

Chromosome 17q21 Gene Variants Are Associated with Asthma and Exacerbations but Not Atopy in Early Childhood

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Rationale: An asthma predisposition locus on chromosome 17q12-q21 has recently been replicated in different ethnic groups.

Objectives: To characterize the asthma and atopy phenotypes in early childhood that associate with the 17q12-21 locus.

Methods: The single nucleotide polymorphism (SNP), rs7216389, was genotyped in 376 of 411 children from the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) birth cohort born to mothers with asthma together with 305 mothers and 224 fathers. Nineteen additional SNPs in the region were genotyped in the children. Investigator-diagnosed clinical endpoints were based on diary cards and clinic visits every 6 months and at acute symptoms from birth. Lung function, bronchial responsiveness, and sensitization were tested longitudinally from early infancy.

Measurements and Main Results: rs7216389 was significantly associated with the development of wheeze (hazard ratio 1.64 [1.05–2.59], *P* value = 0.03), asthma (hazard ratio, 1.88 [1.15–3.07], *P* = 0.01), and acute severe exacerbations (hazard ratio 2.66 [1.58–4.48], *P* value = 0.0002). The effect on wheeze and asthma was observed for early onset but not late onset of disease. The increased risk of exacerbations persisted from 1 to 6 years of age (incidence ratio 2.48 [1.42–4.32], *P* value = 0.001), and increased bronchial responsiveness was present in infancy and at 4 years of age, but not at 6 years. In contrast, rs7216389 conferred no risk of eczema, rhinitis, or allergic sensitization.

Conclusions: Variation at the chromosome 17q12-q21 locus was associated with approximately twofold increased risk of recurrent wheeze, asthma, asthma exacerbations, and bronchial hyperresponsiveness from early infancy to school age but without conferring risk of eczema, rhinitis, or allergic sensitization. These longitudinal clinical data show this locus to be an important genetic determinant of nonatopic asthma in children.

Keywords: polymorphism; asthma; child; exacerbations; hyperresponsiveness; ORMDL3

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AT A GLANCE COMMENTARY

Scientific Knowledge on This Subject

Chromosome 17q12-q21 is a well-replicated predisposition locus for childhood asthma.

What This Study Adds to the Field

Variation at the chromosome 17q12-21 locus was associated with approximately twofold increased risk of recurrent wheeze, asthma, asthma exacerbations, and bronchial hyperresponsiveness from early infancy to school age but without conferring risk of eczema, rhinitis, or allergic sensitization. These longitudinal clinical data show this locus to be an important genetic determinant of nonatopic asthma in children.

Asthmatic symptoms represent the most common chronic illness in infants and preschool children (1). Hospitalization and other health care use are highest in preschool children, reflecting inadequate disease control (2, 3). Likewise, clinical trials often show incomplete control of exacerbations in young children compared with older children (4, 5). Together this suggests heterogeneity of the underlying disease with the very common asthmatic symptoms in early life probably reflecting different diseases with little to differentiate their clinical presentations (6).

The discovery of new asthma genes opens the possibility of defining phenotypes from causal characteristics, which may improve prevention and treatment of this difficult to control disease entity. A recently reported genome-wide association (GWA) study of 317,000 single-nucleotide polymorphisms (SNPs) in a cohort of children with asthma from the U.K. and Germany resulted in the identification of a novel susceptibility locus on chromosome 17q12-q21 (7). Multiple-associated markers defined an interval that spanned 206 Kb and contained 19 genes. Differential expression of one gene, ORMDL3, was highly correlated with the asthma-associated SNPs, notably rs7216389, leading the authors to suggest ORMDL3 as the most likely disease candidate. The ORMDL genes encode transmembrane proteins anchored in the endoplasmic reticulum, but their physiological role is unknown (8). We recently replicated the association in North American white subjects (9) as have others in Scottish, French Canadian, African American, Puerto Rican, Mexican, and Japanese populations (10–13). The original observation suggested that the associated phenotype was particular to early-onset asthma, which was recently confirmed (14).

The longitudinal impact on phenotypic expression in pre-school-aged children has not previously been studied. Using this

well-replicated asthma predisposition locus on chromosome 17q12-q21, we described the associated asthma and atopic phenotypes assessed longitudinally from birth to school age. We analyzed rs7216389 against the longitudinal clinical assessments of recurrent wheeze, asthma, acute severe exacerbations, eczema, and rhinitis, together with objective measurements of lung function, bronchial responsiveness, and sensitization from early infancy to school age in the high-risk birth-cohort “Copenhagen Prospective Study on Asthma in Childhood” (COPSAC) (15–18). None of the results of these studies has been previously reported.

METHODS

The COPSAC birth cohort study is a prospective clinical study of a birth cohort of 411 infants born to mothers with a history of asthma. The newborns were enrolled at the age of 1 month, the recruitment of which was previously described in detail (15–18). The study was approved by the Ethics Committee for Copenhagen (KF 01-289/96) and The Danish Data Protection Agency (2008-41-1754) and informed consent was obtained from both parents.

The families used doctors employed at the clinical research unit, and not the family practitioner, for diagnosis and treatment of any respiratory or skin-related symptoms. Participants were assessed at the COPSAC clinical research unit at six monthly intervals; additional visits were arranged immediately upon the onset of symptoms. At every visit, the infants were given a full physical examination, and history was obtained using structured questions and closed response categories focusing on the child's lung and skin symptoms, medication, healthcare use, lifestyle, and home environment.

Investigator-Diagnosed Clinical Endpoints

Recurrent wheeze. Respiratory symptoms from birth to 6 years of age were recorded in daily diaries by parents. Symptoms were defined as wheeze or whistling sounds, breathlessness, or a persistent and troublesome cough severely affecting the well being of the infant, as previously described (16, 17). The doctor at the clinical research unit reviewed symptom definitions and the diary entries with the parents at the six monthly clinical sessions as well as at acute severe exacerbations. Recurrent wheeze was diagnosed from the diaries as five episodes within 6 months, each episode lasting at least 3 consecutive days, or daily symptoms for 4 consecutive weeks (16, 17).

Asthma was diagnosed according to the international guidelines, as previously detailed (17), and was based on recurrent wheeze as defined above. The character of symptoms, judged by the clinical research unit doctor, were considered to be typical of asthma with discrete exacerbations, but they also included symptoms between episodes, such as exercise-induced symptoms, prolonged nocturnal cough, persistent cough outside common cold, symptoms causing wakening at night (recently termed “multi-trigger wheeze” [19]) and in need of intermittent rescue with inhaled β_2 -agonist and responding to a 3-month course of inhaled corticosteroids but relapsing when stopping treatment.

Episodic viral wheeze (19) is characterized as children who suffer from discrete wheezy episodes, feeling well between episodes, and treated intermittently with inhaled β_2 -agonist only.

Temporal patterns of wheeze were defined as “early transient” if the child fulfilled the above criteria for recurrent wheeze in the first 3 years of life but not thereafter, “persistent” if the child also fulfilled the criteria from 5 to 6 years of age, and “late onset” if the child had a debut after 3 years of age.

Acute severe exacerbations were defined from need of oral prednisolone or high-dose inhaled corticosteroid for wheezy symptoms prescribed at the discretion of the doctor at the clinical research unit or acute hospitalization at the local hospital for such symptoms.

Rhinitis was diagnosed in children by 6 years of age by the clinical research unit doctor and was based on symptoms during the previous year of sneezing or a runny or blocked nose in periods when the child did not have a cold or flu (20).

Eczema. Skin lesions were described at both scheduled and acute visits according to predefined morphology and localization; eczema was defined based on the Hanifin-Rajka criteria, as previously detailed (21, 22).

Treatment Algorithms

Wheezy symptoms were treated by the clinical research unit doctors in accordance with a strict predefined algorithm, as previously described (16, 17). All subjects with episodes of wheeze participated in the randomized controlled clinical trial of intermittent treatment with inhaled budesonide 400 μ g per day versus placebo for 2 weeks during wheezy episodes in the first 3 years of life, showing no short-term or long-term treatment effect on the primary endpoint or on the current endpoints of exacerbations (16). **Episodic wheeze** was treated with terbutaline (Bricanyl AstraZeneca, Lund, Sweden) in a pressurized metered dose inhaler (pMDI) with a spacer to be administered as needed. Asthma symptoms, as defined above, marked the threshold for starting daily treatment with 400 μ g of inhaled budesonide administered by a pMDI with a spacer for 3 months increasing to 6 and 12 months at subsequent relapses. Montelukast administered 4 mg daily was given to children with recurrent wheeze, despite budesonide maintenance treatment. Acute severe exacerbation of wheeze was treated with 1600 μ g of budesonide for 2 weeks or oral prednisolone 1–2 mg/kg daily for 3 days. No other treatment was allowed for wheezy symptoms.

Objective Measurements

Neonatal lung function was tested by the Raised Volume Rapid Thoraco-Abdominal Compression technique (RVRTC) (23). The infant was sedated with an oral dose of chloral hydrate and monitored continuously by both pulse-oximetry and trans-cutaneous oxygen pressure (PtcO₂) (TCM3 from Radiometer, Copenhagen, Denmark). A nonexpandable outer coat was wrapped around the infant's chest and abdomen with an inflatable “balloon” inside. Inflation through the pneumotacograph to a trans-respiratory pressure of 2 kPa raised the infant's lung volume prior to a forced expiration. A compression force transmitting an additional pressure of 2 kPa was then applied via the squeeze-jacket to the thorax and abdomen at the end of the third inspiration, leading to an airway opening pressure of 4 kPa for the forced expirations. Forced expiratory volume was estimated at 0.5 seconds (FEV0.5).

Bronchial responsiveness in infants was determined as the responsiveness to methacholine. The aerosol was administered with a dosimeter. After initial inhalation of saline, methacholine chloride was given in quadrupling dose steps from 0.04–16.67 μ mol. The test procedure aimed at 20% fall in FEV0.5 or reaching the maximum dose. FEV0.5 was chosen as the endpoint for the baseline lung function and PD₁₅(TcO₂) as endpoint for bronchial responsiveness based on previous sensitivity analyses of known indices (23).

Specific airway resistance (sRaw) was measured at 4 and 6 years by whole body plethysmography (24, 25). Bronchial responsiveness at the ages of 4 and 6 years was determined as the relative change in sRaw after hyperventilation of cold-dry air (26).

Atopic sensitization was determined from specific IgE at the ages of 6 months, 1.5 years, 4 years, and 6 years by ImmunoCAP (27) (Phadia AB, Uppsala, Sweden) against the most common food and inhalant allergens (28). Values of 0.35 kU/L or greater were analyzed as the dichotomized index of any sensitization.

Genotyping

rs7216389. Allelic discrimination at rs7216389 was performed using an Applied Biosystems Custom Taqman SNP Genotyping assay (c/n 4332072) on a 7700 Sequence Detection System. The variant was in Hardy-Weinberg equilibrium ($P > 0.05$).

Multiple SNP genotyping. A high throughput genome-wide single nucleotide polymorphism (SNP) genotyping, using the Illumina Infinium II HumanHap550 BeadChip technology (29) (Illumina, San Diego, CA), was performed at the Center for Applied Genomics of the Children's Hospital of Philadelphia, as previously described (30). Of 561,466 SNPs genotyped, 7,068 had call rates less than 95%, 22,327 had minor allele frequency (MAF) less than 1%, and 2,234 SNPs had Hardy Weinberg equilibrium $P < 10^{-5}$ and were rejected. After quality-control measures were completed, 514,386 SNPs remained in the analysis. To gain further information about the genetic variability at the *ORMDL3* locus, nineteen SNPs spanning 186 Kb (35,175–35,361 Kb) around rs7216389 were chosen for extended analyses in the present study.

Statistical Analyses

Cumulative risk of age at onset was estimated using Kaplan-Meier estimates and comparisons were made by log rank tests. The effect of the rs7216389 SNP on the age of onset outcomes was quantified in terms of hazard ratios (HR) by Cox proportional hazards regression (*P* values correspond to Wald tests). The effect on a binary outcome (sensitization, rhinitis, and current asthma by 6 years of age) was modeled by logistic regression. The incidences of exacerbations were calculated in six different age spans (0–1, 1–2, 2–3, 3–4, 4–5, 5–6) for each level of the rs7216389 SNP. Age-adjusted incidence ratios for exacerbations were analyzed by a log-linear GEE model with working independence correlation structure (*P* values correspond to robust Wald tests). The odds of rs7216389 levels were compared among traditional subgroups of wheezers by logistic regression. For β_2 we analyzed the odds of a β_2 by robust logistic regression taking into account within-child correlation. The association between the multiple SNPs and asthma-related events was tested by Cox regression (log-rank test).

For the transmission disequilibrium testing (TDT) parental discordance testing was performed as described (37) considering transmissions from heterozygous mothers versus heterozygous fathers to affected offspring separately.

Analyses were done using R version 2.7.0 (www.r-project.org) and SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

The rs7216389 SNP was genotyped in 376 of 411 children who had good quality DNA available. Genotyping success rate was greater than 98%. The genotype distribution was: CC, 23%; CT, 48%; TT, 29%. The clinical follow-up rate of the COPSAC cohort was 95% at age 1 year; 90% at age 2 years; 85% at age 3 years; 79% at age 4 years; and 76% at age 5 and 6 years.

Investigator-Diagnosed Clinical Endpoints

Recurrent wheeze. Eighty of 376 children developed recurrent wheeze during follow-up. The Kaplan-Meier curves suggest a recessive genetic model with increased risk in subjects with the TT genotype (see Figure EA in the online supplement). A log-rank test comparing CC and CT genotype yielded a *P* value of 0.89. The overall HR in a recessive model for the T allele was 1.64 [1.05–2.59] (*P* value = 0.03). The Kaplan-Meier curves suggest a time-varying effect with the TT genotype being associated with early onset, but not late onset, of wheeze. Accordingly, the HR was significantly increased during the ages of 0 to 3 years (2.06 [1.24–3.39]; *P* value = 0.005), but not during the ages of 3 to 6 years (0.60 [0.17–2.08]; *P* value = 0.42), using a recessive genetic model.

Asthma. Sixty-six of 376 children were diagnosed with asthma during follow-up. The Kaplan-Meier curves suggest a recessive genetic model with increased risk in subjects with the TT genotype (Figure 1). A log-rank test comparing CC and CT genotypes yielded a *P* value of 0.78. The overall HR in a recessive model for the T allele was significantly increased (1.88 [1.15–3.07]; *P* value = 0.01). The Kaplan-Meier curves suggest a time-varying effect with the TT genotype being associated with early onset but not late onset of asthma. Accordingly, the HR during the ages of 0 to 3 years was increased approximately twofold (2.03 [1.18–3.50]; *P* value = 0.01), but not during the ages of 3 to 6 years (1.33 [0.41–4.31]; *P* value = 0.64). The population attributable cumulative 6-year risk in the recessive model was estimated to be 19.4–16.2 = 3.2%.

Acute severe exacerbations. Fifty-seven of 376 children developed acute severe exacerbations on one or more occasions during follow-up. The Kaplan-Meier curves suggest a recessive genetic model with increased risk in subjects with the TT genotype (Figure EB). A log-rank test comparing CC and CT geno-

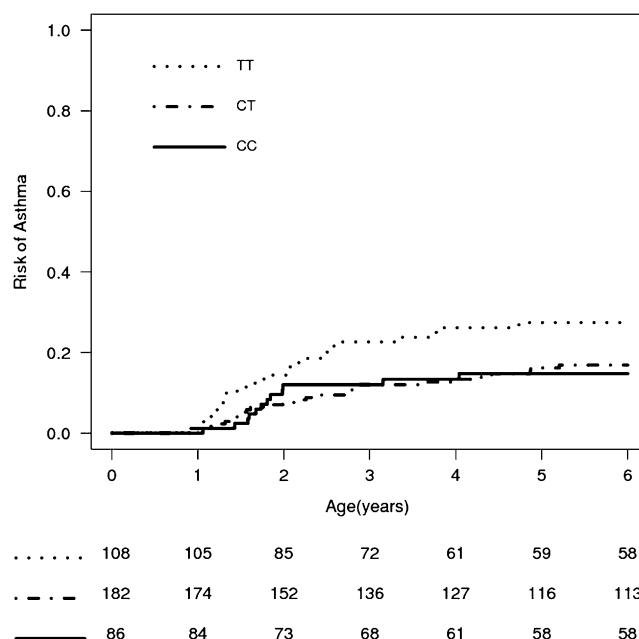


Figure 1. Asthma development during the first 6 years of life. Kaplan-Meier estimates of cumulative risk of asthma are indicated for children with and without rs7216389 polymorphisms. Numbers at risk at are given below the graph.

types yielded a *P* value of 0.81. The overall HR in a recessive model for the T allele was significantly increased (2.66 [1.58–4.48]; *P* value = 0.0002). The Kaplan-Meier curves suggest a stable effect during the time of follow-up. Accordingly, the HR increased 2.5-fold during ages 0 to 3 years (2.46 [1.38–4.38]; *P* value = 0.002) and over threefold during ages 3 to 6 years (3.73 [1.14–12.23]; *P* value = 0.03). The population attributable cumulative 6-year risk in the recessive model was estimated to be 16.5 – 11.8 = 4.6%.

The yearly incidence of having at least one wheezy exacerbation requiring high-dose steroid intervention or hospitalization per child in the study during the first 6 years of life is depicted in Figure 2 for the three genotypes. A recessive model could be assumed for age-adjusted incidence ratios (*P* value = 0.61). The incidence ratio for the TT versus CC or CT genotypes was 2.73 [1.49–5.00] (*P* value = 0.001).

Sensitization from 6 months to 6 years (37% of children) did not modify the effect on “asthma-related events” (*P* = 0.98).

We also analyzed the association of the TT variant with three traditional subgroups of “wheezers” based on (1) temporal pattern of symptoms (31) showing a significant association to early transient but not late onset or persistent wheeze; (2) “atopic wheeze,” i.e., wheeze with concomitant sensitization suggesting nonsignificant trends of association predominantly with the non-sensitized phenotype; and (3) the “episodic viral wheeze” (19) showing the odds of β_2 -agonist use was also significantly increased in children with TT genotype (OR = 1.62 [1.08–2.43], *P* value = 0.02) (see Table E1 in the online supplement).

Atopy. The rs7216389 polymorphism was not significantly associated with the cross-sectional diagnoses of rhinitis by the age of 6 years (OR TT versus CT or CC: 0.64 [0.38–1.09], *P* value using Fishers exact test = 0.12); sensitization ever by the age of 6 years (OR of TT versus CC 0.682 [0.329–1.412], CT versus CC 1.306 [0.706–2.417]; *P* value Fischer’s exact test: *P* = 0.12), or the risk of developing eczema (HR of TT versus CT or CC: 1.02 [0.73–1.43], *P* value log-rank = 0.90) (Figure EC).

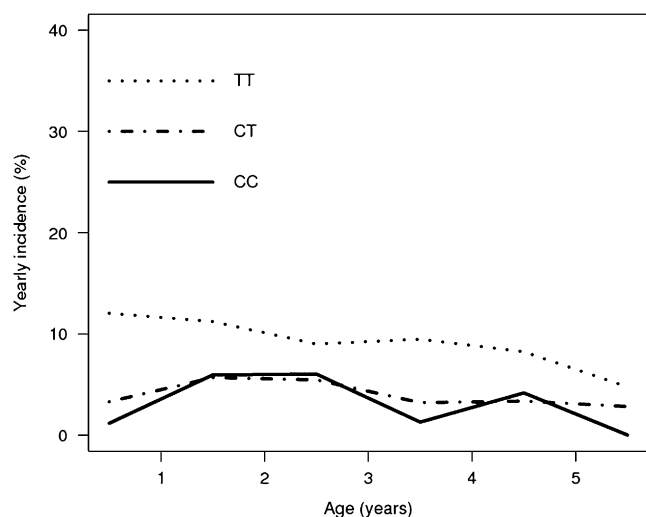


Figure 2. Yearly incidence of one or more acute wheezy exacerbations requiring high-dose steroid intervention or hospitalization stratified on age and R57216389 levels.

Objective Endpoints. Lung function values were tested in a recessive model as there were no differences between CC and CT genotypes. Baseline lung function values were not significantly associated with the rs7216389. Bronchial responsiveness was significantly increased by 1 month of age (P value = 0.035), by the age of 4 years (P = 0.011) and a similar but non-significant trend at the age of 6 years (P = 0.251) (Table 1).

Parental and Family-based Analyses

In addition to genotyping the rs7216389 SNP in 376 COPSAC children we also genotyped it in 305 COPSAC mothers and 224 COPSAC fathers. Of those, 316 unrelated individuals had physician-diagnosed asthma during childhood. To determine if this phenotype also associates with the *ORMDL3* locus, we performed a case-control analysis of the unrelated affected COPSAC individuals indicated, using the publically available genotypes from the 1958 birth cohort (n = 1500) (32) as controls. We performed Eigenstrat analysis on the sample set to control for population stratification. We observed a significant association of rs7216389 with asthma (minor allele frequency in cases 53% and 46% in controls; P value = 0.0002; OR = 1.3), thereby corroborating the original finding of Moffat and colleagues (7) to physician-diagnosed asthma in the Danish population.

Because genotypes for the at-risk allele were available for the COPSAC family trios, we examined the parent-of-origin effect through transmission disequilibrium testing in the COPSAC children. We observed over-transmission of the at-risk T allele from the affected mothers to the affected offspring (maternal minor allele C transmitted:untransmitted 12.5:21.5; paternal minor allele C transmitted:untransmitted 14.5:14.5). Although preliminary, these results lend support to previous reports suggesting maternal origin of allelic effects in asthma and other atopic disorders (33).

Extended SNP Analyses at the *ORMDL3* Locus

The onset of recurrent wheeze, asthma, and acute severe asthma exacerbations were closely correlated. To further assess the genetic association and variability at the *ORMDL3* locus, we next analyzed the composite endpoint “asthma-related event” using all 20 SNPs that were genotyped at this locus. Figure 3 shows the significance of the association between the set of SNPs and the outcome ($-\log [P$ values] of the log-rank test) according to the codominant and recessive models. The recessive model was defined by choosing as reference in the Cox proportional hazards model the homozygote genotype, which differs most from the two other genotypes. Ten SNPs of 18 (including rs7216389) were significantly associated with the age at onset of an asthma-related event. These SNPs were all located in the upstream end of the region and showed similar levels of significance to that of rs7216389, thereby effectively ruling out genotyping error or other spurious reasons for the associations reported. The figure additionally shows that the association was stronger in the recessive model.

DISCUSSION

Key Results

Here, we define for the first time, the asthma and atopy phenotype associated with the gene variants at the *ORMDL3* locus on 17q12-q21 based on prospective longitudinal assessment of asthma symptoms and lung function from birth. In the COPSAC birth cohort, children homozygous for the T allele of rs7216389 were phenotypically characterized by early onset of asthma symptoms and increased risk of severe exacerbations as well as bronchial hyperresponsiveness assessed objectively from infancy to school age. No effect was observed on bronchial responsiveness or lung function at school age (6 years) or on eczema, allergic rhinitis, or allergic sensitization. This is the first example of a genetically defined nonatopic asthma phenotype of the early childhood.

Asthma symptoms and asthma exacerbations in early life represent a severe disease burden with major impact on quality

TABLE 1. ASSOCIATIONS BETWEEN rs7216389 POLYMORPHISM AND BASELINE LUNG FUNCTION AND BRONCHIAL RESPONSIVENESS AT 1 MONTH, 4 YEARS, AND 6 YEARS OF AGE

	TT		CC + CT		TT versus CC + CT
	N	Median	N	Median	Wilcoxon
Baseline lung function					
1 mo of age (FEV 0.5; ml)	107	67	264	66	0.604
4 yrs of age (sRaw; kPa-s)	80	1.26	191	1.27	0.495
6 yrs of age (sRaw; kPa-s)	83	1.31	205	1.23	0.117
Bronchial responsiveness					
1 mo of age (PD ₁₅ TcO ₂ ; μ mol methacholine)	93	0.26	239	0.37	0.035
4 y of age (percent change in sRaw after cold-air hyperventilation)	66	10%	161	6%	0.011
6 y of age (percent change in sRaw after cold-air hyperventilation)	69	9%	173	7%	0.251

Definitions of abbreviations: PD₁₅TcO₂ = provocative dose causing 15% drop in transcutaneous oxygen pressure; sRaw = specific airway resistance

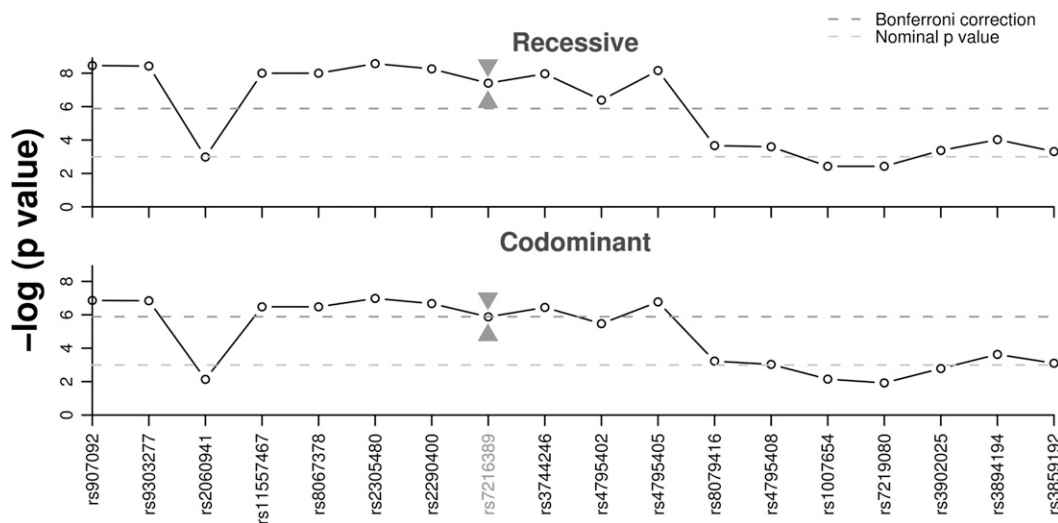


Figure 3. Significance of the associations between the age at onset of an asthma-related event and a set of single nucleotide polymorphism (SNPs) located in a larger region spanning 186 Kb around rs7216389. The $-\log(P)$ values are displayed for both the codominant and “most likely recessive” models. The SNP rs7216389 (arrowheads) is located within a region including several SNPs, which are significantly associated with the outcome.

of life for patients and socioeconomic costs for the health care system. The TT genotype of the rs7216389 variant at the *ORMDL3* locus was strongly associated with recurrent wheeze, asthma, severe asthma exacerbations, and hospitalization from infancy. Furthermore, the population impact of this genetic polymorphism was substantial due to the high allele frequency of the disease variant. The population attributable cumulative 6-year risk suggests that elimination of the risk associated with the T allele in a similar population of children born to mothers with a history of asthma should reduce the proportion of children with asthma and severe exacerbations before the age of 6 years by 16 and 28%, respectively.

The effect of the at-risk allele of rs7216389 on the physician-diagnosed asthma phenotype, as originally reported by Moffat and colleagues (7), was also observed in the Danish population in a case-control analysis of mothers with asthma in the COPSAC birth cohort compared with publically available genotypes from the 1958 birth cohort (32). The strength of the association here is in keeping with the original report and our recent replication (9). Finally, several tagging SNPs extracted from a genome-wide genotyping analysis for the 17q12-q21 locus were also strongly associated with the same phenotypes as the rs7216389 variant, thereby further substantiating the association signals in the COPSAC children.

Strength and Limitations

The strength of the genetic association is underlined by the full disease continuum in the COPSAC cohort from children without lung symptoms to children with intermittent, persistent and severe symptoms.

A well-defined phenotype is essential in genetic association studies. This is particularly difficult in the clinical evaluation of the early childhood wheeze where interobserver variation is a significant problem due to inaccurate use of terms among clinicians and caregivers (34–37).

The major strength of the COPSAC study is the meticulous prospective clinical monitoring, diagnosing, and treatment of lung and skin symptoms based on standard operating procedures by the investigators from this single clinical research unit through the first 6 years of life of the cohort. The cohort was seen regularly at 6-month intervals as well as for acute lung and skin manifestations by the doctor in the COPSAC clinic, who controlled diagnosis and treatment according to predefined algorithms, (i.e., diagnoses and treatments were not made by doctors outside our research unit). Additionally, the longitudinal objective assess-

ments of lung function and bronchial responsiveness from birth through preschool age assure robust objective endpoints. Together this prospective clinical monitoring in a single center is the key difference to other cohorts often based on questionnaires and parents history of diagnoses made by doctors in the community.

Asthma was diagnosed prospectively at the clinical research unit according to a rigid algorithm based on predefined recurrence of diary-recorded wheeze episodes, symptoms typical of asthma, need of short-acting bronchodilator treatments, response to inhaled corticosteroids, and relapse after stopping treatment (17). The accuracy of such pragmatic diagnosis was strengthened by the history being reported by mothers all experienced with asthma and by consistency with the independent intermediary endpoints recurrent wheeze and acute severe exacerbations as well as the repeated objective assessments of bronchial hyper-responsiveness.

The power of the statistics was improved from the longitudinal data set with the time of onset clearly distinguishing these populations. Complex human diseases have variable ages of onset. Because the age of onset is likely to be genetically mediated, the subject's age of onset carries more information about the etiology of the disease than the case-control status. Cross-sectional analyses have less statistical power.

The external validity of this study is limited by the high-risk nature of the cohort and would benefit from replication in population-based studies. Atopic sensitization did not modify the association between rs7216389 and asthma-related events suggesting that the increased frequency of sensitization in this cohort did not modify the effect of the gene variant. Although the parent of origin transmission disequilibrium testing in COPSAC demonstrated overtransmission of the risk T allele from the affected mothers to the affected offspring, our study is too small to accurately estimate the risk ratio of such overtransmission from the affected parent.

Data from both the initial discovery study and a follow-up association study in African Americans, Puerto Ricans, and Mexicans is suggestive of genetic complexity at the 17q12-q21 interval with the possibility of multiple independent effects in Northern Europeans and varying patterns of association in the non-European populations. In line with the discovery study (7), we showed significant linkage disequilibrium between several SNPs in the region and our results confirm rs7216389 as a relevant tagging SNP in populations of Northern European ancestry. We used this surrogate to tag at least one of the major asthma predisposition variants at this locus.

Interpretation

Asthma is the most common chronic disease in children with peak prevalence during preschool age. Many outgrow the disease, but the disease burden is high during the early years of life with higher risk of severe exacerbations and hospitalization than in older children and adults. The prevention of asthma-related exacerbations is a main goal of management (38, 39), yet exacerbations remain the most common cause of hospitalizations in children and accounts for up to three-quarters of the total direct cost of asthma management in the U.S. and Europe (2, 3). A major reason for the limited success in prevention and treatment of preschool asthma is probably the heterogeneous nature of the disease (6).

Different phenotypes of young children with asthma-like symptoms have been proposed based on the temporal patterns of symptoms and their relation to markers of atopy of which the TT genotype of the rs7216389 variant resembles mostly “transient wheezer” (31) and “the nonatopic wheezing phenotype” (40). However, phenotyping based on symptom course and relation to atopy have had little influence on the treatment of preschool wheeze and the understanding of the underlying pathogenesis, whereas phenotypes defined from genotypes are likely to improve targeted treatment and prevention because of the causal relation. Therefore, the recognition of this common gene variant as a major risk factor for nonatopic asthma and severe exacerbations in young children of Northern European descent has the potential of being an important step for the urgently needed improved treatment and prevention of this disease as well as research into environmental risk factors.

Bronchial responsiveness was increased in newborns and maintained during preschool age in children homozygous for the T allele of the rs7216389 polymorphism. This lends biological plausibility to the findings of increased exacerbations rate and suggests that increased bronchial responsiveness is a hallmark and maybe a causal intermediate step between this genetic variation and symptoms of the associated asthma phenotype. In contrast, the gene variant was not associated with bronchial responsiveness or lung function in children at 6 years of age. This is in line with a recent study suggesting that reduced lung function at school age is associated with the atopic and not the nonatopic asthma phenotype (41).

The underlying mechanism of the association between genetic variation on chromosome 17q12-q21 and asthma is not yet understood. Multiple variants within the region have been shown by us and others to contribute independent effects, indicating the potential for several functional SNPs in the region. Among the potentially functional variants within the interval, rs7216389, which lies within an intron of the *GSDML* gene, was shown to be associated with the transcript levels of the neighboring *ORMDL3* gene. Although the functional effects of the disease-associated variant of rs7216389 on *ORMDL3* expression renders it a strong candidate for the disease-causal variation, one needs to keep in mind that the association between rs7216389 and *ORMDL3* expression may be coincidental to asthma predisposition and one or more of the other associated SNPs in the interval may underlie the reported association with the disease trait. Resequencing of the region will be needed to pinpoint the actual functional variant(s).

The discovery cohort of adults recalling “asthma ever” reported an OR of 1.21 [1.04–1.40]. Recall of “asthma attacks” before the age of 8 years was associated with rs3894194 (OR 1.68 [1.25–2.26]) (7). The increased risk of early childhood asthma and exacerbations was confirmed in recent studies (10–12, 14). Our data are consistent with these observations, and, by longitudinal assessment from birth, we extended the observations by showing

a strong association with severe symptoms from the first year of life.

The recessive model for the T allele of the rs7216389 SNP was appropriate for all objective and clinical outcomes. A current case-control study also reports a T allele recessive model for asthma exacerbations but finds a T allele dominant model for asthma in a cross-sectional study of 3- to 22-year-old individuals (10). It is plausible that differences in the genetic mechanism may vary between disease outcomes and over time, and that heterozygous CT individuals in our cohort may develop asthma more frequently than those homozygous for the C allele.

Conclusions

In conclusion, we have firmly established that genetic variation at 17q12-q21 is as a major and independent predisposing factor for asthma but not atopy in young children of Northern European descent. This genotype defines an asthma phenotype with recurrent wheeze, asthma, and acute severe exacerbations, together with the objective correlate, bronchial hyperresponsiveness, from neonatal to school age, and no increased risk was detected for atopy (eczema, rhinitis, and sensitization). The population attributable risk suggests that this genetic variant is responsible for a considerable part of the asthmatic disease burden in preschool years. Although the biologic mechanism behind these associations remains to be explained, it proposes a focus for a possible new molecular mechanism underlying early asthma and exacerbations that might, in the future, provide an alternative pathway for the targeting of therapeutics for the management of asthma and its exacerbations. Furthermore, the polymorphism provides a predictor which may be useful for future targeted research into prevention of early asthma and represents a first example of a genetically defined particular non-atopic asthmatic phenotype of early childhood.

Conflict of Interest Statement: H.B. has been a consultant and advisor to NeoLab, Nycomed, and Merck and paid lecturer for AstraZeneca and Merck; he holds sponsored grants from NeoLab and Medimmune. K.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. P.M.A.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. B.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.K.-M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. C.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. R.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. F.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. C.B.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. C.N.A.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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Chromosome 17q21 Gene Variants Are Associated with Asthma and Exacerbations but Not Atopy in Early Childhood

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ONLINE DATA SUPPLEMENT

ON-LINE REPOSITORY

Table E1. On-line.

	TT	CC or CT	OR (95% CI)	P-value (Wald)
Asthma + sensitization	2	10	0.51 (0.11-2.36)	0.39
Asthma - sensitization	10	15	1.69 (0.73-3.91)	0.22
None	86	218	1	-
Persistent	11	19	1.59 (0.71-3.55)	0.26
Early Transient	13	16	2.23 (1.01-4.94)	0.05
Late onset	4	24	0.46 (0.15-1.38)	0.16
None	55	151	1	-

Figure EA: Wheeze development during the first 6 years of life. Kaplan-Meier estimates of cumulative risk recurrent wheeze is indicated for neonates with and without RS7216389 polymorphisms. Numbers at risk at are given below the graph.

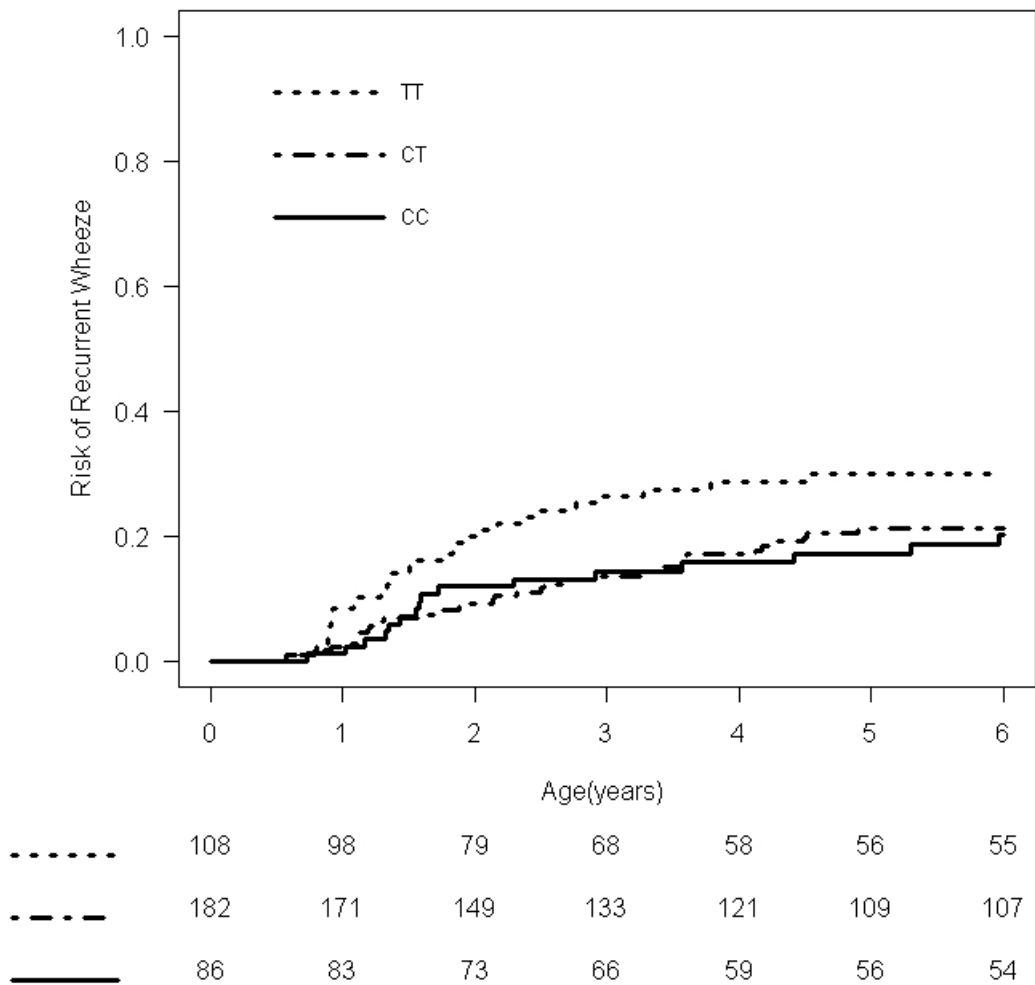


Figure EB: Acute severe exacerbations requiring high-dose steroid intervention or hospitalization during the first 6 years of life. Kaplan-Meier estimates of cumulative risk of exacerbations are indicated for neonates with and without RS7216389 polymorphisms. Numbers at risk at are given below the graph.

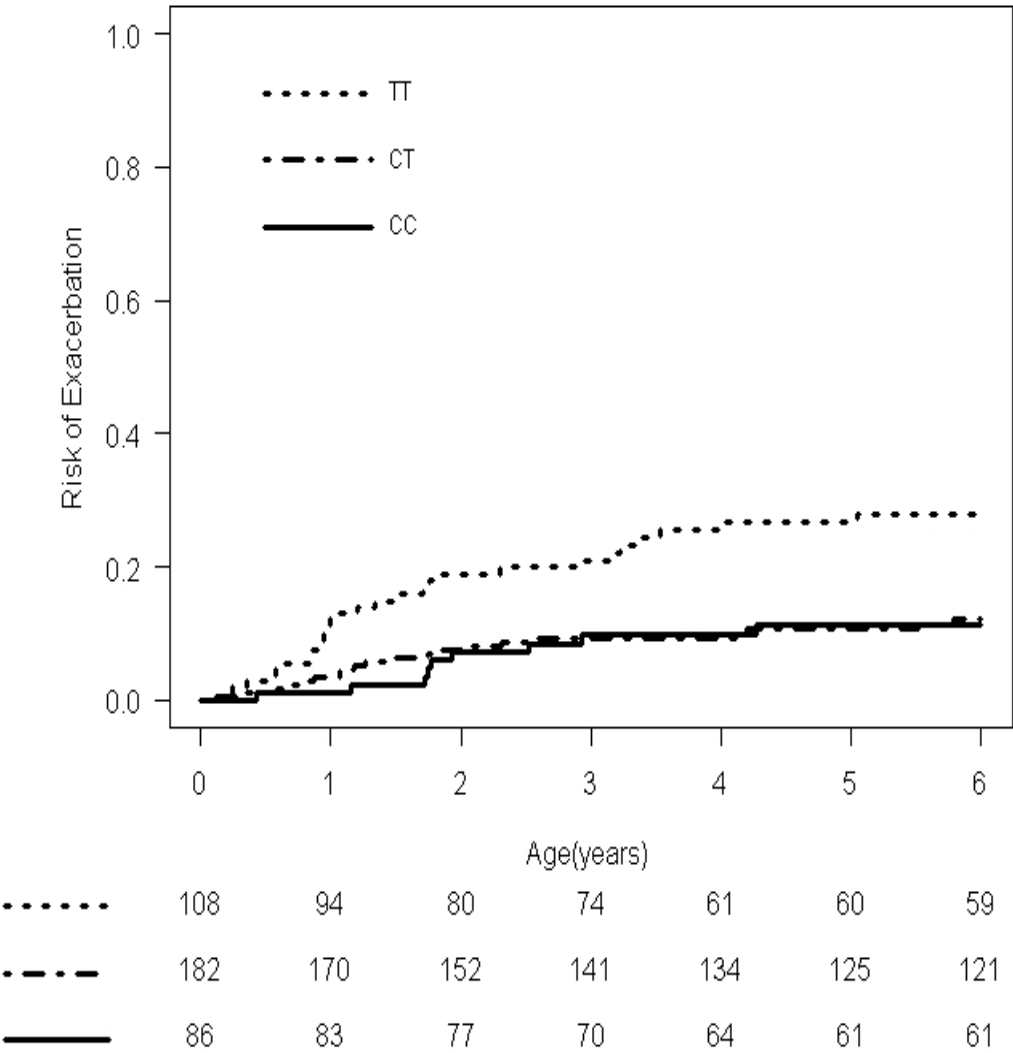
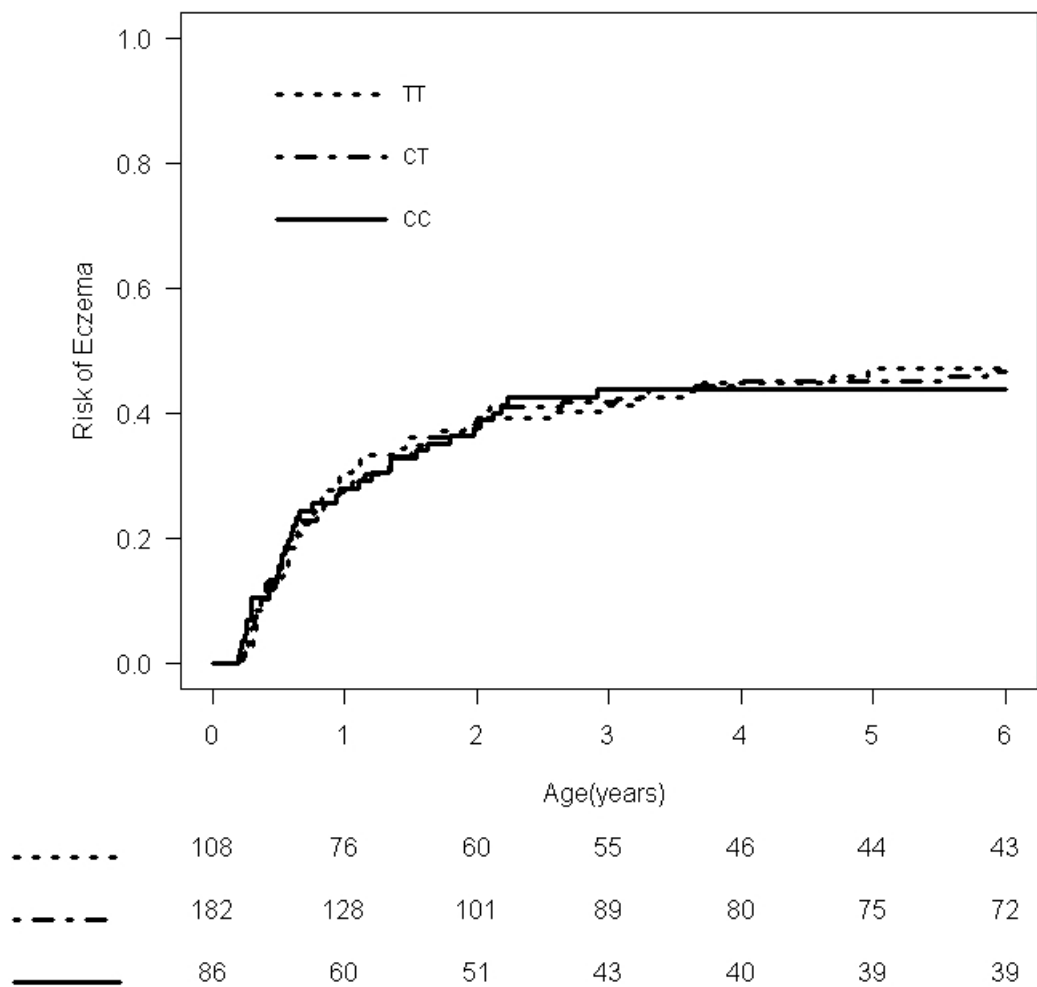


Figure EC: Eczema development during the first 6 years of life. Kaplan-Meier estimates of cumulative risk recurrent wheeze is indicated for neonates with and without RS7216389 polymorphisms.



Numbers at risk at are given below the graph.