194. POLYMORPHISM SCREENING OF THE T LYMPHOCYTE SPECIFIC GLUCOCORTICOID RECEPTOR PROMOTER

A Stevens\(^1\), D Ray\(^1\), R Donn\(^2\), \(^2\) Endocrine Sciences Research Group, University of Manchester, Manchester, United Kingdom; \(^3\) ARC/EU, University of Manchester, Manchester, United Kingdom

Background: Glucocorticoids (Gc) are the most potent anti-inflammatory agents known, and are important for the treatment of multiple inflammatory conditions, including rheumatoid arthritis. However, their use is limited by side effects such as hypertension and osteoporosis. Population based variation in response to Gc therapy has been described, but the genetic basis underlying this response has yet to be determined. The glucocorticoid receptor (GR) gene is a principle candidate for investigation of such variable Gc response. A polymorphism at codon 363 of GR is known to be associated with minimal changes in Gc sensitivity (1). Expression of the GR is determined by multiple tissue specific promoters. Recently, Breslin et al described a T lymphocyte specific promoter/enhancer, 31kb upstream of the GR coding region, which, uniquely, is upregulated by Gc (2). As T cells are critical regulators of the immune response, and are key targets for Gc immunosuppression, nucleotide variation within this region may significantly influence the sensitivity of T lymphocytes to Gc, and as such be important in determining an individual's response to Gc therapy.

Aim: To determine if the T lymphocyte-specific GR promoter contains polymorphisms which may influence Gc response.

Methods: Polymorphism screening was performed using dHPLC (Wave, Transgenicom) in 36 unrelated UK caucasians.

Overlapping PCR fragments were generated to cover the entire 2028bp of the lymphocyte-specific GR promoter. Each fragment was -700bp so allowing a 99% sensitivity for heteroduplex detection. The entire 2028bp region was also sequenced (BigDye ddNTP) for 20 of the individuals studied.

Results: No nucleotide variation was found across the lymphocyte specific GR promoter in any of the 36 UK caucasian individuals investigated.

Conclusions: No genetic variation was seen in the UK caucasian sample set studied. Single nucleotide polymorphism (SNP) frequency varies throughout the genome, but on average a SNP is thought to occur every 1kb (3). If any nucleotide changes do exist within the T lymphocyte specific promoter of the GR they will be present at a frequency of <2.7%, so limiting their potential informativeness in genetic studies of Gc response.

References
[1] Huizenga et al. (1998) J Clin Endocrinol Metab. 83(1);144-151

195. NON-HLA GENETIC INFLUENCES ON OUTCOME OF INFLAMMATORY POLYARTHRITIS: PILOT DATA USING PRESENCE OF THROMBOCYTOPY 5 YEARS AS THE OUTCOME MEASURE

A Barton\(^1\), H Platt\(^1\), P Salfay\(^2\), E Barrett\(^2\), D Symmons\(^1\), E John\(^1\), A Sliman\(^1\), \(^1\) ARC/EU, University of Manchester, Manchester, United Kingdom; \(^2\)Norfolk Arthritis Register, Norfolk and Norwich University Hospital, Norfolk, United Kingdom; \(^3\)Centre for Integrated Genomic Medicine, University of Manchester, Manchester, United Kingdom

Background: A major goal in the management of inflammatory arthritis (IP) is to identify, at presentation, patients who will develop more severe disease in order to target aggressive therapies appropriately. Previous studies have reported associations of the TNF-α and CCR5 genes with erosive outcome in hospital recruited patients with rheumatoid arthritis but, in order to separate susceptibility from severity factors, ideally the study group should comprise an inception cohort of patients with early IP followed prospectively.

Aim: To investigate genetic determinants of presence, extent or progression of erosive change by 5 years in IP patients recruited from the Norfolk Arthritis Register.

Methods: DNA was available for 438 patients with IP (swelling of 2 or more joints for > 4 weeks) all of whom were recruited within 6 months of symptom onset. Hand and feet radiographs (scored by the Larsen method) were available for all patients at 5 years and for 66% patients at 1 year. Single nucleotide polymorphisms (SNPs) in the TNF-α gene were genotyped using a newer extension technique (SNAPshot, ABI). A 32bp deletion in the CCR5 gene was genotyped by size. As disease modifying treatment may mask the influence of genetic determinants of outcome, this was adjusted for in the analysis.

Results: No association was detected with any of the polymorphisms tested with either presence of erosions nor the extent (Larsen score) (Table1) either at 1 or 5 years or with progression of change.

Larsen score by TNF-α allele and carriage of 32 bp CCR5 deletion. OR (95%CI)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Non-erosive (n=244)</th>
<th>Less than median (n=101)</th>
<th>Greater than median (n=93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32bp CCR5 deletion</td>
<td>1.0</td>
<td>1.3(0.8-2.3)</td>
<td>0.6(0.8-1.2)</td>
</tr>
<tr>
<td>TNF-α alleles:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–1031T</td>
<td>1.0</td>
<td>1.2(0.7-2.0)</td>
<td>1.5(0.9-2.6)</td>
</tr>
<tr>
<td>–863C</td>
<td>1.0</td>
<td>1.3(0.7-2.4)</td>
<td>1.7(0.9-3.3)</td>
</tr>
<tr>
<td>–857T</td>
<td>1.0</td>
<td>1.0(0.5-2.1)</td>
<td>0.7(0.3-1.5)</td>
</tr>
<tr>
<td>–376G</td>
<td>1.0</td>
<td>2.1(0.5-9.0)</td>
<td>2.3(0.5-9.8)</td>
</tr>
<tr>
<td>–308G</td>
<td>1.0</td>
<td>1.5(0.8-2.7)</td>
<td>1.5(0.8-2.8)</td>
</tr>
<tr>
<td>–308A</td>
<td>1.0</td>
<td>1.5(0.8-2.7)</td>
<td>1.5(0.8-2.8)</td>
</tr>
<tr>
<td>–238A</td>
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<td>1.1(0.5-2.5)</td>
</tr>
<tr>
<td>–489G</td>
<td>1.0</td>
<td>0.9(0.4-2.6)</td>
<td>1.5(0.6-3.6)</td>
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<tr>
<td>–489T</td>
<td>1.0</td>
<td>1.2(0.6-2.4)</td>
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<tr>
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<td>1.5(0.8-2.8)</td>
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<tr>
<td>–851G</td>
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<td>1.3(0.5-5.1)</td>
<td>1.1(0.4-2.8)</td>
</tr>
<tr>
<td>–1304A</td>
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<td>1.0(0.5-2.2)</td>
<td>1.1(0.5-2.5)</td>
</tr>
<tr>
<td>–1304G</td>
<td>1.0</td>
<td>1.3(0.5-5.1)</td>
<td>1.1(0.4-2.8)</td>
</tr>
</tbody>
</table>

OR = odds ratio, CI = confidence interval

Conclusions: We have not demonstrated a role of these genes with the development of erosions. Further modelling combining other genetic with clinical and environmental factors may improve the ability to identify individuals at high risk of this outcome.

Background: The CCR5 locus was funded by Astra Zeneca Pharmaceuticals Ltd.

196. TRANSFORMING GROWTH FACTOR BETA-1 GENE POLYMORPHISMS AND ANKYLOSING SPONDYLITIS

E Jaakeläinen\(^1\), I Herzberg-Walttani\(^1\), A-M Sims\(^1\), L Bradbury\(^1\), A Catn\(^1\), K Lahko\(^1\), A Crane\(^1\), P Wordsworth\(^1\), J Tuomilehto\(^2\), M Brown\(^1\), \(^1\)Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom; \(^2\)RHV/RHD, Bath, United Kingdom; \(^3\)Rheumatism Foundation Hospital, Heinola, Finland; \(^4\)Department of Public Health, University of Helsinki, Helsinki, Finland

Background: Ankylosing Spondylitis (AS) exhibits a strong genetic component determining both the susceptibility and the severity of the disease and, except for HLA-B27, the genes responsible have not been definitely identified. Transforming Growth Factor Beta-1 (TGF-β1) gene is located on chromosome 19q13 where significant evidence of linkage was reported in both our whole genome screen [1] and the North American Spondylitis Cohort genome screen [2]. We have investigated the effect of this positional and functional candidate gene on susceptibility to and severity of AS in an English and a Finnish population.

Methods: The following five polymorphisms located in the promoter and coding region of the TGF-β1 gene: -800 G/A, -509 C/T, +869 T/C, +915 G/C and +1632 T/C were genotyped using the Polymere Chain Reaction - Sequence Specific Primer (PCR-SSP) approach. 1002 individuals from 212 English and 170 Finnish families with AS were genotyped for all the five SNPs and 762 individuals from 184 multiplex families with AS were typed for the two polymorphisms in the promoter region and the +869 T/C polymorphism. All the probands and the families were HLA-B27-positive. A structured questionnaire was used to assess the age of symptom onset, disease duration and disease severity scores including BASDAI and BASFI. TDT analysis was performed using "TRANSMIT" for the qualitative data and "QDT" for the quantitative data. Nonparametric linkage analysis was performed using ‘Genehunter Plus’.

Results: There were four common haplotypes with a frequency greater than 5%. There was no distortion in the transmission rates to affected siblings of any of the four haplotypes. A marginal association was noted between the rare +1632 T allele and AS in a Finnish population (p=0.04) and in the combined data set (p=0.03), but this was not of sufficient magnitude to explain the
observed linkage at this locus (LOD=2.6). No association was noted between any other SNPs and AS. No association was noted among the subset of families which showed non-parametric linkage score. A marginally significant association between age of onset and +915 polymorphism and +1632 polymorphism was noted (p=0.03 and p=0.01, respectively). No linkage was found between quantitative traits and the SNPs.

Conclusions: This study suggests that the polymorphisms within the TGF-

Background: RA and other autoimmune diseases exhibit a female predominance in disease susceptibility. Sex-linked genetic factors are one of a number of theories proposed to explain this gender difference. We have found previous evidence for linkage of Xp21-p11 region with RA in 252 affected sibling pairs (ASPs) (Hinks et al, 2002). This region also shows previous evidence for linkage to a number of other autoimmune diseases. The Xp21-p11 region spans 25Mbases, therefore to narrow down the region for investigation, fine-mapping with additional microsatellite markers has been performed.

Methods: DNA was available for 183 RA ASP families, which contained 252 affected sibling pairs, and 176 RA simplex families. Twenty-eight microsatellite markers spanning the Xp21-p11 region with an average spacing of 0.9Mb were genotyped using fluorescence-based genotyping technology. Data was analysed using extended transmission disequilibrium test (ETDT) (Sham and Curtis, 1995).

Results: One marker, DXS991, showed evidence for linkage disequilibrium with RA in the total dataset (p=0.04). An adjacent marker, DXST044 showed significant evidence for linkage disequilibrium with RA in RA ASP families (p=0.02) but not in the RA simplex families (p=0.16) or in the combined dataset (p=0.21). These markers are at the distal end of the linkage peak.

Conclusions: Evidence for linkage disequilibrium of two markers with RA has been identified. A number of genes are situated close to these markers, which require further investigation, including the gene APR-1 that may play a role in apoptosis.

References

198. FAMILY-BASED ASSOCIATION STUDY OF CORTICOTROPIN-RELEASENING HORMONE GENOMIC REGION AND RHEUMATOID ARTHRITIS IN SPANISH POPULATION
A Julia1, D Gallardo2, JJ de Agustin1, F Vidal1, P Barcelo1, S Marsal1, 1Unitat de Reumatologia, Hospital General i Universitari Vall d’Hebron, Barcelona, Barcelona, Spain; 2Unitat de Diagnostico y Terapia Molecular, Centre de Transfusió i Banc de Teixits, Barcelona, Barcelona, Spain

Background: A large number of studies have demonstrated that HLA genetic region accounts for one third of the genetic component of Rheumatoid Arthritis (RA). Many other gene locus have been proposed as candidates for the remaining susceptibility to RA but to date none of them has shown consistent association. A recent study has shown and replicated positive association of CRHR2 genomic region markers in UK RA families. In order to validate these findings and assess the relevance of this locus we have performed a family-based association study with Spanish RA families.

Methods: A total number of 121 simplex RA families plus 100 healthy controls from the general population were typed for CRH genomic region using high polymorphic markers (microsatellite, CRHR1 and CRHR2) closely located to the structural gene sequence. For this purpose we developed a multiplex fluorescence-based PCR that allows simultaneous amplification and subsequent electrophoretic analysis of both CRH1 and CRHR2. Family-based association tests for each marker and for both as haplotype were performed by Transmission Disequilibrium Testing (TDT).

Results: With our multiplex PCR method 99% of the samples were conclusively genotyped. From the general population sample we could determine that both markers are in Hardy-Weinberg Equilibrium and that there is significant linkage disequilibrium between them. When performing TDT tests we found that alleles CRHR1a-14 and CRHR2a-15 were positively associated with RA (p<0.05 for both alleles) whereas its haplotype combination was not significantly associated. Interestingly, CRHR1a-10+CRHR2a-14 haplotype, which was positively associated with RA susceptibility in UK population, was undertransmitted in our study, thus indicating an opposite effect although p-value did not reach statistical significance.

Conclusions: Our results suggest a weak association between CRH genomic region and RA in Spanish population. Further replicates of this study in other European populations should help clarify the importance of this region in the susceptibility to RA. In this sense we propose a simple and cost-effective procedure to analyse CRH genomic region markers.

199. LINKAGE AND ASSOCIATION OF MACROPHAGE MIGRATION INHIBITOR FACTOR (MIF) WITH JUVENILE IDIOPATHIC ARTHRITIS
RP Dorr1, E Zeggini1, R Lamb2, E Shelley1, WER Ollier2, BPRG Study Group, W Thomson1, 1ARC/EU, University of Manchester, Manchester, United Kingdom; 2CIGMR, University of Manchester, Manchester, United Kingdom

Background: Macrophage migration inhibitor factor (MIF) is a unique molecule with pro-inflammatory and enzymatic properties. Raised serum and synovial fluid levels of MIF have been demonstrated in juvenile idiopathic arthritis (JIA) patients (1). We have previously described an association of the MIF-173-C allele with JIA (2). As well as identifying the MIF-173C/C polymorphism, we have also recently described polymorphism of a CATT repeat element in the MIF promoter, and two novel intronic polymorphisms (3).

In this present study our aim was to replicate the association and look for evidence linking MIF to JIA.

Aim: To establish linkage and association of the MIF gene to juvenile idiopathic arthritis (JIA).

Methods: 330 simplex families (each containing one affected JIA proband) were used. 220 of the families had 2 parents, and 110 had 1 parent available for genotyping. This study group was independent of the JIA children previously genotyped for the MIF -173 polymorphism (2,3). Genotyping for the MIF-173C/C single nucleotide polymorphism was by SNaPshot ddNTP primer extension and capillary electrophoresis, and for the CATT repeat element by fluorescently labelled PCR primer and capillary electrophoresis (3).

Analysis: The software EPhplus was used to determine linkage disequilibrium (LD). Two point haplotypes were analysed using TDT phase. Finally, conditional ETDT (CETDT) was used to determine if the MIF-173 C/G and the CATT each contribute to the observed result (4).

Results: Strong LD exists between the two loci (p<10^-5), in the main due to the combination of MIF-173-C with the longer length CATT repeat (n=7). Linkage and association was found (p=0.0016), with the MIF-173C-C /CATT haplotype being transmitted 38 times and not transmitted 21 times (from a total of 192 informative transmissions). CETDT showed that both the MIF-173C-C and the CATT, repeat contribute to the observed effect, and suggests an interactive effect between these two polymorphic positions.

Conclusions: This is the first demonstration of linkage of MIF with JIA. Both the -173 and the CATT polymorphisms contribute to this effect. The functional significance of the MIF-173-C/CATT haplotypes is currently being determined. An understanding of the biological significance of these polymorphic positions of MIF will enhance our understanding of JIA autoimmunopathogenesis. This work may also help to determine if the use of anti-MIF antibody treatment, or more targeted anti-MIF therapy would be of value in the treatment of JIA patients.

References
MIF has been shown to regulate the innate immune response through modulation of TNFα expression in JIA susceptibility.

**Methods:** 330 (each containing one affected JIA proband) were studied. Two hundred and twenty families had 2 parents, and one hundred and ten 1 parent available for genotyping. Of the 330 affected probands 52 had systemic onset JIA, 84 persistent oligoarticular, 53 extended oligoarticular, 59 rheumatoid factor (RF) negative polyarticular, 11 RF positive polyarticular, 22 enthesitis related, 20 juvenile psoriatic arthritis, and 29 were unclassified.

Genotyping was performed using genomic DNA (10ng/ul) for the two TLR4 SNPs, at aa positions 299 (nt 896 A to G) and 399 (nt 1196 C to T), by SNAPSHOT ddNTP primer extension and capillary electrophoresis. The data was analyzed and manually typed using Genotype 3.1 software.

**Statistical Methods:** Single point analysis was carried out using ETDT and haplotype transmissions investigated using TRANSMIT.

**Results:** No distortion from random inheritance was observed by single point analysis for TLR4 aa 299 p=0.8895 (44 informative transmissions) or for TLR4 aa 399 p=0.3987 (50 informative transmissions). Similarly, no distortion in transmission was seen when the 2 point haplotypes were studied p=0.536. However, both the 299 and 399 polymorphisms were found to be of low heterozygosity (10%).

**Conclusions:** No linkage or association has been found with JIA for TLR4 aa 299 and 399. There was a limited number of transmissions, due to low heterozygosity of the SNPs investigated, so preventing any meaningful JIA subgroup analysis to be carried out.

Further investigation of polymorphisms within functionally relevant domains of TLR4 will be carried out to determine the possible interactive relationship between TLR4 and MIF in JIA. Additionally, since multiple TLRs recognize antigens in a cross-reactive manner polymorphisms within other TLR genes could be of importance in JIA susceptibility.

**References**


### 201. ANALYSIS OF CYTOKINE GENE EXPRESSION IN A GENETICALLY DEPLETED RHEUMATOID ARTHRITIS (RA) POPULATION DURING ANTI-TNF TREATMENT

**H Mulcahy**, **M Daly**, **C Adams**, **C Molloy**, **C Joyce**, **K O’Rourke**, **G O’Donoghue**, **MG Molloy**, **F O’Gara**, **1 Biometrie Research Centre, Department of Microbiology, University College Cork, Cork, Ireland; 2Department of Rheumatology, Cork University Hospital, Cork, Ireland; 3Biochemistry Laboratory, Cork University Hospital, Cork, Ireland**

**Background:** Rheumatoid Arthritis (RA) is a multifactorial disease with both genetic and environmental factors contributing to disease susceptibility. Tumour Necrosis Factor (TNFα) is a key proinflammatory cytokine involved in the pathophysiology of RA. TNFα blockers appear to improve the disease status by at least 20% in the majority of patients. However, it is not clear why up to 40% of patients do not respond well to anti-TNF therapy. The TNFα-Lymphotoxin (LT) locus is located in the Class IV region of the Major Histocompatibility Complex (MHC) and has been shown to be associated with RA in selected subsets of RA patients. The TNF-LT locus is flanked by five microsatellite markers, and it has been demonstrated in this laboratory that two TNF microsatellite haplotypes are associated with RA susceptibility. Furthermore, single nucleotide polymorphisms (SNPs), located in the promoter region of the TNFα gene and which have the potential to alter TNFα transcription, have been found to be associated with RA.

**Methods:** RT-PCR and ELISA techniques are being used to monitor the expression of TNFα and LTα in unstimulated and LPS-stimulated blood from patients (n=10) undergoing anti-TNF therapy. These patients have been genotyped for previously reported SNPs in the TNF-LT locus. However, polymorphisms in the TNF-LT locus do not appear to influence the inducible TNFα levels observed. 100% (6/6) of patients on Infliximab show a decrease in TNFα protein levels during treatment, in comparison to only 25% (1/4) of patients on Etanercept therapy.

**Results:** 90% (9/10) of patients carry at least one polymorphism in the TNF-LT locus. However, polymorphisms in the TNF-LT locus do not appear to influence the inducible TNFα levels observed. 100% (6/6) of patients on Infliximab show a decrease in TNFα protein levels during treatment, in comparison to only 25% (1/4) of patients on Etanercept therapy.

**Conclusions:** These results illustrate the complexity of TNFα expression in RA, and the non-uniform nature of the disease process, even in patients of similar genetic backgrounds.
204. ASSOCIATION OF POLYMORPHISM IN EXON 1 OF THE TUMOR NECROSIS FACTOR RECEPTOR SUPER FAMILY 1A GENE WITH HAEOMOGLOBIN LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS


Background: A common finding in rheumatoid arthritis (RA) patients is a reduction in haemoglobin (Hb) levels. Tumor necrosis factor alpha (TNF-α), a powerful mediator of the inflammation observed in RA, has been demonstrated to suppress erythropoiesis in vitro and TNF-α is a powerful mediator of the inflammation observed in RA. Therefore, the observed HLA effects could be due to LD with the true disease susceptibility genes.

Results: A trend of decreased number of +36 A/A genotype was observed at position +36 in exon 1 of TNFRSF1A is associated with low haemoglobin (Hb) levels. Tumor necrosis factor alpha (TNF-α), a powerful mediator of the inflammation observed in RA, has been demonstrated to suppress erythropoiesis in vitro and TNF-α blockade has been shown to lead to an increase in Hb levels in vivo. The actions of TNF-α are mediated upon binding to either of two cell surface receptors, tumor necrosis factor receptor (TNFR) super family 1A (TNFRSF1A) or TNFRSF1B. Serum levels of TNFRs have been found to be elevated in patients with active RA and correlate inversely with Hb levels. The objective of this study was to investigate whether single nucleotide polymorphisms (SNPs) in the TNFRSF1A and TNFRSF1B genes are associated with differences in Hb levels in RA patients.

Methods: A group of 145 Caucasian RA patients with established disease was studied. DNA was isolated from patient blood samples and subsequently used to genotype both the position +36 exon 1 TNFRSF1A SNP and the position +196 exon 6 TNFRSF1B SNP by PCR-RFLP analysis. Hb levels measured over a period of 5 years were used to generate mean area under the curve (MAUC) values. Data were analysed by two-tailed T-test analysis with P values < 0.05 considered as significant.

Results: Patients homozgyous for the G allele of the TNFRSF1A SNP had significantly lower MAUC Hb levels than either of the other genotypes individually or when they were combined (see Table). Similar trends were seen in male and female patients separately, and in a second cohort of patients with early disease. No differences in Hb levels were found between the genotypes of the TNFRSF1B SNP.

Conclusions: The evidence presented here suggests that the GG genotype at position +36 in exon 1 of TNFRSF1A is associated with low haemoglobin levels in a population of UK Caucasian RA patients. However, the mechanism behind this association remains to be identified. Further studies are needed on other populations to confirm these findings and to elucidate the role of TNFRs in anaemia and erythropoiesis, in RA.

Supported by the Haywood Rheumatism Research and Development Foundation

205. GENETIC DISSECTION OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN JUVENILE OLGARIOARTHRITIS

E. Zeggini1,2, RP Donn1, WER Ollier2, W Thomson1. 1ARC Epidemiology Unit, University of Manchester, Manchester, United Kingdom; 2CIGMR, University of Manchester, Manchester, United Kingdom

Background: Juvenile oligoarthritis is the commonest subgroup of JIA and can be subdivided into persistent and extended subtypes. HLA-A and HLA-DRB1 have been found to be independently linked and associated with the disease. The MHC region is governed by strong linkage disequilibrium. Therefore, the observed HLA effects could be due to LD with the true disease gene(s).

Results: A trend of decreased number of +36 A/A genotype was observed in sporadic RA (33%) compared to controls (41%) and reached significance in familial RA (24%) (P=0.012). This negative association was most marked in the context of TNFR1 “twin-like” RA sibs, 11% compared to 41% in the controls (P=0.008).

Conclusions: This study provides evidence of the involvement of TNFR1 in RA genetic heterogeneity. Our data suggest that a TNFR1 recessive factor, in linkage disequilibrium with the +36A allele, plays a protective role in a subset of families with multiple RA cases.

206. CANDIDATE GENE ANALYSIS OF THE DISTAL EXTENDED MHC REGION IN RHEUMATOID ARTHRITIS

B. Brinthen1, E. Zeggini1, A. Barton1, A. Hinks1, S. Eyste1, N. Shephard1, D. Ward1, AJ Silman1, WER Ollier2, W Thomson1, J Worthington1. 1ARC-EU, University of Manchester, Manchester, United Kingdom; 2CIGMR, University of Manchester, Manchester, United Kingdom

Background: Previous, linkage and association analysis provided evidence implicating a second genetic effect in RA that localised distal to the extended class I region of the major histocompatibility complex. Two adjacent microsatellite repeat markers from this analysis identified a region ~11.2 Mb distal to the known RA susceptibility gene HLA-DRB1.

Aim: We aim to determine the role of specific candidate genes within the MHC region across 6p21.2-22.3.

Methods: Seven genes (DEK, ID4, E2F3, Flj20342, SOX4, PRL, and AD022) were investigated in a case/control SNP association analysis. Affected siblings from up to 374 families forming part of the UK national register for juvenile idiopathic arthritis were included in the study. Genotyping used an analysis method (Slager and Schaid, 2001 Am. J. Hum. Genet. 68:1457-1462) that accounts for relatedness of cases in a case/control cohort. Haplotype analysis of 16 SNPs, 35 microsatellite repeat markers, and molecular weight data of 4 HLA genes across a 20 Mb region was performed on one affected individual per pedigree through a variety of statistical packages including Ehh+, GOLDF, HELIXTREE, and Haplotype Pattern Mining.

Results: No associations were observed for any SNP selected in the candidate gene analysis. Evidence supporting findings in other studies of a block pattern for LD was observed. An attempt was made to identify disease gene localisations were identified across the 20 Mb region. Three regions of interest as they either included markers that tested positive by ETDT in the original study, were also implicated in JIA, or both. The first of these consisted of a haplotype of the SNPs represented in the haplotype of RA pedigrees and a like number of unaffected unrelated controls were available for genotyping. Association analysis of SNPs was performed using an analysis method (Slager and Schaid, 2001 Am. J. Hum. Genet. 68:1457-1462) that accounts for relatedness of cases in a case/control cohort. Haplotype analysis of 16 SNP's, 35 microsatellite repeat markers, and molecular weight data of 4 HLA genes across a 20 Mb region was performed on one affected individual per pedigree through a variety of statistical packages including Ehh+, GOLDF, HELIXTREE, and Haplotype Pattern Mining.

Results: No associations were observed for any SNP selected in the candidate gene analysis. Evidence supporting findings in other studies of a block pattern for LD was observed. An attempt was made to identify disease gene localisations were identified across the 20 Mb region. Three regions of interest as they either included markers that tested positive by ETDT in the original study, were also implicated in JIA, or both. The first of these consisted of a haplotype of the SNPs represented in the haplotype of RA pedigrees and a like number of unaffected unrelated controls were available for genotyping. Association analysis of SNPs was performed using an analysis method (Slager and Schaid, 2001 Am. J. Hum. Genet. 68:1457-1462) that accounts for relatedness of cases in a case/control cohort. Haplotype analysis of 16 SNP's, 35 microsatellite repeat markers, and molecular weight data of 4 HLA genes across a 20 Mb region was performed on one affected individual per pedigree through a variety of statistical packages including Ehh+, GOLDF, HELIXTREE, and Haplotype Pattern Mining.

Conclusions: Association of a haplotype containing DS61665 and 219091-4 provides additional support for a RA locus ~11 Mb telomeric to HLA-DRB1. Although none of the candidate genes selected were found to be associated, no one gene was intensively studied. Flj20342 in particular deserves further investigation as at present the function of the gene is unknown, and it contains the microsatellite repeat markers DS61665 and 219091-4.