HLA-DRB1 and HLA-DQA1 associated with immunogenicity to adalimumab therapy in patients with rheumatoid arthritis

Advanced targeted therapies including tumour necrosis factor inhibitors (TNFis) have transformed the clinical management of rheumatoid arthritis (RA). However, monoclonal antibody (MAb)-derived TNFis are associated with development of immunogenicity resulting in low circulating drug levels (online supplemental figure S5).[1] A genetic predictor of immunogenicity would have clinical utility by providing a pretreatment biomarker that could be used to inform therapy selection. Previous genetic studies of TNFi immunogenicity have focused on alleles within the HLA locus on chromosome 6.[2–4]

Patients were followed for 12 months with serum samples collected at 3 months, 6 months and 12 months following commencement on adalimumab (TNFi) therapy. Neutralising antidrug antibodies (ADAs) were detected using a drug-sensitive/drug-tolerant radioimmunoassay (Sanquin, NL). The presence of ADAs was determined by radioimmunoassay. A positive ADA titre was defined as >12 arbitrary units/mL. If a patient developed ADA at any time in the study, they were classed as ADA positive. Genotyping was carried out using the Illumina array, and HLA alleles were imputed using SNP2HLA and the T1DGC reference panel following standard data quality control (full details in online supplemental S1). Drug immunogenicity rates were determined using Kaplan-Meier analysis, and Cox proportional hazards regression, which was used to adjust genetic models for biological sex, age, concurrent conventional synthetic disease-modifying antirheumatic drug (csDMARD) use, disease duration and first within-sample principal component from the genetic dataset.

In total 445 patients were studied, of whom 96 (21.6%) became ADA positive during treatment. A total of 377 (85.3%) patients received cotherapy with csDMARDs of which 302 (81.4%) patients received methotrexate (MTX, online supplemental table S1). Disease duration modestly increased the rate of immunogenicity for every year since RA diagnosis (HR=1.02, p=0.01, table 1). Compared with TNFi monotherapy, combination therapy with csDMARD reduced the rate of ADA development by more than twofold (HR=0.379, p=1.27e−07). Importantly, a statistically significant difference in the rate of immunogenicity was observed when MTX cotherapy was compared with cotherapy with alternative csDMARDs; MTX conferring higher protection from immunogenicity (HR=0.425, p=1.27e−05). However, non-MTX csDMARD use also trended towards a reduced rate of immunogenicity (HR=0.66; 95% CI 0.429 to 1.012, p=0.056).

Following quality control of the genetic data, 166 HLA alleles were available for analysis in 435 patients with non-missing covariate data. The most statistically significant association with immunogenicity was observed for HLA-DQA1*03 (HR 0.6; 95% CI 0.474 to 0.775, p=6.4e−05) and HLA-DRB1*04 (HR 0.6; 95% CI 0.476 to 0.775, p=6.3e−05) (4-digit and amino-acid results are reported in online supplemental material S1). In the Kaplan-Meier analysis, carriage of HLA-DQA1*03 and HLA-DRB1*04 alleles under an additive model was associated with reduced rate of immunogenicity (figure 1A–C). The two HLA alleles were in LD (R^2: 0.94),[5] suggesting a single protective effect. In carriers of at least one copy of HLA-DQA1*03 or HLA-DRB1*04, MTX was observed to provide stronger protection against ADA development compared with other csDMARDs (HR 0.44; 95% CI 0.24 to 0.78, p=5.7e−03, figure 1B–D). We also investigated HLA alleles that have previously been reported on in RA and Crohn's disease and provide support for alleles at HLA-DQA1*05, HLA-DRB1*11 and HLA-DRB1*03 (online supplemental figure S4).

In conclusion, in the largest study of its type in RA to date, carriage of HLA-DQA1*03 and HLA-DRB1*04 reduced the rate of drug immunogenicity to adalimumab. The strongest protection from immunogenicity was conferred by csDMARD cotherapy, particularly in combination with MTX. Our results suggest that the use of alternative csDMARDs should be encouraged for patients treated with MAb TNFi who are MTX intolerant. Larger studies are now needed to determine if genetic testing could optimise

Table 1 Cox regression output for the clinical attributes, where N is the number of samples available within each variable

	N	P value	HR	ADA negative	ADA positive
Concurrent csDMARD usage	442	1.27e−07	0.38 (0.26–0.54)	354 (80%)	88 (20%)
Methotrexate (MTX) usage*	371	1.93e−05	0.41 (0.28–0.62)	312 (84%)	59 (16%)
MTX versus other csDMARD†	377	1.27e−05	0.43 (0.29–0.62)	315 (84%)	62 (16%)
Concurrent csDMARD (excluding MTX)	143	0.06	0.66 (0.43–1.01)	95 (66%)	48 (34%)
First biologic	444	0.88	0.95 (0.53–1.73)	356 (80%)	88 (20%)
Age	445	0.18	0.99 (0.97–1.00)	357 (80%)	88 (20%)
Sex	445	0.29	1.21 (0.85–1.73)	357 (80%)	88 (20%)
BMI	364	0.87	1.00 (0.97–1.03)	296 (81%)	68 (19%)
ACPA status	239	0.83	1.06 (0.65–1.72)	192 (80%)	47 (20%)
Never versus current smoker	151	0.27	0.66 (0.32–1.37)	125 (83%)	26 (17%)
Never versus ever smoker‡	254	0.47	0.85 (0.54–1.33)	207 (82%)	47 (18%)
Disease duration	438	0.01	1.02 (1.00–1.04)	351 (80%)	87 (20%)
Baseline DAS28 score	439	0.59	0.95 (0.78–1.15)	353 (80%)	86 (20%)

*Comparison within patients with complete MTX information, those with missing information were not included in this analysis.
†Comparison within recorded patients of having known combination therapy, as well as complete MTX information.
‡Ever smoker refers to ex smokers and current smokers.
ACPA, anti-citrullinated peptide antibody; ADA, antidrug-antibody; BMI, body mass index; csDMARD, conventional synthetic disease-modifying antirheumatic drug ; DAS28, disease activity score in 28-joints.

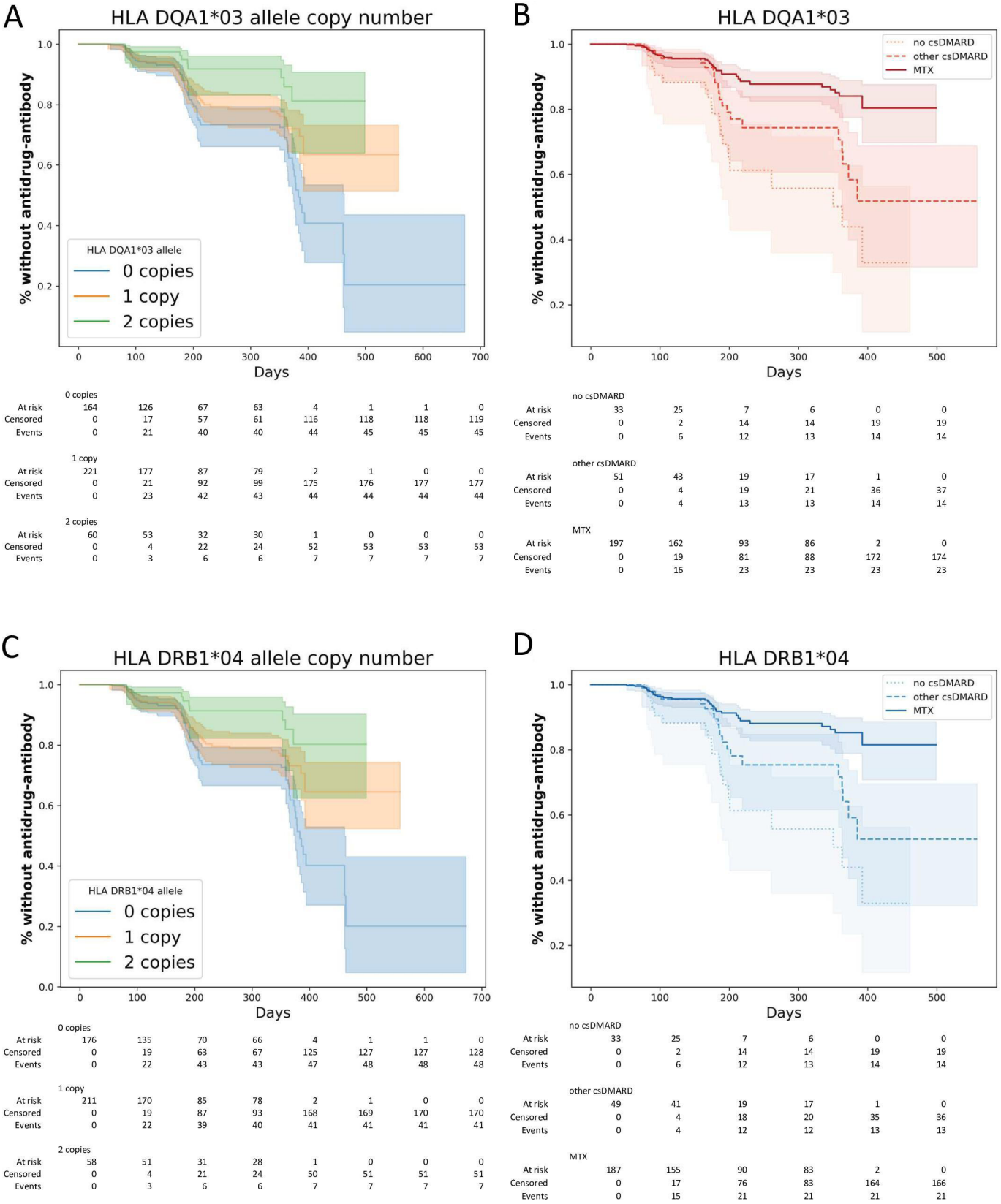

Figure 1 (A, C) Kaplan-Meier (KM) plot showing rate of drug antidrug antibody development, stratified by the number of HLA alleles carried (A, HLA-DQA1*03; C, HLA-DRB1*04). The tables presented underneath the KM plots represents the number of participants at risk over time. Blue, orange and green indicate 0, 1 and 2 copies of the alleles respectively. (B, D) Kaplan-Meier plot of drug immunogenicity rate for carriers of at least one copy of HLA-DQA1*03 and HLA-DRB1*04, respectively, for different types of csDMARD cotherapy. Solid line and darkest shade of colour represent cotherapy with MTX, dashed line and middle shade represents non-MTX csDMARD, dotted line with the lightest shade represents monotherapy with only adalimumab. csDMARD, conventional synthetic disease modifying antirheumatic drug; MTX, methotrexate.

selection of treatment and to quantify effects of non-MTX csDMARDs on immunogenicity.

Chuan Fu Yap ,[1] **Nisha Nair**,[1,2] **Annick de Vries**,[3] **Floris C Loeff**,[3] **Ann W Morgan** ,[4,5,6] **John D Isaacs** ,[7,8] **Anthony G Wilson** ,[9] **Kimme L Hyrich**,[2,10] **Anne Barton** ,[1,2] **Darren Plant** [1]

[1]Centre for Genetics and Genomics Versus Arthritis, Centre for Musculoskeletal Research, The University of Manchester, Manchester, UK
[2]NIHR Biomedical Research Centre, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK
[3]Diagnostic Services, Sanquin, Amsterdam, The Netherlands
[4]School of Medicine, University of Leeds, Leeds, UK
[5]NIHR Leeds Biomedical Research Centre, Leeds Teaching Hospitals NHS Trust, Leeds, UK
[6]NIHR In Vitro Diagnostic Co-operative, Leeds Teaching Hospitals NHS Trust, Leeds, UK
[7]Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK
[8]Musculoskeletal Unit, Newcastle-upon-Tyne Hospitals NHS Foundation Trust, Newcastle-upon-Tyne, UK
[9]School of Medicine and Medical Science, Conway Institute, University College Dublin, University College Dublin, Dublin, Ireland
[10]Centre for Epidemiology Versus Arthritis, Centre for Musculoskeletal Research, The University of Manchester, Manchester, UK

Correspondence to Dr Darren Plant, Centre for Musculoskeletal Research, The University of Manchester Centre for Genetics and Genomics Versus Arthritis, Manchester, UK; Darren.Plant@manchester.ac.uk

Handling editor Josef S Smolen

Twitter Chuan Fu Yap @chuanfuyap and John D Isaacs @ProfJohnIsaacs

Acknowledgements We thank Asma Kalei for carefully coordinating sample analysis at Sanquin Diagnostic Services.

Contributors DP and CFY conceived or designed the study and data analyses. NN, AdV and FCL acquired the data. CFY analysed the data. CFY, NN, AB and DP had access to the data. All authors were involved in interpretation of data and reviewed and approved the manuscript's content before submission. CFY accepts final responsibility for this work and controlled the decision to publish.

Funding This research was supported by the NIHR Manchester Biomedical Research Centre. We thank Versus Arthritis (grant number 21173, grant number 21754 and grant number 21755) for support. This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No. 831434 (3TR). The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

Disclaimer The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Competing interests AWM is supported National Institute for Health and Care Research (NIHR) and Medical Research Council (MRC). AWM has acted as consultant for Roche/Chugai, Vifor and AstraZeneca. AWM is member of speakers' bureaus for Roche/Chugai. AWM is on Data Safety Monitoring Board for GSK and Regeneron/Sanofi. AWM is on the board for MRC and Vasculitis UK. KH has received grant/research support from Pfizer, Bristol Myers Squibb (BMS). KH has received honoraria for speaking at educational meeting by Abbvie. AB is supported by the NIHR Manchester Biomedical Research Centre. AB has received grant/research support from Pfizer, BMS, Scipher Medicine and Galapagos (paid to host institution). AB is member of speakers' bureaus for Galapagos (paid to host institution). DP has received grant/research support from BMS, Versus Arthritis and European Commission.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and ethics was approved by the North West 6 Central Manchester South Research Ethics Committee (COREC 04/Q1403/37) and all patients provided written consent. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/ard-2023-223955).

To cite Yap CF, Nair N, de Vries A, *et al*. *Ann Rheum Dis* 2024;**83**:263–265.

Received 30 January 2023
Accepted 22 July 2023
Published Online First 12 September 2023

Ann Rheum Dis 2024;**83**:263–265. doi:10.1136/ard-2023-223955

ORCID iDs
Chuan Fu Yap http://orcid.org/0000-0001-5256-5642
Ann W Morgan http://orcid.org/0000-0003-1109-624X
John D Isaacs http://orcid.org/0000-0002-6103-7056
Anthony G Wilson http://orcid.org/0000-0003-4855-3926
Anne Barton http://orcid.org/0000-0003-3316-2527
Darren Plant http://orcid.org/0000-0003-1395-9344

REFERENCES

1 Jani M, Chinoy H, Warren RB, *et al*. Clinical utility of random anti-tumor necrosis factor drug-level testing and measurement of antidrug antibodies on the long-term treatment response in rheumatoid arthritis. *Arthritis Rheumatol* 2015;67:2011–9.
2 Billiet T, Vande Casteele N, Van Stappen T, *et al*. Immunogenicity to Infliximab is associated with HLA-Drb1. *Gut* 2015;64:1344–5.
3 Sazonovs A, Kennedy NA, Moutsianas L, *et al*. HLA-Dqa1*05 carriage associated with development of anti-drug antibodies to Infliximab and Adalimumab in patients with Crohn's disease. *Gastroenterology* 2020;158:189–99.
4 Liu M, Degner J, Davis JW, *et al*. Identification of HLA-Drb1 Association to Adalimumab Immunogenicity. *PLoS One* 2018;13:e0195325.
5 Rogers AR, Huff C. Linkage disequilibrium between Loci with unknown phase. *Genetics* 2009;182:839–44.

Supplementary Document

Table of contents

Patients

The patients in this study were already taking part in the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate (BRAGGSS, Research Ethics Committee (REC) reference: 04/Q1403/37), which is a prospective multi-centre observation study cohort based in the UK. BRAGGSS patients included in the current study had a diagnosis of RA according to the American College of Rheumatology 1987 revised criteria for the classification of RA [1], were of European ancestry and were about to receive treatment with adalimumab for their RA symptoms. Adalimumab was the anti-TNF agents most commonly prescribed for the treatment of RA in the national UK cohort at the time that this study was designed. In total 671 adalimumab treated patients were recruited from 2008-2019 where a genetic sample was available for genotyping, 1 patient was withdrawn from the study due to non-compliance. Of these 671's patients, 445 of had a serum sample at the 6-month follow-up visit of sufficient quality and quantity to permit testing for anti-drug antibodies. The category of non-MTX csDMARDs included: leftlunomide (n=6), sulphasalazine (n=8), Azathioprine (n=4) and hydryxocyhloroquinne (n=1). These csDMARDs were grouped together in the analysis due to missing data and, where the csDMARD was known, low numbers of individuals receiving the different drugs.

Genotype sample processing

Genotyping was carried out using the Illumina Infinium HumanCoreExome 24 BeadChip kit (Illumina, San Diego, California, USA). 250 ng of DNA was used, according to the manufacturer's guidance. Genotype calling was carried out using GenomeStudio software (Illumina, San Diego, California, USA). Standard QC was conducted on each individual array using PLINK v1.9 [2]: SNPs and samples were excluded if there was >2% missing data, and SNPs with MAF < 0.01 and Hardy Weinberg Equilibrium (HWE) $p < 1 \times 10^{-4}$ were also excluded. Population stratification adjustment was done using HapMap 3 reference panel [3], that includes individuals of European descent, to determine genetic ancestry of each individual, followed by Principal Component Analysis (PCA) analysis. Only individuals of European descent were kept in the dataset. HLA information (types and amino acid) were imputed using SNP2HLA using T1DGC reference panel; imputation refers process of assigning SNP that were not genotyped in the array using a reference panel, the SNPs would then be assigned amino acids, and subsequently allele types [4].

Cox Regression Model

Cox proportional hazards regression model was used to determine immunogenicity rate association to HLA alleles using an additive genetic model. The final genetic model was adjusted for biological sex, age, concurrent conventional synthetic disease modifying anti-rheumatic drug (csDMARD) use, disease duration, and first within-sample principal component from the genetic dataset. After accounting for all available data that includes the above covariates, there were only 435 samples left. Smoking information which could be informative was excluded as there was high number of missing relative to the entire cohort (43%); inclusion of this variable would greatly reduce the power of the study. Another potentially informative variable to be excluded was BMI, this was also due to high number of missing data (18%). However, a final model that included BMI was built, the inclusion of

BMI information did not alter the results for either HLA-DRB1 or HLA-DQA1 in comparison to the same subset of patients where BMI was excluded from the model. For statistical testing the p-value threshold for significance was set to 3E-04. This value is derived from dividing p=0.05 by the number of alleles tested (n=166).

			Grouped by Antidrug-Antibody Status		
		Missing	**Overall**	**Negative**	**Positive**
n			445	349 (78.4)	96 (21.6)
Sex, n (%)	**Female**	0	337 (75.7)	263 (75.4)	74 (77.1)
	Male		108 (24.3)	86 (24.6)	22 (22.9)
Age, mean (SD)		0	57.2 (11.9)	57.5 (11.7)	56.0 (12.9)
Disease Duration, median [Q1,Q3]		7	6.6 [2.5,15.4]	5.9 [2.5,14.1]	9.3 [3.0,17.9]
First Biologic, n (%)	**No**	1	66 (14.9)	57 (16.4)	9 (9.4)
	Yes		378 (85.1)	291 (83.6)	87 (90.6)
Baseline DAS, mean (SD)		6	5.2 (0.9)	5.2 (0.9)	5.2 (0.9)
Concurrent csDMARD usage, n (%)	**No**	3	65 (14.7)	36 (10.4)	29 (30.2)
	Yes		377 (85.3)	310 (89.6)	67 (69.8)
MTX treatment, n (%)	**No**	74	69 (18.6)	47 (15.3)	22 (34.9)
	Yes		302 (81.4)	261 (84.7)	41 (65.1)
Smoking Status, n (%)	**Current smoker**	191	43 (16.9)	37 (18.3)	6 (11.5)
	Ex-smoker		103 (40.6)	82 (40.6)	21 (40.4)
	Never smoked		108 (42.5)	83 (41.1)	25 (48.1)
BMI, median [Q1,Q3]		81	27.8 [24.2,32.5]	28.1 [24.6,32.6]	26.1 [23.6,31.2]
ACPA+ve, n (%)	**No**	206	62 (25.9)	50 (26.3)	12 (24.5)
	Yes		177 (74.1)	140 (73.7)	37 (75.5)
Time Points, n (%)	**3 months**	0	144 (32.4)	114 (32.7)	30 (31.2)
	6 months		107 (24.0)	76 (21.8)	31 (32.3)
	12 months		194 (43.6)	159 (45.6)	35 (36.5)

Table S1: Patient characteristics summary for this study. *tableone* package was used to generate patient characteristics table [5].

VARIANT	GENE	*p*-value	HR	Lower CI	Higher CI	Patients with 1 Copy	Patients with 2 Copies
HLA-DRB1*04	DRB1	0.000063	0.607	0.476	0.775	204	57
HLA-DRB1*0404	DRB1	0.000241	0.327	0.18	0.594	31	2
HLA-DRB1*0401	DRB1	0.004429	0.626	0.453	0.864	136	3
HLA-DQA1*0301^{+}	DQA1	0.000064	0.606	0.474	0.775	214	59
HLA-DQA1*03^{+}	DQA1	0.000064	0.606	0.474	0.775	214	59
HLA-DQB1*02	DQB1	0.000326	1.622	1.246	2.111	115	18

HLA-DQB1*03	DQB1	0.001195	0.685	0.545	0.861	207	94
HLA-DQB1*0302	DQB1	0.005412	0.637	0.463	0.875	152	14
HLA-DQB1*0202	DQB1	0.006217	1.692	1.161	2.467	57	0
HLA-DQB1*0201	DQB1	0.025259	1.468	1.049	2.054	73	6

Table S2: Statistical output for final Cox regression model for HLA-DRB1, DQA1. Given that DQA1 and DQB1 form a heterodimer, the results for tested alleles at the DQB1 locus are also presented. However, the results for DQB1*02 or DQB1*03 did not meet the p-values the threshold for multiple testing (p=3E-04). ⁺The results for HLA-DQA1*03 and HLA-DQA*0301 were identical because there is only one 4-digit allele for HLA-DQA1*03.

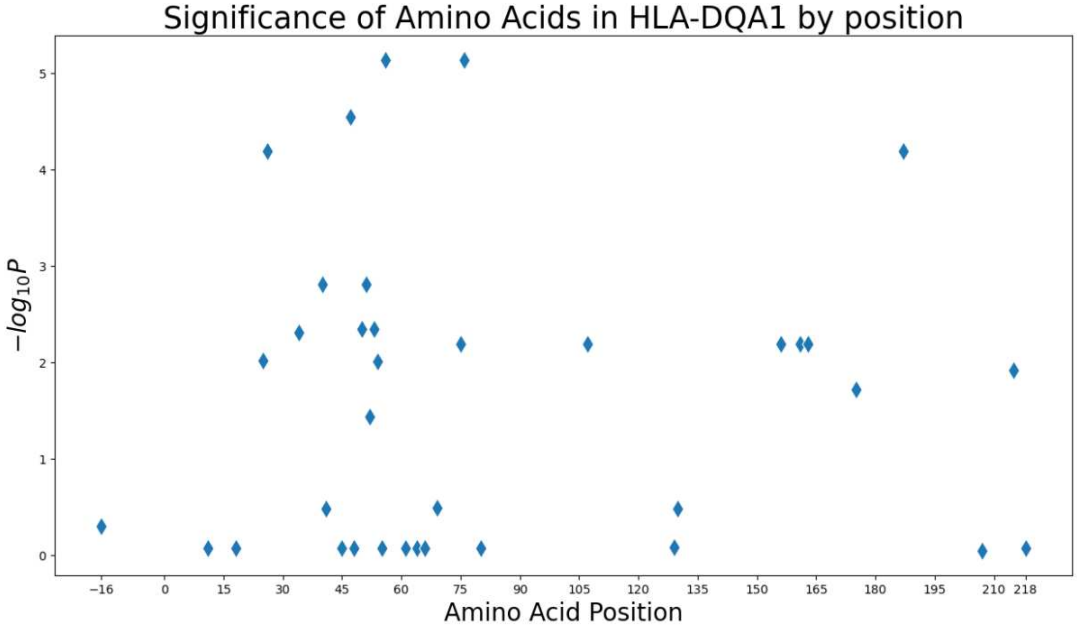

Figure S1: Manhattan plot for HLA-DQA1 amino acids. Most significant positions are 56 and 76.

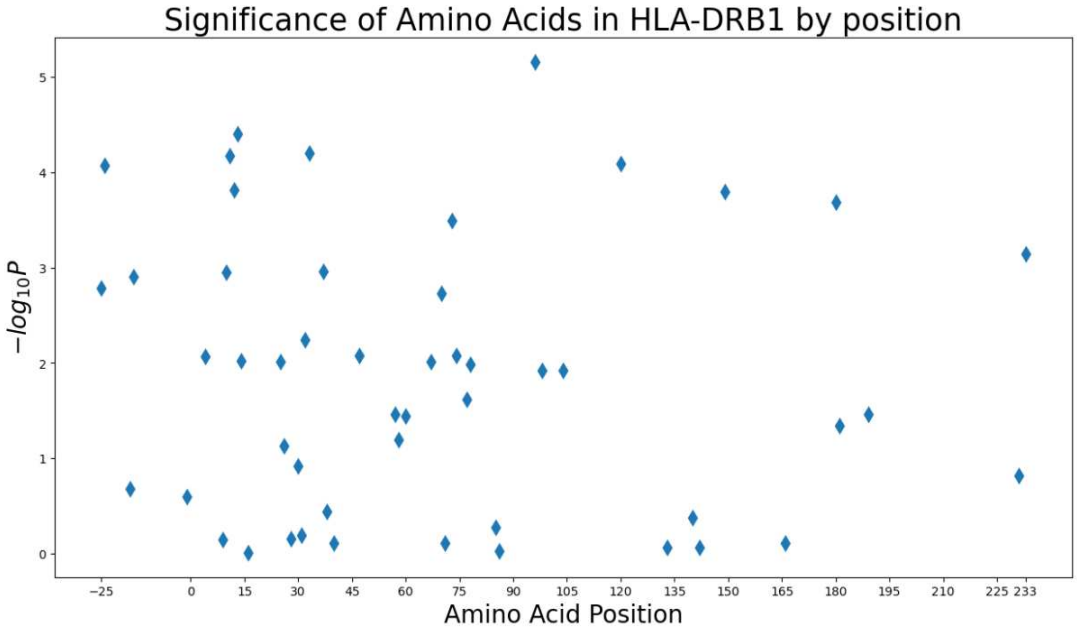

Figure S2: Manhattan plot for HLA-DRB1 amino acids. Most significant positions is 96.

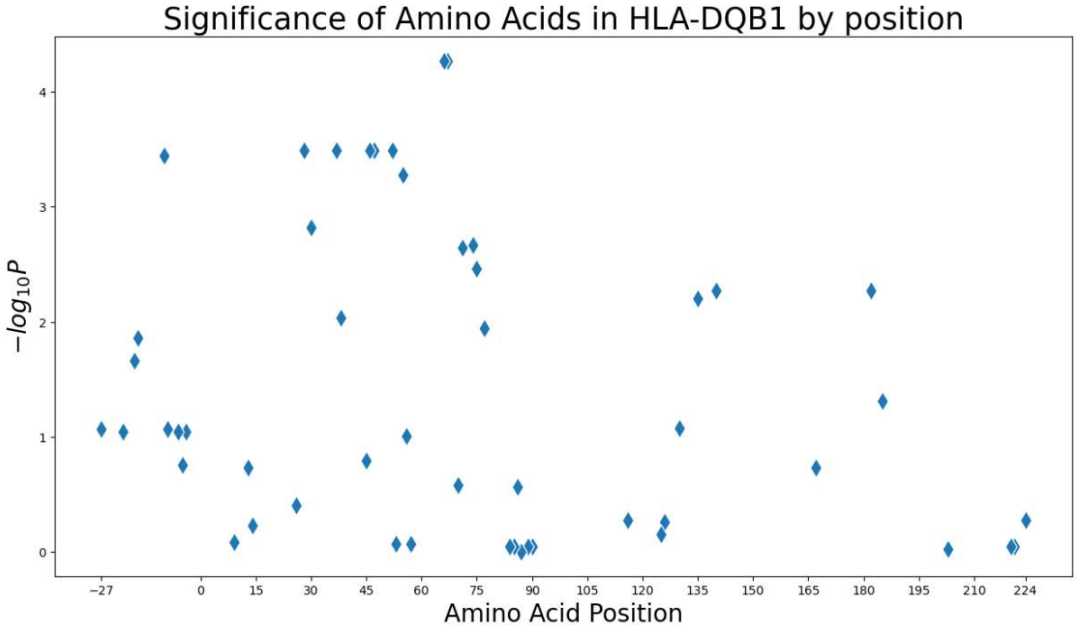

Figure S3: Manhattan plot for HLA-DQB1 amino acids. Most significant positions are 66 and 67.

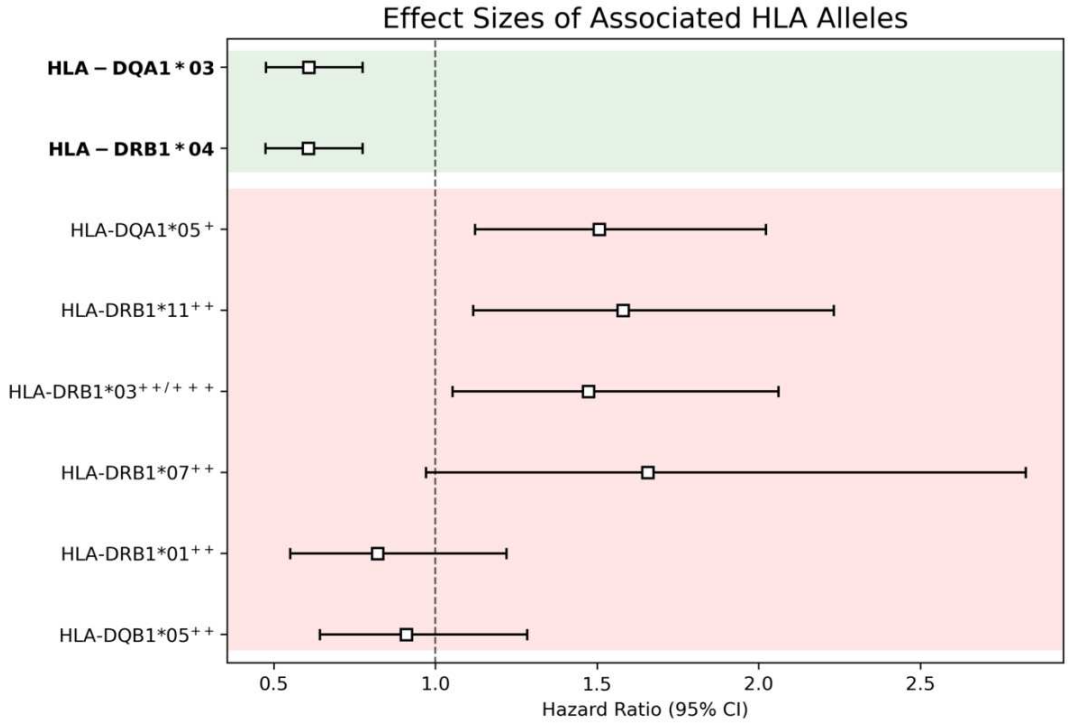

Figure S4: Effect sizes of HLA alleles on time to immunogenicity. The region marked in green are the alleles with the strongest association found in this study. The region marked in red are associations found in other studies but with effect sizes (and standard error) measured from the current study. For alleles found in other study, all alleles except for HLA-DRB1*07, were in the same direction, i.e. those found to be risk, were also found to be risk here. [+]From Sazonovs et al [6], [++]From Liu et al [7] and [+++]From Billiet et al [8].

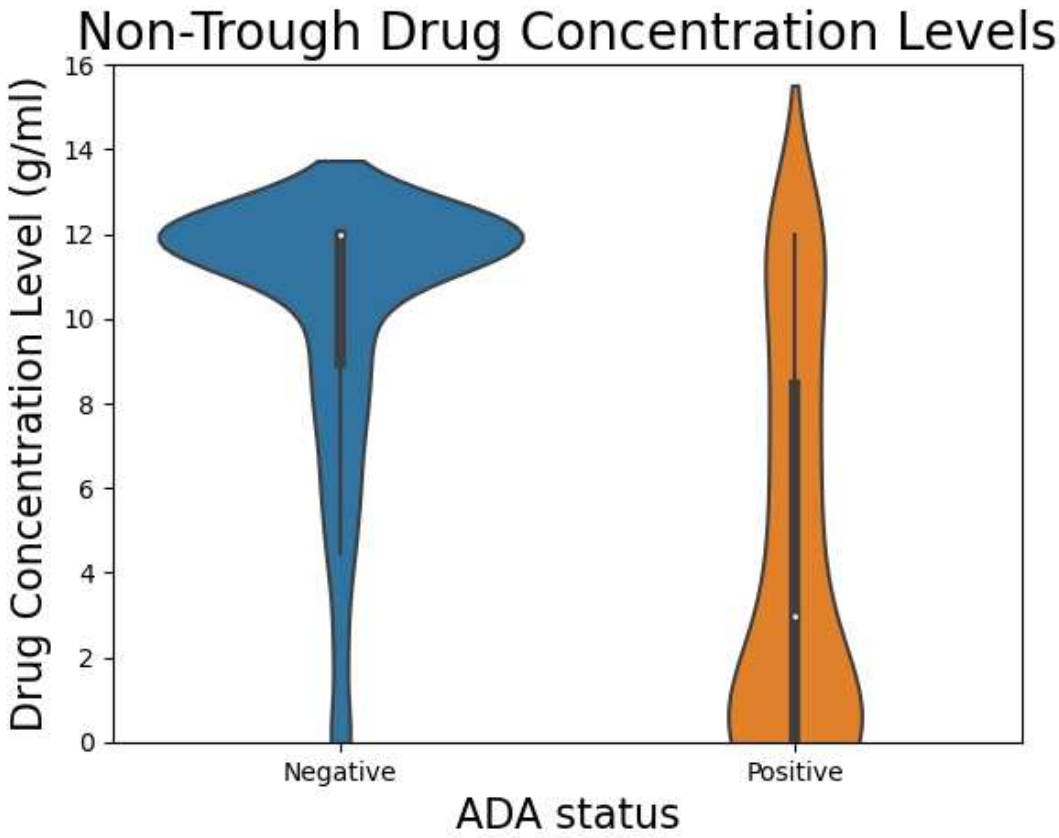

Figure S5: Violin plot for non-trough drug levels for ADA negative and positive samples. Mann-Whitney test indicated statistically significant difference with *p*: 5.7e-33.

References

1 Arnett FC, Edworthy SM, Bloch DA, *et al.* The american rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315–24. doi:10.1002/art.1780310302

2 Purcell S, Neale B, Todd-Brown K, *et al.* PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *Am J Hum Genet* 2007;**81**:559–75. doi:10.1086/519795

3 HapMap3. https://www.sanger.ac.uk/resources/downloads/human/hapmap3.html

4 Jia X, Han B, Onengut-Gumuscu S, *et al.* Imputing Amino Acid Polymorphisms in Human Leukocyte Antigens. *PLoS One* 2013;**8**:e64683. doi:10.1371/journal.pone.0064683

5 Pollard TJ, Johnson AEW, Raffa JD, *et al.* tableone: An open source Python package for producing summary statistics for research papers. *JAMIA Open* 2018;**1**:26–31. doi:10.1093/jamiaopen/ooy012

6 Sazonovs A, Kennedy NA, Moutsianas L, *et al.* HLA-DQA1*05 Carriage Associated With Development of Anti-Drug Antibodies to Infliximab and Adalimumab in Patients With Crohn's Disease. *Gastroenterology* 2020;**158**:189–99.

doi:10.1053/j.gastro.2019.09.041

7 Liu M, Degner J, Davis JW, *et al.* Identification of HLA-DRB1 association to adalimumab immunogenicity. *PLoS One* 2018;**13**:e0195325. doi:10.1371/journal.pone.0195325

8 Billiet T, Vande Casteele N, Van Stappen T, *et al.* Immunogenicity to infliximab is associated with HLA-DRB1. *Gut* 2015;**64**:1344–5. doi:10.1136/gutjnl-2015-309698