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IBD Candidate Genes and Intestinal Barrier Regulation

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Abstract

Technological advances in the large scale analysis of human genetics have generated profound insights into possible genetic contributions to chronic diseases including the inflammatory bowel diseases (IBDs), Crohn's disease and ulcerative colitis. To date, 163 distinct genetic risk loci have been associated with either Crohn's disease or ulcerative colitis, with a substantial degree of genetic overlap between these 2 conditions. Although many risk variants show a reproducible correlation with disease, individual gene associations only affect a subset of patients, and the functional contribution(s) of these risk variants to the onset of IBD is largely undetermined. Although studies in twins have demonstrated that the development of IBD is not mediated solely by genetic risk, it is nevertheless important to elucidate the functional consequences of risk variants for gene function in relevant cell types known to regulate key physiological processes that are compromised in IBD. This article will discuss IBD candidate genes that are known to be, or are suspected of being, involved in regulating the intestinal epithelial barrier and several of the physiological processes presided over by this dynamic and versatile layer of cells. This will include assembly and regulation of tight junctions, cell adhesion and polarity, mucus and glycoprotein regulation, bacterial sensing, membrane transport, epithelial differentiation, and restitution.

Keywords

epithelial; permeability; inflammation; tight junctions

The use of genome-wide association studies (GWAS) has transformed our understanding of genetic risk factors for inflammatory bowel disease (IBD) and many other chronic inflammatory conditions. These studies have opened new avenues of investigation into pathologic mechanisms involved in IBD pathogenesis such as bacterial sensing, autophagy, and dysregulated immune response signaling pathways as well as the variance of disease phenotypes found in Crohn's disease (CD) and ulcerative colitis (UC).[1] GWAS have also identified susceptibility genes involved in the function of various aspects of the intestinal barrier. A number of excellent review articles have been published describing IBD candidate genes and their potential roles in the pathogenesis of IBD.[2–5] This article attempts to update

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the list of IBD candidate genes that have been identified as having a role in regulating intestinal barrier properties and how they serve to link IBD genetics with this major pathophysiological series of events involved in the onset and progression of IBD. In the interests of clarity, the focus of this article will primarily be on candidate genes affecting barrier function. However, the use of the term “intestinal barrier properties” in this article will incorporate broader aspects of the epithelial barrier including, but not limited to, the strictest definition of “intestinal barrier function,” which reflects changes in intestinal permeability and refers exclusively to altered flux of solutes and fluid across the epithelium.[6]

INTESTINAL EPITHELIAL BARRIER

The epithelial barrier plays a critical role in maintaining intestinal homeostasis because it lies at the interface between luminal microbes and the host immune system, while also being the first site of exposure to many of the environmental factors that can act as triggers of disease activity.[7,8] The epithelium communicates in a bidirectional manner with luminal microbes and the immune system; and therefore, the integrity of the epithelial lining is critical for the appropriate partitioning of interactions between immune cells and microbes.[9] The epithelium therefore has the capacity to sense luminal contents i.e., microbial products and through activation of surface receptors such as toll-like receptor signaling pathways, secrete biochemical signals to adjacent lamina propria immune cells.[10] Furthermore, luminal antigens are sampled across the epithelial layer thus contributing to the immune surveillance role of the mucosal barrier.[11,12] However, excessive uptake of antigens can result in overactivation of the immune system, thus leading to mucosal inflammation.[11] In addition to its wider role in innate immune signaling, an essential function of the epithelial lining that allows it to perform a vast array of sophisticated tasks is its ability to form a tightly regulated and selectively permeable barrier. The “barrier” function of the epithelium is absolutely essential to intestinal function. It is required for the ability of the epithelium to absorb nutrients and water under the direction of solute-couple uptake mechanisms. By the same token, a regulated barrier is a prerequisite for the regulated secretion of fluid and digestive enzymes to break down ingested foods and allow digestion and assimilation of nutrients, as well as fluid secretion that serves to lubricate the epithelial surface or wash away noxious agents or pathogens from the epithelial surface.[13,14]

MUCUS LAYERS AND EPITHELIAL SECRETORY FUNCTIONS

Another essential component of intestinal homeostasis is provided by the secretion of mucus from goblet cells and dispersal of tightly-packed mucus droplets by bicarbonate secretion.[15,16] Mucus particle dispersal facilitates formation of appropriately hydrated and expanded mucus layers. The stomach and colon have 2 mucus layers with an inner mucus layer that is 50 to 200 μm thick, firmly attached to the epithelium, and is built around the gel-forming MUC5A mucin protein in the stomach and MUC2 in the intestine that act as a structural skeleton for the inner mucus layer.[17] This inner layer can act as a filter to prevent bacteria from accessing the epithelial surface. The outer colonic mucus layer is generated through endogenous protease cleavage of MUC2 resulting in a 4- to 5-fold expansion in volume. This layer is easily removed, and although it has a less defined outer border, it has

been reported to be approximately 700 μm in thickness.[17–19] In contrast to the colon, the small intestine has only 1 layer of mucus.[18] However, the small intestine does exhibit more pronounced release of Paneth cell-derived antimicrobial peptides including cathelicidins, defensins, and bactericidal lectins such as RegIII proteins that help to maintain a relative bacterial sterility on the epithelial surface.[2,20,21] Expression of antimicrobial peptides is mostly reduced in IBD. A possible influence of IBD candidate genes on antimicrobial peptide expression was suggested by the decrease in human defensin 5 and human defensin 6 peptides that occurred in both adult and pediatric ileal patients with CD particularly in those with mutations in the *CARD15* gene that encodes the bacterial-sensing protein, NOD2.[2] In contrast, the cathelicidin LL-37 was observed to be upregulated in active and quiescent UC but not in CD.[22] An additional link between antimicrobial peptides and IBD genetics was also identified in one study that reported that a SNP in the gene locus of the C-type lectin, CLEC16A, was associated with patients with CD harboring 3 established CD-associated NOD2/CARD15 mutations.[23] Due to space limitations and the scope of the topic, IBD candidate genes involved in Paneth cell function (i.e., *KCNN4*, *XBP1*, *ATG16L1*, etc.) will not be highlighted in this article. Many excellent articles have covered the roles of Paneth cell dysfunction and associated candidate genes in considerable depth, and the reader is referred to a selection of those articles.[2,3,24]

Secretory IgA

Another important component of the intestinal barrier involves enterocyte transcytosis of secretory immunoglobulin A (sIgA) into the lumen through the polymeric immunoglobulin receptor, which binds to the dimeric (or polymeric) form of IgA on the basal membrane of intestinal epithelial cells (IECs).[25] IgA is the most abundant antibody isotype produced in the body and provides mucosal protection through interaction with the pIgR.[26] The sIgA promotes the clearance of antigens and pathogenic microorganisms from the intestinal lumen by impeding access to epithelial receptors, thus leading to their entrapment in mucus, and subsequent removal by peristaltic and mucociliary activities. This mechanism has been referred to as “immune exclusion.” Moreover, sIgA has been shown to negate bacterial virulence factors, modify composition of the intestinal microflora, and dampen inflammatory responses to allergens and pathogenic bacteria.[9,27–30]

Electrolyte and Fluid Transport

Epithelial electrolyte and fluid transport plays a crucial role in the maintenance of intestinal homeostasis.[13] As previously mentioned, fluid secretion is required for dispersal of mucus, flushing of intestinal crypts, and dispersal of Paneth cell secretions and antimicrobial peptides. The overall balance of fluid and electrolyte absorption and secretion is fundamental for pH regulation of the luminal microenvironment and can also influence composition of the microbiome.[14,31] Moreover, loss of transporters such as sodium-hydrogen exchanger 3 or the Cl-/HCO3-exchanger, downregulated in adenoma, in mouse intestine can alter baseline inflammatory status and predispose to more severe injury after challenge.[32–36] Regarding genetic links between electrolyte transporters and IBD, there is 1 report of a SNP in the *KCNN4* gene that is associated with ileal CD.[37] This gene encodes a calcium-activated K^+ channel (KCNN4) that is widely distributed in IECs and has many important transport functions. These include recycling of K^+ across the basolateral

membrane during colonocyte Cl^- secretion, mediating colonocyte apical K^+ secretion, and an apparent role in regulating Paneth cell secretion of antimicrobial peptides.[37,38] KCNN4 (KCa3.1) is also a major calcium-activated K^+ channel in T cells, and inhibition of this channel has been shown to have efficacy in limiting T-lymphocyte–mediated murine colitis.[39]

EPITHELIAL JUNCTIONS

The intestinal epithelium is composed of a single layer of polarized columnar epithelial cells that are laterally affixed to adjacent epithelial cells by apicolateral tight junctions, adherens junctions, and desmosomes located toward the basal aspect of lateral space.[40,41] Although formation of the adherens junctions is essential for appropriate polarization and formation of tight junctions, the tight junctions themselves are the primary regulators of paracellular permeability.[42,43] Many excellent review articles have been published describing adherens junctions and tight junctions in great depth, and we refer the reader to a sample of those articles for a more comprehensive appreciation of these structures.[41,44–49] Briefly, adherens junctions are formed through interactions between a family of cadherin transmembrane proteins, i.e., E-cadherin that form strong interactions with molecules on adjacent cells such as p120 catenin and β-catenin. These molecules in turn regulate local actin assembly and perijunctional actomyosin ring development.[41,50] Adherens junctions are required for assembly of the tight junction, which seals the paracellular space. Tight junctions are composed of at least 2 functionally distinct pathways. First, there is a high-capacity, charge-selective pore pathway that allows passage of small ions and uncharged molecules. In addition, there is a low-capacity leak pathway that permits the flux of larger ions and molecules, regardless of charge. Therefore, the tight junction operates as a selectively permeable barrier, and this seems to operate independently of the number of tight junction strands based on comparative studies in Madin-Darby canine kidney (MDCK) epithelial cell clones with markedly different electrical resistances but identical tight junction strand numbers.[49,51] Tight junctions are comprised of integral membrane-spanning proteins such as members of the claudin family, occludin, and immunoglobulin superfamily members such as the junctional adhesion molecules. A variety of other tight junction proteins including integral membrane, peripheral membrane tight junction proteins, and signaling proteins, including a number of kinases involved in tight junction regulation, have also been identified.[52,53] A critical feature of these proteins is that many interact with the actomyosin perijunctional ring that forms the key stabilizing structure necessary for tight junction and adherens junction integrity. Arguably, the most important of the transmembrane proteins are members of the claudin family, because they define various aspects of tight junction permeability in a tissue-specific manner. The great variety of claudin family members lends great flexibility to the composition of the tight junctions and their overall influence on tight junction function. For example, although one might intuitively consider expression of a leaky claudin as detrimental to barrier function, loss of expression of claudins 2 and 15 in mice caused deficient Na^+ paracellular flow and nutrient uptake, resulting in death from malnutrition.[54]

Occludin is another integral transmembrane tight junction protein, and it can interact directly with claudins and actin. The role of occludin in tight junction regulation is less clearly

understood as occludin knockout (KO) mice show no obvious defect in barrier function.[55] However, occludin has been implicated as a protective factor in barrier regulation in a number of studies.[56–59] Peripheral membrane proteins, such as zonula occludens 1 and 2 (ZO-1, ZO-2), are crucial to tight junction assembly and maintenance. This is due in part to their multiple domains that facilitate interaction with other integral or regulatory proteins, including claudins, occludin, and actin and thus allow them to act as scaffolding proteins.[60–62] Furthermore, these proteins are dynamic and a typical marker of barrier dysfunction altered membrane localization or internalization of tight junction proteins, with or without changes in overall expression.[56] Therefore, appropriate localization of tight junction proteins is an essential factor in epithelial homeostasis. Disruption of epithelial barrier function can therefore affect many key physiological functions of the intestine, from protection against infection, to nutrient uptake to appropriate hydration. Thus, it should come as no surprise that a compromised epithelial barrier is a feature of many chronic intestinal conditions including IBD, celiac disease, and type 1 diabetes.[63]

BARRIER DYSFUNCTION IN IBD

CD and UC, collectively referred to as IBD are believed to arise from disrupted intestinal homeostasis caused by a combination of genetic susceptibility, altered immune interactions with intestinal microbiota, and environmental factors that can initiate or reactivate disease.[4,64] A number of studies in patients with IBD have identified that intestinal barrier function is disrupted both in active and in quiescent disease states.[65] Indeed, landmark studies identified that increased intestinal permeability to inert tracer molecules such as PEG molecules or ^{51}Cr-EDTA occurs before inflammation and can predict disease relapse in patients with CD.[66,67] Moreover, increased permeability has also been documented in siblings of patients with IBD while there is also a case report of an IBD sibling who had increased intestinal permeability without any symptoms of IBD but later went on to develop CD.[67–70] Additional methods used to quantify intestinal barrier function in humans have used measuring the intestinal uptake and urinary excretion of the inert sugars probes such as lactulose and mannitol. A number of studies have shown an increase in the lactulose:mannitol ratio in patients with IBD indicative of barrier defects that reflect abnormal tight junction permeability. Increased permeability to lactulose is associated with elevated permeability of the small intestine because lactulose is broken down by colonic bacteria and therefore is not a marker for colonic permeability.[71,72] Functional measures of increased intestinal permeability were supported by freeze fracture electron microscopy studies showing a reduction in the overall number of tight junction contacts in addition to disjointed tight junction strand patterns. In addition, another well-documented feature of epithelial tight junctions in tissues from patients with IBD is the altered expression or localization of claudin proteins and occludin in mild to moderately active IBD.[73–75]

Studies in a number of animal models of IBD have also provided strong supporting evidence for increased intestinal permeability preceding the onset of intestinal inflammation, thus suggesting a prominent role early in disease development. Madsen et al described increased permeability before the onset of intestinal inflammation in the IL-10$^{-/-}$ mouse, whereas a similar phenomenon has been shown in the Samp1/Yit model of Crohn's ileitis and mdr1a$^{-/-}$ mice.[76–78] Intriguingly, increased permeability is necessary but not sufficient for

the onset of inflammation as shown in elegant studies using a constitutively active myosin light chain kinase transgenic mouse. Myosin light chain kinase has been shown to represent a final common pathway for activation of barrier "leaks" that are large enough to allow macromolecules to pass through tight junctions between epithelial cells.[79–81] These studies support the "two-hit" hypothesis that a barrier defect and a trigger of an inappropriate immune response are required for manifestation of intestinal inflammation.[41,81] Indeed, the importance of an intact barrier was further emphasized in studies showing that restoration of barrier function, as assessed by reducing small molecule permeability with the zonulin inhibitor AT-1001, alleviated inflammation in an IL-10$^{-/-}$ mouse model.[82] The success of this intervention suggests that this approach of specifically improving barrier function before onset of severe inflammation may have viability as a clinical approach in patients in remission. In addition to established effects in paracellular permeability, there is also evidence that suggests dysfunctional transcellular pathways in IBD. This includes studies on excised intestinal tissues from UC and CD patients mounted in Ussing chambers that demonstrate increased transcellular passage of large molecules such as horseradish peroxidase and ovalbumin.[83–85] These studies suggest that increased translocation of luminal contents and bacterial products could be elevated in IBD and thus facilitate increased exposure of lamina propria immune cells to antigenic stimuli that may propagate an inflammatory response. Defects in intestinal barrier function are not restricted to IBD, however, because they are also associated with early stages of disease in celiac disease, type 1 diabetes, and in microscopic colitis, specifically collagenous colitis.[63,86]

REGULATION OF INTESTINAL BARRIER PROPERTIES BY IBD CANDIDATE GENES

IBD candidate genes are involved in regulating multiple components of the epithelial barrier. Although most of the genes listed have been linked to overall modulation of epithelial and/or intestinal permeability, for the purposes of functional relevance, genes have been broadly grouped according to their primary functions in regulating epithelial properties with the caveat that some genes fall into multiple groups. Genes are presented in Table 1 in the order of their discussion in the text.

CELL ADHESION

CDH1

The CDH1 locus at 16q22 comprises 3 genes of which *CDH1* is the most plausible candidate gene.[87] This locus was identified by the UK IBD Genetics Consortium in 2009 as being associated with UC, and the CDH1 gene encodes the adherens junction protein E-cadherin.[88] As mentioned above, E-cadherin plays a critical role in cell–cell adhesion and is also required for tight junction formation. The association with UC was confirmed by McGovern et al in 2010 and in a Dutch cohort by van Sommeren et al.[87,89] Muise et al[90] identified an association between the CDH1 locus risk haplotypes rs12597188, rs10431923, and rs9935563 with CD. This study also identified that these haplotypes were associated with increased E-cadherin cytoplasmic accumulation in the intestinal epithelium that was likely because of the presence of a novel truncated form of E-cadherin. In complementary in

vitro studies using the Caco-2 IEC line and the MDCK renal epithelial cell line, transfection of the truncated E-cadherin in polarized epithelial cells resulted in abnormal intra-cellular accumulation and reduced plasma membrane localization of both E-cadherin and β-catenin. The manifestation of a CDH1 mutation as a functional defect in the form of loss of E-cadherin membrane localization is consistent with our understanding of cytoplasmic internalization of junctional proteins being a common mode of junctional disruption in the setting of inflammation or in response to infection. Evidence for a genetic defect in cadherins and in E-cadherin function leading to altered intestinal homeostasis was generated by studies in mice expressing a dominant-negative N-cadherin, where the function of E-cadherin was perturbed and this resulted in Crohn's-like inflammation in the small intestine.[91]

E-cadherin is also a target for invasion by intestinal pathogens such as *Candida albicans* and *Bacteroides fragilis* that can cleave E-cadherin. In the case of *B. fragilis*, this occurs through release of a toxin called fragilysin that cleaves the extracellular domain of E-cadherin thus disrupting adherens junctions and compromising TJ integrity.[92] Moreover, *Listeria monocytogenes* is capable of binding to E-cadherin through the surface protein internalin-A that interacts with E-cadherin and triggers the same signaling cascade induced by homotypic E-cadherin interactions, including the recruitment of α- and β-catenin, myosin VIIa, and the AJ-associated protein vezatin at bacterial sites of entry. Regarding patients harboring CDH1 mutations, defective E-cadherin localization could conceivably facilitate bacterial penetrance across the epithelium through generalized AJ and TJ defects rather than specific targeting of the extracellular domain of E-cadherin as an entry portal because it is likely internalized in these patients and therefore not as accessible as wild-type E-cadherin.

A study by Demetter et al investigating E-cadherin expression at various sites in active IBD indicated a complex pattern of expression where E-cadherin was upregulated in active IBD at sites of focal inflammation and in sites adjacent to ulcerated areas but was actually reduced in areas of ulceration.[93] Although it has been speculated that downregulation of E-cadherin expression in cells located at the margins of mucosal ulceration could be an important part of epithelial restitution and repair as cell migration is facilitated by reduced cell adhesion, however, this remains to be verified.[5,94] Furthermore, *CDH1* mutations are associated with increased proliferation, invasion, and metastasis of multiple epithelial tumors including gastric and colorectal cancer.[95–98] The separate associations of CDH1 with IBD and colorectal cancer are intriguing given the significantly higher risk of patients with IBD for the development of colorectal cancer.[99,100] Moreover, promoter methylation of the CDH1 locus has been associated with dysplasia in patients with UC. Therefore, epigenetic regulation of the CDH1 promoter by methylation may potentially serve as a biomarker to identify patients at high risk of progression to colorectal cancer.[5,101]

LAMB1

A UC-specific susceptibility locus was identified in a region on chromosome 7q31, indicating the *LAMB1* gene as the strongest corresponding candidate.[88,102] The UK study observed peak association at rs886774, whereas the North American study reported a cluster of *LAMB1* SNPs at 7q31 with the rs4598195 SNP having the highest association.[88,102]

However, a study in a Dutch cohort of patients with UC failed to establish an association of the rs6949033 SNP (after correction).[87] *LAMB1* encodes the laminin β1 subunit, a light chain present in the heterotrimers laminin-1, laminin-2, and laminin-10. Laminins represent the most abundant glycoprotein component of the basement membrane. These αβγ heterotrimeric molecules interact with integrins to create a key cell adhesion network in the intestinal epithelium.[103,104] Consequently, laminins are involved in multiple cell functions including adhesion, migration, differentiation, and survival.[105]

Laminin expression occurs in a heterogeneous manner because segregated expression of laminins has been reported along the crypt-villus axis in human small intestinal basement membrane, with laminin-10 occurring in the upper-crypt/villus and laminin-2 expressed deeper in the crypts.[5,106,107] *In vitro* studies identified that laminin-10 promotes crypt-villus differentiation as assessed by expression of the epithelial brush border enzyme, sucrose-isomaltase.[108] In IBD, laminin expression seems to be increased in serum in both UC and CD, and this correlates positively with disease activity.[109] Intriguingly, laminin levels in the epithelial basement membrane of colonic crypts in inflamed UC was reduced.[110] Although the rs886774 variant of *LAMB1* was not associated with CD, a previous study identified altered expression profiles of laminins in ileal crypts of patients with CD with reduced laminin-2 but increased laminin-5 and laminin-10.[111,112] These data further suggest heterogeneous modes of regulation of laminin expression in response to inflammatory cytokines. Indeed, regulation of laminin expression in an inflamed environment seems to be quite complex. In vitro studies using the normal embryonic human IEC line, HIEC-6, demonstrated that the proinflammatory cytokines TNF-α and IFN-γ can synergistically potentiate laminin-5 and -10 secretion from these cells and this is in contrast to the overall reduced laminin expression in active UC mentioned above.[110,113] The human UC study may suggest that loss of laminin from basement membrane could contribute to overall barrier impairment in UC but in addition that the appearance of laminin in the serum may serve as a biomarker for intestinal barrier dysfunction, although this requires confirmation. *LAMB1* also shows a genetic association with uterine leiomyoma (fibroids) with altered expression of *LAMB1* identified in the leiomyomatous region, thus suggesting a role for *LAMB1* in the genesis and development of leiomyoma.[114] Of greater relevance to intestinal cancers is that serum laminin has been identified as a predictive marker in colorectal cancer.[115]

CELL POLARITY

PARD3

Wapenaar et al uncovered a suggestive association between a cell polarity gene, *PARD3*, and UC having made a clear association of this gene with celiac disease.[116] The SNPs in the *PARD3* gene differed between the conditions with rs10763976 significantly associated with celiac disease and rs4379776 falling just short of significance.[116] In contrast, Wolters et al[117] found no association between *PARD3* and either UC or CD in a Canadian cohort. Par-3 regulation of tight junctions was apparent from a study showing that altered Par-3 polarization in vitro or in biopsies from patients with celiac disease was associated with increased expression of pore-forming claudins like claudin-2 and a reduction in the sealing claudins, 3, 5 and 7, and reduced membrane localization of the tight junction-associated

regulatory protein ZO-1.[118] Interestingly, there was no increase in apoptosis in epithelial cells with impaired Par-3 localization. Although the association of SNPs in the *PARD3* gene is stronger with celiac disease rather than IBD, the functional studies on the role of the *PARD3* protein product, Par-3, in regulating tight junction protein organization provide valuable insights into the consequences of dysregulation of these genes involved in epithelial cell polarity for intestinal barrier function in disease. Par3 is thought to be a scaffolding protein required for the recruitment of the binding complex of an additional PDZ containing protein, Par6, with an atypical protein kinase C (aPKC) isoform that activates Par6, to sites where its activity is required. For example, Par3 binds directly to junctional adhesion molecules, and this recruits it to the tight junctions in simple epithelial cells.[119] In simple epithelial cells, the Par3/Par6/aPKC complex is localized to tight junctions, and it can be recruited to these structures either by the interaction of Par3 with junctional adhesion molecule-1 or by binding of Par6 to the Crumbs (Crb) transmembrane protein complex.[119–122] In contrast to Par6, studies in keratinocytes did not implicate a role for Par3 in epithelial wound healing.[120] However, a Par3-aPKC complex can also be recruited to the leading edge of migrating MDCK II epithelial cells through association with the multi-PDZ protein PATJ (PALS1-associated TJ protein) and in regulating the polarization of microtubule organizing centers during wound healing.[123] Moreover, the Par3-Par6-aPKC polarity complex interacts with the TJ protein occludin to facilitate directional movement at the leading edge of epithelial cells engaged in wound repair.[124] This suggests that mutations in *PARD3* may also affect the signaling outcomes of occludin-regulated events and compromise wound repair thus weakening the recovery response of the epithelial barrier to injury or inflammation.

TIGHT JUNCTION ASSEMBLY

GNAI2

Heterotrimeric G proteins regulate fundamental cellular properties through their Gα and Gβγ subunits.[125] In addition to their well-recognized roles in signaling from plasma membrane receptors, G proteins have been identified as having roles in TJ assembly and barrier function regulation.[126] Gα12 is the protein product of *GNA12* and is a membrane-bound GTPase that has been shown to directly interact with the TJ accessory protein ZO-1.[127] Follow-on studies showed that Gα12 activates Src kinase through a mechanism that uses the heat shock protein 90 (Hsp90) chaperone.[128] Src activation causes phosphorylation of ZO-1 and ZO-2, resulting in loss of claudin 1 and occludin interactions with ZO-1. They also showed that endogenous Gα12 was activated during TJ assembly in the absence of receptor agonists, and that thrombin stimulation of endogenous Gα12 can play a role in thrombin-induced permeability increases in intact MDCK monolayers and in delaying TJ assembly after calcium-switch assays.[128] *GNA12* (rs798502) was identified as the most likely candidate at the 7p22 locus that exhibited a significant association with UC by Anderson et al.[129] Although the functional studies cited above suggest that mutations in *GNA12* may contribute directly to an epithelial barrier defect, the contribution of *GNA12* mutations to UC pathogenesis may also extend to dysregulation of adaptive immune function. Herroeder et al showed that T-cell-specific inactivation of Gα12 and Gα13 in vivo resulted in an increased activity of integrin leukocyte function antigen-1 in murine $CD4^+$ T cells and

lymphadenopathy because of increased lymph node entry and enhanced T-cell proliferation. In addition, they observed increased susceptibility of T-cell Gα12 and Gα13 double-deficient mice toward 2 models of T-cell–mediated pathology: contact hypersensitivity to dinitrofluorobenzene as a model for responses to foreign antigen and low-dose streptozotocin-induced diabetes mellitus as a model of autoimmune disease.[130]

MAGI2

The first study identifying a significant association of the *MAGI2* gene with UC was made by Wapenaar et al[116] in 2008 in a Dutch cohort. This study had the added significance of identifying a risk association of this gene in celiac disease, and thus an additional shared association between these 2 intestinal inflammatory conditions. The shared genetic overlap between IBD and celiac disease is well recognized, and patients with celiac disease, or gluten sensitive enteropathy, have an approximately 5-fold increased risk of developing IBD.[116,131–133] The Wapenaar study focused on 41 genes from the tight junction pathway, including those encoding transmembrane, adaptor, signal transduction, and transcriptional regulatory proteins.[116] The association of *MAGI2* with both UC and celiac disease occurred at rs6962966 suggesting a similar functional defect in this gene in both conditions. In conflict with these findings, and similar to their data with *PARD3* in the same cohort, Wolters et al[117] found no association between *MAGI2* and either UC or CD. McGovern et al made a very comprehensive study of the *MAGI2* gene and its possible association with both CD and UC.[134] They genotyped 113 *MAGI2* SNPs in 681 cases of CD, 259 cases of UC and 195 controls. The most significant IBD association was in intron 6 (rs2160322) and both UC and CD contributed to this association. This article also made the first associations of *MAGI2* with CD in contrast to Wapenaar et al[116] who found no significant association with CD. The most significant CD association was with an intron 2 haplotype (rs7785088/rs323149/rs13246026). They also observed highly significant associations with UC in intron 6 (rs7803276/rs7803705) and also significant associations in introns 2, 6 and 20. In assessing links with other IBD biomarkers, they identified significant associations with IgG ASCA, anti-CBir1, and anti-OMPc positive CD. The UC-associated SNP identified by Wapenaar was not tested in the McGovern paper.[116,134] The *MAGI2* gene is roughly 1.4 megabases consisting of 21 exons that encode a protein of 2410 amino acids, MAGI-2, also known as synaptic scaffolding molecule.[134] The authors concluded that they likely identified SNPs associated with disease rather than actual “causative” polymorphisms.[134]

The MAGI-2 scaffolding protein contains 9 potential protein–protein interaction domains. These include a guanylate kinase-like (Guk) domain, 6 PDZ domains (modular protein interaction domains that bind in a sequence-specific manner to short C-terminal peptides), and 2 WW domains that mediate protein–protein interaction by recognition of proline rich peptide motifs and/or phosphoserine/phosphothreonine containing motifs.[135] MAGI-2 is involved in epithelial tight junction assembly.[136] Consequently, a defect in MAGI-2 expression or function may contribute to disassembly of protein networks involved in tight junction or adherens junction function with resultant barrier defects. The association of *MAGI2* SNPS with immunophenotypic subgroups of CD is intriguing, and the loss of function of this structural scaffolding protein and consequent compromise in barrier properties against intestinal microbes, may explain the association between *MAGI2* SNPs

and antibodies to microbial antigens.[134] In addition, MAGI-2 can inhibit cell migration and proliferation by increasing the stability of the enzyme PTEN (phosphatase and tensin homolog) after binding.[137,138] PTEN plays a critical role in the regulation of cell growth and apoptosis, and defects in apoptosis have been implicated in IBD pathogenesis.[139] Therefore, in addition to effects on junctional assembly, alterations in the balance of epithelial cell growth versus apoptosis may represent an additional consequence of *MAGI2* gene mutations associated with IBD.

MYO9B

The *MYO9B* gene encodes a member of the class IX myosin family of actin-based molecular motor heavy chain proteins. The MYO9B protein contains 4 Isoleucine–glutamine motifs located in the neck domain that bind calmodulin, which serves as a light chain.[140,141] The MYO9B complex has a single-headed structure, and it exhibits processive movement on actin filaments. The protein also has rho-GTPase activity that converts active Rho-GTP into inactive Rho-GDP, thereby downregulating Rho-dependent signaling pathways.[142] Rho family GTPases have been shown to play important roles in regulating actin filament remodeling and tight junction integrity.[52,143,144] In addition to maintaining regulation of paracellular permeability in established TJs, they also have roles in the assembly of intercellular associations and recovery of barrier function.[145] Therefore, mutations in *MYO9B* could affect multiple facets of myosin and actin filament regulation of barrier function. The initial connection between *MYO9B* and impaired barrier function in chronic intestinal inflammatory disease was made by Monsuur et al[146] when they associated *MYO9B* mutations, in particular, rs2305764 (intron 28) with increased risk of celiac disease. However, a number of smaller follow-up studies failed to show *MYO9B* association with celiac disease in different European cohorts.[147,148] The proposed site of genetic variation on chromosome 19p13 also contains an IBD linkage locus (*IBD6*). Although a number of studies have investigated the association between *MYO9B* SNPs and IBD, results have been conflicting. Van Bodegraven et al[149] identified association of a nonsynonymous coding SNP (rs1545620) in UC and less strongly in CD across 3 cohorts of European descent (United Kingdom, Dutch, Italian/Canadian). A weaker association with the *MYO9B* SNP (rs2305764) was also observed. In contrast, Cooney et al[150] found that the coding rs1545620 SNP had no significant association with CD or UC, however, a noncoding intronic SNP (rs2305767) had significant association with CD only. Interestingly, evidence of an interaction between multiple *MYO9B* SNPs and another IBD candidate gene, *IL-23R*, was observed, whereas epistasis was not observed with *ATG16L1*, *CARD15* genes, or the *IBD5* haplotype.[150] Further corroboration for an association of 3 *MYO9B* SNPS (rs2305764, rs2305767, and rs1457092) with IBD and UC but not CD was determined in a Spanish cohort.[151]

A study in a Canadian cohort found no association of *MYO9B* SNPs with UC but identified an association of 3 *MYO9B* SNPs, with rs1457092 being the most prominent, with ileal CD only. Intriguingly, the odds ratio association was in the opposite direction to those identified in the previous studies. By way of possible explanation, Wolters et al[117] proposed that these non-GWAS studies may have been underpowered. An additional concern raised by the authors was that none of the *MYO9B* variants were associated in GWAS.[129,152] This

suggests that if the disease association with *MYO9B* is not a false positive, it must be associated with a subphenotype that was not part of the large-scale GWAS. However, the authors advocated that regardless of the direction of the association of *MYO9B* between the different IBD cohorts discussed, the data from these smaller genetic studies do suggest a possible role for *MYO9B* in IBD pathogenesis. Functional studies indicated a correlation between MYO9B protein expression and intestinal permeability in patients with type 1 diabetes. This seemed to be related to elevated levels of the protein zonulin in patients with diabetes. Zonulin can promote TJ disassemby and increased levels correlated with increased intestinal permeability in vivo and with an increased *MYO9B* gene expression in intestinal tissue.[153,154] This suggested a possible link between MYO9B expression and intestinal permeability changes that were also supported by data identifying a trend towards abnormal intestinal permeability in patients with CD carrying the rs1545620 risk allele.[155,156] More recent studies also indicate a key role for MYO9B in TJ integrity and wound healing. In wounded monolayers of the $Caco2_{BBe}$ IEC line, MYO9B protein localized to the leading edge of lamellipodia of migrating cells. Loss of MYO9B expression by RNAi, or by transfection with a dominant-negative MYO9B C-terminal, results in a decrease in lamellipodia and failure of cells to migrate into the wound.[157] These cells also exhibit mislocalization of ZO-1, occludin, and claudin-1 TJ proteins, and increased TRITC-dextran permeability as determined in a microscopy-based assay, thus indicating a barrier defect. Although the possible role of MYO9B in barrier dysfunction in IBD remains to be conclusively demonstrated, however, the provoking findings from genetic studies and indicators from functional studies in other chronic inflammatory diseases make it an attractive candidate for further investigation.

TIGHT JUNCTION REGULATION

PTPN2

The protein tyrosine phosphatase nonreceptor type 2 (PTPN2) gene locus represents the strongest candidate at the IBD-associated locus 18p11. The first identification of *PTPN2* as an IBD candidate gene was made in the landmark WTCCC article in 2007.[152] The rs2542151 SNP was significantly associated with CD, and this SNP has been the most widely identified *PTPN2* SNP associated with IBD. The *PTPN2* association with CD was confirmed by a number of studies while Waterman et al observed an association between the rs2542151 SNP and colonic CD thus providing some insight into the association of *PTPN2* with inflammation in different intestinal regions.[111,129,158–160] Franke et al[161] made the initial association of the rs2542151 *PTPN2* SNP with UC, although in their study they only observed a weak association of this SNP with CD. Additional insight into associations between *PTPN2* SNPs and disease outcomes was provided by Glas et al[162] In this study, genotype–phenotype analysis could not identify a clear phenotypic outcome associated with *PTPN2* SNPs; however, there was a potential association between the rs7234029 SNP with a stricturing disease phenotype in patients with CD and weak associations of the same SNP with an early onset of CD and UC.[162] In silico analysis, it is predicted that the increased CD risk mediated by rs7234029 may be related to a stronger activation of proinflammatory transcription factors such as NF-κB, C/EBP, and E4BP4. A weak association was described between *PTPN2* and the autophagy gene, ATG16L1, being a strong IBD candidate gene.

Although a functional interaction between these genes and their protein products has not been confirmed, *PTPN2* has been shown to play a role in autophagosome formation in IEC lines.[163] Furthermore, the rs1893217 SNP, an intronic noncoding SNP that is in complete linkage disequilibrium with the GWAS lead SNP rs2542151, has been shown to exert a functional defect in muramyl dipeptide signaling and autophagosome formation in fibroblasts isolated from patients with CD harboring this SNP.[164] This suggests that genes integral to particular pathways may actually represent points of convergence between multiple IBD-associated physiological events and thus increase patient susceptibility through a number of routes. The convergence of *PTPN2* with other IBD-associated genes was also highlighted in a study by Yu et al[165] who identified association of a putative protective *PTPN2* SNP (rs2542152) with CD but also showed that PTPN2 in B-lymphocytes and intestinal tissues was regulated by the IBD-associated transcription factor gene, NK2 transcription factor related, locus 3 (*NKX2-3*) in vitro. Moreover, the authors also described the presence of putative NKX2-3 binding sites upstream of the *PTPN2* transcription start site. The nature of the proposed interaction between NKX2-3 and its regulation of *PTPN2* remains to be determined; however, *NKX2-3* KO mice exhibit disturbed small intestinal development and reduced epithelial proliferation.[165,166]

The protein product of *PTPN2* is the enzyme T-cell protein tyrosine phosphatase (TCPTP), so called because it was originally cloned from a T-cell cDNA library, although it is expressed ubiquitously.[167,168] Colonic TCPTP expression is increased in active CD and particularly so in the epithelium.[169–171] A study of potential causal variants in the *PTPN2* gene locus identified that 5 associated intronic SNPs affected TCPTP protein expression and transcription factor binding.[169] This included rs2542170, rs2847288, and rs1893217, which showed the strongest association in genotyped patients tested in this study. Given that TCPTP expression is upregulated in CD, this may suggest that levels of TCPTP are either insufficient to adequately regulate signaling substrates in an inflamed environment, or that TCPTP enzymatic activity is functionally compromised. The rs1893217 SNP is associated with both CD and UC, but was originally strongly associated with type 1 diabetes.[129,172] In addition, this SNP was associated with early-onset pediatric CD.[173] *PTPN2* SNPs have also been associated with celiac disease thus emphasizing the potential for disruption of major signaling pathways in multiple chronic inflammatory diseases through shared genetic mutations.[174] A striking feature of the pathology of these 4 chronic inflammatory diseases, CD, UC, type 1 diabetes, and celiac disease, is that they all feature increased intestinal permeability before the onset of inflammation.[71]

Two functional variants of TCPTP are expressed that arise from alternative splicing of *PTPN2* message. The 48 kD form is targeted to the endoplasmic reticulum by a hydrophobic C-terminus, whereas the 45 kD variant lacks the hydrophobic C-terminus and is targeted to the nucleus by a bipartite nuclear localization sequence that is also expressed in the 48kD form, but this sequence is masked by the hydrophobic C-terminus.[175,176] The 45 kD form can exit the nucleus in response to a variety of stimuli to access substrates in the cytoplasm and at the plasma membrane.[177] TCPTP substrates include: (1) receptor protein tyrosine kinases such as the epidermal growth factor receptor (EGFR) and the insulin receptor; (2) non-receptor protein tyrosine kinases such as members of the Src family and JAK-1 and -3;

and (3) Protein tyrosine kinase substrates such as p52Shc and signal transducers and activators of transcription (STAT)-1, -3, -5 and -6.[177–183] Mice globally deficient for *Ptpn2* develop a systemic inflammation and die at approximately 5 weeks of age from severe anemia, hematopoietic defects, and the development of progressive systemic inflammatory disease, characterized by increases in circulating proinflammatory cytokines, splenomegaly, diarrhea, and lymphocytic infiltrates in nonlymphoid tissues.[184,185] However, features of the inflammation in *Ptpn2*-deficient mice are strain-dependent.[186] Bone marrow chimeric studies indicate that the loss of *Ptpn2* in the nonhematopoietic compartment is the critical factor in the severe inflammation these mice develop.[185] Furthermore, heterozygous mice lacking 1 *Ptpn2* allele show increased susceptibility to challenge with dextran sulfate sodium (DSS).[187]

Regarding the role(s) of *PTPN2*/TCPTP in the regulation of barrier function, we have previously shown that knockdown of TCPTP in IECs facilitates a greater barrier defect induced by the proinflammatory cytokine, IFN-γ.[171] This was associated with expression of the cation-selective claudin-2 pore that mediates increased paracellular passage of sodium ions. Furthermore, TCPTP-deficient cells also exhibited an enhanced permeability to 10 kD FITC-dextran suggesting additional mechanisms involved in regulating a leak barrier defect, as distinct from a claudin-2 pore effect, are also likely affected. Although TCPTP regulates a number of substrates, it does seem to exert a discreet level of control depending on the signaling context. Specifically, TCPTP regulation of EGFR phosphorylation was studied in response to direct versus indirect activation of the EGFR by epidermal growth factor (EGF) and IFN-γ, that can transactivate the EGFR, respectively.[188] Knockdown of TCPTP in T_{84} colonic epithelial cells accentuated EGF but not IFN-γ-induced EGFR tyrosine residue phosphorylation and enhanced EGF inhibition of Ca^{2+}-stimulated chloride secretion.[189] Therefore, this capacity of TCPTP to discriminate between different modes of activation of the same substrate may be of particular consequence about identifying the functional consequences of IBD-associated *PTPN2* SNPs. Indeed, this will likely be of great importance in studying the effects of *PTPN2* SNPs on signaling events initiated by IBD-associated inflammatory cytokines such as IFN-γ and TNF-α that disrupt barrier function and whose activity is negatively regulated by TCPTP.[56,170,171,190]

EPITHELIAL DIFFERENTIATION

HNF4A

The gene locus for Hepatocyte nuclear factor 4-alpha (HNF4A) has been positively associated with UC in a number of GWAS. The rs6017342 SNP had the strongest new association in the large Wellcome Trust Case Control Consortium 2 (WTCCC2) study.[88] Furthermore, rs6017342 mapped within a recombination hotspot on chromosome 20q13, containing the 3′ untranslated region of the *HNF4A* gene alone.[5] This SNP was also strongly associated with UC in a Dutch cohort.[87] Although common variants of *HNF4A* have previously been shown to affect the risk of type 2 diabetes (rs2144908) and polygenic dyslipidemia (rs1800961), rs6017342 was not in linkage disequilibrium with either of these.[152,191] Although *HNF4A* was not found to be associated with CD in the original WTCCC study, Marcil et al[192] identified 6 *HNF4A* SNPs that were associated with a

Canadian pediatric CD cohort, whereas the rs1884613 SNP was significantly associated with both the discovery cohort and a second distinct cohort after correction for multiple testing. This study concluded that the *HNF4A* gene locus may be a common genetic determinant of childhood-onset CD.

The *HNF4A* gene has previously been identified as being associated with the disease, maturity onset diabetes of the young. Maturity onset diabetes of the young is a genetically heterogeneous monogenic form of noninsulin-dependent (type 2) diabetes mellitus, characterized by early onset, usually before 25 years of age and often in adolescence or childhood and is distinct from type 1 or juvenile diabetes.[193] The *HNF4A* gene belongs to the nuclear hormone receptor family of ligand-dependent transcription factors, and it encodes the transcription factor hepatocyte nuclear factor-4 alpha, which is expressed in the liver, pancreas, kidney, stomach, and intestine.[194] It has a crucial role in mammalian development of the gastrointestinal tract, and expression is found in both absorptive and secretory cell lineages of the intestinal epithelium.[195] *Hnf4a*$^{-/-}$ mouse embryos die before embryonic day (E) 10.5 and show arrested and disrupted gastrulation with the primary defect thought to be impaired visceral endoderm function.[196] KO mice lacking functional HNF4α in the developing colon were generated to circumvent embryonic lethality, and although these mice were subject to neonatal lethality, recovery of colonic tissue at E18.5 showed no expression of HNF4α.[195] Histologically, these tissues showed absent crypt formation and reduced formation of smooth muscle. However, there was no alteration in transition from pseudostratified to simple columnar epithelium in the *Hnf4a* KO mice. In support of the development of a normal epithelium, the cell-adhesion protein, E-cadherin, was readily detected between adjoining epithelial cells regardless of the presence of HNF4α with only a modest reduction occurring in *Hnf4a* KO mouse intestine. This is in contrast to the fetal liver, where loss of HNF4α results in a dramatic reduction of E-cadherin expression.[197] Interestingly, although laminin extended throughout the mucosa occupying the spaces between the crypts of control mouse colons, in *Hnf4a*-null colons, laminin was still clearly detectable but was organized as a lining at the base of the epithelium.[195] The authors concluded that the altered localization of laminin was a consequence of defective crypt formation rather than complete absence of the lamina propria.[195] This may have relevance to patients with IBD with mutations in *HNF4A* and an additional IBD candidate gene, *LAMB1* (discussed below), that encodes for the laminin β1 subunit. In addition, colonic tissue from *Hnf4a*-null mice exhibited a reduction in enterocyte cell numbers due to impairment of proliferation and also in goblet cell numbers. The observed loss of goblet cell morphology likely reflected disrupted maturation of mucus granules as opposed to a significant loss of expression of secretory mucin core protein genes. Conditional KO of *Hnf4a* in IECs using villin-Cre transgenic mice avoided embryonic and neonatal lethality but increased susceptibility to chemical colitis and increased intestinal permeability after treatment with DSS.[198] A separate study using a conditional tamoxifen-inducible *Hnf4a*-villin-Cre KO mouse showed that loss of HNF4α in the small intestines altered Wnt/b-catenin signaling and the balance of crypt proliferation and differentiation. This resulted in increased IEC crypt proliferation, increased permeability, distended tight junctions, and altered tight junction and adherens junction formation. This was associated with decreased ZO-1, claudin-4, and claudin-7 mRNA expression, increased claudin-2 and E-cadherin

mislocalization to the cytoplasm.[199] Another important function of HNF4A is its role as a transcriptional regulator of ion transporters. Darsigny et al[200] demonstrated that loss of HNF4α in mice initiated loss of mucosal homeostasis through a decline in mucosal ion transport rather than the expected increase in permeability. The loss of mucosal homeostasis triggered a chronic inflammatory response in the colon of the mice and a more severe degree of tissue damage in experimental colitis. This served to emphasize the importance of ion transport function in regulating overall intestinal inflammatory status. These mice also displayed increased goblet and enteroendocrine cell numbers in contrast to observations in the developing colon described by Garrison et al.[195,201] Regarding patient studies, Ahn et al[198] described a decrease in HNF4A in colonic tissues from UC and CD patients using a commercially available IBD tissue qPCR array panel. This correlated with their findings of decreased HNF4α expression in murine DSS colitis.

HNF4A is also linked to colon cancer as 3 SNPs in the human HNF4α protein, 2 of which are in the HNF4α F domain that interacts with the Src kinase SH3 domain, increase phosphor-ylation by Src tyrosine kinase. Src activation is associated with the early stages as well as progression and metastasis.[202] The 3 *HNF4A* SNPs can decrease HNF4α protein stability and function, suggesting that individuals with those variants may be more susceptible to Src-mediated effects.[203] Therefore, although *HNF4A* SNPs may influence the onset of both UC and CD through disruption of mucosal homeostasis through effects on IEC crypt formation and differentiation, as well as mucosal permeability and ion transport, they may in addition further increase the susceptibility of patients with IBD to the development of colorectal cancer.

MUCUS AND GLYCOPROTEIN REGULATION

ECM1

A number of IBD candidate genes have been identified that are involved in glycoprotein regulation. The 1q21.2 gene locus spans 290 kb and contains the gene encoding extracellular matrix protein 1 (ECM1). This gene locus was identified as being selectively associated with UC rather than CD with the rs3737240 and rs13294 SNPs reaching significance.[204,205] Further confirmation that the rs13294 SNP in the ECM1 locus was selective for UC was provided in a study of a Dutch patient cohort.[206] Regarding the biology of ECM1 and its role in the mucosal barrier, ECM1 has been best characterized in the skin. ECM1 is a secreted glycoprotein that plays an important role in the structural and functional biology of the skin because loss-of-function mutations in ECM1 can lead to the development of genodermatosis lipoid proteinosis. This condition is characterized by reduplication of the skin basement membrane and hyalinization of the underlying dermis.[207] ECM1 has been proposed to act as a “biological glue” in the dermis, helping to regulate basement membrane and interstitial collagen fibril macroassembly and growth factor binding.[208] Although the function of ECM1 in the intestine is not as well understood, it is possible that it may have a role to play in gut epithelial basement membrane integrity and function. The basement membrane is a specialized structure composed of extracellular matrix components that separates the epithelium from the underlying connective tissue or lamina propria. Moreover, basement membrane can also influence the behavior of epithelial cells by controlling their

shape, gene expression, adhesion, migration, proliferation, and apoptosis.[209] Therefore, ECM1 may be required for basement membrane structural or functional support of the intestinal crypt niche and consequently the ability of the intestinal epithelium to form an effective barrier. Indeed, the Wnt/beta-catenin signal transduction pathway, which governs epithelial differentiation and has also been implicated in tumorigenesis, has been shown to influence ECM1 expression.[210] ECM1 has also been shown to inhibit the activity of matrix metalloproteinase-9, a tissue degrading enzyme that is elevated in active IBD.[211] Furthermore, the destructive capacity of MMP-9 outcompetes the beneficial properties of the barrier protective gelatinase, MMP-2.[212] Thus in patients with ECM1 SNPs, loss-of-function mutations may facilitate increased tissue destruction by MMP-9 and thus exacerbate intestinal ulceration or scarring in IBD. ECM1 is also associated with cell proliferation, angiogenesis, and differentiation and has been shown to be overexpressed in metastatic epithelial tumors including gastric and colorectal cancer.[213] It will be of interest to determine if patients with UC with ECM1 SNPs are at increased risk for development of colorectal cancer versus other UC-associated candidate genes.

MUC3A

A genetic association for mucin genes with IBD has been studied for many years. Satsangi et al[214] performed linkage analysis for susceptibility genes in IBDs involving 186 affected sibling pairs and generated evidence for linkage to markers *D12S83*, *D7S669*, and *D3S1573*. The marker *D7S669* was located near the human intestinal mucin gene "*MUC3*," which is located on chromosome 7q212, and previous reports had indicated a possible mucin abnormality in patients with IBD.[215,216] Kyo et al then examined a correlation between "*MUC3*" and IBD by genotyping of variable number of tandem repeat in the "*MUC3*" gene.[217] Analysis of the 3′ terminal structure showed that MUC3 consists of 2 genes, *MUC3A* and *MUC3B* each containing 2 EGF-like domains.[218] In addition, their data implied that rare alleles of *MUC3A*, which have an unusual number of 51-bp repeat units, are associated with risk of UC and CD.[217,218] The authors made the interesting postulation that these rare alleles in *MUC3A* could encode mucin proteins with conformational alterations. Given that O-glycosylation and N-glycosylation are dependent on the structure of the mucin protein, a conformational alteration of the protein could result in decreased glycosylation.[219] This in turn could lead to increased sensitivity to intestinal bacterial proteases and mucin degradation thus contributing to barrier disruption.[219,220] This gains particular importance given that MUC3A is now recognized as a membrane-bound mucin as opposed to the original belief that MUC3 was secreted. In addition, colonic mucin is heavily sulfated and this helps prevent degradation by bacterial proteases. A significant loss of mucin sulfation has been reported in patients with UC, and this may also be related to the possible under-glycosylation of MUC3A protein associated with rare alleles.[217,221] MUC3A and MUC3B are expressed in the small intestine, colon, and the gall bladder.[222] In addition to other established barrier properties of mucin proteins such as epithelial restitution, an interesting degree of cross-regulation has been observed for MUC3 and the cystic fibrosis transmembrane conductance regulator (CFTR) transporter that is responsible for secretion of chloride and bicarbonate ions.[223] Therefore, it is possible that defects in the *MUC3* gene may have consequences for other proteins critically involved in additional aspects of intestinal epithelial homeostasis such as ion transporters.

MUC19

Danoy et al[224] first postulated a potential link between a SNP in the *MUC19* gene locus and IBD. The rs11175593 SNP was identified in the locus of *MUC19* and *LRRK2* genes. Follow-up studies investigated nonsynonymous coding variants in the *MUC19* and *LRRK2* genes in a case-control sample comprising CD cases aged younger than 18 years at diagnosis.[225] Three *MUC19* SNPS (rs11564245, Asp/His; rs4768261, Ser/Phe; and rs2933353, Glu/Ala) were confirmed as being risk associated with CD with rs4768261 retaining significance after corrections. None of the *LRRK2* SNPs were associated with CD, thus leading the authors to conclude that the GWAS signal at chr 12 position 22 could be accounted for by the *MUC19* gene locus. Rivas et al[226] also identified a rare variant highlighted in *MUC19* (p.V56M on chromosome 12, position 39226476) that was independent of the common associated GWAS variants. Muc19 protein was originally identified as a secretory product of murine sublingual mucous cells.[227] The association of *MUC19* with CD and UC is intriguing as a detailed study by Das et al[228] in the mouse demonstrated that although MUC19 transcript and protein are readily detectable in mucus glands of the oral cavity, MUC19 is not detectable in the stomach, small intestine, or colon. This raises the question to the relevance and pathophysiological role of *MUC19* mutations. Although patients with CD can exhibit extraintestinal manifestations including oral ulcers, the contribution of mutations in this gene to UC are more puzzling given the lack of transcript and protein expression in normal colon, albeit these studies were conducted using mouse rather than human colon.[229] Whether a defect in MUC19 at mucosal sites proximal to the GI tract may have an influence on the pathology of UC remains to be determined.

MEMBRANE TRANSPORT

ITLN1

Human intelectin-1 (hITLN-1) is a Ca^{2+}-dependent, D-galactosyl-specific lectin (120 kD) expressed in Paneth and goblet cells of the small intestine and is believed to serve a protective role in the innate immune response to parasite infection.[230] It recognizes galactofuranosyl residues found in the cell wall of various microorganisms but not in mammalian tissues.[231] Human ITLN1 (120-kDa) seems to have quite different properties from its murine counterpart. Although both hITLN-1 and mITLN-1 recognize galactofuranosyl residues, these intelectins have different quaternary structures and saccharide-binding specificities with human intelectin-1, a disulfide-linked trimer, whereas the mouse homolog is a monomer.[232] The intelectin gene was first cloned from intestinal paneth cells and was suggested to be involved in host defense against microorganisms including *Trichinella spiralis*.[233,234] Intelectin has since been identified as a receptor for the iron-binding glycoprotein, lactoferrin (LF) and is involved in regulating uptake, subcellular localization, and release of immunochemically detectable LF by intestinal epithelial Caco-2 cells.[235,236] Intelectin-1 has been identified as sharing 100% structural homology with an adipokine, Omentin, a secretory protein that is highly expressed in visceral adipose tissue relative to subcutaneous adipose tissue.[237] *In vitro* studies have shown that intelectin/omentin increases insulin signal transduction and enhances insulin-stimulated glucose transport in isolated human adipocytes. Furthermore, low levels of circulating intelectin/

omentin are associated with obesity-induced metabolic dysfunction such as insulin resistance and glucose intolerance while it has also been indicated to play an anti-inflammatory role by reducing TNF-α induction of COX-2 in vascular endothelial cells.[238,239] Therefore, it may play a paracrine or endocrine role in modulating insulin sensitivity and is consequently of great interest in the study of diabetes.[238] A small patient-based study indicated intelectin-1 as an autoantigen in intestinal mucosa isolated from patients with CD perhaps providing additional avenues of investigation for the functional contribution of this gene to IBD.[240] A follow-up study identified that both serum and colonic omentin levels (protein and mRNA, respectively) were reduced in active CD, and it was suggested that reduced omentin may represent a more robust biomarker of active disease than C-reactive protein.[241] A genetic association of the *ITLN1* gene with IBD was identified in a GWAS study by Barrett et al[111] who uncovered a risk association of the rs2274910 SNP at position 1q23 with CD. Interestingly, this SNP was associated with a positive outcome of biologic therapy in the GOIA2 study from a Portuguese CD cohort.[242] However, Shaffer et al[243] did not identify an association of a novel single nucleotide missense polymorphism (Val109Asp) in white patients with IBD in a German cohort. Moreover, an *ITLN1* SNP was also associated with increased risk of asthma.[244] Although the exact contribution of Intelectin-1 to barrier properties of the gut remains to be resolved, its role in the uptake of LF may be critical given the anti-inflammatory effects of LF and its capacity to reduce both bacterial infection, and the effects of LPS, in murine models.[245,246]

Vitamin D Receptor

The vitamin D receptor (VDR) is a member of the steroid receptor family and mediates the effects of the active metabolite 1,25(OH)2 vitamin D3 by regulating transcription of a number of different cellular genes. An 1,25(OH)2 vitamin D3 acts as a pleiotropic endocrine hormone and influences many physiological processes.[247,248] An example of the essential role of vitamin D in human health is that severe vitamin D deficiency leads to rickets because 1,25(OH)2 vitamin D3 is essential for adequate calcium and phosphate absorption from the intestine and hence for bone formation.[249] Vitamin D also has numerous effects on the immune system, and the VDR is expressed by monocytes and activated B and T lymphocytes, whereas 1,25 (OH)2 vitamin D3 is synthesized by activated macrophages and can act in a local paracrine manner similar to other cytokines.[250] Of note, 1,25(OH)2 vitamin D3 activates monocytes and macrophages but suppresses lymphocyte proliferation, immunoglobulin production, NF-κB activation, and the production of IL-2, IL-12, and IFN-γ cytokines.[251–253] The linked, commonly occurring, single nucleotide polymorphisms at the 3′ end of *VDR* (*Bsm*I, *Apa*I, and *Taq*I) 11 and the exon 2 splice site *Fok*I polymorphism12 were initially associated with bone mineral density and osteoporosis in a number of different populations. The *VDR* gene is localized on chromosome 12 at a region linked to IBD in a number of populations.[214,254] Simmons et al[248] were the first to identify a genetic association between CD susceptibility and the *VDR* gene. Although the *Taq*I "*tt*" genotype was found to be associated with CD, no association was found with UC. The *Taq*I polymorphism is an A to C base substitution at codon 352 of exon 8 of *VDR* but this does not produce an amino acid coding change. The functional consequence of this polymorphism for IBD remains to be determined. Although controversial, it has been shown using a *VDR*-luciferase reporter gene construct that the "*t*" allele may be associated with

increased levels of mRNA production in vitro; however, conflicting data also suggest that this polymorphism leads to reduced VDR mRNA or has no effect on VDR expression.[255,256]

Subsequent studies have confirmed the *Taq*I association with IBD and identified associations of other polymorphisms with IBD. The *Bsm*I VDR gene polymorphism has been associated with UC in Jewish Ashkenazi patients.[257] Xue et al conducted a meta-analysis in Asian and European subjects and identified that a significant increase in CD risk for Europeans carrying *Taq*I "tt" genotype and a significant decrease in CD risk for all carriers of the *Apa*l "a" allele.[258] In Asian subjects, the *VDR Fok*I polymorphism seemed to confer susceptibility to UC, whereas in males, the *Taq*I "tt" genotype was associated with susceptibilities to both UC and CD. Studies in an Iranian IBD cohort showed significant association of the *FokI* polymorphism with CD and UC.[259] In contrast to the observations described above, a study in a large cohort of 1359 Irish patients showed no significant association of any of the 4 common *VDR* polymorphisms with IBD, although the *Fok*l allele did approach significance.[260] Although VDR plays a crucial role in calcium uptake and consequent bone formation, *VDR* polymorphisms were not indicators for osteoporosis in patients with IBD with low body mass index being the only independent risk factor.[261]

VDR was confirmed as a protective factor in intestinal homeostasis in studies where *Vdr* KO mice were crossed with other transgenic mouse models of colitis. In the CD45RB transfer model of IBD, $CD4^+/CD45RB^{high}$ T cells from *Vdr* KO mice induced more severe colitis than wild-type $CD4^+/CD45RB^{high}$ T cells. The second model of IBD investigated was the spontaneous colitis that occurs in IL-10 KO mice. *Vdr*/IL-10 double KO mice developed accelerated IBD and 100% mortality by 8 weeks of age. At 8 weeks of age, all of the *Vdr* and IL-10 single KO mice were healthy.[262] A clear influence of vitamin D on intestinal epithelial barrier function was demonstrated by Kong et al[263] who showed that *Vdr* ko mice were more susceptible to DSS and developed more severe mucosal barrier defects. Intriguingly, the colonic ulceration observed in $Vdr^{-/-}$ mice was preceded by a greater loss of intestinal transepithelial electric resistance (TER) compared with $Vdr^{+/+}$ mice after 3 days of DSS. Confocal and electron microscopy analysis also revealed disruption of epithelial junctions in $Vdr^{-/-}$ mice after 3-day DSS treatment. Therefore, $Vdr^{-/-}$ mice were much more susceptible to DSS-induced mucosal injury than $Vdr^{+/+}$ mice. In cell cultures, 1,25(OH)2 vitamin D3 increased expression of ZO-1, claudin-1 and E-cadherin in Caco-2 monolayers, increased TER, and restricted TER loss and E-cadherin relocalization by DSS. The effect of 1,25(OH)2 vitamin D3 was shown to be VDR-dependent because these effects were lost in VDR siRNA-transfected cells. These observations suggest that VDR plays a critical role in mucosal barrier homeostasis by preserving the integrity of junction complexes and the healing capacity of the colonic epithelium.[263] Confirmation that epithelial VDR is important for mucosal integrity was demonstrated in a study showing that intestinal epithelial-specific *Vdr* KO mice were more susceptible to DSS colitis characterized by greater loss of body weight, increased tissue damage, and inflammation. These mice also displayed decreased levels of bile acids in feces.[264] Although the contribution of VDR dysfunction to intestinal inflammation is yet to be determined, there is suggestive evidence that a loss of calcium uptake could, in part, explain some of the defects

associated with loss of VDR activity. The influence of calcium on intestinal homeostasis was demonstrated in a study where calcium supplementation reduced inflammation and strengthened the mucosal barrier in the transgenic HLA-B27 rat model of colitis.[265] Regarding targeting of VDR for therapeutic purposes, a synthetic form of 1,25(OH)2 vitamin D3, 1alpha,25 (OH)2-16-ene-20-cyclopropyl-vitamin D(3) (BXL-62) is a VDR agonist that has shown potent anti-inflammatory activity with the benefit of low-calcemic activity. BXL-62 reduced the severity of DSS colitis in mice and caused a greater induction of VDR target genes, and inhibition of proinflammatory cytokine production, than 1,25(OH)2 vitamin D3 in peripheral blood mononuclear cells isolated from patients with IBD.[266]

EPITHELIAL RESTITUTION

PTGER4

A number of GWAS have identified an association of *PTGER4* with IBD. In a GWAS analyzing more than 318,000 SNPs, Libioulle et al[267] identified a 250 kb region on chromosome 5p13.1 contributing to CD susceptibility. The gene located closest to the associated region is *PTGER4*, which encodes for the EP4 prostaglandin receptor that binds prostaglandin E_2 (PGE_2). The disease-associated alleles (rs4495224 and rs7720838) strongly correlated with increased EP4 expression levels. The 5p13.1 association was validated in a follow-up GWAS identifying the rs1553575 SNP as the most prominent, while also indicating a higher frequency of the 5p13.1 signal in females versus males.[267,268] An association of *PTGER4*, specifically the rs4613763 variant in the 5p13.1 region, with UC was first reported by McGovern et al.[89] In a large study of a German cohort, Glas et al confirmed association of the risk alleles rs4495224 and rs7720838 with CD, whereas *PTGER4* association with CD was also identified in the large meta-analysis by Jostins et al.[1,269] Furthermore, *in silico* analysis identified predicted rs4495224 and rs7720838 as essential parts of binding sites for the transcription factors NF-κB and XBP1 with higher binding scores for carriers of the CD risk alleles. The authors suggested that this may provide an explanation of how these SNPs might contribute to increased *PTGER4* expression.[269] EP4 receptors are expressed in lamina propria immune cells and while constitutively expressed in human colonic epithelium, they are upregulated in IBD.[270,271] *In vivo* studies with *Ptger4*$^{-/-}$ mice showed that they develop more severe DSS colitis than wild-type mice. Moreover, treatment with EP4-selective agonists enhanced epithelial viability and regeneration thus exerting protection against colitis in these mice.[270,272,273] However, the roles of EP4 receptors in inflammation are likely cell-type specific as a proinflammatory role for EP4 has been described in models of rheumatoid arthritis or experimental autoimmune encephalitis, although EP4 has also been shown to drive the differentiation of Th1 cells and proliferation of Th17.[274–276] In addition, PGE_2 upregulates IL-8 production in T_{84} colonic epithelial cells through EP4 interaction, further suggesting a proinflammatory role of PGE_2 activation of EP4.277

Regarding the possible influence of EP4 on barrier function, an in vitro study using Caco-2 IECs showed that PGE_2 can increase paracellular permeability through EP4 or EP1 receptor activation.[278] The EP4-dependent effect occurred in a cAMP-Protein kinase A-dependent

manner and resulted in redistribution of the tight junction proteins occludin and ZO-1, as well as the perijunctional actin ring. These effects were also associated with an increase in myosin light chain kinase activity. Lejeune et al showed that in healthy colonic mucosa and T_{84} IECs, EP4 receptors were localized on apical plasma membrane of epithelial cells at the tip of mucosal folds, whereas, in patients with IBD and in rats with DSS-induced colitis, they were diffusely overexpressed throughout the mucosa. Apical administration of PGE_2 to T_{84} monolayers caused a decrease in barrier function, which was alleviated by an EP4 receptor antagonist.[279] However, PGE2 may also have a protective/restorative role on barrier function after injury. Gookin et al showed in an ex vivo chemical injury model of Ussing chamber-mounted porcine ileum that endogenous prostaglandin production or exogenous PGE_2 administration rescued TER by osmotic-driven collapse of the paracellular space, and this required Cl^- secretion. These studies suggested that mucosal restitution is ineffective in the absence of PG-mediated paracellular space closure.[280] Therefore, the exact role(s) of the EP4 receptor in the regulation of barrier function are likely context-dependent and will require careful elucidation as to the suitability of this target for therapeutic intervention in IBD.

BACTERIAL SENSING

CARD15

Mutations in the *CARD15* gene were the first to achieve significant association with IBD and have been associated most strongly with ileal CD.[281,282] The protein product of *CARD15*, nucleotide-binding oligomerization domain-containing 2 (NOD2), is known to play a critical role as a microbial sensor in innate immune cells including epithelial cells and has been extensively reviewed elsewhere.[283] Regarding a role for *CARD15*/NOD2 in barrier function, Buhner et al[284] identified that *CARD15* mutations were associated with increased intestinal permeability in vivo. In a study of 128 patients with quiescent CD, 129 first degree relatives (CD-R), 66 nonrelated household members (CD-NR), and 96 healthy controls, they analyzed the 3 most common *CARD15* polymorphisms (R702W, G908R, and 3020insC) and correlated their subjects genetic profile with intestinal permeability as determined by the lactulose/mannitol ratio. Intestinal permeability was significantly increased in CD and CD-R groups compared with CD-NR and controls. Perhaps most strikingly, in healthy first degree relatives, high mucosal permeability was associated with the *CARD15* 3020insC mutation. These data provided the first indication that an established IBD-associated genetic mutation could contribute to, or was at least associated with, increased intestinal permeability in patients with IBD. This conclusion was in part supported by a study identifying that patients with CD with NOD2 variants had increased levels of tissue endotoxin as determined by immunohistochemistry compared with NOD2 wild-type patients with CD. Although a decrease in adherens junction proteins and altered molecular ratios of tight junction proteins were observed in inflamed versus noninflamed CD tissues, an association between *CARD15* SNPs and altered junctional protein levels was not investigated.[285] Because a number of mechanisms could be involved in the increased tissue endotoxin levels, including translocation across intestinal epithelium, the impact of *CARD15* mutations on junctional proteins is yet to be fully defined.

MEMBRANE RECEPTOR KINASE REGULATION

ERRFI1

A significant association of the ErbB receptor feedback inhibitor 1 (*ERRFI1*) gene with UC was indicated in a GWAS by Anderson et al.[129] The identified SNP, rs35675666, lies on chromosome 1p36. The UC-associated locus is shared by a number of genes in addition to *ERRFI1*. These include *TNFRSF9* (tumor necrosis factor receptor superfamily member 9 or CD137), *UTS2* (Urotensin-2), and *PARK7* (Parkinson disease autosomal recessive, early onset 7).[129] Of these genes, *ERRFI1* is the most viable candidate to have an impact on epithelial barrier function. The *ERR-FI1* gene encodes mitogen-inducible gene 6 (Mig-6) protein. Mig-6 selectively controls EGFR-family signaling and downstream MAPK activation by a negative feedback mechanism because EGF can actually induce Mig-6 expression. Impairment of Mig-6 attenuation of EGFR family signaling may lead to increased cell proliferation and tumor formation.[286–288] Furthermore, on ErbB receptor family ligand deprivation, Mig6 dissociates from the ErbB receptor and binds to and activates the tyrosine kinase c-Abl to trigger p73-dependent apoptosis in mammary epithelial cells. This was also shown to occur in ErrfI1 siRNA knockdown epithelial cells.[289] These data suggest that ErrfI1 loss-of-function may predispose to epithelial apoptosis, which could in turn compromise barrier function. In addition, EGFR family members play critical roles in intestinal epithelial homeostasis including growth, restitution, wound healing, and transport and barrier functions.[290–294] Although EGFR activation has been shown to have “positive” roles about the outcomes of these physiological processes for intestinal homeostasis, EGFR activation has been shown in a number of studies to participate in deleterious events that compromise barrier function. This involves transactivation of the EGFR in response to agents such as zonulin, oxidants, and bile acids.[295–297] Therefore, context-dependent altered regulation of EGFR signaling may have important consequences for epithelial barrier function.

CONCLUSIONS

GWAS have generated profound insights into the genetic contribution to IBD and the genomic regions that contain risk factors for IBD. Translation of these genetic markers into identification of their influence on biological processes that contribute to the pathogenesis of IBD is the next challenge to an increased understanding of how IBD occurs. IBD risk involves multigenic contributions, each with a relatively modest effect size. Therefore, identification of functional consequences of multiple risk loci that collectively contribute to affect individual or intersecting signaling pathways will lead to significant advances in determining how broad physiological events such as regulation of barrier function and autophagy are affected in IBD. Functional studies of IBD genes associated with barrier function will also help to identify specific barrier regulatory mechanisms affected in CD versus UC (Fig. 1). Although GWAS, fine-mapping, and epigenetic characterization together with follow-up functional studies will greatly enhance our understanding of IBD pathology, an additional goal is to use these risk markers for better patient classification, diagnosis, and potentially treatment. Initial efforts offer promise because a recent study successfully generated a diagnostic tool incorporating serological markers, genetic variants,

and markers of inflammation into a computational algorithm to examine patterns of combinations of markers to first identify patients with IBD and second to differentiate patients with CD from those with UC. Two of the 4 genetic markers used, *ECM1* and *NKX2-3*, are associated with aspects of barrier function regulation as described above. The incorporation of genetic markers, along with markers of inflammation, gave superior efficacy in diagnosing patients with IBD and discriminating between CD and UC than serological markers alone.[298] In addition, expression of tight junction proteins such as claudin-2 and claudin-8 serves as a valuable indicator of the treatment response to the anti–TNF-α agent, Infliximab, in responder versus nonresponder UC patients.[299] Therefore, studies of barrier function-associated IBD genetic risk factors will likely enhance not just our understanding of IBD pathophysiology, but also contribute to better diagnosis and treatment.

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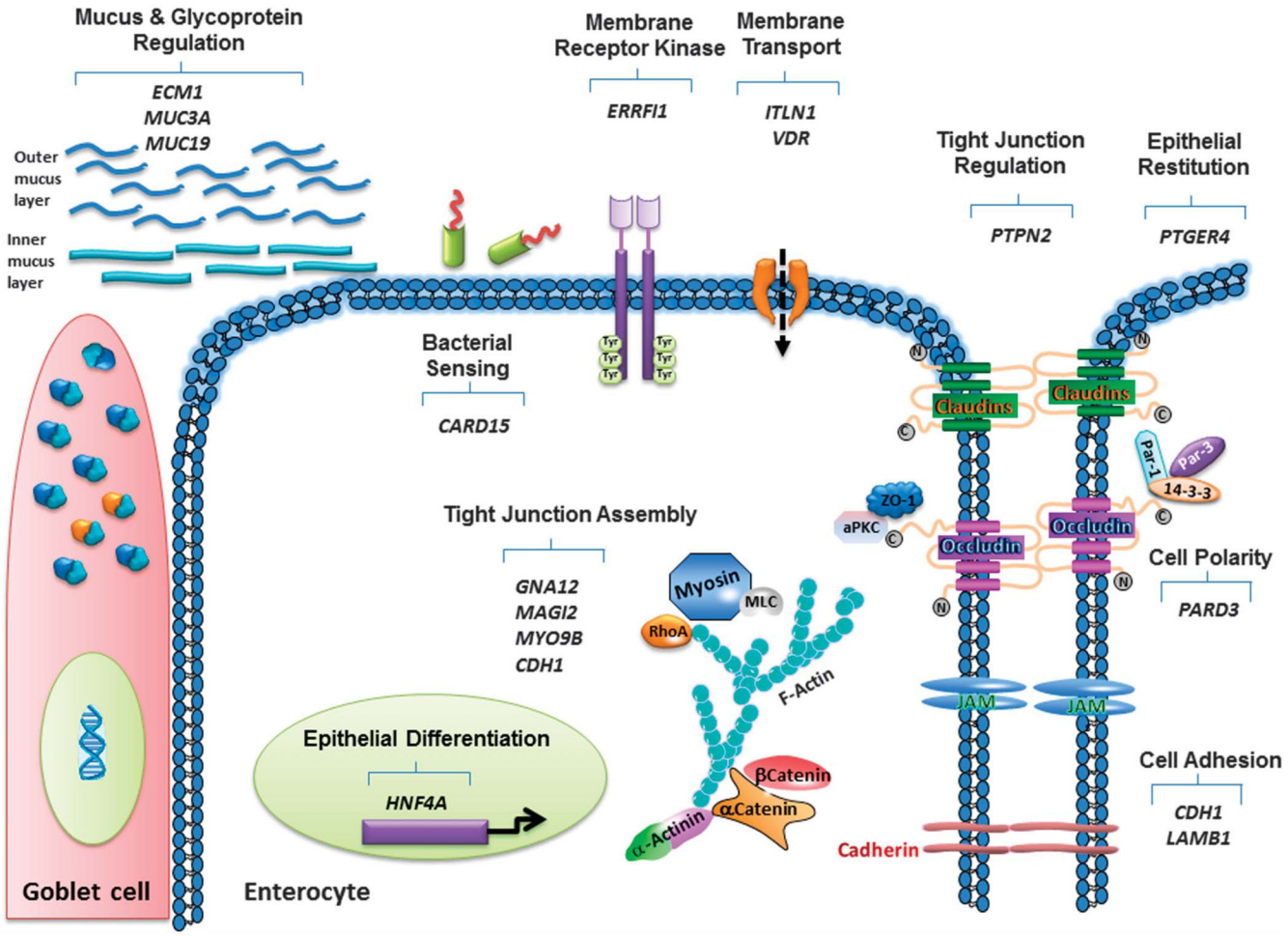

FIGURE 1.

IBD candidate genes regulate multiple aspects of epithelial barrier integrity. IBD candidate genes are involved in regulating multiple components of the epithelial barrier. Although most of the genes listed have been linked to overall modulation of epithelial/intestinal permeability; here, genes are broadly categorized with specific aspects of epithelial barrier properties that they have been identified as influencing.

TABLE 1

IBD Candidate Genes Modify Multiple Barrier Components

Gene	Protein	Function	SNP ID	Locus	CD	UC	Other Diseases	IBD References
CDH1	E-cadherin	Major epithelial adherens junction protein	rs12597188; rs10431923; rs9935563; rs1728785	16q22	+	+	GI Cancers; breast cancer	87–89
LAMB1	Laminin β1	Secreted protein involved in adhesion and differentiation	rs886774	7q31		+	Leiomyoma	87,90
PARD3	Par-3 (partition defective-3)	Cell polarity	rs10763976[a]; rs4379776[b]	10p11		+	Celiac disease; breast cancer[a]	91–93
GNA12	Guanine nucleotide-binding protein α12	Inhibition of TJ assembly through Src and HSP90 phosphorylation of ZO-1, ZO-2	rs798502	7p22		+	Schizophrenia	94,95
MAGI2	Membrane-associated guanylate kinase, WW, and PDZ domain-containing protein 2	Acts as scaffold for assembly of junction proteins. Enhances PTEN dephosphorylation of AKT1	rs6962966	7q21	+	+	Celiac disease, early; infantile epileptic; encephalopathy	92,96
MYO9B	Myosin IXB	Actin-based molecular motor	rs1545620; rs2305767	19p13	+	+	Celiac disease	93,97–99
PTPN2	TCPTP	Dephosphorylates Tyr residues on kinases. Inhibits IFN-γ induction of claudin-2	rs2542151; rs1893217; rs7234029	18p11	+	+	Celiac disease; type 1 diabetes	100–105
HNF4A	Hepatocyte nuclear factor 4α	Transcription factor. Regulates differentiation along crypt-villus axis	rs6017342; rs1884613	20q13	+	+	Mature onset diabetes of the young	87,106,107
ECM1	Extracellular matrix protein 1	Secreted glycoprotein involved in cell proliferation	rs3737240; rs13294	1q21		+	Hyalinosis cutis et mucosae	108–110
MUC3A	Mucin 3A	Transmembrane mucin in the protective inner mucus layer	D7S669	7q22	+	+	Epithelial cancers	111–113
MUC19	Mucin 19	Essential secreted mucin in outer mucus layer	rs11175593	12q12	+		Ankylosing spondylitis	114–116
ITLN1	Intelectin-1	Lectin involved in brush border protection, lactoferrin transport	rs2274910; rs9286879; rs11584383	1q23	+		Type 2 diabetes; Asthma	117–119
VDR	Vitamin D receptor	Ca^{2+} and phosphate absorption; immune regulation	*Taq*I	12q12-q14	+	+	Osteoporosis	120
PTGER4	EP4 receptor	Epithelial restitution	rs4495224; rs7720838; rs4613763	5p13.1	+	+	Rheumatoid arthritis	121–123
CARD15	NOD2	Recognition of pathogen-associated molecular patterns, bacterial sensing	3020insC	5q31	+		Graft versus Host disease, Blau Syndrome	124–126
ERRFI1	ErBB receptor feedback inhibitor 1	Protein product RALT/MIG6 suppresses EGFR signaling	rs35675666	1p36		+	Various cancers	94,127

[a] rs10763976—Strong association with Celiac disease.

[b] rs4379776—Suggestive association with UC.