Do alopecia Areata and Atopic Dermatitis share HLA alleles and cytokine profiles?

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Alopecia areata (AA) is hypothesized to be an organ-specific autoimmune disease mediated by T cells directed to the hair follicle. The severity of the clinical phenotype varies from discrete patchy hair loss in one or several areas (alopecia areata; AA) to total loss of scalp hair (alopecia totalis; AT) and total scalp and body hair loss (alopecia universalis; AU). Although most cases of AA are sporadic, a genetic basis for AA has been suggested.
Atopic dermatitis (AD) is a hereditary, and chronically relapsing inflammatory skin disease characterized by a perivascular infiltrate of activated T-cells. Like AA, AD also may be triggered by environmental factors such as stress or viral infections. The incidence of having both AA and AD has been reported to be 10-60%. Patients with atopy usually have the triad of atopic dermatitis, allergic asthma, and/or allergic rhinoconjunctivitis.

We proposed to study the samples collected from National Alopecia Areata Registry (NAAR) to study the genetic and immunological pattern of both diseases. Our goal was to study the following specific aims:

Specific Aim 1: To evaluate the incidence of AA severity phenotypes with AD in the United States. Two hundred eighty seven sporadic AA cases were collected from NAAR. Among those, 152 had atopy (atopic dermatitis and/or asthma and/or allergic rhinoconjunctivitis). Most of the patients with atopy had at least two symptoms together. AA patients were classified as AAT (AA transient; if they had a patchy hair loss phenomenon for less than 1 year), AAP (AA persistent; if they had patchy hair loss phenomenon for a year or more than 1 year), AT (alopecia totalis; if they had a complete scalp hair loss), AU (alopecia universalis; if they had a complete scalp and body hair loss). Eighteen unaffected, non blood related samples were collected as control. The percentages of atopy in AAT, AAP, and AT/AU types were as below:

Table 1: Frequency of atopy in AA phenotypes

	Atopic dermatitis	Asthma	Allergic rhinoconjunctivitis	No allergy
AAT(n=27)	8(29.63%)	5(18.52%)	10(37.04%)	4(14.8%)
AAP(n=89)	25(28.09%)	18(20.22%)	42(47.19%)	4(4.49%)
AT/AU(n=153)	48(53.93%)	26(16.99%)	59(38.56%)	20(13.07%)

To date, we have 3189 AA patients registered as a 1st tier participants. Among those, 835 have AAT, 926 have AAP, and 1428 have AT/AU. The percentage of having atopy (atopic dermatitis, asthma, allergic rhinoconjuctivitis) in different AA phenotypes were as below:

Table 2: Frequency of atopy in AA phenotypes among 1st tier participants

	Atopic dermatitis	Asthma	Allergic rhinoconjunctivitis	No allergy
AAT (n=835)	163 (19.52%)	105 (12.57%)	198 (23.19%)	369 (44.2%)
AAP (n=926)	195 (21.06%)	143 (15.44%)	261 (28.19%)	327 (35.31%)
AT/AU (n=1428)	355 (24.86%)	253 (17.72%)	431 (30.18%)	389 (27.24%)

Specific Aim 2: To determine whether HLA alleles differ among AA patients with or without atopy and with severity of AA phenotypes.

Because the NAAR registry was short on control samples (only 18 samples at present), the department of epidemiology at UT-MD Anderson Cancer Center provided us 152 DNA samples for further analysis. Polymerase chain reaction and dot blot hybridization were performed on 480 DNA samples to genotype HLA-DRB, HLA-DQA, and HLA-DQB. Samples were divided into five 96-well plates for analysis. Two samples on each plate were analyzed in duplicate in different locations to ensure well-to-well variation was minimal. Three samples were included on all plates to verify there was no plate-to-plate variation. A total of 443 samples was informative for analysis. Because our sample group for AAT numbered only 30, which was too few, we did not report the results for this particular group. Also, because these patients had patchy AA for less than a year, we are planning to follow up on these patients to see if they remain with patchy AA (AAP) or progress to the severe form (AT/AU).

Chi-square test was performed to analyze the data. Results were obtained for alleles with p-value <0.05, but because the numbers of people for some alleles were too small (less than 10), the results for those alleles are not displayed below.

Table 3: The association of HLA-DRB1 alleles with AAP and AT/AU versus Controls

	DRB1*0301 (p value, number of samples)	DRB1*0701 (p value, number of samples)	DRB1*1101 (p value, number of samples)	DRB1*1104 (p value, number of samples)	DRB1*1501 (p value, number of samples)
AAP (n=99) vs Controls	0.03, 12	0.022, 31	0.06, 16	0.012, 13	0.016, 16
AT/AU (n=163) vs Controls	0.000066, 12	0.136, 42	0.01, 28	0.000032, 31	0.049, 32

Table 4: The association of HLA-DRB3 alleles with AAP and AT/AU versus Controls

	DRB3*52b (p value, number of samples)	DRB3*53 (p value, number of samples)
AAP (n=99) vs Controls	0.0099, 38	0.053, 59
AT/AU (n=163) vs Controls	0.000084, 82	0.0096, 100

Table 5: The association of HLA-DQA1 alleles with AAP and AT/AU versus Controls

	DQA1*0102 (p value, number of samples)	DQA1*0501 (p value, number of samples)
AAP (n=99) vs Controls	0.14, 32	0.24, 44
AT/AU (n=163) vs Controls	0.046, 50	0.018, 82

Table 6: The association of HLA-DQB1 alleles with AAP and AT/AU versus Controls

	DQB1*0201 (p value, number of samples)	DQB1*0202 (p value, number of samples)	DQB1*0301 (p value, number of samples)	DQB1*0602 (p value, number of samples)
AAP (n=99) vs Controls	0.0015, 11	0.00015, 27	0.0045, 45	0.014, 20
AT/AU (n=163) vs Controls	0.000018, 15	0.0025, 34	0.000000016, 97	0.033, 30

Table 7: The association of MICA alleles with AAP and AT/AU versus Controls

	MICA*4 (p value, number of samples)	MICA*5 (p value, number of samples)	MICA*5.1 (p value, number of samples)	MICA*6 (p value, number of samples)	MICA*9 (p value, number of samples)
AA (n= 291) overall vs Controls	0.64, 65	0.15, 72	0.18, 183	0.59, 104	0.57, 72
AAP (n=99) vs Controls	0.65, 21	0.51, 22	**0.03,** 55	0.22, 40	0.77, 29
AT/AU (n=163) vs Controls	0.59, 35	0.14, 42	0.40, 106	0.81, 56	0.36, 37

There were no significant association of MICA alleles between AA patients with atopy versus AA patients without atopy; MICA*4 (p=0.20), MICA*5 (p=0.22), MICA*5.1(p=0.54), MICA*6 (p=1.00), MICA*9 (p=0.79). However, MICA*5.1 was significantly associated with AAP versus controls (Table 7).

We also studied if the association of HLA alleles differ in AA patients with atopy versus AA patients without atopy:

Table 8: Association of HLA alleles in AA atopic patients versus AA non-atopic

	DRB1*4 (*0401-*0411) alleles (p value, number of atopic , nonatopic samples)	DRB3*53 allele (p value, number of atopic , nonatopic samples)	DQA1*03 allele (p value, number of atopic , nonatopic samples)
AA atopic (n=154) vs AA non-atopic (n=137)	0.05, 65, 42	0.02, 103,72	0.05, 63, 40

Specific Aim 3: To study cytokine profiles in AA with and without atopy.
SearchLight™ Proteome Array (Pierce Biotechnology, Inc., Rockford, IL) was used to study the cytokine levels in the sera samples collected from NAAR. This multi-analyte assay allowed us to study 17 different cytokines at the same time under the same conditions. Sera from 287 sporadic cases were studied for IL-1a, IL-1b, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-18, RANTES, IFN-gamma, and TNF-alpha. Some of the samples were analyzed in duplicate to ensure well-to-well variation was minimal. Among those, 27 were AAT, 89 were AAP, 153 were AT/AU, and 18 were unaffected, non-blood related participants (control). Because the amount of control samples was small, Pierce Biotechnology, Inc. provided us control samples, but not equally for each cytokine. Therefore we ended up with different numbers of controls for each cytokine [IL-1b (total number of controls, n=39), IL-1ra (n=68), IL-2 (n=39), IL-4 (n=38), IL-6 (n=68), IL-8 (n=62), IL-10 (n=39), IL-12p70 (n=39), IL-15 (n=68), IL-18 (n=39), TNF-a (n=68), IFN-g (n=68)]. The provided samples came from donors unaffected by AA, as per Pierce Biotechnology, but since we did not have medical histories of these donors, we could only assume that the donors were non-atopic. The SearchLight™ Proteome Array had a minimum detectable value for each cytokine, and consequently we were not able to obtain values below these levels. The minimum cytokine level for each cytokine is shown in Table 9.

Cytokine results were compared among different AA phenotypes alone and also among phenotypes with and without association with atopy. If at least 50% of samples for a particular cytokine resulted in a less than minimal detectable value, the cytokine was not included in the analysis due to lack of useful information. If 10-50% of samples for a particular cytokine resulted in cytokine levels less than or equal to the minimal detectable value, the Wilcoxon Rank Sum test were performed. For cytokines where less than 10% of samples resulted in cytokine levels less than or equal to the minimal detectable value, *t*-tests were used. A p-value less than 0.05 was considered significant.

Table 9: Minimum detectable value of the SearchLight™ Proteome Array

	IL-1a	IL-1b	IL-1ra	IL-4	IL-5	IL-6	IL-8	IL-9	IL-10	IL-12p70	IL-13	IL-15	IL-18	IFN-g	TNF-a	RANTES
Minimum detectable range (pg/ml)	2	1	7.8	3.9	2	2	2	30.5	2	2.9	39.1	2	10	2	11.7	2

Following results were observed:

1- Median serum cytokine levels of IL-1ra, IL-8, IL-10, RANTES were higher in AA group and median serum cytokine levels of IL-6, IL-12, and IFN-g were lower in AA group in comparison with Control group (Table 10).

2- Median serum cytokine levels of IL-1ra, IL-8, IL-10, IL-18, and RANTES are higher in AAT group and median serum cytokine levels of IL-15 is lower in AAT group versus Controls (Table 11).

3- Median serum cytokine levels of IL-1ra, IL-8, IL-10, and RANTES are higher in AAP group and median serum cytokine levels of IFN-g is lower in AAP group versus Controls (Table 12).

4- Median serum cytokine levels of IL-1ra, IL-8, IL-10, and RANTES are higher in AT/AU group and median serum cytokine levels of IL-12, INF-g, and TNF-a are lower in AT/AU group versus Controls (Table 13).

5- We compared the AA patients with atopy versus Control group with atopy and we have observed that the median serum cytokine levels of IL-2, IL-6, IL-8, IL-10, IFN-g, and RANTES were higher in the atopic AA group versus atopic Controls (Table 14).

6- We compared 144 AA patients with atopy versus 125 AA patients without atopy. Median serum cytokine levels of IL-1a and IL-12 were higher in the atopic AA group versus nonatopic AA group (Table 15).

7- From a group of 153 AT/AU patients we compared 74 patients with no atopy to 79 patients with atopy and we observed that the median serum cytokine level of IL-1a was higher in atopic AT/AU group versus non-atopic AT/AU group (Table 16).

8- From a group of 89 patients with AAP we compared 38 patients with no atopy to 51 patients with atopy and we observed that the median serum cytokine levels of TNF-a and IFN-g were higher in the atopic AAP group versus non atopic AAP (Table 17).

9- From a group of 27 patients with AAT we compared 13 patients with no atopy to 14 patients with atopy. There was no difference between median serum cytokine levels of 17 cytokines that were examined (data not shown).

Table 10: Median serum cytokine levels in AA patients vs Controls

Cytokine markers	Control group (median) (n)	AA group (median) (n)	P-value
IL-1ra	(29.56) (68)	(162.32) (268)	<0.00001
IL-6*	(3.80) (68)	(3.65) (268)	0.011
IL-8	(8.37) (62)	(166.64) (269)	<0.0001
IL-10	(2.00) (39)	(2.35) (269)	0.00025
IL-12	(6.18) (39)	(3.57) (269)	0.040
IFN-g	(10.73) (68)	(7.74) (269)	0.00713
RANTES*	(42979.50) (38)	(67425.96) (269)	<0.0001

Wilcoxon test
* t- test
Serum cytokine levels of IL-4, IL-1b, IL-5, IL-9, and IL-13 were not informative enough to include in the analysis.

Median serum cytokine levels of IL-1ra, IL-8, IL-10, RANTES are higher in AA group and median serum cytokine levels of IL-6, IL-12, and IFN-g are lower in AA group versus Controls.

Table 11: Median serum cytokine levels in AAT patients vs Controls

Cytokine markers	Control group (median) (n)	AA group (median) (n)	P-value
IL-1ra	(29.56) (68)	(101.26) (27)	0.00053
IL-8	(8.37) (62)	(130.26) (27)	0.05
IL-10	(2.00) (39)	(2.46) (27)	0.012
IL-15	(2.95) (68)	(2.00) (27)	<0.0001
IL-18	(190.74) (39)	(239.44) (27)	0.057
RANTES	(42979.50) (38)	(60962.21) (27)	0.014

Wilcoxon test
Serum cytokine levels of IL-4, IL-1b, IL-5, IL-9, and IL-13 were not informative enough to include in the analysis.

Median serum cytokine levels of IL-1ra, IL-8, IL-10, IL-18, and RANTES are higher in AAT group and median serum cytokine levels of IL-15 is lower in AAT group versus Controls.

Table 12: Median serum cytokine levels in AAP patients vs Controls

Cytokine markers	Control group (median) (n)	AA group (median) (n)	P-value
IL-1ra	(29.56) (68)	(167.87) (88)	<0.0001
IL-8	(8.37) (62)	(257.79) (89)	0.00008
IL-10	(2.00) (39)	(2.35) (89)	0.001
IFN-g	(10.73) (68)	(7.57) (89)	0.026
RANTES*	(42979.50) (38)	(74454.11) (89)	<0.0001

Wilcoxon test
*t-test
Serum cytokine levels of IL-4, IL-5, IL-9, and IL-13 were not informative enough to include in the analysis.

Median serum cytokine levels of IL-1ra, IL-8, IL-10, and RANTES are higher in AAP group and median serum cytokine levels of IFN-g is lower in AAP group versus Controls.

Table 13: Median serum cytokine levels in AT/AU patients vs Controls

Cytokine markers	Control group (median) (n)	AA group (median) (n)	P-value
IL-1ra*	(29.56) (68)	(159.62) (153)	<0.0001
IL-8	(8.37) (62)	(166.64) (153)	<0.0001
IL-10	(2.00) (39)	(2.34) (153)	0.00053
IL-12	(6.18) (39)	(3.18) (153)	0.018
IFN-g	(10.73) (68)	(7.74) (153)	0.00756
TNF-a	(43.49) (68)	(19.59) (153)	0.020
RANTES*	(42979.50) (38)	(63666.61) (153)	0.0001

Wilcoxon test
*t-test
Serum cytokine levels of IL-4, IL-1b, IL-5, IL-9, and IL-13 were not informative enough to include in the analysis.

Median serum cytokine levels of IL-1ra, IL-8, IL-10, and RANTES are higher in AT/AU group and median serum cytokine levels of IL-12, IFN-g, and TNF-a are lower in AT/AU group versus Controls.

Table 14: Median serum cytokine levels of AA with atopy vs control with atopy

Cytokine markers	Control with atopy (median) (n)	AA with atopy (median) (n)	P-value
IL-2	(5.65) (8)	(15.1) (144)	0.05
IL-6	(2.00) (8)	(3.59) (143)	0.028
IL-8	(2.00) (8)	(145.26) (144)	0.0017
IL-10	(2.00) (8)	(2.34) (144)	0.0055
IFN-g	(3.74) (8)	(8.05) (144)	0.0139
RANTES	(42272.59) (8)	(67451.26) (144)	0.013

Wilcoxon test

Serum cytokine levels of IL-4, IL-1b, IL-5, IL-9, IL-12, IL-13, and TNF-a were not informative enough to include in the analysis.

Median serum cytokine levels of IL-2, IL-6, IL-8, IL-10, IFN-g, and RANTES are higher in atopic AA versus atopic Controls.

Table 15: Median serum cytokine levels of atopic AA patients versus nonatopic AA

Cytokine markers	AA with atopy (median) (n)	AA without atopy (median) (n)	P-value
IL-1a	(8.62) (144)	(5.00) (125)	0.00306
IL-12	(4.20) (144)	(2.90) (125)	0.055

Wilcoxon tests

Serum cytokine levels of IL-4, IL-5, IL-9, IL-13, and TNF-a were not informative enough to include in the analysis.

Median serum cytokine levels of IL-1a and IL-12 are higher in atopic AA verus non atopic AA.

Table 16: Median serum cytokine levels of atopic versus nonatopic patients with AT/AU

Cytokine markers	AA with atopy (median) (n)	AA without atopy (median) (n)	P-value
IL-1a	(9.69) (79)	(5.04) (74)	0.0036

Wilcoxon test

Serum cytokine levels of IL-4, IL-1b, IL-5, IL-9, IL-12, IL-13, and TNF-a were not informative enough to include in the analysis.

Median serum cytokine level of IL-1a is higher in AT/AU with atopy verus AT/AU without atopy.

Table 17: Median serum cytokine levels of atopic versus non-atopic patients with AAP

Cytokine markers	AA with atopy (median) (n)	AA without atopy (median) (n)	P-value
TNF-a	(51.13) (51)	(19.88) (38)	0.053
IFN-g*	(8.41) (51)	(6.38) (38)	0.069

Wilcoxon test
* t-test
Serum cytokine levels of IL-4, IL-5, IL-9, and IL-13 were not informative enough to include in the analysis.

Median serum cytokine levels of TNF-a and IFN-g are higher in AAP with atopy verus AAP without atopy.

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1-Abstracts:

Nazila Barahmani, Mariza de Andrade, Joshua Slusser, Qing Zhang, Madeleine Duvic. HLA Class II and MICA alleles separate different phenotypes in sporadic alopecia areata patients. 64th Annual Meeting of American Academy of Dermatology. March 2006.

Nazila Barahmani, Ying Yang , Adriana Lopez, Madeleine Duvic: Atopic alopecia areata patients have increased serum Th1 Cytokine profiles. 66th Annual Meeting of Society for Investigative Dermatology. May 2005.

Mariza de Andrade, Nazila Barahmani, Kathleen Anne Hunzicker, Qing Zhang, John Reveille, Madeleine Duvic: HLA class II associations confirm alopecia areata phenotypic subtypes. 66th Annual Meeting of Society for Investigative Dermatology. May 2005.

Nazila Barahmani, Sara Donley, Ying Yang, Madeleine Duvic: Cytokine profiling of AA phenotypic subsets. 63th Annual Meeting of American Academy of Dermatology. Feb 2005.

2- Manuscripts:

Nazila Barahmani, Mariza de Andrade, Joshua Slusser, Qing Zhang, Madeleine Duvic: Major Histocompatibility Complex Class I Chain-Related Gene A (MICA) Polymorphisms and Extended Haplotypes Are Associated with Familial Alopecia Areata. JID-2005-0227.r1 (accpeted).

Serum cytokine profiles of AA patients (in preparation).