

Maternal microchimerism protects against the development of asthma

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Background: Maternal asthma and child's sex are among the most significant and reproducible risk factors for the development of asthma. Although the mechanisms for these effects are unknown, they likely involve nonclassical genetic mechanisms. One such mechanism could involve the transfer and persistence of maternal cells to her offspring, a common occurrence known as maternal microchimerism (MMc). MMc has been associated with many autoimmune diseases but has not been investigated for a role in asthma or allergic disease. **Objective:** We hypothesized that some of the observed risks for asthma may be due to different rates of transmission or persistence of maternal cells to children of mothers with asthma compared with children of mothers without asthma, or to sons compared with daughters. We further hypothesized that rates of MMc differ between children with and without asthma.

Methods: We tested these hypotheses in 317 subjects from 3 independent cohorts by using a real-time quantitative PCR assay to detect a noninherited HLA allele in the child. **Results:** MMc was detected in 20.5% of the subjects (range 16.8%-27.1% in the 3 cohorts). We observed lower rates of asthma among MMc-positive subjects than among MMc-negative subjects (odds ratio, 0.38; 95% CI, 0.19-0.79; $P = .029$). Neither maternal asthma nor sex of the child was a significant predictor of MMc in the child ($P = .81$ and $.15$, respectively). **Conclusions:** Our results suggest for the first time that MMc may protect against the development of asthma. (*J Allergy Clin Immunol* 2013;■■■:■■■-■■■.)

Key words: Asthma, maternal, microchimerism

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Asthma is a common disease with a multifactorial etiology that involves both genetic and environmental risk factors.¹⁻⁵ Epidemiologic and birth cohort studies have revealed intriguing sex-specific effects on risk that are poorly understood.⁶⁻¹¹ For example, maternal asthma and male sex are among the most significant and reproducible risk factors for the development of childhood-onset asthma, whereas paternal asthma and female sex are more significant risk factors for asthma with onset at older ages,^{6,7,11-13} and these risk factors can vary in sons compared to daughters.¹⁴ These effects are unlikely to be due to classical genetic mechanisms, and have been variously attributed to X-linked¹⁵ or mitochondrial^{16,17} inheritance, imprinting,^{18,19} and hormonal factors.^{20,21} Yet none has provided satisfactory explanations for these sex-specific patterns, suggesting that other as yet unidentified mechanisms play a role.

Microchimerism (Mc) refers to the presence of small numbers of nonself cells in an individual. The normal exchange of cells between the mother and the fetus during pregnancy is the most common source of Mc. The presence of maternal cells in her offspring is referred to as maternal Mc (MMc), while the presence of fetal cells in the mother is referred to as fetal Mc. MMc is detectable in approximately 17% to 55% of healthy children,^{22,23} and can persist in the offspring well into adulthood (at least to age 69 years).²⁴ Fetal Mc is detectable in 22% to 31% of healthy women with at least 1 previous pregnancy,²⁵⁻²⁷ and has been detected up to 40 years after childbirth.²⁵ The roles that these foreign cells play in human health and disease are controversial, with evidence supporting both beneficial and harmful effects to the host.^{28,29} In addition, although the precise mechanisms through which foreign cells influence either health or disease are unknown, a study of 31 healthy women characterized peripheral blood MMc cells as T cells in 25% of women, B cells in 14% of women, monocytes/macrophages in 16% of women, and natural killer cells in 28% of women.³⁰ The investigators suggested

Abbreviations used

COAST: Childhood Origins of ASThma
 GE: Genome equivalents
 IIS: Infant Immune Study
 Mc: Microchimerism
 MMc: Maternal Mc
 NIMA: Noninherited maternal allele
 ORs: Odds ratios
 PBMCs: Peripheral blood mononuclear cells
 T1D: Type 1 diabetes
 WB: Whole blood

that persistent chimeric cells may be stem cell-like and able to differentiate into cell types that perform diverse immunological, and possibly other, functions.

Both fetal^{28,29,31,32} and maternal^{23,29} Mc has been associated with many autoimmune diseases and, more recently, with nonautoimmune diseases, such as cancer.^{28,33} However, to date, a role for MMc in asthma or any allergic disease has not been investigated. We hypothesized that rates of MMc differ between children of asthmatic and nonasthmatic mothers, between sons and daughters, and between children with asthma and children without asthma. To test these hypotheses, we studied 91 asthmatic and 220 nonasthmatic subjects from 3 cohorts. Our study suggests that MMc protects against the development of asthma in childhood, but does not support a role for MMc in the increased risks associated with maternal asthma or for the observed sex ratio skewing in asthma.

METHODS**Subjects**

This study was performed in subjects from 3 independent cohorts, described below.

Childhood Origins of ASThma Cohort. The Childhood Origins of ASThma (COAST) study is a prospective birth cohort study conducted in Madison, Wis. The cohort included 289 children with at least 1 parent with respiratory allergies and/or a history of physician-diagnosed asthma who were enrolled between 1998 and 2000, as previously described.³⁴ DNA, isolated from whole blood (WB), was available from 107 mother-child pairs with an informative noninherited maternal allele (NIMA; for description, see Detection of MMc section); all children were sampled between the age of 1 and 7 years.

Asthma in the child was diagnosed at age 6 and at age 8 years on the basis of the presence of at least 1 of the following in the previous year: (1) physician-diagnosed wheezing, (2) use of albuterol for coughing or wheezing as prescribed by a physician, (3) use of daily asthma medication, (4) step-up plan including use of albuterol or short-term use of inhaled corticosteroids during illness, or (5) use of prednisone for asthma exacerbation. Subjects were classified as positive for atopy by skin prick testing when the mean diameter of the wheal (half the sum of the largest diameter and its perpendicular measurement) was 3 mm or more in response to any of the following allergens: eastern tree mix, grass mix, weed mix, ragweed, *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog epithelium, cat hair, and American/German cockroach. EDTA-anticoagulated WB was collected during yearly visits, and DNA was isolated by using Gentra Puregene DNA extraction reagents from the WB (QIAGEN, Inc, Germantown, Md; 158389). One DNA sample, collected from each child between the ages of 1 and 7 years, was selected for the studies. The study was approved by the University of Wisconsin Human Subjects Committee and the University of Chicago Institutional Review Board.

HLA genotypes in the COAST children and their mothers were determined by using reverse strip blots.³⁵ The 107 mother-child pairs in this study included 24 pairs in which only the mother had asthma, 25 pairs in which only the child had asthma, 17 pairs in which both the mother and the child had asthma, and 41 pairs in which neither the mother nor the child had asthma. This sample is 86% European American, 8% African American, 4% Latino, and 2% Native American.

Tucson Infant Immune Study. The Tucson Infant Immune Study (IIS) is a prospective birth cohort study of 482 children who were enrolled between 1996 and 2004, without regard to asthma or allergy status in the parents.³⁶⁻³⁸ DNA, isolated from peripheral blood mononuclear cells (PBMCs), was available from 107 mother-child pairs with an informative NIMA; all children were sampled between age 1 and 8 years.

Asthma in the child was based on a doctor diagnosis at age 5 years in addition to either asthma symptoms or use of medication since the age of 4 years. Asthma was physician-diagnosed in the mothers. A diagnosis of atopy in the child was based on skin prick test reactivity to 18 allergens. Wheal sizes were measured by using the longest diameter plus the perpendicular diameter and the 2 diameters were summed. Wheal sizes of 3 mm or more after subtraction of the control wheal size were considered positive. Heparinized blood samples were collected during doctor's visits between age 1 and 8 years; DNA was isolated from ficoll-separated PBMCs by using QiaAMP DNA Blood Mini Kit (QIAGEN, Inc; 51106). The Tucson IIS was approved by the University of Arizona Institutional Review Board.

The Tucson IIS children and mothers were genotyped for the 10 HLA alleles that had validated quantitative PCR assays for Mc studies, as described above, using allele-specific primers.³⁹ The 107 mother-child pairs in this study included 13 pairs in which only the mother had asthma, 9 pairs in which only the child had asthma, 2 pairs in which both the mother and the child had asthma, and 83 pairs in which neither the mother nor the child had asthma. This sample was 71% European American, 22% Hispanic, 2% African American, and 5% other ethnicities.

Hutterites. The Hutterites are a religious isolate who originated in Europe in the 16th century and now live in the northern plains states (United States) and western provinces (Canada).^{40,41} Our asthma studies in approximately 1200 South Dakota Hutterites are population-based, and include all individuals age 6 years and older who were home during our visits to their communal farms in South Dakota and were able to perform lung function and undergo methacholine challenge testing, as described.^{42,43} To best match the Hutterite sample to the COAST and Tucson IIS samples and to maximize the number of children with asthma in our study, we first selected all Hutterite children who were 12 years or younger at the time of our studies (N = 66) and then selected from that sample those with both a diagnosis of asthma and an informative NIMA (N = 18). We further included all their siblings with an informative NIMA, except for married sisters who were excluded to avoid the possibility of fetal Mc. This yielded a sample of 63 individuals from 18 families, only 3 of whom had a mother with asthma. Therefore, to increase the number of families with an asthmatic mother, we included 12 additional families (40 children) with a mother with asthma and an informative NIMA, also excluding married daughters. The final sample was composed of 103 offspring from 30 families (range of 1-10 children per family) and included 15 mothers and 38 children (range of 1-7 per family) with asthma. DNA for these individuals was isolated from WB.

Phenotyping studies were conducted in South Dakota between 1996 and 1997 and again between 2006 and 2009^{43,44}; the criteria for a diagnosis of asthma were (1) the presence of at least 2 symptoms (wheezing, cough, or shortness of breath), (2) a more than 20% fall in baseline FEV₁ at 25 mg/mL or less or a 15% improvement in baseline FEV₁ following inhalation of albuterol, and (3) a doctor's diagnosis of asthma. A diagnosis of atopy was based on a positive skin prick test result for reactivity to at least 1 of the 14 tested allergens. A reaction was considered positive if the mean diameter of the wheal size was 3 mm or more larger than the mean diameter of the negative control reaction. EDTA-anticoagulated WB was collected, and DNA was isolated by using an isopropanol precipitation-based protocol using an Autogen AGF3000 DNA Extractor (Autogen, Inc, Holliston, Mass). These studies were approved by The University of Chicago's Institutional Review Board.

TABLE I. Characteristics of the study samples

	Sample size (no. of mother-child pairs)	Male:female (% males)	No. with asthma (%)	No. with maternal asthma (%)	Age (y), mean $\pm$ SD (range)	Birth order, mean $\pm$ SD (range)	Samples of European descent (%)	DNA source
COAST	107	60:47 (56.1)	42 (39.2)	41 (38.3)	5.1 $\pm$ 1.6 (1-7)	2.0 $\pm$ 1.1 (1-6)	92 (85.9)	WB
Tucson IIS	107	50:57 (46.7)	11 (10.3)	15 (14.2)	4.2 $\pm$ 1.5 (1-8)	1.6 $\pm$ 0.92 (1-6)	76 (71.0)	PBMC
Hutterites	103	55:48 (53.4)	36 (37.1)	48 (46.6)	13.9 $\pm$ 5.8 (6-35)	3.8 $\pm$ 2.3 (1-10)	103 (100)	WB
Combined sample	317	165:152 (52.1)	91 (29.2)	104 (32.8)	7.7 $\pm$ 5.6 (1-35)	2.4 $\pm$ 1.8 (1-10)	271 (85.5)	–

Asthma status was unavailable for 6 offspring in the Hutterite families; these individuals were therefore included only in analyses of variables that could influence MMc rates but not in analyses of asthma. HLA typing in the Hutterites was performed by using a combination of serological and molecular methods, as previously described.⁴⁵

Detection of MMc

MMc detection was based on the presence of the noninherited maternal (HLA) allele, referred to as NIMA, as described previously.⁴⁶ We used validated assays for 8 common HLA alleles (DRB1*01, DRB1*15/16, DRB1*04, DRB1*07, DRB1*08, DRB1*14, DQA1*01, DQA1*05),³⁹ and considered the NIMA to be informative if it differed from both alleles present in the child. All NIMAs were confirmed to be present in the mother and absent in the child by allele-specific PCR in mother-child pairs prior to inclusion in studies of Mc.

Real-time quantitative PCR was performed by using a 7900HT Fast Real Time PCR System to detect the presence and quantity of maternal cells in the child, following established protocols (see Lambert et al³⁹ for details). Briefly, all reactions were performed in a final volume of 20 μ L by using TaqMan Universal Master Mix (Applied Biosystems, Foster City, Calif) and 300 μ mol/L of each amplification primer and 100 μ mol/L of the allele-specific probe. Each DNA sample was tested for the presence of the NIMA in 6 aliquots of approximately 20,000 genome equivalents (GE) each; the sensitivity of the assay is 1 cell/20,000 GE as determined by testing dilutions of 0.5, 1, 5, and 10 GEs of DNA that is homozygous for a specific allele, each in a background of 10,000, 15,000, 20,000, 25,000, and 30,000 GEs of DNA that is negative for that allele. Using a conversion factor of 6.6 pg of DNA per cell, 1 cell in a background of 20,000 GEs could be detected. The number of cells/GEs surveyed is calculated on the basis of a standard curve by using DNA from source DNA that is homozygous for the HLA allele (NIMA) being tested and amplified with control (β -globin) primers. GEs of NIMA per million host cells are determined by using standard curves generated with the same control DNA amplified with the HLA-specific primer. We required that a sample test positive in at least 1 out of 6 wells to be considered positive for Mc. The range of Mc detected is expressed as the number of maternal GEs within a sample containing 1 million host (child's) cells. All studies were performed in 1 laboratory and by a single individual (E.E.T.) who was blind to affection status, sex, and maternal asthma status of the samples at the time of the testing.

Statistical analyses

All analyses were performed by using mixed logistic models (SAS PROC GLMMIX), which allowed us to take into account the relatedness between Hutterite subjects. A random genetic effect for each Hutterite subject was modeled with a mean of zero and a covariance structure that is dependent on the relationships between pedigree members (using the kinship coefficient between them). The restricted pseudo-likelihood estimation method was applied to estimate the variance components. The best linear unbiased estimators of fixed effects were obtained with the generalized least square method.

Because the extent to which a number of potential variables influence rates of MMc is unknown, we first evaluated the effects of age of child, birth order, NIMA, sample (COAST, Tucson IIS, Hutterite), and DNA source (WB or PBMCs) on rates of MMc in the offspring by using a mixed logistic model. We

TABLE II. Number of MMc-positive (+) individuals and range of GEs in each cohort and the combined sample

	Sample size	MMc+, n (%)	Range of GE
COAST	107	18 (16.8)	4.1-925.2
Tucson IIS	107	29 (27.1)	0.2-312.0
Hutterites	103	18 (17.5)	1.5-374.7
Combined	317	65 (20.8)	0.2-925.2

then tested our hypotheses that the prevalence of MMc differs between (1) children of asthmatic mothers and children of nonasthmatic mothers, (2) sons and daughters, or (3) children with asthma and children without asthma by using logistic regression, and included in these analyses the significant covariate (DNA source) identified in the first analyses described above.

Power analyses

We used 100,000 permutations to assess the power of logistic regression to detect a significant result given odds ratios (ORs) of 1.5, 2.0, and 2.5, an average rate of MMc of 26.5%,^{22,23,39,47,48} and prevalence rates of 0.2 for maternal asthma, 0.15 for asthma, and 0.5 for female/male sex.

RESULTS

The composition of the 3 study samples is described in [Table I](#) and in [Table E1](#) in this article's Online Repository available at www.jacionline.org. In the sample of 317 mother-child pairs, we had power of 0.25, 0.22, and 0.33 to detect an OR of 1.5 for maternal asthma, asthma, and sex after 100,000 permutations; 0.60, 0.56, and 0.78 to detect an OR of 2.0; and 0.85, 0.81, and 0.96 to detect an OR of 2.5, respectively. Overall, we detected MMc in 65 of the 317 (20.5%) subjects, with rates of 16.8% in COAST subjects (GE range, 4.1-925.2), 27.1% in Tucson IIS subjects (GE range, 0.2-312.0), and 17.5% in Hutterite subjects (GE range, 1.5-374.7) ([Table II](#); see [Table E2](#) in this article's Online Repository at www.jacionline.org). These rates of MMc are within the range of those previously reported.^{23,39,49}

Before testing our main hypotheses, we first examined 6 variables that could potentially influence rates of MMc by using a multivariate logistic model ([Table III](#)). Rates of MMc did not significantly differ with respect to birth order ($P = .42$; see [Fig E1, A](#), in this article's Online Repository at www.jacionline.org), age ($P = .95$; [Fig E1, B](#)), ethnicity (European American, African American, Hispanic, other; $P = .62$), or HLA type of NIMA ($P = .30$; see [Table E3](#) in this article's Online Repository at www.jacionline.org). Both cohort (COAST, Tucson, Hutterite) and source of DNA (WB, PBMCs) were each significant predictors of MMc rates ($P = .039$ and $.036$, respectively), which were higher in the Tucson IIS cohort and in DNA derived from PBMCs. Because the only DNA derived from PBMCs was

TABLE III. Results of multivariate logistic regression analysis of potential covariates

Variable	β (SE)	P value
Variables with potential effects on the MMc rate		
Birth order	0.077 (0.096)	.42
Age	−0.003 (0.047)	.95
Ethnicity (European American as reference)		
African American	0.274 (0.748)	.71
Hispanic	−0.275 (0.537)	.61
Other	−1.339 (1.104)	.23
DNA source (WB as reference) [†]	0.785 (0.374)	.036
HLA type of NIMA (DRB1*07 as reference)		
DRB1*01	−0.357 (0.696)	.61
DRB1*15/16	0.671 (0.543)	.22
DRB1*04	0.22 (0.528)	.68
DQA1*05	0.798 (0.506)	.12
Other*	0.617 (0.593)	.29

*HLA alleles that occur in less than 10% of the sample were pooled (DRB1*01, DRB1*08, DRB1*14).

[†]DNA source (PBMc) is included as a surrogate for the Tucson IIS sample, which has the same effect on MMc rates (see text for details).

from the Tucson cohort, these 2 covariates were completely confounded. As a result, we could not determine whether the higher rate of MMc in the Tucson IIS cohort was due to the source of DNA (PBMcs) or due to other features of this sample that differ from the other 2, and we included DNA source (as a surrogate for Tucson) as a covariate in all subsequent analyses in the 317 mother-child pairs.

We first tested the null hypothesis that maternal asthma status was not a significant predictor of MMc in her offspring. We could not reject the null hypothesis because rates of MMc did not differ significantly between offspring of mothers with and without asthma (19.2% and 21.1%, respectively; $P = .81$; Table IV). We next tested the null hypothesis that sex of the offspring was not a significant predictor of MMc. MMc rates were higher in daughters (24.3%) than in sons (16.9%), but these differences were not significant (OR for MMc in daughters compared with sons: 1.57; 95% CI, 0.91–2.73; $P = .15$; Table IV) and we could not reject the null hypothesis.

Last, we tested the null hypothesis that MMc was not a significant predictor of asthma, including DNA source as a covariate. The rate of asthma was significantly lower among MMc-positive subjects than among MMc-negative subjects (15.4% and 32.1%, respectively; OR for asthma among MMc-positive compared with MMc-negative individuals: OR, 0.38; 95% CI, 0.19–0.79; $P = .029$; Table V and Fig 1). DNA source was also a significant predictor of asthma in this model (OR, 0.18; 95% CI, 0.09–0.37; $P = 4.4 \times 10^{-7}$), reflecting the lower prevalence of asthma in the Tucson IIS cohort in which the DNA source was PBMcs compared with the COAST and Hutterite cohorts in which the DNA source was WB. The different prevalence rates in the 3 cohorts reflect the different ascertainment schemes (see Methods section), and there was no interaction between MMc and DNA source on asthma risk ($P = .23$). The proportion of atopic subjects did not differ between MMc-positive and MMc-negative individuals, or between MMc-positive and MMc-negative individuals with asthma ($P = .17$ and $P = .73$, respectively; Table E1). Among the offspring with detectable MMc, the mean GEs were not significantly different between MMc-positive children with and without asthma (41.8 and 105.9,

respectively; $P = .43$) or between MMc-positive sons and daughters (88.2 and 102.0, respectively; $P = .62$).

DISCUSSION

The long-term survival of maternal cells in healthy individuals is a common occurrence,^{22,39} but the immunologic consequences of harboring maternal cells well into adulthood are still largely unknown. Our study implicates MMc for the first time in risk for asthma, suggesting a protective role for persisting maternal cells in her offspring.

Although both asthma and autoimmune diseases are immune-mediated conditions, their underlying immune aberrations may be complementary, or even opposite with respect to T-cell polarization and subsequent disease risk. For example, a meta-analysis of studies in more than 1000 European school children reported significantly reduced risk for the development of type 1 diabetes (T1D) by age 15 years among children with asthma (OR, 0.70; 95% CI, 0.54–0.91); interestingly, the protective effect was stronger with respect to asthma compared with other allergic conditions (atopy, atopic dermatitis).⁵⁰ The converse association was reported in study of 181 British school children with T1D, which showed a significantly reduced risk for self-reported ever wheezing compared with control children from the same study (OR, 0.36; 95% CI, 0.25–0.52).⁵¹ In the latter study, more frequent or more severe wheezing attacks were associated with a lower risk of T1D (trend $P < .01$). A recent meta-analysis of studies in almost 4000 children (including the 2 studies discussed above) reported a significantly reduced risk for asthma by age 18 years among children with T1D (OR, 0.82; 95% CI, 0.68–0.99).⁵² Moreover, while variants in many of the same genes are associated with both asthma and autoimmune diseases,⁵³ the effect is often in opposite directions, so that the allele associated with the risk for autoimmune disease is associated with protection from asthma (or allergic diseases), and vice versa.^{54–56} Those observations, combined with the results of our study, would be consistent with a model of opposite immune dysregulation in autoimmune disease and asthma, in which persistent MMc in the children predisposes to autoimmune diseases and protects from asthma and allergic diseases.

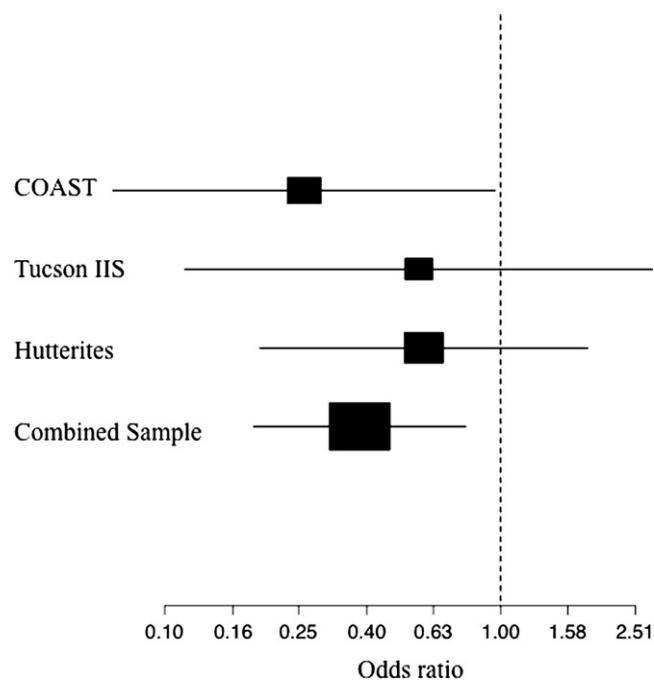
Further studies are warranted to both validate the results presented here and to elucidate the mechanism(s) by which persistent maternal cells in her offspring modulate the risk for asthma and other immune-mediated diseases. Moreover, although the higher rates of MMc in daughters than in sons (24.3% vs 16.9%) were not significant in this study, it is a potentially intriguing observation because it could suggest a mechanism for the higher prevalence of asthma in boys during childhood^{10,21} and the higher rates of autoimmune diseases in females throughout life.⁵⁷ Larger studies would be needed to further evaluate this observation, although studies of MMc and disease are challenging for a number of reasons. First, the availability of samples suitable for these studies is limiting because DNA must be available from mother-child pairs and informative markers with highly specific assays are required. Although these studies could be performed by using other informative genetic markers, HLA offers a highly informative and robust system for detecting Mc. However, HLA typing is not only expensive but also requires specialized assays that are not available in all laboratories. Moreover, validated assays for Mc studies are currently available for a limited number of HLA alleles. As a result, only approximately half of the

TABLE IV. Results of multivariate logistic analyses on maternal asthma and sex of child as predictors of MMc

	Sample size	MMc rates (%)	OR (95% CI)	DNA source		Main effect	
				β (SE)	<i>P</i> value	β (SE)	<i>P</i> value
Model 1: Maternal asthma (MA) as a predictor of MMc							
MA—No	213	21.1	1.12 (0.62-2.03)	0.57 (0.29)	.06	0.08 (0.32)	.81
MA—Yes	104	19.2					
Model 2: Male sex as a predictor of MMc							
Sons	165	16.9	1.57 (0.91-2.73)	0.52 (0.29)	.07	0.41 (0.28)	.15
Daughters	152	24.3					

TABLE V. Results of multivariate logistic analysis of MMc as a predictor of asthma

	Sample size	Asthma rate (%)	OR (95% CI)	DNA source		Main effect	
				β (SE)	<i>P</i> value	β (SE)	<i>P</i> value
Model 3: MMc as a predictor of asthma							
MMc—No	246	32.1	0.38 (0.19-0.79)	0.27 (0.052)	4.4×10^{-7}	0.13 (0.06)	.029
MMc—Yes	65	15.4					

**FIG 1.** Forest plot showing the ORs and 95% CIs for asthma risk among MMc-positive subjects. The box sizes are proportional to the sample sizes.

mother-child pairs in the cohorts in our study had an informative NIMA. Thus, although this is one of the largest studies of MMc to date, it is still relatively small. Second, the presence of Mc can vary in DNA derived from different sources (ie, peripheral blood vs tissues),^{39,48,58} and possibly by DNA isolation methods. As a result, it is essential that DNA samples from cases and controls within a single study are collected from the same blood components or tissues and processed by using identical protocols. The higher rate of MMc in the Tucson IIS cohort in this study could be due to DNA source (PBMc vs WB), although comparative studies of Mc rates in paired DNA samples from PBMcs and WB from the same individual are ambiguous, suggesting that

relative rates may differ depending on disease status or possibly other variables.⁵⁹

Last, while MMc in the periphery may have direct effects on immune development, it is likely that the presence and/or abundance of maternal cells in tissues, that is, the lung in this case, may differ between individuals with and without asthma, as has been observed in pancreatic beta cells in T1D²³ and muscle tissue in juvenile dermatomyositis.⁴⁷ However, performing MMc studies directly in lung tissues from asthmatic and non-asthmatic individuals has not been possible because maternal DNA (or HLA information) is rarely, if ever, available for adults who participate in studies of lung-derived cells. Nonetheless, future efforts to study MMc in lung-derived tissues from individuals with and without asthma may provide valuable insights into the mechanisms by which persistent maternal chimeric cells in her children protect them against the development of childhood asthma, and possibly suggest novel therapeutic interventions.

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Clinical implications: The transfer and persistence of maternal cells to her offspring during pregnancy may protect against the development of asthma, suggesting a previously unexplored mechanism that influences asthma risk.

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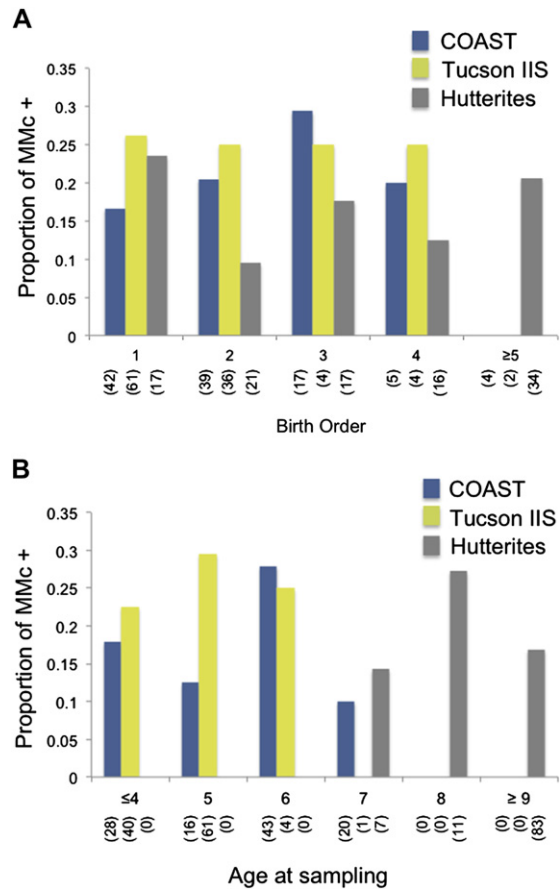


FIG E1. MMc rates by birth order (**A**) and age (**B**) in the COAST cohort (N = 107), the Tucson IIS cohort (N = 107), and the Hutterites (N = 103). Numbers of subjects are listed on the *x* axis for each category, and the proportion of MMc-positive subjects is shown on the *y* axis.

TABLE E1. Maternal asthma, sex ratios, and asthma and atopy status of COAST, Tucson IIS, Hutterite, and the combined samples

	COAST				Tucson IIS				Hutterites				Combined sample			
	Total (%)	M:F	MMc+ (%)	MMc+ M:F	Total (%)	M:F	MMc+ (%)	MMc+ M:F	Total (%)	M:F	MMc+ (%)	MMc+ M:F	Total (%)	M:F	MMc+ (%)	MMc+ M:F
Maternal asthma																
Yes	41/107 (38.3)	24:17	7/41 (17.1)	4:3	15/107 (14.0)	3:12	6/15 (40.0)	1:5	48/103 (46.6)	25:23	8/48 (16.7)	2:6	104/317 (32.8)	52:52	21/104 (20.2)	7:14
No	66/107 (61.7)	36:30	11/66 (16.7)	5:6	92/107 (85.9)	47:45	23/92 (25.0)	10:13	55/103 (53.4)	30:25	11/55 (20.0)	7:4	213/317 (67.2)	113:100	45/213 (21.1)	22:23
Asthma																
Yes	42/107 (39.3)	27:15	3/42 (7.1)	2:1	11/107 (10.3)	6:5	2/11 (18.1)	0:2	36/97 (37.1)	19:17	5/36 (13.9)	3:2	89/311 (28.6)	54:37	10/89 (11.2)	5:5
No	65/107 (60.7)	33:32	15/65 (23.1)	7:8	96/107 (89.7)	44:52	27/96 (28.1)	11:16	61/97 (62.9)	32:29	13/61 (21.3)	5:8	222/311 (71.4)	107:113	55/222 (24.8)	23:32
Atopy																
Yes	42/88 (47.7)	28:14	5/42 (11.9)	3:2	41/87 (47.1)	20:21	12/41 (29.3)	6:6	45/78 (57.7)	24:21	6/45 (13.3)	2:4	128/253 (50.6)	72:56	23/128 (18.0)	11:12
No	46/88 (52.3)	23:23	12/46 (26.1)	12:12	46/87 (52.9)	21:25	14/46 (30.4)	5:9	33/78 (42.3)	16:17	6/33 (18.2)	2:4	125/253 (49.4)	60:65	32/125 (25.6)	19:25

F, Female; M, male.

TABLE E2. Summary of quantitative PCR results in MMc+ subjects

Sample	MMc range (GE/million host cells tested)	Mean $\pm$ SD of GEs surveyed		Range of total GEs surveyed	
		In MMc+ subjects	In the total sample	In MMc+ subjects	In the total sample
COAST	4.1-925.2	83,136 $\pm$ 23,864	82,723 $\pm$ 30,475	41,325-127,165	20,513-149,245
Tucson IIS	0.2-312.0	89,806 $\pm$ 43,862	94,284 $\pm$ 34,387	12,453-142,641	12,453-148,527
Hutterites	1.5-374.7	74,241 $\pm$ 16,636	89,203 $\pm$ 27,075	53,999-101,266	32,560-131,522

TABLE E3. Number of subjects tested for each NIMA and corresponding number of MMc-positive subjects per sample

NIMA	COAST		Tucson IIS		Hutterites		Combined sample	
	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
DRB1*01	8	1	12	1	15	2	35	4
DRB1*15/16	19	4	19	7	9	1	47	10
DRB1*04	23	3	26	7	24	3	73	13
DRB1*07	21	3	16	4	9	0	46	7
DRB1*08	3	0	3	0	2	0	8	0
DRB1*14	1	0	0	0	1	0	2	0
DQA1*01	4	1	16	7	4	1	24	9
DQA1*05	28	5	15	3	39	11	82	20