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Integrative genetic and metabolite profiling analysis suggests altered phosphatidylcholine metabolism in asthma

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Abstract

Background: Genome-wide association studies (GWAS) have identified many risk loci for asthma, but effect sizes are small, and in most cases, the biological mechanisms are unclear. Targeted metabolite quantification that provides information about a whole range of pathways of intermediary metabolism can help to identify biomarkers and investigate disease mechanisms. Combining genetic and metabolic information can aid in characterizing genetic association signals with high resolution. This work aimed to investigate the interrelation of current asthma, candidate asthma risk alleles and a panel of metabolites.

Methods: We investigated 151 metabolites, quantified by targeted mass spectrometry, in fasting serum of asthmatic and nonasthmatic individuals from the population-based KORA F4 study ($N = 2925$). In addition, we analysed effects of single-nucleotide polymorphisms (SNPs) at 24 asthma risk loci on these metabolites.

Results: Increased levels of various phosphatidylcholines and decreased levels of various lyso-phosphatidylcholines were associated with asthma. Likewise, asthma risk alleles from the *PDED3* and *MED24* genes at the asthma susceptibility locus 17q21 were associated with increased concentrations of various phosphatidylcholines with consistent effect directions.

Conclusions: Our study demonstrated the potential of metabolomics to infer asthma-related biomarkers by the identification of potentially deregulated phospholipids that associate with asthma and asthma risk alleles.

In past years, enormous progress has been made in the identification of complex-trait susceptibility loci through the application of genome-wide association studies (GWAS). For asthma, several GWAS (1–9) and two large meta-analyses of GWAS (10, 11) reported numerous new risk SNPs. While some of these associated alleles seem to be specific for asthma, others appear to affect pathways implicated in a broad spectrum of diseases (11). Similar to GWAS in other diseases, most of the asthma susceptibility alleles have small effects, and information on the underlying biological processes is scarce. Systems biology approaches that combine existing biological data from different hierarchies are one way to address this problem (12).

Targeted metabolite profiling is a rapidly emerging field that is based on the quantitative measurement of a panel of key metabolites representing a whole range of pathways of intermediary metabolism in a biological sample. The analysis of the metabolome – ideally all small molecules in a given sample – provides a functional read-out of the combined effect of environmental and genomic influences reflecting the endpoints of complex chronic diseases. The simultaneous and highly standardized measurement of single-nucleotide polymorphisms (SNPs) and serum concentrations of endogenous metabolites in a single population enables the integrated analysis of these datasets. The use of metabolomics as a read-out of molecular phenotypes has allowed the discovery of previously undetected associations between disease, signalling and metabolic pathways. In addition, combining GWAS and metabolomic information (mGWAS) allows the simultaneous analysis of the genetic and environmental impact on physiological homoeostasis (13). The mGWAS was shown to be a powerful tool to explore gene regulatory networks and to gain insights into effects of genetic variation at the molecular level (14–17).

To gain a deeper understanding of the mechanisms and pathways involved in asthma, we carried out an integrative systems biology approach combining biomarkers from genomics and metabolomics. To this end, we investigated the association of asthma, established asthma risk alleles and a range of 151 serum metabolites assessed by electrospray ionization tandem mass spectrometry in fasting serum samples from a large population-based cohort (Fig. 1).

Methods

Study population

The KORA studies are several cross-sectional cohorts representative of the general population in the city Augsburg, Southern Germany, and the two surrounding counties (Augsburg and Aichach-Friedberg) that were initiated as part of the WHO MONICA study (18). Follow-up for the S4 survey carried out from 1999 to 2001 (4261 participants) was performed in 3080 individuals in 2006–2008 (KORA F4), comprising individuals (at that time aged 32–81 years). All study participants underwent a standardized face-to-face interview by certified medical staff and a standardized medical examination including blood draw and anthropometric measurements. We defined three asthma phenotypes: 'current asthma', 'ever asthma' and 'medicated asthma'; as controls 'never asthma' according to the answers given in the questionnaire was used. The asthma phenotypes and their definition are summarized in Table 1. All subjects defined as 'ever asthma' had responded positively to the question 'Have you ever had asthma?'. Current asthma was based on a further positive response to the question 'Do you still suffer from asthma?', while 'current medicated asthma' required a positive response to the question 'Are you currently taking any asthma drugs including inhalers, aerosols or tablets for asthma?' Individuals who had reported no asthma in the past were used as controls. To evaluate asthma specificity of observed associations, metabolites associated with asthma were additionally analysed

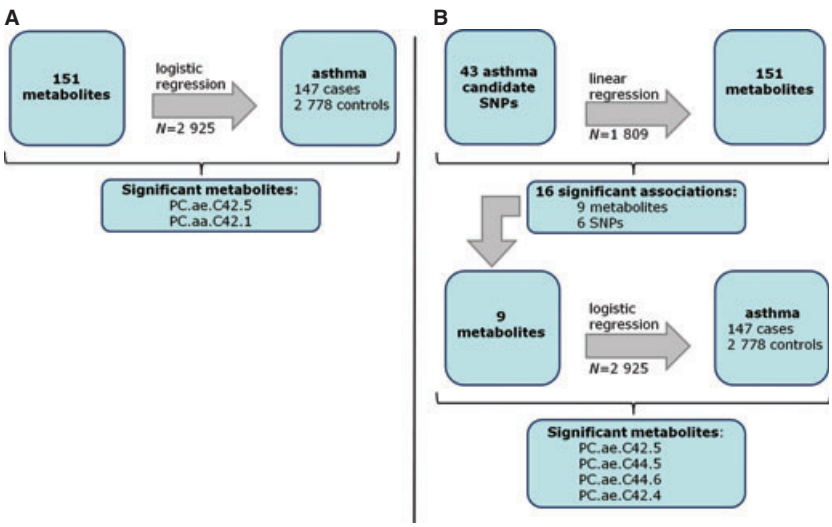

Figure 1 Summary of analysis strategy and results. (A) Nongenetic analysis of association of metabolites on asthma. (B) Analysis of asthma risk loci.

Table 1 Definition and frequency of different asthma phenotypes in this study. The characteristics of the three asthma types and controls are given together with the absolute counts of each phenotype and the frequency in the analysed KORA F4 individuals. Six individuals (0.2%) had no valid asthma definition

	Questionnaire variable			
	Have you ever had asthma?	Do you still suffer from asthma?	Are you currently taking any asthma drugs?*	Number of individuals (frequency in analysed sample, $N = 3044$)
Current asthma	Yes	Yes	–	147 (4.83%)
Ever asthma	Yes	–	–	260 (8.54%)
Medicated asthma	Yes	Yes	Yes	104 (3.42%)
Never asthma	No	–	–	2778 (91.26%)

*Including inhalers, aerosols or tablets for asthma.

for dermatologist-diagnosed atopic dermatitis (AD) (54 cases/379 controls, case frequency: 12.5%) and prebronchodilator spirometry-based diagnosis of chronic obstructive pulmonary disease (COPD) (130 cases/1175 controls, case frequency: 10%) (19). COPD was classified with the GOLD standard using the FEV1/FVC ratio (FEV1: forced expiratory volume, FVC: forced vital capacity) and the FEV1% predicted, which is the FEV1 divided by the average FEV1 in an age-, sex- and body composition-matched population. More details of the definition are given in the supplementary Table S1. We classified a person as COPD case if the GOLD classification identifies a mild or severer COPD.

Written informed consent was given by each participant. The study, including the protocols for subject recruitment, assessment and the informed consent, was approved by the ethics committee of the Bavarian Medical Chamber.

Metabolomic measurements

A panel of 163 metabolites were measured in 3061 participants of KORA F4 using electrospray ionization tandem mass spectrometry with the Absolute*IDQ*™ p150 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) in fasting serum of individuals of KORA F4. Details of the measurement methods and explanations for abbreviations were described previously (15). The samples were processed in three nonoverlapping batches at different time points. To correct for machine calibration differences and possible environmental effects, further analyses had to be adjusted by the batch. Metabolites that were not stable in repeated measurements of control samples (experimental variance >25%) were excluded. Metabolites with more than 5% missing were removed from the analyses. A metabolite value was defined as data outlier if it differed more than five standard deviation from the mean of all measurements of this metabolite. Samples that showed one or more data outlier for more than three independent metabolites (correlation <70%) were excluded. For samples that showed less than three or three independent data outliers, only the data outliers were excluded. All missing values were imputed with the MICE algorithm (Multivariate Imputation by Chained Equations; http://cran.r-project.org/web/packages/mice/index.html, standard method of predictive mean matching), which is implemented in R (http://www.r-project.org/). After quality control, 151 metabolites and 3044 individuals remained for further analyses. A full list of all metabolite names (abbreviations and full names) is given in the Supporting Information (Table S2), as well as population statistics for all metabolites (Table S3). A number of 2925 individuals had both measurements of metabolites and information on asthma status.

Genotyping and imputation

A total of 1814 randomly selected individuals from KORA F4 were genotyped with Affymetrix 6.0 SNP-arrays (Affymetrix, Santa Clara, CA, USA). After SNP-wise filtering, genotypes were imputed with Impute v. 0.4.2 (20) with reference HapMap II release 22 (21). Thousand eight hundred and nine individuals had both genotypes and metabolite measurements available.

Selection of SNPs

As asthma candidate SNPs, we used all hits from GWAS and meta-analyses on asthma and childhood onset asthma reported by the catalogue of published genome-wide association studies that had a *P*-value lower than 5×10^{-8} (http://www.genome.gov/GWAStudies/; date of access: 2012-09-20) (13 SNPs). Only results from studies on Caucasians were considered. Additionally, we selected 72 SNPs reported by the GABRIEL consortium (10) that had a meta-analysis *P*-value $<5 \times 10^{-8}$ [random effects meta-analyses; meta-analysis summary results from the webpage (http://www.cng.fr/gabriel/results.html)]. Twenty-six of these 85 SNPs were not available in KORA F4, and 16 SNPs did not pass quality control criteria (minor allele frequency >5%, rsqhat (calculated by quick test) >0.4, *P*-value for deviation from Hardy–Weinberg Equilibrium $>10^{-6}$). Therefore, 43 SNPs remained in the analyses. LD analyses of these candidate SNPs showed that 17 were not in LD to any other candidate SNPs, whereas 26 SNP could be grouped into seven independent groups (LD >0.8 to any other SNP of the group). Therefore, SNPs of 24 independent loci were analysed. Detailed information on asthma SNPs is given in Table S4.

Statistical analysis

Analysis of metabolites

A logistic regression approach was performed to investigate differences in the metabolite profile of asthmatic and

nonasthmatic individuals. 'Current asthma' was used as the primary outcome, one of the standardized metabolites as explanatory variables, and age, sex, BMI, smoking and an internal batch variable as covariates. The same logistic regressions were calculated for 'medicated asthma', 'ever asthma', AD and COPD. The significance level was adjusted for the number of metabolites ($N = 151$). As the metabolites were highly correlated within well-defined biological groups, this correction was very conservative. This analysis is visualized in Fig. 1.

The dependencies between all measured metabolites were analysed by generating a network of pairwise metabolite associations by Gaussian graphical modelling (GGM) (22). Briefly, each edge in the network corresponds to a partial correlation coefficient above a certain threshold. Partial correlations represent pairwise correlations between metabolites after the effects of all other metabolites and covariables age, sex, BMI, internal batch and medication status have been removed. Each node in the network was colour-coded with *P*-values and beta coefficients from the logistic regression of metabolites on asthma status. GGM analysis was performed using Matlab, version 7.14 (MathWorks, Natick, MA, USA), and visualized using the free yEd graph editor (http://www.yworks.com/).

Association analysis of asthma risk variants and metabolites

The selected SNPs were tested for association with metabolites. We used residuals of log-transformed metabolites concentrations in a linear regression concerning age, sex and internal batch as outcome variables for testing the association with SNPs. Additive SNP effects were assumed. The SNP association test was performed with the mean method of QUICKTEST v. 0.95 (http://toby.freeshell.org/software/quicktest.shtml). The significance level was corrected for the number of independent loci ($0.05/24 = 0.00208$). The consideration of the logistic regression results on asthma status for metabolites with significant SNP association links the effect of asthma risk SNPs on metabolites to the effect of these metabolites on asthma. A graphical overview of this two-stage analysis is given in Fig. 1.

If not stated otherwise, additional analyses were performed in R (http://www.r-project.org/).

Results

Analysis of asthma and metabolites

Two unsaturated phosphatidylcholines (PC.ae.C42:1, PC.ae.C42:5) were significantly associated with 'current asthma' after correction for multiple testing (8.16×10^{-5}, OR = 1.38; P7.35×10^{-5}, OR = 1.39). Multiple related phosphatidylcholines also showed higher levels in asthmatics, while various lyso-phosphatidylcholines and acylcarnitines were decreased (Tables 2 and S5; Bonferroni-corrected significance level: $0.05/151 \approx 0.00033$). For 'medicated asthma', similar effects of metabolites were observed (Table S5). Effects were smaller but consistent for 'ever asthma' (Table S5). None of these metabolites was associated with AD or COPD (Table S6). Thus, further analyses were restricted to 'current asthma'. To systematically investigate how asthma effects propagate through the metabolic network, we estimated a Gaussian graphical model from the metabolomics data corrected for asthma effects (Fig. 2). GGMs are based on partial correlation coefficients and have previously been demonstrated to reconstruct biochemical reactions from metabolomics data (22). We observed high partial correlations between metabolites for which asthma coincides with higher concentrations (e.g. phosphatidylcholines with chain length of 40–44) as well as between metabolites for which asthma coincides with lower concentrations (lyso-phosphatidylcholines with chain length 16–18). Two metabolites of which a higher concentration was associated with asthma were not highly correlated (partial correlation = 0.147) with any other metabolites (PC.aa.C42:2 and PC.aa.C42:4).

Table 2 Results of logistic regression of metabolites on current asthma (147 cases/2778 controls) adjusted for age, sex, BMI, smoking and internal batch. We present all results with a *P*-value below 0.05; significant results after Bonferroni correction are highlighted in bold (significance level $0.05/151 \approx 0.00033$)

Metabolite	OR	*P*-value
PC.ae.C42:5	**1.39**	**7.35×10^{-5}**
PC.aa.C42:1	**1.38**	**8.16×10^{-5}**
PC.ae.C44:5	1.33	4.37×10^{-4}
PC.ae.C40:4	1.34	5.66×10^{-4}
PC.aa.C42:4	1.31	8.76×10^{-4}
PC.ae.C44:6	1.31	9.91×10^{-4}
PC.ae.C40:5	1.31	1.18×10^{-3}
lysoPC.a.C18:2	0.7	1.69×10^{-3}
PC.aa.C42:0	1.3	1.84×10^{-3}
lysoPC.a.C16:0	0.74	2.69×10^{-3}
PC.ae.C42:4	1.29	3.30×10^{-3}
lysoPC.a.C17:0	0.76	6.17×10^{-3}
PC.aa.C40:4	1.22	8.85×10^{-3}
PC.ae.C44:4	1.25	7.89×10^{-3}
PC.aa.C42:2	1.24	8.19×10^{-3}
lysoPC.a.C18:0	0.78	9.08×10^{-3}
PC.ae.C36:5	1.24	1.20×10^{-2}
PC.aa.C36:0	1.22	1.87×10^{-2}
PC.aa.C40:1	1.22	2.15×10^{-2}
C7.DC	0.79	2.60×10^{-2}
PC.aa.C32:0	1.21	2.95×10^{-2}
PC.aa.C42:6	1.18	3.12×10^{-2}
C18	0.81	3.30×10^{-2}
PC.ae.C40:3	1.22	2.85×10^{-2}
lysoPC.a.C18:1	0.8	3.17×10^{-2}
C14.1.OH	0.81	3.70×10^{-2}
PC.ae.C36:0	1.16	4.54×10^{-2}
PC.aa.C38:4	1.18	4.35×10^{-2}

Association analysis of asthma risk variants and metabolites

After correction for 24 independent loci (corrected significance level 2.08×10^{-3}), significant effects of six SNPs on metabolite concentrations were observed. Two SNPs

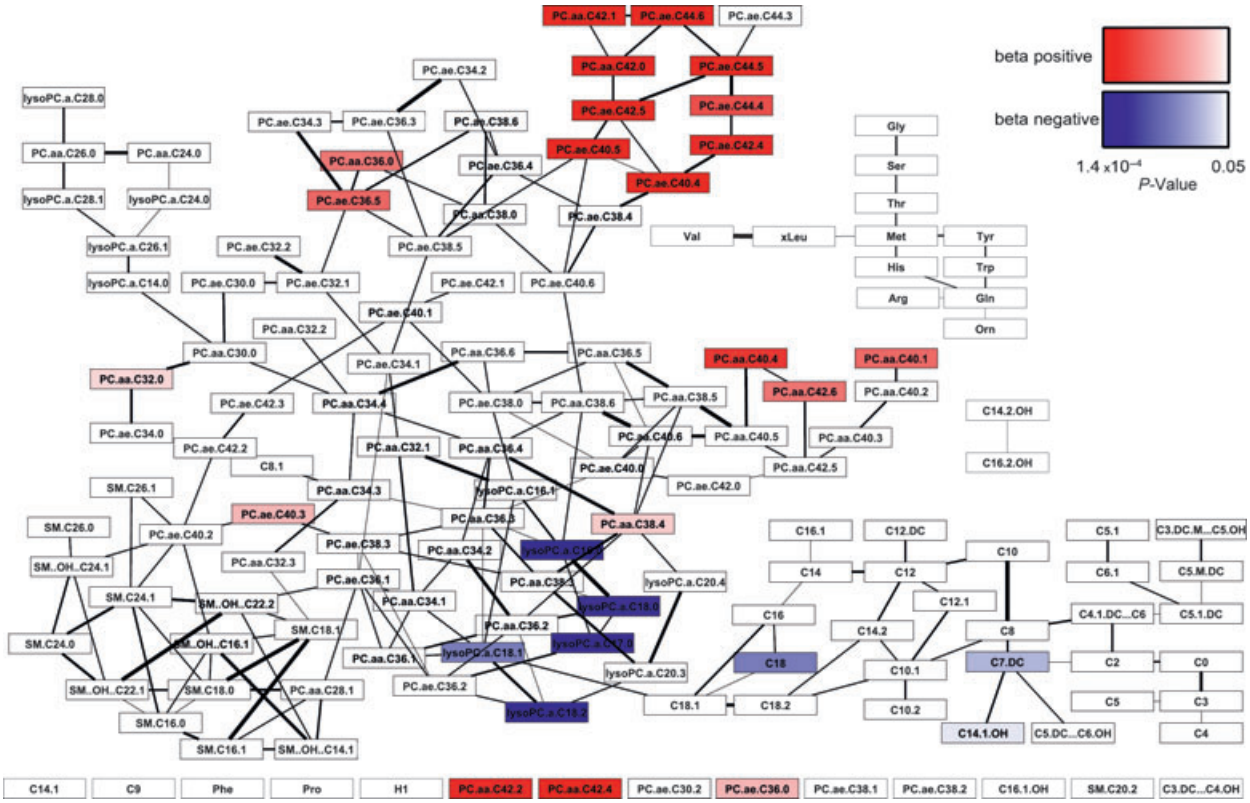

Figure 2 Gaussian graphical model of all measured metabolites coloured according to the association with asthma status. Each node represents one metabolite, and the edge weights correspond to the strength of partial correlation. Only edges with a partial correlation above $r = 0.2$ are shown. Metabolites are highlighted with red colour if asthma coincided with higher concentrations and with blue colour if asthma coincided with lower concentrations. No colour is used if the *P*-value was above 0.05.

(rs8075668/*PSMD3*, rs11078936/*MED24*) at 17q21 and multiple polyunsaturated PC.ae species with a chain length of 42–44 including the asthma-associated PC.ae.42:5 were observed. Further, the asthma risk allele of a SNP located upstream the *TSLP* gene was associated with both increased levels of the PC.aa.C34:4 and decreased levels of the sphingomyelin concentrations (SM.C20:2), and altered levels of the amino acid serine were associated with a risk allele in the *IL1RL1* gene (Tables 3 and S7).

Asthma risk coincided significantly with higher concentrations of four of these PC.ae species (acyl-alkyl-phosphatidylcholines PC.ae.C42:4, PC.ae.C42:5, PC.ae.C44:5, PC.ae.C44:6) that were significantly associated with 17q21 variants rs8075668 (*PSMD3*) and rs11078936/rs9916158 (*MED24*), respectively; the other metabolites that were identified to be associated with asthma risk variants did not show association with asthma (Table 4).

Discussion

To the best of our knowledge, this study represents the first attempt to delineate the relationship between asthma, a panel of asthma risk gene variants and global metabolite levels. We analysed 151 phenotypic measures obtained from a mass spectrometry-based screen of fasting serum samples in a large population-based cohort of 3044 German adults. We identified positive associations of various polyunsaturated phosphatidylcholines (PC) levels and negative associations with several lyso-phosphatidylcholines (LPC) levels in fasting serum with current asthma. PCs make up approximately half of the total cellular phospholipids and comprise a substantial fraction of the lipid membranes. They contain two saturated and/or (poly-) unsaturated fatty acid chains. Polyunsaturated fatty acids are precursors of multiple pro- and anti-inflammatory molecules like prostanoids, which play an important role in asthma exacerbation (23). Analysis of the metabolite network using GGM suggested that asthma-specific concentration differences can be detected in PC metabolism with strong interclass associations between diacyl-phosphatidylcholine (PC.aa) and acyl-alkyl-phosphatidylcholine (PC.ae) species (Fig. 2). Likewise, risk alleles of known asthma-associated polymorphisms in the *PDED3* and *MED24* gene at 17q21 were associated with increased circulating levels of PCs (PC.ae.C42:4, PC.ae.C42:5, PC.ae.C44:4, PC.ae.C44:5, PC.ae.C44:6), which in turn showed direct associations with asthma risk. Our observations thus indicate that changes in circulating levels of polyunsaturated PCs are associated with asthma and influenced by asthma risk alleles. Thus, asthma

Table 3 Significant results of previously published asthma risk SNPs tested for association with metabolites. The significance level was adjusted for the number of independent loci ($0.05/24 = 2.08 \times 10^{-3}$)

Metabolite	SNP	Chromosome	Position	Gene (nearest gene)	Asthma risk/other allele	Effect	se(effect)	*P*-value
Ser	rs3771180	2	102320049	*IL1RL1*	G/T	−0.033	0.009	4.2×10^{-4}
C3	rs1588265	5	59405551	*(PDE4D)*	G/A	−0.031	0.010	1.6×10^{-3}
PC.aa.C34:4	rs1837253	5	110429771	*(TSLP)*	C/T	−0.046	0.013	3.6×10^{-4}
SM.C20:2	rs1837253	5	110429771	*(TSLP)*	C/T	0.049	0.013	1.6×10^{-4}
PC.ae.C44:6	rs8075668	17	35391149	*PSMD3*	C/T	0.029	0.009	1.9×10^{-3}
PC.ae.C44:5	rs8075668	17	35391149	*PSMD3*	C/T	0.033	0.009	2.3×10^{-4}
PC.ae.C42:4	rs8075668	17	35391149	*PSMD3*	C/T	0.034	0.008	4.3×10^{-5}
PC.ae.C44:4	rs8075668	17	35391149	*PSMD3*	C/T	0.034	0.009	7.6×10^{-5}
PC.ae.C42:4	rs11078936	17	35451440	*MED24*	T/C	0.028	0.009	1.3×10^{-3}
PC.ae.C44:5	rs11078936	17	35451440	*MED24*	T/C	0.032	0.010	7.5×10^{-4}
PC.ae.C44:4	rs11078936	17	35451440	*MED24*	T/C	0.030	0.009	1.2×10^{-3}
PC.ae.C42:4	rs9916158	17	35435755	*MED24*	G/T	0.034	0.008	6.6×10^{-5}
PC.ae.C44:4	rs9916158	17	35435755	*MED24*	G/T	0.035	0.009	7.9×10^{-5}
PC.ae.C42:5	rs9916158	17	35435755	*MED24*	G/T	0.026	0.007	4.9×10^{-4}
PC.ae.C44:6	rs9916158	17	35435755	*MED24*	G/T	0.030	0.010	1.7×10^{-3}
PC.ae.C44:5	rs9916158	17	35435755	*MED24*	G/T	0.037	0.009	5.5×10^{-5}

Table 4 Results of logistic regression of metabolites on asthma (adjusted for age, sex, BMI, smoking, batch) for all metabolites on which a previously published asthma SNP had a significant effect. Significant results printed in bold (significance level $0.05/9 = 0.0056$)

Metabolites	OR	*P*-value	Significant associated SNPs (chr)(SNP effect direction)	Gene (nearest gene)
PC.ae.C42:5	**1.39**	**7.35×10^{-5}**	**rs9916158(17)(+)**	*MED24*
PC.ae.C44:5	**1.33**	**4.37×10^{-4}**	**rs8075668(17)(+), rs11078936(17)(+) rs9916158(17)(+)**	*MED24, PSMD3*
PC.ae.C44:6	**1.31**	**9.91×10^{-4}**	**rs8075668(17)(+), rs9916158(17)(+)**	*MED24, PSMD3*
PC.ae.C42:4	**1.29**	**3.30×10^{-3}**	**rs8075668(17)(+), rs11078936(17)(+), rs9916158(17)(+)**	*MED24, PSMD3*
PC.ae.C44:4	1.25	7.89×10^{-3}	rs8075668(17)(+), rs11078936(17)(+), rs9916158(17)(+)	*MED24, PSMD3*
PC.aa.C34:4	1.12	0.17	rs1837253(5)(−)	*(TSLP)*
Serine	0.89	0.22	rs3771180(2)(−)	*IL1RL1*
C3	1.10	0.31	rs1588265(5)(−)	*(PDE4D)*
SM.C20:2	0.95	0.61	rs1837253(5)(+)	*(TSLP)*

risk variants at 17q21 might either act through influences on PC metabolism or influence PC metabolism through asthma. In contrast, there were no significant associations of sphingolipid metabolites with 17q variants regulating expression of *ORMDL3*, which is thought to be a major regulator of sphingolipid metabolism (24). However, we further observed a significant association of an asthma risk SNP (rs1837253) located upstream the *TSLP* gene with both increased levels of the PC.aa.C34:4 and decreased levels of the sphingomyelin concentrations (SM.C20:2). Changes in SM levels can be interpreted as a result of a changed homoeostasis of phosphatidylcholines, as sphingomyelin can be produced from phosphatidylcholine by the action of the sphingomyelin synthase. While rs1837253 is predicted to be functional (http://pupasuite.bioinfo.cipf.es/PupaSNP (cited 2007 11/15/07), it is not yet clear whether *TSLP* is the affected gene (product).

Our findings are in line with previous observations on abnormal lipid metabolism and alterations in the phospholipid composition of serum, bronchoalveolar lavage fluid and exhaled breath in asthmatics (25–27). They confirm crucial genetic influences on metabolite levels, in particular lipids and lipoproteins (15, 16, 28). We also observed altered levels of the amino acid serine in association with a risk allele in the *IL1RL1* gene; no associations were seen for arginine, which is a key metabolite in the synthesis of exhaled nitric oxide. Data on amino acid profiles in patients with asthma are scarce. Interestingly, results from a recent study on excreted urine metabolites indicated an altered protein and amino acid metabolism in children with asthma (29). Specific abnormalities in amino acid concentrations have also recently been reported to be associated with a predisposition to diabetes (30).

Our analyses suggest that metabolite profiles in serum are associated with the condition of current/active asthma rather than reflecting medication effects or an overall inflammatory state. However, more detailed comparisons with chronic inflammatory disease states other than asthma, COPD and more specifically asthma subtypes are needed to confirm and expand our findings. In particular, validation and complementation of our findings in prospective and intervention settings, with increased numbers of cases, and for more homogeneous asthmatic subpopulations are needed. Such studies could test whether PCs are useful as biomarkers, for example predicting asthma or treatment responses or classify-

ing asthma subtypes, and to elucidate the biological mechanisms by which certain PCs influence asthma risk. Our study should be considered a first attempt and pilot study, but it demonstrates the utility of large-scale metabolic profiling and the combination with genomic information to investigate pathophysiological mechanisms. It is anticipated that such approaches will be increasingly important for a better understanding of disease mechanisms, for the functional investigation of genetic variants and for predicting, diagnosing and treating diseases.

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Authors' contributions

JSR and SW performed data analysis, interpretation and writing of the manuscript. FS and JK performed GGM analyses and contributed to interpretation of the results. CG, JH, AP, KSt, HEW, HS and TI contributed to study design, collection of phenotype information and genotyping. CG, KSu, RWS, TI, JA and CP designed and planned the experiment. JA and CP performed metabolite measurements of metabolites. FJT, MK, ER and HB contributed to interpretation of results. All authors approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. COPD classification.

Table S2. Full list of metabolite names and metabolite classes.

Table S3. Metabolite population statistics.

Table S4. Selected candidate asthma SNPs for analysis.

Table S5. Results of logistic regression of metabolites on current, medicated and ever asthma status in KORA F4.

Table S6. Results of logistic regression of metabolites on asthma, COPD and atopic dermatitis in KORA F4.

Table S7. Further information on significant SNPs.

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