Genetic markers in ulcerative colitis. Why are the results different?

Leading article

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Significant advances in molecular biological techniques permit the accurate typing of patients at the DNA levels by using small amounts of blood. The diagnosis of ulcerative colitis is straightforward if clinical history, laboratory results, sigmoidoscopic/colonoscopic features, histopathological characteristics, and imaging techniques have been applied. Most of the investigators are also aware that ethnic differences are important and therefore care is taken to compare a patient group with an appropriate control. With the exception of the Basques, the Gypsies, the Jews, and possibly the Hungarian and the Finnish populations, no major differences should be encountered among the people of the European continent. Why then is there a difference in results when gene polymorphisms and pANCA antibodies are studied in patients with ulcerative colitis between different countries and occasionally, within one country? Are differences real? What do they mean? Let's first discuss the results obtained with cytokine gene polymorphisms since these results are probably not affected by technological pitfalls, in contrast to the methodology used for the detection of pANCA antibodies (immunofluorescence technique).

In the present issue of this journal, Papo et al. from Tarragona and Barcelona, Catalonia, Spain report the allelic distribution of the genes encoding the cytokine genes interleukin-1 receptor antagonist (IL-1RN), tumour necrosis alpha (TNFA), and TNFbeta (or lymphotoxin alpha, LTA) in a case-control study of 95 non-operated patients with ulcerative colitis attending two referral hospitals. The authors found no significant differences between UC patients and controls in any polymorphism studied. Contrary to other studies, no differences were found in UC subgroups as a function of the clinical pattern. One explanation for the different results could be that only non-operated patients were studied. No information is given on time of follow-up. The fact, that 41% of their patients had extensive colitis, however without any need for surgery during their clinical course, suggests that patients were good responders to the standard therapy, or that only a minority of them belongs to the 20% of the patients with chronic-relapsing type of UC. It may also suggest, that the follow up time period has been relatively short. To compare therefore these patients with those of our department and those reported by Roussomoustakaki et al. from Oxford seems somewhat risky. The aim of the Oxford study was to test the hypothesis that the IL-1RN allele 2 predicts severe and extensive ulcerative colitis in patients undergoing surgery for their disease (3). It should be taken into account that patients with pancolitis have a variable prognosis since the disease is dynamic. For example, a total of 1161 patients with UC in Denmark were followed up from diagnosis up to 25 years. After 10 years, the colectomy rate was 24%. The cumulative probability of a relapsing course is 90% after 25 years of

follow-up (4). The rates of colectomy in Oxford and Denmark (a community-based study) are probably different due to different indications of colectomy since tertiary referral centres have more patients with aggressive, extensive disease, requiring surgery. There is evidence that carriers of allele 2 of the IL-1RN have more chances to belong to these groups of patients (3, 5-8). A study from Rennes and Lille in France, clearly shows a higher frequency of allele 2 of IL-1RN gene polymorphism in the surgically treated, than in the non-operated patients (7). Another interesting observation linking the presence of this allele to severe UC has been reported by Brett et al. (9), who found an increased frequency of the IL-1RN allele 2 in a group of 53 patients with restorative proctocolectomy for UC with the majority arising from the pouchitis group; this observation suggests that carriers of this allele have an increased chance of developing pouchitis after a restorative colectomy for UC. However, there is no strong association between this allele and ulcerative colitis and one therefore needs to study large numbers of patients. Carter and colleagues (10) studied 357 unrelated British Caucasian patients with UC and 827 healthy controls and noticed that patients with UC had a statistically significant increased carriage rate of allele 2 (53.2%) when compared with controls (44.9%). This difference was highest for pancolitis. Carriage of allele 2 is associated with an Odds Ratio (OR) of 1.4 (95% CI: 1.1-1.8) for UC. For pancolitis, the OR is 1.78 (95% CI: 1.2-2.7).

To a certain extent, the biological mechanism of this association is now better understood. German and British investigators have provided evidence that carriers of allele 2 produce significant lesser amount of

the IL-1ra in their inflamed intestinal mucosa, in particular in relation to the production of IL-1 (11, 12). Earlier studies in healthy individuals have shown, that inter-individual variations in *in vitro* production of IL-1 protein were to some extent determined as inherited inter-individual differences (13). The VNTR gene polymorphism in the IL-1RN gene as well as polymorphisms at positions -511 and +3953 in the nearby IL-1B gene were shown to influence the level of production of their respective protein. Recently it has been demonstrated, that production of IL-1β by mononuclear cells of blood donors is increased in those individuals who are carriers of allele 2 of the IL-1RN gene (14). This strongly suggests that those individuals who produce high amounts of IL-1β and lower amounts of the IL-1ra are at a clear advantage in case of a bacterial infection but at a disadvantage in autoimmune disease. Papo et al., have not typed their patients for polymorphisms in the IL-1B gene. Therefore we cannot judge, whether in Spain the presence of the IL-1RN allele 2 is linked to a high secretor allele of the IL-1B gene. The authors have typed for bi-allelic polymorphisms of the TNFA and LTA genes and found no differences in the polymorphisms analysed between their patients and controls. Undeniably, similar considerations concerning the relationship with subgroups and gene polymorphism as made above in relation to the IL-1RN gene polymorphisms, apply to the TNFA and LTA genes.

This holds perhaps even more strongly for these genes because of the solid linkage disequilibrium with genes of the MHC, and it is well know that certain subgroups of patients with UC are associated with distinct MHC haplotypes. It has been shown recently that a specific

combination of four alleles in the TNFA and LTA genes, haplotype TNF-C (15), was increased in patients with extensive colitis. This haplotype was significantly positively associated with progression in the extent of disease in 98 clinically well-characterised UC patients with a mean period of follow-up period of 10 years (8).

Papo et al., report that 52% of their patients were pANCA positive. This is in contrast with the more than 70% of pANCA positive UC patients, reported in a previous study in Barcelona (16). Sera from 112 patients from a large UC outpatient clinic from "Hospital de la Santa Creu i Sant Pau" were compared with the sera from 501 patients from the Free University Hospital in Amsterdam. The sensitivity of pANCA was 75% for ulcerative colitis with a specificity of 88% in Amsterdam, while in the Barcelona group, sensitivity was 71% and the specificity was 87.5% (16). It is likely, that he study from Papo et al. deals with a smaller number of patients with more severe outcome than the Barcelona-Amsterdam study. Papo et al., have observed that "the combination of a subclinical (pANCA) and a genetic (the IL-1RN gene) marker identified a distinct UC subgroup (pANCA; genotype IL-1RN 1,2)". This finding would provide further evidence of genetic heterogeneity within UC, and would support the concept that pANCA may represent a subclinical marker of genetic heterogeneity. However, in view of the above mentioned functional studies of the IL-1RN allele 2, it is difficult to understand why the group homozygous for allele 2 is included in the same group of patients with the genotypes 1,1; 1,3; and 3,3 (Papo et al., Table 4).

The genetic analysis of complex diseases, such as ulcerative colitis and Crohn's disease, is difficult. The difficulties encountered when identifying disease susceptibility loci are the presence of disease heterogeneity, the relatively low frequency of the disease in the population, the degree to which first-degree relatives of patients are affected (approximately 10%), the presence of genes with minor phenotypic effects, as well as ethnic differences (17). The working hypothesis is that the contributions of genetic polymorphisms that are involved in the regulation of inflammation, such as cytokine gene polymorphisms, HLA, pANCA will determine the heterogeneity and prognosis of the disease. These studies will probably be of great importance to the tailoring of specific treatment in the future. A premise for the success of these studies is to use a solid clinical and genetic classification, which should take into account the dynamic process and the natural evolution of the disease on the one hand, and the use of the advances of molecular biological typing on the other.

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