

A common mitochondrial haplogroup is associated with elevated total serum IgE levels

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Background: Maternal history of asthma or atopy is among the most consistently reported risk factors for asthma and atopy in children, yet the molecular basis for this observation remains unclear. Mitochondria are inherited exclusively through the maternal line, raising the possibility that sequence variation in the mitochondrial genome contributes to the pathogenesis of asthma and atopy.

Objective: We set out to determine whether common European mitochondrial haplogroups are associated with asthma-related atopic phenotypes.

Methods: We studied 654 self-reported white children age 5 to 12 years with mild to moderate asthma participating in the Childhood Asthma Management Program. Eight haplogroup-tagging polymorphisms were genotyped with TaqMan probe hybridization assays, and mitochondrial haplogroup tests of association with asthma-related and atopy-related phenotypes were performed with haplo.stats.

Results: We found significant evidence of mitochondrial haplogroup association with total serum IgE levels (global significance, $P = .04$), with carriers of European haplogroup U (frequency 11%) having higher total serum IgE levels (median level, 684 IU/L) compared with noncarriers (389 IU/L; $P = .001$). Haplogroup U carriers also had trends of greater skin

prick test reactivity ($P = .03$) and higher frequency of atopic dermatitis ($P = .07$), although global haplogroup tests for these later 2 phenotypes were not significant at an α level of 0.05.

Conclusion: These data are the first to suggest that common mitochondrial haplogroups influence the atopic diathesis.

Clinical implications: These findings may provide a molecular explanation for the prominent influence of maternal history of atopy on the development of atopy in offspring. (J Allergy Clin Immunol 2007;120:351-8.)

Key words: Mitochondria, haplogroup, genetics, asthma, atopy, immunoglobulin E, atopic dermatitis, single nucleotide polymorphism, susceptibility

The preferential importance of maternal history of atopy or asthma (compared with paternal history) as a risk factor for asthma and atopy in the child is among the most consistent and reproducible observations in asthma epidemiology. Although it is clear that genetic contributions from the father are important, particularly regarding airway hyperresponsiveness,^{1,2} maternal history of asthma or atopy more strongly influence development of asthma and atopy per se.³⁻⁶ Data from several prospective birth cohorts suggest that a maternal (but not paternal) history of asthma confers substantial risk for development of persistent wheeze or asthma.^{7,8} This preferential maternal effect is also observed with atopic phenotypes, including atopic dermatitis,^{9,10} allergic rhinitis,^{6,11} and specific allergic sensitization.¹² Several groups have demonstrated that maternal history of atopy is an important modifier of the effects of various environmental factors on the development of asthma and atopy in the child, including early life exposure to cat, daycare, and endotoxin.¹³⁻¹⁵ Although these phenomena could be a result of *in utero* effects or shared postnatal environmental exposures, the consistency of these associations and their relatively early onset suggest a genetic contribution. Imprinting, a specific epigenetic phenomenon of preferential activation or silencing of autosomal loci depending on the parent of origin, is a potential explanation, yet evidence for it is scant and generally not reproducible.¹⁶⁻¹⁸ We propose another genetic mechanism explaining this predominant maternal influence: common sequence variation of the mitochondrial genome.

The mitochondrial genome is a circular double-stranded DNA structure of ~16.6 kb that replicates autonomously in mammalian mitochondria.¹⁹ Because spermatozoan mitochondria are not introduced into the ovum cytoplasm during fertilization, the mitochondrial

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Abbreviations used

CAMP: Childhood Asthma Management Program
 mtDNA: Mitochondrial DNA
 mtSNP: Mitochondrial single nucleotide polymorphism
 rCRS: Revised Cambridge Reference Sequence
 SNP: Single nucleotide polymorphism

genome is inherited exclusively (at least in human beings) through the maternal line.²⁰ Thus, genetic disorders caused by mutations mapping to the mitochondrial genome exhibit characteristic maternal inheritance. The mitochondrial genome encodes for 37 genes, including 13 mitochondrial proteins involved in the oxidative phosphorylation reaction in the mitochondria, as well as 22 transfer RNAs and 2 ribosomal RNAs required for protein synthesis. All of some 14 inherited disorders associated with rare mitochondrial DNA (mtDNA) coding mutations demonstrate a maternal pattern of inheritance.¹⁹ Common mitochondrial single nucleotide polymorphisms (mtSNPs) can be broadly organized into mitochondrial haplogroup clades that reflect the ancestral matrilineal composition of populations. In populations of European ancestry, 9 such haplogroups with frequencies of at least 1% have been described,^{21,22} which can be characterized using 8 haplogroup-tagging polymorphisms (Fig 1).²³ These haplogroups have been associated with several disorders that demonstrate maternal-predominant (but not exclusive) patterns of inheritance, including Parkinson and Alzheimer diseases.^{23,24} Although consistent replication of these findings remains elusive, these early associations suggest that common mitochondrial sequence variation may influence other common complex diseases such as asthma. Given the strong epidemiologic observations of a predominant maternal influence on asthma and atopy susceptibility, we hypothesize that common mitochondrial sequence variants affect the development of atopic phenotypes, and we have tested this hypothesis in a well characterized cohort of children with mild to moderate asthma. Herein we demonstrate evidence of association of a common mitochondrial haplogroup with total serum IgE levels, and trends of association with skin test reactivity and atopic dermatitis, supporting a role for mitochondrial sequence variation in the pathogenesis of atopic diathesis.

METHODS

Human subjects

The Institutional Review Boards of the Brigham and Women's Hospital and of the other Childhood Asthma Management Program (CAMP) study centers approved this study. Informed assent and consent were obtained from the study participants and their parents to collect DNA for genetic studies.

Population

The CAMP Study is a multicenter North American clinical trial designed to investigate the long-term effects of inhaled anti-

inflammatory medications in 1041 children with mild-to-moderate asthma.^{25,26} Enrollment criteria and study protocols are described elsewhere.^{25,26} A total of 968 children and 1518 parents contributed DNA samples, including those of self-reported white (654 children and 950 parents), African American (131 and 128), and Hispanic (86 and 94) ancestry. Given the relatively small sample sizes of the nonwhite ethnic groups in CAMP and the inability of European haplogroup-tagging variants to capture the common mitochondrial variation in these later groups (see Results), and to avoid spurious association caused by population stratification, association analyses were restricted to white probands. Total serum IgE was measured with radioimmunosorbent assays from blood samples collected during the CAMP screening sessions. Atopic dermatitis was defined as an affirmative response to the question, "Has your child ever had atopic dermatitis (eczema) for 2 consecutive years?" Ten core skin prick tests (see the legend to Table III) were administered, each considered positive if a wheal and/or flare developed with a mean diameter ≥ 3 mm and/or ≥ 10 mm, respectively.

Single nucleotide polymorphism genotyping

TaqMan 5'-3' assays (ABI, Foster City, Calif) based on the Revised Cambridge Reference Sequence (rCRS; GenBank accession J01415.2)²⁷ were designed for 8 mtSNPs that capture the common European mitochondrial haplogroup variation.²³ Fluorescent output was measured with the 7900HT Sequence Detection System (ABI) and processed with Sequence Detection Software v.2.1. We assessed genotype quality by (1) separation of genotype clusters, (2) genotype completion rate, (3) genotype concordance rates among 276 random samples genotyped twice for all 8 markers (2208 comparisons), and (4) genotype concordance rates for all 8 markers between all 687 available mother-offspring pairs (5496 comparisons).

Statistical analysis

Baseline frequencies and distributions were determined using R (v.2.4.1). Total serum IgE levels were log-transformed to achieve normal distribution. Haplogroup analysis was performed by using haplo.stats (v.1.2.1).²⁸ Haplogroup enumeration was performed by using the progressive insertion haplo.em algorithm with default settings (loci.insert.order=1:8, insert.batch.size=min(4,8), min.posterior=0.0001, tol=0.00001, max.iter=500, random.start=0). Haplogroup-phenotype association testing was performed by using the haplo.score function for total serum IgE levels, number of positive core skin prick tests, and atopic dermatitis (trait.type settings Gaussian, ordinal, and binomial, respectively) and restricting analysis to haplotypes with frequency ≥ 0.01 (skip.haplo=0.01). Analysis was restricted to 92% of subjects with complete genotype information. To reduce type I error resulting from testing all haplogroup-phenotype combinations individually, we adopted a conservative testing strategy in which haplogroup-specific associations were deemed significant only if first a global haplogroup test and subsequently the haplogroup-specific test were each significant at P values $< .05$. The global test simultaneously evaluates the trait distribution across all haplogroups, thus reducing the number of comparisons to 1 screening test per phenotype evaluated. All P values were empirically derived by permutation of phenotype data in 5000 simulated data sets.

RESULTS

Mitochondrial single nucleotide polymorphism genotyping

Torroni et al²¹ previously defined the common mitochondrial haplogroups among Western Europeans, describing 9 common haplogroups with frequencies $> .01$.

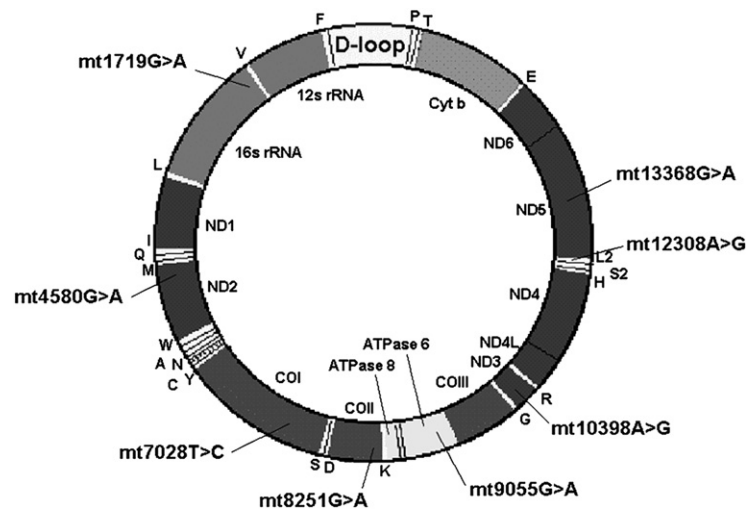


FIG 1. Mitochondrial DNA and European haplogroup-tagging polymorphisms. Positions (based on rCRS)²⁷ of European mitochondrial haplogroup-tagging SNPs. *Single letters* represent transfer RNA. *ATPase 6 and 8*, ATP synthase 6 and 8; *COI, COII, and COIII*, cytochrome C oxidase I-III; *Cyt b*, cytochrome B gene; *D-loop*, hypervariable region; *ND1-6 and ND4L*, NADH dehydrogenase, subunits 1-6 and 4L; *rRNA*, ribosomal RNA. Figure modified from mitomap.org.

TABLE I. Results of genotype quality control analysis for mitochondrial SNP

MtSNP	MAF	Genotype completion rate	Genotype discordances (n = 276)	Mother-child discordances (n = 687)
mt1719 G>A	0.064	0.98	0	0
mt4580 G>A	0.044	0.99	0	0
mt7028 T>C	0.400	0.99	0	5 (0.72%)
mt8251 G>A	0.056	0.98	0	0
mt9055 G>A	0.089	0.95	0	1 (0.15%)
mt10398 A>G	0.206	0.97	0	2 (0.30%)
mt12308 A>G	0.219	0.99	0	1 (0.43%)
mt13368 G>A	0.102	0.98	0	0
Overall	N/A	0.98	0	11 (0.18%)

mtSNP numbering based on their location in the rCRS.²⁷ MAF denotes minor allele frequency, that of the second allele listed, for 950 parents of self-reported white racial designation. Genotype discordance rates (replicate genotyping vs mother-child comparisons) are significantly different (Fisher exact test *P* value = .04).

For this initial survey of mitochondrial genetic variation in relation to atopic phenotypes, we selected 8 single nucleotide polymorphisms (SNPs) with minor allele frequencies of at least 0.05 that in combination efficiently tag these common European mitochondrial haplogroups.²³ Genotype completion rates were at least 95% at all 8 loci (Table I), and complete genotype information at all 8 loci was available in 92% of subjects. For each marker, genotype clusters separated into 2 distinct groups, with no observed instances of mitochondrial heteroplasmy at any of the 8 loci tested (Fig 2). Although no discordant genotypes were found on repeat genotyping of a random subset of 276 samples, we observed 11 instances of maternal-offspring genotype discordance (overall discordance rate of 0.18%). These discrepancies possibly suggest a low genotype error rate, but they may also represent novel mutation in the offspring, a possibility supported by the fact that mutation rates are higher in the mitochondrial genome²⁹ and the discordance rate among maternal-

offspring comparisons (11 of 5496 comparisons = 0.18%) was significantly higher than that in repeat genotyping (no discordances out of 2208 comparisons; Fisher exact test *P* = .04). However, because typical mitochondrial nucleotide substitution rates are several orders of magnitude lower ($\sim 2.4 \times 10^{-8}$)³⁰ than that implied from our data, it is reasonable to conclude that these 11 discordances reflect a very low genotype error rate of ~ 1.8 per 1000 genotypes rather than novel mutation. Regardless, these data demonstrate the high quality of the mitochondrial SNP genotyping generated in this survey.

Mitochondrial haplogroups in CAMP

The haplogroup frequency distributions in the CAMP parents of self-reported white, African American, and Hispanic ancestry are presented in Table II. Eleven haplogroups were observed at a frequency of ≥ 0.01 among the CAMP white parents, and in general, haplogroup

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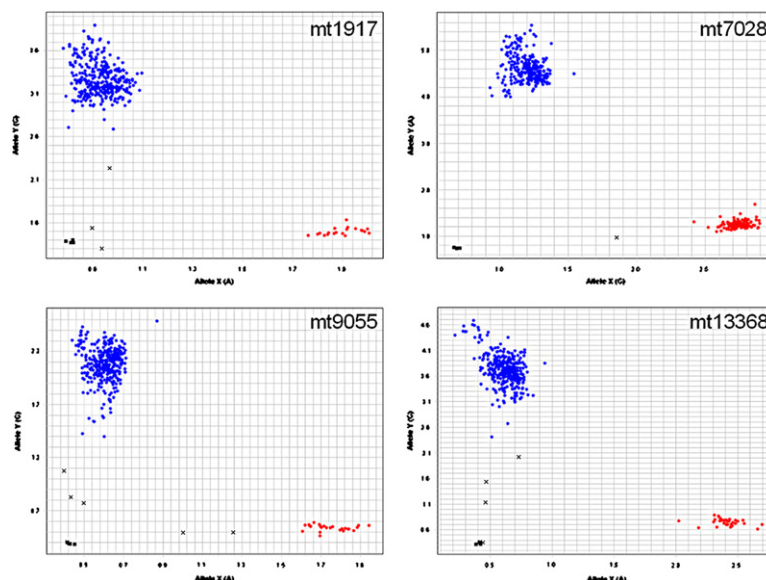


FIG 2. TaqMan genotype cluster plots for mitochondrial polymorphism. Representative plots of fluorescence intensities for minor allele (x-axis) and major allele (y-axis) for 4 genotyped mtSNPs. Circles represent allele calls. Boxes represent water-negative controls. X's represent ambiguous DNA calls and are considered missing for analysis. There was no evidence of heteroplasmy (as reflected by the absence of genotype calls in the upper right corner of each plot).

frequencies were similar to those observed in other studies of more homogenous populations of European ancestry,^{21,31} suggesting this cohort is also of European origin. In contrast, stark differences in mitochondrial haplogroup distribution were observed among individuals of African American or Hispanic descent. To study these populations would thus require entirely different panels of polymorphisms. Given the relatively small sample sizes of these ethnic groups in CAMP, and the inability of European haplogroup-tagging variants to capture the common mitochondrial variation in these groups, association analyses were restricted to the white probands.

Mitochondrial haplogroup association with atopic phenotypes

We next evaluated whether mitochondrial genetic variation influences asthma-related phenotypes in the population-based sampling of white asthmatic children participating in the CAMP study. These children exhibited characteristics consistent with the known demographic distributions of childhood asthma: there were a larger proportion of boys (60.1%), the majority of children had a maternal history of asthma or allergy (54.7%), and most were atopic, with only 12% having both total serum IgE levels < 100 IU/L and no evidence of skin test reactivity (Table III).

Because the CAMP study is a case-only family-based study, and because trio-based association studies of mitochondrial variation are not feasible given the mandatory transmission of maternal alleles, we were unable to test directly the hypothesis that mitochondrial genetic variation influences asthma-susceptibility *per se*. However, the CAMP study included detailed phenotypic evaluation at

baseline, including measurement of total serum IgE levels, skin test reactivity, total serum eosinophil levels, and measurements of airway responsiveness and lung function. Given the particularly strong relationship of maternal history of atopy as a determinant of subsequent atopy in the child, our primary hypothesis was that mitochondrial variation would most strongly influence atopy-related phenotypes. Indeed, we found strong evidence of mitochondrial haplogroup association with total serum IgE levels (global simulated empiric *P* value = .04, 11 *df*, based on 5000 permutations; Table IV). The highest total serum IgE levels were observed among individuals harboring the common haplogroup U (frequency, 10.6%), with a median level (interquartile range) of 684 IU/L (283-1505) among haplogroup U carriers compared with 389 IU/L (144-1047) among noncarriers (empiric *P* = .001). Importantly, *post hoc* analysis demonstrated that no haplogroup-tagging SNP alone was independently associated with any of these phenotypes (data not shown), suggesting that none of the tested haplogroup-tagging SNPs are causal, but that a putative causal variant is in linkage disequilibrium with the U haplogroup. We also note that although mitochondrial haplogroup global tests of association were observed for neither skin test reactivity (omnibus *P* value = .12) nor atopic dermatitis (*P* = .46), carriers of the elevated IgE-associated U haplogroup also tended to have greater skin test reactivity (haplogroup-specific *P* = .03) and report a history of atopic dermatitis more frequently (haplogroup-specific *P* = .07) compared with noncarriers. Other specific haplogroups demonstrated nonsignificant protective trends of association with skin test reactivity and atopic dermatitis, with carriers of the less common K2 haplogroup having lesser skin test reactivity (mean number

TABLE II. Mitochondrial haplogroup frequencies in CAMP*

Haplogroup	MtSNP								CAMP		
	mt1719 G>A	mt4580 G>A	mt7028 T>C	mt8251 G>A	mt9055 G>A	mt10398 A>G	mt12308 A>G	mt13368 G>A	White (n = 950)	African American (n = 128)	Hispanic American (n = 94)
H	G	G	C	G	G	A	A	G	0.396	0.031	0.043
I	A	•	T	A	•	G	•	•	0.026	0.016	—
J	•	•	T	•	•	G	•	•	0.117	0.873	0.183
K	•	•	T	•	A	G	G	•	0.063	—	—
K2	•	•	T	•	A	•	G	•	0.021	—	—
T	•	•	T	•	•	•	•	A	0.100	—	—
U	•	•	T	•	•	•	G	•	0.130	0.016	0.085
V	•	A	T	•	•	•	•	•	0.043	—	—
W	•	•	T	A	•	•	•	•	0.015	—	—
X	A	•	T	•	•	•	•	•	0.025	—	0.011
Z	•	•	T	•	•	•	•	•	0.039	0.024	0.667

*Haplogroup frequencies estimated using available parental genotype data. Haplogroups observed at greater than 1% in parents of self-reported European ancestry are presented. SNP names according to nucleotide position in rCRS.²⁷ • denotes allele is that observed in most common haplogroup (H).

of positive skin tests, 1.58; $P = .02$) and carriers of the most common haplogroup H (frequency, 44%) having a lower frequency of atopic dermatitis (24.6%; $P = .02$). Although we stress that only the haplogroup U association with elevated total serum IgE levels is statistically significant (ie, both the global test and the haplotype-specific test were significant), and the latter findings relating to atopic dermatitis and skin test reactivity are not (and should therefore be interpreted prudently), together they suggest that mitochondrial variation influences the atopic diathesis. In contrast to these findings, *post hoc* analysis of phenotypes more specifically related to lung function and inflammation (methacholine airways responsiveness, FEV₁, or blood eosinophil counts) showed no significant relationships with mitochondrial variation (global and individual P values $>.05$), suggesting that mitochondrial genetic variation specifically influences atopic features of asthma.

DISCUSSION

Despite substantial epidemiologic evidence supporting maternal inheritance of asthma and atopy, this is the first report exploring the relationship between these phenotypes and mitochondrial genomic sequence variation. Our preliminary evidence shows an association between common European mitochondrial haplogroups and atopic phenotypes in a cohort of children with mild to moderate asthma. The common mitochondrial haplogroup U, present in 11% of the cohort, was associated with significantly higher total serum IgE levels ($P = .001$), with haplogroup U-carriers having median IgE levels that were 78% higher than among carriers of all other haplogroups. Weaker, nonsignificant trends of association of this haplogroup were also observed with a higher incidence of atopic dermatitis ($P = .07$) and a greater degree of skin-test reactivity ($P = .03$). In contrast, mitochondrial haplogroups were not associated with lung function or airway

TABLE III. Baseline characteristics of self-reported white CAMP probands in this study*

Variables	Values
Sex, N (%)	
Male	393 (60.1%)
Female	261 (39.9%)
Age, y, mean (SD)	8.9 (2.1)
Age asthma onset, y, mean (SD)	3.0 (2.4)
Atopic dermatitis, N (%)	
Yes	187 (28.6%)
No	467 (71.4%)
FEV ₁ , postbronchodilator	
L, mean (std)	1.82 (0.49)
Percent predicted, mean (SD)	105.6 (13.5)
Methacholine PC ₂₀ , mg/dL, geometric mean (IQR)	1.08 (0.48-2.78)
Total serum IgE levels, IU/L, geometric mean (IQR)	403 (156-1075)
Blood eosinophil levels, cells/L, geometric mean (IQR)	400 (200-669)
No. of core positive skin prick tests, N (%)	
0	120 (18.4%)
1-2	162 (24.8%)
3-4	167 (25.5%)
5-6	116 (17.7%)
7 or more	89 (13.6%)
Parental history of asthma or atopy, N (%)	
Maternal	358 (54.7%)
Paternal	280 (42.8%)

*Ten core skin prick tests were performed that included *Dermatophagoides pteronyssinus* (Der p 1), *Dermatophagoides farinae* (Der f 1), cat and dog dander, American cockroach, German cockroach, *Penicillium*, *Aspergillus*, timothy grass, and short ragweed. IQR, Interquartile range.

responsiveness phenotypes. Taken together, these findings suggest that mitochondrial variation preferentially affects atopic diathesis and not lung function—a finding entirely consistent with the repeated observations of preferential influence of maternal history of atopy on atopic phenotypes,³⁻¹² rather than with lung function phenotypes.^{1,2}

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TABLE IV. Results of population-based association analysis of mitochondrial haplogroups with atopic phenotypes in the CAMP self-reported white participants with asthma

Haplogroup (frequency)	Total serum IgE levels			Atopic dermatitis			No. of positive skin tests		
	Median (interquartile range)	Score	<i>P</i> value*	Percent affected (n)	Score	<i>P</i> value*	Mean (interquartile range)	Score	<i>P</i> value*
H (0.444)	407 (159-1195)	−0.3	.74	24.6% (71)	−2.18	.02	3.21 (1-5)	−0.93	.35
I (0.025)	191 (123-497)	−0.78	.42	37.5% (6)	0.38	.72	2.80 (1-5)	−0.08	.93
J (0.118)	380 (135-1012)	−1.36	.18	30% (24)	0.2	.83	3.50 (1-5)	0.14	.91
K (0.044)	437 (229-724)	−0.02	.98	27.6% (8)	−0.1	.93	3.66 (1-5)	1.02	.32
K2 (0.019)	182 (67-421)	−1.82	.07	16.7% (2)	−0.86	.45	1.58 (0-2.5)	−2.36	.02
T (0.105)	553 (170-1392)	1.17	.25	28.6% (18)	0.22	.82	3.64 (1-5)	0.5	.63
U (0.106)	684 (283-1505)	3.14	.001	36.6% (26)	1.86	.07	3.79 (2-6)	2.27	.03
V (0.039)	253 (117-777)	−0.69	.5	29.2% (7)	0.27	.78	3.87 (1-8)	1.23	.21
W (0.015)	661 (138-4571)	0.38	.7	55.6% (5)	1.74	.11	3.50 (1-5.5)	−0.44	.65
X (0.027)	544 (345-912)	0.92	.36	25% (4)	−0.29	.77	3.00 (1-5)	−0.28	.79
Z (0.037)	355 (148-933)	−1.37	.17	33.3% (7)	0.94	.36	2.79 (0-4)	−1.78	.08

**P* values based on 5000 permutations (of phenotype). Global simulated *P* values are .04 for total serum IgE levels, .46 for atopic dermatitis, and .12 for number of positive skin prick tests. Statistical tests performed by using log-transformed total serum IgE levels. Score refers to haplo.score test statistic for log-transformed total serum IgE (Gaussian), atopic dermatitis (binomial), and number of positive skin prick tests (ordinal). Analysis restricted to 598 subjects with complete genotype data at all 8 loci (91.7% of cohort). Similar results were observed when all subjects were included.

Mitochondria are the primary source of endogenous production of free radicals, generated through oxidative phosphorylation as a byproduct of ATP synthesis.^{32,33} Thus, mitochondrial dysfunction has been implicated in the pathogenesis of several diseases characterized by free radical-induced damage,³³ including the specific patterns of neuronal degeneration characteristic of Parkinson and Alzheimer diseases.³² Genetic associations of specific mitochondrial haplogroups have been demonstrated with these disorders^{23,24} and with aging,³⁴ suggesting haplogroup-specific differences in mitochondrial free radical production. More specific to our study are several lines of evidence supporting a role for free radical generation and damage in the pathobiology of the allergic response. The production of endogenous reactive oxygen species is required for normal functioning of the acquired immune response, including normal T-cell activation,³⁵ B-cell differentiation, and T-cell and B-cell proliferation.³⁶ Chemically enhanced induction of intracellular oxidants in the Brown Norway rat results in T_H2-mediated autoimmunity typified by eosinophilic vasculitis, increased total serum IgE production,³⁷ and enhanced mast cell sensitization to IgE-triggered cytokine release.³⁸ Pretreatment with 1 of several antioxidants reduces this response, implicating reactive oxygen species in the T_H2 inflammatory response. Boldogh et al³⁹ have also demonstrated that allergen-specific IgE production is enhanced by simultaneous exposure to environmental free radicals. Finally, several studies have suggested therapeutic benefit of antioxidants in the treatment of allergic disease, including vitamin E supplementation and N-acetylcysteine.^{40,41} Although the precise mechanisms underlying the associations of mitochondrial haplogroups with atopy-related phenotypes remain speculative, haplogroup-specific differences in free radical production during oxidative phosphorylation may be 1 such mechanism.

The development of high-throughput genotyping technologies has greatly increased the feasibility of comprehensive association studies of the mitochondrial genome. Compared with studies of autosomal loci, mitochondrial haplotype enumeration for association testing is a relatively trivial process, particularly when no genotype data are missing, because most individuals are homoplasmic (that is, only 1 sequence type represented among the 100s to 1000s of genome copies per cell) and mitochondrial DNA recombination in human populations does not occur (or is extremely rare).⁴² However, the study of mitochondrial sequence in genetic association studies presents several unique challenges. First, the mitochondrial genome is highly polymorphic. A recent comparison by Saxena et al³¹ of 928 European mitochondrial DNA sequences identified 2349 variant sites, of which 144 had a minor allele frequency of $\geq 1\%$, suggesting a common polymorphism rate of 0.009 per base pair—approximately 10 times reported estimates of the autosomal genome.²⁹ However, this common variation explains only a small proportion of the total mitochondrial heterozygosity in the European population (72% for mitochondrial variants compared with 90% for autosomal variants). Thus, most studies of mitochondrial variation in common disease are underpowered to detect associations with rare variants. Saxena et al³¹ also demonstrated that surveys limited to testing the common European haplogroup-tagging SNPs capture a relatively small amount of this total variation, substantially reducing the likelihood of capturing associations with more common variants. Accordingly, this preliminary survey is also relatively underpowered: given the parameters of our study design (sample size of 593 subjects, 11 haplogroups, assuming that 1 haplogroup at 10% frequency increased log-transformed IgE levels by 0.28 SDs from the mean), the power to detect an association based on a global haplogroup test *P* value $<.05$ was

only 28%. We are therefore fortunate to have demonstrated the association of haplogroup U with total serum IgE levels. However, it is possible that other associations were missed, such as those with atopic dermatitis and count of positive skin tests that demonstrated trends of association but did not reach a threshold of statistical significance. Another challenge posed by the mitochondrial genome is the unusually high rate of spontaneous mutation in the noncoding control region (the hypervariable D-loop)—a 400-bp noncoding sequence implicated in the initiation of mtDNA replication and transcription. This high mutation rate currently precludes genotyping of D-loop variation other than by direct resequencing. Thus, all mtDNA association studies have ignored this region. A third complexity, with relevance to all genetic association studies, but perhaps particularly important in mitochondrial genetic studies, is the potential for false-positive association caused by occult population stratification.⁴³ Because mitochondrial haplogroup distributions are sensitive to differences in matrilineal heritage of populations, the presence of subtle admixture of 2 or more populations of distinct ancestral background can result in spurious associations with unlinked phenotypes. The problem is compounded because the transmission of mitochondrial variation is exclusively matrilineal, thus precluding the use of family-based designs, which can assess for differential nonrandom transmission of autosomal alleles from parent to child and are immune to population stratification.⁴⁴ We cannot conclusively prove that our findings are not a result of occult substructure; however, the strong similarity of the observed haplogroup distributions in CAMP with those in other surveys of more homogeneous European populations^{21,22,31} suggests that the self-reported white CAMP subjects are of European ancestry. Finally, we note that the haplo.em algorithm of in haplo.stats, like most haplotype-imputation methods, assume Hardy-Weinberg equilibrium²⁸—an assumption that does not hold in the context of mitochondrial homoplasmy. However, the Hardy-Weinberg equilibrium assumption is required only for haplotype imputation when marker phase is ambiguous. In the case of homoplasmic populations like CAMP, haplogroup phase is never ambiguous (there is only 1 haplogroup observed per subject), particularly when considering samples without any missing data. Under these conditions, haplo.em simply enumerates (rather than imputes) the observed haplotypes (which we confirmed by manual inspection of our data). We also note that because of the high genotype completion rates (>95% for each marker, with 92% of sample with complete data at all 8 markers), the small amount of missing data did not significantly alter our results, even though some haplogroup imputation was required: there was no significant difference in the estimated haplogroup frequency distributions or differences in the observed strength of the haplogroup U-IgE association whether the analysis was restricted to complete cases or not.

In summary, despite substantial epidemiologic evidence supporting maternal inheritance of asthma and atopy, this is the first demonstration that mitochondrial

genetic variation may influence atopy-related phenotypes, providing a novel biologic explanation for the strong maternal genetic influence on the inheritance patterns of atopic phenotypes. Although further replication in additional populations is warranted to assess the generalizability of these findings, testing of additional mitochondrial variation known to reside on the atopy-associated U-haplogroup should also be initiated in an attempt to identify causative variation.

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