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## HLA Class I polymorphisms influencing both, peptide binding and KIR interactions are associated with remission among children with atopic dermatitis: A longitudinal study.

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### Abstract

Atopic dermatitis (AD) is a disease of immune dysregulation and skin barrier dysfunction with a relapsing, remitting course and has been associated with several different genetic risk variants. Human leukocyte antigens (HLA) represent a highly variable set of genes that code for cell surface protein molecules involved in the antigen-specific immune response, including the regulation or functioning of T-cells, natural killer (NK) cells, and antigen presenting cells. The purpose of this study was to evaluate associations between HLA Class I polymorphisms and the progression of AD over time. We evaluated the associations of AD symptoms and HLA Class I polymorphisms

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**Potential Conflicts of Interest:** David Margolis is or recently has been a consultant for Pfizer, Leo, and Sanofi with respect to studies of atopic dermatitis and serves on an advisory board for the National Eczema Association. Dimitri S. Monos is Chair of the Scientific Advisory Board of Omixon and owns options in Omixon. Dimitri Monos, Deborah Ferriola, and Jamie Duke receive royalties from Omixon.

based on high resolution two-field typing in a longitudinal cohort of children with AD (up to 10-years of follow-up). Seven hundred ninety-two children were evaluated every 6 months resulting in 12,752 AD evaluations. Using generalized estimating equations, B\*44:02 was found to be associated with AD remission (1.83 [1.35, 2.47];  $p_{\text{corr}}=0.0015$ ). The HLA-B residues at position 116 (D-aspartate) and 80 (T-threonine) were associated with remission (1.42 [1.13, 1.76]  $p=0.003$ ,  $p_{\text{corr}}=0.028$ ) and (1.45 [1.17, 1.80]  $p=0.0008$ ,  $p_{\text{corr}}=0.0024$ ) respectively. B80T is a killer-cell immunoglobulin-like receptor (KIR) site. Our findings reveal that two axes of immune response (T-cell and NK cell) may influence disease progression. Identifying binding pocket changes in addition to other factors (e.g. allergens) that increase the risk or severity of AD can improve our understanding of the immunologic mechanisms associated with AD and may lead to personalized therapies for improving patient care.

## Keywords

Human; Skin; Allergy; MHC; T Cells; Natural Killer Cells

## Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease that affects about 10% of the US population.(1, 2) At one time, it was thought to primarily affect children and resolve in late childhood, but recently several studies have shown that it affects individuals of all ages. (1, 3, 4) For those with childhood onset, AD can persist into adulthood.(3) Genetic variation has been associated with the risk of having AD as well as with its severity and persistence. (3, 5, 6) The most commonly identified genetic association is with filaggrin (*FLG*), a skin barrier protein.(5) However, AD is often thought of as an immune mediated illness and has been associated with genes controlling inflammatory processes such as IL-4 and IL-13.(7–9) A recently approved therapy for AD blocks IL-4 and IL-13 signaling.(10)

Human leukocyte antigens (HLA) represent a highly variable set of genes found on chromosome 6 that code for cell surface protein molecules involved in the antigen-specific immune response, including the regulation or functioning of T-cells, natural killer (NK) cells, and antigen presenting cells.(11) Previous studies, including genome-wide association studies (GWAS) and studies using immunophenotyping techniques, have found associations between HLA genes and the risk of AD, as well as other atopic illness such as asthma, seasonal allergies, and food allergies.(12–18) With respect to AD, GWAS of Europeans and Asians have identified a number of single nucleotide polymorphisms (SNPs) within the MHC region to be associated with AD including rs9368677 (between *HLA-B* and *HLA-C*) (14), rs2251396 (between *HLA-B* and *MICA*)(19), rs6474 (complement region)(19), rs9469099 (*TSBP1*)(14), rs4713555 and rs28383201 (between *DRB1* and *DQA1*)(13, 20) (21). However, the RSID numbers listed above, merely designate regions of potential relevance to the onset of AD but they do not identify actual HLA genes or alleles, that could be relevant to the onset of AD.

Independently, Natural Killer (NK) cell function has also been shown to be important in the pathogenesis of AD.(22–26) This observation was first noted in 1985.(25) A recent study by

Mack *et al* revealed that the number of circulating NK cells in adults with moderate to severe AD were significantly less than in a healthy control population.(22) NK cell function is dependent on the interaction of HLA class I molecules with killer-cell immunoglobulin-like receptor (KIR) molecules on NK cells. A prior study of a Polish population, demonstrated that *KIR2DS1* was associated with a decreased risk of AD.(23, 24) Studying HLA class I regions that interact with KIR may shed light on the innate immune system's role in the pathophysiology of AD as well as interactions with the adaptive immune system (through T cell engagement).

In this study we focused on HLA class I genes and their polymorphisms that influence critical functionalities of the HLA molecule, such as interactions with peptides or other receptors (T-cell receptor or KIR). Our rationale for focusing on HLAs is based upon past studies evaluating risk of developing AD, as summarized above. We leveraged the availability of up to 10 years of longitudinal data on 792 children with AD(5, 27) to assess whether polymorphisms of HLA class I molecules are associated with clinical course of AD over time. To our knowledge, no previous studies have ever explored the role of HLA class I variation in disease progression of AD.

## Methods:

Study subjects were enrolled in the *Pediatric Eczema Elective Registry* (PEER; [www.thepeerprogram.com](http://www.thepeerprogram.com)) which is a United States nationwide cohort of more than 8,000 subjects with pediatric-onset AD. All subjects had a diagnosis of AD confirmed by a physician. The current study represents a sub-cohort of PEER children, those who provided a genetic sample (PEER DNA cohort).(27, 28) In the PEER DNA cohort, both self-described race/ethnicity and ancestry informative markers were used to define ancestry and were previously found to be highly correlated.(27) PEER DNA enrollment occurred between November of 2004 and January 2015. At the time of enrollment, children were two to seventeen years old, had a physician-confirmed diagnosis of AD, and had used pimecrolimus cream for at least six months, but may have used many other therapies.(27) With respect to outcome measures (e.g., the persistence of AD), subjects are followed for up to 10 years and during that time were not required to (and most did not) continue therapy with pimecrolimus.(29) Follow-up is still ongoing.

All subjects who provided DNA or their legal guardians provided written informed consent approved by the University of Pennsylvania's Institutional Review Board.

## Outcome:

The clinical course of AD was determined periodically and defined by whether a child was in remission based on the self-reported outcome of whether or not a child's skin was AD symptom-free during the previous six-months while the child was not using medication to treat their AD. (27) AD disease activity was based on the survey question: "*During the last six months would you say that your child's skin disease (AD) has shown: complete disease control, good disease control, limited disease control, or uncontrolled disease*". Symptom free was defined as an affirmative response to "*complete disease control*". This response has

been shown to correlate with other tools used to evaluate symptom control and is likely a marker of long-term disease severity.(30–32) Outcome data collection for PEER is ongoing. For this study, the last date that the outcome was reported for a study subject occurred either at the 10-years of follow-up or up to their last response before March 2020, whichever date occurred first.

## HLA Genotyping

DNA was collected using Oragene DNA collection kits (DNA Genotek, Ottawa Canada) as previously reported.(27) The classic Class HLA I genes -A, -B and -C for individuals in the PEER DNA cohort were fully sequenced using one of two next generation sequencing (NGS) approaches. Three hundred fifty-seven subjects were genotyped using the Omixon HLA Holotype kit and 424 subjects were typed using a combination of probes generated by SureDesign and a custom design of HLA probes based on the complete collection of IMGT HLA sequences (version 3.09). Using these NGS methods we report high resolution two-field (HR-2F) typing that was used to determine amino acid variation in HLA Class I molecules.

## Analysis:

Analyses were based on HLA amino acid residue variation described by HLA allele HR-2F typing per gene. Our primary analysis plan focused on assessing the association between HLA amino acid residue variation based on the HR-2F HLA genotyping (assuming an additive genetic model) and the likelihood of remission at any given timepoint. We used a generalized estimating equations (GEE) approach for the binary outcome of remission using a logistic model and independence working correlation structure to account for the correlation among repeated measures per participant (outcomes were determined by survey every six months for up to 10 years). GEE belong to a class of semiparametric regression models that measure the average response over the population and are frequently used to evaluate longitudinal data that has repeated outcome measures. The odds ratio measured in this study reflects the odds that a subject with an exposure of interest (e.g., a specific HLA polymorphism) as compared to one who does not have that exposure will be in remission at any given time that the outcome was measured. The primary analysis was of the full cohort. A secondary analysis was conducted based on European or African ancestry in order to confirm the stability of the effect estimates. Odds ratios (OR) for association between variant of interest and AD clinical course (remission (OR >1.0) or persistence (OR = 1.0)) and 95% confidence intervals were estimated from these models.

HR-2F typing was conducted for all HLA-A, HLA-B, and HLA-C variants (results available in the supplement). We used the allelic genotyping to determine protein sequence (residue) variation (IMGT version 3.09). Allelic frequency for all the variants is presented in the supplement as well as the association with our outcome for those with an AF of  $\geq 0.05$ . For residue analysis and based on our HR-2F typing, we used the Simpson reciprocal index (SRI)(33) for each polymorphic residue of the binding cleft in our PEER cohort. The SRI is a metric reflecting the influence particular pocket residues exert in peptide binding.(34) We focused only on those pocket residues with the highest SRI index ( $\geq 3.0$ ), i.e. the residues

that exert the highest influence on peptide binding(33). As a result, positions 9, 97 and 114 of HLA-A, 67 and 116 of HLA-B and positions 156 of HLA-C were evaluated with respect to our outcome, remission or persistence to AD.

In addition, based on the potential role of NK cells in AD, known polymorphic HLA residues that constitute contact points with KIR receptors (and not associated with SRI) were explored. These contact residues are 80 and 83 of HLA-A and HLA-B (i.e., Bw4 KIR binding site), -21T and -21M of HLA-B (areas associated with NK education), and 80 of HLA-C (i.e., C1/C2 KIR binding site) and their associations with respect to AD remission were evaluated using the GEE models described above. (35–39)

Additional models were adjusted for the following baseline variables: sex, analysis of African ancestry race based on ancestral informative markers(27), age of onset of AD, and the presence of asthma and/or seasonal allergies. Missingness was previously evaluated and felt to be completely at random and consistent with GEE assumptions.(9) The Bonferroni procedure was used per gene for multiple testing correction based on the number of amino acid residue variants tested after screening. Hypothesis testing of KIR binding sites were adjusted based on the number of KIR analyses however the association between NK cell function and these specific sites were known *a priori*.

All statistical analyses were conducted using STATA Version 16.1 (College Station, TX)

## Results:

Seven hundred and ninety-two children from the PEER DNA cohort were HLA genotyped using NGS-based technologies. The primary outcome of AD remission/persistence was surveyed 12,752 times in this cohort and on average each child had nine evaluations. At the time of this study, PEER DNA children had been followed for an average of 102 months (sd: 48.36). The 792 children were of African-American ancestry (41.2%), European ancestry (55.4%), and of other ancestries or not reported (3.4%), and 52.6% of the children were female. The average age of onset of AD was reported as 1.92 (sd: 2.66) years with a median age of onset of 0.75 years (IQR: 0.25–2). Overall remission was reported in 16.3% of surveys and on average children reported 13.70 (sd: 23.15) months in remission.

In the PEER DNA cohort, 65 HLA-A, 115 HLA-B and 65 HLA-C unique alleles were identified (supplement Table 1). However, only 5 HLA-A, 4 HLA-B and 7 HLA-C had AF 0.05 and these alleles were included in the outcome analysis (see supplement Tables 1 and 2). Several alleles appear to be associated with AD remission or persistence but only HLA-B, B\*44:02 (Supplement Table 2) was found to be associated with remission after correction (1.83 (1.35, 2.47);  $p_{\text{corr}}=0.0015$ ).

After filtering HLA Class I isotypes by SRI  $\geq 3.0$ , we analyzed residues 9, 97 and 114 for HLA-A, residues 67 and 116 for HLA-B, and residues 156 for HLA-C (Table 1). The associations between amino acid residue variation and remission are presented in Table 2. Several residue sites are potentially associated with remission, however only a single residue of HLA-B at position 116 (D-aspartate), (1.42 (1.13, 1.76)  $p=0.003$ ,  $p_{\text{corr}}=0.028$ ) was significant after correction (Table 2). Residue 116 of HLA-B is part of pocket F of the HLA

Class I molecule. The association of the probability of remission by this variant by ancestry over time is shown in Figure 1. The ORs of association was similar in those of both European or African ancestry (Table 2).

Regarding residues that interact with KIR, residues A80 (G/I) and A83 (on HLA-A) were not associated with AD remission (Table 3). On HLA-B, residue B80T (threonine) was associated with remission (1.45 (1.17, 1.80)  $p=0.0008$ ,  $p_{\text{corr}}=0.0024$ ), while B80I (isoleucine) was not associated with remission (0.84 (0.65,1.08)  $p=0.1694$ ). The probability of remission by ancestry due to the presence of B80T over time is presented in Figure 2. The B80T residue is part of pocket F. Residue B83R (arginine) was not associated with remission. HLA-C position 80 with N (asparagine) or K (lysine) was not associated with remission (Table 3). The NK cell education sites of HLA-B, -21M (methionine) (1.12(0.92,1.30) and -21T (0.93(0.76,1.15) were also not associated with remission.

As noted, B80 and B116, are both part of pocket F. B80T and B116D are in linkage disequilibrium (LD) ( $R^2 = 0.71$ ), suggesting a possible functional role and that these two residues may have co-evolved. B116D was overall highly associated with Bw4 (B80T or B80I). When B116D did not occur with B80T, B116D occurred with B80I (but never occurred with B80N(Bw6)).

## Discussion:

This study is the largest to date assessing the variation of Class I HLA loci of children with AD that uniquely focuses on the *clinical course* of disease over time. Prior studies have shown that HLAs are associated with the *risk of having* AD and that there is a role for NK cell activity in the pathogenesis of AD.(12–14, 20, 25, 40) Unlike previous studies, we longitudinally evaluated the *clinical course* of AD over time. We identified two residues that are both found in HLA Class I binding pocket F. One of the HLA residues is at a location known to be an interaction site for KIR ligand (B80T/I), which is part of Bw4 epitope, and the other HLA molecular variant is at position B116, which is highly likely to be associated with variation in antigen presentation to T-cells. Therefore, the identified associations suggest a role for both the T and NK cells, implying an effect that is T-cell mediated through specific HLA-peptide/TCR interactions and also an NK effect that is HLA/KIR dependent. Based on our findings, we hypothesized that both adaptive and innate immune responses play roles in the clinical course of AD.

B116D and B80T are shared epitopes formed by several HLA-B alleles including B\*44:02 (the only allele in our cohort significantly associated with remission that also had an AF  $>0.05$ ). However, these polymorphisms are also associated with the less common alleles like B\*44:03, B\*44:27, B\*27:02, B\*27:05 and B\*15:17. By analyzing the amino acid polymorphisms involved in the HLA binding pockets (e.g., composites of many alleles), we were able to include all of the alleles (both rare and common) in our analyses and evaluate their biologically relevant functionality. Our analysis was consistent with the widely accepted notion that HLA disease associations can be defined much more sensitively at the HLA amino acid level than at the classical HLA allelic level.(33, 41)



The B116D and B80T polymorphisms are in LD, so it is not clear whether their functional effects are independent or coordinated. However, it is very likely that two axes of immune response (innate and adaptive) influence longitudinal disease activity (i.e., the clinical course) of AD. B116D can influence interactions with incoming side chains of peptides bound to the HLA binding pockets, but the same B116 residue is also known to influence the optimization of peptide editing/selection by Tapasin, a protein known to influence the presentation of selected peptides.(42) B116 variation mismatch in bone marrow transplantation has also been shown to be associated with transplant related morbidity like graft versus host disease.(43, 44) Overall, the residue B116D possibly influences more than one function contributing to the formation of the HLA-peptide complex, which may influence the odds of remission from AD symptoms.

There are approximately 15 KIR genes on chromosome 19 and they have important regulatory function for NK cells.(45, 46) Similarly to HLA, the KIR allelic variation results in receptor variation. KIR recognize HLA Class-I molecules and two KIR variants are known to interact with Bw4, *KIR3DL1*, which inhibits NK cell function, and *KIR3DS1*, which activates NK cell function.(46) *KIR3DL1* and *KIR3DS1* are allelic variants of the same gene called *KIR3DL1/S1*, however *3DL1* variant is most commonly expressed.(35) The association between the HLA-B Bw4 epitope and the KIR receptor variant occurs primarily at amino acid location 80. The Bw4 epitope is represented by HLA-B allelic variation at location 80 represented by amino acids threonine (T) or an isoleucine (I). Threonine is polar and therefore hydrophilic while isoleucine is non-polar and hydrophobic. As a result, biochemically, it is likely that receptor interactions with amino acid T and I differ. In fact, differential KIR interactions by residue 80 (i.e. I or T) have been previously reported.(40, 47) Furthermore, the crystal structure of the receptor/ligand interactions for *KIR3DL1*, which is centered on location 80, shows more enhanced binding between *KIR3DL1* and B80I.(45) This hypothesis of differential Bw4 activity based on the presence of T or I is also substantiated by our observed results. Final confirmation would require KIR genotyping, which was beyond the focus of this study. However, based on our data, future studies evaluating KIR genes and AD are warranted.

In prior studies, we demonstrated that *FLG* variation, which codes for a skin barrier protein, and *TSLP*, which codes for a protein initiator of inflammation at barrier surfaces are associated with remission.(7, 9, 27) We now show that HLA Class I proteins found on antigen presenting cells, T-cells, and NK cells are involved in the clinical course of AD thereby creating a supportable and coherent model of genetic variation associated with both immunologic and skin barrier aspects of AD. A simple explanation for the involvement of these genetic variants is that antigen enters through an impaired skin barrier (FLG), is presented by an antigen presenting cell (HLA Class II) resulting in the TSLP activation of T cells and the subsequent production of IgE by plasma cells or the direct interaction of NK cells and HLA Class I KIR ligand. With the inclusion of data from this study, HLA allelic variants may have now been shown to alter the clinical course of AD following this simple pathway. The effects we note in HLA Class I in part could help explain hypothesized “endotypes” of atopic dermatitis base on person-specific varying immunologic dysfunction. (48–51)

In fact, previous studies have shown that polygenic combinations of KIR and HLA Class I receptors are associated with disease outcomes.(45, 46) For example, the protective role of B\*53:01 in HIV-1 infection is primarily, because of the KIR binding properties at B80I that cause NK cell activation.(36, 47) However, the B\*53:01 HIV-1 protective effect is also related to TCR binding properties.(52) Our finding regarding B116D and B80T may be mechanistically similar with respect to AD disease remission in that HLA disease associations with AD should be evaluated through the perspective that HLAs influence not only antigen presentation to TCR but also NK activation.

As with all epidemiologic studies, this study has limitations. Although this study is the largest study of its kind, we are under-powered to evaluate less common residue variations and for this reason we *a priori* limited our analyses to residue variants most likely to affect the binding pocket based on SRI. Restricting our analyses to more common variants may limit a fuller understanding of the underlying mechanisms. However, our initial evaluation of allelic variants with frequencies  $> 0.05$  is arguably clinically meaningful for a common disease that has a prevalence of about 10%. Furthermore, we ultimately evaluated the protein residue altered by the alleles thereby allowing for the inclusion of both common and rarer HLA allelic variants. The PEER DNA study is a subset of the PEER study, a national study that broadly sampled the population of the US. As purposeful sampling techniques were not used for the PEER study and the PEER DNA cohort was composed of volunteers from the original PEER, the sample includes children from across the US and is likely generalizable.

In summary, our findings demonstrate that HLA variation is associated with likelihood of remission of AD over time. Our findings add to the general body of evidence about the genetics of AD and, specifically, the association of HLA Class I residue variation with the likelihood of disease remission. Specifically, in the context of AD disease susceptibility but not disease prognosis, previous studies have shown an associations of HLA variation as well as reports suggesting a role for NK cells in the onset of AD.(12, 20, 24, 25) Further characterization of HLA binding pocket changes and potential allergens that increase the risk or severity of AD can expand our understanding of the immunologic mechanisms associated with AD and hold promise for developing more efficient and personalized therapeutic approaches for AD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations:

<b>AF</b>	Allele frequency
<b>AD</b>	Atopic dermatitis



<b>CI</b>	Confidence interval
<b>DNA</b>	Deoxyribonucleic acid
<b>FLG</b>	Filaggrin
<b>GWAS</b>	Genome wide association study
<b>HR-2F</b>	High resolution two-field typing
<b>HLA</b>	Human Lymphocyte Antigen
<b>IMGT</b>	Immunogenetics database
<b>KIR</b>	Killer-cell immunoglobulin-like receptor
<b>LD</b>	Linkage disequilibrium
<b>NMDP</b>	National Marrow Donors Program
<b>NK</b>	Natural killer cell
<b>NGS</b>	Next generation sequencing
<b>OR</b>	Odds Ratio
<b>p</b>	p-value
<b>PCR</b>	Polymerize chain reaction
<b>PEER</b>	Pediatric Eczema Elective Registry
<b>SNP</b>	Single nucleotide polymorphisms

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**Key Points:**

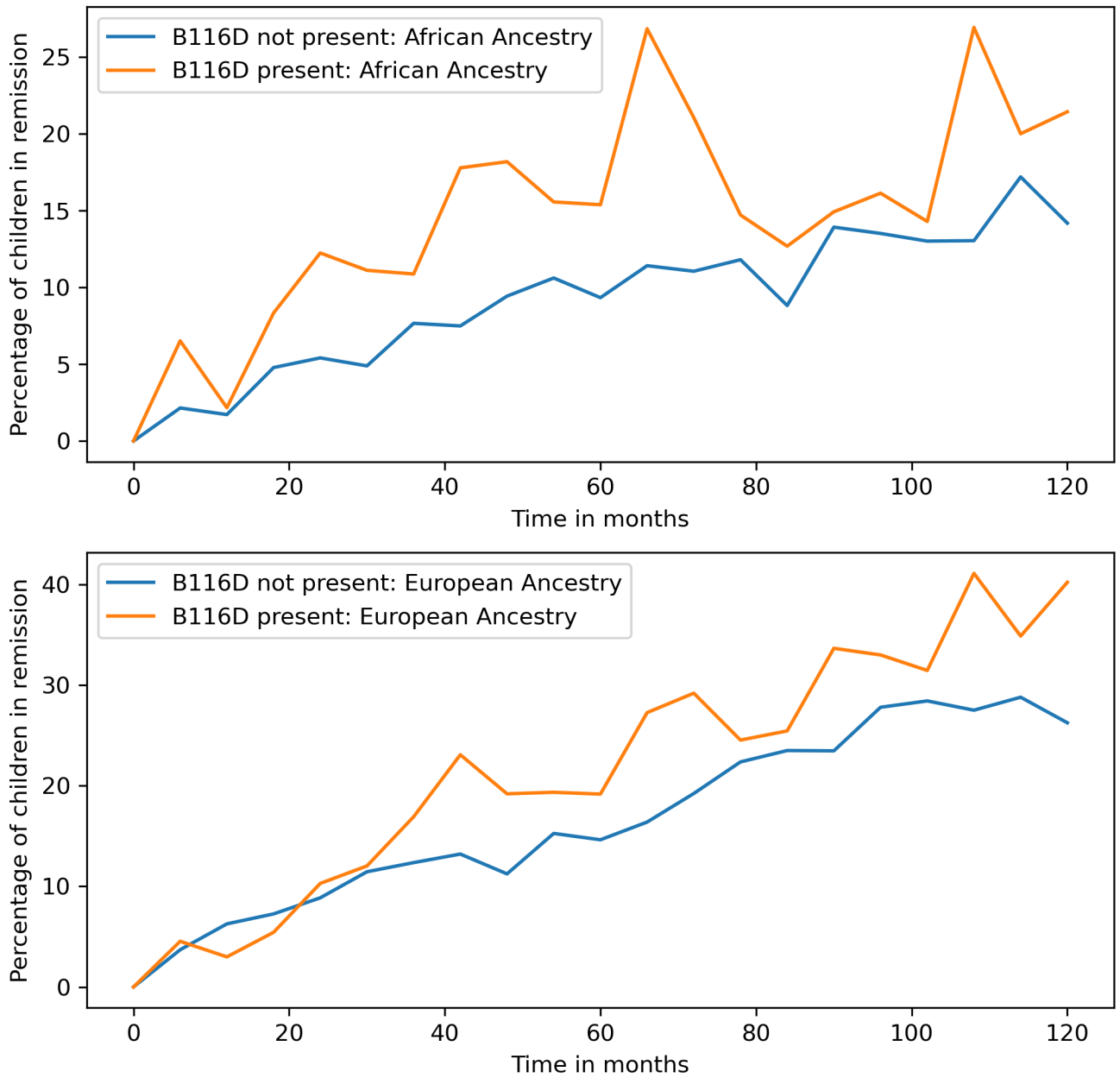
Atopic dermatitis (AD) is a chronic waxing and waning illness.

HLA Class I polymorphisms are associated with less severe AD.

AD progression is associated with HLA polymorphisms engaging both T and NK cells



### B116D by ancestry



**Figure 1:** Graph of HLA Class I residue B116D versus other B116 variants over time with respect to the percentage of children symptom and medication free at any evaluation timepoint by ancestry.

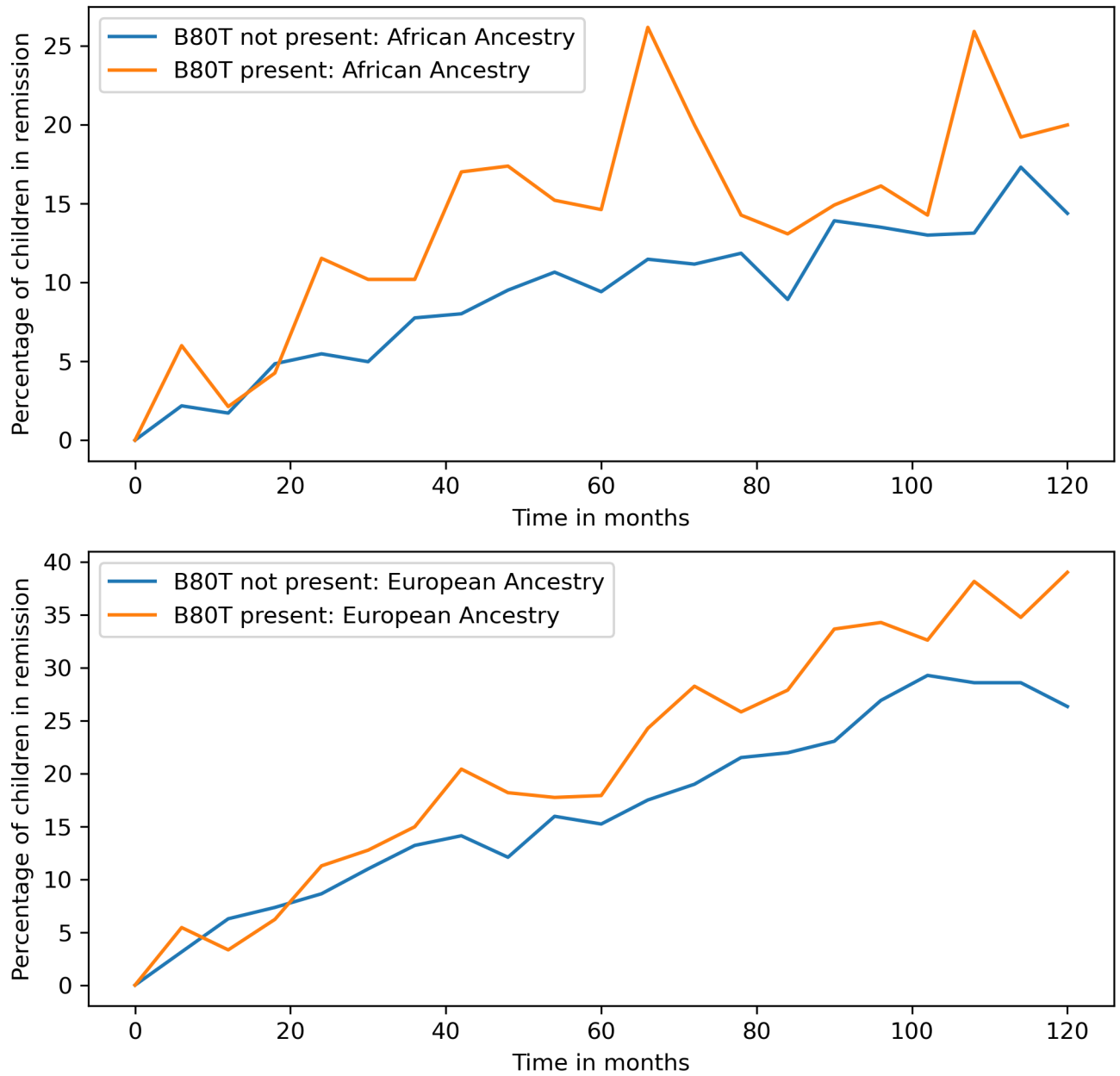
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### B80T by ancestry



**Figure 2:** Graph of HLA Class I residue B80T versus other B80 variants over time with respect to the percentage of children symptom and medication free at any evaluation timepoint by ancestry.

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**Table 1:**

Simpson reciprocal index (SRI) is presented for the full PEER DNA cohort for residues known to occur within HLA Class I binding pockets. Variants within a position are presented by amino acid single letter abbreviation.

position	HLA-A		HLA-B		HLA-C	
	SRI	Variants	SRI	Variant	SRI	Variants
<b>9</b>	<b>3.0</b>	F; S; T; Y	1.8	D; H; Y	2.6	D; F; S; Y
<b>63</b>	1.5	E; N; Q	2.0	E; N	1.0	E
<b>66</b>	1.9	K; N	1.17	I; K; N	1.3	K; N
<b>67</b>	1.3	M; V	<b>3.7</b>	C; F; M; S; Y	1.0	Y
<b>70</b>	1.8	H; Q	1.7	K; N; Q; S	1.0	Q
<b>74</b>	1.6	D; H; N	1.8	D; Y	1.00	D
<b>77</b>	2.0	D; N; S	2.0	D; N; S	2.0	N; S
<b>80</b>	1.4	I; T	2.2	I; N; T	2.0	K; N
<b>81</b>	1.4	A; L	1.8	A; L	1.0	L
<b>97</b>	<b>3.0</b>	I; M; R	2.7	N; R; S; T; V; W	1.5	N; R; W
<b>99</b>	1.3	C; F; Y	1.0	S; Y	1.9	C; F; S; Y
<b>114</b>	<b>3.2</b>	E; H; Q; R	2.1	D; H; N	1.8	D; N; V
<b>116</b>	2.3	D; H; Y	<b>3.7</b>	D; F; L; S; Y	2.6	F; L; S; Y
<b>152</b>	2.5	A; E; R; V; W	1.8	E; V	1.8	A; E; T; V
<b>156</b>	2.3	L; Q; R; W	2.2	D; L; R; W	<b>3.2</b>	D; L; Q; R; W
<b>163</b>	1.5	E; R; T	2.6	E; L; T	1.6	E; L; T
<b>167</b>	1.6	G; W	1.3	L; S; W	1.0	W

**Table 2:**  
**Amino acid residues associations with remission outcome by HLA-A gene.**

Amino acids and their letter abbreviation (For example, A97T is HLA-A, position 97, amino acid R (arginine)) are presented with odds ratios and 95% CI estimated by generalized estimating equation (family binomial link logit correlation exchangeable). Positions were selected for evaluation based on SRI 3.0. Odds ratios are presented for the full cohort and by ancestry with 95% confidence intervals and p-values (and when appropriate corrected p-value).

Position/Variant	Variant Frequency	Full cohort (OR)	European	African
A9T	0.12 (0.10,0.14)	0.91 (0.67, 1.23) p=0.054	0.94 (0.59,1.50) p=0.802	1.40 (0.90,2.17) p=0.132
A9Y	0.18 (0.16,0.20)	1.03 (0.80, 1.32) p=0.842	1.00 (0.73,1.37) p=0.995	1.21 (0.76,1.92) p=0.420
A9S	0.20(0.18,0.22)	0.88 (0.68, 1.12) p=0.135	1.13 (0.82,1.55) p=0.451	1.01 (0.66,1.56) p=0.956
A9F	0.48 (0.45,0.50)	1.16 (0.96, 1.40) p=0.135	1.16 (0.92,1.48) p=0.210	0.70 (0.47,1.04) p=0.081
A97M	0.29 (0.27,0.32)	1.02 (0.83, 1.25) p=0.852	1.11 (0.87,1.42) p=0.393	1.20 (0.83,1.75) p=0.337
A97R	0.31 (0.29,0.34)	1.26 (1.03, 1.55) p=0.024	1.24(0.98,1.58) p=0.077	1.03(0.68, 1.55) p=0.894
A97I	0.35 (0.33,0.38)	0.84 (0.69, 1.03) p=0.097	0.85 (0.67,1.08) p=0.178	0.83 (0.57,1.20) p=0.330
A114E	0.08 (0.07,0.10)	0.66 (0.45,0.98) p=0.040	0.89 (0.44,1.82) p=0.757	1.02 (0.61,1.69) p=0.943
A114H	0.38 (0.35,0.40)	1.25 (1.03,1.53) p=0.023	1.31 (1.03,1.67) p=0.026	0.93 (0.64,1.35) p=0.712
A114Q	0.17 (0.16,0.19)	1.02 (0.81,1.31) p=0.812	0.97 (0.71,1.33) p=0.857	1.39 (0.94,2.05) p=0.101
A114R	0.32 (0.30,0.34)	0.93 (0.76,1.14) p=0.496	0.87 (0.69,1.10) p=0.248	0.77 (0.50,1.20) p=0.255
B67C	0.09 (0.07,0.10)	1.34 (0.98, 1.82) p=0.062	1.05 (0.74,1.48) p=0.781	2.09(0.108,4.02)p=0.028
B67F	0.23 (0.21,0.25)	0.79 (0.63, 1.00) p=0.051	0.77 (0.58,1.01) p=0.059	0.93 (0.61,1.42) p=0.732
B67M	0.06 (0.05,0.07)	0.94 (0.62, 1.42) p=0.770	1.38 (0.81,2.36) p=0.229	0.57 (0.26,1.21) p=0.144
B67S	0.36 (0.33,0.38)	1.23 (1.02, 1.48) p=0.034	1.21(0.98, 1.51) p=0.083	1.18(0.82, 1.70) p=0.376
B67Y	0.14 (0.13,0.16)	0.98 (0.74, 1.29) p=0.900	1.00 (0.70,1.41) p=0.981	1.01 (0.63,1.62) p=0.949
B116D	0.14 (0.12,0.16)	1.42 (1.13, 1.79) p=0.003 p <sub>corr</sub> =0.028	1.29(0.99, 1.67) p=0.055	1.69(1.02, 2.78) p=0.040
B116S	0.25 (0.23,0.28)	0.89 (0.73, 1.09) p=0.259	0.97 (0.77,1.23) p=0.820	0.91 (0.63,0.32) p=0.629
B116F	0.08 (0.07,0.09)	1.07 (0.76, 1.51) p=0.682	0.84 (0.57,1.24) p=0.380	1.34 (0.62,2.90) p=0.453
B116L	0.08 (0.07,0.10)	1.13 (0.82, 1.56) p=0.454	1.13 (0.77,1.67) p=0.529	1.38 (0.79,2.39) p=0.257
B116Y	0.34 (0.31,0.36)	0.91 (0.75, 1.11) p=0.360	0.88 (0.70,1.11) p=0.291	0.79 (0.53,1.18) p=0.254
C156D	0.02 (0.02,0.03)	1.71 (1.02, 2.84) p=0.040	2.12 (1.21,3.73) p=0.009	0.79 (0.21, 3.03)p=0.731
C156L	0.39 (0.36,0.41)	0.87 (0.72,1.06) p=0.172	0.89 (0.71,1.12) p=0.327	0.66 (0.45,0.97) p=0.038
C156Q	0.06(0.05,0.08)	0.65 (0.34,1.25) p=0.199	1.25 (0.59,2.62) p=0.559	0.30 (0.08,1.19) p=0.087
C156R	0.28 (0.26,0.30)	1.05 (0.86,1.29) p=0.632	1.05 (0.82,1.36) p=0.663	1.10 (0.74,1.62) p=0.636
C156W	0.15 (0.13,0.17)	1.19 (0.92,1.53) p=0.175	1.13 (0.83,1.53) p=0.448	1.38 (0.90,2.12) p=0.136

**Table 3:**

KIR binding sites as represented by amino acid residues association. Frequency with 95% confidence intervals and OR and 95% confidence intervals. Odds ratios are presented for the full cohort and by ancestry with 95% confidence intervals and p-values (and when appropriate corrected p-value).

Residue	Variant Frequency	Full cohort (OR)	European Ancestry	African Ancestry
A80I	0.34 (0.31,0.36)	1.03 (0.84,1.26) p=0.762	0.78(0.58,1.04) p=0.092	0.78 (0.50,1.20) p=0.261
B80T (Bw4)	0.16(0.14,0.18)	1.45 (1.17,1.80) <b>p=0.0008399</b>	1.34 (0.93, 1.92) p=0.114	1.57 (0.95,2.59) p=0.077
B80I (Bw4)	0.34(0.31, 0.36)	0.84(0.65, 1.08) p=0.169	0.92(0.63,1.34) p=0.657	0.66 (0.42,1.03) p=0.066
B83G (Bw4)	0.53(0.50,0.55)	0.97 (0.81, 1.17) p=0.772	0.82(0.62, 1.10) p=0.185	1.25 (0.64,2.92) p=0.411
C80K (C2)	0.41(0.39,0.44)	1.01(0.83, 1.22) p=0.932	0.88(0.66,1.67) p=0.365	1.17 (0.82,1.67) p=0.383
C80N (C1)	0.48(0.45,0.50)	1.05 (0.87,1.27) p=0.585	1.12 (0.85,1.49) p=0.409	0.72 (0.49,1.05) p=0.086