Genome-wide association studies in Behçet’s disease: 
expectations and promises

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Behçet’s disease (BD), a multisystem inflammatory disorder characterised by recurrent exacerbations in mucocutaneous, intraocular, vascular or other tissues, has a multifactorial etiology. A genetic tendency to BD has long been noted, and a relatively high sibling recurrence risk ratio ($\lambda_s$ value between 11.4 and 52.5) has been estimated with the documentation of familial aggregation (1). Association of HLA-B51 with BD has been recognised as the strongest contribution to the disease tendency so far. This association was identified in 1973 by investigation of just 21 patients with BD and 78 healthy controls (2). It was later replicated in various ethnic groups, and a recent meta-analysis of all available HLA-B51 studies in BD from different parts of the world revealed a pooled odds ratio of 5.78 for HLA-B5/B51 allele for the development of BD (3). On the other hand, HLA-B51-related pathogenic mechanism(s) in BD pathogenesis has yet to be elucidated. Analysis of multicase families suggests an important contribution of non-HLA genes to the BD pathogenesis (3). Until very recently, case-control studies conducted in relatively small number of patients and controls revealed many association findings of various candidate genes with BD. Unfortunately only a few of them (e.g. polymorphisms in the TNF, MEFV, ICAM1 and eNOS genes) were replicated in different ethnic groups (4). Most of the association studies did not provide reliable data which is needed to understand the underlying pathology of the disease, mainly due to problems resulting from the lack of enough statistical power, the recruitment methods of the patient and control groups, and the absence of robust data on the disease pathogenesis to help the selection of the right candidate genes. With the help of Human Genome and HapMap Projects, as well as the availability of low cost high-throughput genotyping platforms for single nucleotide polymorphisms, genome-wide association studies have become a realistic approach of searching susceptibility genes for complex diseases. Recently, BD has been added to the enlarging list of the diseases screened by these high-throughput analysis methods at the genome-wide level for the role of common polymorphisms in its pathogenesis (5, 6).

Two independent studies conducted in the populations of the opposite ends of the Silk Road enabled the discovery of genetic risk factors and provided new insights into the basic biological mechanisms in BD. Both studies revealed similar results, which confirm the strong influence of the shared genetic factors between Turkish and Japanese patients, as well as the BD patients of other ethnicities in the development of the “BD phenotype”.

The MHC region polymorphisms in chromosome 6 showed the most significant association findings in BD genome-wide association studies, resulting in the tallest peak in the HLA-B locus in the so-called “Manhattan plot” (5, 6). A second peak was also observed in the telomeric end of the class I region around the HLA-A gene, with a strikingly similar pattern in both Turkish and Japanese cohorts. These findings support the results of Meguro and colleagues, which reported the independent association of HLA-A26 and neighbouring non-classical Class I HLA antigens with BD (HLA-A*26-F*010101-G*010102 haplotype) (7),
adding further difficulties to the HLA and BD conundrum.

The second most important finding of the genome-wide association studies was discovery of the IL-10 gene association with BD. Remmers and colleagues identified an intronic polymorphism in a high linkage disequilibrium (LD) block of the IL-10 gene region (rs15181111), achieving the genome-wide significance (OR=1.41, p=1.88 × 10⁻⁹). Mizuki and colleagues found two SNPs (rs1800871 and rs1800872) in the promoter region of the IL-10 gene giving the strongest association result in Japanese patients (OR=1.64, 9.5 × 10⁻⁹). Remmers et al. demonstrated that the BD-associated intronic rs15181111 variation was associated with lower mRNA expression as well as lower production of IL-10 from mononuclear cells/monocytes following stimulation with lipopolysaccharide (LPS) or MDP+PAM₃Cys, respectively (5). This association between a functional IL-10 polymorphism and BD helps at least partially explaining the dysregulated/exaggerated inflammatory response observed in our patients. IL-10 is an essential cytokine in the regulation of both innate and adaptive immune responses. The IL-10 gene knockout mouse models have been associated with development of uncontrolled enterocolic inflammation induced by intestinal flora (8). Common polymorphisms in the IL-10 gene were identified as risk factors for ulcerative colitis and Crohn disease (9, 10). Also, rare variants of the IL-10 receptor chains (the IL-10RA and IL-10RB genes) were found in patients with early-onset enterocolitis, which are thought to be associated with hyperinflammatory immune responses resulting from disruption of counter-regulatory interleukin-10-dependent “negative feedback” mechanisms (11). Any intervention increasing the IL-10 production may restore the imbalance in favour of controlling hyper-inflammatory state, and clinical efficacy of interferon-alpha treatment in BD may be associated with its potential to induce IL-10 production in appropriate settings (12).

Another shared genetic association in Turkish and Japanese BD patients was located within an intergenic region between the IL-23R and IL-12RB2 genes, which was shown to be in LD block of the IL-23R gene. The IL-23R polymorphisms were found to be associated with diseases comprising the “spondyloarthritides (SpA)” group, ankylosing spondylitis, inflammatory bowel disease, and psoriasis (13,15). Along with the IL-10 association, these findings position BD much closer to inflammatory bowel diseases, and fuels further the long-disputed issue of classification of BD within the SpA. On the other hand, despite all similarities in the shared pathways, BD-associated IL-10 and IL-23R genetic variants do not overlap with those observed in SpA. The differences in the associated sequences may help to understand the functional consequences of these variations in the regulation of innate and Th1/Th17 type adaptive immune responses (16), which may be associated with the development of BD phenotype with its unique features. The findings of the genome-wide studies could not explain all of the heritability observed in our patients. Both studies also provided a long list of weaker associations waiting to be replicated in larger groups of patients, which emphasises the need for collaborations of researchers from different parts of the world. Larger collections of phenotypically well-defined patients and matched controls will help to discover new genes associated with BD, subsets of patients with particular manifestations such as uveitis, as well as genetic risk factors associated with copy number variations. It is also necessary to look for the “rare variants” using alternative approaches, such as exome sequencing or whole genome sequencing in patients with different ancestral background. Therefore, familial cases from extended pedigrees would become much more valuable than previously thought in the discovery of rare, but more penetrant variants associated with the BD phenotype. On the other hand, our expectations from genetics should not be “great”. None of the approaches would be expected to provide data enabling us to develop a genetic test useful in the diagnosis of BD and prediction of prognosis in our patients. Disease-associated polymorphisms are either quite common in the healthy population or very rare, contributing to the pathogenesis in only a very small number of patients despite their larger impacts.

So what to expect from the genetic studies? Advances in the understanding of the pathogenesis of BD are very promising as the consequence of the discovery of genetic risk factors and basic biological mechanisms underlying BD. GWAS and other approaches would help to elucidate the inflammatory pathways further as well as yet unknown networks between the associated genes and/or pathways. Similar to other complex disorders, BD is not a single-gene disease. Therefore, each disease associated functional polymorphism, such as HLA-B51 and IL-10 gene variations may have a distinct role within the context of other associated genes, all interacting in a network; and their interaction with yet unknown environmental factors may complete the complex nature of the disease.

It would not be surprising to identify involvement of more than one pathogenic mechanism in the development of BD and/or different subsets of disease manifestations. In that case, development of diagnostic tools to define the dominant inflammatory pathways may provide insights to therapeutic targets and development of new treatments tailored according to needs of our patients.

Hence, the future of genetic studies in BD seems very promising if we keep our expectations realistic.

References
5. Remmers EF, Cosan F, Kiriño Y et al.
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