

# Genetics of Sjögren´s syndrome

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Autoimmune diseases cluster in families (1-8). Population, family and twin studies have shown that genetic factors exert a significant influence on susceptibility to autoimmune diseases of which insulin dependent diabetes mellitus (IDDM), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and primary Sjögren´s syndrome (pSS) are typical examples. Having solved many of the single gene disorders showing a Mendelian mode of inheritance, time has now come to focus on the genetics of common diseases like the autoimmune, psychiatric and cardiovascular diseases with a multifactorial background. Although monogenic diseases often show extreme clinical phenotypes, the major burden of genetic illness lies in the more prevalent polygenic disorders. These conditions affect thousands of individuals seriously, and their management consumes vast amounts of health care resources. Fortunately, new DNA technology with microsatellite analysis and automatic sequencing procedures, has now made it possible to approach this challenge with greater effort.

## Genetics and autoimmune diseases

A major feature of pSS is its genetic predisposition. Several families containing two or more cases of SS have been described (3,9-14), and a family history with relatives having other autoimmune diseases is quite common for SS patients ( 30-35%) (3,4). Most often this is SS ( 12%), thyroid disease ( 14%), RA ( 14%), or SLE ( 5-10%) (3,4). Evidence of implicating genetic factors in the etiology of autoimmune diseases includes higher frequencies of autoimmune diseases in monozygote than dizygote twins, higher frequencies in dizygote twins than more distantly related family members (15), strong associations of autoimmune disease with specific genes and gene products (16-18), and animal models in which spontaneous autosomal dominant transmission of autoimmune disease occurs. However, genetic twin studies in pSS patients have not been performed. Only a few case reports describing pSS twins have hitherto been published (13,19), and twins with pSS seem to be infrequent.

Most autoimmune diseases are multifactorial disorders where more than one gene is involved in the genetic predisposition, in concert with some environmental factors [16,20,21]. Today there is only one autoimmune disease described with established monogenetic background and that is autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). This first report of a single-gene defect causing a systemic human autoimmune disease came in 1997, and the novel gene was named AIRE (autoimmune regulator) (22,23).

## The role of MHC

The polymorphic major histocompatibility complex (MHC) genes are the best documented genetic risk factors for the development of autoimmune diseases (21,24,25). The most relevant MHC complex genes involved in susceptibility to SS are the MHC class II genes, most specifically HLA-DR and DQ alleles (5). Patients of different origin show different HLA gene association. In Caucasians of northern and western European descent, including North American Caucasians, pSS is one of several autoimmune diseases associated with the haplotypes HLA-B8, DRw52, and DR3. An association with HLA-DR2 has been reported in Scandinavians (26) and DR5 in Greeks (27).

HLA class II allele association has been reported to differ among anti-Ro/SSA positive subjects according to the presence or absence of coexisting anti-La SSB (28). Distinct HLA haplotypes have been associated with different degree of autoantibody diversification in pSS patients (29). Two HLA haplotypes, DR3-DQ2 and DR2-DQ1, have frequently been reported to be associated with autoantibody responses to both Ro and La autoantigens (30). It is noteworthy that stronger

correlation has been found with anti-Ro/SSA autoantibodies to HLA DR3 and DR2 than to the disease itself (1,30). Fei et al. (31) found autoantibodies to Ro/SSA and La/SSB to be associated with DR3 and DQA alleles. SS patients with DQ1/DQ2 alleles have a much more severe autoimmune disease than patients with any other allelic combination at HLA-DQ (32). Recently DR3-DQ2 haplotype has been indicated as a possible marker for a more active immune response in Finnish pSS patients (33). The previously mentioned studies indicate that the MHC plays an important role in the predisposition to SLE and SS. However, studies of HLA haplotypes in multiplex SLE and SS families, and differences in HLA associations observed between different ethnic groups, suggest that HLA-DR alleles are probably not the sole or primary disease-associated alleles. Other alleles may be better candidates for conferring disease susceptibility.

### **“Autoimmunity gene”**

Mapping has revealed that many autoimmune disease genes colocalise with the genes of other autoimmune diseases. It has been suggested that there may exist shared “autoimmunity genes”, a common core of genetic loci which confer susceptibility to autoimmunity. This core of autoimmunity genes increases the risk for the development of many autoimmune disorders, while other genes and environmental factors determine the susceptibility of the target organ (6,34). Each of the autoimmune conditions may be associated with a different confluence of multiple genes and environmental exposures that may need to exceed some threshold for the development of disease (18).

Genome wide linkage searches of autoimmune disorders have identified a large number of non-major histocompatibility complex loci that collectively contribute to disease susceptibility. Genes encoding complement components, transporters associated with antigen processing (TAP), and immunoglobulin genes are strongly linked loci to a number of autoimmune diseases (16). Mutation in TAP2 (Val<sup>577</sup>) may be involved in Ro/SSA autoantibody production and could be a factor of importance for the susceptibility to SS (35). This is in line with what is found in SLE (36).

### **New technology enables new strategies**

Before 1980, only a few genes had been identified as disease loci. This had largely been possible because of previously established biochemical basis of the disease so that purification of the gene product could be performed. The application of recombinant DNA technology in the 1980's and subsequent use of the PCR technique for linkage studies and mutation screening, offered new approaches to mapping and identifying the genes underlying inherited disorders, and the number of disease genes identified started to increase rapidly (37).

Problems with polygenic or multifactorial diseases are i.e. 1) that different constellation of genes may lead to the same disease phenotype; 2) high incidence of disease-prone genes in the population may entail that an identified gene may not be contributing to the disease process in all affected individuals; 3) small contributions by any given gene to the disease process will signify that large numbers of samples may be required, and biases in patient selection may obscure the contribution of a gene; 4) diagnoses are based on observations far removed from the basic physiologic process causing false classification of individuals; 5) an identified gene may be linked or in linkage disequilibrium with other genes in the area, and 6) replication of results may be difficult and erratic (38).

In the search for susceptibility genes of pSS, two main strategies are utilised, the position independent candidate gene approach with mutation screening of suspected disease related genes, and microsatellite analysis on humans as well as on animal models to determine susceptible chromosomal regions which later on will be used in a positional candidate gene strategy.

## **The candidate gene approach**

Because pSS affects the immune system, there are a number of topical candidate genes, as the genes encoding T-cell receptors (TCRs), cytokines, Ro/SSA and La/SSB autoantigens, Fas and FasL and a cascade of other molecules involved in the apoptotic-signalling pathway. We have been screening for mutations in genes encoding Fas, FasL, Ro/SSA and La/SSB in pSS patients of the following reasons: It has been postulated that the accumulation of lymphocytes in the salivary glands of SS patients may be the result of defect apoptosis (39). Fas and its ligand, FasL, are cell surface molecules that transduce the cell death signal (40-43), and probably participate in autoimmune disease development (44,45). Abnormally expressed Fas is observed in the *lpr* mutant mouse strain and a mutation in FasL is seen in *gld* mutant mice which display lymphoproliferative disorders and high production of autoantibodies (46-48). Mutations in Fas and FasL have been found in patients with SLE and lymphoproliferative disease (45-52).

The increased level of autoantibodies against Ro/SSA and La/SSB antigens is another characteristic of pSS, although not specific only for this disease. Anti-Ro/SSA antibodies are found in 50-80% and anti-La/SSB in 30-60% of the patients. The significance of these antibodies is unclear, but changes in disease activity have been found to be associated with change in autoantibody levels in pSS and SLE (53). In pSS, the presence of anti-Ro/SSA and anti-La/SSB antibodies correlates with cytopenias, as well as with vasculitis, hypergammaglobulinemia and rheumatoid factor (54). The known association between these autoantibodies and the specific HLA alleles also makes the genes encoding these autoantibodies interesting for candidate gene analysis.

## **Microsatellites and linkage analysis**

The standard tools for linkage analysis of complex inherited diseases are now microsatellites. The basis for making this approach possible lies in the organisation of the human genome in a large amount of blocks of tandemly repeated non-coding DNA sequences spread throughout the whole genome. These repetitive arrays exhibit high degrees of polymorphisms both between individuals and in the number of repeats at a given chromosomal site. A set of about 400 commercially available microsatellite genetic markers placed evenly over the genome are tested on families having at least two affected individuals. Linkage analysis is performed by different statistical methods, and regions where linkage is found in the genome scan are analysed for candidate genes. When the disease has been mapped to a chromosomal region, confidence in a particular candidate disease gene is increased substantially if it can be shown to map to the same subchromosomal region as the disease gene, and this is called positional candidate gene approach.

Gene scan on pSS families is ready to start. In order to reach significance and to understand the relative value of such genes in different populations, extensive family material is required. pSS families are continuously collected, and a set of about 370 microsatellite markers will be used for a genome wide search for susceptibility genes. A similar approach is at present applied on other autoimmune diseases at several laboratories around the world. Of outmost interest are the genomewide scanning being performed on the related disease SLE (55-57). Potential SLE loci have been identified on several chromosomes and there are as expected ethnic differences. Association studies have implicated numerous candidate loci for SLE such as HLA DR3 and DR2, Fc $\gamma$ -receptors IIA and IIIA and hereditary complement deficiencies. An effect for the Fc RIIA candidate polymorphism is syntenic with linkage in a murine model of lupus.

## **Conclusion**

In conclusion, genetic studies on autoimmune diseases are expanding. A great effort is put into the collection of affected families, microsatellite analysis of the human genome for susceptible chromosomal regions, and candidate gene analysis. In diseases such as MS, IDDM, RA, and SLE interesting results have already appeared. It is the intention that similar methods applied to pSS will reveal

more information of the genetics behind the disease and the pathogenesis. This will help in identifying risk factors, improve diagnosis and achieve better therapy.

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(A complete reference list is available from corresponding author AI Bolstad)

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