# Supplementary Information

Decreased sphingolipid synthesis in children with 17q21 asthma-risk genotypes

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## Supplementary Methods

### Study Design/Subject enrollment

Parents of children between the ages of 5 and 17 years were recruited for enrollment in this case-control study with prevalent cases, from two ambulatory pediatric practices in New York City, NY which provide both pediatric primary care and pediatric pulmonology subspecialty care between 2/5/2013 and 5/25/2017. The pediatric practices are located in the boroughs of Manhattan and Queens and provide outpatient care for primarily low-income families. Case and Control subjects were recruited at the time of arrival at their pediatric appointment (routine pediatric primary care visit for controls, or asthma-specific pulmonology visits for cases) and were enrolled in the study if they agreed to participate and were able to undergo data collection either that day or scheduled within 2 weeks. Case and Control subjects were enrolled concomitantly and sequentially over time if they met inclusion/exclusion criteria (described below) without consideration of gender or age, with a goal to enroll a minimum of 50 subjects in each group based on a pre-enrollment power analysis. A schematic of the subject recruitment and enrollment process is shown in **Supplementary Figure 6**.

Children were classified as having asthma if they were given a diagnosis of asthma by the pediatric pulmonologist with whom they were an established patient and have been symptomatic within the last 2 years. Children with asthma were enrolled in the study at the time of their visit with the pulmonologist. Healthy control subjects were recruited out of the general pediatric practices at routine annual visits, and were provided a screening questionnaire to evaluate for any prior history of wheezing, bronchodilator administration by a provider or parent, or any history of breathing concerns. An affirmative history of difficult breathing in any context or ever bronchodilator use lead to the exclusion of the subject. Subjects for both case or control groups were additionally excluded if they reported via parental questionnaire, any acute illness or fever at the time of enrollment, a history of autoimmune or chronic inflammatory disease, sickle-cell disease, history of past or active malignancy, immunodeficiency, congenital lung malformations, chronic lung disease of prematurity or any history of prematurity of a gestational age of less than 35 weeks. For subjects with asthma, any parent who identified their child as being on oral steroids at the time of enrollment or were having acute asthma symptoms were excluded. Additional information was collected regarding any other co-exiting medical conditions which did not fall into exclusion criteria. One control subject and one asthma subject were excluded from final analysis after enrollment following review of medical record. The control subject was excluded for a history of prematurity (gestational age 29 weeks) and the asthma subject due to a history of systemic lupus erythematosus. Weight and BMI was obtained at time of enrollment; obesity was defined as a BMI $\geq$ the 95$^{th}$ percentile for age and was compared between both groups.

The study was approved by the Weill Cornell Institutional Review Board (protocol #1206012409) and the Western Institutional Review Board (protocol #20150736) for human subjects. Following informed consent, all subjects underwent collection of peripheral blood for

whole blood and plasma sphingolipid quantification, measurement of total IgE (IU/ml) and absolute eosinophil counts for characterization of allergic status, DNA isolation and genotyping at five 17q21 associated SNPs, and isolation of PBMCs for measurement of newly synthesized products of the *de novo* sphingolipid synthesis pathway. All subjects with absolute eosinophil counts of $\geq$300 cells/ml or a total IgE of $\geq$ 200 (IU/ml) at the time of data collection were classified as "allergic" for purposes of analysis.

**Quantitative Sphingolipid Determination**

Sphingolipids were quantified in plasma, whole blood, PBMCs and airway epithelial cells by high pressure liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-MS/MS) using minor modification of a described method (1). The method is validated for 5 dihydroceramides: (d18:0/16:0 d18:0/18:0, d18:0/18:1, d18:0/24:0, d18:0/24:1), 6 ceramides (d18:1/C16:0, d18:1/C18:0, d18:1/C20:0, d18:1/C22:0, d18:1/C24:0, d18:1/C24:1), 4 sphingomyelins (SM d18:1/C16:0, SM d18:1/C18:0, SM d18:1/C18:1, SM d18:1/C24:1), and 4 long-chain bases: sphingosine (SO d18:1), sphinganine (SA d18:0), sphingosine-1-phosphate (S1P d18:1), sphinganine-1-phosphate (Sa-1-P d18:0). 25 ul serum or whole blood were extracted by vortexing overnight in 900 ul dichloromethane / methanol (1:1) with addition of internal standard (N-lauroyl-D-erythro-sphingosylphosphorylcholine). After centrifugation to precipitate cell debris, an aliquot was transferred into an Agilent 1200 HPLC (Agilent Poroshell 120 column) linked to an Agilent 6430 triple quadrupole mass spectrometer. Mobile phase A consisted of methanol/water/chloroform/formic acid (55:40:5:0.4 v/v); Mobile phase B consisted of methanol/acetonitrile/chloroform/formic acid (48:48:4:0.4 v/v). After pre-equilibration for 6 sec, the gradient was increased gradually to 60% mobile phase B and 100% mobile phase B that was held for 1.9 min. With a flow rate is 0.6 mL/min, the duration of the entire run was 9.65 min. We used the Mass Hunter optimizer and pure synthetic standards (Avanti Polar Lipids) to

determine optimum fragmentation voltage, precursor/ product ions and m/z values. Peak calls and abundance calculations were obtained with MassHunter Workstation Software Version B.06.00 SP01/Build 6.0.388.1 (Agilent). Final concentrations were calculated from a standard curve for each sphingolipid run in parallel. For plasma, equal volumes of sample were used for quantification. For whole blood, each sample was normalized to the subjects' hematocrit measured at the time of sample collection. All whole blood and plasma samples were analyzed in one HPLC-MS/MS run per specimen type; the investigator performing the quantification was blinded to case or control status and SNP genotypes. The quantification of newly produced products of de novo synthesis in PBMCs was performed in two HPLC-MS/MS groups of individual subjects based on time of enrollment, and the investigator performing the quantification was blinded as to group or genotype of the individual samples.

**SNP genotyping**

SNP genotyping was performed in the core laboratory of the Clinical and Translational Science Center of Weill Cornell Medicine. DNA was isolated and genotyping for 5 SNPs was performed on whole blood from both asthma and control subjects. Briefly, genomic DNA was extracted from 200µl whole blood using QIAamp DNA blood mini kit (QIAGEN Inc., cat# 51106) according to manufacturer's instructions, and the concentration quantitated by UV absorbance. The SNP genotyping was performed using the TaqMan® SNP Genotyping Assays (SNP ID: rs8067378, rs4065275, rs8076131, rs12603332, rs7216389) following the manufacturer's instructions. Each SNP genotyping reaction was carried out in duplicate and three positive controls were included for each SNP genotype. The thermal cycling conditions included an initial incubation at 50°C for 2 min, then 95°C for 10 min, and 40 cycles of 95°C for 15 second and 60°C for one min. The SNP genotyping reaction was run in an Illumina Eco Real-Time PCR system and the data was analyzed using EcoStudy (version 5.0.4890) software.

**Metabolic labelling and measurement of *de novo* synthesized sphinganine and sphinganine-1-phosphate**

Sphingolipid *de novo* synthesis was measured in PBMCs isolated from whole blood of control and asthma subjects by metabolic labelling with the SPT substrate serine. This method can reliably assess SPT activity (2, 3). Following Ficoll gradient separation (CPT Mononuclear Cell Separation Tubes, BD Biosciences #362761), PBMC were incubated at 37˚C for 30 min with 2.2 mM stable isotope labeled L-[U-$^{13}$C, $^{15}$N) serine (Cambridge Isotope Laboratories #CNLM-474). Sphinganine and sphinganine-1-phosphate were extracted and quantitated by monitoring [M+3] isotope in precursor and product ions used in corresponding non-labeled sphinganine and Sa-1-P. Concentrations were calculated through extrapolation from a standard curve with internal standards and then normalized to the number of PBMC.

**Primary Airway Epithelial cells**

To assess sphingolipid synthesis of human primary airway epithelial cells based on the 17q21 rs7216389 genotype, we used banked primary airway epithelial cells that were screened for the 17q21 rs7216389 SNP. These cell lines were from two studies: (1) Part of ES015482 from lung transplants at University of North Carolina; and (2) Lung resections to remove cancerous lung areas at the University of New Mexico Lung Cancer Center as part of funding from Lovelace Respiratory Research Institute. Four cell lines with the rs7216389-TT genotype (minor allele) and four cell lines with the rs7216389-CC genotype (major allele) were selected and the cells were expanded using Bronchial Epithelial Growth Media (BEGM) and S-ALI growth media (Lonza, Walkersville, MD), respectively, and then cultured in air-liquid interface to generate differentiated airway epithelium following established protocols (4). Cells were then washed with PBS and sphingolipids in the cells were quantified by high pressure liquid

chromatography electrospray ionization tandem mass spectrometry (HPLC-MS/MS) as described in the methods section.

**Statistics**

The normality of the data was checked by histogram and D'agostino-Pearson tests for normality. Log-transformation was performed on the original scale as necessary. Two-sample t-test or chi-square test/Fisher's exact test were used to compare two groups depending on the data types (continuous or categorical); and ANOVA and logistic regressions were used to perform univariable or multivariable association analyses. Non-parametric tests between 2 comparisons (Mann-Whitney) or more than 2 (Kruskal-Wallis) were performed to compare groups where data was not normally distributed. To address the potential for overinflating statistical significance due to multiple comparisons and control for false discovery rate, we applied the Benjamini and Hochberg (BH) procedure (5) to sphingolipid analyses where 3 or more comparisons were made, adjusting for a maximum false discovery rate of 0.05; critical p-values for each analysis indicating the maximum unadjusted p-value which was statistically significant after adjusting for an FDR of 0.05 are reported. Sphingosine was not measurable in the majority of subjects in whole blood and was excluded from the analyses. For the genetic biomarkers, analyses were conducted initially on individual SNP levels. To avoid misspecification due to strong correlation among predictors, we further assessed the correlation among SNPs by Pearson Correlation Coefficient and then performed Principal Component Analysis (PCA) to assess for combined genetic features. Both parameter estimation and p-values are reported. The analyses and visualization of the data were conducted in R Version 3.5.2 (R Core Team, Vienna, Austria), SPSS version 23 (SPSS, Chicago, IL) and Graphpad Prism version 8.0.2 (GraphPad Software).

## Supplementary Results

### Study Group Characteristics

In total, 120 subjects with (n=59) and without asthma (n=61) between 5-17 years were enrolled. We successfully obtained blood from 116 subjects from which all were genotyped at the 5 asthma-associated 17q21 SNPs. 113 were available for plasma and whole blood sphingolipid analysis of which 13 plasma and 8 whole blood samples were excluded from the final sphingolipid analysis due to technical difficulties with the sample and missing data. PBMCs were successfully isolated for 85 subjects with 9 participants excluded due to technical difficulties with sample analysis and missing data. Allergic characterization by absolute eosinophil count and Total IgE was available for 107 and 113 respectively.

Age and sex distributions were similar between groups (**Supplemental Tables 1 and 2** for full cohort details). Compared with control subjects, subjects with asthma were more likely to have a reported history of atopy (eczema, allergies, allergic rhinitis) and had higher mean total IgE and eosinophils in peripheral blood. 70.5% of subjects with asthma reported being prescribed daily inhaled corticosteroid therapy, and 74% of the asthma subjects received oral steroids at least once within the year of enrollment (**Supplemental Table 2)**. The number of subjects with comorbid chronic medical conditions as well as obesity and overall BMI percentiles for age was similar in each group. The following comorbid medical conditions were reported in controls subjects: autism, attention-deficit hyperactivity disorder (ADHD), and depression. Five subjects with asthma reported obstructive sleep apnea, low vitamin D, autism, ADHD, irritable bowel syndrome and neurofibromatosis type 1.

### Genotype Frequencies in Study Cohort

All five SNP genotypes were available for 116 subjects (96.7%). For rs7216389, rs4065275 and rs8076131, the proportions of subjects having one of three genotypes did not

significantly differ between control and asthma groups (**Supplemental Table 3**). For rs8067378 where the G allele is asthma-protective (6), rs8067378-AA was associated with a higher proportion of asthma subjects (62.5%) compared to controls (37.5%, not significant), and rs8067378-AG was associated with higher proportions of control subjects (61.4% controls, 38.6% asthma, p=0.026). There were no differences in proportions of rs8067378-GG between groups. For rs1260332 where C is the asthma-risk allele (7), asthma was associated with higher proportion of rs1260332-CC (37.8% controls, 62.2% asthma, not significant) and control subjects associated with higher proportions of rs1260332-CT (63.0% controls, 37.0% asthma, p=0.014). There were no differences in proportions of rs1260332-TT between groups (**Supplemental Table 3**).

There was strong collinearity among the five SNPs analyzed, and to avoid misspecification under regression modeling framework on the asthma clinical phenotype, we performed principal component analysis to combine the SNPs and adjust for collinearity. The first component explained 80% of the overall variance and was used to determine the relationship between genetic variation at the 17q21 SNPs and asthma. Overall there were no significant associations in this cohort between the principal component and having asthma, allergic asthma (defined by absolute eosinophil count or total IgE level), absolute eosinophil count or total IgE levels in plasma/serum.

## Supplementary Tables

**Supplemental Table 1.** Subject characteristics enrolled at two sites[a].

| | **Control n=59** | **Asthma n=61** | |
|---|---|---|---|
| Sex (male) | 36/59 (61%) | 34/61 (55.7%) | p=0.424 |
| Age in years at enrollment mean (+/- sd) | 10.6 (3.9) range: 5-17 | 10.0 (3.7) range 5-17 | p=0.125 |
| History of Eczema | 11/58 (19.0%) | 23/61 (37.7%) | p=0.024 |
| History of Allergies | 15/58 (25.9%) | 47/61 (77.0%) | p=0.0001 |
| History of Allergic Rhinitis | 2/58 (3.4%) | 23/61 (37.7%) | p=0.0001 |
| Total IgE mean (+/- sd) | 207 (538) | 609 (588) | p=0.005 |
| Absolute Eosinophils mean (+/- sd) | 262 (482) | 440 (289) | p=0.033 |
| Insurance (Medicaid) | 51/59 (86.4%) | 59/61 (96.7%) | p=0.095 |
| BMI Percentile for age mean (+/- sd) | 73.85 % (28.1) | 73.97 % (24.1) | p=0.980 |
| Number of obese subjects | 30/59 (50.1%) | 27/61 (44.3%) | p=0.357 |
| Number of subjects with co-existing chronic medical conditions[b] | 5/59 (8.5%) | 5/61 (8.2%) | p=0.549 |
| Enrollment Site 1 (total N= 53) | 21/59 (35.6%) | 32/61 (52.5%) | |
| Enrollment Site 2 (total N= 67) | 38/59 (64.4%) | 29/61 (47.5%) | |

[a] Enrollment Site 1 = New York Presbyterian Hospital Weill Cornell Medicine, New York NY; Enrollment Site 2 = New York Presbyterian Hospital Queens, Queens, NY.

[b] Reported chronic medical conditions in control group: Autism, ADHD, and depression; in asthma group: Obstructive sleep apnea, low Vitamin D, autism, ADHD, irritable bowel syndrome/constipation, Ehlers-Danlos syndrome, and neurofibromatosis type1.

**Supplemental Table 2.** Characteristics of pediatric asthma subjects. Data was obtained through parent and patient in-person survey at time of enrollment.

| **Characteristic** | **Number of subjects (percent of total)** |
|---|---|
| Sex (M) | 34/61 (54.8%) |
| Median age of asthma diagnosis (range) | 2.5 years (4 months -10 years) |
| Currently on an inhaled corticosteroid | 43/61 (70.5%) |
| Use of oral steroids in last 12 months | 45/61 (74%) |
| Visited ED in lifetime | 51/61 (84%) |
| Visited ED at least once in the last month | 32/51 (63%) |
| Admitted to hospital in lifetime | 40/61 (65.6%) |
| Admitted to hospital at least once in last 12 months | 15/40 (37.5%) |
| Required O2 during admission | 32/40 (80%) |
| Admitted to ICU in lifetime | 25/61 (41%) |
| Admitted to ICU at least once in last 12months | 14/25 (56%) |

**Supplemental Table 3.** Proportion of genotypes at each of 5 SNPs in control and asthma subjects[a].

| | **Total[1] N=116** | **Control N=59** | **Asthma N=57** | **Chi Square p-value** |
|---|---|---|---|---|
| **rs7216389** | | | | p=0.169 |
| CC | 8 (6.9%) | 4 (50.0%) | 4 (50.0%) | |
| CT | 56 (47.4%) | 30 (55.4%) | 25 (44.6%) | |
| TT | 52 (44.8%) | 24 (46.2%) | 28 (53.8%) | |
| **rs8067378** | | | | p=0.026 |
| AA | 32 (27.6%) | 12 (37.5%) | 20 (62.5%) | |
| AG | 57 (49.6%) | 35 (61.4%) | 22 (38.6%) [b] | |
| GG | 27 (22.4%) | 12 (40.7%) | 15 (55.6%) | |
| **rs4065275** | | | | p=0.144 |
| AA | 15 (12.9%) | 8 (53.3%) | 7 (46.7%) | |
| AG | 54 (45.7%) | 29 (53.7%) | 24 (44.4%) | |
| GG | 47 (40.5%) | 21 (44.7) | 26 (55.3%) | |
| **rs8076131** | | | | p=0.197 |
| AA | 57 (49.1%) | 27 (47.4%) | 30 (52.6%) | |
| AG | 53 (44.8%) | 28 (52.8%) | 24 (45.3%) | |
| GG | 6 (5.2%) | 3 (50.0%) | 3 (50.0%) | |
| **rs1260332** | | | | p=0.014 |
| CC | 37 (31.9%) | 14 (37.8%) | 23 (62.2%) | |
| CT | 54 (45.7%) | 34 (63.0%) | 20 (37.0%) [b] | |
| TT | 25 (21.7%) | 11 (44.0%) | 14 (56.0%) | |

[a] Cohort population composition is self-described as 16.7% African American, 0.8% Asian, 5% White, 42.5% Hispanic/Latino, 9.2% South Asian, 11.7% Other/Unknown, 14.2% Mixed.

[b] Differs from expected frequencies between groups.

**Supplemental Table 4.** Associations between genotype at five 17q21 SNPs and sphingolipid levels in whole blood. Adjusted for race/ethnicity and sex. Pearson's correlations determined using additive coding of genotypes in order of lowest to highest associated asthma risk. Asthma-risk associated alleles are underlined.

| **Dihydroceramides** | **rs7216389 (C/<u>T</u>) Pearson R (p-value)** | **rs12603332 (T/<u>C</u>) Pearson R (p-value)** | **rs8076131 (G/<u>A</u>) Pearson R (p-value)** | **rs8067378 (G/<u>A</u>) Pearson R (p-value)** | **rs4065275 (A/<u>G</u>) Pearson R (p-value)** |
|---|---|---|---|---|---|
| DH C16 (n=111) | -0.25 (p=0.01) | -0.11 (p=0.25) | -0.26 (p=0.01) | -0.06 (p=0.56) | -0.14 (p=0.14) |
| DH C18 (n=111) | -0.23 (p=0.02) | -0.07 (p=0.45) | -0.22 (p=0.02) | -0.03 (p=0.77) | -0.11 (p=0.25) |
| DH C18:1 (n=111) | -0.20 (p=0.04) | -0.07 (p=0.46) | -0.2 (p=0.07) | -0.04 (p=0.66) | -0.13 (p=0.16) |
| DH C24 (n=109) | -0.20 (p=0.04) | -0.06 (p=0.56) | -0.22 (p=0.02) | -0.01 (p=0.92) | -0.09 (p=0.35) |
| DH C24:1 (n=111) | -0.22 (p=0.02) | 0 (p= 0.98) | -0.23 (p=0.02) | 0.03 (p=0.79) | -0.08 (p=0.40) |

| **Ceramides** | **rs7216389 (C/<u>T</u>) Pearson R (p-value)** | **rs12603332 (T/<u>C</u>) Pearson R (p-value)** | **rs8076131 (G/<u>A</u>) Pearson R (p-value)** | **rs8067378 (G/<u>A</u>) Pearson R (p-value)** | **rs4065275 (A/<u>G</u>) Pearson R (p-value)** |
|---|---|---|---|---|---|
| Cer C16 (n=111) | -0.20 (p=0.04) | -0.08 (p=0.38) | -0.21 (p=0.03) | -0.06 (p=0.5) | -0.14 (p=0.16) |
| Cer C18 (n=111) | -0.22 (p=0.02) | -0.10 (p=0.28) | -0.22 (p=0.02) | -0.05 (p=0.61) | -0.17 (p=0.07) |
| Cer C20 (n=111) | -0.24 (p=0.01) | -0.11 (p=0.25) | -0.24 (p=0.01) | -0.10 (p=0.30) | -0.15 (p=0.11) |
| Cer C22 (n=111) | -0.18 (p=0.06) | -0.07 (p=0.47) | -0.19 (p=0.05) | -0.06 (p=0.17) | -0.12 (p=0.23) |
| Cer C24 (n=111) | -0.16 (p= 0.10) | -0.03 (p=0.75) | -0.16 (p=0.10) | 0.00 (p=0.96) | -0.08 (p=0.40) |
| Cer C24:1 (n=111) | -0.14 (p=0.14) | 0 (p=1) | -0.16 (p=0.09) | 0.03 (p=0.72) | -0.08 (p=0.39) |

| **Long-chain Bases** | **rs7216389 (C/<u>T</u>) Pearson R (p-value)** | **rs12603332 (T/<u>C</u>) Pearson R (p-value)** | **rs8076131 (G/<u>A</u>) Pearson R (p-value)** | **rs8067378 (G/<u>A</u>) Pearson R (p-value)** | **rs4065275 (A/<u>G</u>) Pearson R (p-value)** |
|---|---|---|---|---|---|
| Sphinganine (n=111) | -0.14 (p=0.16) | -0.06 (p=0.53) | -0.031 (p=0.95) | -0.09 (p=0.37) | -0.11 (p=0.24) |
| Sphinganine-1-P (n=111) | -0.07 (p=0.47) | -0.06 (p=0.52) | -0.01 (p=0.9) | -0.01 (p=0.37) | -0.14 (p=0.14) |

| | | | | | |
|---|---|---|---|---|---|
| Sphingosine (n=75) | 0.08 (p=0.53) | 0.06 (p=0.59) | 0.1 (p=0.41) | 0.11 (p=0.34) | 0.05 (p=0.7) |
| Sphingosine-1-P (n=111) | -0.06 (p=0.57) | 0.00 (p=0.99) | 0.00 (p=0.98) | 0.05 (p=0.58) | -0.12 (p=0.22) |

| **Sphingomyelins** | **rs7216389 (C/<u>T</u>) Pearson R (p-value)** | **rs12603332 (T/<u>C</u>) Pearson R (p-value)** | **rs8076131 (G/<u>A</u>) Pearson R (p-value)** | **rs8067378 (G/<u>A</u>) Pearson R (p-value)** | **rs4065275 (A/<u>G</u>) Pearson R (p-value)** |
|---|---|---|---|---|---|
| SM C16 (n=111) | -0.14 (p=0.13) | -0.12 (p=0.21) | -0.11 (p=0.28) | -0.06 (p=0.52) | -0.15 (p=0.11) |
| SM C18 (n=111) | -0.16 (p=0.09) | -0.05 (p=0.63) | -0.14 (p=0.15) | 0.01 (p=0.94) | -0.13 (p=0.17) |
| SM C18:1 (n=111) | -0.13 (p=0.18) | -0.04 (p=0.64) | -0.10 (p=0.31) | 0.00 (p=0.97) | -0.13 (p=0.19) |
| SM C24:1 (n=111) | -0.17 (p=0.08) | -0.04 (p=0.72) | -0.13 (p=0.16) | 0.00 (p=0.94) | -0.14 (p=0.14) |

**Supplemental Table 5.** Pearson correlations of genotypes at rs7216389 and rs8076131 with whole blood dihydroceramides and ceramides in subjects with asthma. Pearson's correlations were determined using additive coding of genotypes in order of lowest to highest associated asthma risk. Asthma-risk associated alleles are underlined.

| **Dihydroceramides (n=54)** | **rs7216389 (C/<u>T</u>) Pearson R (p-value)** | **rs8076131 (G/<u>A</u>) Pearson R (p-value)** |
|---|---|---|
| DH C16 | -0.40 (p<0.0001) | -0.51 (p<0.0001) |
| DH C18 | -0.28 (p=0.04) | -0.32 (p=0.02) |
| DH C18:1 | -0.18 (p=0.21) | -0.23 (p=0.10) |
| DH C24 | -0.24 (p=0.10) | -0.32 (p=0.02) |
| DH C24:1 | -0.25 (p=0.07) | -0.32 (p=0.02) |
| **Ceramides (n=54)** | | |
| Cer C16 | -0.26 (p=0.06) | -0.34 (p=0.01) |
| Cer C18 | -0.21 (p=0.14) | -0.28 (p=0.04) |
| Cer C20 | -0.26 (p=0.06) | -0.34 (p=0.01) |
| Cer C22 | -0.09 (p=0.51) | -0.17 (p=0.24) |
| Cer C24 | -0.04 (p=0.80) | -0.10 (p=0.48) |
| Cer C24:1 | -0.07 (p=0.64) | -0.18 (p=0.20) |

**Supplemental Table 6.** Comparison of sphingolipid masses in whole blood by genotype at rs7216389. Analysis of masses of dihydroceramides, long-chain bases, ceramides and sphingomyelins in whole blood of combined control and asthma subjects by genotype.

| Dihydroceramides & Long-chain Bases | | Mean (picomoles) | SD | F(2, 109)[a] | p-value |
|---|---|---|---|---|---|
| DH C16 | | | | 4.510 | 0.013 |
| | CC (n= 8) | 0.330 | 0.199 | | |
| | CT (n= 53) | 0.254 | 0.100 | | |
| | TT (n= 51) | 0.214 | 0.104 | | |
| DH C18 | | | | 9.228[b] | 0.001[b] (*) |
| | CC (n= 8) | 0.538 | 0.352 | | |
| | CT (n= 53) | 0.411 | 0.198 | | |
| | TT (n= 51) | 0.326 | 0.233 | | |
| DH C18:1 | | | | 4.276[b] | 0.118[b] |
| | CC (n= 8) | 2.330 | 0.907 | | |
| | CT (n= 53) | 2.014 | 0.717 | | |
| | TT (n= 51) | 1.758 | 0.666 | | |
| DH C24 | | | | 7.388[b] | 0.025[b] |
| | CC (n= 8) | 0.752 | 0.281 | | |
| | CT (n= 53) | 0.600 | 0.353 | | |
| | TT (n= 51) | 0.487 | 0.352 | | |
| DH C24:1 | | | | 3.690 | 0.028 |
| | CC (n= 8) | 1.493 | 0.323 | | |
| | CT (n= 53) | 1.375 | 0.445 | | |
| | TT (n= 51) | 1.177 | 0.429 | | |
| Sphinganine | | | | | |
| | CC (n= 8) | 0.130 | 0.055 | 1.620[b] | 0.445[b] |
| | CT (n= 53) | 0.124 | 0.039 | | |
| | TT (n= 51) | 0.114 | 0.030 | | |
| Sphinganine-1-P | | | | 5.154[c] | 0.013 |
| | CC (n= 8) | 0.832 | 0.230 | | |
| | CT (n= 53) | 1.208 | 0.622 | | |
| | TT (n= 51) | 0.963 | 0.360 | | |
| Sphingosine | | | | 0.182[d] | 0.876 |
| | CC (n= 4) | 33.790 | 26.960 | | |
| | CT (n= 40) | 29.430 | 25.960 | | |
| | TT (n= 31) | 33.650 | 35.480 | | |
| Sphingosine-1-P | | | | 2.847 | 0.062 |
| | CC (n= 8) | 2.225 | 0.789 | | |
| | CT (n= 53) | 2.976 | 1.399 | | |
| | TT (n= 51) | 2.501 | 0.942 | | |

| Ceramides & Sphingomyelins | | Mean (picomoles) | SD | F(2, 109)[a] | p-value |
|---|---|---|---|---|---|
| Cer C16 | | | | 3.349 | 0.039 |
| | CC (n= 8) | 6.495 | 4.324 | | |

| | | | | | |
|---|---|---|---|---|---|
| | CT (n= 53) | 5.231 | 1.82 | | |
| | TT (n= 51) | 4.634 | 1.677 | | |
| Cer C18 | | | | 3.875 | 0.024 |
| | CC (n= 8) | 2.366 | 1.102 | | |
| | CT (n= 53) | 2.001 | 0.6809 | | |
| | TT (n= 51) | 1.726 | 0.6517 | | |
| Cer C20 | | | | 4.356 | 0.015 |
| | CC (n= 8) | 1.628 | 0.5914 | | |
| | CT (n= 53) | 1.382 | 0.501 | | |
| | TT (n= 51) | 1.168 | 0.4664 | | |
| Cer C22 | | | | 2.548 | 0.080 |
| | CC (n= 8) | 23.81 | 8.277 | | |
| | CT (n= 53) | 22.08 | 6.576 | | |
| | TT (n= 51) | 19.67 | 6.053 | | |
| Cer C24 | | | | 2.076 | 0.130 |
| | CC (n= 8) | 30.43 | 9.637 | | |
| | CT (n= 53) | 28.54 | 8.625 | | |
| | TT (n= 51) | 25.8 | 7.235 | | |
| Cer C24:1 | | | | 1.719 | 0.184 |
| | CC (n= 8) | 24.44 | 8.064 | | |
| | CT (n= 53) | 22.16 | 8.592 | | |
| | TT (n= 51) | 19.88 | 7.322 | | |
| SM C16 | | | | 1.928 | 0.1504 |
| | CC (n= 8) | 472.0 | 114.0 | | |
| | CT (n= 53) | 429.0 | 97.2 | | |
| | TT (n= 51) | 405.6 | 94.3 | | |
| SM C18 | | | | 2.949 | 0.0566 |
| | CC (n= 8) | 127.5 | 37.81 | | |
| | CT (n= 53) | 107.4 | 26.38 | | |
| | TT (n= 51) | 103.1 | 24.67 | | |
| SM C18:1 | | | | 2.303 | 0.1048 |
| | CC (n= 8) | 33.94 | 8.475 | | |
| | CT (n= 53) | 29.31 | 6.798 | | |
| | TT (n= 51) | 28.29 | 6.875 | | |
| SM C24:1 | | | | 2.845 | 0.0625 |
| | CC (n= 8) | 430.4 | 83.11 | | |
| | CT (n= 53) | 370.5 | 95.69 | | |
| | TT (n= 51) | 345.9 | 101.3 | | |

[a] One-way ANOVA F-statistic (degrees of freedom, between and within groups)
[b] Kruskal-Wallis Statistic H(3,109) and associated p-value
[c] Refers to Welsch F statistic due to imbalanced SD
[d] One-way ANOVA F-statistic: F(2,72)
*indicates significance following adjustment for FDR; critical p-value =0.009.

**Supplemental Table 7.** Comparison of sphingolipid masses in whole blood by genotype at rs8076131. Analysis of masses of dihydroceramides, long-chain bases, ceramides and sphingomyelins in whole blood of combined control and asthma subjects by genotype.

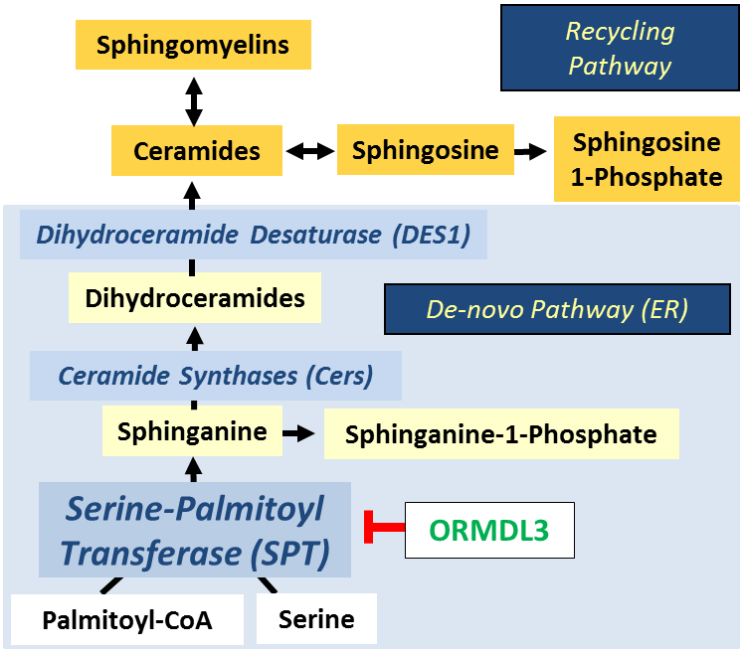
| Dihydroceramides & Long-chain Bases | | Mean (picomoles) | SD | F(2, 109)[a] | p-value |
|---|---|---|---|---|---|
| DH C16 | | | | 4.484 | 0.013 |
| | GG (n= 6) | 0.316 | 0.194 | | |
| | AG (n= 51) | 0.264 | 0.109 | | |
| | AA (n= 55) | 0.211 | 0.101 | | |
| DH C18 | | | | 9.498[b] | 0.009[b] |
| | GG (n= 6) | 0.502 | 0.377 | | |
| | AG (n= 51) | 0.455 | 0.288 | | |
| | AA (n= 55) | 0.326 | 0.225 | | |
| DH C18:1 | | | | 2.816[b] | 0.245[b] |
| | GG (n= 6) | 2.240 | 0.864 | | |
| | AG (n= 51) | 2.040 | 0.761 | | |
| | AA (n= 55) | 1.780 | 0.647 | | |
| DH C24 | | | | 8.851[b] | 0.012[b] |
| | GG (n= 6) | 0.720 | 0.274 | | |
| | AG (n= 51) | 0.631 | 0.353 | | |
| | AA (n= 55) | 0.474 | 0.346 | | |
| DH C24:1 | | | | 4.151 | 0.018 |
| | GG (n= 6) | 1.450 | 0.294 | | |
| | AG (n= 51) | 1.400 | 0.449 | | |
| | AA (n= 55) | 1.180 | 0.421 | | |
| Sphinganine | | | | 1.427[b] | 0.490[b] |
| | GG (n= 6) | 0.110 | 0.033 | | |
| | AG (n= 51) | 0.124 | 0.040 | | |
| | AA (n= 55) | 0.118 | 0.034 | | |
| Sphinganine-1-P | | | | 4.432[b] | 0.109[b] |
| | GG (n= 6) | 0.763 | 0.224 | | |
| | AG (n= 51) | 1.170 | 0.589 | | |
| | AA (n= 55) | 1.070 | 0.431 | | |
| Sphingosine | | | | | |
| | GG (n= 2) | 18.050 | 17.030 | 0.248[c] | 0.781 |
| | AG (n= 40) | 30.770 | 26.320 | | |
| | AA (n= 33) | 32.990 | 34.820 | | |
| Sphingosine-1-P | | | | | |
| | GG (n=6) | 1.920 | 0.581 | 2.505 | 0.086 |
| | AG (n= 51) | 2.930 | 1.350 | | |
| | AA (n= 55) | 2.590 | 1.040 | | |

| Ceramides & Sphingomyelins | | Mean (picomoles) | SD | F(2, 109)[a] | p-value |
|---|---|---|---|---|---|
| Cer C16 | | | | 4.183 | 0.018 |

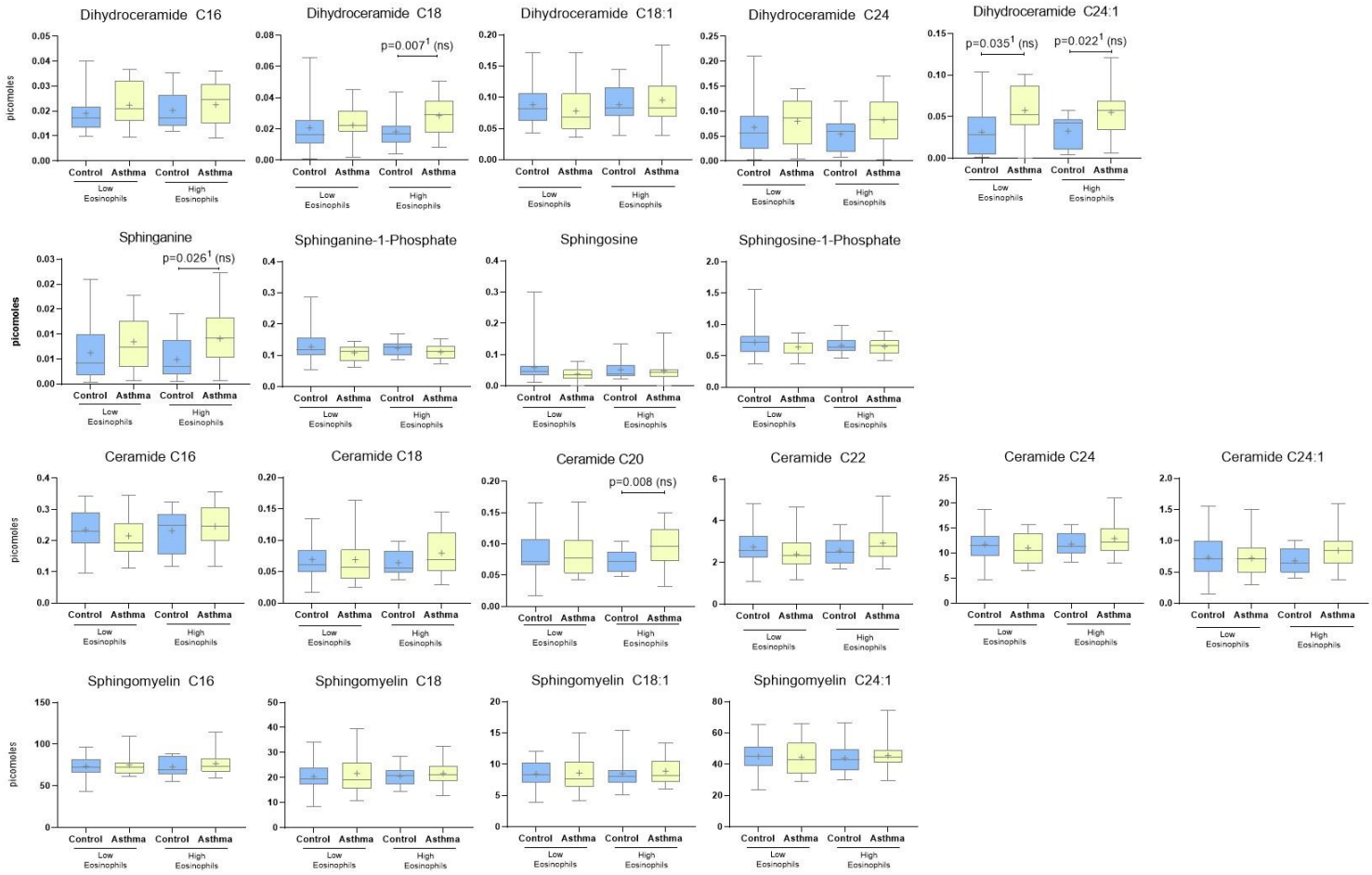
| | | | | | |
|---|---|---|---|---|---|
| | GG (n= 6) | 7.02 | 4.870 | | |
| | AG (n= 51) | 5.24 | 1.880 | | |
| | AA (n= 55) | 4.66 | 1.620 | | |
| Cer C18 | | | | 3.589 | 0.031 |
| | GG (n= 6) | 2.37 | 1.170 | | |
| | AG (n= 51) | 2.02 | 0.719 | | |
| | AA (n= 55) | 1.74 | 0.630 | | |
| Cer C20 | | | | 4.001 | 0.021 |
| | GG (n= 6) | 1.61 | 0.577 | | |
| | AG (n= 51) | 1.4 | 0.527 | | |
| | AA (n= 55) | 1.18 | 0.451 | | |
| Cer C22 | | | | 2.462 | 0.090 |
| | GG (n= 6) | 24.88 | 8.780 | | |
| | AG (n= 51) | 21.98 | 6.780 | | |
| | AA (n= 55) | 19.88 | 5.950 | | |
| Cer C24 | | | | 1.970 | 0.144 |
| | GG (n= 6) | 31.08 | 9.710 | | |
| | AG (n= 51) | 28.55 | 8.890 | | |
| | AA (n= 55) | 25.99 | 7.110 | | |
| Cer C24:1 | | | | 2.087 | 0.129 |
| | GG (n= 6) | 25.27 | 7.930 | | |
| | AG (n= 51) | 22.36 | 8.680 | | |
| | AA (n= 55) | 19.86 | 7.260 | | |
| SM C16 | | | | 1.090 | 0.340 |
| | GG (n= 6) | 464.19 | 114.35 | | |
| | AG (n= 51) | 428.68 | 97.95 | | |
| | AA (n= 55) | 410.01 | 95.89 | | |
| SM C18 | | | | 2.271 | 0.108 |
| | GG (n= 6) | 127.96 | 39.40 | | |
| | AG (n= 51) | 107.74 | 27.57 | | |
| | AA (n= 55) | 103.78 | 24.25 | | |
| SM C18:1 | | | | 2.128 | 0.124 |
| | GG (n= 6) | 34.76 | 8.50 | | |
| | AG (n= 51) | 29.16 | 6.84 | | |
| | AA (n= 55) | 28.58 | 6.92 | | |
| SM C24:1 | | | | 1.753 | 0.178 |
| | GG (n= 6) | 416.57 | 78.74 | | |
| | AG (n= 51) | 373.44 | 96.77 | | |
| | AA (n= 55) | 348.68 | 101.65 | | |

[a] One-way ANOVA F-statistic (degrees of freedom, between and within groups)
[b] Kruskal-Wallis Statistic H(3,109) and associated p-value
c One-way ANOVA F-statistic: F(2,72)

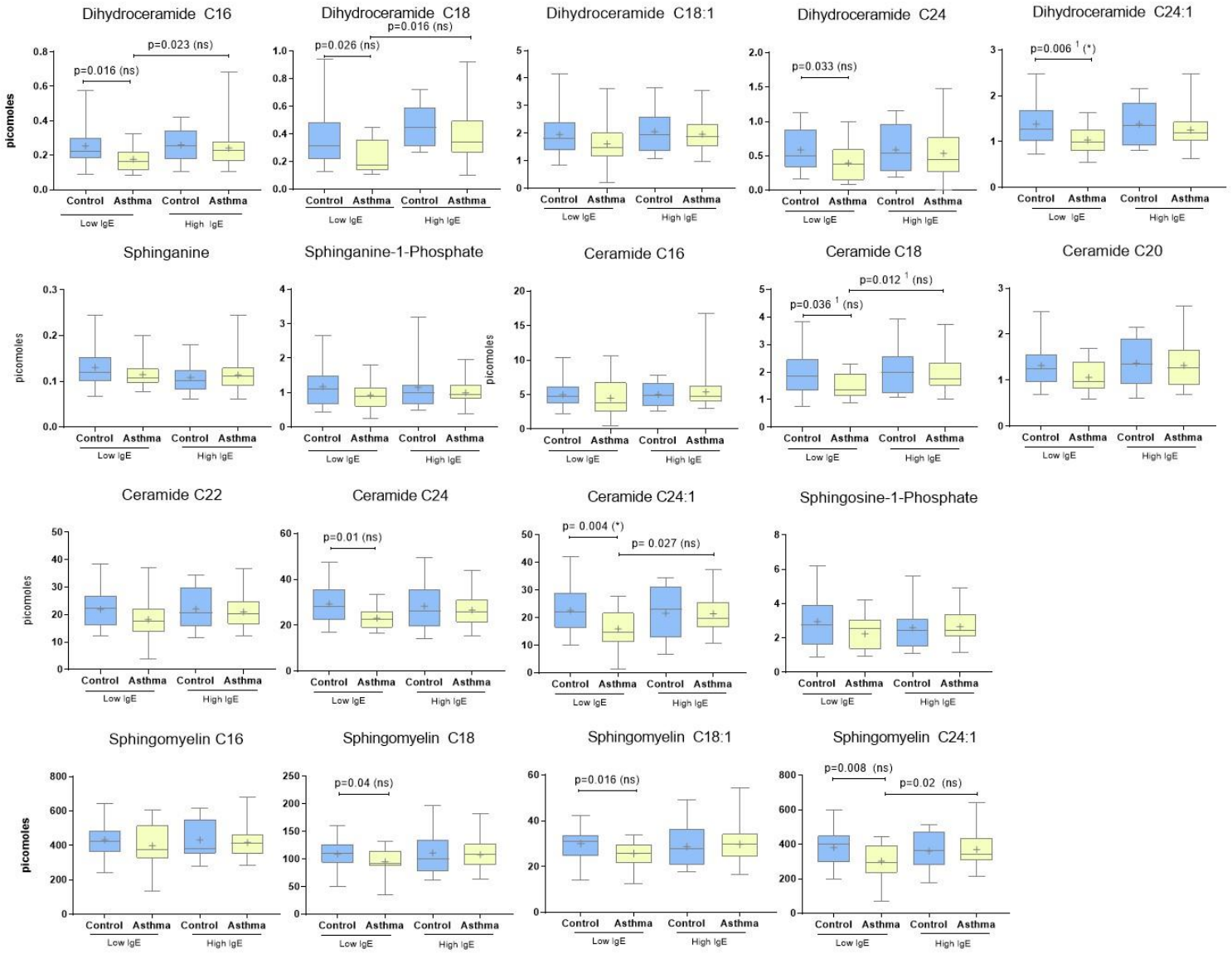
## Supplementary Figures




**Supplemental Figure 1.** The sphingolipid synthesis pathways highlighting products of the *de novo* and recycling pathways. Serine and palmitoyl-CoA are substrates for SPT which is inhibited by ORMDL3. The blue box represents the *de novo* pathway of sphingolipid synthesis and shows sphingolipid products which are directly reliant on SPT activity and the *de novo* pathway for synthesis.

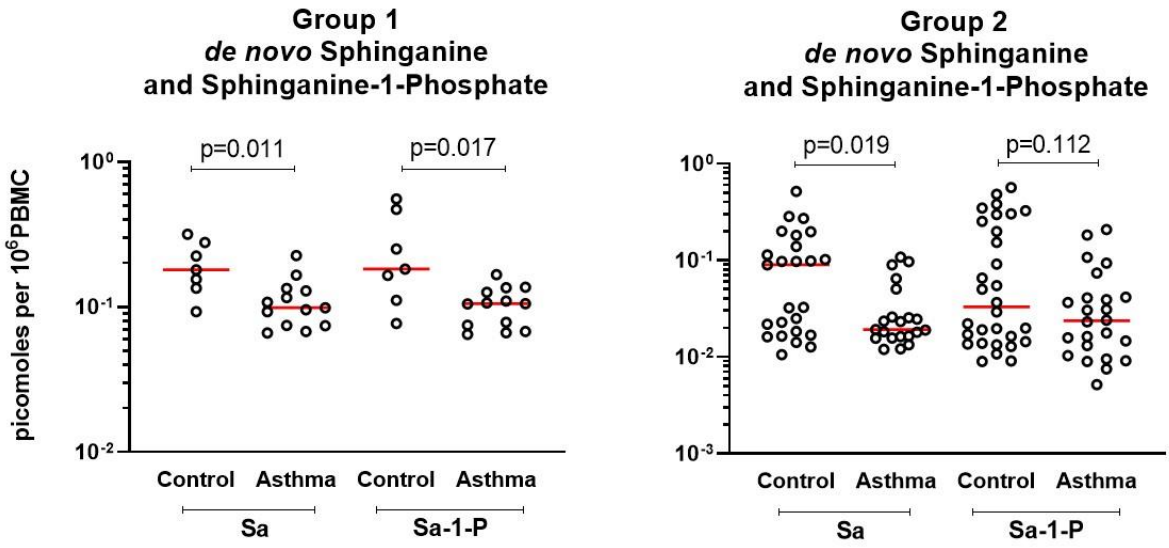



**Supplemental Figure 2. Sphingolipid composition in plasma.** Sphingolipids in control and asthma subjects, stratified by allergic group. Boxes represent interquartile ranges with median; whiskers indicate range of observations. Means indicated (+) and compared by two-sample t-test for normally distributed data; for non-normal data, comparison of median values indicated ([1]) with corresponding p-value. P-values reported if <0.05. Significant p-values following the BH procedure for an FDR of 0.05 are indicated (*) and fall below the BH-critical value of 0.001. Low-eosinophils: <300/ml, High-eosinophils: ≥ 300/ml in peripheral blood. Low-eosinophil control (n=39), asthma (n=14); High-eosinophil control (n=15); asthma (n=32).

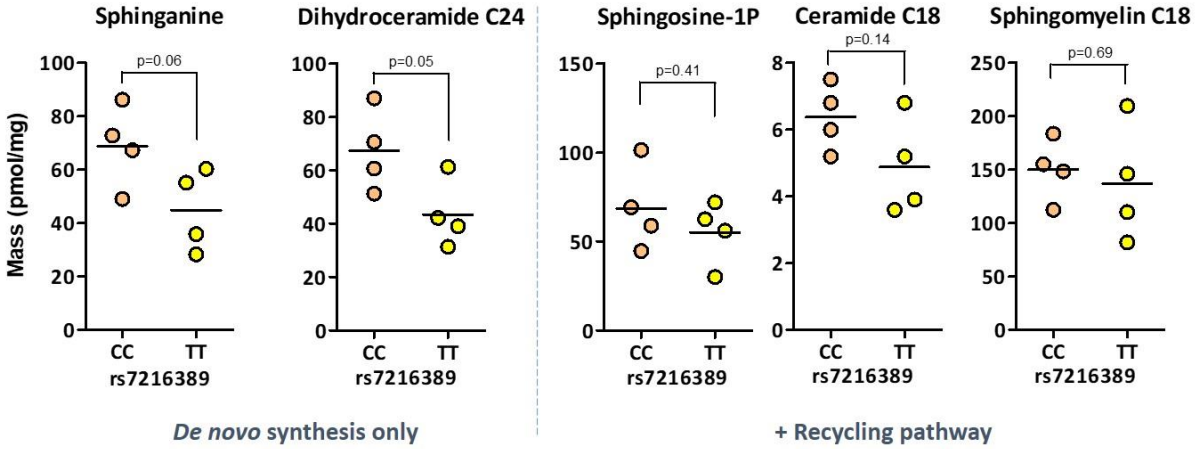



**Supplemental Figure 3. Sphingolipid composition in whole blood stratified for total IgE level.**

Sphingolipids measured in control and asthma subjects, stratified by total IgE group. Boxes represent interquartile ranges with median; whiskers indicate range of observations. Means are indicated by +; compared by two-sample t-test for normally distributed data; for non-normal data, comparison of median values indicated ([1]) with corresponding p-value. p-values reported if <0.05. Significant p-values following the BH procedure for an FDR of 0.05 are indicated (*) and fall below the BH-critical value of 0.007. Low-IgE: <200 IU/ml, High-IgE: ≥ 200 IU/ml in peripheral blood. Low-IgE control (n=45), asthma (n=12), high-IgE control (n=15), asthma (n=41).

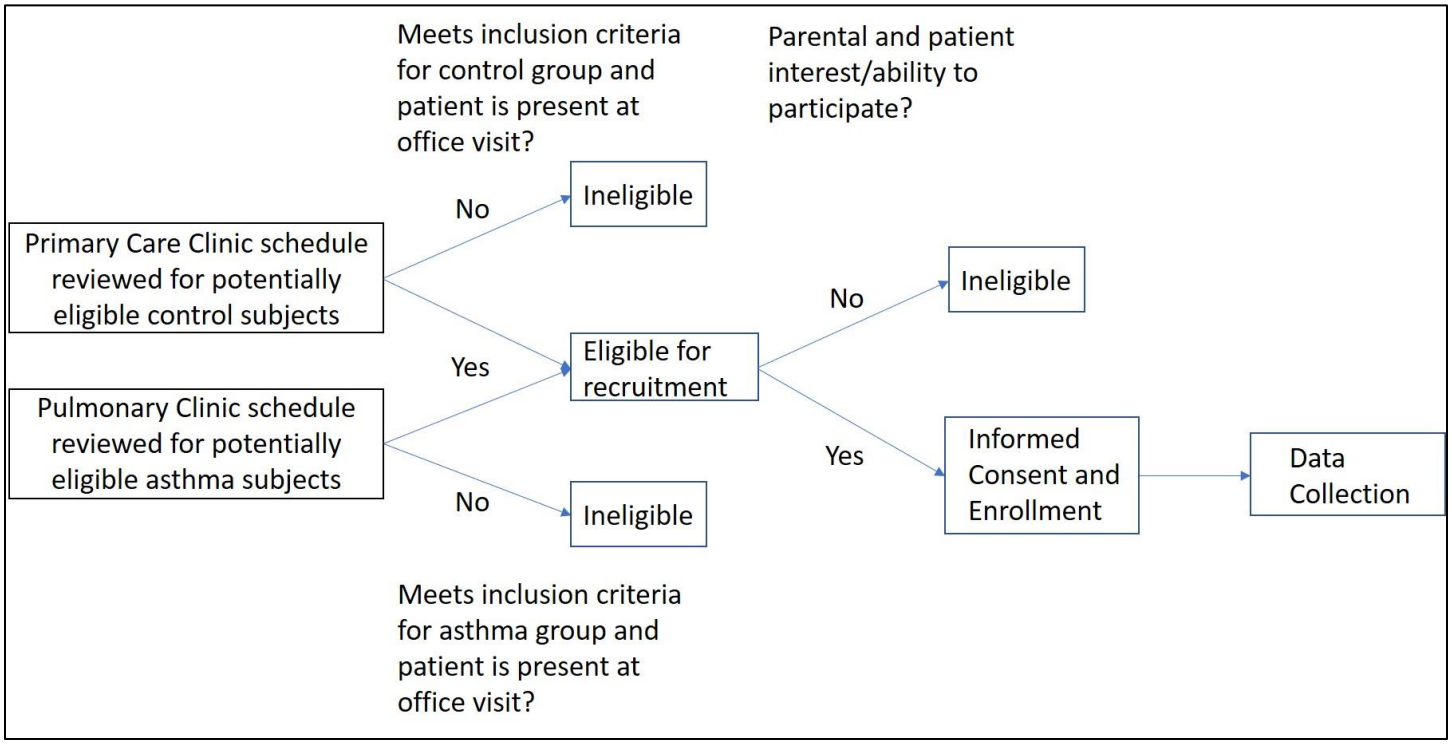



**Supplemental Figure 4. *De novo* sphingolipid synthesis is decreased in children with asthma.** Newly synthesized sphinganine (Sa) and sphinganine-1-phosphate (Sa-1-P) in control and asthma following metabolic labelling of substrate ($C^{13}N^{15}$–serine) in PBMCs. Samples were analyzed in two groups over subject enrollment period, and displayed from each HPLC/mass spectrometry analysis (Group 1 n= 22, Group 2 n=54). Individual data points shown, compared by Mann-Whitney test.




**Supplemental Figure 5**. Newly synthesized sphinganine and dihydroceramide C24 measured following metabolic labelling with $C^{13}N^{15}$–serine, and naturally occurring sphingosine-1-phosphate, ceramide C18 and sphingomyelin C18 were measured in primary airway epithelial cells cultured at air-liquid interface with either rs7213389-CC or rs7216389-TT genotypes. Masses expressed in picomoles per mg protein.

**Supplemental Figure 6.** Diagram of the subject recruitment and enrollment process.



## Supplemental References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