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Maternal asthma and microRNA regulation of soluble HLA-G in the airway.

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Abstract

Background—We previously reported an interaction between maternal asthma and the child's *HLA-G* genotype on the child's subsequent risk for asthma. The implicated SNP at +3142 disrupted a target site for the microRNA (miR)-152 family. We hypothesized that the interaction effect may be mediated by these miRs

Objective—The objective of this study was to test this hypothesis in adults with asthma who are a subset of the same subjects who participated in our earlier family-based studies.

Methods—We measured soluble (s)HLA-G concentrations in bronchial alveolar lavage (BAL) fluid (N=36) and plasma (N=57) from adult asthmatics with and without a mother with asthma, and *HLA-G* and miR-152 family (*miR-148a*, *-148b*, and *-152*) transcript levels in airway epithelial cells from the same individuals.

Results—*miR-148b* levels were significantly elevated in airway epithelial cells from asthmatics with an asthmatic mother compared to asthmatics without an asthmatic mother, and +3142 genotypes were associated with sHLA-G concentrations in BAL fluid among asthmatic individuals with an asthmatic mother but not among those with a non-asthmatic mother. Neither effect was observed in the plasma (sHLA-G) or white blood cells (miRNA).

Conclusion—These combined results are consistent with +3142 allele-specific targeting of HLA-G by the miR-152 family, and support our hypothesis that miRNA regulation of sHLA-G in the airway is influenced by both the asthma status of the subject's mother and the subject's genotype. Moreover, we demonstrate that the effects of maternal asthma on the gene regulatory landscape in the airways of her children persist into adulthood.

Keywords

Asthma; maternal asthma; microRNA; Human Leukocyte Antigen

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INTRODUCTION

Asthma is a chronic lung disease characterized by persistent airway inflammation, bronchial hyperresponsiveness, and structural remodeling^{1, 2}. The onset and exacerbation of asthma symptoms are associated with viral respiratory infections^{3, 4}, bacteria⁵, and exposure to airborne allergens^{6–8}. Among the many epidemiologic risk factors for asthma, the presence of asthma in the mother is one of the most significant^{4, 9–11}, although the mechanism(s) underlying this association are unknown.

We previously identified human leukocyte antigen G (*HLA-G*) as an asthma-susceptibility gene in a positional cloning study¹², and demonstrated that a soluble form (sHLA-G) was expressed in airway epithelial cells¹² and elevated in bronchoalveolar lavage (BAL) fluid from adults with asthma compared to non-asthmatic controls¹³. The genetic association was complex however, revealing an interaction between the child's *HLA-G* genotype at promoter single nucleotide polymorphisms (SNPs) (–964 G/A [rs1632947], or a SNP in perfect LD with –964) and the mother's asthma status on her child's risk for asthma.

The interaction effects could not be attributed to any one particular SNP because of the strong linkage disequilibrium (LD) between SNPs in this gene and the functionality of most SNPs were unknown. Therefore, we more thoroughly characterized variation throughout this gene in children who are participants in a birth cohort from Madison, Wisconsin¹⁴. In addition to promoter and coding SNPs, we included variants in the *HLA-G* 3' UTR, including a SNP (+3142 C/G; rs1063320) that disrupts a target site for the microRNA (miR)-152 family (*miR-148a*, *-148b*, and *-152*). An interaction between the asthma status of the mother and her child's *HLA-G* genotype on asthma risk was replicated in this cohort¹⁵. We further showed that homozygosity for the +3142G allele, which preserved the miRNA target site, was protective against asthma only in children of asthmatic mothers. This suggested that allele-specific targeting of the *HLA-G* transcript by the miR-152 family could contribute to the interaction between maternal asthma status and the risk for asthma to her child if the levels of these miRNAs differed in airway cells from individuals with an asthmatic mother compared to individuals with a non-asthmatic mother¹⁵.

In this study, we directly tested this hypothesis in 36 adults with asthma who are a subset of the same subjects who participated in our earlier family-based studies¹². We collected BAL fluid and airway epithelial cell brushings by bronchoscopy, and measured sHLA-G protein concentrations and miR-152 family levels (*miR-148a*, *-148b*, *-152*), respectively. We show both increased *miR-148b* levels in airway epithelial cells and genotype-specific effects on sHLA-G levels in BAL fluid from adult asthmatics with an asthmatic mother. The latter associations were not observed in adult asthmatics with a non-asthmatic mother. These results support our hypothesis that the interaction between the child's *HLA-G* genotype and maternal asthma status are mediated by miRNAs in her adult child.

METHODS

Sample Composition

Adult subjects with asthma were recruited from among the participants in our asthma genetic studies that were originally conducted between 1993 and 2003¹². Asthma was initially diagnosed using the following criteria: (1) age ≥ 6 years; (2) either (a) a fall in baseline forced expiratory volume at one second (FEV₁) ≥ 20% at 25 mg/ml methacholine, in subjects whose FEV₁ predicted was ≥ 70% or (b) a ≥ 15% increase in baseline FEV₁ after inhalation of a bronchodilator (albuterol) or over time with treatment, in subjects whose FEV₁ predicted was <70%; (3) at least two symptoms (cough, wheeze, and dyspnea); (4)

fewer than three pack years of cigarette exposure; and (5) a physician's diagnosis of asthma and no conflicting pulmonary diagnoses¹⁶. We attempted to contact all asthmatic probands (n=496) to participate in this study, which required a screening visit to determine eligibility for bronchoscopy and to perform the studies described below. Of the 109 subjects that we were able to contact, 57 completed the screening visit and 37 completed both the screening and bronchoscopy visits. Eleven subjects were ineligible for bronchoscopy due to medical contraindications, and nine subjects declined further participation after the first visit. One individual was excluded after bronchoscopy due to an insufficient collection of BAL fluid. The final sample consisted of 36 subjects for studies in lung-derived tissues and 57 subjects for studies in peripheral blood. Maternal asthma status was determined by interviewing the mother during our earlier studies and the child during the current studies. Any discrepancies in responses were clarified by follow up phone calls when possible. The clinical characteristics of the 57 subjects at the time of the current studies are described in Table I.

Clinical Evaluation of Subjects

Spirometry was repeated at the time of the present study. Methacholine challenge was done when baseline FEV₁ was ≥ 60% predicted; measurement of reversibility after inhalation of albuterol was done when baseline FEV₁ was < 60% predicted, following guidelines recommended by the American Thoracic Society¹⁷. Atopy was assessed by skin prick tests for 14 allergens (European dust mites [*Dermatophagoides pteronyssinus*], American dust mites [*Dermatophagoides farinae*], cat [*Felis catus(domesticus)*], dog [*Canis familiaris*], mold [*Alternaria alternaria*, *Cladosporium herbarum*, *Aspergillus fumigatus*], German cockroach [*Blattella germanica*], American cockroach [*Periplaneta Americana*], Kentucky bluegrass [*Periplaneta Americana*], short ragweed [*Ambrosia artemisiifolia*], common mugwort [*Artemisia vulgaris*], white oak [*Quercus alba*], red birch [*Betula nigra*]), and defined as a positive reaction to at least one allergen. Fractional exhaled nitric oxide (FeNO) was measured on a NIOX MINO device (Aerocrine, Inc., Morrisville, NC) during exhalations at 50 mL/second following the American Thoracic Society/European Respiratory Society protocol¹⁸. The lower limit of sensitivity for the device is 5 ppb. Blood samples were collected for measurements of total serum IgE and whole blood eosinophils. These studies were approved by the University of Chicago Institutional Review Board. Written informed consent was obtained from all research participants.

Specimen collections

Endobronchial brushings and BAL fluid were obtained during bronchoscopy, as previously described^{13, 19}. White blood cells and plasma were obtained from EDTA-anticoagulated whole blood collected during the screening visit, and stored at -80°C until use. We isolated RNA and small RNAs (including miRNA) from endobronchial brushings from 20 of the 37 subjects who underwent bronchoscopy, using the miRNeasy Mini kit (QIAGEN, Germantown, MD) and the RNeasy MinElute cleanup kit (QIAGEN, Germantown, MD), respectively.

Genotyping

HLA-G -964 G/A, the SNP used in our earlier studies¹², is in perfect LD ($r^2=1$) with the +36 C/T SNP²⁰. Therefore, in this study we genotyped the +36 C/T SNP, in addition to +3142 C/G. +36 tags the two major HLA-G promoter haplotypes²⁰ and +3142 resides in the miR-152 family target site in the *HLA-G* 3' UTR. These two SNPs are in strong, but not perfect, LD ($r^2=0.9$)¹⁵. Genotyping was performed using SNaPshot (Invitrogen, Carlsbad, CA), as previously described²¹.

sHLA-G protein quantitation

sHLA-G protein concentrations were assayed in plasma and concentrated BAL fluid using an HLA-G ELISA kit that detects the shed G1 and secreted G5 soluble isoforms²² (BioVendor, Candler, NC). BAL fluid was concentrated approximately 20–30X using the Amicon Ultra-15 Centrifugal Filter Unit (Millipore, Billerica, MA). All samples were analyzed in duplicate, according to the manufacturer's instructions. A sHLA-G standard curve was generated on each plate. BAL fluid measurements that were below the minimum standard value (2.5 U/mL) were randomly assigned a number between the minimal detectable unit (2.5 U/mL) and 0.

HLA-G transcript quantitation

One microgram of airway epithelial cell mRNA obtained from endobronchial brushing was converted to cDNA using the Superscript III cDNA synthesis kit (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. *HLA-G* transcript levels were quantitated by qPCR using the Platinum SYBR Green UDG qPCR with ROX (Invitrogen, Carlsbad, CA). Primers, specific for the *HLA-G* promoter/exon 1 were designed: F: 5' - CTGACCGAGACCTGGGCGGGCT -3' and R: 5' - GGCTCCATCTCGGACACGCCGA -3'. PCR products were sequenced to verify the specificity of primers for *HLA-G*. *POLR2C* was used as the reference gene: F: 5' - GAGACCTCATCTCCAACAGC -3' and R: 5' - ATAGGCTCGAAGTCTCAGCTC -3'. One microliter of 10 uM primer stocks and 24ng of cDNA were added to the Platinum SYBR green master mix. qPCR reactions were run on an ABI 7900 HT (Applied Biosystems, Carlsbad, CA) using the following conditions. Step 1: 96°C 10 min, Step 2: 96°C 30 sec, Step 3: 70°C 30 sec. Steps 2–3 were repeated 40 times. Due to the number of samples tested and the timing of collection, *HLA-G* qPCR assays were run on two 384 well plates. cDNA from JEG3, a choriocarcinoma cell line that expresses HLA-G at high levels, was diluted and run on each plate to verify linear amplification of each run. Amplified cDNA from one airway epithelial cell sample was run on both plates and used as the reference for comparison across plates. We analyzed the expression data using the delta delta Ct method²³.

miRNA quantitation

One hundred nanograms of small RNAs were converted to cDNA using the TaqMan miRNA reverse transcription kit (Applied Biosystems, Foster City, CA). Small RNA quantitation of *miR-148a*, *miR-148b*, *miR-152*, and *U6* levels were performed using Taqman Small RNA assays (Applied Biosystems, Foster City, CA). RT synthesis and TaqMan quantitation were carried out following the manufacturer's instructions. The delta delta Ct method (using U6 as the reference gene) was used to analyze our data²³.

Statistical analysis

Data were analyzed using R (V2.11.1). *HLA-G* transcript, sHLA-G protein data, and miRNA measurements were normalized by quantile transformation. We used linear regression to test for associations between transcript levels or protein concentrations and race, gender, and processing batch or plate effects. Significant variables were included as covariates in subsequent studies of genetic associations with each SNP. Linear regression was used to test for additive genetic effects and for genotype by maternal asthma interaction effects on *HLA-G* transcript and protein data, as well as for differences in miRNA levels by maternal asthma status. A t-test or ANOVA was used to compare mean values between genotype and maternal asthma subgroups.

Differences in clinical characteristics between subjects with and without a mother with asthma were analyzed using the Wilcoxon Rank-Sum test for continuous variables and Fisher's test for categorical (discrete) variables.

RESULTS

Clinical characteristics of adult asthmatics with and without an asthmatic mother

The clinical profiles of asthmatic individuals with and without an asthmatic mother are shown in Table I. There were no significant differences between the two groups after accounting for multiple testing, although the asthmatics with an asthmatic mother were younger at the time of the current studies (mean age 30.8 years vs. 38.7 years, $P=0.04$) and had a younger age at the time of asthma diagnosis (mean age 4.0 years vs. 9.5 years, $P=0.05$).

sHLA-G levels in African American and European American subjects

Because our sample is comprised of both African American ($n=47$) and European American ($n=10$) subjects, we first compared sHLA-G concentrations in the plasma and BAL fluid between these two groups. Normalized sHLA-G plasma concentrations were significantly elevated in African American (median=0.17; $N=47$) compared to European American (median=-1.16; $N=10$) subjects ($P=0.002$) (Figure E1a). Neither concentrations of sHLA-G in BAL fluid nor the levels of airway epithelial cell miR-152 family differed between these groups (Figures S1b and S2). As a result, we included race as a covariate only in analyses of sHLA-G concentrations in plasma.

We tested for differences in +3142 and +36 allele frequencies by ethnicity in asthmatic individuals from our entire original asthma genetic studies population¹², using a chi square test. This population includes all 57 individuals within the current study. The minor allele frequencies (MAF) for +3142 is 0.42 and 0.48 and +36 is 0.50 and 0.49 in African American and Caucasian individuals. We did not detect significant differences in +3142 or +36 allele frequencies by ethnicity (chisquare test $P=0.2$ and 0.8 for +3142 and +36 respectively).

sHLA-G concentrations vary by HLA-G +36 and +3142 genotypes in the lung but not in plasma

To determine if the *HLA-G* promoter (+36) and 3' UTR (+3142) polymorphisms are associated with sHLA-G concentrations, we stratified sHLA-G protein levels measured in BAL fluid and in plasma by genotype at these SNPs. Concentrations differed significantly by genotype in the BAL fluid, with decreasing concentrations of sHLA-G associated with increasing numbers of +3142G or +36T alleles (+3142, $P=0.046$; +36, $P=0.007$) (Figure 1a). This association was not present in the plasma (+3142 $P=0.13$; +36 $P=0.14$) (Figure 1b). Trends were similar in the African American only sample (Figure E3). In contrast, *HLA-G* transcript levels in airway epithelial cells did not vary by +3142 or +36 genotype ($P=0.34$ and 0.94 , respectively) (Figures 1c).

sHLA-G concentrations do not vary by maternal asthma status

Mean levels of sHLA-G protein did not differ significantly by maternal asthma status in BAL fluid (Figure 2a) or in plasma (Figure 2b) ($P=0.46$ and $P=0.87$, respectively).

HLA-G +3142 genotype interacts with maternal asthma status to influence sHLA-G protein concentrations in the lung

We next tested for an interaction between the *HLA-G* genotype of the subject and the asthma status of his/her mother on sHLA-G protein concentrations in the subject's BAL

fluid and plasma. Because there were no +3142 CC subjects who had a non-asthmatic mother in this sample, we combined the +3142 CC and CG genotypes at +3142 for comparison against the +3142 GG genotype group. For consistency, we also combined the +36 CC and CT genotypes (results for all three genotypes at both SNPs are shown in Figure E4). We observed higher concentrations of sHLA-G in subjects with an asthmatic mother who carried the +3142C or +36C alleles compared to those with the +3142 GG or +36 TT genotypes ($P=0.004$ and 0.004 , respectively). Concentrations of sHLA-G did not differ by genotype at either SNP among subjects with a non-asthmatic mother (0.94 and $P=0.37$ respectively). This resulted in a significant interaction between +3142 genotype and maternal asthma status on sHLA-G concentrations ($P=0.034$) (Figure 3a). The interaction with +36 genotype was not significant ($P=0.16$), although the trend was similar to that observed for +3142 (Figure 3b). Concentrations of sHLA-G did not differ between subjects with the CG or GG genotypes and a non-asthmatic mother. Surprisingly, sHLA-G concentrations in individuals with the C allele and an asthmatic mother were the highest of the four groups (ANOVA, $P=0.02$). Specifically, sHLA-G levels in BAL fluid from individuals with the C allele and an asthmatic mother were significantly elevated compared to individuals with the C allele and a non-asthmatic mother (t-test, $P=0.02$). There were no significant differences in sHLA-G concentrations by maternal asthma status in individuals with the GG genotype (t-test; $P=0.45$). This pattern suggests that the observed interaction is due in large part to elevated concentrations of sHLA-G in subjects with a +3142C allele and an asthmatic mother.

The miR-152 family is elevated in airway epithelial cells from asthmatic individuals with an asthmatic mother

To determine whether the genotype effects on sHLA-G protein levels in the airways of children of asthmatic mothers only is associated with the level of miR-152 family of miRNAs in airway epithelial cells from these subjects, we measured *miR-148a*, *-148b*, and *-152* levels. A significant batch effect was detected for *miR-148b* levels ($P=0.03$), thus batch was included as a covariate in all analyses of airway epithelial cell miR-152 family levels. *miR-148b* was significantly elevated in the airways of subjects with an asthmatic mother compared to those with a non-asthmatic mother ($P=0.04$; Figure 4a). A similar, but non-significant, trend was observed for *miR-148a* and *miR-152* levels ($P=0.52$ and 0.31 , respectively; Figures 4b, 4c, and S2).

To determine if the miR-152 family is also elevated in blood cells from asthmatics with a history of maternal asthma, we measured miR-152 family levels in white blood cells from a subset of the individuals tested above. Because white blood cell *miR-148a* levels were significantly higher in female subjects compared to male subjects ($P=0.03$), we included gender as a covariate in these analyses. White blood cell miR-152 family levels did not differ between subjects with and without a mother with asthma (*miR-148b*; $P=0.77$, *miR-148a*; $P=0.42$, *miR-152*; $P=0.21$) (Figure 4D–F).

DISCUSSION

The prenatal environment is influential in determining fetal risk for diseases with onset in childhood and throughout adult life^{9, 10, 24, 25}. We and others have suggested that the well-established increased risk for asthma among children of asthmatic mothers is due, at least in part, to their unique prenatal exposures^{26–31}. We previously reported an interaction between fetal *HLA-G* genotypes and maternal asthma status on subsequent risk for the development of asthma in childhood^{12, 15}, and hypothesized that the mechanism might be mediated by miRNAs¹⁵. Here, we provide evidence supporting this hypothesis, and demonstrate that prenatal effects of maternal asthma on the regulation of fetal genes in airway cells persist well into adulthood.

In this study we demonstrated that the *HLA-G* +3142 genotype is associated with sHLA-G levels in the airways, but not in peripheral blood, of individuals with asthma. Although sHLA-G concentrations did not vary significantly by maternal asthma status, a significant interaction was observed when evaluated in combination with *HLA-G* genotype. Importantly, the association between +3142 genotype and sHLA-G concentrations in the airways is only present in individuals with an asthmatic mother. We also observed elevated levels of *miR-148b* in freshly-isolated airway epithelial cells from these individuals compared to those collected from subjects with a non-asthmatic mother. A similar trend is observed in the additional miR-152 family members (*miR-148a* and *miR-152*). In contrast, there is no evidence for associations between genotype and sHLA-G protein concentrations in plasma, and no differences in levels of the miR-152 family in white blood cells of individuals with or without a mother with asthma. These data provide support for the miR-152 family regulation of sHLA-G concentrations in a +3142 genotype-specific manner in the airways of individuals with an asthmatic mother, and are consistent with our previous studies demonstrating miR-152 family targeting of the *HLA-G*+3142 G allele using luciferase constructs in an airway epithelial cell line¹⁵. Moreover, the lack of evidence for genotype effects on *HLA-G* transcript levels in airway epithelial cells suggests that miR-152 family members regulate HLA-G by inhibiting translation and not by the more common mechanism of degrading mRNA³². This is in agreement with studies showing decreased expression of HLA-G protein, but not *HLA-G* RNA, in JEG3 cells over-expressing *miR-152*³³. The JEG3 cell line has the +3142 GG genotype, and therefore has an intact miR-152 family target site. The absence of genotype associations with transcript levels in our studies also suggests that the association between sHLA-G protein levels in BAL fluid and the promoter-tagging SNP at +36 is likely due to LD with +3142 and not an independent effect on transcription.

Collectively, these results support a role for the miR-152 family in the molecular mechanism that underlies the interaction between maternal asthma status and the child's *HLA-G* genotype on the subsequent asthma risk in her children^{12, 15, 20}. We report here significantly higher levels of *miR-148b* in airway epithelial cells of individuals with an asthmatic mother compared to individuals with a non-asthmatic mother, and significant +3142 genotype effects on sHLA-G protein concentrations only in individuals with an asthmatic mother. The lower concentrations of sHLA-G among +3142 GG individuals with an asthmatic mother, compared to individuals with the C allele and an asthmatic mother, could promote the asthma protective effect observed in our earlier study¹⁵.

Our results also provide unexpected insights into the previously observed interactions^{12, 15}. In particular, we observed overall low concentrations of sHLA-G among asthmatics with a non-asthmatic mother, regardless of *HLA-G* +3142 genotype. Although the lack of a genotype effect is consistent with minimal or no effects of miR-152 family targeting in these individuals (due to lower levels of the miR-152 family), we expected to observe sHLA-G concentrations similar to those of the +3142 CC individuals with an asthmatic mother, which would also be unaffected by the miR targeting. Instead, sHLA-G concentrations are highest among +3142 CC asthmatics with an asthmatic mother, and are similar and low among the other maternal asthma-genotype groupings (Figure 3a). These findings suggest that sHLA-G concentrations may be higher overall among individuals with an asthmatic mother due to an as yet unidentified mechanism that is likely independent of *HLA-G* genotype. The higher levels of the miR-152 family in individuals with an asthmatic mother reduce sHLA-G concentrations among individuals with the +3142 GG genotype to levels equivalent to those seen in individuals with a non-asthmatic mother. This likely also contributes to the previously observed interaction effects on asthma risk, in which risks were highest among children of asthmatic mothers with a +3142C allele (or the promoter SNP in LD with +3142)^{12, 15}. Moreover, these data would suggest that the pathogenesis of asthma

may be different among offspring of asthmatic versus non-asthmatic mothers, with HLA-G-mediated effects playing a more important role in the former group and HLA-G-independent effects being more important in the latter group.

The role of HLA-G in the onset or promotion of asthma is currently unknown. This protein is best known for its role in pregnancy, during which time it is highly expressed by fetal placental cells and is thought to promote maternal tolerance of the genetically foreign fetus³⁴. However sHLA-G is expressed in adult tissues where it is thought to have pro-angiogenic and pro-inflammatory effects^{35–37}, and is variably expressed during development of the fetal lung⁴³. Moreover, sHLA-G levels are elevated in subjects with different inflammatory diseases^{38–42}. Of relevance to this study is that sHLA-G was elevated in the lung of adults¹³ and in the plasma of children⁴⁴ with asthma in two independent studies. Although our data is supportive of these findings, our study was not designed to address this question. In addition, we could not assess interaction effects between maternal asthma status and *HLA-G* genotype on sHLA-G levels in the airways, or between maternal asthma status and miR-152 family levels, in non-asthmatic (control) subjects. As a result we do not know if the effects reported here are specific to individuals with asthma. Lastly, we did not address the mechanism for higher expression of the miR-152 family in the airways of offspring of asthmatic mothers or whether this effect is specific to this family of miRNAs or more global, questions that we are currently evaluating.

In summary, we present data that is consistent with a miRNA-mediated mechanism of regulation of sHLA-G concentrations that could explain our previous observations of statistical interactions between maternal asthma status and the *HLA-G* genotype of her child on the child's subsequent risk for asthma. Our studies provide compelling evidence that *HLA-G*+3142 is the causal variant for the observed association in airway epithelial cells, an abundant cell type in the lung that is the primary site of HLA-G expression in the airways, and that prenatal exposures in fetuses of asthmatic mothers have life-long effects on the regulatory landscape in specialized cells in the airways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

HLA-G	Human Leukocyte Antigen
BAL	bronchial alveolar lavage
SNP	single nucleotide polymorphism
LD	linkage disequilibrium
miRNA	microRNA
FEV₁	forced expiratory volume
MAF	minor allele frequency

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KEY MESSAGES

- *miR-148b* is elevated in asthmatics with a history of maternal asthma, and correlates with HLA-G levels in the BAL fluid of these individuals in a +3142 genotype specific manner.
- Variations in miR-148b, and subsequently HLA-G concentrations in BAL fluid, is a possible mechanism via which maternal asthma influences the gene regulatory landscape of adult children with asthma.

Figure 1.

sHLA-G concentrations stratified by genotype in BAL fluid and plasma. *HLA-G* +3142 (left panels, x-axis) and +36 (right panels) genotypes and normalized sHLA-G concentrations in BAL fluid (A) and in plasma (B). +3142 and +36 *HLA-G* genotypes and transcript levels in airway epithelial cells (C). Grey boxes represent the interquartile range, encompassing the first through third quartile; the horizontal bar shows the median value. Values greater than 1.5 times the interquartile range are plotted outside of the whiskers. P-values from linear regression under an additive model.

Figure 2. sHLA-G concentrations in BAL fluid and plasma stratified by maternal asthma status. Normalized sHLA-G concentrations in BAL fluid (A) and in plasma (B).

Figure 3. sHLA-G concentrations stratified by genotype in individuals with and without a history of maternal asthma. *HLA-G* genotypes at +3142 (A) and +36 (B) are associated with concentrations of sHLA-G in BAL fluid from individuals with an asthmatic mother but not in individuals with a non-asthmatic mother. The P-values for an interaction effect between maternal asthma and *HLA-G* genotype in the child on sHLA-G concentrations in BAL fluid is 0.036 for *HLA-G*+3142 and 0.12 for *HLA-G*+36.

Figure 4. miRNA-152 family levels stratified by maternal asthma status. Levels of *miR-148* family in the airways of adult children with an asthmatic mother (A–C), and in circulating white blood cells (D–F).

Table I
Clinical characteristics of the subjects at the time of the current study

None of the subjects in our studies were current smokers or had a history of smoking (>5 pack years). P values correspond to comparisons between adult asthmatics with an asthmatic mother and adult asthmatics without an asthmatic mother.

	All ⁺ (N=57)	Maternal Asthma (N=22)	No Maternal Asthma (N=32)	P value
Age at time of current study (mean years $\pm$ SD)	35.9 $\pm$ 13.8	30.8 $\pm$ 13.7	38.7 $\pm$ 13.6	0.04
Age Asthma onset (mean years $\pm$ SD)	7.6 $\pm$ 8.8	4.0 $\pm$ 4.3	9.5 $\pm$ 10.5	0.05
Ethnicity (# Af Am/# Eur Am)	47/10	17/5	27/5	0.72
Gender (%Male)	36	41	34	0.77
Atopy (%)	95	93	95	0.79
Blood eosinophil count (K/uL) (median(upper quartile, lower quartile))	0.2(0.1,0.4)	0.3(0.1,0.3)	0.2(0.1,0.6)	1.0
IgE (IU/ml) (median (upper quartile, lower quartile))	99.5(42.5,415.5)	123(73,276)	76(35.5,356.5)	0.23
FeNO (ppb) (median (upper quartile, lower quartile))	19.0(10.5,38.0)	23.0(11.0,43.0)	19.0(12.5,36.5)	0.90
FEV ₁ % predicted (mean $\pm$ SD)	72.3 $\pm$ 20.2	72.9 $\pm$ 17.8	73.6 $\pm$ 21.8	0.94
PC20 [‡] (median (upper quartile, lower quartile))	2.0(0.5,7.1)	2.2(1.3,12.1)	1.2(0.1,7.0)	0.20
Bronchodilator reversibility [§] (median (upper quartile, lower quartile))	16.6(11.6,31.8)	12.3(11.4,33.8)	16.5(11.8,29.9)	0.85

⁺Maternal asthma status is not known for three individuals

[‡]37 subjects underwent methacholine challenge studies, 16 of 37 had a PC20 (Maternal Asthma, n= 8; No Maternal Asthma, n = 8)

[§]20 subjects had measurements of FEV₁ pre- and post-bronchodilator (Maternal Asthma, n=5; No Maternal Asthma, n = 13)