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Signe Vedel-Krogh, Katrine L. Rasmussen, Børge G. Nordestgaard, Sune F. Nielsen

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Complement C3 and allergic asthma: A cohort study of the general population

Signe Vedel-Krogh, PhD[1,2,3], Katrine L. Rasmussen, PhD[1,2,3], Børge G. Nordestgaard, DMSc[1,3,4], and Sune F. Nielsen, PhD[1,3*]

[1]Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark.

[2]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Denmark.

[3]Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark.

[4]Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.

**Corresponding author*

Sune Fallgaard Nielsen, senior scientist, PhD, Department of Clinical Biochemistry, 54M1, Herlev and Gentofte Hospital, Copenhagen University Hospital, Borgmester Ib Juuls Vej 73, DK-2730 Herlev, Denmark, Phone: +45 3868 3846, Fax: +45 3868 3311.

E-mail: sune.fallgaard.nielsen@regionh.dk

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Take-Home Message:

High concentrations of plasma complement C3 are associated with asthma hospitalization and exacerbation risk among individuals from the general population; genetic analyses support that complement C3 is involved in asthma pathogenesis and severity.

Abstract

Complement C3 plays a role in asthma development and severity. We tested the hypothesis that high plasma complement C3 concentration was associated with high risk of asthma hospitalizations and exacerbations.

We prospectively assessed the risk of asthma hospitalizations in 101 029 individuals from the Copenhagen General Population Study with baseline measurements of plasma complement C3, and genotyped for rs1065489, rs429608, and rs448260 determining levels of complement C3. Risk of asthma exacerbations was further assessed in 2248 individuals with allergic asthma.

The multivariable adjusted hazard ratio of asthma hospitalisations was 1.23(95% confidence interval 1.04-1.45) for individuals in the highest tertile(>1.19 g/L) of plasma complement C3 compared with those in the lowest tertile(<1.03 g/L). The *C3* rs448260 genotype was associated with risk of asthma hospitalizations with an observed hazard ratio of 1.17(1.06-1.28) for the CC genotype compared with the AA genotype. High plasma complement C3 was associated with high levels of blood eosinophils and IgE(p for trends $\leq 6 \cdot 10^{-9}$), but only the *SKIV2L* rs429608 genotype was positively associated with blood eosinophil count($p=3 \cdot 10^{-4}$) and level of IgE($p=3 \cdot 10^{-4}$). In allergic asthma, the multivariable adjusted incidence rate ratio for risk of exacerbations was 1.69(1.06-2.72) for individuals in the highest plasma complement C3 tertile(>1.24 g/L) versus the lowest(<1.06 g/L).

In conclusion, high concentration of plasma complement C3 was associated with high risk of asthma hospitalizations in the general population and with high risk of asthma exacerbations in individuals with allergic asthma. Our findings support a causal role of the complement system in asthma severity.

Introduction

Asthma is a chronic inflammatory airway disease[1, 2] driven by allergen-specific T helper type 2 (Th2) cells and accompanying cytokines[3], allergen-specific immunoglobulin E (IgE) antibodies, mast cell degranulation, eosinophil infiltration[4], but perhaps also the innate immune system[5-7]. Here, the complement system may act as key regulator of the adaptive immune responses[8] and effectors of an allergen driven response[9, 10], but also as a bridge between innate and adaptive immune responses in asthma[7].

Complement C3 is an acute-phase reactant in the center of the complement activation pathway[11]. In a model of pulmonary allergy, mice deficient of plasma complement C3 show reduced airway hyperresponsiveness with lower numbers of lung eosinophils and cells producing interleukin (IL) 4, a Th2 cytokine[12]. In patients with allergic asthma, the amount of biologically active complement C3 fragments, C3a anaphylatoxin, increase in bronchoalveolar lavage fluid after allergen challenge[10]. As C3a is capable of eosinophil and mast cell activation[13, 14], and may regulate recruitment and activation of Th2 cells as well as promote IL17 production, this suggest a role for C3 in asthma and particular in severe asthma[15, 16]. Finally, variants in the complement C3 and C3 receptor gene have been associated with higher frequency of asthma and severity of childhood asthma, respectively[17, 18]. Collectively, the above support a role for the complement system and C3 in the pathogenesis and severity of allergic asthma, although to date most data on complement C3 in asthma derives from animal studies and small sample size studies in humans. Therefore, in this large-scale study we tested the hypothesis that high levels of plasma complement C3 are associated with high risk of hospitalizations due to asthma in individuals from the general population. Furthermore, we tested

whether plasma complement C3 determining genotypes are associated with asthma hospitalizations. Finally, we tested the hypothesis that higher levels of plasma complement C3 are associated with more respiratory symptoms and a higher frequency of asthma exacerbations in individuals with allergic asthma. For this purpose, we included 101 029 individuals from the general population including 2248 individuals with allergic asthma and prospectively assessed the risk of asthma hospitalizations and exacerbations. All individuals had baseline measurements of plasma complement C3 and were genotyped for rs1065489, rs429608, and rs448260 determining levels of complement C3.

Methods

Copenhagen General Population Study

The Copenhagen General Population study (CGPS) is prospective study of the general population residing in Greater Copenhagen[19, 20]. Individuals were included from 2003 to 2013 and written informed consent was obtained from all participants. The study was conducted according to the Declaration of Helsinki and all individuals were of Danish descent. Participation rate was 43%.

In total, we included 101 029 individuals with a baseline measurement of plasma complement C3 and 100 003 of these had been genotyped for C3 (*C3*) rs448260, complement factor H (*CFH*) rs1065489, and superkiller viralicidic activity 2-like RNA helicase region of the class III gene region of the major histocompatibility complex (*SKIV2L*) rs429608. To further investigate the association between plasma complement C3 and asthma, we identified 2248 individuals with allergic asthma and baseline measurement of plasma complement C3, of these 2225 were genotyped for *C3* rs448260, *CFH* rs1065489, and *SKIV2L* rs429608.

Allergic asthma

Allergic asthma was defined as asthma with less than 10 pack-years of smoking, a ratio of the forced expiratory volume in one second divided by the forced vital capacity (FEV_1/FVC) above the lower limit of normal, and the reporting of allergy. Asthma was defined as an affirmative answer to the question “Do you have asthma”. Allergy was self-reported according to the CGPS questionnaire if the

participants reported asthma, hay fever, or eczema as a reaction to food, medication, grass, flowers, animal hair, or other allergens.

Biochemical measurements and genotyping

Plasma complement C3 was measured turbidimetrically using polyclonal antibodies (Complement C3 antiserum 981931, Thermo Scientific) on fresh samples with a Kone autoanalyzer (Konelab, Thermo Fischer Scientific, Waltham, Massachusetts, USA). C-reactive protein, leucocytes, eosinophils, fibrinogen, and IgE were measured using standard hospital assays. Measurement of IgE had only been done in a subset of individuals at the time of conducting this study (n=49 328).

We genotyped for *C3* rs448260, *CFH* rs1065489, and *SKIV2L* rs429608 using Applied Biosystems ViiA 7 (Life Technologies, Thermo Fischer Scientific) and TaqMan-based assay with a call rate of >99.9% after reruns. Hardy-Weinberg equilibrium was fulfilled for all three individual single-nucleotide polymorphisms. The three variants have previously been found in a genome-wide association scan to identify high concentrations of plasma complement C3.[21]

Asthma hospitalizations and exacerbations

We included time to first asthma hospitalization as an indicator of asthma severity. Information on asthma hospitalization (World Health Organization International Classification of Diseases ICD8: 493 and ICD10: J45–J46) from 1977 until November 10th, 2014 was obtained by linking CGPS to the national Danish Patient Registry, while the date of death or emigration was obtained from the Danish

Civil Registration System. The national Danish Civil Registration System records all births, immigrations, emigrations, and death in Denmark and the national Danish Patient Registry records all hospital contacts.

In individuals with allergic asthma, we prospectively analysed risk of asthma exacerbations from 2003–2013. Exacerbation was defined as a short-course treatment with prednisolone or a hospitalization due to asthma. Information on hospitalization and medication was obtained by linking the CGPS to the National Danish Patient Registry (ICD10 J45-46) and to the Danish Registry of Medicinal Product Statistics as done previously[20]. Treatment with prednisolone (H02AB06) was identified using the Anatomic Therapeutics Chemical code. All exacerbations during follow-up was recorded, that is, one individual could have more than one exacerbation during follow-up.

For more information on covariates see Methods in online Data Supplement.

Statistical analyses

We used Stata/SE version 15 for Windows. Using prediction equation estimates from a Danish, healthy, non-smoking reference population (CGPS) of similar sex derived by Løkke et al.[22], we calculated FEV_1 in percent of the predicted value and for FEV_1/FVC, the lower limit of normal, the fifth percentile of a frequency distribution. A $\chi 2$ test was used to evaluate Hardy-Weinberg equilibrium. P values for trends were estimated using Cuzick's non-parametric trend test. All reported P values were two sided. Plasma C-reactive protein was logarithmically transformed due a skewed distribution. Data were >98% complete, and missing values for covariates were imputed according to age and sex for which no values were missing. We used multivariate normal imputation for continuous variables and

chained equation for categorical variables. However, if individuals with any missing data were excluded, results were similar to those reported.

For more information on statistical methods see Methods in online Data Supplement.

Results

Mean plasma complement C3 was 1.10 g/L (interquartile range 0.97-1.25) in the CGPS (supplementary figure S1). Plasma complement C3 concentration was associated with all included baseline characteristics, with the exception of sex (table 1). Importantly, this included a positive association between high concentrations of plasma complement C3 and low FEV_1 in percent of the predicted value, a high frequency of familial disposition to asthma, a higher frequency of inhaled medication, high body mass index, low physical activity during leisure time, high levels of IgE, high blood eosinophil count, and high levels of inflammatory biomarkers including blood neutrophils. High concentrations of plasma complement C3 were also associated with a high frequency of previous respiratory infections. *C3* rs448260, *CFH* rs1065489, and *SKIV2L* rs429608 were not associated with any of the included characteristics, with the exception that *SKIV2L* rs429608 was significantly associated with blood eosinophil count and plasma IgE concentration (supplementary table S1).

Plasma complement C3 and risk of asthma hospitalizations

Median follow-up time in observational analyses was 6 years (range 0-11 years) during which time 1238 individuals were hospitalized due to asthma. The cumulative incidence of asthma hospitalizations was higher for individuals in the middle (1.03-1.19 g/L) and highest (>1.19 g/L) tertile of plasma complement C3 compared with individuals in the lowest tertile (<1.03 g/L) (log-rank $P = 3 \cdot 10^{-8}$) (figure 1). Results were similar after exclusion of individuals with ischemic heart disease, rheumatoid arteritis, diabetes mellitus, cancer, or obesity defined as body mass index ≥ 30 kg/m^2 (supplementary figure S2). After multivariable adjustment, we found a hazard ratio (HR) of 1.14 (95% confidence interval (CI)

0.98-1.33) for asthma hospitalization for those in the middle tertile and 1.23 (95% CI 1.04-1.45) for those in the highest tertile compared with individuals in the lowest tertile of plasma complement C3 (figure 2).

Genetically high plasma complement C3 and risk of asthma hospitalizations

C3 rs448260, *CFH* rs1065489, and *SKIV2L* rs429608 showed a stepwise per genotype increase in plasma complement C3 level (all P values $\leq 6 \cdot 10^{-76}$) (figure 3). For *C3* rs448260, a 3.5% higher plasma concentration of complement C3 for genotype CC versus AA theoretically predicted a HR of 1.02 (95% CI 1.01-1.03) for risk of asthma hospitalizations. The corresponding observed HR was 1.17 (95% CI 1.06-1.28) (P for trend = 0.002). *CFH* rs1065489 and *SKIV2L* rs429608 were not associated with risk of asthma hospitalizations.

Observationally, high plasma complement C3 was associated with both high blood eosinophil count, high level of IgE, and high blood neutrophil count in all individuals (figure 4). In genetic analyses, only *SKIV2L* rs429608 was positively associated blood eosinophil counts and level of IgE. In cross-sectional sensitivity analyses, *SKIV2L* rs429608 genotype AA was also associated with a high risk of allergic asthma with an odds ratio (OR) of 1.36 (95% CI 1.01-1.84) (supplementary figure S3) compared to genotype GG. *C3* rs448260 and *CFH* rs1065489 were not associated with risk of allergic asthma.

Plasma complement C3 in allergic asthma

High concentrations of plasma complement C3 was associated with increased risk of allergic asthma ($P=4 \cdot 10^{-6}$) in a multivariable adjusted cross-sectional analysis.

In individuals with allergic asthma, mean plasma complement C3 concentration was 1.14 g/L (interquartile range 1.00-1.31) (supplementary figure S4). Like in the entire CGPS, high concentrations of plasma complement C3 were associated with low FEV_1 in percent of the predicted value, a higher percentage with use of inhaled medication, high body mass index, low physical activity during leisure time, and high levels of inflammatory biomarkers including blood neutrophil counts (table 2). We also found a higher frequency of individuals with a history of respiratory infections among individuals with allergic asthma in the highest tertile of plasma complement C3. In contrast to the whole population, blood eosinophil count and level of plasma IgE were not associated with the level of complement C3. There was a lower percentage of individuals in the highest tertile of plasma complement C3 with asthma diagnosed before the age of 15; none of the three plasma complement C3 determining genotypes were associated with any of the included baseline characteristics (supplementary table S2).

Individuals with allergic asthma in the highest tertile (>1.24 g/L) of plasma complement C3 had a higher frequency of respiratory symptoms (table 3). However, after multivariable adjustments only the risk of dyspnoea remained significant with an OR of 1.44 (95% CI 1.13-1.84) and 1.68 (95% CI 1.26-2.24) for individuals in the middle (1.06-1.24 g/L) and highest tertile (>1.24 g/L) compared with individuals in the lowest complement C3 tertile (<1.06 g/L).

During prospective follow-up, 350 individuals with allergic asthma had one or more asthma exacerbations. In total, we captured 927 exacerbations and 148 individuals had more one exacerbation during follow-up. Individuals with high concentrations of plasma complement C3 had a higher risk of

asthma exacerbations with a multivariable adjusted incidence rate ratio (IRR) of 1.69 (95% CI 1.06-2.72) for individuals in the highest tertile of plasma complement C3 compared to individuals in the lowest tertile (figure 5). The corresponding risk was 1.52 (95% CI 1.36-1.70) in all individuals. In supplementary genetic analyses of asthma exacerbation risk in individuals with allergic asthma, *C3* rs448260 was associated with an IRR of 1.75 (95% CI 1.11-2.76) for genotype AC and 1.27 (95% CI 0.79-2.04) for genotype CC compared with genotype AA (supplementary figure S5). *SKIV2L* rs429608 was associated with higher exacerbation risk with an IRR of 2.51 (95% CI 1.16-5.45) for asthma exacerbations comparing genotype AA with genotype GG, however, P for trend was 0.17.

Discussion

In this study of 101 029 individuals from the general population, our principal finding is that high concentrations of plasma complement C3 were associated with increased risk of asthma hospitalizations. Additionally, we found that *C3* rs448260 was associated with risk of asthma hospitalizations while *SKIV2L* rs429608 was associated with higher blood eosinophil count and higher levels of IgE. Further investigating the role of plasma complement C3 in 2248 individuals with allergic asthma, we found that high concentrations of plasma complement C3 were associated increased risk asthma exacerbations. These findings are novel.

Several studies indicate a role for the complement system in the pathogenesis of asthma. When activated, complement C3 is a potent pro-inflammatory mediator involved in leukocyte activation[13], smooth muscle cell contraction, and regulation of vascular permeability[23]. In allergic asthma, complement activation via the classical pathway could be triggered by allergen specific IgE immune complexes, via the alternative pathway through recognition of structures from dust mite, fungi, or pollen, or via the lectin pathway and recognition of polysaccharide structures of allergens. Additionally, studies have indicated that mast cell proteases released after binding of allergen specific IgE could potentially generate C3a from C3 without initiation of the entire complement cascade[9, 24]. Thus, higher concentrations of plasma complement C3 in individuals with allergic asthma, might lead to higher risk of exacerbations through the formation of C3a driven by IgE. In the present study, we found that high concentration of plasma complement C3 was associated with high risk of asthma hospitalizations and exacerbations. Previously, a study of 52 patients with acute asthma have reported increased levels of C3a in patients hospitalized with asthma exacerbations compared with patients with

mild exacerbations not requiring hospitalization[16]. Given that the level of C3 can be seen as a proxy for the potential of C3 activation, C3 could be a marker of asthma severity. This is supported by data from a study in mice in which complement C3 was associated with asthma severity through a mixed Th2/Th17 response following allergen challenge[15]; when found in the airways of asthma patients such a mixed response characterize a population with severe asthma [25]. The Th2/Th17-predominant asthma subtype is associated with a mixed eosinophilic/neutrophilic phenotype and insensitivity to treatment with corticosteroids. Furthermore, in bronchoalveolar lavage from patients with this subtype, the level of C3a is positively correlated with neutrophils, an additional parameter of asthma severity [26]. In our study, the association between plasma complement C3 and eosinophils and IgE was not reproduced in the population with allergic asthma. This could be due to lower statistical power in this small population, due to influence from the much higher use of inhaled medication among individuals with allergic asthma; however, it could also be due to potentially different roles of the complement system in the susceptibility to develop asthma in the general population versus the influence on severity among individual with already established allergic asthma. Interestingly, we found that high plasma complement C3 concentration was associated with high blood neutrophil count in both individuals from the general population and individuals with allergic asthma in support of complement C3 involvement. A dysregulated immune response with Th2 cells producing IL-4, IL-5 and IL-13 orchestrating a pulmonary allergic response is a well-known contributor to asthma pathogenesis, and interestingly here IL-4 and IL-13 are known to stimulate complement C3 production [27]. Furthermore, complement activation stimulates the activation of granulocytes, the rapid production and release of histamines, leukotrienes and platelet-activating factors, as well as release of IL-1, IL-6 and TNF-α contributing to a proallergic environment [27]. Measurement of complement factors C3 and C5

together with IL-1, IL-4, IL-5, IL-6, IL-13, IL-17 and TNF-α in future studies may help to further refute or confirm the role of the complement system in asthma pathogenesis.

In the present study, we observed an association between *C3* rs448260 and asthma hospitalizations further strengthening a causal involvement of C3 in asthma severity. Previously, two studies have reported an association between variants in the *C3* gene and asthma[17, 18]. A Japanese study of 864 asthma patients and controls, reported a variant in the *C3* gene to be associated with childhood and adult asthma[17]. This association was more pronounced when analyses were stratified according to IgE levels. Furthermore, a variant in the C3a receptor 1 was associated with severe childhood asthma, again supportive of a role for C3 in severe asthma. While in the present study, *C3* rs448260 and *CHF* rs1065489 were not associated with allergic asthma in cross-sectional analyses, we found an association between *SKIV2L* rs429608 and risk of allergic asthma. In the general population, *SKIV2L* rs429608 was associated with higher blood eosinophil count and level of IgE and when assessing risk of exacerbations in the subpopulation with allergic asthma, *SKIV2L* rs429608 AA homozygosity was associated with 2.5 higher risk of exacerbation compared to wildtype. As *SKIV2L* is involved in innate immune responses against viral infection[28], our results could possibly be explained by a higher susceptibility to exacerbations triggered by viral infections. However, as data from the national Danish registries do not contain information on the cause of exacerbations our study cannot conclude on this. Nonetheless, *the complement factor 2/factor B* gene is adjacent to *SKIV2L* and involved in regulation of the alternative pathway of the complement system. Factor B may be of critical importance for the development of asthma as showed in a previous study which found significantly reduced airway responsiveness and less airway inflammation following allergen sensitization in factor B deficient mice[29]. In the present study, we found an association between asthma severity and both *C3* and

SKIV2L, but only *SKIV2L* was associated with blood eosinophil count and IgE. As activation of the alternative pathway leads to activation of both factor C3 and C5, activation of the latter may be what is driving the association between *SKIV2L* and eosinophils and IgE as *C3* was not associated with these. This is however in contrast to data based on animal models suggesting opposite roles of C3 and C5 in asthma pathogenesis[7]. Here, high C5 in combination with low C3 have a protective effect on the development of asthma during initial allergen exposure. The role of C5 is however dual as once the Th2- mediated response has been initiated C5 acts pro-allergic by recruiting eosinophils and mast cells which is more in line with the findings in our study. Taken together, our results strengthen the understanding that several components of the complement system could be causally involved in the severity of asthma, and as mentioned above that the role of C3 and C5 in patients with severe asthma needs further investigation.

Strengths of the present study include the large sample size from a homogenous general population and no losses to follow-up. Potential limitations include, first, as complement C3 is an acute-phase reactant, our baseline measurement may not necessarily be a marker of the general individual complement C3 concentration, although in healthy individuals, the plasma complement C3 concentration is relatively stable over time, and plasma complement C3 levels were measured in a large general population cohort of individuals not hospitalized at blood sampling[30]. Second, we are limited by only having prebronchodilator spirometry measurements, and as asthma and allergy were self-reported, we cannot exclude that some of the individuals classified as allergic asthma in fact did have chronic obstructive pulmonary disease. However, by excluding individuals with FEV_1/FVC below the lower limit of normal we believe this is unlikely. Third, as the CGPS does not include information on asthma control we were not able to include this for assessment of asthma severity. Likewise, we cannot conclude on

the frequency of medication use, which could be a proxy for asthma severity, however data on the number of individuals with use of asthma medication at baseline indicated a higher frequency of use with higher plasma complement C3. Fourth, as all participants were of Danish descent, our results may not necessarily apply to other races; however, this feature also minimized risk of population stratification affecting genetic analyses and we are not aware of results indicating that our findings should not be applicable to other ethnic groups.

In conclusion, high concentrations of complement C3 were associated with increased risk of asthma hospitalizations in 101 029 individuals from the general population and with increased risk of asthma exacerbations in 2248 individuals with allergic asthma. Furthermore, genetic analyses imply that variants in *C3* and *SKIV2L* are involved in asthma severity. Our findings support a role for the complement system in asthma pathogenesis, and more importantly suggest a role for involvement of the complement system in the susceptibility to asthma exacerbations.

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Contributors

Signe Vedel-Krogh, Katrine L. Rasmussen, Børge G. Nordestgaard, and Sune F. Nielsen designed the study together. Signe Vedel-Krogh analyzed the data. Sune F. Nielsen oversaw all analyses and contributed to the interpretation of data. Signe Vedel-Krogh wrote the first draft of the paper and Katrine L. Rasmussen, Børge G. Nordestgaard and Sune F. Nielsen edited the paper. All authors approved this paper in its final form.

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Table 1. Table of characteristics in the Copenhagen General Population Study.

	Plasma complement C3 tertile			
	First	**Second**	**Third**	
				P for trend
Plasma complement C3, g/L	<1.03	1.03 – 1.19	>1.19	
No. of individuals	35 460	32 308	33 261	
Age, years	56 (47-66)	58 (49-68)	59 (49-68)	$4 \cdot 10^{-85}$
Sex, male	15 519 (44)	15 582 (48)	14 347 (43)	0.16
Low level of education	16 269 (46)	17 878 (55)	21 431 (64)	$4 \cdot 10^{-300}$
FEV_1 %	100 (90-109)	97 (87-106)	93 (83-103)	$1 \cdot 10^{-300}$
Smoking status				$3 \cdot 10^{-19}$
Never	15 666 (44)	13 216 (41)	13 296 (40)	
Current	5818 (16)	5899 (18)	6212 (19)	
Former	13 976 (40)	13 193 (41)	13 753 (41)	
Pack-years of smoking*	13 (5-25)	17 (7-30)	20 (8-34)	$8 \cdot 10^{-254}$
Body mass index, kg/m^2	24 (22-26)	26 (24-28)	28 (26-31)	$1 \cdot 10^{-300}$
No. of individuals with				
<18.5 kg/m^2	609 (71)	179 (20)	74 (9)	
18.5 – 24.9 kg/m^2	23 305 (54)	13 448 (31)	6759 (16)	
25 – 29.9 kg/m^2	10 329 (26)	14 932 (37)	15 074 (37)	
30 – 39.9 kg/m^2	1202 (8)	3718 (24)	10 587 (68)	
>40 kg/m^2	15 (2)	40 (5)	776 (93)	
Occupational exposure to dust and fumes	2693 (8)	3404 (11)	4245 (13)	$1 \cdot 10^{-111}$
Familial disposition to asthma	6165 (17)	5584 (17)	6241 (19)	$3 \cdot 10^{-6}$
Asthma, hay fever, or eczema during childhood	5143 (15)	4386 (14)	4358 (13)	$9 \cdot 10^{-8}$
Low physical activity during leisure time	13 832 (39)	15 418 (48)	19 939 (60)	$1 \cdot 10^{-300}$
C- reactive protein, mg/L	1.1 (0.7-1.4)	1.4 (1.0-2.0)	2.2 (1.4-4.0)	$1 \cdot 10^{-300}$
Fibrinogen, µmol/L	9.5(8.5-10.8)	10.6(9.5-12.1)	12.0(10.5-13.9)	$1 \cdot 10^{-300}$
Leucocytes, $\cdot 10^9$ cells/L	6.6 (5.6-7.6)	7.0 (6.0-8.1)	7.5 (6.5-8.8)	$1 \cdot 10^{-300}$

Eosinophils, $\cdot 10^9$ cells/L	0.15 (0.10-0.23)	0.16(0.11-0.25)	0.17(0.12-0.26)	$1 \cdot 10^{-169}$
Neutrophils, $\cdot 10^9$ cells/L	3.8 (3.1-4.6)	4.0 (3.3-4.9)	4.4 (3.6-5.3)	$1 \cdot 10^{-300}$
IgE, g/L#	21 (4-52)	21 (4-56)	22 (5-63)	$6 \cdot 10^{-9}$
History of respiratory infections	7180 (20)	7432 (23)	8935 (27)	$5 \cdot 10^{-93}$
Rheumatoid arthritis	225 (0.7)	264 (0.8)	390 (1.2)	$4 \cdot 10^{-10}$
Diabetes mellitus	882 (2)	1085 (3)	2417 (7)	$2 \cdot 10^{-222}$
Ischemic heart disease	1429 (4)	1901 (6)	2435 (7)	$3 \cdot 10^{-77}$
Any cancer	3756 (11)	3492 (11)	3685 (11)	0.04
Any inhaled medication	1491 (4)	1824 (6)	2641 (8)	$2 \cdot 10^{-95}$

Data are median and interquartile range for continuous variables and number and percentage for categorical variables. FEV_1 %: Forced expiratory volume in one second in percent of the predicted value. Information on inhaled medications dispensed in the year prior to baseline was obtained from the Danish Registry of Medicinal Product Statistics, which records information on all prescriptions dispensed in Danish pharmacies.

*Only in ever-smokers

#IgE measurements only available in 49 328 individuals

C3 rs448260, *CFH* rs1065489, and *SKIV2L* rs429608 were not associated with any of the included baseline characteristics apart from *SKIV2L* rs429608 which was associated with blood eosinophil count ($P=3 \cdot 10^{-4}$) and IgE ($P=3 \cdot 10^{-4}$).

Table 2. Table of characteristics in individuals with allergic asthma.

	Plasma complement C3 tertile			
	First	**Second**	**Third**	
				P for trend
Plasma complement C3, g/L	<1.06	1.06-1.24	>1.24	
No. of individuals	777	727	744	
Age, years	49 (43-58)	52 (44-63)	54 (44-63)	$5 \cdot 10^{-7}$
Sex, male	272 (35)	278 (38)	218 (29)	0.02
Low level of education	290 (37)	353 (49)	422 (57)	$3 \cdot 10^{-14}$
FEV_1 %	97 (90-105)	95 (86-104)	93 (83-101)	$6 \cdot 10^{-14}$
Smoking status				0.20
Never	519 (67)	514 (71)	521 (70)	
Current	31 (4)	26 (4)	22 (3)	
Former	227 (29)	187 (26)	201 (27)	
Pack-years of smoking*	3.8 (1.5-6.0)	3.8 (1.4-6.0)	4.0 (1.5-6.1)	0.73
Body mass index, kg/m^2	24 (22-26)	26 (24-28)	29 (27-33)	$2 \cdot 10^{-138}$
No. of individuals with				
<18.5 kg/m^2	14 (78)	4 (22)	0 (0)	
18.5 – 24.9 kg/m^2	499 (56)	276 (31)	110 (13)	
25 – 29.9 kg/m^2	231 (26)	344 (39)	313 (35)	
30 – 39.9 kg/m^2	33 (8)	100 (24)	278 (67)	
>40 kg/m^2	0 (0)	3 (7)	42 (93)	
Occupational exposure to dust and fumes	60 (8)	81 (11)	108 (15)	$2 \cdot 10^{-4}$
Familial disposition to asthma	305 (40)	306 (42)	322 (43)	0.11
Asthma, hay fever, or eczema during childhood	415 (54)	378 (52)	343 (46)	0.005
Low physical activity during leisure time	283 (36)	346 (48)	460 (62)	$4 \cdot 10^{-23}$
C- reactive protein, mg/L	1.1 (0.6-1.5)	1.5 (1.0-2.0)	2.5 (1.5-4.7)	$6 \cdot 10^{-126}$
Fibrinogen, µmol/L	9.4 (8.3-10.5)	10.3 (9.3-11.8)	12.1 (10.6-13.9)	$6 \cdot 10^{-117}$
Leucocytes, $\cdot 10^9$ cells/L	6.6 (5.7-7.5)	7.0 (6.1-7.9)	7.7 (6.7-9.0)	$6 \cdot 10^{-47}$
Eosinophils, $\cdot 10^9$ cells/L	0.20 (0.13-0.32)	0.22 (0.14-0.33)	0.21 (0.13-0.31)	0.57
Neutrophils, $\cdot 10^9$ cells/L	3.7 (3.1-4.5)	3.9 (3.2-4.6)	4.4 (3.7-5.5)	$6 \cdot 10^{-34}$
IgE, g/L#	54 (20-128)	44 (14-121)	53 (18-126)	0.50
History of respiratory infections	309 (40)	320 (44)	393 (53)	$3 \cdot 10^{-7}$
Asthma diagnosed before age 15	180 (27)	164 (24)	176 (20)	0.002
Rheumatoid arthritis	5 (0.8)	3 (0.4)	12 (1.3)	0.18
Diabetes mellitus	9 (1)	14 (2)	52 (6)	$5 \cdot 10^{-7}$
Ischemic heart disease	15 (2)	27 (4)	47 (5)	$3 \cdot 10^{-3}$
Any cancer	42 (6)	45 (7)	70 (8)	0.24

Any inhaled medication	345 (44)	376 (42)	362 (49)	$2 \cdot 10^{-6}$

Data are median and interquartile range for continuous variables and number and percentage for categorical variables. FEV_1 %: Forced expiratory volume in one second in percent of the predicted value. Information on inhaled medications dispensed in the year prior to baseline was obtained from the Danish Registry of Medicinal Product Statistics, which records information on all prescriptions dispensed in Danish pharmacies. For full information on asthma medications, see Supplementary table S3.

*Only in ever-smokers

#IgE measurements only available in 1093 individuals with allergic asthma.

C3 rs448260, *CFH* rs1065489, and *SKIV2L* rs429608 were not associated with any of the included baseline characteristics in the allergic asthma population.

Table 3. Respiratory symptoms in allergic asthma.

Respiratory symptom	Plasma complement C3 tertiles			P for trend	Plasma complement C3 tertiles (multivariable adjusted)			P for trend
	First	Second	Third		First (Ref)	Second OR (95%CI)	Third OR (95%CI)	
Plasma complement C3 (g/L)	<1.06	1.06-1.24	>1.24					
No. of individuals	766	716	738					
Dyspnea (mMRC≥2)	204 (26)	299 (42)	429 (58)	$6 \cdot 10^{-34}$	1.00	1.44 (1.13-1.84)	1.68 (1.26-2.24)	$3 \cdot 10^{-4}$
Sputum production	93 (12)	109 (15)	140 (19)	$1 \cdot 10^{-3}$	1.00	1.08 (0.79-1.48)	1.17 (0.82-1.67)	0.62
Cough during exercise	265 (34)	288 (40)	334 (45)	$1 \cdot 10^{-5}$	1.00	1.18 (0.95-1.47)	1.18 (0.90-1.51)	0.15
Wheezing	421 (55)	426 (59)	486 (67)	$4 \cdot 10^{-5}$	1.00	1.00 (0.80-1.24)	0.98 (0.75-1.28)	0.94
Any respiratory symptom	553 (71)	560 (77)	636 (85)	$1 \cdot 10^{-10}$	1.00	1.09 (0.85-1.40)	1.30 (0.95-1.79)	0.51

Categorical variables are shown as N and percentage. P is from Cuzick's non-parametric trend test. P for trend is from logistic regression with plasma complement C3 tertiles as a continuous variable. Multivariable adjusted was for age, sex, education, body mass index, smoking status, C-reactive protein, and FEV_1 % predicted. mMRC, modified Medical Research Council Dyspnea Scale. OR: Odds ratio. 95% CI: 95% confidence interval.

Figure legends

Figure 1. Cumulative incidence of asthma hospitalizations as a function of age and tertiles of plasma complement C3 concentrations.

Individuals hospitalized due to asthma before the day of plasma complement C3 measurement (n=2710) were excluded from the prospective, observational analyses.

C3; Plasma complement C3 concentration.

Figure 2. Multivariable-adjusted HRs for asthma hospitalizations according to plasma complement C3 percentiles.

Solid lines are multivariable-adjusted HRs using a polynomial smoother and dashed lines indicate 95% CI derived from restricted cubic spline regression. The analyses were multivariable adjusted for age, sex, education, plasma C-reactive protein, body mass index, smoking status, and physical inactivity.

Figure 3. Plasma concentrations of complement C3 and corresponding theoretically predicted and observed hazard ratios for asthma hospitalization for *C3* rs448260, *CFH* rs1065489, and *SKIV2L* rs429608.

As exposure is a genetic instrument, individuals entered at date of birth or 1977 (start of the Danish Patient Registry), that is, with delayed entry for those born before 1977, as genotypes are present at

birth and therefore precede all events. Thus, 100 003 individuals were included and 3900 were hospitalized due to asthma. Mean with 95% CIs are given for plasma complement C3 (left). Theoretically predicted HR (middle) were calculated using delta mean concentrations of plasma complement C3 for each genetic variant and adjusted only for age and sex. Observed HRs (right) were also only adjusted for age and sex.

CI: Confidence interval, HR: Hazard ratio.

Figure 4. Plasma complement C3, blood eosinophil count, IgE, and blood neutrophil count.

Top panel, left: Observational data showing blood eosinophil counts by tertiles of plasma complement C3. Boxes with medians, 25th, and 75th percentiles.

Top panel, right: Genetic data showing complement C3 determining genetic variants and levels of blood eosinophils with mean and 95% confidence intervals.

Middle panel, left: Observational data showing IgE by tertiles of plasma complement C3. Boxes with medians, 25th, and 75th percentiles.

Middle panel, right: Genetic data showing complement C3 determining genetic variants and level of IgE with mean and 95% confidence intervals (right).

Bottom panel, left: Observational data showing blood neutrophil counts by tertiles of plasma complement C3. Boxes with medians, 25th, and 75th percentiles.

Bottom panel, right: Genetic data showing complement C3 determining genetic variants and levels of blood neutrophils with mean and 95% confidence intervals.

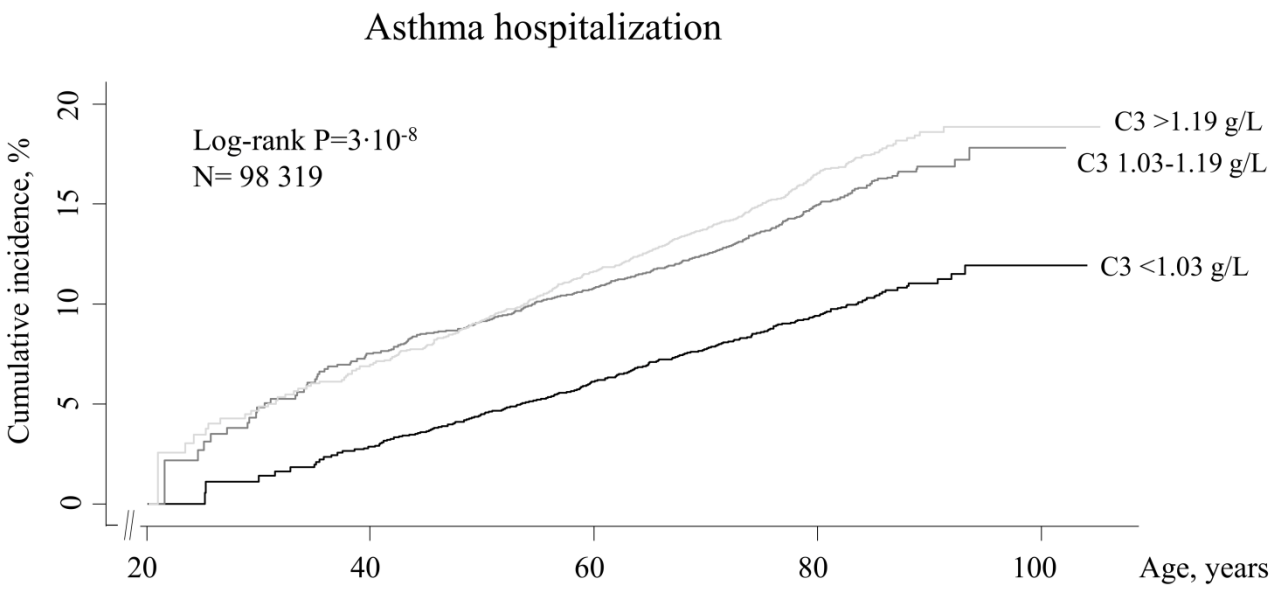
IgE measurements were only available in 49 328 individuals.

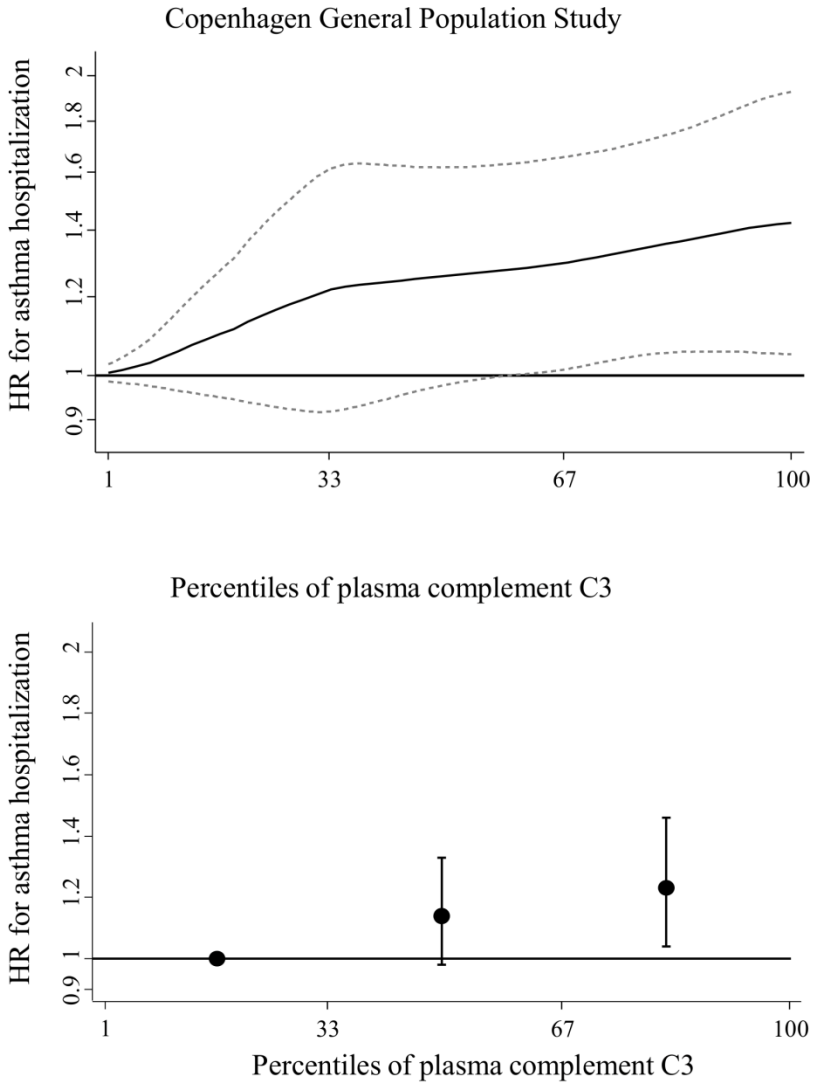
[NS]Not significant after Bonferroni correction for multiple comparisons (required P value < $4 \cdot 10^{-3}$).

Figure 5. Risk of exacerbations according to plasma complement C3 tertiles in individuals with allergic asthma and in all individuals.

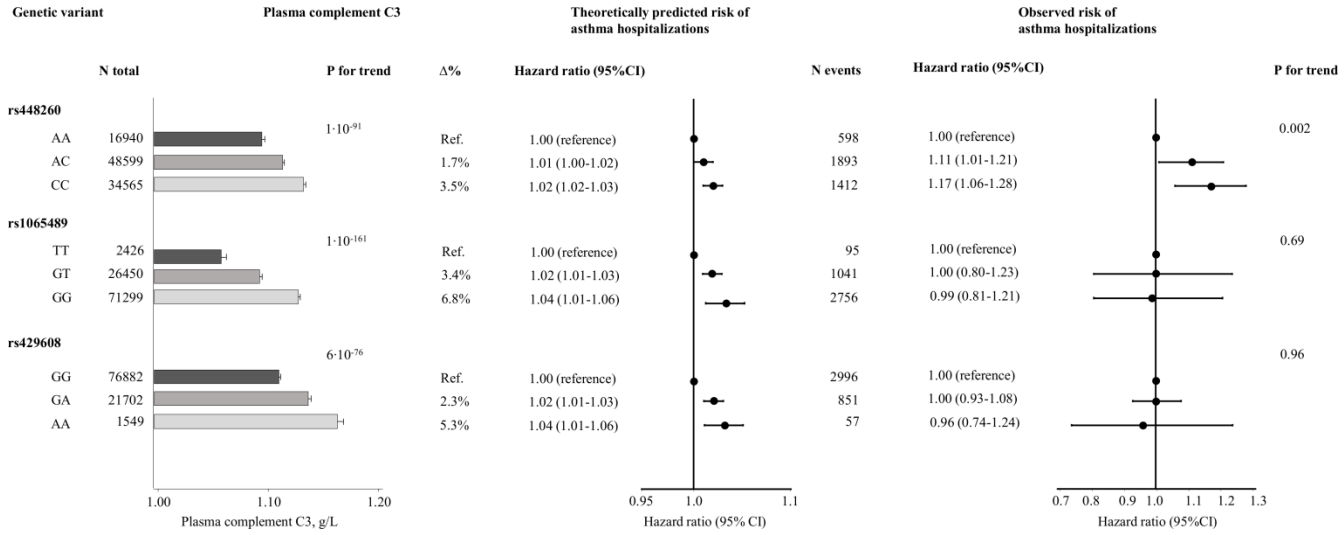
Multivariable adjustments were for age, sex, education, plasma C-reactive protein, body mass index, smoking status, low physical activity, and FEV_1 in percent of the predicted value.

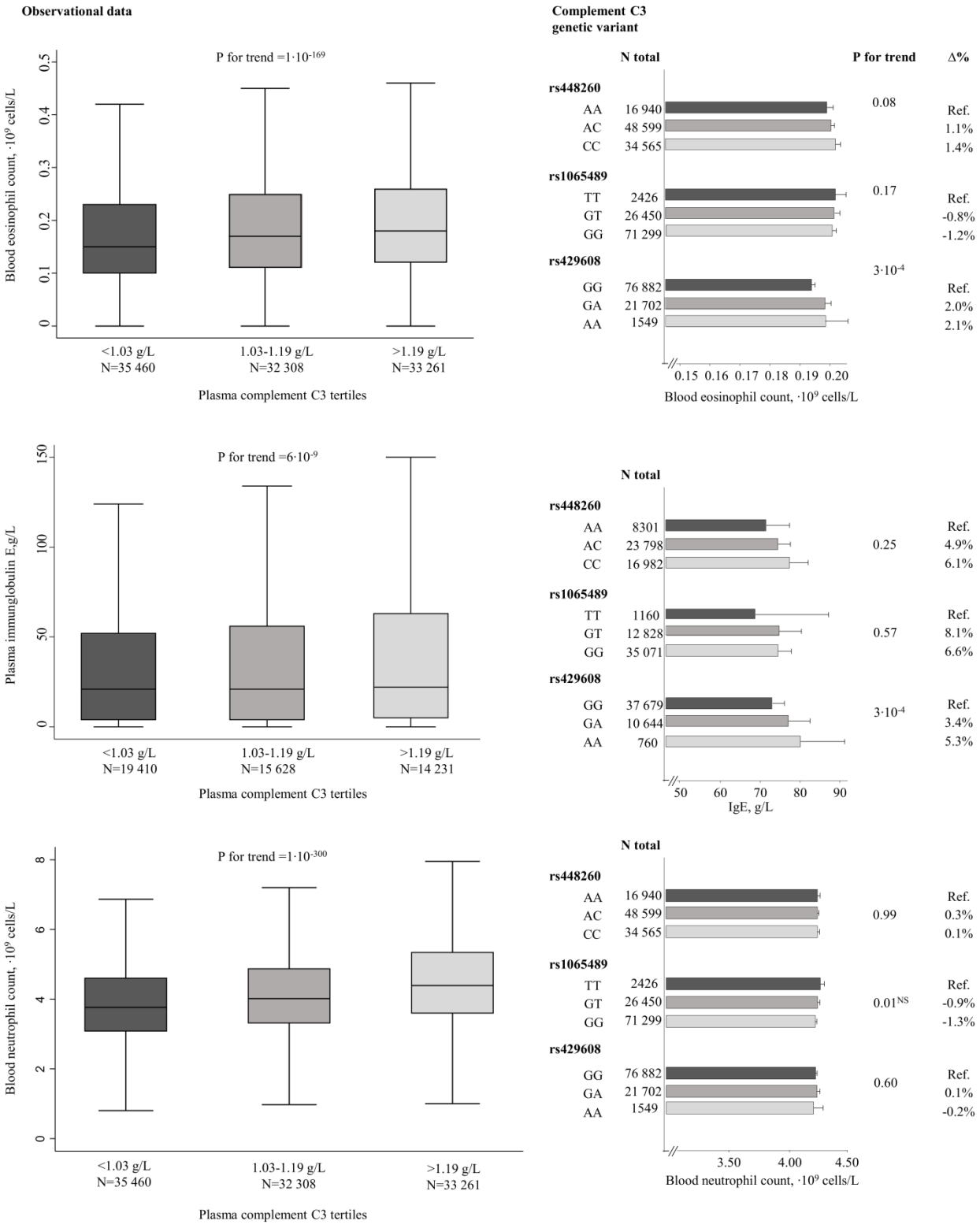
CI: confidence interval., IRR: Incidence rate ratio.


Asthma hospitalization
Log-rank P=3·10-8
N= 98 319
C3 >1.19 g/L
C3 1.03-1.19 g/L
C3 <1.03 g/L
Cumulative incidence, %
0
5
10
15
20
20
40
60
80
100
Age, years

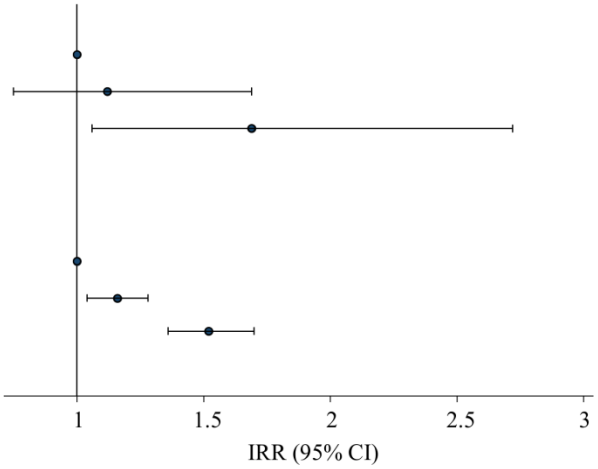


No. of participants	32 455	32 986	31 642
No. of asthma hospitalizations	302	413	523


Genetic variant
Plasma complement C3
Theoretically predicted risk of asthma hospitalizations
Observed risk of asthma hospitalizations
N total
P for trend
Δ%
Hazard ratio (95%CI)
N events
Hazard ratio (95%CI)
P for trend
rs448260
1·10-91
0.002
AA 16940 Ref. 1.00 (reference) 598 1.00 (reference)
AC 48599 1.7% 1.01 (1.00-1.02) 1893 1.11 (1.01-1.21)
CC 34565 3.5% 1.02 (1.02-1.03) 1412 1.17 (1.06-1.28)
rs1065489
1·10-161
0.69
TT 2426 Ref. 1.00 (reference) 95 1.00 (reference)
GT 26450 3.4% 1.02 (1.01-1.03) 1041 1.00 (0.80-1.23)
GG 71299 6.8% 1.04 (1.01-1.06) 2756 0.99 (0.81-1.21)
rs429608
6·10-76
0.96
GG 76882 Ref. 1.00 (reference) 2996 1.00 (reference)
GA 21702 2.3% 1.02 (1.01-1.03) 851 1.00 (0.93-1.08)
AA 1549 5.3% 1.04 (1.01-1.06) 57 0.96 (0.74-1.24)
1.00 1.10 1.20
Plasma complement C3, g/L
0.95 1.0 1.1
Hazard ratio (95% CI)
0.7 0.8 0.9 1.0 1.1 1.2 1.3
Hazard ratio (95%CI)


Observational data
Complement C3 genetic variant
P for trend =1·10-169
Blood eosinophil count, ·10⁹ cells/L
<1.03 g/L N=35 460
1.03-1.19 g/L N=32 308
>1.19 g/L N=33 261
Plasma complement C3 tertiles
N total
P for trend
Δ%
rs448260
AA 16 940 0.08 Ref.
AC 48 599 1.1%
CC 34 565 1.4%
rs1065489
TT 2426 0.17 Ref.
GT 26 450 -0.8%
GG 71 299 -1.2%
rs429608
GG 76 882 3·10-4 Ref.
GA 21 702 2.0%
AA 1549 2.1%
0.15 0.16 0.17 0.18 0.19 0.20
Blood eosinophil count, ·10⁹ cells/L
P for trend =6·10-9
Plasma immunglobulin E,g/L
<1.03 g/L N=19 410
1.03-1.19 g/L N=15 628
>1.19 g/L N=14 231
Plasma complement C3 tertiles
N total
rs448260
AA 8301 Ref.
AC 23 798 0.25 4.9%
CC 16 982 6.1%
rs1065489
TT 1160 Ref.
GT 12 828 0.57 8.1%
GG 35 071 6.6%
rs429608
GG 37 679 Ref.
GA 10 644 3·10-4 3.4%
AA 760 5.3%
50 60 70 80 90
IgE, g/L
P for trend =1·10-300
Blood neutrophil count, ·10⁹ cells/L
<1.03 g/L N=35 460
1.03-1.19 g/L N=32 308
>1.19 g/L N=33 261
Plasma complement C3 tertiles
N total
rs448260
AA 16 940 Ref.
AC 48 599 0.99 0.3%
CC 34 565 0.1%
rs1065489
TT 2426 Ref.
GT 26 450 0.01NS -0.9%
GG 71 299 -1.3%
rs429608
GG 76 882 0.60 Ref.
GA 21 702 0.1%
AA 1549 -0.2%
3.50 4.00 4.50
Blood neutrophil count, ·10⁹ cells/L

Tertiles of plasma complement C3	No. individuals/ exacerbations	Incidence rate ratio (95%CI)
Allergic asthma, N=2248		
<1.06	777/242	1.00 (reference)
1.06 – 1.24	727/232	1.12 (0.75-1.69)
>1.24	744/453	1.69 (1.06-2.72)
All, N=101 029		
<1.03	35 460/6466	1.00 (reference)
1.03 – 1.19	32 308/8432	1.16 (1.04-1.28)
>1.19	33 261/13 739	1.52 (1.36-1.70)



Online Data Supplement

Complement C3 and allergic asthma: A cohort study of the general population

Signe Vedel-Krogh, Katrine L. Rasmussen, Børge G. Nordestgaard, and Sune F. Nielsen,

Methods

The Copenhagen General Population Study was initiated in 2003 with ongoing recruitment. Using the national Danish Civil Registration System, individuals aged 20–100 years were randomly selected to reflect the general population. At study attendance, participants filled out an extensive questionnaire, performed spirometry, were physically examined, and had blood drawn for biochemical tests and DNA analyses

Respiratory symptoms

All respiratory symptoms were self-reported. Dyspnoea was defined according to the Modified Medical Research Council dyspnea scale as equal to or above two. Sputum (>3 months) was sputum production for at least 3 consecutive months/year. Wheezing was defined according to the question: "Do you occasionally experience whistling or wheezing while breathing?"

Covariates

Measured weight (kg) and height (m) were used to calculate body mass index (kg/m^2). Education was dichotomized and low education was less than three years of education following the 7-9 years of Danish mandatory primary school. Smoking was self-reported and participants were grouped as never, former, or current smokers according to the questions "Do you smoke?" and "Have you previously smoked?". Low physical activity was defined as being physical active for 4 hours or less per week during leisure time. Individuals with asthma was asked when they were diagnosed with asthma and this information was used to define childhood asthma.

Information on diagnoses of rheumatoid arteritis, diabetes mellitus, and ischemic heart disease at baseline were obtained by linking information from the Copenhagen General Population Study to the national Danish Patient Registry using the World Health Organization International Classification of Diseases (rheumatoid arteritis: ICD8: 712.1, 712.2, 712.3 and ICD10: M05-M06, diabetes mellitus: ICD8: 250 and ICD10: E11, E13, and E14, ischemic heart disease: ICD8: 410-414 and ICD10: I20-I25). In addition, diabetes mellitus was defined as self-reported disease, having a non-fasting blood glucose>11 mmol/L, or with use of anti-diabetic medication. Information on cancer prior to baseline was drawn from the national Danish Cancer Registry, which contains data on the incidence of cancer in the Danish population from 1943.

Information on inhaled medication was drawn from the Danish Registry of Medicinal Product Statistics, which records information on all prescriptions dispensed in Danish pharmacies. From here we obtained information on inhaled medications dispensed in the year prior to baseline (see supplementary table S1).

Statistical analyses

To evaluate cumulative incidence of asthma hospitalizations, we used Kaplan-Meier curves and log-rank trend test. To test if plasma complement C3 was associated with asthma hospitalizations, we used Cox proportional hazards regression models to obtain multivariable adjusted hazard ratios (HR) with 95% confidence intervals (CI) as a function of complement C3 in tertiles. For Cox regression models, proportionality over time was assessed by plotting -ln(-ln(survival)) vs. ln(analysis time) with no sign of non-proportionality. Furthermore, we performed multivariable adjusted, restricted cubic spline Cox

regression for graphical representation using plasma complement C3 and three knots[1]. For this purpose, we used percentiles of plasma complement C3. Analyses were multivariable adjusted for age, sex, education, plasma C-reactive protein, body mass index, smoking status, and physical activity. Individuals hospitalized due to asthma before the day of plasma complement C3 measurement (n=2710) were excluded from the prospective, observational analyses.

To test whether the three plasma complement C3 determining genotypes were associated with plasma complement C3 concentration, we used Cuzick's non-parametic test for trend. Cox regression models were adjusted only for age and sex with entry at date of birth or 1977 (start of the Danish Patient Registry), that is, with delayed entry for those born before 1977, as genotypes are present at birth and therefore precede all events. In supplementary cross-sectional analyses, we used logistic regression models to test whether high plasma complement C3 and C3 determining genotypes were associated with allergic asthma. In these analyses, only individuals without chronic airway disease were included as controls (n=81 957) and analyses were adjusted for the same confounders as in observational regression analyses.

To test whether plasma complement C3 levels were associated with respiratory symptoms, we used Cuzick's non-parametric trend test and multivariable-adjusted logistic regression. Multivariable adjustment was for age, sex, education, plasma C-reactive protein, body mass index, smoking status, low physical activity, and FEV_1 in percent of the predicted value.

For risk of asthma exacerbations, we calculated multivariable adjusted incidence rate ratios (IRRs) with 95% CI in a negative binomial regression model assessing the risk of exacerbations according to tertiles of plasma complement C3 and genetic variants in a model including all exacerbations during

follow-up. The analysis was adjusted for age, sex, education, plasma C-reactive protein, body mass index, smoking status, physical activity, and FEV_1 in percent of the predicted value while genetic analyses were adjusted only for sex and age. In sensitivity analyses, we calculated age and sex adjusted incidence rate ratios (IRRs) with 95% CI in a negative binomial regression model assessing risk of asthma exacerbations in individuals with allergic asthma according to plasma complement C3 genotype. Only exacerbations from the day of study attendance until end of follow-up were included.

References

1. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med* 1989: 8(5): 551-561.

Supplementary table S1. Table of characteristics in the Copenhagen General Population Study including genetic data.

	Plasma complement C3 tertile				Complement C3 genetics		
	First	**Second**	**Third**		***C3* rs448260**	***CFH* rs1065589**	***SKIV2L* rs429608**
				P for trend	**P for trend**	**P for trend**	**P for trend**
Plasma complement C3, g/L	<1.03	1.03 – 1.19	>1.19				
No. of individuals	35 460	32 308	33 261				
Age, years	56 (47-66)	58 (49-68)	59 (49-68)	$4 \cdot 10^{-85}$	0.65	0.55	0.10
Sex, male	15 519 (44)	15 582 (48)	14 347 (43)	0.16	0.83	0.85	0.06
Low level of education	16 269 (46)	17 878 (55)	21 431 (64)	$4 \cdot 10^{-300}$	0.27	0.76	0.08
FEV_1 %	100 (90-109)	97 (87-106)	93 (83-103)	$1 \cdot 10^{-300}$	0.62	0.12	0.22
Smoking status				$3 \cdot 10^{-19}$	0.22	0.74	0.04^{NS}
Never	15 666 (44)	13 216 (41)	13 296 (40)				
Current	5818 (16)	5899 (18)	6212 (19)				
Former	13 976 (40)	13 193 (41)	13 753 (41)				
Pack-years of smoking*	13 (5-25)	17 (7-30)	20 (8-34)	$8 \cdot 10^{-254}$	0.67	0.32	0.93
Body mass index, kg/m^2	24 (22-26)	26 (24-28)	28 (26-31)	$1 \cdot 10^{-300}$	0.96	0.06	0.59
No. of individuals with							
<18.5 kg/m^2	609 (71)	179 (20)	74 (9)				
18.5 – 24.9 kg/m^2	23 305 (54)	13 448 (31)	6759 (16)				
25 – 29.9 kg/m^2	10 329 (26)	14 932 (37)	15 074 (37)				
30 – 39.9 kg/m^2	1202 (8)	3718 (24)	10 587 (68)				
>40 kg/m^2	15 (2)	40 (5)	776 (93)				
Occupational exposure to dust and fumes	2693 (8)	3404 (11)	4245 (13)	$1 \cdot 10^{-111}$	0.13	0.32	0.05

Familial disposition to asthma	6165 (17)	5584 (17)	6241 (19)	$3 \cdot 10^{-6}$	0.18	0.69	0.47
Asthma, hay fever, or eczema during childhood	5143 (15)	4386 (14)	4358 (13)	$9 \cdot 10^{-8}$	0.07	0.01[NS]	0.06
Low physical activity during leisure time	13 832 (39)	15 418 (48)	19 939 (60)	$1 \cdot 10^{-300}$	0.17	0.15	0.04[NS]
C- reactive protein, mg/L	1.1 (0.7-1.4)	1.4 (1.0-2.0)	2.2 (1.4-4.0)	$1 \cdot 10^{-300}$	0.65	0.29	0.99
Fibrinogen, µmol/L	9.5 (8.5-10.8)	10.6 (9.5-12.1)	12.0 (10.5-13.9)	$1 \cdot 10^{-300}$	0.60	0.37	0.05
Leucocytes, $\cdot 10^9$ cells/L	6.6 (5.6-7.6)	7.0 (6.0-8.1)	7.5 (6.5-8.8)	$1 \cdot 10^{-300}$	0.27	0.01[NS]	0.81
Eosinophils, $\cdot 10^9$ cells/L	0.15 (0.10-0.23)	0.16 (0.11-0.25)	0.17 (0.12-0.26)	$1 \cdot 10^{-169}$	0.08	0.18	$3 \cdot 10^{-4}$
Neutrophils, $\cdot 10^9$ cells/L	3.8 (3.1-4.6)	4.0 (3.3-4.9)	4.4 (3.6-5.3)	$1 \cdot 10^{-300}$	0.99	0.01[NS]	0.60
IgE, g/L#	21 (4-52)	21 (4-56)	22 (5-63)	$6 \cdot 10^{-9}$	0.25	0.57	$3 \cdot 10^{-4}$
History of respiratory infections	7180 (20)	7432 (23)	8935 (27)	$5 \cdot 10^{-93}$	0.26	0.33	0.99
Rheumatoid arthritis	225 (0.7)	264 (0.8)	340 (1.2)	$4 \cdot 10^{-10}$	0.38	0.73	0.01 [NS]
Diabetes mellitus	882 (2)	1085 (3)	2417 (7)	$2 \cdot 10^{-222}$	0.74	0.27	0.78
Ischemic heart disease	1429 (4)	1901 (6)	2435 (7)	$3 \cdot 10^{-77}$	0.72	0.25	0.18
Any cancer	3756 (11)	3492 (11)	3685 (11)	0.04	0.42	0.04 [NS]	0.28
Any inhaled medication	1491 (4)	1824 (6)	2641 (8)	$2 \cdot 10^{-95}$	0.11	0.54	0.62

Data are median and interquartile range for continuous variables and number and percentage for categorical variables. FEV_1 %: Forced expiratory volume in one second in percent of the predicted value. Information on inhaled medications dispensed in the year prior to baseline was obtained from the Danish Registry of Medicinal Product Statistics, which records information on all prescriptions dispensed in Danish pharmacies.

*Only in ever-smokers

#IgE measurements only available in 49 328 individuals

[NS]Not significant after Bonferroni correction for multiple comparisons (required P value < $5 \cdot 10^{-4}$)

Supplementary table S2. Table of characteristics in individuals with allergic asthma including genetic data.

	Plasma complement C3 tertile				Complement C3 genetics		
	First	**Second**	**Third**		***C3* rs448260**	***CFH* rs1065589**	***SKIV2L* rs429608**
				P for trend	**P for trend**	**P for trend**	**P for trend**
Plasma complement C3, g/L	<1.06	1.06-1.24	>1.24				
No. of individuals	777	727	744				
Age, years	49 (43-58)	52 (44-63)	54 (44-63)	$5 \cdot 10^{-7}$	0.02^{NS}	0.68	0.57
Sex, male	272 (35)	278 (38)	218 (29)	0.02	0.54	0.88	0.40
Low level of education	290 (37)	353 (49)	422 (57)	$3 \cdot 10^{-14}$	0.20	0.52	0.49
FEV_1 %	97 (90-105)	95 (86-104)	93 (83-101)	$6 \cdot 10^{-14}$	0.19	0.59	0.37
Smoking status				0.20	0.21	0.30	0.10
Never	519 (67)	514 (71)	521 (70)				
Current	31 (4)	26 (4)	22 (3)				
Former	227 (29)	187 (26)	201 (27)				
Pack-years of smoking*	3.8 (1.5-6.0)	3.8 (1.4-6.0)	4.0 (1.5-6.1)	0.73	0.53	0.98	0.88
Body mass index, kg/m^2	24 (22-26)	26 (24-28)	29 (27-33)	$2 \cdot 10^{-138}$	0.09	0.96	0.12
<18.5 kg/m^2	14 (78)	4 (22)	0 (0)				
18.5 – 24.9 kg/m^2	499 (56)	276 (31)	110 (13)				
25 – 29.9 kg/m^2	231 (26)	344 (39)	313 (35)				
30 – 39.9 kg/m^2	33 (8)	100 (24)	278 (67)				

>40 kg/m^2	0 (0)	3 (7)	42 (93)				
Occupational exposure to dust and fumes	60 (8)	81 (11)	108 (15)	$2 \cdot 10^{-4}$	0.88	0.54	0.98
Familial disposition to asthma	305 (40)	306 (42)	322 (43)	0.11	0.21	0.85	0.41
Asthma, hay fever, or eczema during childhood	415 (54)	378 (52)	343 (46)	0.005	0.78	0.75	0.41
Low physical activity during leisure time	283 (36)	346 (48)	460 (62)	$4 \cdot 10^{-23}$	0.60	0.53	0.25
C- reactive protein, mg/L	1.1 (0.6-1.5)	1.5 (1.0-2.0)	2.5 (1.5-4.7)	$6 \cdot 10^{-126}$	0.45	0.79	0.57
Fibrinogen, µmol/L	9.4 (8.3-10.5)	10.3 (9.3-11.8)	12.1 (10.6-13.9)	$6 \cdot 10^{-117}$	0.90	0.29	0.54
Leucocytes, $\cdot 10^9$ cells/L	6.6 (5.7-7.5)	7.0 (6.1-7.9)	7.7 (6.7-9.0)	$6 \cdot 10^{-47}$	0.81	0.10	0.88
Eosinophils, $\cdot 10^9$ cells/L	0.20 (0.13-0.32)	0.22 (0.14-0.33)	0.21 (0.13-0.31)	0.57	0.04^{NS}	0.44	0.85
Neutrophils, $\cdot 10^9$ cells/L	3.7 (3.1-4.5)	3.9 (3.2-4.6)	4.4 (3.7-5.5)	$6 \cdot 10^{-34}$	0.45	0.05	0.89
IgE, g/L#	54 (20-128)	44 (14-121)	53 (18-126)	0.50	0.86	0.26	0.84
History of respiratory infections	309 (40)	320 (44)	393 (53)	$3 \cdot 10^{-7}$	0.43	0.44	0.37
Asthma diagnosed before age 15	180 (27)	164 (24)	176 (20)	0.002	0.85	0.48	0.39
Rheumatoid arthritis	5 (0.8)	3 (0.4)	12 (1.3)	0.18	0.45	0.23	0.31
Diabetes mellitus	9 (1)	14 (2)	52 (6)	5·10-7	0.11	0.63	0.60
Ischemic heart disease	15 (2)	27 (4)	47 (5)	3·10-3	0.79	0.22	0.26
Any cancer	42 (6)	45 (7)	70 (8)	0.24	0.08	0.16	0.95
Any inhaled medication	345 (44)	376 (42)	362 (49)	$2 \cdot 10^{-6}$	0.41	0.04^{NS}	0.38

Data are median and interquartile range for continuous variables and number and percentage for categorical variables. FEV_1 %: Forced expiratory volume in one second in percent of the predicted value. Information on inhaled medications dispensed in the

year prior to baseline was obtained from the Danish Registry of Medicinal Product Statistics, which records information on all prescriptions dispensed in Danish pharmacies. For full information on asthma medications, see Supplementary table S3.

*Only in ever-smokers

#IgE measurements only available in 1093 individuals with allergic asthma.

[NS]Not significant after Bonferroni correction for multiple comparisons (required P value < $5 \cdot 10^{-4}$).

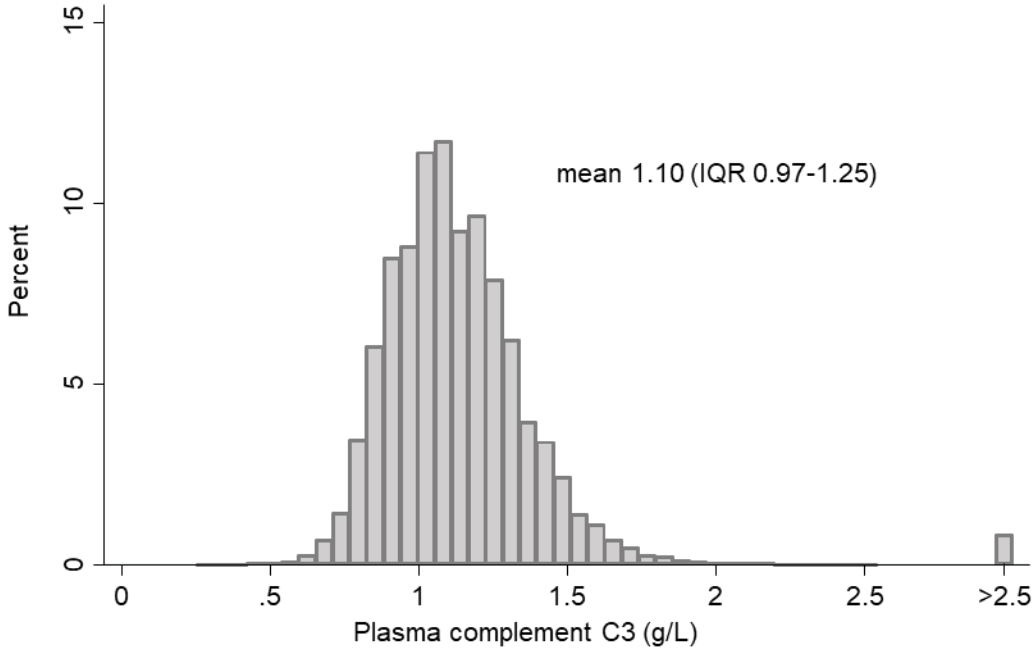
Supplementary table S3. Number of participants using asthma medication at baseline and corresponding Anatomic Therapeutic Chemical codes in the allergic asthma population (N=2248).

Medication	No. of participants (%)	Corresponding ATC codes
Short-acting β2-agonists	547 (24%)	R03AC02, R03AC03, R03CC02, R03CC03
Long- acting β2-agonists	137 (6%)	R03AC12, R03AC13, R03CC12, R03AC18
Short-acting anticholinergics	5 (0.2%)	R03BB01
Long-acting anticholinergics	12 (0.5%)	R03BB04
Inhaled corticosteroids	603 (27%)	R03BA01, R03BA02, R03BA05, R03BA07
Combination products	403 (18%)	R03AK03, R03AK04, R03AK06, R03AK07
Xanthines	8 (0.4%)	R03DA04
Leukotriene receptor antagonists	83 (4%)	R03DC03
Any medication	1083 (48%)	Any of the above

Number of participants with use of asthma medication in the year prior to examination. Corresponding codes are Anatomic Therapeutic Chemical codes used in the Danish Registry of Medicinal Products Statistics.

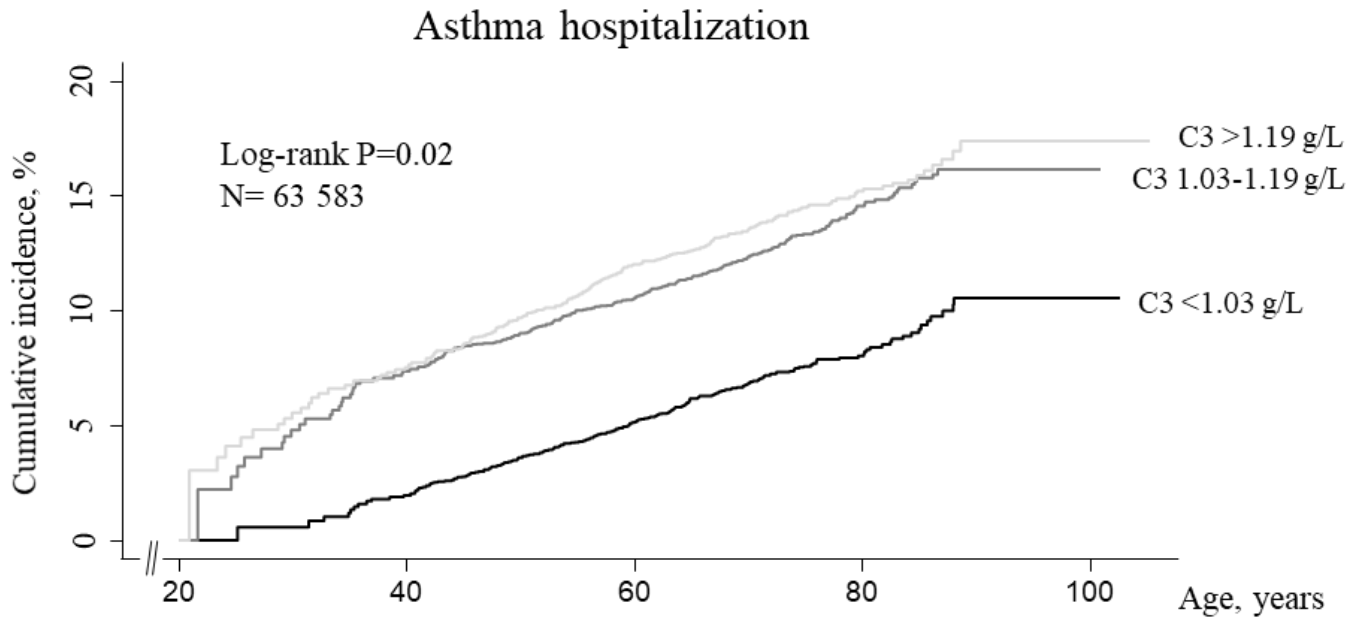
ATC: Anatomical Therapeutic Chemical Classification System.

Supplementary figure S1. Plasma complement C3 concentrations in 101 029 individuals from the Copenhagen General Population Study.



IQR: Interquartile range.

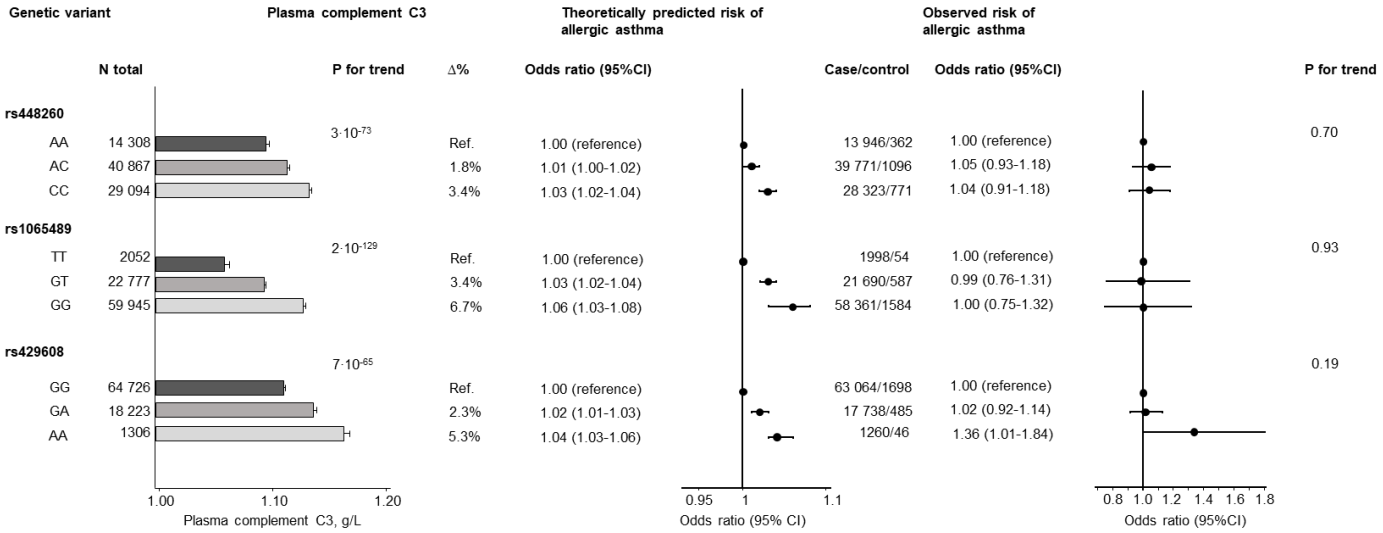
Supplementary figure S2. Cumulative incidence of asthma hospitalizations as a function of age and tertiles of plasma complement C3 concentrations in the Copenhagen General Population Study excluding individuals with ischemic heart disease, rheumatoid arteritis, diabetes mellitus, cancer, or body mass index ≥ 30 kg/m^2.



C3; Plasma complement C3 concentration.

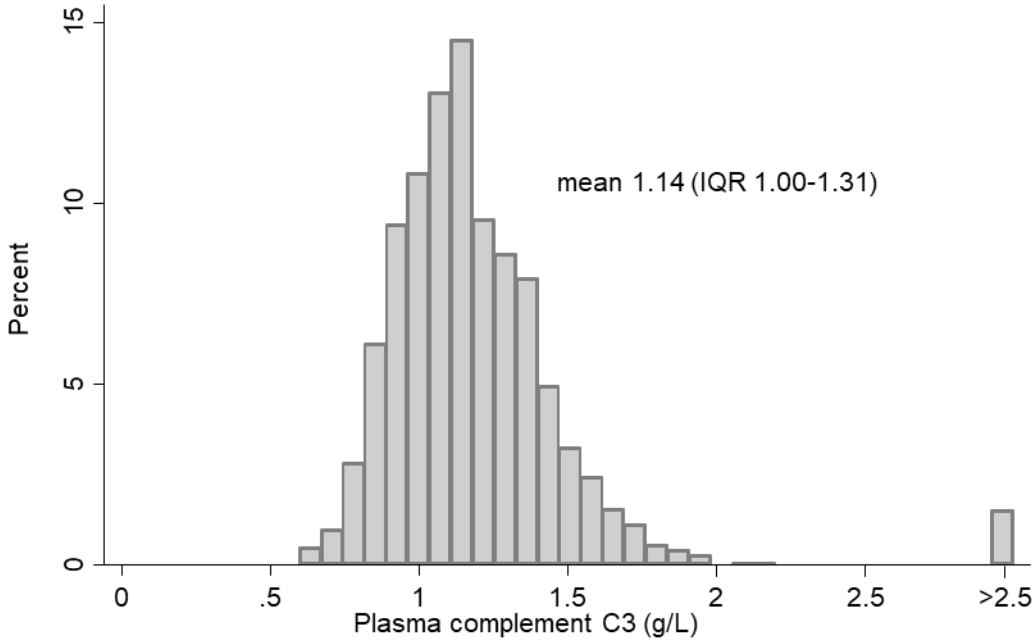
As obesity (body max ≥ 30 kg/m2) has been identified as an independent risk for asthma and asthma severity *(Peter et al. Obesity and Asthma. J Allergy Clin Immunol. 2018;141(4):1169*), it was investigated whether the correlation between obesity and plasma complement C3 could explain the main finding of a higher risk of exacerbations with higher C3 concentration by excluding individuals with BMI>30 kg/m2.

Supplementary figure S3. Plasma concentrations of complement C3 and corresponding theoretically predicted and observed odds ratios for allergic asthma for rs448260 (*C3*), rs1065489 (*CFH*), and rs429608 (*SKIV2L*).



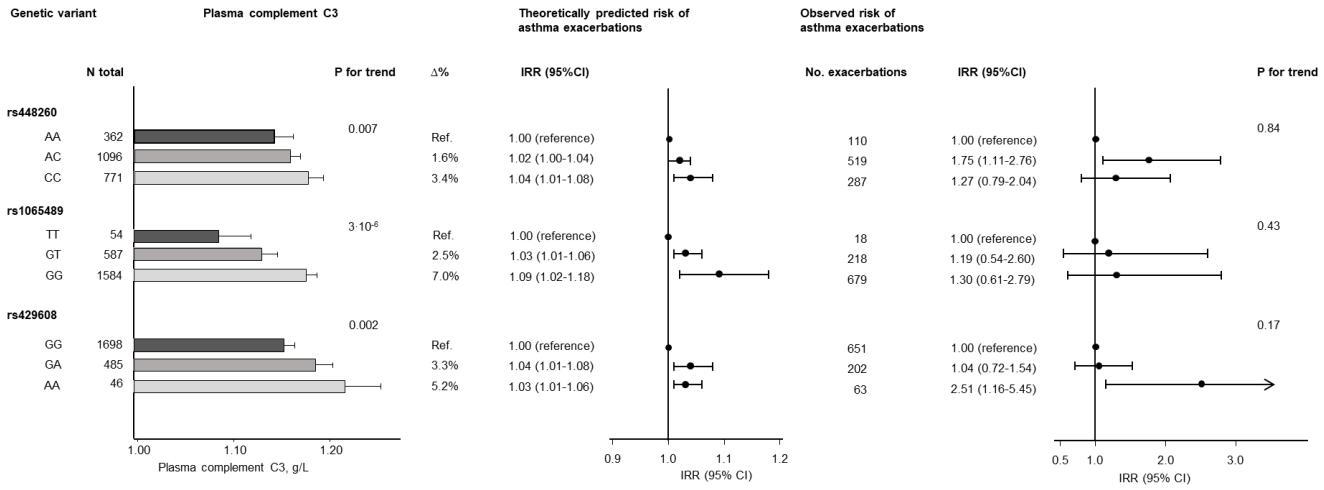
Controls were individuals without chronic airway disease. Mean with 95% CIs are given for plasma complement C3 (left). Theoretically predicted odds ratios (middle) were calculated using delta mean concentrations of plasma complement C3 for each genetic variant and adjusted only for age and sex. Observed odds ratios (right) were also only adjusted for age and sex.

Supplementary figure S4. Plasma complement C3 concentrations in 2248 individuals with allergic asthma.



IQR: Interquartile range

Supplementary figure S5. Plasma concentrations of complement C3 and corresponding theoretically predicted and observed incidence rate ratios for asthma hospitalization for *C3* rs448260, *CFH* rs1065489, and *SKIV2L* rs429608 in allergic asthma.



Mean with 95% CIs are given for plasma complement C3 (left). Theoretically predicted incidence rate ratios (middle) were calculated using delta mean concentrations of plasma complement C3 for each genetic variant and adjusted only for age and sex. Observed incidence rate ratios (right) were also only adjusted for age and sex.

CI: Confidence interval, IRR: Incidence rate ratio.