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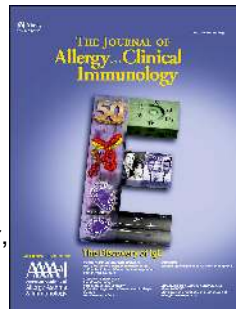

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Managing Asthma in Pregnancy (MAP) trial: FeNO levels and childhood asthma

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Abstract:

Background: The single-centre double-blind, randomised controlled Managing Asthma in Pregnancy (MAP) trial in Newcastle, Australia, compared a treatment algorithm using the fraction of exhaled nitric oxide (FeNO) in combination with asthma symptoms (FeNO group) against a treatment algorithm using clinical symptoms only (clinical group) in pregnant asthmatic women (ANZ Clinical Trials Registry, number 12607000561482). The primary outcome was a 50% reduction in asthma exacerbations during pregnancy in the FeNO group. However, the effect of FeNO-guided management on the development of asthma in the offspring is unknown.

Objective: We sought to investigate the effect of FeNO-guided asthma management during pregnancy on asthma incidence in childhood. Methods: 179 mothers consented to participate in the Growing Into Asthma (GIA) double-blind follow-up study with the primary aim to determine the effect of FeNO-guided asthma management on childhood asthma incidence. Results: 140 children (78%) were followed up at 4 to 6 years of age. FeNO-guided as compared to symptoms only based approach significantly reduced doctor diagnosed asthma (25·9% versus 43·2%; odds ratio [OR] 0.46, 95% confidence interval [CI] 0.22 to 0.96, p=0.04). Furthermore frequent wheeze (OR 0.27; CI 0.09 to 0.87, p=0.03), use of short-acting beta agonists (OR 0.49; CI 0.25 to 0.97; p=0.04), and emergency department visits for asthma (OR 0.17, CI 0.04 to 0.76; p=0.02) in the past 12 months were less common in children born to mothers from the FeNO group. Doctor diagnosed asthma was associated with common risk alleles for early-onset asthma at gene locus 17q21 (p=0·01 for rs8069176; p=0·03 for rs8076131), and higher airways resistance (p=0·02) and FeNO levels (p=0·03). A causal mediation analysis suggested natural indirect effects of FeNO-guided asthma management on childhood asthma through "any use" and "time to first change in dose" of inhaled corticosteroids during the MAP trial (OR 0.83; CI 0.59 to 0.99 and OR 0.90, CI 0.70 to 1.03, respectively). Conclusion: FeNO-guided asthma management during pregnancy prevented doctor diagnosed asthma in the offspring at preschool age, in part mediated through changes in use and dosing of inhaled corticosteroids during the MAP trial.

Introduction:

A history of maternal asthma remains among the most robust predictors of virus-induced lower respiratory tract infections in infancy, and childhood-onset asthma with an odds ratio of 3.0 (95% confidence interval [CI], 2.6-3.6) for asthma in a pooled analysis of 33 studies(1). Surprisingly, the impact of asthma control, exacerbations and asthma management during pregnancy on the offspring's asthma risk remains poorly studied. However, an increased risk for asthma has been reported in a retrospective case-control study of children whose mothers had moderate-to-severe uncontrolled asthma during pregnancy(2). Asthma during pregnancy may shape the future child's future asthma risk trajectory via an interaction between heritability and *in utero* exposures at the feto-maternal interface. Notably very common exposures such as asthma medications and exacerbations during pregnancy have not been investigated for an association with the child's asthma risk.

The Managing Asthma in Pregnancy (MAP) study was a double-blind randomised controlled trial (RCT) in asthmatic women(3). It compared the efficacy of a management algorithm that combined the non-invasive assessment of T helper 2 (TH2) cell cytokine (IL-4 and IL-13) induced airways inflammation by means of measuring the fractional exhaled nitric oxide (FeNO) and asthma symptoms (FeNO group) against a symptoms only based approach (clinical group). Total asthma exacerbations were reduced (incidence rate ratio 0.50, 95% CI 0.33-0.76) in the FeNO group, compared to the clinical group(3). At the end of the study, more pregnant women took inhaled corticosteroids (ICS) and ICS plus long-acting beta agonists (ICS/LABA) -but in a lower mean ICS dose- in the FeNO group as a result of the treatment algorithm (68.5% versus 42.2%, $p<0.0001$ and 40.5% versus 17.4%, $p<0.0001$). In contrast, fewer women in the FeNO group as compared to the clinical group needed short acting beta agonist treatment in the last RCT week (median [interquartile range, IQR] 0 [0-3] days versus 1 [0-5] days, $p=0.024$). There were no differences in FeNO levels, FEV1 and FEV1% between the groups(3).

During the MAP RCT, mothers were invited to have their offspring participate in the Growing into Asthma (GIA) birth cohort study. At 12 months of age, we conducted the first follow-up visit to investigate the parent-reported incidence of virus-induced wheezy lower respiratory tract infections ("bronchiolitis"). We found that infants born to mothers from the FeNO group had a reduced risk for recurrent bronchiolitis episodes in the first year of life (odds ratio [OR] 0.08, 95% confidence intervals [CI] 0.01-0.66)(4). Notably, the Childhood

Origins of Asthma (COAST) birth cohort of high-risk children born to parents with asthma or allergy reported an association between moderate-to-severe wheezy illnesses in the first three years of life and asthma by age six years. Odds ratios ranged between 2.6 (95% CI 1.0-6.3) for respiratory syncytial virus (RSV)–induced and 9.8 (95% CI, 4.3-22.0) for rhinovirus (RV)-induced wheezing illnesses(5). Furthermore a single nucleotide polymorphism (SNP) at a highly replicated asthma locus on chromosome 17q21 (rs7216389)(6) was associated with a genotype-specific risk for asthma only in children with a RV-induced wheezing illness in early life (p=0.004)(7). The ORM (yeast)-like protein isoform 3 (ORMDL3) protein, coded at the 17q21 locus, has been shown to be associated with a cellular stress response and release of pro-inflammatory molecules involved in airways remodelling and inflammation(8),(9),(10).

The results from the COAST study in conjunction with our findings provided a strong rationale to prospectively test the hypothesis in the GIA cohort proposed earlier(4) that 'asthma in pregnancy is a potentially modifiable determinant in the prenatal origins of bronchiolitis with the prospect to be evaluated as a potential primary preventative strategy that could modulate the risk of childhood asthma'. The primary aim of the GIA study was to determine the effect of FeNO-guided asthma management during pregnancy on asthma incidence in childhood, and the secondary aim was to determine asthma-related symptoms and healthcare utilisation. In further explorative analyses, we aimed at investigating the role of ICS in mediating, and the relevance of 17q21 variants in modifying the effect of FeNO-guided asthma management on childhood asthma.

Methods

Study design and participants

The GIA study is a prospective, longitudinal, observational birth cohort study following the offspring of women with asthma during pregnancy who participated in the MAP study, a double-blind RCT conducted in Newcastle, Australia (Australian and New Zealand Clinical Trials Registry, number 12607000561482)(3).

Children were born from February 6, 2008, to November 25, 2010. 174 mothers with 179 children (one set of triplets and 3 twins) were subsequently recruited to the GIA study, of whom 146 were assessed at 12 months (82% participation)(4) and 140 (78%) at 4 to 6 years of age (Supplement Fig 1). The predefined primary aim(4) of the follow-up at 4 to 6 years of age was to determine the effect of FeNO-guided asthma management during pregnancy on the incidence of childhood asthma. The mothers, children and all study personnel involved in the follow-up visits and collection of biosamples were blinded as to which treatment group the mother was allocated during pregnancy. The study was approved by the Hunter New England Health and University of Newcastle Human Research Ethics Committees (ref no 12/06/20/4.03), and written informed consent was obtained before inclusion into the study.

Procedures and outcomes in the MAP study

The primary aim of the MAP study was to test the efficacy of a treatment algorithm using monthly FeNO measurements in combination with asthma symptoms (defined by asthma control questionnaire [ACQ](11)(12), FeNO group) against a symptoms only based approach (clinical group) for the adjustment of maintenance therapy with inhaled corticosteroids (ICS) with and without long-acting beta agonists in pregnant women with asthma. Eligible women were randomised before 22 weeks' gestation, and those with uncontrolled asthma who were not using ICS as maintenance treatment (n=31) were started on budesonide (200 μg twice per day) before randomisation. Women using ICS continued with their current dose, delivered as budesonide turbuhaler (Astra Zeneca, North Ryde, NSW, Australia), with dose equivalence determined from guidelines. Women were reviewed monthly at the antenatal clinic until delivery. The research assistant collected data for the clinical symptoms, ACQ score, current treatments, FeNO, and forced expiratory volume in 1 s (FEV1) in both groups. A statistician applied the relevant algorithm, and sent the treatment recommendation to the research assistant in the antenatal clinic, who informed the participant. Participants were seen by an investigator (PG) in the antenatal clinic if their asthma remained uncontrolled despite being at maximum treatment level in the algorithm. Telephone assessments were done 2 weeks after

each clinic visit to assess symptoms and to encourage drug adherence. Maternal asthma exacerbations during pregnancy were defined as events for which the patient sought medical attention (an unscheduled visit to a doctor, presentation to the emergency room or admission to hospital, or when oral corticosteroids were used for treatment of asthma). All exacerbations that occurred after randomisation were recorded prospectively.

Procedures and outcomes in the GIA study

Questionnaires completed by the parent/carer were the ISAAC core questionnaire (wheezing, rhinitis and eczema modules for 6-7 year old children)(13) and additional standardised respiratory symptom questions validated for pre-schoolers(14, 15). A clinical examination of the child and standardised interview of the primary carer was conducted by a senior medical doctor (advanced trainee level in paediatrics or greater), to confirm the appropriateness of a doctor diagnosis of asthma in accordance with national asthma guidelines(16).

Buccal swabs were collected for DNA extraction and atopy was defined as a positive response (≥3mm wheal) to any one of the common aeroallergens dermatophagoides pteronyssinus, dermatophagoides farinae, cat, dog, aspergillus fumigatus, 5 grass mix, and the standard food allergens whole peanut extract, egg yolk and egg white. Blood sample collection was offered as an opt-in investigation.

Lung function measurement was attempted in all willing participants during attendance at the preschool age check-up. Multiple breath nitrogen washout ($MB_{N2}W$) was performed using commercial $MB_{N2}W$ (Exhalyzer D, EcoMedics AG, Switzerland) in accordance with the consensus statement for inert gas washout measurements using multiple- and single-breath washout(17), and was successfully measured (at least two measurements with a coefficient of variance [CV] within 10%) in 99 out of 128 (77%) children who were willing to be tested. Impulse oscillometry (IOS) was conducted using a combined commercial system (MasterScreen system, Jaeger Co, Wuerzberg, Germany) and successful in 107/122 (88%). Successful measurement was classified as at least three measurements (CV within 10%) with a best coherence threshold ≥0.6 at 5Hz and ≥0.9 at 20Hz. Single breath FeNO was also performed according to ATS/ERS recommendations(18) in order to familiarise the child with the test procedure for future follow-up visits, even though the expiratory manoeuvre can be challenging at preschool age. We used the CLD88 instrument (EcoMedics AG, Switzerland) and acceptable measurements (at least two measurements with a CV within 5% or three measurements within 10%) were recorded in 51/125 (41%).

DNA extraction and genotyping of 17q21 variants

DNA was successfully extracted from 139 of the 140 buccal swabs from participating children collected at one of the follow-up visits (either preschool or 12 month visit) using the Gentra Puregene Buccal Cell kit (QIAGEN). DNA concentration and quality were measured using a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific).

Five previously reported childhood onset asthma-associated single-nucleotide polymorphisms (SNPs) at locus 17q21 were genotyped (rs8069176, rs7216389, rs8076131, rs9303277, rs2290400). Genotyping was performed using the TaqMan (Applied Biosystems Inc) assays and previously described primers and probes(7),(19). Genotyping success rates were 100% for all variants.

RNA extraction from PBMCs and measurement of ORMDL3 gene expression

Five millilitres of whole blood was collected from 72 (51%) consenting children. PBMCs were isolated from whole blood samples using the Lymphoprep^{TM} separation protocol (Stemcell technologies). After PBMC isolation, cells were counted on a hemocytometer and resuspended in 10% FCS RPMI at $1x10^6$cells/ml. Cells were plated into 96 well round bottom plates at $2x10^5$/well and incubated at 37°C for five days. Total mRNA was extracted using TRIzol (Ambion). All steps were performed according to manufacturer's instructions.

ORMDL3 and HPRT primers (Supplementary table 1) were obtained from Jomar Biosciences and qPCR was run using SYBR green (QIAGEN). ORMDL3 was successfully amplified in 71 out of 72 PBMC samples. Each qPCR reaction was run in duplicate and only duplicates with a coefficient of variance of less than 0.05 were included in the analysis. We quantified mRNA copies using cDNA standards for all genes. We normalised expression levels to the house-keeping gene HPRT.

Statistical analysis

Categorical measures were summarised using counts and percentages while continuous measures were summarised using means and standard deviations for normally distributed data, or alternatively medians and interquartile ranges. Data were analysed according to the treatment intervention group to which the mothers were randomised. Due to the very low amount of missing data we present a complete case analysis under the missing completely at random assumption. Statistical significance for continuous secondary outcome variables was determined using a nonparametric, two-tailed Mann-Whitney U-test or the parametric Student's t-test as appropriate. Categorical variables were compared using Chi^2 or Fisher's exact test as appropriate. Estimates of the effect size of the pregnancy intervention on

childhood asthma risk (primary outcome) were calculated as unadjusted odds ratio and obtained using logistic regression models. We also adjusted the models for sex, birth weight, rs8076131, rs8069176, rs9303277, rs7216389, rs2290400, and inhaled corticosteroid use prior to the first RCT screening visit because these variables were demonstrated previously to be associated with childhood asthma. In further explorative analyses, the estimates of the effect size of medications (ICS or LABA at screening visit, randomisation and last visit) on childhood asthma risk were also calculated as odds ratios and obtained using logistic regression models. These models were adjusted for sex, birth weight, rs8076131, rs8069176, rs9303277, rs7216389, and rs2290400. In order to investigate for interaction between 17q21 variants and intervention interaction terms were included in the logistic regression analysis along with confounders. Predictor variables were tested for collinearity using variance inflation factors post estimation. The cut-off for statistical significance was set at 5% for all analyses.

A causal mediation analysis (parametric regression approach described by VanderWeele(20)·(21)) was used to decompose the effect of the FeNO intervention versus ICS use during pregnancy on childhood asthma. We considered "ICS use at any visit during the study" as a mediator (with a logistic regression model), as well as "time to first change in ICS dose" (imperfectly modelled as a linear regression since there are no methods capable of dealing with a censored time to event mediator variable). Smoking status was accounted for as a confounder of the mediator outcome relationship under the assumption that there are no unmeasured confounders of this relationship. We present the natural indirect effect (on the odds ratio scale), representing the effect what a treatment induced change in mediator has on asthma. Standard errors and confidence intervals for the indirect effects were obtained via non-parametric bootstrapping (with n=500 replicates sampled with replacement); lower and upper bounds of the interval are presented as the 2.5^{th} and 97.5^{th} percentiles of the bootstrapped distribution. To complete the mediation analysis, we also assessed the associations of each path separately. The association between treatment group and any use of ICS was assessed using logistic regression; the association between treatment group and time from baseline to first change in ICS dose was assessed using discrete time survival analysis. The association between any ICS use (or time to first change in ICS dosage) and asthma was assessed using logistic regression. A Kaplan-Meier plot for the differences between treatment groups in time to first ICS dosage change is also provided. Hazard ratios (or odds ratios) with 95% confidence intervals are presented. All analyses were programmed in Stata 13

(StataCorp, College Station Texas, USA) and plots were produced using Prism Graph 6 (GraphPad software, La Jolla, USA).

Results

Subject characteristics, prenatal and perinatal history

140 children (78%) attended the follow-up visit between the ages of 4 and 6 years (mean age 59 months, table 1). The intervention groups were balanced regarding the child's sex, age, height and weight, allergic sensitisation and five 17q21 variants (table 1).

Childhood asthma, respiratory symptoms and asthma management during pregnancy

Prevalence of doctor diagnosed asthma was 36% (50/139) in this high-risk cohort. A significantly lower rate of asthma was observed in the offspring of mothers from the FeNO group, compared to the clinical management group (table 2, unadjusted OR 0.46, 95% CI 0.22 to 0.96, p=0.037). The prevalences for wheeze ever, use of short acting beta agonists in the past 12 months, emergency department visits for wheezing or asthma in the last 12 months, and frequent wheeze past 12 months were also significantly lower in the FeNO group (table 2). Significantly fewer children from the FeNO group had a past history of recurrent episodes of bronchiolitis at preschool age (table 2).

Risk factors of childhood asthma

Children diagnosed with asthma were less likely to be female and more likely to have a past history of bronchiolitis and a lower birth weight (table 3). Maternal ICS use at the screening, randomisation and final study visit reduced the odds ratio for childhood asthma (table 3, OR 0.24, 0.35 and 0.46, p=0.002, 0.005 and 0.032, respectively). Average maternal FeNO levels during the RCT and FeNO levels at the final study visit were not significantly associated with childhood asthma (table 3). Doctor diagnosed asthma was significantly associated with risk alleles in the 17q21 variants rs8069176 and rs8076131 (table 3, OR 1.98 and 1.80, p=0.013 and 0.033, respectively), as well as increased airway resistance at 5Hz and FeNO levels but not baseline FEV1 and lung clearance index in the children (Supplementary table 2).

Childhood asthma, asthma management and ICS use during pregnancy

In further explorative analyses we stratified the cohort into four groups of children based on the maternal asthma management group (FeNO or clinical) and the use of ICS (yes or no) at the RCT randomisation and final RCT visit (table 4). The highest prevalence of asthma was observed in those children whose mothers were in the clinical group and did not use ICS at randomisation or final study visit (reference group). The odds ratios for doctor diagnosed asthma (Fig 1 and table 4), wheeze ever, and short acting bronchodilator use in past 12

months (table 4) were significantly lower in children whose mothers were in the FeNO group and whose mothers were in the clinical group and using ICS.

A causal mediation analysis was performed in order to determine to what extent the FeNO intervention caused a reduction in childhood asthma via "ICS use at any visit during the study" (natural indirect effect). This analysis confirmed a significant natural indirect effect of the FeNO intervention on asthma prevalence through "ICS use at any visit during the study" (OR 0.83; 95% CI 0.59 to 0.99). This amounts to approximately 30% of the total effect of FeNO intervention while the remainder was through other pathways. We hypothesized that the FeNO intervention may also have affected the time to the first change in ICS dose. This hypothesis was consistent with Kaplan-Meier estimates of survival probabilities for "time to first change in ICS dose" that showed that the clinical group had a significantly longer time to first change in ICS dose compared to the FeNO group (Fig 2, hazard ratio 1.87, 95% CI 1.22 to 2.84, p=0.004). The natural indirect effect of FeNO intervention on childhood asthma through decreasing time to first ICS dosage change also appeared important in a subsequent causal mediation analysis (OR 0.90, 95% CI 0.70 to 1.03). The natural indirect effects of FeNO intervention through "ICS use at any visit during the study" and "time to first change in ICS dose" are consistent with Figure 1; among children who were born to women that did not use ICS the absolute difference between intervention groups in asthma prevalence was greater; among children who were born to women that did use ICS the absolute difference between intervention groups in asthma prevalence was smaller.

Childhood asthma, 17q21 variants and maternal asthma management during pregnancy

Asthma risk was significantly increased by risk alleles in all five 17q21 variants among those children (n=90) with a prior history of bronchiolitis in accordance with previous reports(7),(22) (Supplementary table 3 and Fig 3 for rs7216389). As expected, none of the 17q21 risk alleles were associated with asthma in those children who never had bronchiolitis (n=78). These gene-environment interactions between bronchiolitis and risk alleles were significant for three out of the five 17q21 variants. We also found a significant association between risk alleles in three out of five 17q21 variants (rs2290400, rs9303277, rs7216389) and the expression patterns of ORMDL3 in PBMCs collected in our cohort (Fig 3 for rs7216389). Although bronchiolitis was a risk factor for childhood asthma (table 3) and recurrent bronchiolitis episodes were reduced in children from the FeNO group (table 2), we could not identify any significant interaction between any risk allele in the five 17q21

variants and the asthma management intervention during pregnancy (Supplementary table 3 and Fig 3 for rs7216389).

Discussion

The results of our study have demonstrated for the first time that FeNO-guided asthma management during pregnancy reduced the odds for doctor diagnosed asthma in preschool children. This intervention was also associated with a significantly reduced risk of frequent wheeze and recurrent bronchiolitis, and less medication use and emergency department visits for wheeze and asthma in the past 12 months prior to the follow up assessment.

A strength of our study is the randomised, controlled design of the MAP study's asthma management intervention along with comprehensive prospective data collection on asthma exacerbations, medications, and FeNO levels throughout pregnancy. Furthermore, our previously published studies on FeNO-guided asthma management in pregnancy and bronchiolitis risks in infancy provided a strong scientific rationale for the primary hypothesis to be tested in the GIA study(4). We acknowledge that a diagnosis of childhood asthma is considered more uncertain the younger the child is. However we demonstrated that childhood asthma diagnosed by the study doctor who was blinded to the mother's pregnancy intervention was associated with common asthma risk factors and asthma-like symptoms, as well as with genetic variants at the 17q21 locus, increased airways resistance at 5Hz and elevated FeNO levels in our preschool cohort. We also confirmed previously described gene-environment interactions at the 17q21 locus(7) (22) in an Australian cohort. Finally we provided functional evidence of variants at locus 17q21 resulting in differences in ORMDL3 expression in a child cohort. This allowed us to demonstrate that FeNO-guided asthma management reduced the odds for childhood asthma independent of risk alleles for early-onset asthma at the 17q21 locus. We found no evidence for a statistical interaction between 17q21 variants in the offspring and FeNO guided management for the risk of asthma. It is possible however that a yet to be determined maternal genotype may interact with FeNO guided management to modify asthma risk. Bisgaard et al observed that supplementation of fish-oil derived fatty acids in pregnancy had a greater effect on wheeze and asthma development in children of mothers with a variant in the gene encoding fatty acid desaturase that was associated with lower blood levels of long-chain polyunsaturated fatty acids (23).

A major effect of FeNO-guided asthma management during pregnancy was a reduction in asthma exacerbations during pregnancy, however this was not associated with childhood asthma risk (table 3). We reported previously(3) that FeNO-guided asthma management also resulted in an increase in ICS and ICS/LABA use during pregnancy. Therefore it was not

unexpected to find a decrease in childhood asthma risk if the mother used ICS (table 3). However, we also found that ICS use at the RCT screening visit -that represents pregnancy exposure prior to any intervention- showed a strong inverse relationship with childhood asthma prevalence (OR 0.24, 95% CI 0.10 to 0.59, p=0.002). This raised the possibility that some of the benefit of the FeNO intervention on childhood asthma may have been mediated by more appropriate ICS use during pregnancy, which was supported by a causal mediation analysis. We also found that time to first change in ICS dose was significantly reduced in the FeNO group compared to the control group (Fig 3). Time to first change in ICS was probably also an important effect mediator, further highlighting the complex effects that FeNO-guided asthma management algorithm had on the use of ICS in pregnancy.

Furthermore, ICS use at the final RCT visit was associated with a lower asthma risk in the clinical group (Fig 1). This suggests that there may be a potential beneficial effect of ICS therapy in pregnancy for reducing childhood asthma risk. We propose that FeNO-guided asthma management resulted in an optimised monthly treatment decision regarding the use and dose of ICS by considering Th2 cytokine-induced airway inflammation along with clinical symptoms. Importantly, our results provide long-term child health outcomes to further support the clinical consensus that the benefits of ICS therapy in pregnancy -when used guided by FeNO levels and clinical symptoms- largely outweigh their potential risks.

FeNO-guided management and ICS maintenance treatment may have resulted in reduced Th2 inflammation during pregnancy and led to changes at the feto-maternal interface that suppressed the development of asthma in early childhood. We previously showed that asthma during pregnancy resulted in differential methylation profiles in the infant's blood DNA(24). Epigenetic changes may have affected airway growth, lung repair and regeneration, or the child's immune response. Interestingly, we found no effect of the pregnancy intervention on atopy suggesting changes that are independent of the child's IgE-mediated immune response.

Our study has limitations. Firstly, despite good participation, our cohort is small however the effect size large and significant in this high-risk cohort. Our results will however enable the design of future appropriately powered RCTs for other outcomes, for instance airway resistance at 5Hz and FeNO levels between the intervention groups that were associated with childhood asthma in our study. In this regard, we are currently conducting the multi-centre Breathing for Life RCT (Australian and New Zealand Clinical Trials Registry number 12613000202763) to test the effect of FeNO-guided asthma management on perinatal

outcomes (primary outcome) and have begun a stage 2 paediatric follow-up study to determine pre-specified childhood outcomes informed by the GIA study(25). A blinded study doctor who took into account parent reported symptoms made the diagnosis of asthma. This can be inaccurate in preschool age although it should have affected both groups equally as mothers and staff remain blinded. In accordance, we found no difference in the association between 17q21 variants and childhood asthma in children from the clinical and FeNO group. Fewer children from the FeNO group participated in the follow-up visit. Investigating the reasons for non-participating at the preschool follow-up visit revealed that more children from the FeNO versus clinical group moved out of the area (14 versus 5 children, respectively) and more did not attend the visit despite scheduling appointments trice (6 versus 2 children) (Supplement Figure 1). We have conducted phone interviews with the parents of children who were unable to attend the follow-up visit but consented to provide information by phone. We could get in contact with 15 parents after attempting on at least three separate occasions. The interviewer and parent remained fully blinded regarding treatment group allocation. The prevalence for doctor diagnosed asthma in the clinical group was 40% (2 out of 5) and in the FeNO group, it was 30% (3 out of 10). When reanalysing the results with all children included, we confirmed a significantly lower rate of doctor diagnosed asthma in the offspring of mothers from the FeNO group, compared to the clinical management group (unadjusted OR 0.497, 95% CI 0.229 to 0.989, p=0.047, n=154). This data further helps to assure that there is no attrition bias.

Our study has important clinical implications because asthma is the most common chronic disease to affect pregnant women, making improvements in this area clinically relevant for a significant number of women and their children. We have demonstrated that management of asthma during pregnancy with a FeNO-guided strategy, which adjusted treatment according to both symptoms and airway inflammation, reduced the odds of asthma at the age of 4-6 years of age by over 50%. Implementation of this approach in clinical practice has the potential to reduce asthma rates among a group of children at high-risk of developing the disease.

Contributors

MM and Joseph Meredith collected data. Joerg Mattes (JM), PGG, VM were responsible for the concept outline, study design, ethics application, funding applications, team meetings, data collection, data interpretation. JM and MM prepared first draft of the manuscript. AC was responsible for the processing and secure storage of re-identifiable bio-specimens, contributed to the study design, data collection and data interpretation. PDR, PDS advised regarding the study design, study methodology, data collection, and provided data interpretation. HP managed data sets, merged data sets, and prepared data for statistical analysis. DB, CO performed statistical analyses. MM and JM prepared tables and figures. JA advised and supervised parts of the statistical analysis. All authors interpreted data, edited and critically reviewed all manuscript drafts.

Declarations of interests

No author has any competing interests to declare.

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Figure 1: Odds ratio and 95% confidence intervals (CI) adjusted for sex, birth weight, rs8076131, rs8069176 for childhood asthma stratified for double-blind asthma management intervention during pregnancy and A) ICS use (yes [+] or no [-]) at randomisation and B) ICS at last RCT visit. *p<0.05

Figure 2: Kaplan-Meir estimates of survival probabilities in FeNO (red) versus clinical (blue) group for time to first change in ICS dose (hazard ratio 1.87, 95% CI 1.22 to 2.84, p=0.004). First visit ("0") is screening visit and second visit ("1") is randomisation visit.

Figure 3: Prevalence of childhood asthma stratified for A) a past history of bronchiolitis or B) double-blind asthma management intervention during pregnancy by rs7216389 alleles (C=cytosine; T=thymidine [risk allele]). C) ORDML3 expression in PBMCs by rs7216389 alleles.

Table 1: Subject characteristics, prenatal/perinatal history by pregnancy intervention in the GIA cohort

	Clinical	FeNO
Total (n=140)	81	59
Female	43/81 (53%)	31/59 (53%)
Age (months)	56 ±10	57 ±9
Mean weight (kg)	19·42 ± 0·30	20·02 ± 0·49
Mean height (cm)	109·2 ± 0·74	110·0 ± 0·99
Atopy (Skin prick test positive <3mm)	21/81 (26%)	15/58 (26%)
Exclusive breastfeeding (weeks)	14 ±24	18 ±21
Prematurity (<37 weeks)	8/81 (10%)	3/59 (6%)
Birth weight (kg)	3·28± 0·07	3·51 ± 0·07
Caesarean section elective	16/78 (21%)	5/56 (9%)
Caesarean section non-elective	10/78 (13%)	8/56 (14%)
rs8069176 (CC)	14/80 (17·5%)	9/58 (15·5%)
(CG)	40/80 (50·0%)	26/58 (44·8%)
(GG)	26/80 (32·5%)	23/58 (39·7%)
rs8076131 (TT)	11/80 (13·8%)	12/58 (20·7%)
(TA)	40/80 (50·0%)	30/58 (51·7%)
(AA)	29/80 (36·3%)	16/58 (27·6%)
rs2290400 (TT)	16/80 (20·0%)	13/58 (22·4%)
(TA)	38/80 (47·5%)	26/58 (44·8%)
(AA)	26/80 (32·5%)	19/58 (32·8%)
rs9303277 (GG)	1/80 (1·3%)	5/58 (8·6%)
(CG)	53/80 (66·3%)	35/58 (60·3%)
(CC)	26/80 (32·5%)	18/58 (31·0%)
rs7216389 (TT)	16/80 (20·0%)	13/58 (24·4%)
(TA)	38/80 (47.5%)	29/58 (50·0%)
(AA)	26/80 (32·5%)	16/58 (27·6%)

Data shown as median ± IQR or mean ± standard error of mean for normally distributed datasets unless otherwise indicated

Table 2: Doctor diagnosed asthma, asthma-associated symptoms and bronchiolitis in the GIA cohort stratified for maternal asthma management during pregnancy

	Clinical group n/N (%)	FeNO group n/N (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
Doctor diagnosed asthma	35/81 (43·2%)	15/58 (25·9%)	**0·46 (0·22 to 0·96) p=0·037**	**0·39 (0·16 to 0·94) p=0·035**
Has your child ever had wheezing or whistling in the chest at any time in the past?	53/81 (65·4%)	28/58 (48·3%)	**0·49 (0·25 to 0·98) p=0·044**	0·45 (0·20 to 1.01) p=0·052
Short-acting beta agonist use in the last 12 months to manage cough, wheeze or asthma	48/79 (61·0%)	23/56 (41·1%)	**0·49 (0·25 to 0·97) p=0·042**	**0·41 (0·18 to 0·93) p=0·033**
Attending the emergency department in the last 12months for wheezing or asthma	14/65 (21·5%)	2/55 (3·6%)	**0·17 (0·04 to 0·76) p=0·021**	**0·15 (0·03 to 0·76) p=0·023**
Frequent wheeze in the last 12 months (greater than four episodes)	18/81 (22·2%)	4/58 (6·9%)	**0·27 (0·09 to 0·87) p=0·027**	**0·24 (0·06 to 0·88) p=0·032**
Bronchiolitis ever	35/77 (45·5%)	18/55 (32·7%)	0·60 (0·29 to 1·24) p=0·167	0·59 (0·27 to 1·29) p=0·186
Frequent bronchiolitis (more than one episode)	21/77 (27·3%)	4/55 (7·3%)	**0·21 (0·07 to 0·65) p=0·007**	**0·15 (0·04 to 0·54) p=0·004**

P values and odds ratios were calculated using unadjusted and adjusted logistic regression models

*adjusted for gender, birth weight, rs8076131, rs8069176, rs9303277, rs7216389, rs2290400, visit 1 ICS use

Table 3: Asthma risk in the GIA cohort according to demographics, genetic risk and perinatal history

	Asthmatic	Non Asthmatic	Unadjusted OR (95% CI)
Female	21/50 (42·0%)	53/89 (59·6%)	**0·49 (0·24 to 0·99) p=0·048**
Atopy (Skin prick test positive <3mm)	17/50 (34·0%)	19/89 (21·3%)	1·90 (0·88 to 4·12) p=0·105
Birth weight (grams)	3194 ± 101·42	3462± 60·77	**1·00 (0·99 to 1·00) p=0·024**
Bronchiolitis ever	29/48 (60·4%)	25/85 (29·4%)	**3·66 (1·74 to 7·70) p=0·001**
Frequent bronchiolitis (more than one episode)	20/48 (41·7%)	6/85 (7·1%)	**9·40 (3·43 to 25·80) p<0·0001**
Maternal self-reported smoking during pregnancy	5/49 (8·5%)	10/89 (11·2%)	0·90 (0·29 to 2·79) p=0·852
Asthma exacerbation during pregnancy	23/49 (46·9%)	37/89 (41·5%)	1·24 (0·62 to 2·51) p=0·543
Maternal ICS use at screening (first) visit	7/50 (14·0%)	36/89 (40·4%)	**0·24 (0·10 to 0·59) p=0·002**
Maternal ICS use at randomisation (second) visit	16/50 (32·0%)	51/89 (57·3%)	**0·35 (0·17 to 0·73) p=0·005**
Maternal ICS use final visit	22/50 (44·0%)	56/89 (62·9%)	**0·46 (0·23 to 0·94) p=0·032**
Maternal ICS+LABA use at at screening (first) visit	7/50 (14·0%)	25/89 (28·1%)	0·42 (0·17 to 1·05) p=0·063
Maternal ICS+LABA use at randomisation (second) visit	10/50 (20·0%)	28/89 (31·4%)	0·54 (0·24 to 1·24) p=0·149
Maternal ICS+LABA use final visit	11/50 (22·0%)	33/89 (37·1%)	0·48 (0·22 to 1·06) p=0·069
Mean maternal FeNO (ppb)	11·32 ±21	11·35 ±13·62	1·00 (0·99 to 1·03) p=0·487
Maternal FeNO final visit (ppb)	8 ±16·5	8·2 ±8·7	1·01 (0·99 to 1·04) p=0·183
rs8069176 (CC)	6/23 (26·1%)	17/23 (73·9%)	**1·98 (1·16 to 3·41) p=0·013**
(CG)	17/65 (26·2%)	48/65 (73·8%)	
(GG)	25/49 (51·0%)	24/49 (49·0%)	
rs8076131 (TT)	4/23 (17·4%)	19/23 (82·6%)	**1·80 (1·05 to 3·09) p=0·033**
(TA)	24/69 (34·8%)	45/69 (65·2%)	
(AA)	20/45 (44·4%)	25/45 (55·5%)	
rs2290400 (TT)	8/29 (27·6%)	21/29 (72·4%)	1·40 (0·85 to 2·29) p=0·185
(TA)	21/63 (33·3%)	42/63 (66·6%)	
(AA)	19/45 (34·5%)	36/45 (65·5%)	
rs9303277 (GG)	1/6 (16·7%)	5/6 (83·3%)	1·69 (0·87 to 3·29) p=0·120
(CG)	28/87 (32·1%)	59/87 (67·8%)	
(CC)	19/44 (43·2%)	25/44 (56·8%)	
rs7216389 (TT)	8/29 (27·6%)	21/29 (72·4%)	1·42 (0·86 to 2·34) p=0·174
(TA)	22/67 (32·8%)	45/67 (67·2%)	
(AA)	18/42 (42·9%)	24/42 (57·1%)	

Data shown as median ± IQR or mean ± standard error of mean for normally distributed datasets unless otherwise indicated
P values and odds ratios were calculated using unadjusted logistic regression models

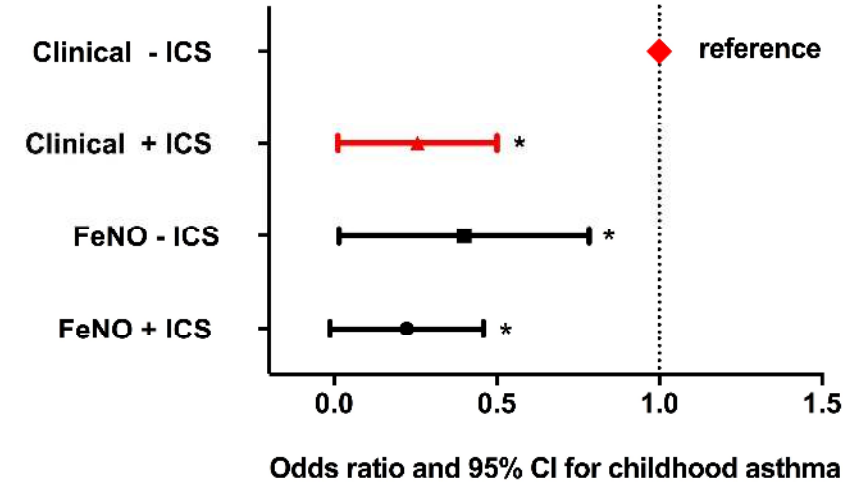
Table 4: Childhood asthma prevalence stratified for ICS use at randomisation visit and for maternal asthma management group

ICS use at randomisation visit					
	Clinical/No ICS (N=40)	**Clinical/ICS (N=41)**	**FeNO/No ICS (N=31)**	**FeNO/ICS (N=28)**	χ^2
Doctor diagnosed asthma	24/41 (59%) Reference	11/40 (28%) **0·19 (0·06 to 0·56) p=0·003**	10/31 (32%) **0·28 (0·09 to 0·87) p=0·028**	5/27 (19%) **0·14 (0·04 to 0·50) p=0·003**	**0·003**
Has your child ever had wheezing or whistling in the chest at any time in the past?	32/41 (78%) Reference	21/40 (52%) **0·24 (0·08 to 0·71) p=0·010**	14/30 (47%) **0·22 (0·07 to 0·71) p=0·011**	14/28 (50%) **0·26 (0·08 to 0·83) p=0·023**	**0·019**
Short-acting beta agonist use in the last 12 months to manage cough, wheeze or asthma	31/40 (78%) Reference	17/39 (44%) **0·20 (0·07 to 0·60) p=0·004**	14/30 (47%) **0·25 (0·08 to 0·79) p=0·018**	9/28 (32%) **0·15 (0·05 to 0·49) p=0·002**	**0·001**
ICS use at final visit					
	Clinical/No ICS (N=47)	**Clinical/ICS (N=34)**	**FeNO/No ICS (N=14)**	**FeNO/ICS (N=44)**	χ^2
Doctor diagnosed asthma	25/47 (53%) Reference	10/34 (29%) **0·23 (0·08 to 0·69) p=0·009**	3/14 (21%) **0·16 (0·03 to 0·90) p=0·038**	12/44 (27%) **0·28 (0·10 to 0·77) p=0·013**	**0·028**
Has your child ever had wheezing or whistling in the chest at any time in the past?	34/47 (72%) Reference	19/34 (56%) 0·37 (0·13 to 1.00) p=0·051	6/14 (43%) 0·27 (0·07 to 1·10) p=0·068	22/44 (50%) **0·36 (0·14 to 0·93) p=0·035**	0·084
Short acting beta agonist use in the last 12 months to manage cough, wheeze or asthma	34/46 (74%) Reference	14/33 (42%) **0·18 (0·06 to 0·52) p=0·002**	4/14 (29%) **0·11 (0·02 to 0·55) p=0·007**	19/44 (43%) **0·24 (0·09 to 0·66) p=0·005**	**0·002**

P values and odds ratios were calculated using adjusted logistic regression models

*adjusted for gender, birth weight, rs8076131, rs8069176, rs9303277, rs7216389, rs2290400

A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Odds ratio and 95% CI for childhood asthma

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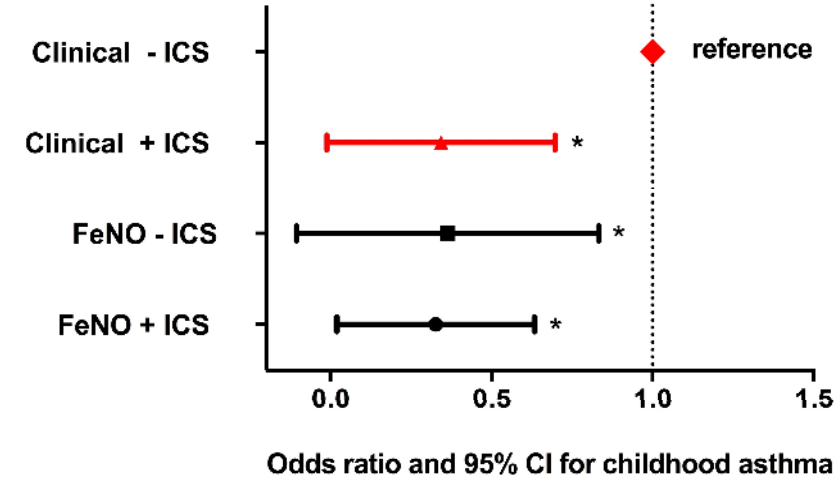
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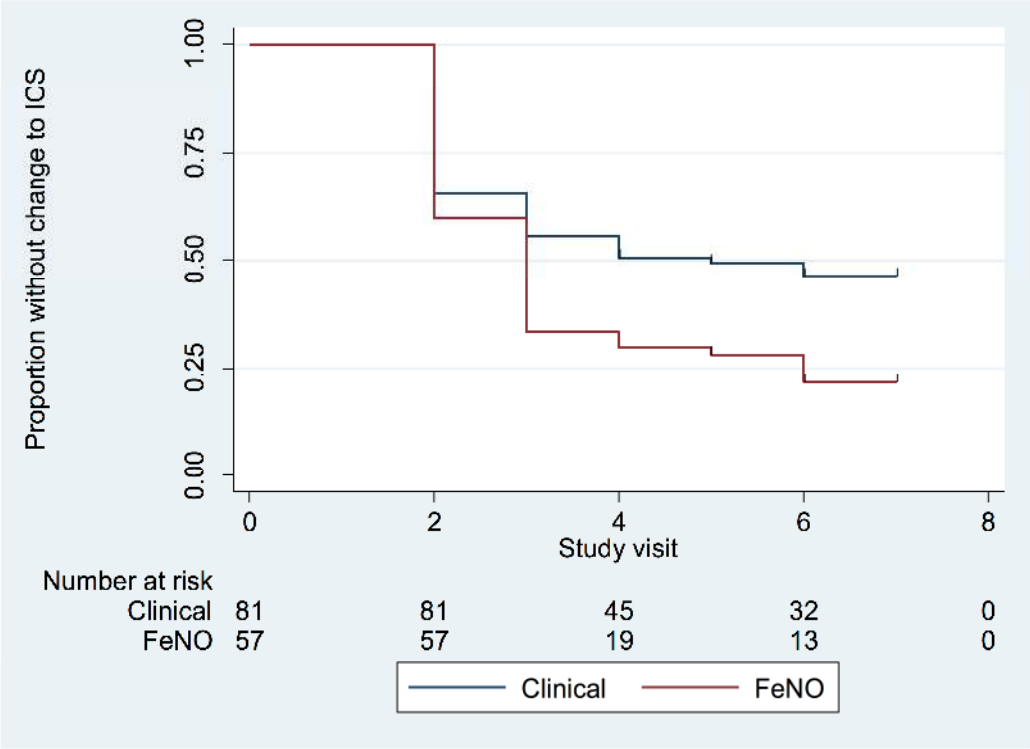

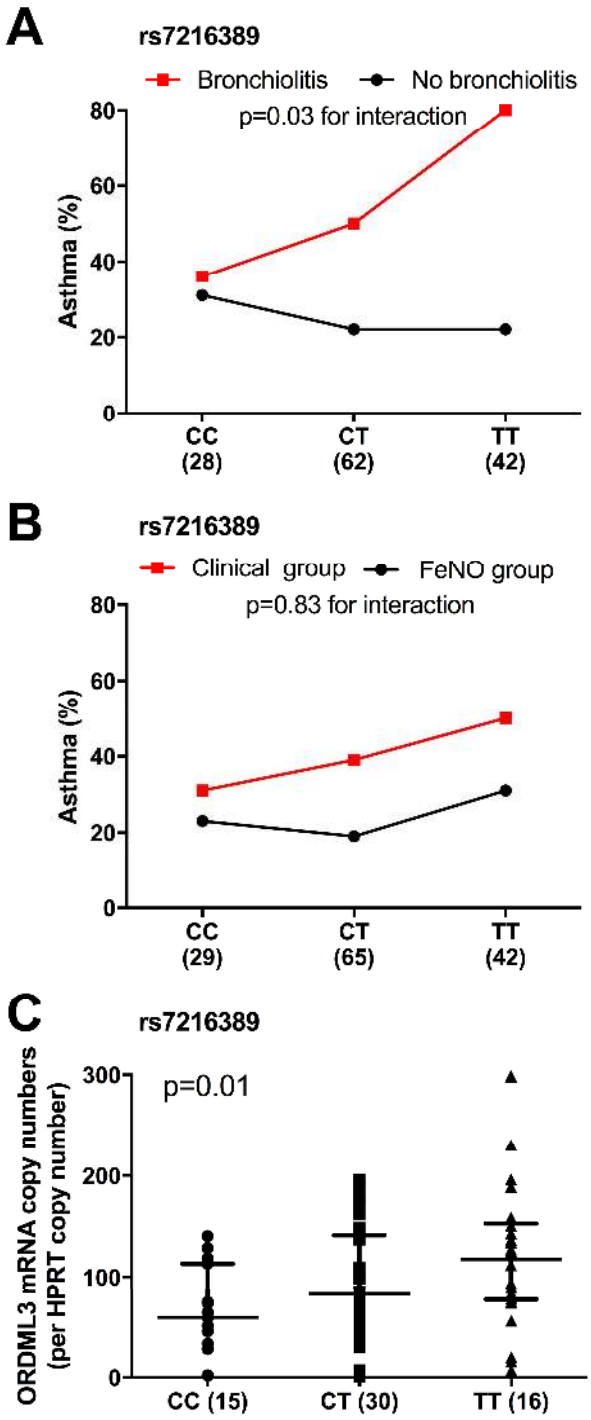

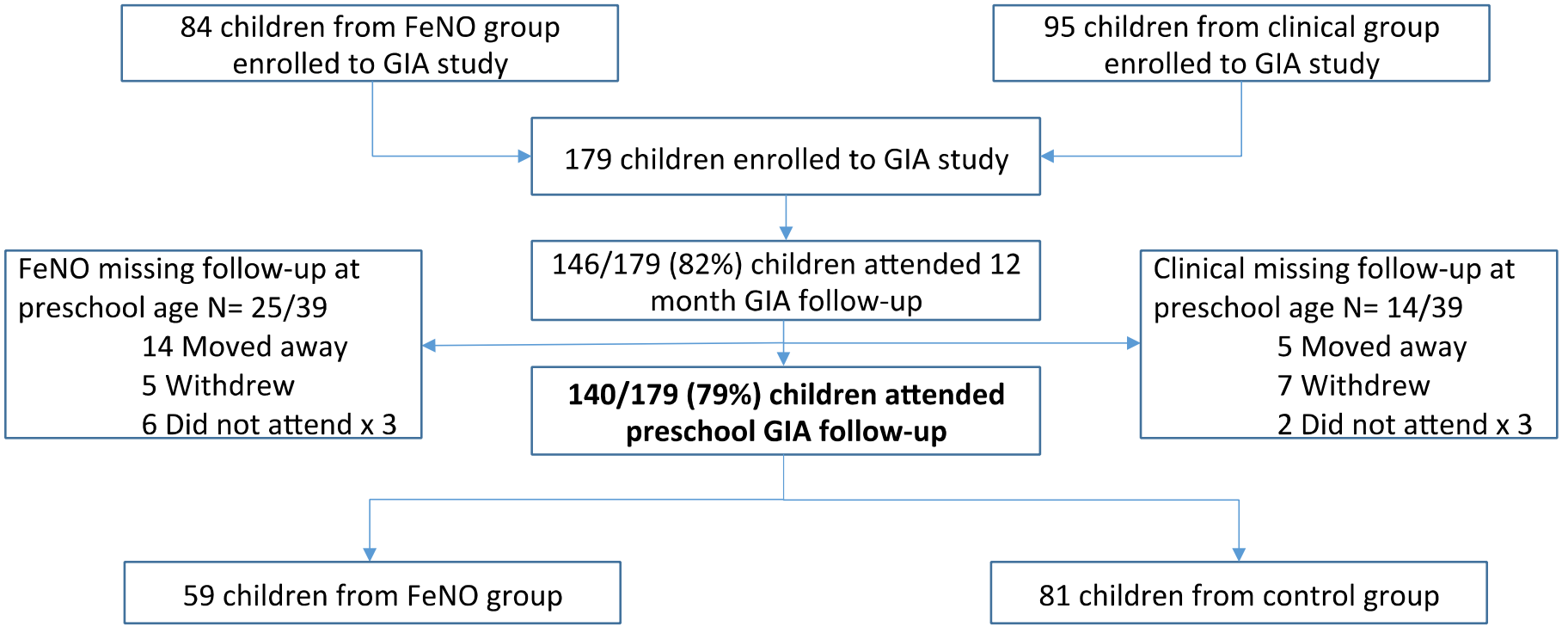

Supplementary Table 1: Primer list

Transcript	Direction	Primer Sequence
ORMDL3	Forward	5'-CAC CAA CCT CAT TCA CAA CA -3'
	Reverse	5'-AAG TAC AGC ACG ATG GGT GT-3'
HPRT	Forward	5'-TGA CAC TGG CAA AAC AAT GCA-3'
	Reverse	5'-GGT CCT TTT CAC CAG CAA GCT-3'

Supplementary Table 2: Lung function according to asthma in GIA cohort

	Asthmatic	Non Asthmatic	Unadjusted OR (95% CI)
Zscore Resistance at 5Hz (N=109)	0·69 ± 0·19	0·19 ± 0·11	**1·64 (1·07 to 2·51) p=0·024**
Lung clearance index (N=96)	7·33 ± 0·14	7·22 ± 0·10	1·19 (0·71 to 1·94) p=0·539
Fraction of exhaled nitric oxide (ppb) (N=51)	5·07 ± 13·63	3·97 ± 3·33	**1·12 (1·01 to 1·25) p=0·028**
FEV1% predicted (N=79)	107.44 ± 14·97	110.36 ± 13·17	0·98 (0·95 to 1·02) p=0·36
FEV1/FVC % predicted (N=79)	102.10 ± 9.17	104.68 ± 8.37	0·97 (0·92 to 1·02) p=0·20

P values and odds ratios were calculated using unadjusted logistic regression models
Data shown as median ± IQR or mean ± standard error of mean for normally distributed datasets

Supplementary Table 3: Association between doctor diagnosed asthma and 17q21 variants stratified for a past history of bronchiolitis and maternal asthma management during pregnancy

SNP	OR (95% CI) for doctor diagnosed asthma*					
	Past history of bronchiolitis (Yes versus No)			Asthma management (Clinical versus FeNO)		
	Yes (N=51)	No (N=78)	Interaction p value	Clinical group (N=80)	FeNO group (N=57)	Interaction p value
rs7216389	**3·38 (1·29 to 8·80) p=0·013**	0·87 (0·39 to 1·92) p=0·729	**0·029**	1·47 (0·71 to 3·06) p=0·298	1·37 (0·54 to 3·48) p=0·513	0·832
rs9303277	**6·75 (1·53 to 29·88) p=0·012**	0·87 (0·32 to 2·35) p=0·775	**0·021**	1·44 (0·51 to 4·04) p=0·494	2·07 (0·65 to 6·59) p=0·219	0·687
rs2290400	**3·41 (1·33 to 8·77) p=0·011**	0·72 (0·32 to 1·60) p=0·423	**0·012**	1·46 (0·70 to 3·05) p=0·309	1·40 (0·56 to 3·51) p=0·468	0·845
rs8076131	**3·18 (1·21 to 8·30) p=0·018**	1·10 (0·46 to 2·60) p=0·834	0·094	1·43 (0·67 to 3·12) p=0·367	1·98 (0·74 to 5·28) p=0·175	0·718
rs8069176	**2·64 (1·09 to 6·39) p=0·031**	1·29 (0·57 to 2·95) p=0·540	0·223	2·11 (0·98 to 4·53) p=0·055	**3·07 (1·03 to 9·14) p=0·044**	0·571

P values and odds ratios were calculated using adjusted logistic regression models

*adjusted for gender, birth weight, visit 1 ICS use