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

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High concentrations of plasma complement C3 are associated with asthma hospitalisation and exacerbation risk among individuals from the general population; genetic analyses indicate a causal role for complement C3 in asthma pathogenesis and severity https://bit.ly/30PIIwg

Cite this article as: Vedel-Krogh S, Rasmussen KL, Nordestgaard BG, *et al*. Complement C3 and allergic asthma: a cohort study of the general population. *Eur Respir J* 2021; 57: 2000645 [https://doi.org/10.1183/13993003.00645-2020].

ABSTRACT Complement C3 plays a role in asthma development and severity. We tested the hypothesis that high plasma complement C3 concentration is associated with high risks of asthma hospitalisation and exacerbation.

We prospectively assessed the risk of asthma hospitalisation 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 exacerbation was further assessed in 2248 individuals with allergic asthma.

The multivariable adjusted hazard ratio of asthma hospitalisation was 1.23 (95% CI 1.04–1.45) for individuals in the highest tertile (>1.19 $g{\cdot}L^{-1}$) of plasma complement C3 compared with those in the lowest tertile (<1.03 $g{\cdot}L^{-1}$). The *C3* rs448260 genotype was associated with risk of asthma hospitalisation with an observed hazard ratio of 1.17 (95% CI 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 $\leqslant 6\times10^{-9}$), but only the *SKIV2L* rs429608 genotype was positively associated with blood eosinophil count ($p=3\times10^{-4}$) and IgE level ($p=3\times10^{-4}$). In allergic asthma, the multivariable adjusted incidence rate ratio for risk of exacerbation was 1.69 (95% CI 1.06–2.72) for individuals in the highest plasma complement C3 tertile (>1.24 $g{\cdot}L^{-1}$) *versus* the lowest (<1.06 $g{\cdot}L^{-1}$).

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

This article has supplementary material available from erj.ersjournals.com

Received: 11 March 2020 | Accepted after revision: 10 Aug 2020

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 IgE antibodies, mast cell degranulation, eosinophil infiltration [4] and also perhaps the innate immune system [5–7]. In the latter, the complement system may act as a key regulator of adaptive immune responses [8] and as an effector 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 at the centre of the complement activation pathway [11]. In a model of pulmonary allergy, mice deficient in 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 a biologically active complement C3 fragment, C3a anaphylatoxin, increases in bronchoalveolar lavage fluid after allergen challenge [10]. Because C3a is capable of eosinophil and mast cell activation [13, 14], and may regulate recruitment and activation of Th2 cells as well as promote IL-17 production, it is possible that C3 has a role in asthma, particularly severe asthma [15, 16]. Variants in the complement C3 and C3 receptor genes have been associated with higher frequency of asthma and severity of childhood asthma, respectively [17, 18]. Collectively, the above evidence supports 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 derive from animal studies and small sample size studies in humans. In this large-scale study, we tested the hypothesis that a high level of plasma complement C3 is associated with a high risk of hospitalisation due to asthma in individuals from the general population. Furthermore, we tested whether plasma complement C3-determining genotypes are associated with asthma hospitalisation. 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 hospitalisation and exacerbation. 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 a 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. The 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 224 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 <10 pack-years of smoking, a ratio of the forced expiratory volume in 1 s (FEV_1) divided by the forced vital capacity (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 981 931; Thermo Fisher Scientific, Waltham, MA, USA) on fresh samples with a Kone autoanalyzer (Konelab, Thermo Fisher Scientific). C-reactive protein, leukocytes, 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 Fisher Scientific) and a 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 hospitalisations and exacerbations

We included time to first asthma hospitalisation as an indicator of asthma severity. Information on asthma hospitalisation (World Health Organization International Classification of Diseases (ICD) 8: 493 and ICD10: J45–J46) from 1977 to November 10, 2014, was obtained by linking CGPS to the Danish National Patient Registry, which records all hospital contacts. The date of death or emigration was obtained from the Danish Civil Registration System, which records all births, immigrations, emigrations and deaths in Denmark.

In individuals with allergic asthma, we prospectively analysed risk of asthma exacerbation from 2003 to 2013. Exacerbation was defined as a short-course treatment with prednisolone or a hospitalisation due to asthma. Information on hospitalisation and medication was obtained by linking the CGPS to the Danish National 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 were recorded, *i.e.* one individual could have more than one exacerbation during follow-up.

More information on covariates can be found in the supplementary material.

Statistical analyses

We used Stata/SE version 15 for Windows. Using prediction equation estimates from a healthy Danish non-smoking reference population (CGPS) of similar sex derived by Løkke *et al.* [22], we calculated FEV_1 % pred and the lower limit of normal for FEV_1/FVC, which is the fifth percentile of a frequency distribution. A Chi-squared 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 owing to 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. If individuals with any missing data were excluded, results were similar to those reported.

More information on statistical methods can be found in the supplementary material.

Results

Mean plasma complement C3 concentration was 1.10 $g{\cdot}L^{-1}$ (interquartile range 0.97–1.25 $g{\cdot}L^{-1}$) 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 % pred, 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 hospitalisation

Median follow-up time in observational analyses was 6 years (range 0–11 years), during which time 1238 individuals were hospitalised due to asthma. The cumulative incidence of asthma hospitalisations was higher for individuals in the middle (1.03–1.19 $g{\cdot}L^{-1}$) and highest (>1.19 $g{\cdot}L^{-1}$) tertiles of plasma complement C3 concentration than for individuals in the lowest tertile (<1.03 $g{\cdot}L^{-1}$) (log-rank $p=3\times10^{-8}$) (figure 1). Results were similar after exclusion of individuals with ischaemic heart disease, rheumatoid arteritis, diabetes mellitus, cancer or obesity defined as a body mass index $\geqslant$30 $kg{\cdot}m^{-2}$ (supplementary figure S2). After multivariable adjustment, we found a hazard ratio (HR) of 1.14 (95% CI 0.98–1.33) for asthma hospitalisation 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 hospitalisation

C3 rs448260, *CFH* rs1065489 and *SKIV2L* rs429608 showed a stepwise per genotype increase in plasma complement C3 level (all p-values $\leqslant6\times10^{-76}$) (figure 3). For *C3* rs448260, a 3.5% higher plasma

TABLE 1 Table of characteristics in the Copenhagen General Population Study

	Plasma complement C3 tertile			
	First	Second	Third	p-value for trend
Plasma complement C3 g·L^{-1}	<1.03	1.03–1.19	>1.19	
Individuals n	35460	32308	33261	
Age years	56 (47–66)	58 (49–68)	59 (49–68)	4×10^{-85}
Male sex	15519 (44)	15582 (48)	14347 (43)	0.16
Low level of education	16269 (46)	17878 (55)	21431 (64)	4×10^{-300}
FEV$_1$ % pred	100 (90–109)	97 (87–106)	93 (83–103)	1×10^{-300}
Smoking status				3×10^{-19}
Never	15666 (44)	13216 (41)	13296 (40)	
Current	5818 (16)	5899 (18)	6212 (19)	
Former	13976 (40)	13193 (41)	13753 (41)	
Pack-years of smoking$^{\#}$	13 (5–25)	17 (7–30)	20 (8–34)	8×10^{-254}
Body mass index kg·m^{-2}	24 (22–26)	26 (24–28)	28 (26–31)	1×10^{-300}
Body mass index n				
<18.5 kg·m^{-2}	609 (71)	179 (20)	74 (9)	
18.5–24.9 kg·m^{-2}	23305 (54)	13448 (31)	6759 (16)	
25–29.9 kg·m^{-2}	10329 (26)	14932 (37)	15074 (37)	
30–39.9 kg·m^{-2}	1202 (8)	3718 (24)	10587 (68)	
>40 kg·m^{-2}	15 (2)	40 (5)	776 (93)	
Occupational exposure to dust and fumes	2693 (8)	3404 (11)	4245 (13)	1×10^{-111}
Familial disposition to asthma	6165 (17)	5584 (17)	6241 (19)	3×10^{-6}
Asthma, hay fever or eczema during childhood	5143 (15)	4386 (14)	4358 (13)	9×10^{-8}
Low physical activity during leisure time	13832 (39)	15418 (48)	19939 (60)	1×10^{-300}
C-reactive protein mg·L^{-1}	1.1 (0.7–1.4)	1.4 (1.0–2.0)	2.2 (1.4–4.0)	1×10^{-300}
Fibrinogen μmol·L^{-1}	9.5 (8.5–10.8)	10.6 (9.5–12.1)	12.0 (10.5–13.9)	1×10^{-300}
Leukocytes ×10^9 cells·L^{-1}	6.6 (5.6–7.6)	7.0 (6.0–8.1)	7.5 (6.5–8.8)	1×10^{-300}
Eosinophils ×10^9 cells·L^{-1}	0.15 (0.10–0.23)	0.16 (0.11–0.25)	0.17 (0.12–0.26)	1×10^{-169}
Neutrophils ×10^9 cells·L^{-1}	3.8 (3.1–4.6)	4.0 (3.3–4.9)	4.4 (3.6–5.3)	1×10^{-300}
IgE g·L^{-1}¶	21 (4–52)	21 (4–56)	22 (5–63)	6×10^{-9}
History of respiratory infections	7180 (20)	7432 (23)	8935 (27)	5×10^{-93}
Rheumatoid arthritis	225 (0.7)	264 (0.8)	390 (1.2)	4×10^{-10}
Diabetes mellitus	882 (2)	1085 (3)	2417 (7)	2×10^{-222}
Ischaemic heart disease	1429 (4)	1901 (6)	2435 (7)	3×10^{-77}
Any cancer	3756 (11)	3492 (11)	3685 (11)	0.04
Any inhaled medication^{+}	1491 (4)	1824 (6)	2641 (8)	2×10^{-95}

Data are presented as median (interquartile range) or n (%), unless otherwise indicated. *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 ($p=3\times10^{-4}$) and IgE ($p=3\times10^{-4}$). FEV$_1$: forced expiratory volume in 1 s. $^{\#}$: only in ever-smokers; ¶: IgE measurements only available in 49328 individuals; $^{+}$: 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.

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 hospitalisation. The 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 hospitalisation.

Observationally, high plasma complement C3 concentration was associated with high blood eosinophil count, high IgE level and high blood neutrophil count in all individuals (figure 4). In genetic analyses, only *SKIV2L* rs429608 was positively associated with blood eosinophil counts and IgE level. In cross-sectional sensitivity analyses, *SKIV2L* rs429608 genotype AA was also associated with a high risk of allergic asthma (OR 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

A high concentration of plasma complement C3 was associated with increased risk of allergic asthma ($p=4\times10^{-6}$) in a multivariable adjusted cross-sectional analysis.

In individuals with allergic asthma, the mean plasma complement C3 concentration was 1.14 g·L^{-1} (interquartile range 1.00–1.31 g·L^{-1}) (supplementary figure S4). As in the entire CGPS, high

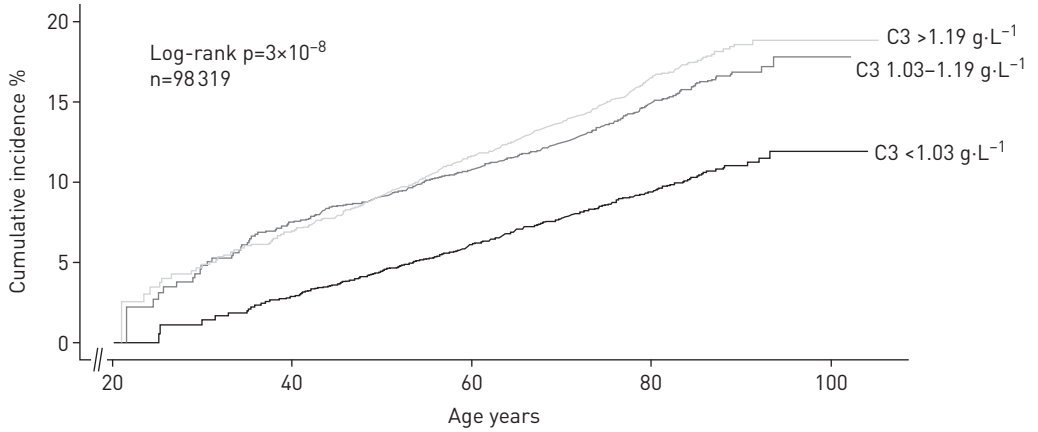
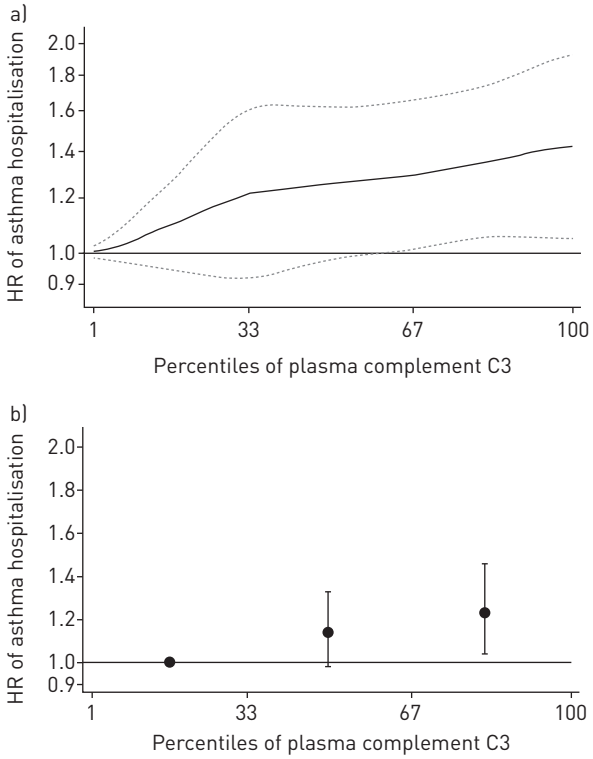


FIGURE 1 Cumulative incidence of asthma hospitalisation as a function of age and tertiles of plasma complement C3 concentration. Individuals hospitalised because of asthma before the day of plasma complement C3 measurement (n=2710) were excluded from the prospective, observational analyses.

concentrations of plasma complement C3 were associated with low FEV_1 % pred, a higher percentage of inhaled medication use, 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 plasma IgE level were not associated with the complement C3 level. There was a lower percentage of individuals in the highest tertile of plasma complement C3 with asthma diagnosed before the age of 15 years; none of the three plasma complement C3-determining genotypes were associated with any of the included baseline characteristics (supplementary table S2).



Participants n	32 455	32 986	31 642
Asthma hospitalisations n	302	413	523

FIGURE 2 Multivariable adjusted hazard ratios (HRs) for asthma hospitalisation according to plasma complement C3 percentile, as splines (a) and as tertiles (b). Solid lines are multivariable adjusted HRs using a polynomial smoother. Dashed lines indicate 95% CI derived from restricted cubic spline regression. The analyses were adjusted for age, sex, education, plasma C-reactive protein, body mass index, smoking status and physical inactivity.

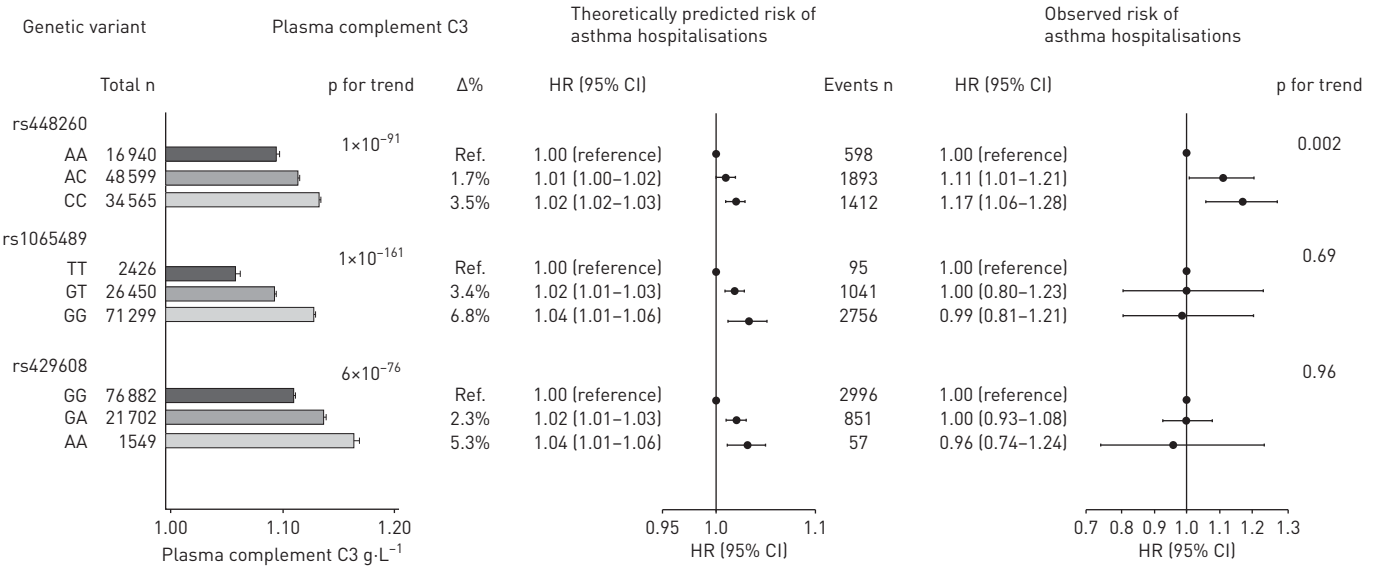


FIGURE 3 Plasma concentrations of complement C3 and corresponding theoretically predicted and observed hazard ratios (HRs) for asthma hospitalisation for *C3* rs448260, *CFH* rs1065489 and *SKIV2L* rs429608. Because exposure is a genetic instrument, individuals entered at date of birth or 1977 (start of the Danish Patient Registry), *i.e.* with delayed entry for those born before 1977, because genotypes are present at birth and therefore precede all events. Thus, 100 003 individuals were included and 3900 were hospitalised due to asthma. Mean (95% CI) is given for plasma complement C3 (left). Theoretically predicted HRs (middle) were calculated using Δ mean concentrations of plasma complement C3 for each genetic variant and adjusted only for age and sex. Likewise observed HRs (right) were also only adjusted for age and sex.

Individuals with allergic asthma in the highest tertile (>1.24 g·L^{-1}) of plasma complement C3 had a higher frequency of respiratory symptoms (table 3). However, after multivariable adjustments only the risk of dyspnoea remained significant for individuals in the middle tertile (1.06–1.24 g·L^{-1}; OR 1.44, 95% CI 1.13–1.84) and highest tertile (>1.24 g·L^{-1}; OR 1.68, 95% CI 1.26–2.24) compared with individuals in the lowest plasma complement C3 tertile (<1.06 g·L^{-1}).

During prospective follow-up, 350 individuals with allergic asthma had one or more asthma exacerbation. In total, we captured 927 exacerbations and 148 individuals had more than one exacerbation during follow-up. Individuals with high concentrations of plasma complement C3 had a higher risk of asthma exacerbation 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 exacerbation 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 was that high concentrations of plasma complement C3 were associated with increased risk of asthma hospitalisation. Additionally, we found that *C3* rs448260 was associated with risk of asthma hospitalisation while *SKIV2L* rs429608 was associated with higher blood eosinophil count and higher IgE levels. On further investigation on the role of plasma complement C3 in 2248 individuals with allergic asthma, we found that high concentrations of plasma complement C3 were associated with increased risk of asthma exacerbation. 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 mites, 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

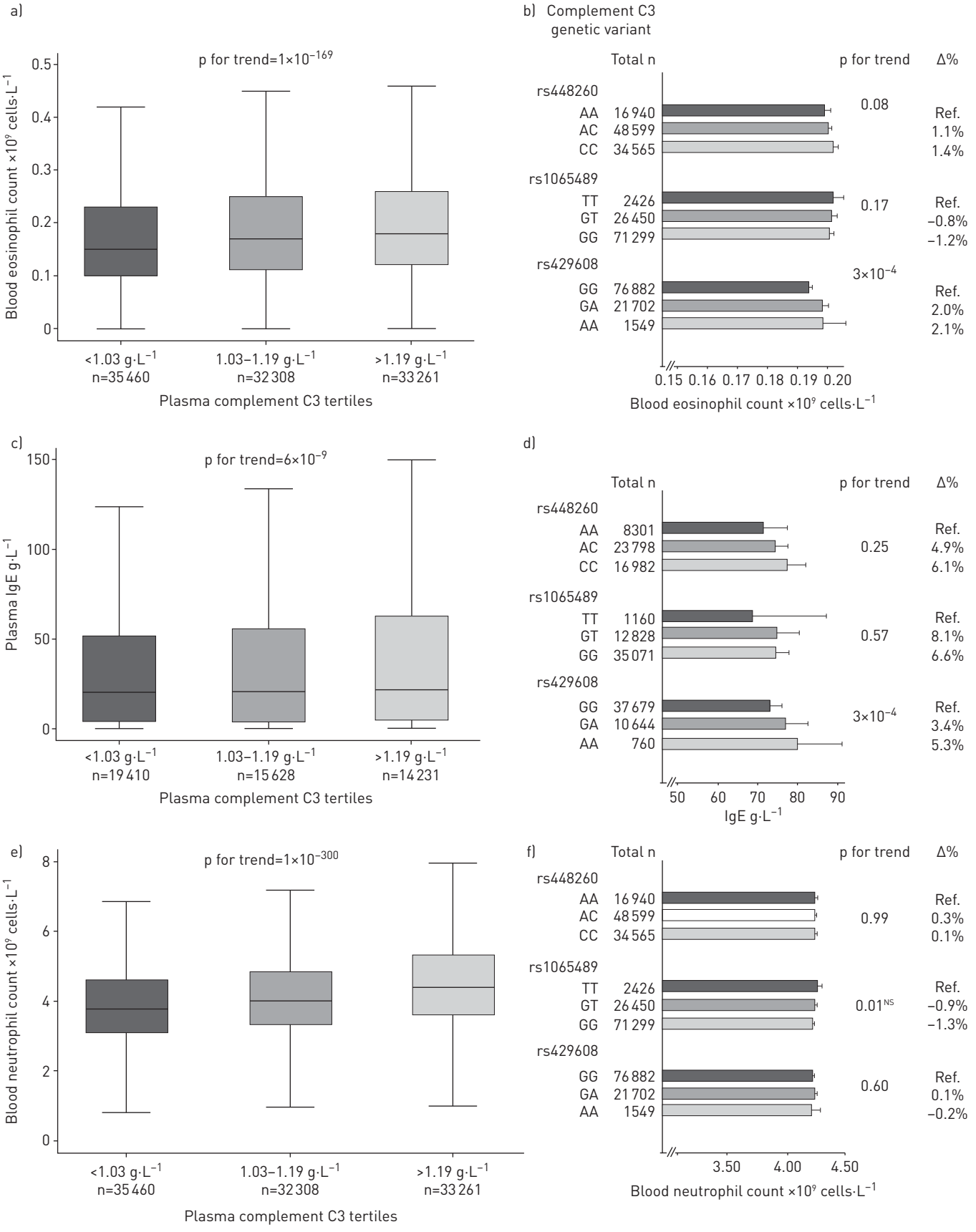


FIGURE 4 Plasma complement C3, blood eosinophil count, IgE and blood neutrophil count. a) Observational data showing blood eosinophil counts by tertiles of plasma complement C3. Boxes indicate medians and 25th and 75th percentiles. b) Genetic data showing complement

C3-determining genetic variants and blood eosinophil counts. Data presented as mean (95% CI). c) Observational data showing IgE by tertiles of plasma complement C3. Boxes indicate medians and 25th and 75th percentiles. d) Genetic data showing complement C3-determining genetic variants and IgE level. Data presented as mean (95% CI). e) Observational data showing blood neutrophil counts by tertiles of plasma complement C3. Boxes indicate medians and 25th and 75th percentiles. f) Genetic data showing complement C3-determining genetic variants and blood neutrophil counts. Data presented as mean (95% CI). IgE measurements were only available in 49 328 individuals. NS: nonsignificant after Bonferroni correction for multiple comparisons (required p-value $<4\times10^{-3}$).

complement C3 in individuals with allergic asthma might lead to higher risk of exacerbation through the formation of C3a driven by IgE. In the present study, we found that a high concentration of plasma complement C3 was associated with a high risk of asthma hospitalisation and exacerbation. Previously, a study of 52 patients with acute asthma reported increased levels of C3a in patients hospitalised with asthma exacerbations compared with patients with mild exacerbations not requiring hospitalisation [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];

TABLE 2 Table of characteristics in individuals with allergic asthma

	Plasma complement C3 tertile			
	First	Second	Third	p-value for trend
Plasma complement C3 g·L^{-1}	<1.06	1.06–1.24	>1.24	
Individuals n	777	727	744	
Age years	49 (43–58)	52 (44–63)	54 (44–63)	5×10^{-7}
Male sex	272 (35)	278 (38)	218 (29)	0.02
Low level of education	290 (37)	353 (49)	422 (57)	3×10^{-14}
FEV_1 % pred	97 (90–105)	95 (86–104)	93 (83–101)	6×10^{-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)	2×10^{-138}
Body mass index n				
<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)	2×10^{-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)	4×10^{-23}
C-reactive protein mg·L^{-1}	1.1 (0.6–1.5)	1.5 (1.0–2.0)	2.5 (1.5–4.7)	6×10^{-126}
Fibrinogen μmol·L^{-1}	9.4 (8.3–10.5)	10.3 (9.3–11.8)	12.1 (10.6–13.9)	6×10^{-117}
Leukocytes ×10^9 cells·L^{-1}	6.6 (5.7–7.5)	7.0 (6.1–7.9)	7.7 (6.7–9.0)	6×10^{-47}
Eosinophils, ×10^9 cells·L^{-1}	0.20 (0.13–0.32)	0.22 (0.14–0.33)	0.21 (0.13–0.31)	0.57
Neutrophils, ×10^9 cells·L^{-1}	3.7 (3.1–4.5)	3.9 (3.2–4.6)	4.4 (3.7–5.5)	6×10^{-34}
IgE g·L^{-1}¶	54 (20–128)	44 (14–121)	53 (18–126)	0.50
History of respiratory infections	309 (40)	320 (44)	393 (53)	3×10^{-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)	5×10^{-7}
Ischaemic heart disease	15 (2)	27 (4)	47 (5)	3×10^{-3}
Any cancer	42 (6)	45 (7)	70 (8)	0.24
Any inhaled medication+	345 (44)	376 (42)	362 (49)	2×10^{-6}

Data are presented as median (interquartile range) or n (%), unless otherwise indicated. *C3* rs448260, *CFH* rs1065489 and *SKIV2L* rs429608 were not associated with any of the included baseline characteristics in the allergic asthma population. FEV_1: forced expiratory volume in 1 s. #: only in ever-smokers; ¶: IgE measurements only available in 1093 individuals with allergic asthma; +: 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.

TABLE 3 Respiratory symptoms in allergic asthma

Respiratory symptom	Plasma complement C3 tertiles			p-value for trend	Plasma complement C3 tertiles (multivariable adjusted)			p-value for trend
	First	Second	Third		First (Reference)	Second OR (95% CI)	Third OR (95% CI)	
Plasma complement C3 g·L^{-1}	<1.06	1.06–1.24	>1.24					
Individuals n	766	716	738					
Dyspnoea (mMRC⩾2)	204 (26)	299 (42)	429 (58)	6×10^{-34}	1.00	1.44 (1.13–1.84)	1.68 (1.26–2.24)	3×10^{-4}
Sputum production	93 (12)	109 (15)	140 (19)	1×10^{-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)	1×10^{-5}	1.00	1.18 (0.95–1.47)	1.18 (0.90–1.51)	0.15
Wheezing	421 (55)	426 (59)	486 (67)	4×10^{-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)	1×10^{-10}	1.00	1.09 (0.85–1.40)	1.30 (0.95–1.79)	0.51

Data are presented as n (%), unless otherwise indicated. Unadjusted p-value for trend is from Cuzick's non-parametric test. Multivariable adjusted p-value for trend is from logistic regression with plasma complement C3 tertiles as a continuous variable. Multivariable adjusted model was for age, sex, education, body mass index, smoking status, C-reactive protein and forced expiratory volume in 1 s % pred. mMRC: modified Medical Research Council Dyspnoea Scale.

when found in the airways of asthma patients, such a mixed response characterises 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 fluid 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, owing to the 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 asthma in the general population *versus* the influence on severity among individuals with already-established allergic asthma. Interestingly, we found that high plasma complement C3 concentration was associated with high blood neutrophil count in individuals from both the general population and with allergic asthma, which supports complement C3 involvement. A dysregulated immune response with Th2 cells producing IL-4, IL-5 and IL-13 that orchestrate a pulmonary allergic response is a well-known contributor to asthma pathogenesis,

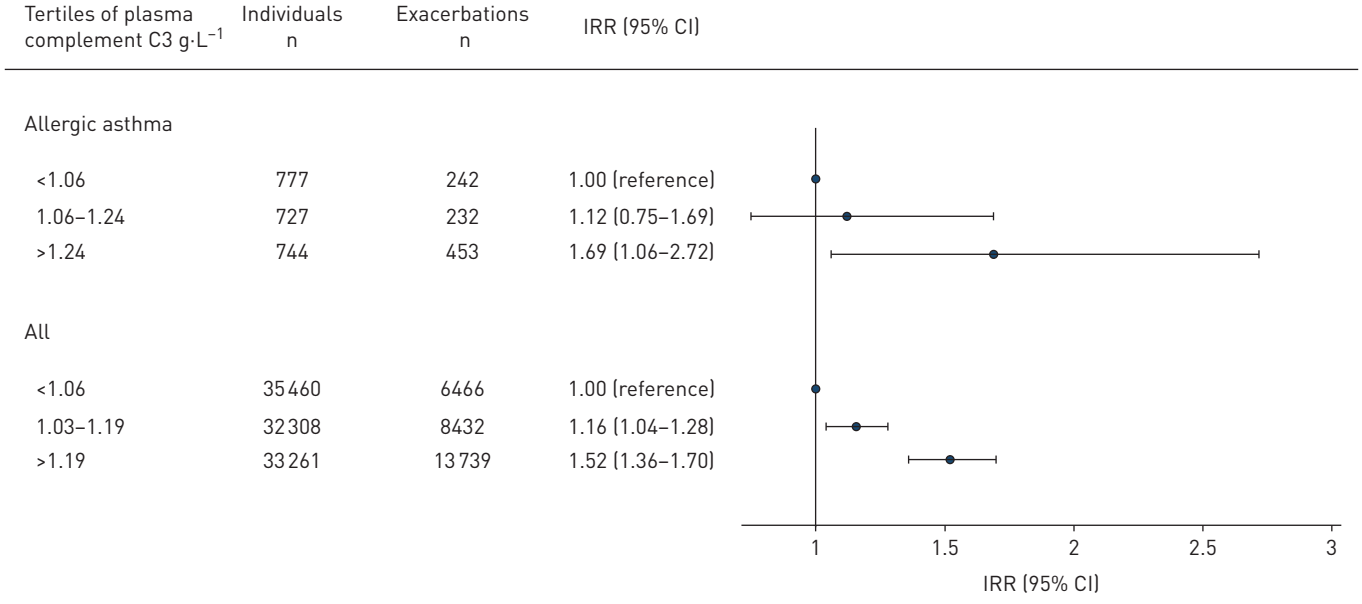


FIGURE 5 Risk of exacerbation according to plasma complement C3 tertiles in individuals with allergic asthma (n=2248) and in all individuals (n=101 029). Multivariable adjustments were for age, sex, education, plasma C-reactive protein, body mass index, smoking status, low physical activity and forced expiratory volume in 1 s % pred. IRR: incidence rate ratio.

and 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; and the release of IL-1, IL-6 and tumor necrosis factor-α (TNF-α). Together these contribute to a pro-allergic 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 hospitalisation, 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 that a variant in the *C3* gene was associated with childhood and adult asthma [17]. This association was more pronounced when analyses were stratified according to IgE levels. Furthermore, a variant in 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 IgE level. When assessing risk of exacerbation in the subpopulation with allergic asthma, *SKIV2L* rs429608 AA homozygosity was associated with a 2.5-times higher risk of exacerbation compared to wild type. Because *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, because data from the national Danish registries do not contain information on the cause of exacerbation, our study cannot conclude on this. Nonetheless, *the complement factor 2/factor B* gene is adjacent to *SKIV2L* and is involved in regulation of the alternative pathway of the complement system. Factor B may be of critical importance for the development of asthma, as shown in a previous study which reported significantly reduced airway responsiveness and less airway inflammation following allergen sensitisation 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 level. Because 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, given that 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]. In that study, high C5 in combination with low C3 had a protective effect on the development of asthma during initial allergen exposure. C5 does, however, have a dual role because once the Th2-mediated response has been initiated, C5 acts pro-allergically by recruiting eosinophils and mast cells. This 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. As mentioned above, 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. This study does have some limitations. First, because 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 hospitalised at blood sampling [30]. Second, we were limited by only having prebronchodilator spirometry measurements, and because asthma and allergy were self-reported, we cannot exclude the possibility that some of the individuals classified as having allergic asthma did in fact have chronic obstructive pulmonary disease. However, by excluding individuals with an FEV_1/FVC below the lower limit of normal we believe this is unlikely. Third, because the CGPS does not include information on asthma control, we were not able to include this to assess asthma severity. Likewise, we cannot draw any conclusions on the frequency of medication use, which could be a proxy for asthma severity; however, data on the number of individuals using asthma medication at baseline indicated a higher frequency of use with higher plasma complement C3. Fourth, because all participants were of Danish descent, our results may not necessarily apply to other races; however, this feature also minimised 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 hospitalisation in 101 029 individuals from the general population and with increased risk of asthma exacerbation 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 exacerbation.

Acknowledgements: We are indebted to the staff and participants of the Copenhagen General Population Study for their important contributions.

Author contributions: S. Vedel-Krogh, K.L. Rasmussen, B.G. Nordestgaard and S.F. Nielsen designed the study together. S. Vedel-Krogh analysed the data. S.F. Nielsen oversaw all analyses and contributed to the interpretation of data. S. Vedel-Krogh wrote the first draft of the paper and K.L. Rasmussen, B.G. Nordestgaard and S.F. Nielsen edited the paper. All authors approved this paper in its final form.

Conflict of interest: None declared.

Support statement: The study was funded by the Dept of Clinical Biochemistry, Herlev and Gentofte Hospital. The sponsor of the study had no role in study design, data collection, data analysis, data interpretation or writing of the paper. Funding information for this article has been deposited with the Crossref Funder Registry.

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