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Association between HLA Inheritance and Asthma MEDICATION USE in HIV+ Children

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Abstract

This study's purpose was to determine whether asthma medication use in HIV+ children is associated with HLA alleles. We reviewed HLA and medication data collected during the Women and Infants Transmission Study for 124 HIV+ children and their mothers. Analysis revealed that HLA-A68 ($P=0.006$) was independent and predictive for time to first asthma medication use. There was a preventive association of Cw6 ($P=0.008$) with AT. HAART was also associated with time to first asthma medication use ($P=0.05$). HLA alleles may modulate risk of developing a need for asthma medications and seem to function independently of the actions of HAART therapy.

Evidence from adult and pediatric studies suggests that CD4+ T cells have a critical role in the development of asthma in children with HIV infection [1-5]. There have been numerous studies of human leukocyte antigen (HLA) genes as predictors for childhood atopy and asthma, including the MHC (major histocompatibility complex) class II restricted T cells. Several studies have found associations between HLA genes and asthma and allergy susceptibility (e.g., Juhn et al) [6].

To examine the possibility of HLA associations with asthma in HIV infection, investigators of the Women and Infants Transmission Study (WITS), a large multicenter pediatric cohort ($n=2,471$) with documented HIV-1 in utero exposure [7], reviewed the HIV+ (HIV-infected $n=193$) cohort for the use of asthma medication [5] and performed HLA typing. The results of that investigation are reported herein.

A subset of 124 HIV+ WITS patients with available data/specimens underwent HLA typing by sequence-specific PCR performed to the two digit allelic level. DNA isolation and

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sequence-based HLA typing was performed using the method as described by Winchester and Johnston-Dow [8-9]. Haplotypes were determined by direct comparison of the typing of the mother and her infant. Maternal and paternal haplotypes were identified.

Asthma was defined in terms of asthma medication use rather than by parental report of wheezing, reactive airways disease, or asthma [10-11]. The time to first asthma medication was defined as asthma time (AT).

A multi-staged approach was used to identify the final association models relating risk factors to the asthma medication use. The population baseline characteristics were compared by asthma medication use (Yes/No) as determined during the WITS follow up. Differences in continuous variables were assessed by using t-tests, and differences in categorical variables were assessed by using the Fisher's exact test.

Univariate analyses were first performed to assess the associations between AT and HLA alleles using the log-rank statistic. All the covariates that were significant at $P=0.10$ level were further evaluated in multivariate analyses using the COX model [12]. The final multivariate model was built using a backward selection technique that forced the HAART variable to remain in the model. To reduce the number of spurious associations due to the large number of tests being performed in this study, a significance level of $P=0.01$ for statistical inference was used as the criterion for keeping a variable in a multivariate model analysis. We evaluated every subject from their birth to the end of WITS follow up. Non-event related interval censoring occurred when a subject was lost to follow up. The Breslow method for handling event/censoring ties was used to establish each contribution to the likelihood ratio. In the COX proportional hazard model analyses, data were arranged using counting process style of input to incorporate time-dependent variables [12-13]. The COX model's proportionality assumption was checked by plotting log negative log survival density functions for the final model. The proportionality assumption was not violated for the two HLA alleles that remained in the final model (HLA-A68 and HLA-Cw6).

Reported P-values from COX proportional hazard models were calculated using likelihood ratio test (comparing the full and reduced likelihoods) since number of events in a risk factor category was small (i.e., HLA Cw6).

HLA typing was performed on 124 infants in the WITS study. Gender, race/ethnicity, and ability to pay for medical costs (socioeconomic status) did not differ by asthma medication status. The study population was predominantly of minority race and ethnic groups and was composed of inner city dwellers [7]. The study cohort is ethnically diverse with 52 African-Americans, 43 Hispanics, 21 Caucasians and 8 infants in other ethnic categories. Children with a history of asthma medication use were more likely to have been treated with HAART therapy.

Analysis of MHC class I A, B, and C and class II DRB1 and DRB345 alleles is shown in the Table I (only HLA surviving alleles associated with asthma shown). There were six HLA Class I alleles that were either associated with or protective against asthma medication use at the $P=0.10$ level in the preliminary association analyses (HLA-A68, HLA-B13, HLA-B39, HLA-B55, HLA-Cw4, and HLA-Cw6 [Table IA]). Thirty-three percent of subjects possessing at least one HLA-A68 allele had a history of asthma medication use vs. 9% of subjects without the allele. Rates of comparison for the other alleles were: 50% vs. 13% for HLA-B13, 50% vs. 13% for HLA-B39, 100% vs. 13% for HLA-B55, 23% vs. 12% for HLA-Cw4. The HLA-Cw6 allele was associated with lack of asthma medication use. The rates of comparisons were 0% vs. 18% for HLA-Cw6.

Candidate alleles, including the long term non-progression alleles HLA-B27 and B57, and HAART medication use were subjected to multivariate analysis using the Cox model to examine the independent associations of HLA allele and HAART treatment with AT (Table IB). After a backward selection and forcing HAART medication use to remain in the final model since earlier results [3-5] have indicated that this variable is related to immunoreconstitution, the final model contained variables for HLA-A68 ($P=0.006$, increased risk for asthma medication use), HLA-Cw6 ($P=0.008$, decreased risk) and HAART therapy ($P=0.05$, increased risk) (Table IB). HLA-B27 and HLA-B57, along with HLA-B13, HLA-B39, HLA-B55 and HLA-Cw4, were all excluded from the final model with a significance level to stay at 0.01. Also, we tested the interaction of HAART use with HLA-A68 and HLA-Cw6 but these tests did not meet the statistical significance criterion to remain in the final model.

In summary, stimulated by the unexpected discovery of a $CD4^+$ T cell dependent increased incidence and prevalence of asthma in HIV+ children treated with HAART, we sought an alternate explanation for this observation. We performed HLA analysis of children with and without asthma in the same cohort WITS study. Our discovery was that inheritance of certain HLA class I alleles, A68 and Cw6, are independent predictors of AT use in HIV+ children; A68 predicts increased incidence and Cw6 predicts decreased incidence, and both are independent of the increased incidence of asthma attributable to HAART itself. Thus, immunoreconstitution of a suppressed immune system in HIV infection may produce immunological events that trigger bronchial hyperresponsiveness and autoimmune disorders, particularly in those individuals with an underlying genetic predisposition. The relatively small number of patients in this study makes us interpret our results with caution. Longer term follow up may have yielded more patients with asthma.

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References

1. Lin RY, Lazarus TS. Asthma and related atopic disorders in outpatients attending an urban HIV clinic. *Ann Allergy Asthma Immunol.* 1995; 74:510–515. [PubMed: 7788519]
2. Galli L, Sabatino G, Zappa M, Barbante E, Chiappini E, de Martino M. Reduced frequency of wheezing respiratory illness in infants with perinatal human immunodeficiency virus-type 1 infection: a model for immunologic and inflammatory mechanisms of airway obstruction? *Pediatr Allergy Immunol.* 2003; 14:42–49. [PubMed: 12603710]
3. Foster SB, Paul ME, Kozinetz CA, Macias CG, Shearer WT. Prevalence of asthma in children and young adults with HIV infection. *J Allergy Clin Immunol.* 2007; 119:750–752. [PubMed: 17336620]
4. Gutin F, Butt A, Alame W, Thomas R, Secord E. Asthma in immune-competent children with human immunodeficiency virus. *Ann Allergy Asthma Immunol.* 2009; 102:438. [PubMed: 19492668]
5. Foster SB, McIntosh K, Thompson B, Lu M, Yin W, Rich KC, et al. Increased incidence of asthma in HIV-infected children treated with highly active antiretroviral therapy in the National Institutes of Health Women and Infants Transmission Study. *J Allergy Clin Immunol.* 2008; 122:159–165. [PubMed: 18547627]

6. Juhn YJ, Kita H, Lee LA, Smith RW, Bagniewski SM, Weaver AL, et al. Childhood asthma and human leukocyte antigen type. *Tissue Antigens*. 2007; 69:38–46. [PubMed: 17212706]
7. Sheon AR, Fox HE, Rich KC, Stratton P, Diaz C, Tuomala RE. The woman and infants transmission study (WTS) of maternal-infant HIV transmission study design methods and baseline data. *J Women's Health*. 1996; 5:69–78.
8. Winchester R, Pitt J, Charurat M, Magder LS, Goring HH, Landay A, et al. Mother-to-child transmission of HIV-1: strong association with certain maternal HLA-B alleles independent of viral load implicates innate immune mechanisms. *J Acquir Immune Defic Syndr*. 2004; 36:659–670. [PubMed: 15167284]
9. Johnston-Dow, I.; Bengtson, A.; Scheltinga, S. DNA sequencing based typing HLA-A. *Proceedings of the Twelfth International Histocompatibility Workshop and Conference*; 1997; p. 250-254.
10. Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. Tucson Children's Respiratory Study: 1980 to present. *J Allergy Clin Immunol*. 2003; 111:661–675. [PubMed: 12704342]
11. Werk LN, Steinbach S, Adams WG, Bauchner H. Beliefs about diagnosing asthma in young children. *Pediatrics*. 2000; 105:585–590. [PubMed: 10699113]
12. Andersen PK, Gill RD. Cox-regression-model for counting processes--a large sample study. *Annals of Statistics*. 1982; 10:1100–1120.
13. Therneau, TM.; Grambsch, PM. *Modeling survival data*. New York: Springer; 2000.

Abbreviations

ART	antiretroviral therapy
AT	time to first asthma medication use
HLA	human leukocyte antigen
HIV	human immunodeficiency virus type 1
HIV+	HIV infected
HIV–	HIV uninfected
HAART	highly active antiretroviral therapy
WITS	Women and Infants Transmission Study

Table I
Univariate Analysis on Associations Between HLA Alleles

IA. Univariate analysis on Associations between HLA Alleles and Asthma Medication among HIV+ Infants					
Allele	Present		Not Present		P-Value *
	Asthma	No Asthma	Asthma	No Asthma	
HLA A68	7	14	6	62	0.003
HLA B13	2	2	15	103	0.09
HLA B39	2	2	15	103	0.02
HLA B55	1	0	16	105	0.05
HLA Cw4	5	17	8	59	0.07
HLA Cw6	0	17	13	59	0.06

IB. Multivariate Analysis on Associations between HLA Alleles and the Presence of Asthma among HIV+ Children Treated with and without HAART			
Predictors	Hazard Ratio	(Lower CI, Upper CI)	P *
HLA A68	4.76	(1.60, 14.21)	0.006
HLA Cw6	0.00	-	0.008
HAART: Yes vs. No	4.50	(0.86, 23.59)	0.05

* P-value from log-rank test

* P-value from likelihood ratio test