Slipping the barrier: how variants in CARD15 could alter permeability of the intestinal wall and population health

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The increased intestinal permeability in Crohn's disease is associated with the presence of variants in the *CARD15* gene that are regarded as causative for Crohn's disease, suggesting a genetic rather than an environmental background for intestinal barrier dysfunction in Crohn's disease

The association of sequence variants in the caspase recruitment domain family, member 15 (*CARD15*) gene that encodes for the protein nucleotide binding oligomerisation domain 2 (NOD2) with Crohn's disease¹⁻³ has been a major breakthrough, illustrating the importance of disturbed innate immune function in the aetiology of chronic intestinal inflammation. Three main variants (R702W, G908R, and 1007fsInsC, originally labelled as single nucleotide polymorphisms 8, 12, and 13, respectively) in addition to a large number of private rare variants⁴ lead to incapacitation of the resulting NOD2 protein product.⁵⁻⁸ However, dissection of the main threads of NOD2 induced pathophysiology has resulted in controversial hypotheses.⁹⁻¹⁰ Unravelling the complex pathophysiology induced by expression of variant NOD2 proteins has therefore become one of the new challenges of clinical research in Crohn's disease. NOD2 is expressed in various cell types, including intestinal epithelial cells,⁷⁻⁸ macrophages/monocytes, and B cells.⁶

Intestinal epithelial cells (IECs) have long been regarded as passive components of the mechanical part of the barrier and were thought to be merely involved in innate immune defence. In recent years it has become clear that IECs express PAMP receptors and are capable of recognising invading and adherent pathogens under specific conditions.⁸⁻¹¹⁻¹⁴ Therefore, IECs are the first line of defence against invading pathogens and have to be considered an integral part of the innate immune system of the intestine (reviewed by Gewirtz¹⁵ and Jobin¹⁶). Contact with invading microorganisms can induce concerted secretion of chemotactic and proinflammatory cytokines (for example, macrophage inflammatory protein 1, interleukin 8)⁸⁻¹² and release of

several families of antimicrobial peptides (for example, β defensins).¹⁷

NOD2 is an intracellular receptor for the bacterial cell wall component muramyl dipeptide.¹⁸⁻¹⁹ It is expressed in low levels in colonic IECs but can be upregulated by proinflammatory cytokines both in vitro and in vivo. Under normal unstimulated conditions, NOD2 is expressed in Paneth cells where constitutive expression of NOD2 appears to regulate expression of bactericidal peptides on the cell membrane, such as cryptdins or defensins, respectively, that probably have a gatekeeper function against the ileocolonic microbiota.⁹ NOD2 itself has also been implicated as a direct antibacterial factor in IECs.¹⁴ NOD2 overexpression sensitises intestinal epithelial cells to bacterial cell wall components and induces release of the chemotactic cytokine interleukin 8,⁸ which is the strongest known activator of neutrophils. Impairment of NOD2 function, as is seen in a subpopulation of patients suffering from Crohn's disease, would therefore lead to a complex defect, including facilitated entry of bacteria into epithelial cells through defective regulation of antibacterial peptide (for example, defensin) expression,²⁰ impaired bactericidal capacity, and reduced epithelial immune defence. A net increase in proinflammatory cytokine release in the mucosa could result in this situation from augmentation of TLR-2 function.²¹

Direct augmentation of nuclear factor κ B (NF κ B) activation and signalling through a defective NOD2, as seen in vitro,¹⁻⁶⁻⁸ has recently been suggested to be of relevance in vivo in a transgenic murine model in which a variant equivalent to the human 1007fsInsC was introduced.¹⁰ Although current hypotheses of the exact mechanism of how impairment of the NOD2 sensor domains result in vast and widespread

NF κ B activation in the mucosa in Crohn's disease are not fully conclusive, agreement exists that defective NOD2 function ultimately translates to NF κ B activation in the mucosa and hence increased expression of NF κ B target genes, including proinflammatory cytokines.

Increased permeability to inert macromolecular proteins has long been described in patients with Crohn's disease but clustering of this phenomenon has also been observed in healthy relatives.²²⁻²⁶ Most interestingly, clinical investigation of large pedigrees has suggested that the pattern of increased permeability in healthy relatives of patients with Crohn's disease follows an autosomal recessive mode of inheritance²⁵⁻²⁷ and that subclinical inflammation could also be an independent underlying trait in these families.²⁸ The causative gene(s) has not been identified. The link between abnormal intestinal permeability and subclinical inflammation is also supported by clinical-epidemiological studies which demonstrate that increased permeability in Crohn's disease precedes clinical relapse²⁹ in a similar fashion to increased mucosal production of proinflammatory cytokines³⁰ and of calprotectin.³¹ The hypothesis that abnormal permeability is driven at least partially by proinflammatory cytokines is supported by in vitro data which demonstrate that tumour necrosis factor (TNF) can negatively influence epithelial integrity by an additive action of apoptosis and apoptosis independent mechanisms.³²⁻³³ Anti-TNF therapy leads therefore, unsurprisingly, to restoration of disturbed barrier function.³⁴ However, as alluded to previously in this journal, it remains scientifically unproven whether permeability is primary to an increase in TNF or vice versa.³⁵

The work presented by Buhner and colleagues³⁶ in this issue of *Gut* is important in linking the animal model and in vitro derived mechanisms for regulation of intestinal permeability to genetic susceptibility in the human system (see page 342). They report that the increased permeability in Crohn's disease is associated with the presence of variants in *CARD15* that are regarded as causative for Crohn's disease. The strongest signal, however, was observed in healthy relatives. The findings suggest that *CARD15* variants could be the underlying genetic defect that causes subclinical inflammation and hence permeability disturbances, as have been described previously in relatives and extended pedigrees of patients with Crohn's disease.

The question still remains, what factors and conditions precipitate the

high prevalence of *CARD15* risk variants (that is, up to 8% of the background population) into manifestations of Crohn's disease (that is, 0.2% of the population and only 20–40% of these are carrying *CARD15* risk variants)? The findings of Buhner and colleagues³⁶ suggest that many of the (healthy) relatives carrying a *CARD15* risk constellation could have increased intestinal permeability and hence might have developed a subclinical inflammation constellation. This constellation could put some of them at risk for developing Crohn's disease, similar to the index patients at a future time in their lives.

It is important to note that those *CARD15* risk alleles which have been primarily associated with Crohn's disease are also related to other diseases (for example, asthma, breast cancer, colonic adenocarcinoma). For example, an association has been reported with colonic cancer,³⁷ which although characterised by an odds ratio much smaller than that reported for Crohn's disease, would be of much larger significance to population health due to the higher prevalence of the condition.

The findings of Buhner and colleagues³⁶ need to be extended to the population level. If a *CARD15* risk genotype translates into increased permeability and subclinical inflammation (for example, calprotectin excretion) on a population level, their observation could give rise to the development of genotype based screening and prevention programmes (for example, for colonic cancer) using an anti-inflammatory approach.

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