LEADER

Common pathways in Crohn's disease and other inflammatory diseases revealed by genomics

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Genetics may not provide all the answers but it will, in highlighting the pathways relevant to the pathogenesis of Crohn's disease and other inflammatory conditions, at least indicate which questions need answering

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The past 12 months have seen a remarkable revolution in our ability to detect the genetic variants that predispose to common disease, largely owing to application of the new methods of genome-wide association (GWA) scanning (box 1). This technique is demanding of cutting-edge genotyping and bioinformatic technologies, and requires robust statistical analysis,^{1,2} but has already proved itself capable of delivering highly resolved, highly replicated data in the select group of diseases to which it has so far been applied. One of the most dramatically successful of these has been Crohn's disease.

Until recently *NOD2* was the only confirmed Crohn's disease susceptibility gene.^{3,4} GWA scanning has added no fewer than six more, with six other confirmed loci identified requiring additional fine-mapping to pinpoint the causative gene or regulatory domain. Most of the genes were previously unsuspected and, intriguingly, some of the strongest hits map to gene deserts, hinting at novel mechanisms of complex disease susceptibility.⁵ The effect size at each gene and locus identified is modest (odds ratios of 1.2–1.7 per risk allele), but their importance is in highlighting the pathways and mechanisms that predispose to chronic intestinal inflammation. As has been observed in Crohn's disease this is particularly credible and exciting where hits occur in separate genes that contribute to the same pathway. Further, GWA scan results have indicated potential points of aetiological overlap between inflammatory diseases, with shared hits suggesting that some genes and pathways play a generic role in modulating inflammation. More of these are likely to come to light as the technology of GWA scanning is applied across the spectrum of inflammatory disorders.

"Some genes and pathways play a generic role in modulating inflammation"

GWA scanning is clearly the molecular technique of the moment. As predicted, it has rendered linkage analysis in complex disease obsolete and will indirectly lead to implementation of much-needed reforms in candidate gene approaches.⁶

These "old" techniques as previously applied did have some successes in inflammatory bowel disease. This was best exemplified by the discovery of an association between *NOD2* variants and Crohn's disease susceptibility and, as with most other inflammatory conditions, identification of an important role for the genes of the major histocompatibility complex (MHC) for both Crohn's disease and ulcerative colitis (UC).⁷ However, these techniques do have inherent limitations—mainly relating to the poor resolution of linkage analysis and poor reproducibility of many of the published candidate gene studies. GWA scanning demonstrably addresses and resolves these problems. It is based on association analysis, which provides high resolution, and uses large case-control panels and stringent statistical thresholds to explicitly account for genome-wide multiple testing and limit generation of false-positive results.⁸ Furthermore GWA scans leverage the information extracted from typing 300–500 000 SNP markers by using knowledge of linkage disequilibrium from the HapMap (<http://www.hapmap.org>, accessed 23 August 2007) to derive data or "coverage" for much of the (common) genetic variation in the genome.⁹

IMPORTANCE OF INNATE IMMUNITY AND THE INTERLEUKIN (IL)23 PATHWAY

Among the major and consistent themes to have emerged from the genetic analyses of Crohn's disease is the importance of the early host immune response to bacterial ingress—particularly with regard to innate immunity and its early recruitment of adaptive immune mechanisms. Thus in addition to *NOD2*, four of the top ten genetic hits converge on two key pathways—one the activation of naïve Crohn's disease4+ T cells by the IL12/IL23 pathway, and the other the cellular process of autophagy (table 1). The role of *NOD2* will not be discussed in detail here but is reviewed elsewhere.¹⁰ Suffice to say its precise role as a mediator of innate immunity remains poorly understood, but it is evidently a key sensor of intracellular bacterial muramyl dipeptide—and abnormalities in the handling of intracellular bacteria are emerging as a key theme in Crohn's disease pathogenesis.

In one of the first GWA scans published for any disease, Duerr *et al* reported strong association

Abbreviations: GWA, genome-wide association; IL, interleukin; IL23R, interleukin 23 receptor; MHC, major histocompatibility complex; SNP, single nucleotide polymorphism; T1D, type 1 diabetes; UC, ulcerative colitis; WTCCC, Wellcome Trust Case Control Consortium

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Box 1: Definitions in genomics

Human genome

The complete DNA sequence of one set of human chromosomes. The human genome consists of 22 pairs of autosomes and two sex chromosomes—linear double-stranded DNA molecules totalling 3200 megabases. Just 1.5% of this codes for about 30 000 genes; the rest is non-coding, but a significant proportion is nonetheless involved in regulation of gene expression

GWA scanning

The complete genomes of a group of people with a disease are scanned for hundreds of thousands of markers of genetic variation and compared with the genomes of a control population. If a genetic variant is significantly more frequent in people with the disease it is said to be *associated* and highlights this region of the genome as one likely to contain a susceptibility gene or a regulatory sequence that predisposes to the disease being studied.

Single nucleotide polymorphism (SNP) markers

A single nucleotide variation (eg, substitution of adenine with thymine). This can occur anywhere along the genome. About five million SNPs have been identified in humans to date. SNPs are chosen as markers for use in genetic association studies.

HapMap project

A worldwide collaboration which developed a map of the human genome that describes the common patterns of genetic variation. Adjacent markers are often inherited in blocks, within which recombination at meiosis is relatively uncommon, separated by recombination hotspots. The HapMap project has identified the boundaries of the blocks and the groups of variants ("haplotypes") that typically lie within them.

Linkage disequilibrium

The relationship between variants at nearby marker loci. This occurs owing to lack of meiotic recombination between these markers, usually as a consequence of their proximity. It means that genotyping one marker will provide information as to which allele is present at the other marker.

Gene desert

Regions of the genome that contain few genes or are only partially characterised. They may exert long-range regulatory effects or encode poorly characterised components such as micro-RNAs.

between Arg381Gln and other variants in the IL23 receptor gene (*IL23R*) and Crohn's disease susceptibility.¹³ This result was rapidly replicated by the UK IBD Genetics consortium,¹⁴ with respective significance in the two studies for the amino acid changing variant of $p = 6.6 \times 10^{-19}$ and $p = 1.1 \times 10^{-12}$. Unsurprisingly, given the strength of these results (and in contrast to experiences where non-conservative statistical thresholds are used to claim association), many other groups have now replicated this finding.

The data for *IL23R* were important, both as one of the first demonstrations of the power of GWA scans to produce highly significant and replicable data, and also in providing indisputable evidence for the importance of the IL23 pathway in Crohn's disease pathogenesis. As critical determinants of naïve Crohn's disease4+ T cell differentiation, IL23 and IL12 have closely interlinked functions as well as a shared subunit within their heterodimeric structures. It is therefore of significant interest that we have recently identified association between variants in the *IL12B* gene (which encodes the shared p40

subunit of IL12 and IL23) and Crohn's disease ($p = 9.2 \times 10^{-6}$).¹¹ This finding focuses the spotlight on the whole IL12/IL23 pathway in Crohn's disease pathogenesis. Furthermore, it also highlights this as a major point of overlap with the Crohn's disease-associated condition psoriasis, which also shows highly significant association with variants in *IL23R* and *IL12B*.¹⁵

Functional studies illustrate how variations in the IL12 and IL23 pathways might lead to an aberrant early immune response to microbial encounter. IL12 drives naïve Crohn's disease4+ T cells to a Th1 phenotype, while in the presence of IL23 they adopt the recently described Th17 phenotype, characterised by the production of IL17, tumour necrosis factor and IL6.^{16,17} Th17 cells play a central role in driving organ-specific autoimmune inflammation in a number of animal models. Thus specific mouse models lacking IL23 do not develop experimental autoimmune encephalitis, collagen-induced arthritis or chronic intestinal inflammation, correlating with an absence of IL17 producing Th17 cells in the target organs.¹⁷⁻¹⁹ It appears that some specific bacterial components such as peptidoglycan exert a differential regulatory effect on antigen presenting cells in increasing IL23 gene expression but not IL12.²⁰ One can therefore see how subtle variation in these early IL12- and IL23-dependent regulatory mechanisms might have a substantial impact on the pattern of the subsequent inflammatory response. Importantly Th1 cell mediated immunity is unaffected by knockdown of the IL23 pathway, suggesting a potentially effective means of controlling intestinal inflammation without affecting systemic immunity.

AUTOPHAGY AS A NEW PATHOGENIC MECHANISM FOR CROHN'S DISEASE

Another major advance deriving from recent genomics approaches is the identification of an important role for autophagy in predisposing to Crohn's disease. The demonstration of highly significant and replicated association between Crohn's disease and variants in two separate autophagy genes *ATG16L1* and *IRGM* has focused the spotlight firmly on this previously unsuspected process. Autophagy is the mechanism by which cells recycle redundant organelles and, more important for Crohn's disease, it is now also recognised as playing a key role in defence against intracellular micro-organisms. Thus any bacteria that enter the cytoplasm either by invasion or escape from an endocytic vesicle are usually engulfed by the mechanism of autophagy. Once compartmentalised within autophagosomes the cell can deploy a number of mechanisms to eliminate the bacteria, generating a locally hostile environment within the vesicle by, for example, fusing with lysosomes and activating respiratory burst cascades.²¹ Within some cells autophagy may also play a role in loading antigen into MHC binding grooves for presentation at the cell surface.²²

ATG16L1

Since the beginning of this year, association with *ATG16L1* has been reported by three independent groups from Germany ($p = 4 \times 10^{-8}$),²³ North America ($p < 10^{-10}$)²⁴ and the UK ($p = 6.5 \times 10^{-13}$).⁵ The signal appears to be completely accounted for by a non-synonymous (amino acid-changing) SNP encoding a threonine to alanine substitution (T300A). The exact functional impact of this variant is currently unknown, although the ATG16L1 protein is a key component of autophagy and the T300A substitution occurs in an evolutionarily conserved domain.²³ The gene is expressed in the intestine and particularly strongly in Crohn's disease4+ T lymphocytes, and functional knockdown abrogates autophagy of the intracellular pathogen *Salmonella typhimurium*.²⁴

Table 1 Replicated single nucleotide polymorphisms (SNPs) and genes identified by genome-wide association scans (GWA) published to date in Crohn's disease

Gene	Chromosome	Gene/locus function	SNPs	Scan*	Index scan p Values
<i>ATG16L1</i>	2q37.1	Autophagosome formation	rs2241880	NA	6.38×10^{-8}
				G	0.0004
<i>IL23R</i>	1p31	Interleukin 23 receptor	rs10210302	UK	7.1×10^{-14}
			rs11209026	NA	5.05×10^{-9}
				B	1.5×10^{-8}
			rs11805303	UK	6.5×10^{-13}
None	5p13	?	rs1002922	B	9.1×10^{-8}
			rs9292777	UK	2.9×10^{-7}
None	10q21	?	rs224136	NA	7.90×10^{-6}
			rs10761659	UK	2.68×10^{-7}
<i>IRGM</i>	5q33	Induces autophagy	rs13361189	UK	4.38×10^{-8}
<i>PHOX2B</i>	4p13	Homeobox protein	rs16853571	NA	7.68×10^{-7}
<i>NCF4</i>	22q12	NADPH oxidase	rs4821544	NA	2.89×10^{-5}
<i>FAM92B</i>	16q11	?	rs8050910	NA	3.28×10^{-5}
<i>PTPN2</i>	18p11	Modulates inflammatory signals	rs2542151	UK	4.6×10^{-8}
<i>NKX2-3</i>	10q24	Transcription factor	rs10883365	UK	1.4×10^{-8}
<i>MST1</i>	3p21	Macrophage stimulation	rs9858542	UK	7.7×10^{-7}
<i>IL12B</i>	5q33	Shared subunit of IL12 and IL23	rs6887695	UK	1×10^{-3}

NA, North American²²; G, German²¹; UK, Wellcome Trust Case Control Consortium^{5 11}; B, Belgian.¹²

IRGM

IRGM encodes another key component of autophagy. In the Wellcome Trust Case Control Consortium (WTCCC) GWA scan we found highly significant association between *IRGM* variants and susceptibility to Crohn's disease, with replication in an independent panel ($p = 2.1 \times 10^{-10}$).^{5 11} Mice rendered deficient in the homologue of this gene are known to show impaired ability to eliminate the intracellular pathogens *Toxoplasma gondii* and *Listeria monocytogenes*.²⁵ Furthermore, recent experiments in human macrophages show that knockdown of *IRGM* leads to markedly prolonged survival of *Mycobacterium tuberculosis*.²⁶

Taken together the genetics data for *ATG16L1* and *IRGM* unequivocally implicate defects in autophagy as predisposing to Crohn's disease. The demonstration of prolonged survival of intracellular micro-organisms within macrophages when autophagy is disrupted has clear resonance with the *Mycobacterium avium* subsp. *paratuberculosis* hypothesis for Crohn's disease pathogenesis.²⁷ It might also partly explain the presence of subpathogenic organisms such as adherent invasive *E coli* within intestinal epithelial cells and macrophages from people with Crohn's disease.²⁸ For a long time it has been postulated that commensal intestinal bacteria predispose to Crohn's disease but without any good understanding as to how they might do so: defects in autophagy might provide this mechanism and be a key part of the pathogenic pathway. Understanding whether chronic intestinal inflammation then results from impaired viability of epithelial cells or from the necessary (or possibly inappropriate) activation of adaptive immune pathways with consequent collateral damage, or to other mechanisms entirely will need to await further functional studies.

OTHER DISEASES, OVERLAPPING GENES

As well as providing evidence converging on particular pathways within Crohn's disease, genomics approaches have also illuminated a number of areas of overlap between different inflammatory disorders. This was an explicit goal of the WTCCC and one which has demonstrated some previously unsuspected associations. One of the strongest of these was the association of both type 1 diabetes (T1D) and Crohn's disease with variants in the gene *PTPN2*.^{5 11 29} The gene product is a protein tyrosine phosphatase important in cytokine signalling. *PTPN2* knockout mice are unable to mount T-cell-dependent B-cell responses and develop widespread inflammation with raised serum

interferon γ within 2 weeks of birth.³⁰ On a similar theme rheumatoid arthritis, T1D, systemic lupus erythematosus and Hashimoto's thyroiditis all show strong association with variants in *PTPN2*,³¹ which is a member the same family, and there is a hint in the WTCCC of a modest effect of this locus in Crohn's disease also. Variation in the *RUNX* (Runt-related transcription factor) genes and particularly their binding site at the *OCTN 1/2* genes have also been associated with rheumatoid arthritis, systemic lupus erythematosus and psoriasis^{32 33}—with variants 250 kb either side of *OCTN* also showing confirmed association with Crohn's disease and UC.^{34–36} Of particular interest in gastrointestinal disease, another non-MHC “auto-immune locus” at the *IL2* gene previously implicated in T1D and autoimmune thyroid disease has recently shown strong association with coeliac disease in a UK GWA scan, replicated in independent datasets.^{37 38} The exact disease-causing variants at these loci have yet to be defined and may well differ between diseases, but it is evident that each of these genes plays a key generic role in regulating the rheostat of systemic inflammation.

Box 2: Future directions for research

- Replication studies and meta-analysis: to identify the additional inflammatory bowel disease susceptibility genes of more modest effect and gain a complete understanding of the genetic architecture of Crohn's disease and ulcerative colitis
- Resequencing and fine mapping: to identify exactly which variants at each locus are disease-causing
- Expression studies: to determine which cells express the genes implicated in the association analyses—is this affected by genetic variation or by inflammation, or by both?
- Functional studies:
 - What is the role of the gene product?
 - Is it up- or downregulated?
 - Is this a future “druggable” target?
 - What does it tell us about environmental triggers?

To date GWA scans have not been reported for UC, but as with most other complex diseases these will surely come. We will then have a much clearer picture of the extent to which Crohn's disease and UC share or differ in their genetic susceptibility, and the commonality or otherwise of their pathogenic mechanisms. Such molecular illumination of this debate, which goes all the way back to Burrill Crohn himself, is an imminent prospect. The suspicion—corroborated by data showing strong association of *DRB1*0103* with both UC and colon-only Crohn's disease^{7,39}—is that Crohn's colitis and UC will be genetically more similar than pure Crohn's colitis is to small bowel Crohn's disease.

CONCLUSIONS

There is a tangible sense of excitement in the field of inflammatory bowel disease genetics for at last—after 15 years of endeavour with little more than *NOD2* and the MHC to show—major and rapid progress is now being made towards a complete understanding of the genetic architecture of Crohn's disease. A meta-analysis of GWA scan data currently being undertaken will take us still closer to this goal.

"Genetics may not provide all the answers but it will at least indicate which questions need answering"

Already there are some clearly "druggable" targets such as the IL23 pathway, and progress in understanding the environmental drive for intestinal inflammation genuinely seems a step closer having identified the key role of autophagy defects (box 2). Not for the first time genetics is posing awkward questions. While susceptibility genes common to classic autoimmune conditions have long been hypothesised the commonality of association at *PTPN2* between Crohn's disease and T1D is more surprising and elucidation of the mechanism is awaited with interest. We still do not fully understand how loss of function mutations in *NOD2* leads to increased inflammation, and must now also work out a functional and evolutionary explanation as to why a rare coding variant of *IL23R* confers protection against Crohn's disease (or how the very common allele can be a risk allele for disease). Genetics may not provide all the answers but it will, in highlighting the pathways relevant to the pathogenesis of Crohn's disease and other inflammatory conditions, at least indicate which questions need answering. This will focus future functional studies on immunological processes of clear relevance.

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