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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.

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