HLA-A 31:01 is not associated with the development of methotrexate pneumonitis in the UK population: results from a genome-wide association study

We read with interest the article by Furukawa *et al*[1] suggesting an association between HLA-A 31:01 and methotrexate (MTX)-induced interstitial lung disease (ILD) in Japanese patients with rheumatoid arthritis (RA). MTX-ILD or MTX-pneumonitis (MTX-P) is an idiosyncratic hypersensitivity reaction to MTX that usually occurs within the first year of MTX therapy, inducing inflammation, cytokine release and the activation of CD4+ T-lymphocytes within the lung parenchyma,[2–4] with a reported prevalence of 1% of the Caucasian RA population prescribed MTX.[5]

To investigate this association further, we conducted a genome-wide association study. Rheumatologists working within the National Health Service in the UK identified Caucasian patients with RA, who developed clinician diagnosed MTX-P (n=65). Caucasian controls, matched for age and gender, were identified from a prospective observational cohort study of patients starting MTX (n=195). In order to be eligible, controls were required to have 1 year of continuous MTX therapy without the development of MTX-P. Assuming HLA-A 31:01 prevalence of 3.6% in the European Caucasian population,[6] this provided 80% power to detect an OR of 3.0. Genotyping was performed using the Illumina Infinium HumanCoreExome 12 BeadChip genome-wide array (Illumina, San Diego, USA); HLA-A 31:01 was imputed using SNP2HLA[7] and a subset of samples (n=24) were directly genotyped for the allele using an established wet-lab technique described previously.[8]

Following quality control, data for 62 cases and 175 controls remained. HLA-A 31:01 was not associated with MTX-P in this cohort (p=0.21). Wet-lab genotyping of a subset of samples confirmed concordance with in silico imputation (κ=1.00). One locus, rs6593803 mapping to an intergenic region between the *GJA5* and *ACP6* genes, was associated with MTX-P, however, the results did not reach genome-wide significance thresholds for claims of confirmed association ($p=1.85\times10^{-7}$, OR=3.13).[9] Nonetheless, rs6593803 is known to affect the expression of *GJA5*.[10] *GJA5* is a member of the connexin gene family and the resulting protein is connexin 40. The connexin 40 protein is a component of gap junctions that act at sites of cell–cell contact allowing diffusion of signalling molecules between cells.[11] Transgenic mice deficient in connexin 40 and 43 ($cx40^{-/-}/cx43^{-/-}$) have a reduced life span due to lung abnormalities including pulmonary fibrosis, alveolar wall thickening and increased lung fibroblasts,[12] histopathological findings similar to MTX-P.[13]

In summary, we have found no evidence of association between HLA-A 31:01 and MTX-P in a European population. Three loci reached suggestive evidence for association with MTX-P (rs6593803 ($p=1.85\times10^{-7}$, OR=3.13), rs9299346 ($p=1.76\times10^{-6}$, OR=2.76) and rs1624005 ($p=6.54\times10^{-6}$, OR=2.59)), but further studies with larger numbers of patients with this rare disease are required to confirm these non-HLA associations with MTX-P.

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Contributors JB recruited patients, NHS sites, co-conducted the GWAS and analysis. S-AO applied to the ethics committee, recruited patients and NHS sites. JM co-conducted the GWAS and analysis. AA co-genotyped the HLA 31:01. MP co-wrote the article. SMMV is PI of the control cohort. AB is the PI of the cases cohort.

Competing interests None declared.

Ethics approval National Research Ethics Service, NRES Comittee North West, Greater Manchester Central.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Bluett J, Owen S-A, Massey J, *et al*. *Ann Rheum Dis* Published Online First: [*please include* Day Month Year]. doi:10.1136/annrheumdis-2017-211512

► http://dx.doi.org/10.1136/annrheumdis-2017-211518

Ann Rheum Dis 2017;**0**:1. doi:10.1136/annrheumdis-2017-211512

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Ann Rheum Dis published online May 12, 2017