Association of HLA-DRB1*13 with susceptibility to uveitis in juvenile idiopathic arthritis in two independent data sets

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Objectives. Juvenile idiopathic arthritis (JIA) is the commonest rheumatic disease of childhood. Uveitis is the commonest eye complication of JIA, potentially leading to eye surgery and/or visual loss. JIA is a complex genetic trait with well-established HLA-DRB1 associations. The aim of this study was to investigate the involvement of HLA-DRB1 in JIA-associated uveitis. Methods. A set of 130 UK Caucasian simplex families consisting of healthy parent(s) and a child affected with juvenile oligoarticular idiopathic arthritis (of which 31 had developed uveitis) had previously been screened for multiple markers in the major histocompatibility complex region. Associations with uveitis were investigated through haplotype pattern mining (HPM) and the extended transmission disequilibrium test (ETDT). A further set of 228 UK Caucasian patients with long-standing JIA were fully genotyped for HLA-DRB1 using PCR with sequence-specific primers. Associations of HLA-DRB1 alleles in patients with uveitis (n=50) were examined individually using the $\chi^2$ test.

Results. In the first cohort, HPM identified significant associations of HLA-DRB1*13 with uveitis in juvenile oligoarthritis ($P=0.002$). The ETDT confirmed overtransmission of this allele in the families (empirical global $P=0.018$). In the second cohort, the significant association of uveitis with HLA-DRB1*13 was replicated ($P=0.0002$, odds ratio 3.4, 95% confidence interval 1.7–6.5).

Conclusions. This study has established the HLA-DRB1*13 association with uveitis in JIA. Further work is necessary in order to explore the prognostic potential of this marker.

KEY WORDS: JIA, Eye complication, DR13, Replication.

Juvenile idiopathic arthritis (JIA) is the commonest chronic rheumatic disease of childhood. Complications associated with JIA include joint deformities, growth retardation, osteopenia and uveitis. Uveitis represents the commonest form of eye involvement in JIA and can lead to significant visual impairment [1]. There is a high incidence of JIA-associated uveitis (commonly posterior and asymptomatic) in the oligoarthritis disease subgroup [2]. In JIA patients with enthesitis-related JIA and psoriatic arthritis, uveitis (more commonly anterior) also occurs, contributing to an overall poor long-term disease outcome [3]. Due to the asymptomatic nature of posterior uveitis, JIA patients in the high-risk oligoarthritis disease subgroup must receive regular ophthalmological examinations. A molecular marker (antineuclear antibody positivity) has already been associated with the development of uveitis in oligoarticular JIA and HLA-B27 is associated with uveitis in the enthesitis-related and psoriatic arthritis groups. However, the elucidation of further specific genetic factors associated with the development of JIA-associated uveitis could provide additional diagnostic and prognostic tools.

JIA is a complex disease [4] with well-established genetic associations with genes residing in the major histocompatibility complex (MHC) on chromosome 6p21.3 [5–7]. The HLA-DRB1 locus has been associated with susceptibility to a wide array of complex diseases, including JIA [6, 8–10]. In addition, previous studies investigating HLA associations with uveitis have identified associations with the HLA-A, HLA-B and HLA-DRB1 loci [11]. However, as the MHC region is characterized by low recombination rates, the observed associations could be due to linkage disequilibrium, the primary genetic association(s) remaining to be identified. The aims of this study were initially to screen multiple markers across the MHC for associations with uveitis in juvenile oligoarthritis, the JIA subgroup with the highest incidence of eye complications, and subsequently to replicate any significant findings in an independent sample set of well-characterized JIA patients.

Patients and methods

The Arthritis Research Campaign Epidemiology Unit (ARC EU) holds the British Society for Paediatric and Adolescent Rheumatology (BSPAR) National JIA Repository. This is composed of a collection of samples from prevalent UK patients and available parents, recruited by the BSPAR with the aid of 17 contributory centres. Patients have been classified according

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to the ILAR (International League of Associations for Rheumatology) criteria [12]. One hundred and thirty UK Caucasian nuclear families, each consisting of an offspring affected with juvenile oligoarthritis and healthy parent(s), were available for study. Of the 130 patients, 31 had developed uveitis prior to recruitment. Ethics committee approval was obtained for the study [North-West Multi-Centre Research Ethics Committee (MREC) 99/8/84] and the University of Manchester Committee on the Ethics of Research on Human Beings. An independent cohort of 228 UK Caucasian patients with long-standing JIA was also available for study. These patients have also been classified according to the ILAR criteria [12] and their characteristics have been described elsewhere [3].

Twenty-seven markers distributed throughout the MHC and including the HLA-A, HLA-B and HLA-DRB1 loci, as well as single-nucleotide polymorphisms (SNPs) in the TNF-α, HLA-E and DIF-2 genes, had previously been genotyped in the simplex families [7, 8, 13]. Briefly, microsatellite markers had been typed using fluorescence-based PCR, SNPs using the SNaPshot™ primer extension method, and the HLA loci using PCR with sequence-specific primers (SSP). The HLA-DRB1 locus was genotyped in the independent sample set of 228 JIA patients using PCR-SSP with 144 primer mixes [14]. Genotype consistency within families was checked and the GENEHUNTER [15] package was used to reconstruct extended MHC inferred haplotypes in the families. Data mining of the extracted haplotypes was carried out using the haplotype pattern mining (HPM) method [16]. HPM was used to screen juvenile oligoarthritis haplotypes for the presence of uveitis associations by finding all phenotype-associated patterns exceeding a χ² threshold value. Linkage and association with the HLA-DRB1 locus was investigated in the families with offspring affected with uveitis, using the extended transmission disequilibrium test (ETDT) [17]. Empirical P values were obtained by running 10,000 Monte Carlo simulations. Recursive partitioning (RP), as implemented in the HelixTree® software package (Golden Helix, Bozeman, MT, USA), was initially used to identify any HLA-DRB1 associations with uveitis in the independent data set of 228 JIA patients. RP is a data-mining method and can be used as an efficient statistical tool to group multi-allelic locus genotypes into homogeneous groups with respect to the outcome variable of interest (in this study, development of uveitis) [18]. HLA-DRB1 phenotype frequencies were also compared between JIA patients with and without uveitis using the χ² test (StatXact 6, Cytel Software, Cambridge, MA, USA). Odds ratios and 95% confidence intervals were calculated.

Results

Family-based study

Screening of juvenile oligoarthritis extended MHC haplotypes for uveitis-associated patterns using HPM identified a significant association with HLA-DRB1*13 (P = 0.002), while none of the other MHC loci were associated with uveitis in juvenile oligoarthritis. The ETDT was subsequently carried out in families with offspring affected with uveitis, in order to confirm involvement of the HLA-DRB1 locus. The total number of informative transmissions was 32 and the global P value for the test was P = 0.009. Linkage and association of the HLA-DRB1 locus to uveitis in juvenile oligoarthritis was confirmed after 10,000 Monte Carlo simulations (empirical P = 0.018). There was a non-significant excess transmission of HLA-DRB1*13 from healthy parents to the affected child (8 transmitted vs 3 not transmitted).

Replication study

The involvement of HLA-DRB1 in JIA-associated uveitis was examined in an independent cohort of 228 patients. Recursive partitioning of the data set identified a significant association of the HLA-DRB1*13 allele with uveitis in JIA (P = 0.01). No other HLA-DRB1 allele was associated with uveitis. Evidence for the involvement of HLA-DRB1*13 in susceptibility to JIA-associated uveitis was additionally obtained when comparing phenotype frequencies between JIA patients with (DR13-positive, 23; DR13-negative, 27) and without (DR13-positive, 36; DR13-negative, 142) uveitis using the χ² test (P = 0.0002). HLA-DRB1*13 conferred an approximately 3-fold risk of developing uveitis in patients with JIA (odds ratio 3.4, 95% confidence interval 1.7–6.5).

Discussion

Associations between the HLA region and oligoarticular JIA are well described, but the immunogenetic basis of JIA-associated uveitis has not been extensively investigated in previous studies. Nevertheless, the HLA-A, HLA-B and HLA-DRB1 loci have been shown to be potentially associated with disease [11]. In addition, a small study (43 juvenile arthritis patients) found that the HLA-DRB1*1301 allele is associated with ANA positivity, a marker of uveitis [19]. The genomic interval encompassing these loci includes many genes of immune relevance that could potentially be involved in pathogenic processes. This study has initially examined multiple markers across the MHC, in order to identify JIA-associated uveitis susceptibility genes. The family-based study design followed for the screening process had the advantage of low false-positive result generation rates and the ability to detect both linkage and association, depending on the analysis method employed. Furthermore, the use of families enabled the construction of haplotypes across the MHC region, by basing phase assignment on parental genotypic information. HPM revealed an association of uveitis in juvenile oligoarthritis with HLA-DRB1*13. None of the other loci examined were found to be associated with uveitis, including HLA-A and HLA-B, as well as SNPs in the TNF-α, HLA-E and DIF-2 genes. Complementary evidence for the involvement of HLA-DRB1*13 in uveitis was additionally obtained from the extended transmission disequilibrium test. Therefore, although further effects in the region cannot be excluded, due to limited power, the involvement of HLA-DRB1*13 in juvenile oligoarthritis-associated uveitis was supported by strong evidence.

The replication of positive findings in genetic studies is important in order to instill confidence in the results generated. This study therefore investigated the association of HLA-DRB1*13 in an independent cohort of patients with long-standing JIA. Confirmation of the significant association was achieved, thereby establishing the involvement of this locus in susceptibility to JIA-associated uveitis. This finding could have implications in the prognosis of the development of uveitis. However, further work is necessary in order to assess the performance and consolidate the use of HLA-DRB1*13 as a prognostic genetic marker. Prospective studies, such as the Childhood Arthritis Prospective Study (CAPS) in the UK, represent ideal settings in which to test the potential of using HLA-DRB1*13 alongside further established high-risk factors, such as ANA, with the ultimate aim of targeting JIA patients genetically predisposed to developing eye complications for intensive screening and early treatment.

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References