. Interrogation of human hematopoiesis at single-cell and single-variant resolution. Nat Genet 2019;51:683–693.
26. Graham DB, Xavier RJ. Pathway paradigms revealed from the genetics of inflammatory bowel disease. Nature 2020;578:527–539.
27. Afonina IS, Zhong Z, Karin M, et al. Limiting inflammation-the negative regulation of NF-kappaB and the NLRP3 inflammasome. Nat Immunol 2017;18:861–869.
28. Kondo T, Kawai T, Akira S. Dissecting negative regulation of Toll-like receptor signaling. Trends Immunol 2012;33:449–458.
29. Rothschild DE, McDaniel DK, Ringel-Scaia VM, et al. Modulating inflammation through the negative regulation of NF-kappaB signaling. J Leukoc Biol 2018;103:1131–1150.
30. Wells CA, Ravasi T, Hume DA. Inflammation suppressor genes: please switch out all the lights. J Leukoc Biol 2005;78:9–13.
31. Hegarty LM, Jones GR, Bain CC. Macrophages in intestinal homeostasis and inflammatory bowel disease. Nat Rev Gastroenterol Hepatol 2023;20:538–553.
32. MacDonald KP, Palmer JS, Cronau S, 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;116:3955–3963.
33. Farro G, Stakenborg M, Gomez-Pinilla PJ, et al. CCR2-dependent monocyte-derived macrophages resolve inflammation and restore gut motility in postoperative ileus. Gut 2017;66:2098–2109.
34. Keshvari S, Genz B, Teakle N, et al. Therapeutic potential of macrophage colony-stimulating factor in chronic liver disease. Dis Model Mech 2022;15:dmm049387.
35. Mirchandani AS, Jenkins SJ, Bain CC, et al. Hypoxia shapes the immune landscape in lung injury and promotes the persistence of inflammation. Nat Immunol 2022;23:927–939.

36. Uhlig HH. Monogenic diseases associated with intestinal inflammation: implications for the understanding of inflammatory bowel disease. Gut 2013;62:1795–1805.
37. Griffin H, Ceron-Gutierrez L, Gharahdaghi N, et al. Neutralizing autoantibodies against interleukin-10 in inflammatory bowel disease. N Engl J Med 2024;391:434–441.
38. Chuang LS, Villaverde N, Hui KY, et al. A frameshift in CSF2RB predominant among Ashkenazi Jews increases risk for Crohn's disease and reduces monocyte signaling via GM-CSF. Gastroenterology 2016;151:710–723.e2.
39. Denson LA, Jurickova I, Karns R, et al. Genetic and transcriptomic variation linked to neutrophil granulocyte-macrophage colony-stimulating factor signaling in pediatric Crohn's disease. Inflamm Bowel Dis 2019;25:547–560.
40. Mortha A, Remark R, Del Valle DM, et al. Neutralizing anti-granulocyte macrophage-colony stimulating factor autoantibodies recognize post-translational glycosylations on granulocyte macrophage-colony stimulating factor years before diagnosis and predict complicated Crohn's disease. Gastroenterology 2022;163:659–670.
41. Egea L, Hirata Y, Kagnoff MF. GM-CSF: a role in immune and inflammatory reactions in the intestine. Expert Rev Gastroenterol Hepatol 2010;4:723–731.
42. Chapuy L, Bsat M, Sarkizova S, et al. Two distinct colonic CD14(+) subsets characterized by single-cell RNA profiling in Crohn's disease. Mucosal Immunol 2019;12:703–719.
43. Klunk J, Vilgalys TP, Demeure CE, et al. Evolution of immune genes is associated with the Black Death. Nature 2022;611:312–319.
44. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 2012;491:119–124.
45. NE II, Heward JA, Roux B, et al. Long non-coding RNAs and enhancer RNAs regulate the lipopolysaccharide-induced inflammatory response in human monocytes. Nat Commun 2014;5:3979.
46. Del Rosario RCH, Poschmann J, Lim C, et al. Histone acetylome-wide associations in immune cells from individuals with active *Mycobacterium tuberculosis* infection. Nat Microbiol 2022;7:312–326.
47. Okai N, Watanabe T, Minaga K, et al. Alterations of autophagic and innate immune responses by the Crohn's disease-associated ATG16L1 mutation. World J Gastroenterol 2022;28:3063–3070.
48. Dobranowski PA, Tang C, Sauve JP, et al. Compositional changes to the ileal microbiome precede the onset of spontaneous ileitis in SHIP deficient mice. Gut Microbes 2019;10:578–598.
49. Fernandes S, Srivastava N, Sudan R, 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.
50. Chun S, Casparino A, Patsopoulos NA, et al. Limited statistical evidence for shared genetic effects of eQTLs and autoimmune-disease-associated loci in three major immune-cell types. Nat Genet 2017;49:600–605.
51. Pereira-Lopes S, Tur J, Calatayud-Subias JA, et al. NBS1 is required for macrophage homeostasis and functional activity in mice. Blood 2015;126:2502–2510.
52. O'Brien CL, Summers KM, Martin NM, et al. The relationship between extreme inter-individual variation in macrophage gene expression and genetic susceptibility to inflammatory bowel disease. Hum Genet 2024;143:233–261.
53. Papoutsopoulou S, Burkitt MD, Bergey F, et al. Macrophage-specific NF-kappaB activation dynamics can segregate inflammatory bowel disease patients. Front Immunol 2019;10:2168.
54. Kim-Hellmuth S, Aguet F, Oliva M, et al. Cell type-specific genetic regulation of gene expression across human tissues. Science 2020;369:eaaz8528.
55. Richard AC, Peters JE, Savinykh N, et al. Reduced monocyte and macrophage TNFSF15/TL1A expression is associated with susceptibility to inflammatory bowel disease. PLoS Genet 2018;14:e1007458.
56. Kobayashi T, Steinbach EC, Russo SM, et al. NFIL3-deficient mice develop microbiota-dependent, IL-12/23-driven spontaneous colitis. J Immunol 2014;192:1918–1927.
57. Sakai M, Troutman TD, Seidman JS, et al. Liver-derived signals sequentially reprogram myeloid enhancers to initiate and maintain Kupffer cell identity. Immunity 2019;51:655–670.e8.
58. Setterblad N, Peterlin BM, Andersson G. Role of the X2 box in activated transcription from the DRA promoter in B cells. Immunogenetics 1997;46:318–325.
59. Guha M, O'Connell MA, Pawlinski R, et al. Lipopolysaccharide activation of the MEK-ERK1/2 pathway in human monocytic cells mediates tissue factor and tumor necrosis factor alpha expression by inducing Elk-1 phosphorylation and Egr-1 expression. Blood 2001;98:1429–1439.
60. Stanley ER, Chitu V. CSF-1 receptor signaling in myeloid cells. Cold Spring Harb Perspect Biol 2014;6:a021857.
61. Gallant S, Gilkeson G. ETS transcription factors and regulation of immunity. Arch Immunol Ther Exp (Warsz) 2006;54:149–163.
62. Tetsu O, McCormick F. ETS-targeted therapy: can it substitute for MEK inhibitors? Clin Transl Med 2017;6:16.
63. Yang BS, Hauser CA, Henkel G, et al. Ras-mediated phosphorylation of a conserved threonine residue enhances the transactivation activities of c-Ets1 and c-Ets2. Mol Cell Biol 1996;16:538–547.
64. Fowles LF, Martin ML, Nelsen L, et al. Persistent activation of mitogen-activated protein kinases p42 and p44 and ets-2 phosphorylation in response to colony-stimulating factor 1/c-fms signaling. Mol Cell Biol 1998;18:5148–5156.
65. Stacey KJ, Fowles LF, Colman MS, et al. Regulation of urokinase-type plasminogen activator gene transcription by macrophage colony-stimulating factor. Mol Cell Biol 1995;15:3430–3441.
66. Zwicker S, Martinez GL, Bosma M, et al. Interleukin 34: a new modulator of human and experimental inflammatory bowel disease. Clin Sci (Lond) 2015;129:281–290.
67. Jubb AW, Young RS, Hume DA, et al. Enhancer turnover is associated with a divergent transcriptional response to glucocorticoid in mouse and human macrophages. J Immunol 2016;196:813–822.

68. De Cassan C, Fiorino G, Danese S. Second-generation corticosteroids for the treatment of Crohn's disease and ulcerative colitis: more effective and less side effects? Dig Dis 2012;30:368–375.
69. Dubois-Camacho K, Ottum PA, Franco-Munoz D, et al. Glucocorticosteroid therapy in inflammatory bowel diseases: from clinical practice to molecular biology. World J Gastroenterol 2017;23:6628–6638.
70. Verstockt B, Bressler B, Martinez-Lozano H, et al. Time to revisit disease classification in inflammatory bowel disease: is the current classification of inflammatory bowel disease good enough for optimal clinical management? Gastroenterology 2022;162:1370–1382.

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