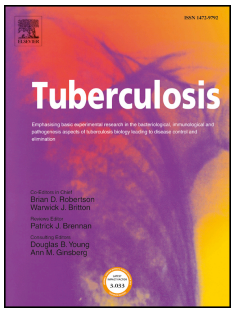

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Human leukocyte antigen associations with protection against tuberculosis infection and disease in human immunodeficiency virus-1 infected individuals, despite household exposure and immune suppression

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Running head

Protective HLA associations against TB in HIV

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Abstract

Background

To determine the association of human leukocyte antigen (HLA) alleles as correlates of risk for and protection against tuberculin skin test (TST) positivity and active TB disease amongst HIV-infected adults.

Methods

Genomic DNA was extracted from 754 HIV-infected adults whole-blood. *HLA-A, -B, -C* and *-DRB1* loci were genotyped by next generation sequencing methods. HLA alleles were analysed by the presence/absence of TST immune conversion and active TB disease and further stratified by exposure to a household TB contact, CD4 T-cell count and, for active TB disease, TST-positivity.

Results

*HLA-A*29:11* and - *B*45:01/07* were associated with TST-positivity, while *HLA-A*24:02, -A*29:02* and *-B*15:16* with TST-negativity. In participants with a household TB contact, *HLA-A*66:01, -A*68:02* and *-B*49:01* were associated with TST-negativity. For TB disease, *HLA-B*41:01, -C*06:02, -DRB1*04:01* and *-DRB1*15:01* were associated with susceptibility, while *HLA-B*07:02* and *-DRB1*11:01* were protective, even for CD4 T-cell count < 350 cells/mm^3. For initial TST-positivity and subsequent TB disease, *HLA-A*01:01* and *-DRB1*11:01* conveyed protection including those with CD4 T-cell count < 350 cells/mm^3.

Conclusion

Several HLA alleles are noted as correlates of TB infection, risk and natural protection in HIV-infected individuals. HLA associations may enable risk stratification of those with HIV

infection. Protective alleles may assist in future TB vaccine development.

Key words

TST immune conversion, Latent TB infection, Active TB disease, HLA alleles, correlates of protection

Abbreviations

Antiretroviral therapy (ART), Human leukocyte antigens (HLA), Killer-cell immunoglobulin-like receptors (KIRs), Latent TB infection (LTBI), Methionine (-21M), *Mycobacterium tuberculosis* (*Mtb*), Natural killer cell (NK cell), Threonine (-21T), Tuberculin skin test (TST), Tuberculosis (TB)

INTRODUCTION

Tuberculosis (TB) is transmitted by respiratory droplets from an infectious case with pulmonary TB[1]. An individual who inhales infectious material containing *Mycobacterium tuberculosis* (*Mtb*) may eliminate bacilli spontaneously or become infected[1]. Most of those who are immunocompetent and latently infected with TB (LTBI) will contain their infection for many years without progression to clinical illness[1], indeed most people with TB infection never develop TB disease. The highest risk of acquiring TB infection in high prevalence settings is during early adolescence, with up to 75% of individuals infected with TB by 25 years of age[2].

In immunocompetent individuals, about 5 - 15% of those latently infected progress to active TB[3–5]. Rates of progression to TB disease are markedly higher in HIV-infected individuals; in whom progression from incipient to active TB disease is frequent, more rapid and, in the absence of antiretroviral therapy (ART), has a poorer prognosis than in HIV-seronegative individuals[6]. There is a growing body of evidence that suggests that LTBI is a continuum of infected states that range from apparently healthy individuals with no symptoms, to those who have subclinical or asymptomatic disease, and finally to active TB disease[7]. Newer data has highlighted that some individuals may have LTBI despite negative tuberculin skin test (TST) or interferon gamma related assay testing, This is evidenced by an expanded adaptive immune response consisting of IgM, class switched IgG antibody responses and non – interferon – y T cell responses to *Mtb* proteins ESAT6 and CFP10[8,9]. Hence, those with a negative TST or interferon-gamma release assay (IGRA) test may still have LTBI, while TST immune conversion has definitively occurred in those with a positive TST[8,9].

South Africa has a high prevalence of both TB and HIV[10] and the high seroprevalence of HIV in South Africa further contributes to high rates of TB infection[11]. Despite the high prevalence of TB disease, particularly in communities with extremely high HIV seroprevalence and apparent ease of TB transmission, there are individuals with a history of close contact with an infectious TB patient yet have no evidence of TB infection, suggesting they may have inherent protection or resistance against TB infection[12,13]. Secondly, although HIV is a marker of far higher risk of progression to TB disease, there are some HIV-infected individuals who appear to have an inherent ability to resist progression to TB disease, despite evidence of exposure to a source patient, and severe immunosuppression.

Human leucocyte antigens (HLA) play an important role in the host–pathogen interaction and response of the immune system to infections. HLA molecules on target cells present immunogenic peptides to the T cell receptor of CD4+ or CD8+ T-cells or mediate natural killer cell (NK cell) activity via interaction with stimulatory or inhibitory receptors, such as killer-cell immunoglobulin-like receptors (KIRs). The genetic loci that encode HLA molecules (the HLA complex) are found on the short arm of chromosome 6 and are divided into 3 classes, with class I and class II coding for molecules that interact with CD8+ and CD4+ T-cells, respectively. There is significant genetic heterogeneity amongst these HLA regions, resulting in HLA allelic variation between individuals. As such the immune response to pathogens may differ based on the HLA profile which may confer either susceptibility to or protection against certain infections, although some alleles confer no risk or protection and are neutral[14]. It is postulated that susceptibility conferred by the HLA class II region against TB infection may be due to reduced presentation of protective *Mtb* antigens to T-cells[15,16]. NK cells require education before they become immunologically functional. This may occur through the KIRs or the inhibitory CD94/NKG2A receptor. HLA-E

molecules consisting of a peptide derived from the leader sequence from HLA-A, -B, -C or -G molecules are ligands for CD94/NKG2A. *HLA-B* allele polymorphisms can affect HLA-E cell surface levels and subsequent peptide presentation and hence modulate NK cell education. These include a -21 polymorphism that encodes Methionine (-21M), conferring greater efficacy of HLA-E binding and NKG2A NK cell education compared to Threonine (-21T) which does not bind effectively to HLA-E and favours KIR-mediated NK cell education[17,18]. Regarding KIRs, all HLA – C molecules are ligands for inhibitory KIRs unlike HLA-A or -B allotypes where only those carrying the Bw4 motif are recognised by KIR[18,19]. HLA – C allotypes are subclassified into two groups, C1 or C2, based on their alpha 1 helix sequence where asparagine at position 80 defines the C1 epitope compared to lysine which defines the C2 epitope[18,19]. A number of genes coding KIRs are identified and particular KIRs expressed recognise either the C1 or C2 epitope with greater affinity. For example, KIR2DL1 recognises HLA-C2 ligands compared to KIR2DL2 and KIR2DL3 which recognise HLA-C1 ligands with greater affinity and may play a role in the KIR mediated immune response of NK cells[20].

HLA allelic variation has previously been shown to play a role in the susceptibility to and protection against TB infection[14,21,22]. Due to the marked heterogeneity of HLA alleles, different populations may demonstrate variability in TB susceptibility or protection based on HLA genotypes. Data describing HLA genotypes to suggest LTBI/TST immune conversion or active TB susceptibility or protection in the South African population, particularly in HIV-positive patients, are limited. This study aims to describe HLA genotypes in South African HIV-infected adults who appeared resistant to TB infection despite being immunocompromised and exposed to a household contact with TB in a high TB prevalence

setting; and those who did not develop TB disease despite evidence of TB infection and a low nadir CD4 T-cell count.

METHODS

In brief, we recruited HIV-infected adults 18 years and older from Soweto, South Africa into a prospective cohort, consisting of 754 adult participants as described elsewhere[23]. To be eligible participants had to have been followed up in one of two prior cohorts of HIV-infected patients recruited and followed up at the same site. At each study visit, demographic details, personal and household socio-economic indicators were recorded including exposure to environmental and occupational air pollutants. Alcohol and other recreational drug use, prior and current medical problems and concomitant medication was recorded. Participants' height, weight, waist and hip circumference was measured. A chest x-ray was performed for participants who have symptoms suggestive of active TB. Other investigations such as sputum smears for acid-fast bacilli, and sputum cultures by MGIT and TB culture were done if clinically indicated to evaluate participants for active TB or other illnesses. Data regarding prior TB infection, TB disease status, receipt of TB preventive treatment and TB treatment, was obtained from the respective study databases. Blood was collected every six months for CD4+ T cell counts and HIV viral load, complete blood count (CBC) and c – reactive protein (CRP). Aspartate transaminase (AST), alanine transaminase (ALT), random cholesterol, haemoglobin A1 (HbA1C) and Quantiferon Gold assay was collected annually. Plasma specimens isolated from 60 ml of blood was taken at enrolment and annually. 3 ml of whole blood will be frozen and stored for subsequent genetic analysis. Events occurring between scheduled visits, typically those requiring acute care was ascertained. Clinical source data from the admission was obtained by abstraction of inpatient medical records. All participants had annual spirometry assessments supervised by a respiratory technologist or study

doctor. An initial flow-volume loop was performed and if suggestive of obstructive lung disease, the test was repeated 15 - 20 minutes following administration of a short-acting beta2-agonist by nebulization, to assess for reversibility**.** Potential participants for this study were re-contacted and then recruited to this prospective cohort between November 2008 and October 2010 with last follow up in July 2012. A total of 7319 person-years of follow up were completed, including time and data accrued when participants were being followed in their respective prior study[24].

For the purpose of analyses the following definitions were used: TB infection or TST immune conversion is defined as a positive tuberculin skin test (TST) result (≥5 mm) transverse diameter read approximately 72 hours after placement. TB disease is defined as ever being diagnosed with active TB – incident, or prevalent. Exposure to TB is defined as the presence of a household contact member with TB disease. Nadir CD4 T-cell count was defined as the lowest recorded CD4 T-cell count over the entire follow up period.

To identify HLA genotypes that appeared to confer particular risk for, or heightened protection against TB infection and disease we stratified patients into groups. First, patients were categorised by TST status into positive or negative, and then by whether they had ever had TB disease or remained TB disease free. All participants received childhood vaccination as per local vaccination protocols. At enrolment all participants' TST status was ascertained (unless previously documented to be positive). Skin test results were read by trained readers 2-3 days later using the ballpoint pen method, either by having the participant return to the clinic, or by a home visit to read the result. For participants with negative results, repeat testing were conducted annually thereafter. For those with initial negative TST a second confirmatory TST was performed and repeated annually. To better understand which HLA

alleles conferred susceptibility to or protection against TB infection and disease, respectively, patients in each group were further stratified into subgroups based on exposure to TB risk factors *(Figure 1).* For TB infection, enhanced risk of documenting TB infections was conferred by either CD4 T-cell count never dropping below 350 cells/mm^3 (to minimise the possibility of a false negative TST response in those with marked immunosuppression) or whether they had a TB household contact with TB disease (a clear demonstration of exposure to someone with TB disease). For TB disease, increased risk was conferred by a CD4 T-cell count (< 350 cells/mm^3), a prior positive TST or a self-report of a household contact with TB disease. Within subgroups, comparisons were made to determine if HLA alleles conferring susceptibility to or protection against the presence or absence of TB infection or active TB disease, respectively.

With an overall TST+ rate of approximately 77%, our sample is sufficient at 5% significance and 80% power to detect an increase in risk to about 92% among those carrying an allele at 8% frequency, or an increase to 89% for an allele carried at 15%. With an overall TB disease positive rate of approximately 11.6%, the corresponding increased risks are approximately 26% and 23%, respectively. While exact Fisher tests were used for individual comparisons to accommodate small subgroups, power to detect associations varies according to carriage frequency.

Informed consent was obtained from all individuals participating in this study and the study was approved by the University of Witwatersrand Human Resource Ethics Committee, clearance certificate number M1706112.

HLA genotyping methods:

High-resolution, full allelic Class I and II HLA typing was performed at a laboratory accredited by the American Society for Histocompatibility and Immunogenetics (ASHI) and the Australian National Association of Testing Authorities (NATA) (Mallal laboratory, Institute for Immunology and Infectious Diseases, Murdoch University, Perth, Australia). HLA class I *A, -B* and *-C* loci were typed by sequencing of exons 2 and 3, while for HLA class II *DRB1,* exon 2 was sequenced. Only exons 2 and 3 for HLA class I A, B and C and exon 2 for DRB1 were sequenced rather than the entire gene as these exons are the most polymorphic and involved in the formation of the peptide binding cleft. Amino acid changes in these coding regions may impact on immunity by variation in antigenic peptide binding. Nucleotide changes in other exons across the entire gene may indeed change the allele nomenclature but in many cases these lead to synonymous amino acid changes or the amino acid change is not involved in antigen binding. All next generation sequencing was performed on the Roche FLX 454 platform. Specific HLA loci on the extracted DNA were PCR amplified using sample specific Molecular indexed primers (MID- tagged) that amplify polymorphic exons from Class I (A, B, C Exons 2 and 3) and Class II (DQ, Exons 2 and 3; DRB and DPB1, Exon 1) HLA genes. Reads with quality of Q20 or below were discarded and a minimum of 10x coverage at each locus was necessary to assign an allele. MID tagged primers have been optimised to minimize allele dropouts and primer bias. Amplified DNA products from unique MID tagged products are pooled in equimolar ratios and subjected to library preparation. Post QC and quantitation the normalised libraries are then sequenced on the Roche FLX 454 platform as this was the only platform providing long enough read lengths at the time. Sequences were separated by MID tags and alleles called using an in-house accredited HLA allele caller software pipeline that minimises the influence of sequencing errors. Alleles are called using the latest IMGT HLA allele database as the allele reference library[25]. Sample to report integrity was tracked and checked using proprietary

and accredited Laboratory Information and Management System (LIMS) and HLA analyse reporting software that performs comprehensive allele balance and contamination checks on the final dataset[26]. The HLA typing data was generated using the G grouping HLA nomenclature system (http://hla.alleles.org/alleles/g_groups.html). In all but five instances, we were able to select the appropriate HLA allele present in the black South African population from the G group list based on published HLA class I and II alleles in this population[27]. For HLA-the B*45:01G group, both HLA-B*45:01 and -45:07 are present in the population, but cannot be discriminated by examination of exons 2 and 3 alone. These alleles were combined under the name HLA-B*45:01/07 in the analysis. Similarly, HLA-C*07:01, -C*07:06 and C*07:18 are all found in our population and fall under HLA-C*07:01G group, as is HLA-C*18:01 and -C*18:02 falling under the HLA-C*18:01G group. Moreover, HLA-A*23:01 and -A*23:17 too are found in our population and fall into the -A*23:01G group. Lastly, HLA-DRB1*14:01 and DRB1*14:54 are both present in the population and fall into the DRB1*14:01G group. All HLA allele names were reported at 4-digit resolution, and where ambiguity was still present the additional alleles are listed together. HLA–B -21M and - 21T dimorphisms amongst the HLA-B groups were also analysed and HLA-C1/C2.

Associations with HLA alleles:

To ascertain associations of HLA allele carriage with TST or TB disease status, we firstly carried out Fisher's exact tests for each allele separately without adjustment for explanatory variables. Those alleles with P-values less than 0.1 were then entered jointly into separate logistic regression models with either TST or TB disease status as outcome, adjusting for gender, age, ART status at baseline, and ART over the study period, baseline viral load and nadir CD4 T-cell count over the study period. Those alleles remaining jointly and

independently significant at P < 0.05 after the adjustment based on backward elimination were retained.

RESULTS

There were 754 HIV-infected adults of black African ethnicity in follow up, of whom 27 without HLA data were excluded. Of those without HLA results, 23 (85.2%) were TST-positive and 4 (14.8%) had TB disease. This analysis is based only on those 727 participants with HLA data. The mean age was 36.8 years and 619 (85.1%) were female. A total of 547 (77.2%) were TST-positive and 84 (11.6%) ever reported TB disease during follow up; 279/727 (38.4%) self-reported a household contact with TB. The mean baseline CD4 T-cell count was 421 cells/mm^3, 221 (30.4%) were on ART at baseline and the mean HIV log RNA viral load was Log 3.2 copies/ml (*Table 1*).

TB INFECTION (TST STATUS)

Susceptible to TST immune conversion (TST-positive)

HLA alleles associated with TST positivity in the whole cohort by univariate analyses suggest that *HLA-A*29:11* (p=0.030) and - *B*45:01/07* (p=0.015) were associated with having a positive TST. Notably, *HLA-B*45:07* is uncommon in the black South African population, while *HLA-B*45:01* is the predominant allele.[27] Those with *HLA-A*24:02* (p=0.014), *-A*29:02* (p=0.043) and *-B*15:16* (p=0.042) were more likely to have a negative TST. In multivariable analysis *HLA-B*45:01/07* (p=0.029, adj. OR 2.7) remained associated with positive TST testing, while *HLA-A*24:02* (p=0.021, adj. OR 0.22) and *-B*15:16* (p=0.0098, adj. OR 0.22) were associated with testing negative. We repeated this overall

analysis to assess the HLA alleles associated with TST positivity restricted to those with a household contact of TB and whose nadir CD4 T-cell count $\geq$ 350 cells/mm^3. In the univariate analyses only *HLA B*57:03* (p=0.023) was significantly associated with being TST-negative. In the joint analyses *HLA-B*57:03* (p=0.006, adj. OR 0.10) and *-A*66:01* (p=0.013, adj. OR 0.12) were associated with a negative TST. *HLA-A*66:02* was also protective to a similar extent but was not significant due to the small numbers, however, when combined with *HLA-A*66:01*, the *-A*66* allele group appeared to be protective (p=0.008, adj. OR 0.13) against being TST-positive (*Table 2; Figure 2).*

*A*29:02* (p=0.043) and *-B*15:16* (p=0.042) were more likely to have a negative TST. In multivariable analysis *HLA-B*45:01/07* (p=0.029, adj. OR 2.7) remained associated with positive TST testing, while *HLA-A*24:02* (p=0.021, adj. OR 0.22) and *-B*15:16* (p=0.0098, adj. OR 0.22) were associated with testing negative. We repeated this overall analysis to assess

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allele group appeared to be protective (p=0.008, adj. OR 0.13) against being TST-positive (*Table 2; Figure 2).*

When considered separately there was no significant association of HLA-B -21 M/T combinations (p=0.87) or HLA-C1/C2 (p=0.85) with TST-positivity. Jointly there was no significant association (p=0.83) and when included in the joint analysis for TST-positive along with the HLA alleles, demographics and HIV variables there was no significant effect of HLA-B -21 M/T and HLA-C1/C2 (p=0.80), nor did their inclusion abrogate the significance of the HLA alleles.

Apparent protection against TST immune conversion (TST-negative)

HLA alleles associated with TST negativity among those with a household contact with TB and also remaining disease-free were evaluated. Among those who remained disease free despite a household contact with TB disease (n=236 with TST result), 45 were TST-negative and 191 TST-positive. In unadjusted analyses, *HLA-A*66:01* (p=0.007), *-A*68:02* (p=0.007) and *-B*49:01* (p=0.034) were significantly associated with TST negativity, though there were only 2 carriers of *HLA-B*49:01*. HLA alleles associated with a negative TST among those with a family history of TB and remaining disease-free with nadir CD4 T-cell count $\geq$ 350 cells/mm^3 were then analysed. In the joint analyses with adjustment, *HLA-A*66:01* (p=0.005, adj. OR 15.9), *-A*68:02* (p=0.03, adj. OR 5.8) and *-B*57:03* (p=0.026, adj. OR 8.2) were independently associated with a negative TST (*Table 2*)(*Figure 2).*

TB DISEASE

Susceptible to TB disease

HLA alleles which were associated with TB disease in the whole cohort individually included: *HLA-B*41:01* (p=0.005), *-C*06:02* (p=0.042), *-DRB1*04:01* (p=0.003) and *-DRB1*15:01* (p=0.029) that were associated with TB disease, while *HLA-B*07:02* (p=0.033)

and -*DRB1*11:01* (p=0.043) were associated with apparent protection against TB disease. In the joint analysis, *HLA-B*41:01* (p=0.008, adj. OR 7.2), -*C*06:02* (p=0.034, adj. OR 1.8), -*C*05:01* (p=0.025, adj. OR 5.9) and -*DRB1*04:01* (p=0.009, adj. OR 2.9) were independently associated with TB disease.

We repeated this analysis to assess HLA alleles associated with TB disease but restricted it to those individuals who were initially TST-positive and then subsequently developed TB disease. When restricted, *HLA-B*41:01* (p=0.05), -*B*42:02* (p=0.026), -*DRB1*04:01* (p=0.045) and -*DRB1*15:01* (p=0.013) were associated with TB disease, while *HLA-A*01:01* (p=0.049), -*A*34:02* (p=0.049) and -*DRB1*11:01* (p=0.028) were associated with protection. In the joint analysis with adjustment, *HLA-C*06:02* (p=0.048, adj. OR 1.9), -*C*05:01* (p=0.018, adj. OR 6.7), -*DRB1*04:01* (p=0.036, adj. OR 2.8) and -*DRB1*15:01* (p=0.048, adj. OR 5.5) were independently associated with TB disease (*Table 3; Figure 2).*

Overall there was no association of HLA-B -21 M/T with TB disease (p=0.23), while HLA-C2/C2 was associated with a higher rate of TB disease (p=0.016, 42/273 vs 42/454). However, in the joint model with HLA alleles the inclusion of HLA-B -21M/T and HLA-C1/C2 was not significant (p=0.24) and slightly abrogated the significance only of *HLA - C*06:02*. Both *HLA - C*06:02* and -*C*05:01* are in the model associated with a higher rate of TB disease, and both are HLA-C2 ligands.

Apparently resistant to TB disease

For HLA alleles associated with apparent protection from TB disease in those whose HIV had progressed to a nadir CD4 T-cell count < 350 cells/mm^3, only *HLA-C*07:02* (p=0.045) and -*DRB1*11:01* (p=0.012) were associated with not progressing to TB disease, whereas

*HLA-B*41:01, -C*05:01, -C*06:02, -DRB1*04:01* and *-DRB1*15:01* were associated with diagnosis of TB disease (*Table 3*). Whilst, HLA alleles associated with not progressing to TB disease, among those who were both TST-positive and whose nadir CD4 T-cell count was below 350 cells/mm^3 in individual analysis were: *HLA-A*01:01* (p=0.021) and *-DRB1*11:01* (p=0.002). Whereas *HLA-B*42:02* (p=0.034) and *-DRB1*15:01* (p=0.01) were associated with TB disease in this sub-group (*Table 3*) (*Figure 2)*. Furthermore, of the HLA alleles associated with no TB disease among those with nadir CD4 T-cell count < 350 cells/mm^3 and a household contact following the individual analyses, only *HLA-C*06:02* (p=0.042) was associated with TB disease. In the joint analysis *HLA-A*30:01* (p=0.004, OR 0.19) and *-C*06:02* (p=0.006, AOR 0.23) were independently associated with TB disease (*Table 3; Figure 2).*

HLA-B -21 M/T was not significantly associated with no TB disease among those with nadir CD4 <350 cells/ul and with household contact (p=0.65). Again here carriage of HLA-C2/C2 was associated with a higher level of TB disease (p=0.017, 16/73 vs 10/112), but once more this was not significant in the joint analysis (p=0.63) while not abrogating the significance of the two HLA alleles included (*HLA - C*06:02* and *-A*30:01*).

DISCUSSION

In this study of South African HIV-infected adults, we report HLA alleles associated with occurrence and protection against TST immune conversion and active TB disease. When patients were stratified by the presence of risk factors for TB infection and disease (a known household member with TB, CD4 T-cell count and additionally in those with active TB the presence of previous TST immune conversion) we show HLA alleles that confer an increased risk of TST immune conversion and TB disease, whilst others specific HLA alleles were

associated with the absence of TST immune conversion or active TB disease, suggesting a protective effect of these HLA genotypes. These HLA genotypes could be utilised as correlates of protection against the development of TST immune conversion or active TB disease amongst HIV positive patients.

Epidemiological data suggests that host genetics play an important role in both the predisposition to and resistance against TB infection[15]. Moreover, twin studies have demonstrated an increased risk of TB disease in monozygotic (66.7%) compared to dizygotic twins (23%), further supporting the relationship between the risk of TB acquisition and host genetics[15,28]. Natural selection of TB resistant genes may play a role in the TB susceptibility. Furthermore, communities with genetic ancestry without TB exposure were at a greater risk of acquiring TB disease than those whose ancestors originated from high TB exposure regions[15].

Other studies also report HLA associations with increased TB susceptibility or protection. Amongst Asian populations a meta-analysis has shown *HLA-DRB1*04, -*09, -*10, -*15 -*16* and *HLA-DQB1*06:01* are associated with increased TB risk[21,29]. A Brazilian study similarly showed *HLA-DRB1*04:07, -*11:01* and -*04:92* were associated with TB susceptibility[30]. In our study we too show an increased susceptibility to TB with *HLA-DRB1*04:01* expression (p = 0.003). Protective alleles that have been identified in other studies include, HLA class I alleles: *HLA-A*26, -A*31,-B*18, -B*14, -B*27* and HLA class II alleles: *HLA-DRB1*03, - DRB1*07, -DRB1*11* and -*DRB1*13*[21,31,32]. We similarly showed that *HLA-DRB1*11* to confers protection against TB disease (p = 0.012). A Ugandan study identified *HLA- DQB1*03:03* as conferring protection against TB[33], however, we did not conduct *HLA-DQB1* genotyping in this study. A West African study identified HLA class

I alleles significantly associated with active TB, which included: *HLA-B*07:02, -B*08:01, -B*14:02, -B*15:03, -B*15:10, -B*18:01, -B*41:01, -B*42:01, -B*42:02, -B*51:01* and -*B*81:01*[34]. Of these, we note the *HLA-B*07:02, -B*41:01* and -*B*42:02* conferred an increased susceptibility to TB in our study population. The variable results reported amongst studies are likely due to differences in ethnicity, small sample sizes and heterogeneity in the populations studied[21].

South African data reporting HLA associations with active TB disease is limited and no data has previously reported on HLA associations of TST immune conversion or the occurrence of active TB in HIV-infected individuals. Early work, by other groups has shown an association between TB susceptibility and *HLA-DRB1*13:02* amongst the Venda population[22], whilst a marginal association of *HLA-DRB1*03* has been shown in people of mixed ancestry in South Africa[22]. More recently, Salie *et al*, have shown associations between HLA class I alleles and susceptibility to specific *Mtb* strains (Beijing, LAM, LCC and Quebec) amongst the mixed ancestry population in Cape Town[35]. Additional HLA class I alleles that are associated with TB susceptibility include *HLA-A*01, -A*02, -B*08, -B*17,-B*27* and -*B*35* . HLA alleles associated with either TST immune conversion or active TB disease in our cohort are not similar to those previously identified[14,22,35]. The possible reason for this is the population we have studied originates from a different geographical area, they are predominantly black Africans but of non-Venda origin compared to previous studies and, moreover, the additional presence of HIV co-infection in our population may influence the differences in susceptibility to and protection against TB that we note. In African populations, specific HLA alleles are associated with increased or reduced susceptibility to HIV acquisition. *HLA–A*68:02* and -*C*07:02* have been shown to increase susceptibility to, whilst *HLA-A*01:01* and -*C*06:02,* confer protection against HIV infection[36–38]. A

Zimbabwean study identified *HLA-A*36:01* as a risk allele for TB disease amongst HIV – infected individuals whilst *HLA-A*68:02* was protective, an observation we too note in our study. When examining HLA alleles that confer susceptibility to or protection against TB in HIV – infected patients, a Brazilian study showed *HLA-A*31* and *-B*41* was associated with HIV and TB co – infection[39]. Furthermore, homozygosity for either *HLA-DRB1* or *HLA-DPB1* was also protective against TB disease[40].

We note a predominance of HLA class I alleles associated with both susceptibility and protection against TST immune conversion and active TB disease. The host immune response to *Mtb* infection necessitates HLA antigen presentation to both CD4+ and CD8+ T-cells via HLA class II and I molecules, respectively[41,42]. Although the immune response of HLA class I restricted CD8+ T-cells to *Mtb* infection is less well understood, it has been shown that CD4+ and CD8+ T-cells work synergistically against *Mtb* and distinct pathways are used to activate CD8+ T-cells following *Mtb* infection[42]. For example, cytotoxic CD8+ T-cell mediated immunity occurs through secretion of granules containing cytotoxic molecules including perforin, granzymes and granulysin, apoptosis of infected target cells though Fas or TNF-R family-related cell death receptors and interferon gamma, tumour necrosis factor-alpha and IL-2 cytokine release[35,42–44]. Furthermore, there is increased interest in the qualitative and quantitative CD8+ T-cell response to *Mtb* infection, and lower percentages of circulating CD8+ T-cells and lower CD8+ T-cell IL-2/interferon-gamma concentrations in patients with TST immune conversion and a higher TB bacillary load have been reported[42,45]. As such, associations with HLA class I alleles may contribute to understanding and predicting susceptibility to and protection from TST immune conversion and active TB determined by the CD8+ T-cell immune response to *Mtb* infection.

The HLA-B -21M dimorphism (M/M genotype), with resultant NK cell education via the NKG2A pathway, is associated with increased susceptibility to HIV infection due to reduced NK cell mediated lysis of HIV-infected CD4 T-cells and macrophages in contrast to cells educated via the KIR pathway which associates with a -21T HLA-B genotype[46]. However, a recent study examining cytomegalovirus (CMV) and HIV co- infection in those of African ethnicity showed no difference in NK cell function regardless of variations in expression of HLA B -21M or -21T dimorphisms. This was postulated to be due to genetic variation in Africans that uniquely alter the supply of NKG2A and KIR ligands[17]. Despite variation in HLA - B 21M and – 21T dimorphisms conveying susceptibility to HIV infection and in line with the lack of effect of HLA – B -21M and – 21T dimorphisms in Africans with HIV and CMV co-infection we too find no association in increased susceptibility to or protection against TB infection conveyed by HLA – B 21M or -21T dimorphisms in this exclusively black African cohort. HLA – C2 expression was notably more common in black African individuals in a South African cohort[47]. We note a higher rate of HLA – C2/C2 expression amongst those with TB disease not described in prior studies, however, this is not significant when adjusted jointly for the HLA alleles. In light of the increased expression of HLA – C2 amongst black Africans this finding merits further study to determine if expression of particular KIRs may alter underlying NK cell function and ultimately influence increased TB susceptibility in black Africans.

We have identified several HLA alleles conferring risk for TST immune conversion and active TB disease. Our cohort is of particular high risk for TB infection due to HIV co-infection. With a greater interest in biomarkers to define correlates of TB infection, risk and disease[48], these HLA associations may prove valuable in identifying individuals at high risk for TB infection and potentially change clinical practice in ensuring earlier or more

intensive screening and follow up for TST immune conversion or active TB disease amongst those patients expressing high risk HLA alleles. Furthermore, as progress in the field of vaccination against TB infection continues the utility of the HLA alleles conferring protection against both TST immune conversion and active TB disease may be of value as correlates of protection. Of note, current vaccine development has focused around HLA class II-restricted CD4+ and HLA class I-restricted CD8+ T-cell responses, however there is growing interest in the role of non-classical HLA class Ib molecules as potential targets for TB vaccination due to their less polymorphic nature[41]. The protective HLA – class I alleles we identified have the potential to allow identification and study of individuals were CD8+ T-cell responses may confer increased resistance to TB infection and further contribute to vaccine development, particularly amongst the HIV positive population with TB co-infection.

Limitations of this study include the lack of IGRA data to define LTBI, false negative results of TST in patients with advanced HIV; and the absence of a HIV-negative control group to delineate the role of HIV co-infection in HLA associations with a TB infection. Furthermore, HLA alleles at the *DQB1* and *DPB1* locus were not evaluated in this study. There is no knowledge of the infecting TB strains in this study, which could have implications on the HLA alleles conferring susceptibility to and protection against TB infection and disease. Moreover, granular detail on adherence to antiretroviral or preventive treatment was not included in our statistical models. Finally, no replication cohort was evaluated in this study, as such our data requires validation in a future prospective cohort.

CONCLUSION

In this study of black South African HIV-infected patients with LTBI and active TB disease, we report several HLA alleles associated with TB infection, TB disease and apparent

resistant to infection and disease. In an era of precision medicine these HLA associations may be of significant value in elucidating factors that may be identified prior to LTBI or the development of active TB disease. It may be possible to risk stratify individuals with HIV infection, whilst protective factors may inform future TB vaccine development.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Figure Legends

Figure 1: Schema of the study cohort stratified by TST and active TB disease status with further subgroups in each category highlighting exposure to risk factors for TB. Subgroups were compared by a single or combination of risk factors. TST: tuberculin skin test.

Figure 2: Summary of significant ($P < 0.05$) susceptible and protective HLA class I (*A, B, C*) and II alleles (*DRB1*) for TB infection and active TB disease stratified by risk factors for TB. TST: tuberculin skin test.

Table Legends

Table 1: Patient demographics

Table 2: *HLA* alleles associated with TST status

Table 3: *HLA* alleles associated with TB disease

Author contributions:

Faheem Seedat - Conceptualization; Data curation; Investigation; Methodology; Project administration; Roles/Writing - original draft, **Ian James** - Data curation; Formal analysis; Software, **Shayne Loubser** - Formal analysis; Methodology; Supervision; Validation; Writing - review & editing, **Ziyaad Waja** - Data curation; Investigation; Methodology; Project administration; Writing - review & editing, **Simon Mallal** - Formal analysis; Investigation; Software; Supervision; Validation; Writing - review & editing, **Christopher Hoffmann** - Data curation; Funding acquisition; Investigation; Methodology, **Caroline T. Tiemessen** - Formal analysis; Methodology; Supervision; Validation; Writing - review & editing, **Richard E Chaisson** - Data curation; Funding acquisition; Supervision and **Neil A Martinson** - Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Resources; Supervision; Writing - review & editing

Table 1: Patient demographics

	Overall	Family history of TB	No family history of TB	*P*-value
N	727	279	448	
Gender				
N (%) Females	619 (85.1%)	255 (91.4%)	364 (81.3%)	**0.0002**
TST+				
N (%)	547[a] (77.2%)	221[b] (81.0%)	326[c] (74.8%)	0.066
Active TB Disease				
N (%)	84 (11.6%)	38 (13.6%)	46 (10.3%)	0.19
Incident TB				
N (%)	36 (5.0%)	14 (5.0%)	22 (4.9%)	1
Baseline ART				
N (%)	221 (30.4%)	82 (29.4%)	139 (31.0%)	0.68
Change of ART				
N (%)	219 (30.1%)	86 (30.8%)	133 (29.7%)	0.8
Age (years)				
Mean (SD)	36.8 (7.1)	36.6 (6.8)	36.9 (7.3)	0.5
BMI (kg/m^2)				
Mean (SD)	27.9 (6.5)	27.9 (6.2)	27.9 (6.7)	0.96
Log VL (RNA copies/ml)				
Mean (SD)	3.2 (1.1)	3.3 (1.1)	3.2 (1.1)	0.62
Baseline CD4 ($cells/mm^3$)				
Mean (SD)	421 (222)	430 (217)	415 (226)	0.39
Nadir CD4 ($cells/mm^3$)				
Mean (SD)	324 (180)	325 (180)	324 (180)	0.94

[a] Count out of 709 with TST status known; [b]Count out of 273 with TST status known; [c]Count out of 436 with TST status known. TST: tuberculin skin test. BMI: body mass index. VL: viral load.
Significant *P* values (<0.05) are indicated in bold

Table 2: *HLA* alleles associated with TST status

A. Susceptible to TST immunoconversion

***HLA* alleles associated with TST positivity in the whole cohort** (n=709: 546 TST+, 163 TST-)				
***HLA* allele**	**TST+ with allele**	**TST+ without allele**	***P*-unadj.**	***P*-adj.**
*A*24:02*	6/13 (46.1%)	540/694 (77.8%)	**0.014**	**0.021**
*A*29:02*	61/89 (68.5%)	485/618 (78.5%)	**0.043**	-
*A*29:11*	16/16 (100%)	530/691 (76.7%)	**0.030**	-
*B*15:16*	10/18 (55.6%)	535/688 (77.8%)	**0.042**	**0.0098**
*B*45:01/07*	53/59 (89.8%)	492/647 (76.0%)	**0.015**	**0.029**
*B*13:02*	25/27 (92.6%)	520/679 (76.6%)	0.060	-
*C*03:02*	26/28 (92.9%)	520/679 (76.6%)	0.062	-

***HLA* alleles associated with TST positivity among those with a family history of TB and with nadir CD4 count ≥ 350 cells/mm^3** (n=91: 73 TST+, 18 TST-)				
HLA allele	**TST+ with allele**	**TST+ without allele**	***P*-unadj.**	***P*-adj.**
*B*57:03*	5/10 (50.0%)	68/81 (84.0%)	**0.023**	**0.006**
*A*30:02*	14/14 (100%)	59/77 (76.6%)	0.064	-
*A*66:01*	5/9 (55.6%)	68/82 (82.9%)	0.072	**0.013**
*A*66:02*	1/3 (33.3%)	72/88(81.8%)	0.099	-

B. Apparently resistant to TB immunoconversion

***HLA* alleles associated with TST negativity among those with a family history of TB and remaining disease-free** (n = 236: 191 TST+, 45 TST -)				
***HLA* allele**	**TST- with allele**	**TST- without allele**	***P*-unadj.**	***P*-adj.**
*A*66:01*	9/21 (42.9%)	35/214 (16.4%)	**0.007**	**0.0013**
*A*68:02*	14/40 (35.0%)	30/195 (15.4%)	**0.007**	**0.0007**
*B*49:01*	2/2 (100%)	42/233 (18.0%)	**0.034**	-

***HLA* alleles associated with negative TST among those with a family history of TB and remaining disease-free with nadir CD4 count ≥ 350 cells/mm^3** (n=80: 64 TST+, 16 TST-)				
***HLA* allele**	**TST- with allele**	**TST- without allele**	***P*-unadj.**	***P*-adj.**
*A*66:01*	4/8 (50.0%)	12/72 (16.4%)	**0.047**	**0.005**
*B*57:03*	4/9 (44.4%)	12/71 (16.9%)	0.073	**0.026**

Significant *P* values (<0.05) are indicated in bold
For P-adj – only significant values are reported
HLA alleles were still analysed if just a single locus was absent, hence denominators may not add up to totals.

Table 3: *HLA* alleles associated with TB disease

A. Susceptible to TB disease

***HLA* alleles associated with TB disease in the whole cohort** (n=727: 643 Disease -, 84 Disease +)				
***HLA* allele**	**Disease + with allele**	**Disease + without allele**	***P*-unadj.**	***P*-adj.**
*B*07:02*	1/47 (2.1%)	82/677 (12.1%)	**0.033**	-
*B*41:01*	5/11 (45.5%)	78/713 (10.9%)	**0.005**	**0.008**
*C*06:02*	28/175 (16.0%)	56/550 (10.2%)	**0.042**	**0.034**
*DRB1*04:01*	12/45 (26.7%)	72/680 (10.6%)	**0.003**	**0.009**
*DRB1*11:01*	13/177 (7.3%)	71/548 (13.0%)	**0.043**	-
*DRB1*15:01*	4/11 (36.4%)	80/714 (11.2%)	**0.029**	-

***HLA* alleles associated with TB disease in those with a positive TST** (n=547: 484 Disease -, 63 Disease +)				
***HLA* allele**	**Disease + with allele**	**Disease + without allele**	***P*-unadj.**	***P*-adj.**
*A*01:01*	1/46 (2.2%)	62/500 (12.4%)	**0.049**	-
*A*34:02*	1/46 (2.2%)	62/500 (12.4%)	**0.049**	-
*B*41:01*	3/8 (37.5%)	59/537 (11.0%)	0.052	-
*B*42:02*	5/16 (31.3%)	57/529 (10.8%)	**0.026**	-
*DRB1*04:01*	8/34 (23.5%)	55/512 (10.7%)	**0.045**	**0.036**
*DRB1*11:01*	8/132 (6.1%)	55/414 (13.3%)	**0.028**	-
*DRB1*15:01*	4/9 (44.4%)	59/537 (11.0%)	**0.013**	**0.048**

***HLA* alleles associated with TB Disease restricted to those participants with household contact** (n=279: 241 Disease -, 38 Disease +)				
***HLA* allele**	**Disease + with allele**	**Disease + without allele**	***P*-unadj.**	***P*-adj.**
*C*04:01*	4/70 (5.7%)	34/208 (16.3%)	**0.026**	-
*C*06:02*	15/66 (22.7%)	23/212 (10.8%)	**0.023**	**0.002**
*C*17:01*	15/75 (20.0%)	23/203 (11.3%)	0.076	**0.012**
*DRB1*15:01*	2/4 (50.0%)	36/274 (13.1%)	0.091	-

B. Apparently resistant to TB Disease

***HLA* alleles associated with no TB Disease restricted to those participants with nadir CD4 count < 350 cells/mm^3** (n=477: 416 Disease -, 61 Disease +)				
***HLA* allele**	**Disease - with allele**	**Disease - without allele**	***P*-unadj.**	***P*-adj.**
*B*41:01*	4/7 (57.1%)	410/467 (87.8%)	**0.047**	**0.04**
*C*05:01*	5/8 (62.5%)	409/467 (87.6%)	0.070	**0.01**
*C*06:02*	92/113 (81.4%)	322/362 (88.9%)	0.052	**0.01**
*C*07:02*	38/39 (97.4%)	376/436 (86.2%)	**0.045**	-
*DRB1*04:01*	21/30 (70.0%)	393/445 (88.3%)	**0.008**	**0.009**
*DRB1*11:01*	116/124 (93.5%)	298/351 (84.9%)	**0.012**	-
*DRB1*15:01*	5/9 (55.6%)	409/466 (87.8%)	**0.019**	**0.04**

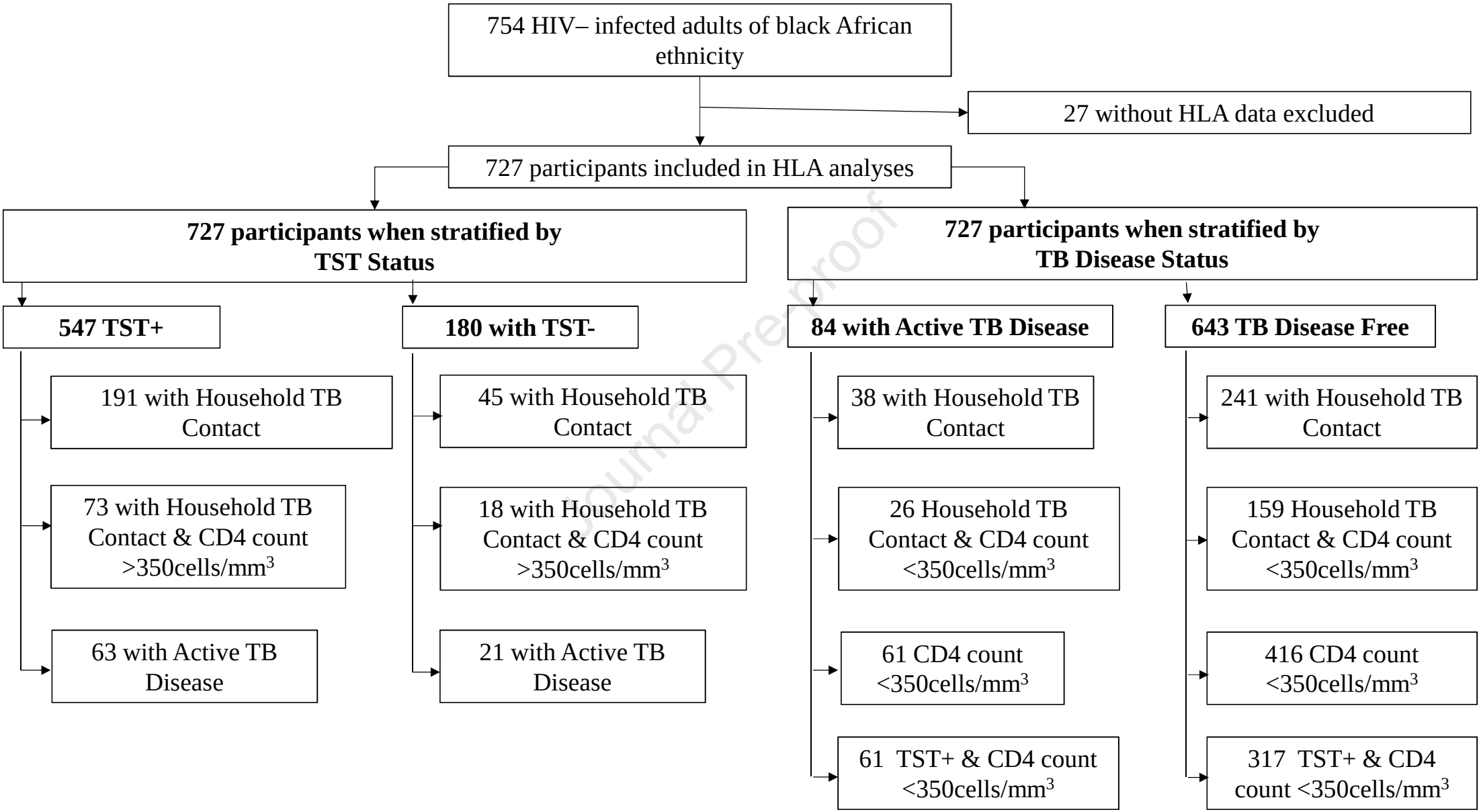
HLA alleles associated with no TB Disease among those who were TST positive and whose nadir CD4 count < 350 cells/mm^3
(n=362: 317 Disease -, 45 Disease +)

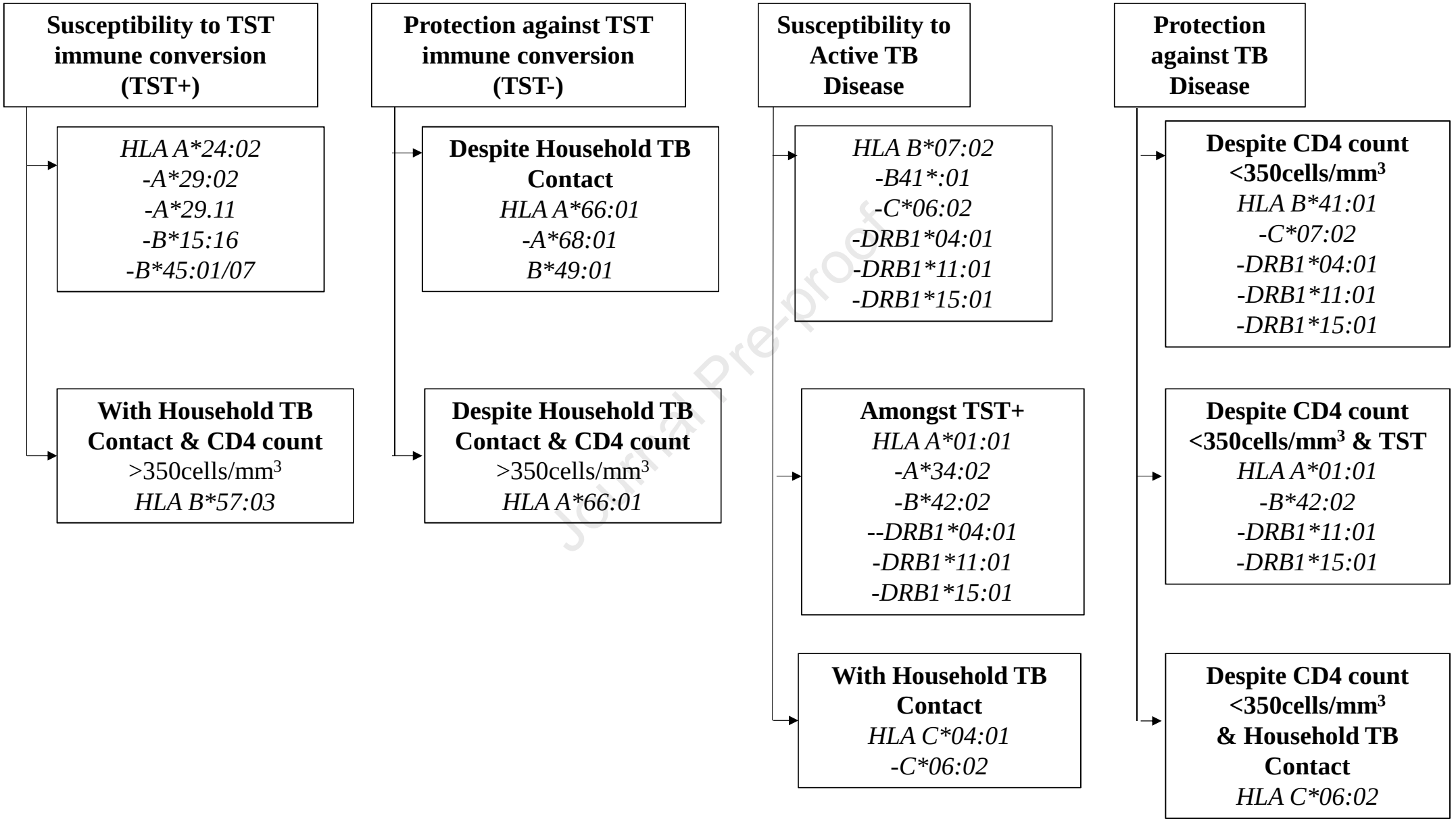
HLA allele	Disease - with allele	Disease - without allele	_P_-unadj.	_P_-adj.
*A*01:01*	31/31 (100%)	285/330 (86.4%)	**0.021**	-
*B*42:02*	11/16 (68.7%)	305/344 (88.7%)	**0.034**	-
*B*57:02*	5/8 (62.5%)	311/352 (88.3%)	0.062	-
*C*05:01*	5/8 (62.5%)	311/353 (88.1%)	0.065	**0.012**
*C*08:04*	25/25 (100%)	291/336 (86.6%)	0.056	-
*DRB1*11:01*	89/92 (96.7%)	227/269 (84.4%)	**0.002**	**0.01**
*DRB1*15:01*	4/8 (50%)	312/353 (88.4%)	**0.010**	**0.04**

HLA alleles associated with no TB Disease among those with nadir CD4 count <350 cells/mm^3 and a household contact
(n=185: 159 Disease -, 26 Disease +)

HLA allele	Disease - with allele	Disease - without allele	_P_-unadj.	_P_-adj.
*A*30:01*	26/35 (74.3%)	132/149 (88.6%)	0.055	**0.003**
*C*06:02*	31/41 (75.6%)	127/143 (88.8%)	**0.042**	**0.006**
*C*17:01*	38/49 (77.5%)	120/135 (88.9%)	0.059	-
*DRB1*15:01*	1/3 (33.3%)	157/181 (86.7%)	0.053	-

Significant *P* values (<0.05) are indicated in bold
For P-adj – only significant values are reported
HLA alleles were still analysed if just a single locus was absent, hence denominators may not add up to totals.





Highlights

- HLA allelic variation affects susceptibility to and protection against TB infection
- Data describing HLA associations with TB in HIV-positive individuals is limited
- Some HLA alleles are associated with protection against TB co-infection in HIV
- Knowledge of protective HLA alleles may assist with future vaccine development