Genetics of Crohn's disease behaviour

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Abstract

Crohn's disease is probably an heterogeneous entity. This heterogeneity may be linked to either genetics or environment. In particular the behaviour of the disease, i.e. the tendency to develop stricturing and/or penetrating lesions, may be linked to the genetic background. While epidemiological and clinical data suggest the relevance of these behavioural classifications, the progresses in the characterization of the immuno-inflammatory reaction in the bowel wall shed a new light on possible candidate genes for these genetic predispositions to various Crohn's disease behaviours. Association studies an linkage analysis focusing on growth factors, metalloproteinases and their tissue inhibitors as well as cytokines may bring new interesting data in this field. (Acta gastroenterol. belg., **2000**, 63, **377-379**).

Introduction

Crohn's disease is a multifactorial polygenic disease. Both linkage analysis and association studies are underway to disclose the genes involved in the predisposition to Crohn's disease. Up to now however many of these studies have been discordant or inconclusive. Methodological and technical problems have been advocated. Beside them, there are probably differences in various ethnic groups and also a certain heterogeneity of Crohn's disease. Therefore, it may be more relevant to perform genetic studies on more homogeneous subgroups of patients defined by clear epidemiological, clinical or biological characteristics. What is called the behaviour of Crohn's disease is one of these clinical characteristics and corresponds to the tendency to develop complicated lesions either fistulizing or stricturing.

Evidence for the existence of different forms of Crohn's disease

Clearly, there are several ways of expression of Crohn's disease. The age at onset, the location and the severity of the disease, the response to treatment, the type of lesions and complications : all these characteristics may vary from one patient to another one. However they may also vary in one given patient over time. To really talk about different forms of Crohn's disease that are relevant for genetic studies, we should be able to define clear phenotypes that tend to remain stable over the history of the patient. This is more difficult to clarify because only few studies have evaluated these characteristics prospectively over a long period of time. As far as the behaviour of Crohn's disease is concerned, the first proposition of distinction between different forms was based on the pattern of the indication for surgery. In a retrospective series, 770 patients with surgery for Crohn's disease were studied (1). In 375 of them the indication was a perforating Crohn's disease while non-perforating indication was present in the 395 others. Perforating Crohn's disease included acute free perforation, subacute perforation with abcess formation and chronic perforation with fistula formation, while non-perforating indications comprised intestinal obstruction, medical intractability, haemorrhage, and toxic dilatation without perforation. Among the perforating Crohn's disease, 128 underwent a second surgery, which was for a perforating indication again in 73% of cases, while among the non-perforating Crohn's disease 164 underwent a second operation which was for non-perforating reason in 71% of cases. There was thus a significant tendency for the indication for surgery to remain the same over time and this even held true for a third operation. It was further suggested that this difference might be linked to the amount of fibrosis leading to various types of lesions and scarring. After this landmark publication, a working group proposed a clinical classification of Crohn's disease based on several points including disease behaviour (2). This was characterized as primarily inflammatory, fistulising or fibrostenotic. In this classification, it was not necessarily the surgical history that determined the behaviour, but rather the dominant pattern at any time. Obviously, this may change over time and the predominant pattern, for example between fistulising and fibrostenotic is often difficult to choose. Although this classification on disease behaviour has been widely used since then, a recent publication has clearly showed the bad reproducibility of this classification between observers (3). To try and solve the problems linked to this first classification, another working group has recently proposed a new classification at the world congress in 1998 in Vienna (4). The behaviour of the disease was here defined as non-penetrating non-stricturing, penetrating or stricturing. Beside the change in words to define similar phenomenons, this classification includes an important change : the penetrating or the stricturing behaviour are no longer chosen on the basis of the predominant pattern since the simple existence of a penetrating lesion automatically classifies the patient as penetrating even if stricturing lesion are also present. This is clearer and will probably lead to a better reproducibility. Using this classification the group of the

Mayo clinic found 37.5% of non-penetrating non- stricturing disease, 9.5% of stricturing and 53% of penetrating disease (5). A problem that remains however is the changing pattern over time. Almost all the patients probably start with a non-penetrating non- sticturing disease although a diagnosis made at a time of complication (penetrating or stricturing) is not seldom. Some of the patients will remain pure inflammatory disease over their history while others will develop strictures and secondary or primary (more seldom) fistulas. The delay between diagnosis and the development of these complications varies between patients and it is difficult to be sure in a given patient that the clinical behaviour will no longer change in the future. In the Vienna classification however, when a patient has developed a fistula, he will allways remain a penetrating disease and it seems that some patients will never develop any fistula despite the existence of stricture remaining thus stricturing disease. On the other hand, some patients even after a long history of Crohn's disease, never develop any complication, remaining thus nonpenetrating non-stricturing disease. However, keeping in mind that characteristics geneticalIy determined must remain completely stable over time, one can speculate that it is rather the propension and the rapidity to develop stricturing and/or penetrating lesions than the lesions themselves which are genetically determined. These clinical impressions should be validated in prospective or at least retrospective studies.

Beside the possible stability of the behaviour of the disease, the conservation of this behaviour or phenotype in families where several individuals are affected is another condition to support the hypthesis of a genetic influence on this behaviour. From this point of view, several studies have reported a statistically significant although not absolute conservation within families (6,7,8). The coefficient of concordance for disease behaviour ranged from 49% to 82%.

Pathophysiology and biology of Crohn's disease behaviour

The biology and pathophysiology of these different behaviours of Crohn's disease are not well known. Indeed, while the inflammatory cascade giving rise to inflammatory lesions and sustained inflammation has been better characterized over the last 10 years, still very little is known about why and how some patients will develop strictures and fistulas. When looking at a transversal section of a strictured intestinal segment, it is striking to see not only large amount of collagen deposition, but also smooth muscle cells hypertrophy and hyperplasia (9,10). When analysing collagen deposition, one finds both quantitative and qualitative changes. The total amount of collagen is increased and the smooth muscle cells are found in a state of individual contraction, encased in dense masses of collagen (9). The turnover of connective tissue and particularly collagen seems to be increased as suggested by increased concentrations of hyaluronic acid and type III procollagen in jejunal perfusates (11). The expression of procollagen gene transcripts for types I, III and V, as assessed by in situ hybridisation, correlates with the density of the inflammatory infiltrate (12). In the strictures, particularly large amounts of collagen type V are produced (13). Imballance between metalloproteinases and their tissue inhibitors have also been suggested. By comparing fibrosed Crohn's disease intestine with inflamed but not fibrosed UC tissue, beside a decreased collagen I :III ratio, RT PCR showed expression of MMP-8 and MMP-9 in ulcerative colitis but not Crohn's disease and a higher expression of TIMP-1 in UC (14). The role of growth factors may also be prominent. Illustrating this, a significant increase of IGF I and IGF II mRNA was found in inflammatory and above all strictured intestine of patients with Crohn's disease (15). In strictures, the increase seemed to be more prominent for IGF I. From a cellular point of vue a recent paper showed an accumulation of mast cells in the hypertrophied and fibrotic muscularis propria. These mast cells colocalised with patches of laminin (16). This suggests a role for an interaction between mast cells and smooth muscle cells in the process of fibrosis and stricture formation in Crohn's disease. The mechanisms of fistulae formation are even less documented. There seems to be a prominent role of mechanical factor. Indeed, the majority of fistulae are probably secondary to strictures and develop either within them or at their oral end (17). Furthermore they seem often to arise at a point of weak resistance, such as along a blood vessel track. However, the majority of strictures, even very narrowed, are not associated with fistulae and the question as to why in the context of a luminal hyperpressure some patients will develop fistulae remains unsolved. At this point, biological factors may also be involved. Supporting this concept, a study using RT PCR showed significant increase in IL-1 beta and IL-lra mRNA levels in resected intestines of patients with nonperforating as compared to perforating disease (18). This point is fascinating and needs to be further studied, particularly looking at important mediators in tissue remodeling, such as metalloproteinases and growth factors.

Genetics of Crohn's disease behaviour

Up to now most of the studies looking at genetics of different forms of Crohn's disease have been association studies. The problem for linkage analysis is to gather a sufficient number of families with homogeneous forms of Crohn's disease to study the transmission of a particular feature. The majority of association studies have actually been primarly designed to study genetic predisposition to Crohn's disease as a whole and subgroups comparisons were secondarly performed, often on small subgroups of patients and sometimes without correcting

the results for the multiple comparisons made. Furthermore, as far as the behaviour of the disease is concerned, different classifications have been used making comparisons between studies difficult.

A negative association has been reported between perianal fistulizing Crohn's disease and the allele DRB1*03 of the HLA (19). In that dutch study, DRB 1 alleles were studied in 35 patients with proved perianal fistulizing Crohn's disease and 2400 healthy controls. The frequencies of the DRB1*03 allele were 3% and 25% in Crohn's and controls respectively. This DRB1*03 allele is usually in strong linkage disequilibrium with TNF2 allele of a single base pair polymorphism located at position -308 in the promoter of TNF gene (20). Surprisingly however, here the TNF2 frequency was not decreased in fistulizing Crohn's disease, suggesting recombination between DRB1 and TNF loci in these patients. In another study performed in our unit, we even found a slightly increased frequency of allele TNF2 in a group of patients including not only perianal, but also intestinal fistulizing Crohn's disease, confirming the absence of decrease of TNF2 allele in this subgroup of patients (21).

As mentioned above, studies specifically designed to assess genetics of various phenotypes of Crohn's disease are awaited. The choice of the candidate genes should then be determined by the biological characteristics of the phenotype studied. While studying the tendency to develop intestinal strictures and fistulas, the genes of the IFG system are certainly interesting candidate genes. As already mentioned, an increased transcription of IGF I and II has been described in strictured intestines in Crohn's disease. Furthermore, the IGFs have been shown to induce both collagen deposition and smooth muscle cells hyperplasia which are both important features of intestinal strictures in Crohn's disease (22). We have therefore undertaken a large genetic study on the implication of IGF system genes in Crohn's disease behaviour. We have already studied several polymorphisms in IGF II gene as well as in type 1 IGF receptor gene (23,24). Our preliminary resuts on about 200 patients with well defined phenotype of Crohn's disease according to Vienna classification, indicate a possible implication of the type 1 IGF receptor gene in the predisposition to develop stricturing and penetrating disease. A work is in progress to try and confirm this data.

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