SHORT COMMUNICATION

Association of the AFF3 gene and IL2/IL21 gene region with juvenile idiopathic arthritis

A Hinks1, S Eyre1, X Ke1, A Barton1, P Martin1, E Flynn1, J Packham2, J Worthington1, Childhood Arthritis Prospective Study (CAPS), UKRAG Consortium, BSPAR Study Group3 and W Thomson1

1arc-EU, Stopford Building, The University of Manchester, Manchester, UK and 2Haywood Hospital, University Hospital of North Staffordshire, Stoke on Trent, Staffordshire, UK

Recent genetic studies have led to identification of numerous loci that are associated with susceptibility to autoimmune diseases. The strategy of using information from these studies has facilitated the identification of novel juvenile idiopathic arthritis (JIA) susceptibility loci, specifically, PTPN22 and IL2RA. Several novel autoimmune susceptibility loci have recently been identified, and we hypothesise that single-nucleotide polymorphisms (SNPs) within these genes may also be JIA susceptibility loci. Five SNPs within the genes AFF3, IL2/IL21, IL7R, CTLA4 and CD226, previously associated with multiple autoimmune diseases were genotyped, in a large data set of Caucasian JIA patients and controls, and tested for association with JIA. We identified two susceptibility loci for JIA, AFF3 and the IL2/IL21 region and additional weak evidence supporting an association with the CTLA4 and IL7R genes, which warrant further investigation. All results require validation in independent JIA data sets. Further characterisation of the specific causal variants will be required before functional studies can be performed.

Keywords: autoimmune; juvenile idiopathic arthritis; CTLA4; AFF3; IL2

Introduction

Autoimmune diseases are caused by dysregulation of the immune system leading to an immune response to self-tissue. Autoimmune diseases are complex genetic diseases and in the last few years great progress has been made in the search for susceptibility loci. As more confirmed autoimmune disease susceptibility loci are identified, an interesting story is emerging in that many of these loci predispose to more than one autoimmune disease. This confirms the hypothesis that shared alleles contribute to a spectrum of diseases and suggests that common immunological pathways are involved in susceptibility to these phenotypically distinct diseases.

Juvenile idiopathic arthritis (JIA) is another complex genetic autoimmune disease characterised by chronic inflammatory disease in children. It is a group of heterogeneous disorders but encompasses all forms of arthritis of unknown aetiology that starts before the age of 16 and which persists for at least 6 weeks. The strategy of using information from autoimmune disease genome-wide association studies or candidate gene studies has facilitated the search for novel JIA susceptibility loci. Indeed two recently identified confirmed JIA susceptibility loci, PTPN22 and IL2RA are putative autoimmune susceptibility genes as they also show association with rheumatoid arthritis (RA), type 1 diabetes (T1D) and Graves’ disease. In addition, using a strategy of examining confirmed RA susceptibility loci in JIA, we have recently reported evidence for association of two further loci (TRAIRC5 and STAT4) with JIA susceptibility.

Several novel putative autoimmune susceptibility loci have recently been identified with association with multiple autoimmune diseases. These include the IL2/21 region on chromosome 4q23 and the genes encoding IL7R, CTLA4, AFF3 and CD226. We hypothesise that these genes may also confer susceptibility to JIA and, therefore, the aim of this study was to determine whether single-nucleotide polymorphisms (SNPs) within these genes are also associated with susceptibility to JIA.

Results and discussion

In this study, using a strategy of examining previously associated autoimmune loci in JIA, we have identified association of two loci with JIA susceptibility (Table 1). First, we show association of a SNP (rs1160542) in the 5’ region of the AFF3 gene (Table 1), a gene that is preferentially expressed in lymphoid cells and has a potential regulatory role in lymphoid development. This SNP has been associated with RA and a perfect proxy SNP (r² = 1), rs9653442 has been associated with...
Association analysis results for those SNPs associated with multiple autoimmune diseases in a cohort of patients with JIA

| Marker     | Chr | Gene | HWE controls | Major allele/minor allele (1/2) | MAF cases | MAF controls | Genotype frequency cases (%) | Genotype frequency controls (%) | P-value | 95% CI | Trend test |
|------------|-----|------|---------------|---------------------------------|-----------|--------------|-----------------------------|-------------------------------|---------|-------|-----------|
| rs1160542  | 2   | AFF3 | 0.32          | A/G                             | 0.5       | 0.45         | 217 (23.7)                  | 483 (52.8)                    | 215 (23.5) | 574 (19.3) | 0.05      |
| rs3087243  | 2   | CTLA4| 0.74          | G/A                             | 0.43      | 0.46         | 180 (19.7)                  | 450 (50.0)                    | 306 (33.5) | 306 (33.5) | 0.006     |
| rs6228644  | 2   | IL2/IL21| 0.19         | G/T                              | 0.15      | 0.18         | 22 (2.3)                    | 408 (45.8)                    | 683 (72.9) | 125 (3.6) | 0.0006    |
| rs2899327  | 2   | IL7R | 0.06          | C/T                             | 0.27      | 0.29         | 62 (6.6)                    | 377 (40.0)                    | 504 (53.4) | 267 (7.6) | 0.06      |
| rs763361   | 18  | CD226| 0.84          | C/T                             | 0.48      | 0.46         | 222 (23.5)                  | 464 (49.2)                    | 257 (27.3) | 745 (21.2) | 0.13      |

Abbreviations: Chr, chromosome; HWE, P-value statistic for Hardy–Weinberg equilibrium test; JIA, juvenile idiopathic arthritis; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

A Bonferroni correction of 5 was applied to correct for the number of loci studied, resulting in a P-value threshold of 0.01 for claims of significance. Genotyping was performed using the Sequenom iPLEX platform. A 90% sample quality control rate and 90% SNP genotyping success rate was imposed on the analysis.

The gene name refers to the nearest gene in the region although SNPs are not necessarily intra-genic.

UK Caucasian JIA patients (n = 1054) from three sources. The British Society for Paediatric and Adolescent Rheumatology (BSPAR) National Repository of JIA (n = 654), a cohort of UK Caucasian patients with long-standing JIA (n = 201), described previously22,23 and a third cohort collected as part of the Childhood Arthritis prospective Study (CAPS), a prospective inception cohort study of JIA cases from five centres across United Kingdom (n = 199). A 90% sample quality control rate and 90% SNP genotyping success rate was imposed on the analysis.

Healthy Caucasian control DNA samples were available from five centres in the United Kingdom as described previously:31 Manchester, 924 controls (including 228 in 1958 birth cohort controls); Sheffield, 995 controls; Leeds 532 controls; Aberdeen 862 controls; Oxford 536 controls, total control sample size = 3531.

Genotype and allele frequencies were compared between cases with JIA and controls using the Cochrane–Armitage trend test implemented in PLINK and allele odds ratios (ORs) and their 95% confidence intervals (CIs) calculated.

Association of the AFF3 gene with JIA

Association of the CTLA4 gene with JIA

IL2/IL21 gene region with JIA

IL7R gene region with JIA

CD226 gene region with JIA

Abbreviations: Chr, chromosome; HWE, P-value statistic for Hardy–Weinberg equilibrium test; JIA, juvenile idiopathic arthritis; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

A Bonferroni correction of 5 was applied to correct for the number of loci studied, resulting in a P-value threshold of 0.01 for claims of significance. Genotyping was performed using the Sequenom iPLEX platform. A 90% sample quality control rate and 90% SNP genotyping success rate was imposed on the analysis.

The gene name refers to the nearest gene in the region although SNPs are not necessarily intra-genic.

UK Caucasian JIA patients (n = 1054) from three sources. The British Society for Paediatric and Adolescent Rheumatology (BSPAR) National Repository of JIA (n = 654), a cohort of UK Caucasian patients with long-standing JIA (n = 201), described previously22,23 and a third cohort collected as part of the Childhood Arthritis prospective Study (CAPS), a prospective inception cohort study of JIA cases from five centres across United Kingdom (n = 199). A 90% sample quality control rate and 90% SNP genotyping success rate was imposed on the analysis.

Healthy Caucasian control DNA samples were available from five centres in the United Kingdom as described previously:31 Manchester, 924 controls (including 228 in 1958 birth cohort controls); Sheffield, 995 controls; Leeds 532 controls; Aberdeen 862 controls; Oxford 536 controls, total control sample size = 3531.

Genotype and allele frequencies were compared between cases with JIA and controls using the Cochrane–Armitage trend test implemented in PLINK and allele odds ratios (ORs) and their 95% confidence intervals (CIs) calculated.

Association of the AFF3 gene with JIA

Association of the CTLA4 gene with JIA

IL2/IL21 gene region with JIA

IL7R gene region with JIA

CD226 gene region with JIA

Abbreviations: Chr, chromosome; HWE, P-value statistic for Hardy–Weinberg equilibrium test; JIA, juvenile idiopathic arthritis; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

A Bonferroni correction of 5 was applied to correct for the number of loci studied, resulting in a P-value threshold of 0.01 for claims of significance. Genotyping was performed using the Sequenom iPLEX platform. A 90% sample quality control rate and 90% SNP genotyping success rate was imposed on the analysis.

The gene name refers to the nearest gene in the region although SNPs are not necessarily intra-genic.

UK Caucasian JIA patients (n = 1054) from three sources. The British Society for Paediatric and Adolescent Rheumatology (BSPAR) National Repository of JIA (n = 654), a cohort of UK Caucasian patients with long-standing JIA (n = 201), described previously22,23 and a third cohort collected as part of the Childhood Arthritis prospective Study (CAPS), a prospective inception cohort study of JIA cases from five centres across United Kingdom (n = 199). A 90% sample quality control rate and 90% SNP genotyping success rate was imposed on the analysis.

Healthy Caucasian control DNA samples were available from five centres in the United Kingdom as described previously:31 Manchester, 924 controls (including 228 in 1958 birth cohort controls); Sheffield, 995 controls; Leeds 532 controls; Aberdeen 862 controls; Oxford 536 controls, total control sample size = 3531.

Genotype and allele frequencies were compared between cases with JIA and controls using the Cochrane–Armitage trend test implemented in PLINK and allele odds ratios (ORs) and their 95% confidence intervals (CIs) calculated.
Differences between subtypes were assessed using a large number of hypothesis tests. Therefore, we first examined whether there was evidence of a difference in the risk of developing RA, JIA, T1D and SLE but is protective for Crohn’s disease. There is also emerging data suggesting that one of the associated SNPs at the locus confers differing risk and protective effects for T1D and multiple sclerosis.26,27

For all the SNPs tested, the same allele was associated with autoimmune disease, comparison with JIA. For example in Table 2). It has not always been the case for the overlap Diagram of disease susceptibility loci, that the same allele is associated. For example in PTPN22, the minor allele of the Rs2056631 SNP is associated with greater risk of developing RA, JIA, T1D and SLE but is protective for Crohn’s disease. There is also emerging data suggesting that one of the associated SNPs at the IL2RA locus confers differing risk and protective effects for T1D and multiple sclerosis.

JIA is a phenotypically heterogeneous disease and can be classified into more clinically homogeneous diseases using the ILAR classification criteria (Supplementary Table 1). However, comparing each of the ILAR subtypes separately against controls would result in a large number of hypothesis tests. Therefore, we first examined whether there was evidence of a difference in allele frequencies between the seven ILAR subtypes. Differences between subtypes were assessed using $\chi^2$ tests on the $7 \times 2$ tables. Only when a difference was found ($P<0.05$) were separate odds ratios and 95% confidence intervals calculated for the subgroups. In all cases, this was not significant ($P>0.05$) (data not shown). Therefore, further stratification by ILAR subtype was not performed. Larger sample sizes will be required to fully examine subgroup differences.

In conclusion, adopting the strategy of targeting loci with previous evidence for association in multiple autoimmune diseases has identified two novel JIA loci, AFF3 and the IL2/IL21 locus.

**Figure 1** Plot of odds ratios for minor allele for SNPs previously associated with autoimmune disease, comparison with JIA.

**References**

1. Lettre G, Rioux JD. Autoimmune diseases: insights from genome-wide association studies. *Hum Mol Genet* 2008; 17 (R2): R116–R121.
2. Zhernakova A, van Diemen CC, Wijmenga C. Detecting shared pathogenesis from the shared genetics of immune-related diseases. *Nat Rev Genet* 2009; 10: 43–55.
3. Vyse TJ, Todd JA. Genetic analysis of autoimmune disease. *Cell* 1996; 85: 311–318.
4. Ravelli A, Martini A. Juvenile idiopathic arthritis. *Lancet* 2007; 369: 767–778.
5. Hinks A, Worthington J, Thomson W. The association of PTPN22 with rheumatoid arthritis and juvenile idiopathic arthritis. *Rheumatology (Oxford)* 2006; 45: 365–368.
6. Hinks A, Ke X, Barton A, Eyre S, Bowes J, Worthington J et al. Association of the IL2RA/CD25 gene with juvenile idiopathic arthritis. *Arthritis Rheum* 2009; 60: 251–257.
7. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls. *Nature* 2007; 447: 661–678.
8. Lowe CE, Cooper JD, Brusko T, Walker NM, Smyth DJ, Bailey R et al. Large-scale genetic fine mapping and genotype-phenotype associations implicate polymorphism in the IL2RA region in type 1 diabetes. *Nat Genet* 2007; 39: 1074–1082.
9. Brand Of, Lowe CE, Heward JM, Franklyn JA, Cooper JD, Todd JA et al. Association of the interleukin-2 receptor alpha (IL2Ralpha)/CD25 gene region with Graves’ disease using a multilocus test and tag SNPs. *Clin Endocrinol (Oxf)* 2007; 66: 508–512.
10. Smyth D, Cooper JD, Collins JE, Heward JM, Franklyn JA, Howson JM et al. Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes* 2004; 53: 3020–3023.
11. Hinks A, Eyre S, Ke X, Barton A, Martin P, Flynn E et al. Overlap of disease susceptibility loci for rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA). *Ann Rheum Dis* 2009; e-pub ahead of print 11 August 2009.
12. van Heel DA, Franke L, Hunt KA, Gwilliam R, Zhernakova A, Inouye M et al. A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. *Nat Genet* 2007; 39: 827–829.
13. Zhernakova A, Alizadeh BZ, Bevova M, van Leeuwen MA, Coenen MJ, Franke B et al. Novel association in chromosome 4q27 region with rheumatoid arthritis and confirmation of type 1 diabetes point to a general risk locus for autoimmune diseases. *Am J Hum Genet* 2007; 81: 1284–1288.
14. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet* 2007; 39: 857–864.
15. Gregory SG, Schmidt S, Seth P, Oklesen JR, Hart J, Prokop A et al. Interleukin 7 receptor alpha chain (IL7R) shows allelic overlap of disease susceptibility loci for rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA). *Ann Rheum Dis* 2009; e-pub ahead of print 11 August 2009.
and functional association with multiple sclerosis. Nat Genet 2007; 39: 1083–1091.

16 Lundmark F, Duvefelt K, Iacobaeus E, Kockum I, Wallstrom E, Khademi M et al. Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. Nat Genet 2007; 39: 1108–1113.

17 Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 2003; 423: 506–511.

18 Hafler JP, Maier LM, Cooper JD, Plagnol V, Hinks A, Simmonds MJ et al. CD226 Gly307Ser association with multiple autoimmune diseases. Genes Immun 2008; 10: 5–10.

19 Ma C, Staudt LM. LAF-4 encodes a lymphoid nuclear protein with transactivation potential that is homologous to AF-4, the gene fused to MLL in t(4;11) leukemias. Blood 1996; 87: 734–745.

20 Barton A, Eyre S, Ke X, Hinks A, Bowes J, Flynn E et al. Identification of AF4/FMR2 family member 3 (AFF3) as a novel rheumatoid arthritis susceptibility locus and confirmation of two further pan-autoimmune susceptibility genes. Hum Mol Genet 2009; 18: 2518–2522.

21 Albers HM, Kurreeman FA, Stoeken-Rijbergen G, Brinkman DM, Kamphijs SS, Van Rossum MA et al. Association of the autoimmuneity locus 4q27 with juvenile idiopathic arthritis. Arthritis Rheum 2009; 60: 901–904.

22 Suppiah V, O’doherty C, Heggarty S, Patterson CC, Rooney M, Prahalad S, Bohnsack JF, Whiting A, Clifford B, Jorde LB, Ireland.

23 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81: 559–575.

This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivative Works 3.0 Licence. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/3.0/

Appendix

Childhood Arthritis Prospective Study (CAPS): Arc Epidemiology Unit, University of Manchester—Kimme Hyrcich, Mark Lay, Sham LAI, Paul Gilbert, Peter Ward; Alderhey—Eileen Baildam, Carol Lydon, Lynsey Brown; Glasgow—Joyce Davidson, Janet Gardner-Medwin, Vicki Price, Jane Sim, Maureen Todd; Great Ormond Street—Lucy Wedderburn, Alexandra Meijer, Julie Jones; Newcastle—Helen Foster, Mark Friswell, Michael Eltringham; Manchester—Alice Chieng, Joanne Buckley; Other—Tauny Southwood

UKRAG Consortium: University of Manchester1: Stephen Eyre, Anne Hinks, Laura J Gibbons, John Bowes, Edward Flynn, Paul Martin, Xiayi Ke, Rachelle Donn, Wendy Thomson, Anne Barton, Jane Worthington University of Leeds2: YEAR Consortium2, Stephen Martin, James I Robinson, Ann W Morgan, Paul Emery University of Sheffield3: Anthony G Wilson University of London4: Sophia Steer University of Aberdeen5: Lynne Hocking, David M Reid University of Oxford6: Pille Harrison, Paul Wordsworth

1arc-Epidemiology Unit, Stopford Building, The University of Manchester, Manchester, UK

2Leeds Institute of Molecular Medicine, Section of Musculoskeletal Disease, University of Leeds, Leeds LS9 7TF, UK

3School of Medicine & Biomedical Sciences, The University of Sheffield, Sheffield S10 2JF, UK

4Clinical and Academic Rheumatology, Kings College Hospital NHS Foundation Trust, Denmark Hill, London SE5 9RS, UK

5Bone Research Group, Department of Medicine & Therapeutics, University of Aberdeen, Aberdeen AB25 2ZD, UK

6University of Oxford Institute of Musculoskeletal Sciences, Botnar Research Centre, Oxford OX3 7LD, UK

YEAR Consortium: Management Team—Professor Paul Emery1, Professor Philip Conaghan2, Dr Mark Quinn3, Dr Ann W Morgan4, Dr Anne-Marie Keenan1, Dr Elizabeth Hensor1, Julie Kitcheman1, Consultants—Dr Andrew Gough1, Dr Michael Green2, Dr Richard Reece4, Dr Lesley Hordon1, Dr Philip Helliiweli5, Dr Richard Melson6, Dr Sheelagh Doherty2, Dr Ade Adabajo6, Dr Andrew Harvey6, Dr Steve Jarrett7, Dr Gareth Huson1, Dr Amanda Isdale2, Dr Mike Martin3, Dr Zunaid Karim1, Prof Dennis McGonagle1, Dr Colin Pease7, Dr Sally Cox1. SpRs—Dr Victoria Bejarano1, Dr Jackie Nam1. Nurses—Claire Brown1, Christine Thomas1, David Pickles1, Alison Hammond1, Beverley Neville3, Alan

Genes and Immunity
Fairclough, Caroline Nunns, Anne Gill, Julie Green,
Belinda Rhys-Evans, Barbara Padwell, Julie Madden,
Lynda Taylor, Sally Smith, Heather King, Jill Firth,
Jayne Heard, Linda Sigsworth. Lab Staff—Diane Cors-
cadden, Karen Henshaw, Lubna-Haroon Rashid, Ste-
phen G Martin, James I Robinson

1Section of Musculoskeletal Disease, LIMM, Leeds, UK
2York District Hospital, York, UK
3Harrogate District Hospital, Harrogate, UK
4Huddersfield Royal Infirmary, Huddersfield, UK
5Dewsbury District and General Hospital, Dewsbury, UK
6St Luke’s Hospital, Bradford, UK
7Hull Royal Infirmary, Hull, UK
8Barnsley District General Hospital, Barnsley, UK
9Pinderfields General Hospital, Wakefield, UK
10Calderdale Royal Hospital, Halifax, UK

British Society of Paediatric and Adolescent Rheumatology
(BSPAR) study group: M Abinum, MD, M Becker, MD, A
Bell, MD, A Craft, MD, E Crawley, MD, J David, MD, H
Foster, MD, J Gardener-Medwin, MD, J Griffin, MD, A
Hall, MD, M Hall, MD, A Herrick, MD, P Hollingworth,
MD, L Holt, MD, S Jones, MD, G Pountain, MD, C Ryder,
MD, T Southwood, MD, I Stewart, MD, H Venning, L
Wedderburn, MD, P Woo, MD and S Wyatt, MD.

Supplementary Information accompanies the paper on Genes and Immunity website (http://www.nature.com/gene)