Rare variants in *PLA2G7* are associated with the age of onset and disease burden of asthma

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Abstract

Understanding of the contributions of rare genetic variants in asthma is limited. In this study, we investigated this contribution in 121 asthma-associated genes to the age of onset and disease burden using targeted next-generation sequencing in 576 cases of asthma. The Sequence Kernel Association Test (SKAT) was used for cumulative rare variant association analysis, with asthma onset as the outcome. The median age of onset was 12 years (IQR 5–21) and 65% of the patients developed asthma before age 16. Ninety-two genes with ≥ 10 rare variants (MAF ≤ 1%) were available for the SKAT analysis. We found a cumulative effect ($p = 0.015$, uncorrected for multiple testing) of rare variants of *PLA2G7 (*phospholipase A2, group VII*)* and asthma onset. *PLA2G7* rare variant carriers showed higher levels of FeNO than *PLA2G7* non-carriers (18 ppb (IQR 14–30) vs. 13 ppb (IQR 8–21), $p = 0.013$). Furthermore, FEV_1 was reduced in the *PLA2G7* carriers compared to the non-carriers (87.6% (12.3) vs. 96.0% (13.6), $p = 0.018$). In conclusion, we found an association between the *PLA2G2* locus and the age of onset in a population of Norwegian patients with asthma. Furthermore, increased FeNO and reduced FEV_1 were observed among the *PLA2G7* carriers.

INTRODUCTION

Asthma is caused by a combination of genetic predisposition and environmental exposure, with variable clinical presentations. Early-onset asthma is associated with atopy, whereas adult-onset asthma is often severe and difficult to treat (1). Heritability estimates of asthma range from 50–90%, with the largest genetic contribution present among younger age groups (2).

Several genome-wide association studies (GWAS) have reported more than 100 asthma susceptibility regions across the genome (3). Interestingly, variants at these loci account for only a small proportion of the genetic liability of asthma (4). Small sample sizes and disease heterogeneity dilute these association signals (5). However, single nucleotide polymorphisms (SNPs) not reaching genome-wide significance contribute to heritability (6). Although many SNPs associated with asthma have modest effect sizes, they also have functional implications by influencing gene expression in the airway epithelial cells (7). Disease heterogeneity may also reflect genetic heterogeneity. Subphenotyping of a trait based on disease severity, age of onset, and similar traits has been shown to improve the power of the association test (8). Childhood-onset asthma has been shown to have both similar and unique genetic risk factors, to adult-onset asthma. Compared to adult-onset asthma, SNP-based heritability is nearly three times higher in childhood-onset asthma (9).

In the present study, we hypothesised that 121 asthma-associated genes contain rare variants that affect the age of onset and clinical presentation in a Norwegian cohort of patients with asthma.

MATERIALS AND METHODS

Study population

The 576 patients with asthma (hereafter termed “cases”) in this study were part of a longitudinal population-based study (10). Information regarding respiratory symptoms, allergies, and age of asthma onset was obtained from a self-administered questionnaire. Measurements of nitric oxide in exhaled air (FeNO) and spirometry tests, both pre- and post-bronchodilator (described previously (10)), were available from the medical examinations of both the cases (n = 576) and controls (n = 635; controls: samples without asthma). The serum chitinase and periostin levels were determined using a Luminex IS 100 instrument (Luminex, Austin, Texas, USA). The plasma concentrations were expressed as ng/ml. High-sensitivity C-reactive protein (hs-CRP) was assessed in the serum using the particle-enhanced immune turbidimetric method on a Cobas 8000 (Roche Diagnostics, Indianapolis, Indiana, USA). The results were expressed as mg/l. Patients with relevant respiratory symptoms in the past 12 months or those using medication for asthma completed the asthma control test (ACT) questionnaire. This questionnaire is based on scoring the answers to five questions regarding asthma control. A total score ≤ 19 indicates poorly controlled asthma (11). Signed informed consent forms were obtained from all the participants, and the study was approved by the Regional Committee for Research Ethics in South-eastern Norway (REC 2012/1665).

Gene panel, sample preparation, and bioinformatic analysis

Genes associated with asthma and asthma-related comorbidities reported in linkage, candidate gene, exome sequencing and GWAS studies were included (Supplementary Table 1). For the inclusion of GWAS genes, the *p*-value threshold for the leading SNP was set at $p \leq 10^{-5}$ with a subsequent replication threshold of $p \leq 0.05$. Linkage disequilibrium (LD) patterns within the GWAS regions were investigated to include additional genes ($r^2 > 0.8$). A custom-designed (Illumina, San Diego, USA) gene panel targeting exons, 5’- and 3’-UTR (untranslated regions) of the 121 selected genes (Supplementary Table 1), were targeted by next-generation sequencing (NGS). Sample preparation and bioinformatics analyses were performed as previously described (12).

Statistical analysis

Differences in the demographic and clinical variables among the cases were assessed using contingency tables and parametric and non-parametric tests, as appropriate (IBM SPSS Statistics 26, Table 2). Sequence kernel association test (SKAT) (13), implemented in the Variant Association Tools software (14), was used for rare variant association analysis. Because the probability of complete independence between the genetic markers was low and as prior evidence of association with the loci was known, the *p*-values < 0.05 not corrected for multiple testing were considered significant. According to an *a priori* power calculation, a sample size of 45 cases entails approximately 80% power to detect a clinically interesting difference of 30 ng/ml of the normally distributed biomarker endpoint periostin at alpha = 0.05.

RESULTS AND DISCUSSION

In the present study, rare variants (minor allele frequency (MAF) ≤ 1%) in 121 asthma-associated loci were investigated and analysed for their cumulative burden of association with asthma onset in a cohort

of 576 cases with physician-diagnosed asthma.

The demographic and clinical characteristics of the Norwegian cases with asthma and the controls are presented in Supplementary Table 2. Briefly, twice as many females as males were present and approximately two-thirds of the cases developed asthma before the age of 16 (n = 361/554, 64.4%). Thirty-three percent of the cases reported poor asthma control, with an ACT (11) score of ≤ 19 (n = 111/337, 32.9%). About half of the cases with an ACT ≤ 19 developed asthma before age 16. Furthermore, spirometric measurements showed noticeable airway obstruction (reduced FEV_1) among the cases. Several clinical variables were significantly different between the cases and controls ($p < 0.05$, Supplementary Table 2). Approximately 1% of the controls had other lung diseases (10).

The mean target coverage was 381 X and 97% of all the targeted bases had coverage of > 20 X. After quality control filtering, 2764 of the variants previously not reported or with a MAF ≤ 1% in ExAC and an in-house database distributed in 92 genes were available for gene-level SKAT analyses (13). The SKAT combines the effects of multiple gene variants. Each of the 92 genes selected for the SKAT analysis contained ≥ 10 variants across the analysed cases. The results of the cumulative burden tests of the 92 genes with the age of onset in the 576 cases are shown in Table 1. Four genes, phospholipase A2, group VII *(PLA2G7)*, elongator acetyltransferase complex subunit 1 (*ELP1)*, hedgehog interacting protein (*HHIP)* and spermatogenesis-associated serine-rich 2 like (*SPATS2L)*, were associated with the age of onset ($p = 0.015$, $p = 0.028$, $p = 0.033$, and $p = 0.034$, respectively, uncorrected for multiple testing) in our Norwegian cohort.

Table 1
Genes associated with asthma onset in the SKAT.

Gene	N cases in total	N rare variants	N cases with rare variants	Q stats	*p*-value
PLA2G7	572	16	16	15.2	0.015
ELP1	572	28	29	25.3	0.028
HHIP	572	16	21	18.8	0.033
SPATS2L	511	24	28	23.4	0.034

The rare variant association analysis included variants located in the exons, splice sites and UTRs with a MAF ≤ 1% in the Exome Aggregation Consortium (ExAC, broadinstitute.org) database and a MAF ≤ 1% in the in-house controls. Frequencies of the included variants were timely checked against the gnomAD v2.1.1 database (broadinstitute.org). The SKAT analyses were adjusted for sex.

Table 2: Demographic and baseline clinical characteristics of the carriers and non-carriers of the rare variants in *PLA2G7*.

	Carriers (N = 16)	Non-carriers (N= 556)	Carriers vs. Non-carriers *p*-value[a]
Female, %	50	62.6	0.31
Age, yrs	49.5 (44.3–54.8)	48 (40–53)	0.23
Asthma onset, yrs	14.5 (3.8–32.3)	12 (5–21)	0.59
Asthma onset ≤ 16 yrs, %	50	64.9	
Asthma in family, %	31.3	27.7	0.78
Allergy positive, %	87.5	73	0.26
ACT score < 20, %	41.7	32.6	0.54
Total IgE (kU/L)	124 (16.8–317)	50 (17–132)	0.10
CRP (mg/L)	0.85 (0–2.1)	1.3 (0.7–2.6)	0.19
FeNO (ppb)	18 (14–30)	13 (8–21)	0.01
CHI3L1 (ng/ml)	28.5 (13.7–46.4)	18.5 (13.1–25.5)	0.05
Periostin (ng/ml)	172.3 (45.2)	188.7 (64.4)	0.31
FEV_1pp	84.1 (14.1)	91.8 (15.1)	0.04
FEV_1pp (pb)	87.6 (12.3)	96.0 (13.6)	0.02

ACT, asthma control test; IgE, immunoglobulin E; CRP, C-reactive protein; FeNO; forced exhaled nitric oxide; CHI3L1, chitinase-3-like protein 1; FEV_1, forced expiratory volume in 1 second; pp, percentage predicted; pb, post-bronchodilator; IQR, interquartile range; SD, standard deviation; a, Fisher Exact Test for categorical data. The normal and non-normal distributed variables were analysed with an independent samples t-test and an independent Mann-Whitney U test, respectively. Continuous data are presented as the mean (SD) or median (IQR 25 %–75 %) as appropriate.

Eight of the 16 variants included in the SKAT for *PLA2G7* have not previously been reported in either ExAC or gnomAD. Three non-synonymous variants and one splice variant were detected (Supplementary Table 3). The pathogenicity of the three nonsynonymous variants Phe110Leu, Ser261Phe, and Val279Phe and the splice variant c.539 + 1G > A is unknown. Other variants in *PLA2G7* are associated with atopic dermatitis and asthma (15). It is hypothesised that these variants might affect the catalytic function of PLA2G7 to hydrolyse PAF; the prolonged presence of PAF might influence IgE levels by increasing the recruitment of inflammatory cells such as B-cells.

Carriers of the rare *PLA2G7* variants had significantly higher FeNO levels than the non-carriers (18 parts per billion (ppb) vs. 13 ppb, $p = 0.013$) (Table 2). The carriers also had a significantly lower FEV_1 (carriers vs. non-carriers: 84.1% of predicted vs. 91.8% of predicted, $p = 0.044$) and an increased effect of broncho-dilation with the β-2-agonist (carriers vs. non-carriers; 87.6% of predicted vs. 96.0% of predicted, $p = 0.018$). Although not significant, a higher proportion of the *PLA2G7* rare variant carriers had poor asthma control according to an ACT score ≤ 19 (carriers vs. non-carriers; 41.7% vs. 32.6%) and experienced allergies along with their asthma (carriers vs. non-carriers; 87.5% vs. 73%). Similarly, the level of total IgE

was more than twice as high in the *PLA2G7* rare variant carriers than in the non-carriers (carriers vs. non-carriers: 124 (16.8–317) vs. 50 (17–132) kU/L).

We also found an association of cumulative burden of rare variants in *ELP1, HHIP* and *SPATS2L* with the onset of asthma ($p = 0.028$, $p = 0.033$, and $p = 0.035$, respectively; Table 1). Few studies have investigated the involvement of these genes in asthma. *ELP1* regulates NF-κB signalling, which influences immunological pathways, and has been linked to childhood asthma outside of Europe (16). *HHIP* is involved in lung development and has been associated with lung function (17), and a homozygous variant of *SPATS2L* was shown to affect the bronchodilator response by increasing the levels of the β2-adrenergic receptor (18). Further statistical analyses did not reveal significant differences between the carriers and non-carriers of the associated genes or variations in the clinical variables in our study (Supplementary Table 4). Supplementary Table 3 shows the distribution of the variants in *ELP1, HHIP* and *SPATS2L* included in the SKAT analyses.

The role of rare variants in complex diseases has been widely discussed owing to variations in results (19). In asthma, studies have shown the effects of rare variants in both the coding and non-coding regions of genes (20). Our study would have benefitted from a larger sample size and replication cohort; however, this was limited by the high cost of sequencing at the time of the study initiation. However, clinical variation among the subgroups of patients is known to be associated with specific variants, and our correlation with the age of onset might strengthen this study.

We found an association between the rare variants in *PLA2G7*, *ELP1*, *HHIP*, and *SPAST2L* and asthma onset ($p < 0.05$, uncorrected for multiple testing). Furthermore, a higher disease burden, with increased FeNO and reduced FEV_1 was observed among the *PLA2G7* rare variant carriers.

Declarations

AUTHOR CONTRIBUTIONS

GBNN drafted the manuscript and was involved in the study design, data collection, data management, data analyses, and data interpretation. ØLB was involved in data management, analysis, and interpretation. CMP and ML were involved in the study design and data analysis. TA was involved in data management. AKMF, ØLH, and JKH were involved in the study design and data interpretation. GK was involved in the data collection and analysis. All the authors participated in the critical revision of the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary Table 1: Genes included in the study (n=121).

Supplementary Table 2: Baseline demographic and clinical characteristics of the Norwegian cases with asthma and controls.

Supplementary Table 3: Distribution of rare variants in *PLA2G7*, *ELP1*, *HHIP* and *SPATS2L* included in the SKAT analyses.

Supplementary Table 4: Baseline demographic and clinical characteristics of carriers and non-carriers of the rare variants in *ELP1*, *HHIP,* and *SPATS2L*.

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Supplementary Files

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