MHC associations of ankylosing spondylitis in East Asians are complex and involve non-HLA-B27 HLA contributions

CURRENT STATUS: UNDER REVISION

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DOI:
10.21203/rs.2.20481/v1

SUBJECT AREAS
Rheumatology

KEYWORDS
Ankylosing spondylitis, HLA, Association
Abstract

Background The association of HLA-B*27 with AS is amongst the strongest of any known association of a common variant with any human disease. Nonetheless, there is strong evidence indicating that other HLA-B alleles are involved in the disease. Large studies in European case-control cohorts have demonstrated risk associations with HLA-B*40 and multiple other HLA-B, -A and Class II alleles, and demonstrated that in that ethnic group the amino acid sequence at position 97 in HLA-B is the key determinant of HLA associations with AS. A recent study in Korean AS cases and controls additionally identified association at HLA-C*15:02. In the current study we examined the MHC associations of AS in an expanded east Asian cohort.

Methods 1,637 Chinese, Taiwanese and Korean AS cases meeting the modified New York Criteria for AS, and 1,589 ethnically matched controls, were genotyped with the Illumina Immunochip, including a dense coverage of the MHC region. HLA genotypes and amino acid composition was imputed using the SNP2HLA program using the Han-MHC reference panel based on the data of Han Chinese subjects (largest Asian MHC reference available (n= 9,689)), and association tested using logistic regression using 10 principal components to control for population stratification effects.

Results Strong association was seen with HLA-B*27 (odds ratio (OR) =205.3, P=5.764×10^-244 ). Controlling for this association, the strongest risk association is seen with HLA-C*15 at genome-wide significant level (OR = 7.62, P=9.30×10^-19 ), and confirmed association is also seen with HLA-B*40 at suggestive level (OR= 1.65, P= 2.54x10^-4 ). At amino acid level the strongest association seen in uncontrolled analysis was with histidine at position 114 in HLA-B (p=7.24 x10^-241 ), but conditional analyses suggest that the primary amino acid associations are with lysine at position 70 and asparagine at position 97. Restriction of the ERAP1 association with HLA-B27-positive AS, previously reported in
European subjects, was confirmed in East Asians.

Conclusions This study confirms in East Asians that the HLA associations of AS are multiple, including previously reported associations at HLA-B*27, -B*40, -C*15, and -DRB1*01, as well as novel associations with -DRB1*13 and -B*39. The HLA-B associations are driven by the amino acids at positions 70 and 97, in the B-pocket of HLA-B.

Background

Ankylosing spondylitis (AS) is a highly heritable rheumatic disease characteristically causing chronic inflammation of spine, sacroiliac joints, as well as in some patients affecting peripheral joints, the anterior uvea, and less commonly other organs. The worldwide distribution of AS is closely related to the prevalence of HLA-B*27, although the underlying mechanism remains unclear. Whilst the HLA-B*27 allele is found in approximately 85% of patients, there is strong evidence indicating that other HLA-B alleles and MHC genes are involved in the disease, as well as non-MHC loci.

Direct genotyping studies in European case-control cohorts have demonstrated risk associations consistently with HLA-B*40, and variably reported associations with multiple other HLA-B, -A, and Class II alleles. The development of accurate HLA-imputation methods from SNP microarray data has enabled far larger case-control studies to be performed, with for the first time, proper control for population stratification effects. Using this approach and studying 22,647 AS cases and controls of European descent, Cortes et al. demonstrated that the amino acid sequence of HLA-B at position 97, in the epitope-binding groove, is the key determinant of HLA associations with AS. After controlling for the associated alleles in HLA-B, independent associations with variants in the HLA-A, HLA- DPB1 and HLA-DRB1 loci were observed [1].

Differences in HLA-B*27 subtype distributions between Asian and European-descent populations have been well reported, and further non-HLA-B*27 HLA Class I associations in
east Asian AS have been reported. Also using HLA imputation methods, a study in 654 Korean cases of AS and 3166 control additionally identified association at HLA-C*15:02[2]. Additionally, using direct genotyping in 360 Han Chinese AS cases and 350 controls with no genomic control for population stratification, risk association of HLA-B*40 and protective association of HLA-B*07 has been demonstrated [3].

In this study, using HLA imputation methods we analyse the associations of AS with major histocompatibility complex (MHC) polymorphisms to identify functional and potentially causal variants using a large cohort of East Asian ancestry AS cases and controls [4]. In addition to our primary analysis of this cohort, we perform fine mapping the MHC region with imputation of SNPs, HLA class I and II classical alleles, and amino acid residues within the classical HLA proteins. In addition to HLA-B*27, we identify further HLA-B and other HLA class I and II alleles associated with AS.

Methods

**Subjects and SNP data**

1,637 Chinese, Taiwanese and Korean AS cases meeting the modified New York Criteria for AS [5], and 1,589 ethnically matched controls (Table 1), were genotyped with the customized SNP array (Illumina Immunochip [6]), including a dense coverage of the MHC region. By standard quality-control procedures, SNPs with a minor allele frequency of at least 1% (MAF>0.01), call rates of ≥0.98, and P-values in Hardy-Weinberg disequilibrium tests ≤10\(^{-7}\) were analysed in this study. To confirm ethnicity, we performed a continental principal components analysis (PCA), merging the study genotype data available data from 51 available populations genotyped by Illumina 650Y from the Human Genome Diversity Panel (HGDP-CEPH) [7]. Cases or controls lying more than 6 standard deviations from the population mean on principal components (PCs) 1-10 were then excluded.
**HLA imputation and association analysis**

We conducted a 2-step imputation. We densely imputed SNPs across the MHC using the Michigan Imputation Server [8] and the 1000 Genomes Phase 3 reference dataset (26 populations across the world), then further using the Han-MHC reference panel [9], to ensure maximum SNP coverage to enable accurate imputation of HLA-B alleles, including of particular interest, HLA-B27. Using this SNP data and the Han Chinese reference panel (N=9869), the program SNP2HLA was used to impute the classic HLA alleles and amino acid residues of the 8 HLA genes (HLA-A, -B, -C, -DPB1, -DQB1, -DRB1, -DPA1, -DQA1) in a total of 3007 East Asian subjects. Association with AS was then tested using logistic regression using 10 principal components to control for population stratification effects. Conditional analyses were performed in AS association tests controlling for HLA-B*27 and other HLA alleles where stated. Only HLA alleles or amino acids with imputation information scores >0.5 were considered.

**Results**

PCA analysis indicated that all study subjects were ethnically East Asian (Supplementary Figure 1). The genomic inflation factor calculated using a set of 1767 negative control SNPs in regions included on Immunochip for studies of reading and writing disabilities, psychosis and schizophrenia was 1.03 (lambda(1000)=1.02). No evidence of statistical inflation is seen in the Q-Q plot (Supplementary Figure 2). After quality control and imputation, 15,748 SNPs across the MHC (from 25-35Mb, hg18) were available for analysis in 1,482 cases and 1,512 controls. Imputed HLA-B allele frequencies amongst controls in the current study were not significantly different from those in previously reported directly genotyped studies (P>0.05), confirming the high accuracy of HLA imputation, particularly at two-digit resolution 3.
HLA-B Associations The strongest SNP association with AS observed was a missense variant of HLA-B, rs1071652 (SNP-B31432180_CG, OR= 180, P= 4.45×10^{-256}, Figure 1). The previously reported east Asian HLA-B27 tagSNP rs13202464 (31452562) 10 was also found significantly associated with AS (OR= 58.73, P= 1.92×10-211). Controlling for rs1071652, residual association is seen with HLA-B27 (4.48×10-22) and SNP rs41553720 (SNP-B31432843_A, P= 3.87×10-31), indicating that combinations of SNPs are currently required to tag HLA-B27 in East Asian populations, in contrast to the situation in European descent populations 11. After SNP imputation in the MHC region, the expected strong association was observed with HLA-B27 (odds ratio (OR) =205, P=5.76×10-244, Table 2). Controlling for the HLA-B27 association and studying other HLA-B alleles, risk association is seen with HLA-B40 at suggestive level (OR= 1.65, P= 2.54×10-4). Controlling for both HLA-B27 and -B40, no association was observed in 2-digit HLA-B allele with MAF > 1% (only rare alleles HLA-B53 and HLA-B38 were associated at suggestive level, P-values were 2.9×10-4 and 3.3×10-4, respectively). At amino acid level the strongest association seen in uncontrolled analysis was with histidine at position 114 in HLA-B (p=7.24×10-241), followed by multiple HLA-B amino acids including lysine at 70 (P=1.49×10-237) and asparagine 97 (P= 2.51×10-237) (Table 3). Asparagine 97 and histidine 114 were previously reported to be the main amino acid determining HLA-B associations with AS in European-descent and Korean populations respectively 1, 2.

Conditional analyses for these individual amino acids and their combinations reveals that only association of histidine 114 can be attenuated by conditioning on asparagine 97 (Table 4). No other individual amino acid explains the association of the other amino acids. For HLA-B alleles, both combinations of lysine 70 + asparagine 97 and lysine 70 + histidine 114, but not asparagine 97 + histidine 114, controlled for association with any other 2-digit HLA-B allele (P>0.0017, correcting for 30 2-digit HLA-B alleles tested).
Controlling for all of lysine 70, asparagine 97 and histidine 114, the strongest HLA amino-acid association remains with several positions including HLA-B position 97 (serine, found on HLA-B7, 8, 15, 2707, 40, 41, 48; OR=2.14, P=2.14×10^-5). Controlling for HLA-B27 alone or in combination with HLA-B40 did not fully control for the association of asparagine 97, lysine 70 or histidine 114 (P<5×10^-8 for both analyses, Table 3). Non-HLA-B susceptibility loci in the MHC. Considering HLA alleles other than HLA-B, several HLA-A, -C and Class II alleles showed significant associations (Table 2). Controlling for the HLA-B27 association, independent risk association was confirmed with HLA-C15 (OR = 2.13, P=9.30×10^-19) and HLA-C1502 (OR= 7.62, P=6.78×10^-23), both associations at amino acid level tagged by a leucine 116 in HLA-C (P=6.61×10^-21) located in the HLA-C epitope binding groove. Stepwise conditional analyses on both HLA-B27 and -C15 demonstrated significant associations with HLA-DQB104 (OR=2.13, P=7.91×10^-5) after correcting for multiple comparisons (499 signals across MHC, as defined by regions with LD r2<0.2, Bonferroni correction threshold = 10^-4). Conditioning on both HLA-B27 and HLA-B40, association was confirmed with HLA-C15 (OR=4.97, P= 9.88×10^-17) and HLA-DQB1*04 (OR= 2.42, P=1.86×10^-6).

ERAP1 variants in association with AS. The key ERAP1 variant associated with AS is rs30187 (ccc-5-96150086-T-C, chr5:96150086hg18, encoding K528R) 4, 12(Table 5). It has previously been observed in European populations that the association with the variant rs30187 in the ERAP1 locus is restricted to HLA-B27-positive subjects, or HLA-B40-positive, HLA-B27-negative subjects, consistent with epistatic interactions. Here we investigated the possibility of interaction between the HLA-B27, HLA-B40 alleles and the previous reported tag SNP of ERAP1 locus (rs30187) 4. When testing for interaction with the HLA-B27 alleles, we found that rs30187-A risk allele increased the risk of disease in the strata where HLA-B27 was present (OR=1.29; P=2.71×10^-6) (Table 5), but no association was
seen in HLA-B27-negative cases (OR=1.06, P=0.61). No evidence of interaction was observed between rs30187 and the HLA-B*40 allele, although the power to identify this was low as the number of HLA-B27-negative cases was low.

Discussion

This study confirms that in East Asians the primary MHC associations with AS are with HLA-B*27 and HLA-B*40, and confirms the risk association of HLA-C*1502 with the disease. The association of HLA-B*40 with AS has been convincingly demonstrated now in both European-descent [1, 13–15] and east Asian studies [3, 16], using both direct genotyping and imputation based methods. HLA-B*4001 has also been shown to be associated with IgA nephropathy (OR = 1.34, P = 5.64 × 10−7) [17], a known though uncommon association of AS. The functional mechanism of association of this allele has been little studied. It does not share the lysine 70, asparagine 97 or histidine 114 residues found in most HLA-B*27 alleles. As with HLA-B*27, it is known to interact with AS-associated ERAP1 variants to cause AS, suggesting that it is likely to operate by the same mechanism. Further studies to compare its properties with HLA-B27, such as its peptide-binding characteristics, folding rate, and whether it forms homodimers, are indicated to investigate its association further.

No protective association was seen with HLA-B*07 as has previously been reported in east Asians [3] and European descent cohorts [1, 14], although the allele frequency was very low and the study may not have had adequate power to detect any association with the allele (frequency = 0.024).

The study indicates that in East Asians the key amino acid drivers of the HLA-B associations in AS are amino acid position 70 and 97. These remain AS-associated controlling for any other HLA-B amino acid. HLA-B position 97 was previously shown in
European descent cohorts to be the key amino acid association in the broad ethnicity, whereas in a Korean study, association of histidine 114 could not be distinguished from associations with lysine 70 and asparagine 97 [2]. The difference in these findings may be explained by three key factors, sample size, ethnicity and the reference haplotype dataset. Cortes et al’s study of European descent subjects involved 9,069 AS cases and 13,578 controls, over seven times as many subjects as involved in the current study (1,637 cases, 1,589 controls) and nearly six times the number involved in the previous Korean study (654 cases, 3,166 controls). Therefore the European descent study had greater power, potentially explaining the absence of signal in the East Asian cohorts for some of the HLA-B allele and the HLA class II associations seen in the European dataset.

The European descent study also has greater power in conditional analyses, potentially explaining the differences in results regarding the role of lysine 70, which remains positively associated with AS after conditioning on asparagine 97 in the current study, but not in the European descent dataset. The different studies have also used different reference haplotype datasets, potentially affecting the accuracy of the imputation data. Ethnic differences could also play a role through differences in HLA-B*27 subtypes or other HLA-B allele frequencies, particularly comparing the European-descent and East Asian cohorts.

Both HLA-B amino acid residues 70 and 97 are found within the B pocket of the HLA-B peptide-binding groove. However, it has been noted that position 70 is tightly coupled with positions 67 and 97 and that position 70 hardly changes the peptide-binding repertoire, suggesting that position 70 is “hitch-hiking” along with positions 67 and 97 in their ability to change the peptide-binding repertoire [18]. Our study and the previous HLA amino-acid imputation studies suggest that other amino acid positions in addition to 70 (like position 97 and 114) are also involved in HLA-B risk attribution. The association of
these amino acids independent of other amino acids found in the HLA-B27 B pocket, and having controlled for HLA-B27, indicates that their effect on disease risk is partially independent of HLA-B27.

Although the HLA-allele frequencies imputed in controls in this study closely match those reported by direct genotyping studies in Han Chinese [3], the accuracy of imputation in such studies is very dependent on the ethnic matching of the imputed and reference datasets. Whilst the Han-MHC reference dataset used here is of large size (n = 9689), but the number of East Asian in 1000 Genomes Phase 3 (n = 524), which we used in Michigan imputation server, is far smaller than the European dataset used in Cortes et al (Type 1 Diabetes Genetics Consortium dataset, n = 5225) [1]. The smaller reference dataset size precluded imputation to four digit levels, and may have affected accuracy of imputation of low frequency alleles in particular. As SNP-based HLA-imputation is a highly efficient method enabling large scale HLA-association studies, there is a clear need for much larger publicly available HLA-imputation reference datasets for Pan-Asian population.

In this study we have also confirmed the interaction between ERAP1 and HLA-B*27, with association only observed of the key ERAP1 variant, rs30187, only seen in HLA-B*27 positive individuals. This confirms the original finding in Europeans [1], and the previous finding in a case only analysis of Taiwanese AS patients of different ERAP1 genotypes in HLA-B*27-positive and -negative cases [19]. We did not see an association of ERAP1 variants in HLA-B*27-negative, HLA-B*40-positive individuals as previously reported [1], although the sample size was not large. The confirmation of the gene-gene interaction in an east Asian population increases the evidence that this is a true positive interaction and is critical to AS pathogenesis.

In conclusion, this study confirms that the HLA-associations of AS are complex, and that multiple non-HLA-B*27 alleles, including both HLA Class I and likely Class II variants, also
contribute to risk and protection from the disease. Further investigation of the mechanisms involved in these associations is likely to assist in determining the pathogenesis of this disease.

Declarations

Ethics approval and consent to participate

This study protocol was reviewed and approved by the relevant ethics committees of the hospitals and institutions involved. All subjects provided written informed consent.

Consent for publication

Not applicable.

Acknowledgements

MAB is funded by a National Health and Medical Research Council (Australia) Senior Principal Research Fellowship (1024879) and Queensland State Premier’s Fellowship for Science. Support for this study was received from a National Health and Medical Research Council (Australia) program grant (566938) and project grant (569829). This research was funded/supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London and/or the NIHR Clinical Research Facility. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Data Availability

Summary data for the datasets used are available at Harvard Dataverse (https://doi.org/10.7910/DVN/NJ7XSO) and on request from the authors.
**Competing Interests**

The authors declare that they have no competing interests.

**Funding**

MAB was funded by a National Health and Medical Research Council (Australia) Senior Principal Research Fellowship (#1024879).

**Author Contribution**

Study design and case recruitment was performed by GW, THK, KK, SYB, MAB and HX.

Data analysis was performed by GW, ZL and AC. The study manuscript was prepared by GW, MAB and HX. All authors read and approved the final manuscript.

**Acknowledgements**

The authors would like to thank Erika de Guzman, Sharon Song and Lisa Anderson from the Australian Translational Genomics Centre (https://research.qut.edu.au/translationalgenomicsgroup/atgc/) for their assistance in SNP microarray genotyping.

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Tables

Table 1 Demographic summary of study cohort.

|                  | Cases | Controls | Total |
|------------------|-------|----------|-------|
| Chinese          | 764   | 683      | 1,447 |
| Taiwanese        | 214   | 179      | 393   |
| Korean           | 659   | 727      | 1,386 |

Table 2 Association of HLA alleles with susceptibility to ankylosing spondylitis (P<0.05 in conditional analysis on HLA-B27).

2-digit HLA haplotypes

| SNP          | FRQ | OR | P       | P_con_B27 | P_con_B27_B40 |
|--------------|-----|----|---------|-----------|---------------|
| HLA-C*15     | 0.04| 1.01| 0.97    | 9.30×10^{-19} | 9.88×10^{-19} |
| HLA-DQBI1*04 | 0.07| 0.96| 0.68    | 5.98×10^{-7}  | 1.86×10^{-7}  |
| HLA-DRBI1*13 | 0.07| 0.37| 6.28×10^{-15}| 2.53×10^{-4}  | 7.85×10^{-4}  |
| HLA-B*40     | 0.12| 0.58| 2.63×10^{-11}| 2.54×10^{-4}  | NA            |
| HLA-C*03     | 0.20| 0.43| 9.19×10^{-33}| 6.08×10^{-4}  | 9.92×10^{-4}  |
| HLA-DQBI1*06 | 0.22| 0.52| 1.50×10^{-20}| 6.32×10^{-4}  | 1.25×10^{-4}  |
| HLA-B*53     | 0.00| 0.62| 0.46    | 7.33×10^{-4}  | 2.87×10^{-4}  |
| HLA-B*38     | 0.01| 2.10| 0.041   | 9.55×10^{-4}  | 3.35×10^{-4}  |
| HLA-C*05     | 0.01| 0.25| 2.75×10^{-3}| 1.77×10^{-3}  | 2.68×10^{-3}  |
| HLA-B*15     | 0.10| 0.40| 1.13×10^{-21}| 0.021       | 0.01          |
| HLA-DRBI1*04 | 0.15| 1.09| 0.26    | 0.028      | 0.01          |
| HLA-DPBI1*45 | 0.00| 0.98| 0.97    | 0.029      | 0.01          |
| HLA-DQAI1*06 | 0.06| 4.09| 3.05×10^{-17}| 0.048      | 0.01          |
### 4-digit HLA haplotypes

| SNP           | FRQ  | OR   | P       | P_con_B27 | P_con_B27_B40 |
|---------------|------|------|---------|-----------|--------------|
| HLA-C*1502    | 0.03 | 1.15 | 0.37    | 6.78×10^{-23} | 4.10×10^{-12} |
| HLA-B*4002    | 0.03 | 0.79 | 0.16    | 9.55×10^{-12} | 6.54×10^{-12} |
| HLA-DRB1*0405 | 0.06 | 0.93 | 0.51    | 2.30×10^{-7}  | 1.06×10^{-7}  |
| HLA-DQ21*0401 | 0.05 | 0.90 | 0.45    | 5.05×10^{-6}  | 1.54×10^{-6}  |
| HLA-B*4005    | 0.00 | 1.72 | 0.55    | 4.21×10^{-4}  | 2.09×10^{-4}  |
| HLA-B*5301    | 0.00 | 0.62 | 0.46    | 7.20×10^{-4}  | 2.82×10^{-4}  |
| HLA-DRB1*1302 | 0.05 | 0.37 | 5.63×10^{-13}| 1.13×10^{-3}  | 2.88×10^{-3}  |
| HLA-B*3802    | 0.01 | 2.29 | 0.036   | 2.16×10^{-3}  | 1.01×10^{-3}  |
| HLA-C*0501    | 0.00 | 0.38 | 0.054   | 3.98×10^{-3}  | 5.60×10^{-3}  |
| HLA-DQA1*0102 | 0.27 | 0.36 | 3.45×10^{-30} | 4.68×10^{-3}  | 0.01 |
| HLA-B*4446    | 0.03 | 0.21 | 5.69×10^{-14} | 6.15×10^{-3}  | 0.01 |
| HLA-C*0304    | 0.08 | 0.53 | 1.72×10^{-9} | 0.012          | 6.95×10^{-9}  |
| HLA-DRB1*1501 | 0.08 | 0.56 | 5.36×10^{-8} | 0.022          | 0.00 |
| HLA-B*4402    | 0.00 | 0.30 | 0.13    | 0.027         | 0.00 |
| HLA-DPB1*4501 | 0.00 | 0.97 | 0.96    | 0.029         | 0.00 |
| HLA-DQB1*0603 | 0.01 | 0.41 | 2.55×10^{-3} | 0.036         | 0.00 |
| HLA-B*1501    | 0.04 | 0.24 | 2.94×10^{-15} | 0.047         | 0.1 |
| HLA-DQA1*0601 | 0.06 | 4.09 | 3.04×10^{-17} | 0.048         | 0.00 |
| HLA-B*5502    | 0.02 | 0.28 | 9.64×10^{-6}  | 0.050         | 0.1 |

**OR**: Odds Ratio in unconditional analysis; **FRQ**: Allele frequency in controls. **P**: P-value in unconditional analysis; **P_con_B27**: P-value in conditional analysis controlling HLA-B*27; **P_con_B27_B40**: P-value in conditional analysis controlling HLA-B*27 and HLA-B*40.

Table 3 Association of amino acid residues in HLA-B with susceptibility to ankylosing spondylitis (P<10^{-100} in unconditional analysis).
| Position | AA | FRQ  | OR   | P       | P_con_B27 | P_con_B27_B40 |
|----------|----|------|------|---------|-----------|---------------|
| 114      | H  | 0.24 | 232.78 | 7.24×10^{241} | 1.86×10^{-8} | 1.18×10^{-8} |
| 70       | K  | 0.25 | 312.90 | 1.49×10^{237} | 1.22×10^{-40} | 1.35×10^{-38} |
| 97       | N  | 0.24 | 255.79 | 2.51×10^{237} | 1.08×10^{-13} | 3.49×10^{-13} |
| 67       | C  | 0.30 | 62.52  | 8.05×10^{-232} | 6.91×10^{-17} | 6.89×10^{-18} |
| 116      | D  | 0.29 | 57.93  | 2.38×10^{-221} | 0.15 | 0.061 |
| 80       | T  | 0.31 | 42.93  | 1.99×10^{-220} | 2.82×10^{-20} | 1.27×10^{-21} |
| 113      | Y  | 0.32 | 25.09  | 5.03×10^{194} | 0.33 | 0.12 |
| 9        | H  | 0.39 | 16.96  | 2.20×10^{-154} | 1.00×10^{-11} | 3.64×10^{-14} |
| 45       | E  | 0.39 | 14.84  | 5.35×10^{-153} | 2.41×10^{-9} | 3.46×10^{-11} |
| 9        | Y  | 0.39 | 16.08  | 1.75×10^{-151} | 3.24×10^{-11} | 2.68×10^{-12} |
| 69       | A  | 0.40 | 11.19  | 1.81×10^{-146} | 8.20×10^{-7} | 1.13×10^{-8} |
| 11       | S  | 0.41 | 13.63  | 4.25×10^{-145} | 8.62×10^{-14} | 1.66×10^{-11} |
| 83       | R  | 0.42 | 13.77  | 8.00×10^{-145} | 1.76×10^{-14} | 7.46×10^{-18} |
| 12       | V  | 0.41 | 12.47  | 9.64×10^{-143} | 6.01×10^{-13} | 9.44×10^{-11} |
| 82       | L  | 0.44 | 10.47  | 2.64×10^{-140} | 8.24×10^{-12} | 3.80×10^{-15} |
| 32       | L  | 0.41 | 11.00  | 7.76×10^{-137} | 3.06×10^{-7} | 1.38×10^{-4} |
| 80       | N  | 0.45 | 8.89   | 3.98×10^{-135} | 8.83×10^{-13} | 1.49×10^{-16} |
| 24       | T  | 0.44 | 9.12   | 2.58×10^{-134} | 3.08×10^{-9} | 8.53×10^{-7} |
| 77       | D  | 0.14 | 27.62  | 5.68×10^{-128} | 0.39 | 0.39 |
| 74       | D  | 0.43 | 7.41   | 6.83×10^{-126} | 7.02×10^{-3} | 2.97×10^{-4} |
| 163      | E  | 0.46 | 6.58   | 1.53×10^{-121} | 2.06×10^{-3} | 0.38 |
| 69       | T  | 0.46 | 5.72   | 4.97×10^{-117} | 5.01×10^{-4} | 5.62×10^{-6} |
| -16      | L  | 0.48 | 5.77   | 4.58×10^{-116} | 1.76×10^{-6} | 1.28×10^{-5} |
| -16      | V  | 0.48 | 5.69   | 2.93×10^{-115} | 1.66×10^{-6} | 1.17×10^{-5} |
| 71       | A  | 0.47 | 5.49   | 5.24×10^{-114} | 1.94×10^{-3} | 3.15×10^{-5} |
| 70       | N  | 0.47 | 5.48   | 5.55×10^{-114} | 1.96×10^{-3} | 3.19×10^{-5} |
| 97       | R  | 0.54 | 5.13   | 9.25×10^{-109} | 7.34×10^{-7} | 2.13×10^{-6} |
| 24       | A  | 0.43 | 0.23   | 6.90×10^{-103} | 3.58×10^{-8} | 1.14×10^{-5} |

**OR:** Odd Ratio in unconditional analysis; **P:** P-value in unconditional analysis; **P_con_B27:** P-value in conditional analysis controlling HLA-B*27; **P_con_B27_B40:** P-value in conditional analysis controlling HLA-B*27 and HLA-B*40. **NS=P>0.05**
Table 4 Conditional analysis p’values of HLA-B amino acid residues. Significance for association of lysine 70 (70K), asparagine 97 (97N) and histidine 114 (114H) are given in columns, either in unconditional analysis, or conditioning on specific amino acid positions (where no letter is given after the HLA-B amino acid position number) or for specific amino acids (where a letter is given after the HLA-B amino acid position number), either individually or in combinations. NS=P>0.05.

|                  | 70K     | 97N     | 114H     |
|------------------|---------|---------|----------|
| **Unconditional**| 1.49x10^{-237} | 2.51x10^{-237} | 7.24x10^{-241} |
| 70               | -       | 1.10x10^{-6} | 1.76x10^{-6} |
| 97               | 1.04x10^{-28} | -       | 0.13     |
| 114              | 4.08x10^{-37} | 2.70x10^{-8} | -        |
| 70+97            | -       | -       | 0.24     |
| 70+114           | -       | 2.94x10^{-1} | -        |
| 97+114           | 5.81x10^{-28} | -       | -        |
| 70+97+114        | -       | -       | -        |
| 70K              | -       | 6.15x10^{-7} | 1.04x10^{-6} |
| 97N              | 1.11x10^{-31} | -       | 0.42     |
| 114H             | 1.70x10^{-38} | 2.02x10^{-8} | -        |
| 70K + 97N        | -       | -       | 0.49     |
| 70K+114H         | -       | 0.30    | -        |
| 97N+114H         | 1.27x10^{-31} | -       | -        |
| 70K +97N+114H    | -       | -       | -        |

Table 5 Association analysis of rs30187 in samples positive and negative for HLA-B*27 and HLA-B*40. Odds ratios are given for the rs30187-risk A allele.
| Group (case/control)                                                                 | OR   | P           |
|-----------------------------------------------------------------------------------|------|-------------|
| ALL (1482/1512)                                                                   | 1.26 | 9.00×10^{-6}|
| HLA^{B27}+cases vs ALL controls (1323/1512)                                       | 1.29 | 2.71×10^{-6}|
| HLA^{B27}-cases vs ALL controls (159/1512)                                        | 1.06 | 0.61        |
| HLA^{B27}+ cases and controls (1323/77)                                            | 1.40 | 0.044       |
| HLA^{B27}-cases and controls (159/1435)                                           | 1.06 | 0.64        |
| HLA^{B27}+cases vs HLA^{B27}-cases (1323/159)                                     | 1.21 | 0.11        |
| HLA^{B27}+/HLA^{B40}+ (207/11)                                                    | 1.25 | 0.60        |
| HLA^{B27}+/HLA^{B40}- (1116/66)                                                   | 1.42 | 0.050       |
| HLA^{B27}/HLA^{B40}+ (89/1027)                                                    | 1.11 | 0.49        |
| HLA^{B27}/HLA^{B40}+ (70/408)                                                     | 1.02 | 0.91        |

OR: Odd Ratio in unconditional analysis; P: P-value in unconditional analysis; SE: Standard error of beta (log-odds) estimate; CHR: chromosome

**Figures**
AS susceptibility associations in the MHC region. Association plots for the extended major histocompatibility complex region. Significance levels of each marker single-nucleotide polymorphisms were calculated by using logistic regression of imputed dosage files, and plotted according to chromosomal locations (based on hg19). A. Top association was identified at HLA-B27. B. There was residual signals with SNPs near HLA-A, -B, -C and class II alleles conditioning on the HLA-B*27.

Supplementary Files

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