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Concise Report

Association between the interleukin 23 receptor and ankylosing spondylitis is confirmed by a new UK case–control study and meta-analysis of published series

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Objectives. It has been shown previously that *IL-23R* variants are associated with AS. We conducted an extended analysis in the UK population and a meta-analysis with the previously published studies, in order to refine these *IL-23R* associations with AS.

Methods. The UK case–control study included 730 new cases and 1331 healthy controls. In the extended study, the 730 cases were combined with 1088 published cases. Allelic associations were analysed using contingency tables. In the meta-analysis, 3482 cases and 3150 controls from four different published studies and the new UK cases were combined. DerSimonian–Laird test was used to calculate random effects pooled odds ratios (ORs).

Results. In the UK case–control study with new cases, four of the eight SNPs showed significant associations, whereas in the extended UK study, seven of the eight *IL-23R* SNPs showed significant associations ($P < 0.05$) with AS, maximal with rs11209032 ($P < 10^{-5}$, OR 1.3), when cases with IBD and/or psoriasis were excluded. The meta-analysis showed significant associations with all eight SNPs; the strongest associations were again seen not only with rs11209032 ($P = 4.06 \times 10^{-9}$, OR $\sim$1.2) but also with rs11209026 ($P < 10^{-10}$, OR $\sim$0.6).

Conclusions. *IL-23R* polymorphisms are clearly associated with AS, but the primary causal association(s) is(are) still not established. These polymorphisms could contribute either increased or decreased susceptibility to AS; functional studies will be required for their full evaluation. Additionally, observed stronger associations with SNPs rs11209026 and rs11465804 upon exclusion of IBD and/or psoriasis cases may represent an independent association with AS.

Key words: IL-23 receptor, AS, Polymorphism, SpA, Genetic association.

Introduction

The heritability of AS estimated from twin studies in the United Kingdom and elsewhere is in excess of 90% [1]. The best known component of this arises from the HLA-B27 immune response gene that contributes 20–30% of the total genetic risk [2]. Substantial progress has now been made in identifying non-MHC genes causing susceptibility to AS; *IL-23R*, encoding one component of the interleukin 23 receptor, is one of these genes identified by genome-wide association studies [3]. IL-23R, a member of haemopoietin receptor superfamily, is the IL-23R-specific component of the heterodimeric receptor for the cytokine IL-23. The other subunit, IL-12Rβ1, is also a component of the IL-12 receptor (together with the IL-12Rβ2 subunit). Cells expressing the IL-23R subunit respond to IL-23 rather than IL-12 [4]. The IL-23 receptor is important in CD4 T-cell differentiation into the T_H17 lymphocyte subset that mediates inflammation in several animal models of autoimmunity [5, 6]. In various experimental models, T_H17 cells producing IL-17 have been shown to cause tissue damage in the brain, joints, heart, lungs and intestines [7]. *IL-23R* variants have also been implicated in susceptibility to human autoimmune disease, such as IBD, psoriasis and multiple sclerosis [8–10]. Thus, it appears to be a common susceptibility locus for conditions that are known to be associated with the seronegative SpAs [11].

In a previous study, we and others have shown that *IL-23R* is strongly associated with AS [11]. Subsequently, this association has been supported by other studies from Canada [12] and Spain [13]. We present here the results of a new UK case–control study, an extended UK study and a meta-analysis of all published series, strengthening the evidence of association between AS and *IL-23R*.

Methods

Study population

All patients were seen by a qualified rheumatologist to confirm AS, as defined by the modified New York criteria and were recruited from those attending the Nuffield Orthopaedic Centre (Oxford, UK) and the Royal National Hospital for Rheumatic Disease (Bath, UK) AS Database, in response to public appeals and by referral from British rheumatologists. All patients gave informed written consent to be included in the study which was carried out with relevant ethical approval (MREC project No. 98/5/23).

New UK case–control study and extended UK study of association between IL-23R and AS

In this study, 730 new AS cases, 1088 published cases and 1331 healthy controls from the UK were compared using a case–control design. None of the new UK cases has been included in previous reports. New cases were genotyped using iPLEX technology (MassArray, Sequenom, San Diego, CA, USA) except for SNP

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rs11209026 that was genotyped using KASPar technology by KBiosciences (Hoddesdon, Herts, UK). SNP genotyping of WTCCC controls and cases was performed with the Infinium I assay (Illumina, San Diego, CA, USA), which is based on allele-specific primer extension [11]. Allelic associations were tested using StatsDirect software (version 2.6.6 03/02/2008). The strength of association was tested using chi-square analysis of contingency tables. As *IL-23R* is known to be associated independently with both IBD and psoriasis, the analyses were performed by both including and excluding AS cases known to have these conditions. A *P*-value <0.05 was considered statistically significant.

Meta-analysis of association between IL-23R and AS

Meta-analysis was conducted for *IL-23R* based on the PubMed database using the terms ‘ankylosing spondylitis’ and ‘interleukin-23R’. Publications in English conducted on Caucasian populations were included. Study selection criteria were: (i) cases satisfying the modified New York criteria for AS; (ii) controls derived from a population within the same geographic area and ethnic background as AS cases; and (iii) AS cases and controls not overlapping between studies. Up to 3482 cases and 3150 controls (depending on the numbers typed for particular SNPs in these studies) from our new series and previously published studies have been combined in this meta-analysis [11–13]. The pooled populations originated from Canada (Alberta, Newfoundland and Toronto as three separate strata presented in the original paper), Spain, United Kingdom [the Wellcome Trust Case Control Consortium (WTCCC)] and United States [the Triple A (Australo/Anglo/American) Spondylitis Consortium—TASC]. The new UK data were combined with the UK cases from the original WTCCC study. Data were analysed using StatsDirect software (version 2.6.6 03/02/2008). The DerSimonian–Laird test was used to calculate random effects pooled odds ratio (OR), chi-square statistic and *P*-values in order to incorporate variation in true effects and to take into account the heterogeneity of the populations [14]. A *P*-value <0.05 was considered statistically significant. Cochran Q and inconsistency (I^2) values were also reported in order to present combinability and consistency of the studies. An I^2–value <50% indicates that less than half of the variation across the pooled studies is due to heterogeneity and they may therefore be combined. Similarly, an insignificant Cochran Q *P*-value (>0.05) indicates that the studies are combinable.

Results

The results of the new UK case–control study (Study I) and the extended UK case–control study (Study II) are shown in Table 1 (A, B, C, D). The results inclusive and exclusive of AS cases known to have concomitant IBD and/or psoriasis were similar. In the extended study, seven of the eight SNPs (rs1004819, rs11465804, rs11209026, rs1343151, rs10889677, rs11209032 and rs1495965) showed significant associations with AS, the strongest being with rs11209032 ($P < 10^{-5}$, OR 1.3) when cases with IBD and/or psoriasis were excluded (Table 1, D). In the case–control study including only new cases, four of the eight SNPs (rs11465804, rs11209026, rs10889677 and rs11209032) showed significant associations with AS, the strongest being with rs11209032 ($P = 0.0037$, OR 1.2) when cases with IBD and/or psoriasis were excluded (Table 1, B). However, the results from individuals with AS who did not have IBD and/or psoriasis led to the observation that when the cases with IBD and/or psoriasis were excluded, SNPs rs11465804 and rs11209026 showed even more significant associations with AS in both studies (Table 1).

The meta-analysis (Table 2) showed significant associations between all eight *IL-23R* SNPs and AS. The strongest associations were seen with rs11209026 ($P < 10^{-10}$, OR ∼0.6) and again

Table 1. Results of the new and extended UK case–control study, combining UK WTCCC cases and new UK cases

		Study I						Study II					
SNP	Minor Allele	MAF cases	MAF controls	Chi-square	*P*-value	OR	95% CI	MAF cases	MAF controls	Chi-square	*P*-value	OR	95% CI
		Case–control study: A. Including all new AS cases only (730)						Extended study: C. Including all new AS cases (730) and WTCCC cases (1088)					
rs1004819	A	0.33	0.3	3.12	0.077	1.13	0.99, 1.30	0.33	0.3	9.83	0.0017	1.19	1.07, 1.32
rs10489629	G	0.43	0.45	2.41	0.12	0.9	0.79, 1.03	0.43	0.45	4.08	0.043	0.9	0.81, 1.00
rs11465804	C	0.04	0.06	6.45	0.011	0.68	0.50, 0.92	0.04	0.06	11.12	0.0009	0.68	0.54, 0.85
rs11209026	A	0.05	0.06	5.41	0.02	0.71	0.53, 0.95	0.05	0.06	12.74	0.0004	0.67	0.53, 0.83
rs1343151	A	0.32	0.34	1.7	0.19	0.91	0.79, 1.05	0.32	0.34	6.05	0.014	0.87	0.79, 0.97
rs10889677	A	0.36	0.31	7.86	0.0051	1.21	1.06, 1.39	0.36	0.31	12.91	0.0003	1.22	1.09, 1.35
rs11209032	A	0.37	0.32	10.78	0.001	1.25	1.09, 1.43	0.37	0.32	22.22	$<10^{-5}$	1.29	1.16, 1.43
rs1495965	G	0.47	0.44	3.33	0.068	1.13	0.99, 1.29	0.47	0.44	9.19	0.0024	1.17	1.06, 1.29
		Case–control study: B. Excluding cases with IBD and/or psoriasis (640 remaining)						Extended study: D. Excluding cases with IBD and/or psoriasis from the new cases only (1728 remaining)					
rs1004819	A	0.33	0.3	2.14	0.14	1.11	0.96, 1.29	0.33	0.3	9.26	0.0023	1.18	1.06, 1.32
rs10489629	G	0.43	0.45	1.56	0.21	0.92	0.80, 1.05	0.43	0.45	3.56	0.059	0.91	0.82, 1.00
rs11465804	C	0.04	0.06	7.88	0.005	0.63	0.45, 0.87	0.04	0.06	12.21	0.0005	0.66	0.53, 0.84
rs11209026	A	0.04	0.06	7.51	0.0061	0.64	0.46, 0.88	0.04	0.06	14.66	0.0001	0.64	0.51, 0.81
rs1343151	A	0.32	0.34	0.98	0.32	0.93	0.80, 1.07	0.32	0.34	5.52	0.019	0.88	0.79, 0.98
rs10889677	A	0.35	0.31	4.53	0.033	1.17	1.01, 1.35	0.35	0.31	10.85	0.001	1.2	1.08, 1.34
rs11209032	A	0.37	0.32	8.4	0.0037	1.23	1.07, 1.42	0.37	0.32	20.99	$<10^{-5}$	1.28	1.15, 1.43
rs1495965	G	0.47	0.44	1.68	0.19	1.1	0.95, 1.26	0.47	0.44	8.01	0.0047	1.16	1.05, 1.28

The combined sample of cases was compared with WTCCC (1958 Birth Cohort) controls ($n = 1331$). MAF: Minor allele frequency.

TABLE 2. Results of meta-analysis for *IL23R* polymorphisms, combining four published studies and new UK data

SNP	Location	No. of cases/controls	MAF cases	MAF controls	OR	95 CI	PAR (%)	Chi-square	*P*-value	Cochran Q *P*-value	I^2 (95% CI)
rs1004819	Intron 5	3482/3150	0.35	0.30	1.21	1.13, 1.31	6	26.7	2.38×10^{-7}	0.73	0 (0, 61)
rs10489629	Intron 7	3482/3150	0.42	0.46	0.85	0.77, 0.94	−7	9.4	0.0021	0.13	41.7 (0, 75.5)
rs11465804	Intron 8	3124/2650	0.04	0.06	0.62	0.50, 0.76	−2	20.4	$<10^{-5}$	0.29	18.2 (0, 70.2)
rs11209026	Exon 9 (NS)	3472/3150	0.04	0.06	0.61	0.52, 0.72	−2	36.6	$<10^{-10}$	0.55	0 (0, 61)
rs1343151	Intron 9	3478/3150	0.30	0.34	0.81	0.72, 0.91	−7	12.1	0.0005	0.08	49.1 (0, 78)
rs11209032	Intergenic	3483/3150	0.37	0.32	1.24	1.16, 1.34	7	34.6	4.06×10^{-9}	0.46	0 (0, 61)
rs1495965	Intergenic	3440/3150	0.48	0.44	1.19	1.10, 1.29	8	18.8	$<10^{-4}$	0.32	15.2 (0, 66.6)

New UK case data and WTCCC case data were combined to form the UK stratum. Canada data were included as three separate strata. For rs11465804, no Spanish data were available. rs10889677 is not presented in the table due to non-combinability of the studies. PAR: population attributable risk; I^2: inconsistency; NS: non-synonymous.

rs11209032 ($P = 4.06 \times 10^{-9}$, OR ~1.2). However, the data for rs10889677 were non-combinable (Cochran Q *P*-value <0.001 and inconsistency = 75.6%) and have therefore not been presented in Table 2.

Discussion

The data from both UK studies confirm the previously published association between *IL-23R* and AS [11]. However, these studies differ as to which SNPs are strongly associated with AS. For instance, in both UK studies, the most strongly associated SNP is rs11209032 (Table 1; Study I $P < 0.001$, Study II $P < 10^{-5}$), which is also the case in the original UK WTCCC study (rs11209032; $P < 2.0 \times 10^{-6}$) [11]. On the other hand, results regarding the order of association magnitude for the other SNPs vary between studies. The findings of the meta-analysis strengthen the evidence that the truly associated variant(s) are more likely tagged by rs11209032 (Table 2; $P = 4.06 \times 10^{-9}$, OR ~1.2). Meta-analysis could be useful in clarifying the *IL-23R* associations.

The results from the meta-analysis follow the same trend as those previously presented from the WTCCC and TASC with regard to the direction and magnitude of associations [11]. However, this is perhaps unsurprising given the relative sizes of the studies included in this meta-analysis. Nevertheless, the Forest plots (data not shown) show consistency in the associations of the most strongly associated SNPs in populations drawn from four different countries (United Kingdom, United States, Canada and Spain).

In the small Spanish study, associations with three SNPs subsequently included in our meta-analysis had been observed (rs1343151, $P < 0.0002$; rs11209026, $P < 0.001$; rs10889677, $P < 0.04$) [13]. However, in the meta-analysis four SNPs (rs1004819, rs10489629, rs11209032 and rs1495965), not apparently associated with AS in the Spanish study, also showed strong associations with AS. Moreover, the rs10889677 allele frequencies differed in the Spanish study and rs10889677 was not therefore included in the meta-analysis due to non-combinability of the studies. Meta-analysis is a valuable tool to detect concordance between studies (i.e. whether the studies are combinable and in agreement with each other) and also to increase the power to refine true associations between polymorphisms and disease. The discordance between the Spanish population and those of more Northern European descent may represent a type II error, variation in linkage disequilibrium patterns in these ethnic groups, or real differences in associations between these different ethnicities.

It is known that *IL-23R* polymorphisms are associated with both IBD and psoriasis [8, 9] and it has been suggested that *IL-23R* variants are more strongly associated with AS in the absence of clinically apparent IBD [3, 11]. In the Spanish study, it was not clear whether AS cases with IBD and/or psoriasis were excluded, whereas in the Canadian study, it was stated that the observed effects were independent of IBD and psoriasis. In our UK studies reported here, associations with rs11209026 and rs11465804 improved when known IBD and psoriasis cases were excluded from the data set (Table 1). There was no improvement in the associations with the rest of the variants and less strong associations were observed upon exclusion of the IBD and psoriasis cases. These results may indicate a pure association between *IL-23R* and AS contrasting the idea that the observed association may be due to IBD and psoriasis being found in the AS population more frequently. The evidence that IBD/psoriasis exerts a major influence on the association of AS with *IL-23R* is not substantial, but it may be prudent to include stratification for these clinical variables in studies.

IL-23R polymorphisms may define a subgroup of inflammatory diseases, including IBD, AS and psoriasis, in which local rather than systemic inflammation is important [13]. Therefore, similar *IL-23R* SNP associations might be expected in each of these diseases. However, to date, the primary associations of *IL-23R* with any of these diseases have not been fully established and appear somewhat variable. For example, in one study of psoriasis, significant association was only observed with rs11209026 [15]. In contrast to our meta-analysis of *IL-23R* and AS, the SNPs rs1004819, rs10489629 and rs1343151 showed no association with psoriasis. Such results are likely due to limited power and the modest effect sizes of various non-MHC genes in psoriasis. The true hierarchy of associations with psoriasis may only become obvious from larger studies or meta-analysis of *IL-23R* associations with psoriasis.

The uncommon coding variant rs11209026 (Arg381Gln) apparently provides protection against Crohn's disease [9]. Since IBD and AS share a common genetic background, it might also be expected that this variant would be associated with AS. Support for this is forthcoming from all studies in AS published to date [11–13]. The SNP rs11209026 also showed one of the strongest associations in our extended UK study (Table 1, D, $P = 0.0001$). The SNP rs11465804 is in linkage disequilibrium with rs11209026 and it showed a similarly strong association (Table 1, D, $P = 0.0005$) with AS in the extended UK study, a result lending support to the strong association observed with rs11209026 in the meta-analysis ($P < 10^{-10}$, Table 2). However, in assessing the strength of the associations with both rs11465804 and rs11209026, the low minor allele frequencies of both (~0.05) should be taken into account, since the observed strong association may, in part, reflect the effect of low minor allele frequency. Furthermore, non-coding variants included in the meta-analysis showed statistically significant associations as expected from the shared genetic background with IBD. It is also known from published studies on IBD that these associations are independent of rs11209026 [9].

IL-23R polymorphisms could theoretically be associated either with increased or decreased susceptibility to AS. The signalling pathways involving the pro-inflammatory cytokine IL-23 and its receptor IL-23R are important potential therapeutic targets in AS, as indicated by previous studies in mouse models of collagen-induced arthritis and experimental autoimmune encephalomyelitis [5, 6]. A more precise definition of the genetic associations of *IL-23R* with AS culminating in the identification of the precise causal variants is of critical importance to the development of new therapies targeting this pathway. Substantial re-sequencing of *IL-23R*, its regulatory sequences and flanking regions is ongoing to achieve this. Functional consequences of these polymorphisms will clearly require investigation and may have implications for inflammatory diseases beyond AS alone.

Rheumatology key messages

- Polymorphisms in the *IL-23R* gene are reproducibly associated with AS.
- Meta-analysis of published data confirms the strength of the associations.
- The IL-23/IL-23R-mediated T_H17 pathway is an important therapeutic target in AS.

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