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Polymorphisms of Chitinases are not Associated with Asthma

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Short Summary

Despite the potential role of chitinases and chitinase-like proteins in the pathogenesis of asthma, variants in their respective genes are not associated with asthma, changes in lung physiology or allergy-related phenotypes in Caucasian children.

Keywords

chitinase; chitinase-like protein; CHIA; CHIT1; CHI13L1; YKL-40; AMCase; SNPs; asthma

To the Editor:

One of the most exciting findings recently in the pathophysiology of asthma is that mammalian chitinases may play an important role in the pathogenesis of asthma. Some researchers hypothesize that mammalian chitinases and a chitinase homologue may contribute to the pathogenesis of type 2 helper immune responses which play an important role in asthma.^{1, 2} Chitinases are enzymes that cleave chitin, a polysaccharide that is present in fungal cells, crustaceans, insects, and parasitic nematodes.³ Although chitin does not exist in humans, two chitinases, acidic mammalian chitinase (AMCase, *CHIA*) and chitotriosidase (*CHIT1*), have been described in humans.³ A third protein, chitinase-like protein YKL-40 (also known as human cartilage glycoprotein 39 [HCgp-39] and chitinase 3-like 1) also appears to be important in asthma.¹

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The objectives of this study were to assess whether single nucleotide polymorphisms (SNPs) in *CHIT1*, *CHIA*, and *CHI3L1* and one *CHIT1* duplication are associated with asthma, changes in lung physiology that are associated with asthma, or allergy-related phenotypes. We used data from the Childhood Asthma Management Program (CAMP), a multi-center trial that enrolled children between the ages of five and 12 years with mild to moderate persistent asthma and their parents.⁴ Subjects were followed every two to four months for four years in order to study the long-term use of the budesonide, nedocromil, and placebo.⁴

SNPs in *CHIT1*, *CHIA*, and *CHI3L1* (see figures E1-3 for linkage disequilibrium plots) were genotyped using the Infinium HumanHap550 genotyping at Illumina (San Diego, CA). Genotyping quality was evaluated using the program PLINK (V1.01). SNPs with low Illumina gencall scores, poor completion rates, or four or more parent-offspring genotyped inconsistencies were dropped. Using the Basic Local Alignment Search Tool (BLAST), SNPs were further limited to those whose flanking sequences were reliably mapped to unique autosomal locations in the hg17 reference genome sequence. The *CHIT1* fragment analysis⁵ was performed utilizing the Applied Biosystems (AB) 3100 Genetic Analyzer platform. Primers CHIT1_A1FGTCTGGATGAGGGGTATCG-FAM and CHIT1 A1RGTTCCTCCCTGCACAGGTCAGCTATC were used to PCR amplify the region containing the 24-bp duplication, and peaks were analyzed with AB GeneMapper software. Genotyping completion rate was 94%.

FBAT-PC is a method that has the ability to use genetic data from family members to assess associations between a disease phenotype and a gene allele, while maximizing the genetic information when multiple phenotypes are tested.⁶ We performed association analyses for each SNP and each phenotype using the FBAT-PC approach. FBAT-PC uses principal components analysis to construct an overall phenotype that amplifies the trait heritability by aggregating the genetic components of all measurements into a single univariate phenotype with maximal heritability.⁶

After the univariate phenotype is generated at each SNP, FBAT-PC uses a screening procedure to select the SNPs to be tested using a univariate quantitative FBAT statistic, the FBAT-PC statistic. For each SNP, the power to detect the association with the generated univariate phenotype is calculated, a group of SNPs with their associated phenotypes are selected based on the power to detect a genetic association, and then the FBAT-PC statistic is calculated on the SNPs and their associated phenotypes. In the analysis for this study, the additive genetic model was used and a minimum of 20 informative families were required. We utilized an FBAT approach with generalized estimating equations (FBAT-GEE)⁷ for our outcomes which were assessed at one time point.

Power calculations were conducted using the FBAT Power Calculator (HelixTree version 6.4.2, 2008; See online repository Table E1). Assuming a significance level of 0.05, we had $\geq 80\%$ power to detect an association between each SNP and a single continuous measure for a heritability of $\geq 4\%$. We had greater power to detect smaller effect sizes for our outcomes with repeated measures. For example, we had $\geq 80\%$ power to detect an association for a heritability of $\geq 4\%$ for the outcomes FEV₁ and FEV₁ percent predicted which had 16 repeated measures.

Table 1 provides the baseline demographic characteristics measured in our study population of 422 children. None of the polymorphisms were associated with asthma after Bonferroni correction. We found no associations between the SNPs and the total number of hospitalizations and ED visits over four years (Table 2). The results were not significantly different after adjusting for age, gender, and treatment group.

Although there is increasing evidence that chitinases play a role in asthma, our analysis suggests that variation in chitinase genes are not associated with asthma. Our study found no associations between SNPs in the genes of *CHIT1*, *CHIA*, and *CHI3L1* and any of the outcomes studied: asthma, changes in lung physiology, or allergy-related phenotypes in subjects with mild to moderate asthma. Another strength of our study was the use of repeated measures of the asthma- and allergy-related phenotypes using a family-based design that accounts for these repeated measures, thus providing greater power to detect associations than a single measurement of the outcome.

Our finding that SNPs in *CHIT1* and *CHI3L1* are not associated with asthma is consistent with the findings of other studies.^{8,9} On the other hand, our study findings are in contrast to previous studies that found that polymorphisms in *CHIA* are associated with asthma and IgE levels^{10,11} and a previous study that concluded that *CHI3L1* is associated with asthma.¹² One potential reason for the discrepant findings may be that the studies that found positive associations used a case-control design, did not evaluate for population stratification, or did not adjust for multiple testing. Alternatively, environmental exposures in our cohort interacting with these genes may be different than in other cohorts.

Despite the strengths of our study, we were limited by our relatively small sample size of 422 subjects. Nevertheless, our power calculations suggest we had adequate power to detect small to moderate effect sizes.

The human chitinases and chitinase-like proteins have received attention in recent years because of their potential role in the pathogenesis of asthma. Nevertheless, polymorphisms in *CHIT1*, *CHIA*, and *CHI3L1* are not associated with asthma, changes in lung physiology, or allergy-related phenotypes, suggesting that the effects of variation in these genes alone are weak without the appropriate environmental exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AMCase, CHIA	Acid mammalian chitinase
BDR	Bronchodilator response
CAMP	Childhood Asthma Management Program
CHIT1	Chitotiosidase
FBAT-PC	Family-Based Association Test-Principal Components

FEV ₁	Forced Expiratory Volume in 1 second
GEE	General Estimating Equations
PC20	Concentration that provoked a 20% decrease from post-diluent FEV ₁

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TABLE 1

Demographics

N=422	Mean [SD] or Percent (n)
Age in years [SD] (range)	8.7 [2.1] (5.2-13.2)
Treatment group	
Budesonide	28% (118)
Nedocromil	29% (122)
Placebo	43% (182)
Gender, n=422	
Male	63% (266)
Female	37% (156)
Weight at baseline (kg), n = 419	31.87 [10.56]
Height at baseline (cm), n = 417	132.45 [13.50]
Total number of hospitalization and ED visits over 4 year period, n=422	
0	69% (291)
1	14% (60)
2	7% (31)
3 or more	9.5% (40)
Baseline PreFEV ₁ , n=421	1.694 [0.473]
Baseline Bronchodilator Response, n = 421	0.104 [0.098]
Baseline FEV ₁ percent predicted, n = 416	93.370 [13.954]
Baseline lnPC ₂₀ , n = 420	0.025 [1.153]
Baseline Log ₁₀ IgE, n = 417	2.616 [0.671]
Baseline Log ₁₀ Eosinophil count, n = 414	2.561 [0.488]

TABLE 2

FBAT-PC Results from the SNPs using the PBAT Power Screen

Gene	Marker	Minor Allele Frequency	Number of Informative Families	pre-FEV ₁	BDR	In PC ₂₀	log ₁₀ Eos	log ₁₀ IgE	Total number of hospitalizations and ED visits
CHIT 1	rs4950934	0.108	161	0.036	0.526	0.879	0.221	0.715	0.144
	rs2486953	0.473	341	0.403	0.483	0.080	0.175	0.084	0.646
	rs2486954	0.196	251	0.233	0.570	0.590	0.776	0.117	0.299
	rs12141375	0.197	252	0.221	0.621	0.573	0.774	0.107	0.282
	rs4950936	0.474	341	0.443	0.524	0.083	0.170	0.121	0.640
	rs4950937	0.280	276	0.697	0.854	0.752	0.089	0.699	0.668
	rs872583	0.195	253	0.262	0.728	0.694	0.796	0.050	0.239
	rs1417149	0.471	340	0.441	0.609	0.079	0.106	0.096	0.679
	rs3831317**	0.174	273	0.913	0.693	0.799	0.490	0.420	0.922
	rs2486958	0.492	340	0.570	0.753	0.102	0.935	0.872	0.591
	rs1556854	0.485	341	0.516	0.697	0.087	0.560	0.815	0.556
	rs2486959	0.174	235	0.263	0.775	0.837	0.702	0.244	0.265
	rs946257	0.309	290	0.387	0.913	0.372	0.860	0.325	0.951
	rs2486068	0.171	234	0.275	0.875	0.724	0.868	0.223	0.253
	rs2297950	0.308	289	0.456	0.886	0.676	0.746	0.233	0.984
	rs2486070	0.171	233	0.312	0.840	0.702	0.799	0.209	0.269
CH1A	rs3766537	0.192	241	0.218	0.148	0.842	0.117	0.106	0.957
	rs1417150	0.467	331	0.781	0.970	0.177	0.237	0.077	0.305
	rs2486072	0.353	323	0.703	0.533	0.784	0.471	0.098	0.844
	rs12747110	0.014	28	0.774	0.567	0.427	0.405	0.896	0.798
	rs2494287	0.128	177	0.149	0.937	0.348	0.602	0.529	0.999
	rs4240529	0.283	222	0.256	0.622	0.538	0.594	0.331	0.589
	rs4272622	0.190	167	0.126	0.900	0.369	0.018	0.049	0.178
	rs11102233	0.258	208	0.052	0.839	0.101	0.167	0.466	0.550

Gene	Marker	Minor Allele Frequency	Number of Informative Families	Unadjusted FBAT-PC p-value*					
				pre-FEV ₁	BDR	In PC ₂₀	log ₁₀ Eos	log ₁₀ IgE	Total number of hospitalizations and ED visits
	rs12401737	0.460	264	0.979	0.078	0.764	0.992	0.028	0.172
	rs10857871	0.212	188	0.847	0.514	0.787	0.493	0.091	0.146
	rs3806448	0.481	261	0.844	0.293	0.891	0.930	0.527	0.666
	rs10494132	0.221	208	0.449	0.263	0.378	0.234	0.257	0.072
	rs3806446	0.450	270	0.590	0.651	0.868	0.891	0.510	0.331
	rs7411387	0.401	239	0.978	0.319	0.775	0.018	0.018	0.064
	rs11584291	0.313	243	0.940	0.502	0.691	0.144	0.191	0.636
	rs4240530	0.288	245	0.533	0.779	0.777	0.060	0.213	0.758
	rs12127313	0.136	149	0.487	0.477	0.625	0.870	0.261	0.214
	rs10494133	0.143	159	0.188	0.417	0.528	0.036	0.039	0.362
	rs3818822	0.101	124	0.547	0.714	0.592	0.395	0.930	0.906
	rs12034576	0.326	242	0.567	0.007	0.610	0.860	0.566	0.524
	rs10494134	0.463	269	0.058	0.000	0.204	0.807	0.631	0.805
	rs2275253	0.288	236	0.603	0.692	0.577	0.028	0.501	0.751
	rs2275254	0.396	261	0.679	0.011	0.400	0.493	0.645	0.718
	rs2256721	0.286	225	0.708	0.764	0.593	0.050	0.432	0.787
	rs2820093	0.102	126	0.663	0.857	0.515	0.457	0.716	0.877
	rs2282290	0.469	267	0.576	0.119	0.767	0.077	0.253	0.268
	rs12034177	0.328	241	0.562	0.007	0.597	0.858	0.558	0.534
	rs10776724	0.454	262	0.815	0.232	0.958	0.088	0.560	0.156
	rs12137697	0.128	141	0.840	0.724	0.594	0.935	0.075	0.105
CHI3LI	rs7542294	0.145	168	0.953	0.616	0.171	0.985	0.257	0.797
	rs880633	0.485	254	0.130	0.436	0.007	0.560	0.272	0.903
	rs10399805	0.126	149	0.448	0.999	0.047	0.757	0.060	0.192
	rs946261	0.401	252	0.200	0.687	0.010	0.811	0.291	0.970

* The results were not significantly different even after adjusting for age, gender, treatment group.

** rs3831317 is a 24-bp duplication in *CHIT1*.