les have been suggested as a minor risk factor in susceptibility to SLE in several populations [8,10,12]. Interleukin-1 receptor antagonist (IL-1Ra) is a naturally occurring competitive inhibitor of IL-1. The IL-1Ra gene contains a polymorphism in intron 2 consisting of a variable number of copies of an 86-base-pair repeat sequence (two, three, four, five, or six copies) [13]. An association has been found between the IL-1Ra 2 allele and SLE [13,14]. Multiple genes are involved in the development of SLE, and the relative importance of these genes may vary between populations and with environmental exposure. We investigated common variant alleles involved in the immune response, immune complex clearance, and regulation of inflammation, with the hypothesis that combinations of polymorphic candidate genes could have synergistic effects on disease susceptibility. Therefore, we have analysed polymorphisms in the genes HLA DR, HLA DQ, C4A, FcγRIIa, FcγRIIIa, MBL, and IL-1Ra and their association with the development of SLE.

Materials and methods

Patients

The study population comprised 124 female and 14 male Caucasian SLE patients, and 200 blood donors (100 men, 100 women) were used as controls. One hundred thirty-eight patients fulfilled four or more criteria of the American College of Rheumatology (ACR) classification for SLE [15]. Five patients with a clinical SLE diagnosis were included in the study even though they fulfilled only three ACR classification criteria; these five patients had multisystemic disease with an immunologic disorder, i.e. presence of anitnuclear antibodies and symptoms characteristic of SLE such as arthritis, photosensitivity, serositis, nephritis, thrombocytopenia, and leucopenia [16]. A breakdown of the ACR criteria is shown in Table 1. There were 129 families with a single case of SLE and 14 families in which multiple cases were recorded. However, from each multicase family, only the first family member with SLE diagnosis, the index case, was included in the statistical analysis. The mean age at diagnosis of the patients was 40 years (range 10–83) and the mean disease duration was 16 years (range 1–42). The mean Systemic Lupus International Collaborating Clinics/ACR-Damage Index score was 1.9 (range 0–9) [17]. The study was approved by the local ethics committee at Lund University.

Genetic analyses

DNA was extracted by the salting-out method described by Miller and colleagues [18]. Analysis of genetic polymorphism was predominantly performed by polymerase chain reaction (PCR).

HLA

HLA DR and DQ alleles were determined with PCR (Olerup SSP™ DQ-DR SSP Combi Tray, Olerup SSP AB, Stockholm, Sweden). However, a minority of the patients had previously been typed with a lymphocytotoxicity test or by restriction fragment length polymorphism as described before [2]. C4A gene deletion was determined by PCR as described by Grant and colleagues [19], or in a few cases by analysis of restriction fragment length polymorphism and determination of MHC haplotypes [2]. With the presence of a DR3 allele together with a DQ2 and a C4AQ0 allele, due to C4A gene deletion, the subject was considered to have the haplotype HLA DR3-DQ2-C4AQ0, although family studies were not uniformly performed to confirm this assumption.

FcγRIIa gene polymorphism

The genetic polymorphism resulting in amino acid R or H in amino acid position 131 was determined as previously described [20].

Analysis of FcγRIIIa gene polymorphism

The analysis of the F/V polymorphism was performed essentially as previously described [21].

MBL gene polymorphism

Variants of MBL due to mutations at codon 52 (D), 54 (B), and 57 (C) in exon 1 of the MBL gene and promotor variants at position -550 (H/L) and -221 (X/Y) were determined by allele-specific PCR amplification, essentially as described before [9]. The wild-type structural allele is designated A, while 0 is a description of the mutant alleles B, C, and D. Based on previously described associations between MBL genotype and serum concentrations, which were confirmed in our 200 healthy controls, the MBL genotypes were divided into three groups. Group 1 (MBL-low) consisted of patients with two structural mutant alleles (0/0) or on one haplotype a structural mutant allele together with another haplotype containing an LX promoter and the wild-type structural allele (ALX/0). Group 2 (MBL-intermediate) consisted of patients with the promoters LX conferring low serum MBL on both haplotypes but with normal structural alleles (ALX/ALX), or, alternatively, haplotypes with one mutant and one wild-type structural allele with a non-LX promoter together with the wild-type allele. Group 3 (MBL-high) included patients with the A/A genotype and at least one non-LX promoter.

IL-1Ra gene polymorphism

Genetic polymorphism in the IL-1Ra gene was determined with a PCR essentially as previously described [13,22], although one primer was modified.

Primers: 5'-CTC AGC AAC ACT CCT AT-3'

5'-TTC CAC CAC ATG GAA C-3'