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Exploring the role of polymorphisms in ficolin genes in respiratory tract infections in children

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Summary

Ficolins are pattern-recognition molecules that appear to be relevant for innate immune defence against infections. The ficolin genes in Caucasians are polymorphic and genetic variations may have functional consequences, both in relation to function and concentration. Low levels of Ficolin-2 have been suggested to associate with recurrent respiratory tract infections (RTI), whereas data on Ficolin-3 are still very limited. We investigated the association between variation in genes encoding Ficolin-2 (*FCN2*) and Ficolin-3 (*FCN3*) and frequency of RTI during the first 4 years of life. The study population consisted of 900 children from a large, population-based birth cohort of Dutch children, followed prospectively from birth to 4 years of age. The number of RTI was assessed by annual parental questionnaires. Nine single nucleotide polymorphisms in *FCN2* and two in *FCN3*, all based on functionality or haplotype-tagging characteristics, were determined and haplotypes constructed. We found that single nucleotide polymorphisms in *FCN2* and *FCN3* were not associated with increased risk of RTI during the first 4 years of life. No difference existed between haplotype-frequencies of *FCN2* and *FCN3* in children grouped according to the reported number of RTI. In conclusion, at a population level, genetic variation in ficolin genes *FCN2* and *FCN3* do not seem to contribute to the risk of RTI in Caucasian children.

Keywords: ficolin, haplotypes, respiratory tract infections, single nucleotide polymorphisms

Introduction

Ficolins are pattern-recognition molecules that bind carbohydrates on the surface of microorganisms. Increasing evidence shows that Ficolin-2 (L-ficolin, encoded by *FCN2*) and Ficolin-3 (H-ficolin, encoded by *FCN3*) act to enhance phagocytosis and activate complement via the lectin pathway, similar to mannose-binding lectin (MBL) [1]. The *FCN2* gene is located on chromosome 9q34, and polymorphisms within this gene have been found to account for inter-individual variation in serum level and function of Ficolin-2 [2–4]. Currently, five common and functionally relevant polymorphisms within this gene have been described in healthy Danish Caucasian populations: three promoter polymorphisms (−986A > G, −602G > A and −4A > G) and two coding polymorphisms in exon 8 (Thr236Met and Ala258Ser) [2,4]. Whereas these five polymorphisms were found to be associated with high or low Ficolin-2 serum levels [3,4], the polymorphisms in exon 8 have also been shown to lead to increased (Ala258Ser) or decreased (Thr236Met) binding of Ficolin-2 to carbohydrates [5].

Ficolin-3 is encoded by the *FCN3* gene located on chromosome 1p36, but further knowledge on this type of ficolin is limited. Large variations in Ficolin-3 serum concentration have been observed in healthy individuals, which might be determined genetically. However, no common polymorphisms causing deficiency or lack of function in *FCN3* have been discovered so far [4,6,7]. Functionally, like Ficolin-2, Ficolin-3 has been shown to interact with the MBL-associated serine proteases enabling activation of the complement system [8,9]. Moreover, a recent comparative study found Ficolin-3 to have the highest complement activating capacity among the lectin pathway initiator molecules [10]. The exact ligands for Ficolin-3 are unknown, although distinct binding to certain strains of bacteria has been demonstrated [11].

The MBL deficiency has been associated with increased respiratory infectious susceptibility [12–15]. Similarly, low Ficolin-2 serum levels have been suggested previously to constitute a risk for recurrent respiratory tract infections (RTI) [16,17]. This might be explained by the fact that Ficolin-2, like MBL, can bind to bacteria such as *Streptococcus pneumoniae*, an important bacterial pathogen causing RTI [18]. To our knowledge, no association studies on the clinical relevance of low Ficolin-3 serum concentrations or any *FCN3* polymorphisms have been performed so far.

Similarities in function between ficolins and MBL and the evidence for a role of Ficolin-2 in the innate immune response to respiratory pathogens led us to hypothesize that polymorphisms and haplotypes of *FCN2* and *FCN3* might be associated with susceptibility to RTI. This was explored in a large population-based birth cohort of children with Dutch ancestry followed prospectively from birth to 4 years. Secondly, this cohort represents the largest (Caucasian) population studied for genetic variations in *FCN2* and *FCN3* so far.

Methods

Study population

Children participated in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study, designed originally to investigate atopy and asthma. Details of the study design have been published previously [19]. Recruitment took place in the years 1996 and 1997. A screening questionnaire was completed by 10 232 pregnant women visiting one of 52 prenatal clinics in the Netherlands [20]. Based on this screening, 7862 women were invited, and 4146 agreed and gave informed consent. After birth the baseline study population consisted of 3963 children. Questionnaires for parental completion, based partly on the International Study of Asthma and Allergies in Childhood core questionnaires, were sent to the parents during pregnancy, at the child's ages of 3 and 12 months, and yearly thereafter to the age of 4 years [21]. DNA was collected successfully from 1037 children. From these children, 987 were of Dutch ancestry and used for analysis. Fifty children of non-Dutch origin were excluded from the current analysis to avoid effects resulting from population stratification. Reportage of frequency of RTI of the children was part of the questionnaire first at 1 year of age and continued annually to 4 years of age. Complete data on frequency of RTI from years 1 to 4 and DNA were available in 900 children of Dutch Caucasian origin.

Table 1. General characteristics of the study population.

	DNA available ($n = 987$)*	
	n	%
Allergic mother†	651	66
Maternal smoking during pregnancy (> 4 weeks from start)	138	14
Breastfeeding ≥ 12 weeks	537	55
Environmental tobacco smoke exposure‡	243	25
Frequency of RTI§		
Frequent¶	55	6
Moderate	715	79
No RTI	130	14
Missing	87	
Dutch Caucasian	987	100

*$n = 987$, DNA available and Dutch Caucasian ethnicity. †Allergic mother according to validated questionnaire. ‡At 1 year of age. §RTI, respiratory tract infections, frequency of RTI based on annual parental questionnaires from 1 to 4 years. ¶Frequent RTI, three or more RTI per year in 3 or 4 years during first 4 years of life.

General characteristics of the study population were comparable between participants and non-participants in the genetic study, apart from allergy in the mother (Table 1). Other variables were also investigated and found to be distributed approximately equally between participants and non-participants: maternal smoking during pregnancy, duration of pregnancy, birth weight, gender, breastfeeding for 12 weeks or more, day care, environmental tobacco smoke exposure and presence of siblings (not shown).

In line with Atkinson *et al.* [16], a subgroup analysis was performed in atopic children from the cohort [$n = 237$, atopy defined as specific immunoglobulin E ≥ 0·7 IU/ml against at least one inhalation allergen and/or positive skin prick test to at least one allergen].

The study protocol was approved by the institutional review board of each participating institute and informed written consent was obtained from all participants.

Polymorphisms and haplotypes of FCN2 and FCN3

Genomic DNA was extracted from buccal swabs or blood by performing chloroform-2-propanol extraction. DNA was amplified by using REPLI-g UltraFast technology (Qiagen™). Single nucleotide polymorphisms (SNPs) of *FCN2* were selected based on known functionality ($n = 5$),

Table 2. Single nucleotide polymorphisms (SNPs) in Ficolin-2 (*FCN2*) and Ficolin-3 (*FCN3*) studied in the Prevention and Incidence of Asthma and Mite Allergy cohort.

Gene	dbSNP identifier	SNP location	SNP type	Minor allele	MAF	HWE *P*-value
FCN2	rs3124952	−986A > G	Promoter, Ht*	G	0·485	0·68
	rs3124953	−602G > A	Promoter, Ht	A	0·208	0·60
	rs17514136†	−4A > G	Promoter/exon 1	G	0·260	0·79
	rs3128626	888	Intron 1, Ht	T	0·291	0·29
	rs3128625	950	Intron 1, Ht	A	0·351	0·68
	rs7037264	2545	Intron 3, Ht	A	0·410	0·65
	rs7041446	3386	Intron 3, Ht	A	0·479	0·98
	rs17549193‡	6359C > T	Thr236Met Exon 8, coding	T	0·278	0·82
	rs7851696	6424G > T	Ala258Ser Exon 8, coding	T	0·122	0·91
FCN3	rs10794501	1861A > T	Intron 5, Ht	A	0·241	0·06
	rs4494157	4473A > C	Intron 7, Ht	A	0·309	0·79

*Ht, haplotype-tagging SNP according to HapMap individuals of northern European ancestry; †rs17514136 = ss32469537; ‡rs17549193 = ss32469544; MAF, minor allele frequency, determined in study population of Dutch Caucasian ethnicity; HWE, Hardy–Weinberg equilibrium.

combined with haplotype-tagging SNPs with minor allele frequency (MAF) > 0·1 ($n = 4$) selected from the publicly available database of the International HapMap Project (International HapMap Consortium, 2005; www.hapmap.org). Because no functionally relevant SNPs of *FCN3* are currently known, only haplotype-tagging SNPs with a minor allele frequency MAF of > 0·1 of this gene ($n = 2$) were selected. The studied SNPs are listed in Table 2. Genotyping was performed by competitive allele-specific polymerase chain reaction using KASPar™ genotyping chemistry, performed under contract by K-Biosciences (http://www.kbioscience.co.uk/). The quality of genotype data was guaranteed by K-Biosciences standards. We verified the genotyping quality by another two steps: (1) inheritance of alleles between parents and children was checked using family-based association tests (FBAT; http://www.biostat.harvard.edu/~fbat/fbat.html); and (2) genotype data were analysed for deviations from Hardy–Weinberg equilibrium.

Previously, haplotypes of *FCN2* based on four functionally relevant SNPs have been related to serum levels of Ficolin-2 in a population of Danish blood donors [2]. We used one extra functionally relevant SNP to construct haplotypes, and serum levels of Ficolin-2 associated with these haplotypes were estimated based on previous data [2]. Schematic representation of SNPs and haplotypes of the *FCN2* and *FCN3* gene that were studied is visualized in Figs 1 and 2.

Respiratory tract infections

Information about frequency of RTI was collected from annual parental questionnaires from 1 to 4 years of age by the following question: 'How often did your child have serious respiratory tract and/or ear–nose–throat infections such as flu, infection of the throat, middle ear or sinuses, bronchitis or pneumonia during the last 12 months?'. Four answers were possible: 'never', '1–2 times', '3–5 times' or '≥ 6 times'. Based on the answers in the four annual consecutive questionnaires, three groups were defined according to the reported frequency of RTI. We defined frequent RTI as three or more RTI per year reported in three or four annual questionnaires (group 1). The second group consisted of children with a low to moderate frequency of RTI (one or more RTI reported in the four annual questionnaires but not according to the definition of frequent RTI). The third group consisted of the remaining children without any reported RTI in 4 years.

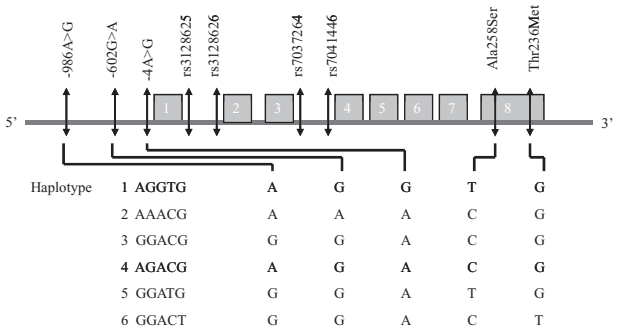

Fig. 1. Schematic representation of single nucleotide polymorphisms (SNPs) and haplotypes studied of the Ficolin-2 (*FCN2*) gene.

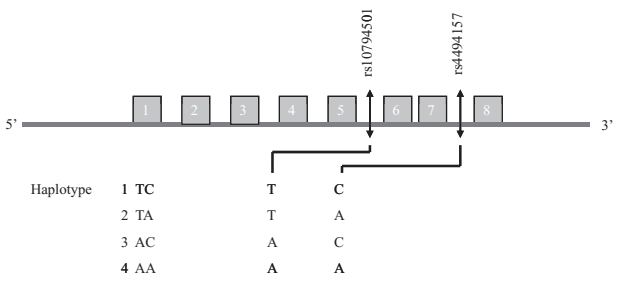

Fig. 2. Schematic representation of single nucleotide polymorphisms (SNPs) and haplotypes studied of the Ficolin-3 (*FCN3*) gene.

Statistical analyses

The genotype data were analysed for deviations from Hardy–Weinberg equilibrium with χ^2 statistics ($P > 0{\cdot}050$). χ^2 statistics were also used to compare genotype frequencies of the SNPs between children with frequent RTI and those without reported RTI. Haplotypes were estimated from unphased genotype data using the Bayasian statistical method in PHASE version 2.1 (http://www.stat.washington.edu/stephens/software.html). Percentage differences in haplotype and genotype frequencies [with corresponding 95% confidence intervals (CI)] between children with frequent RTI and those without reported RTI or those with moderate frequency of reported RTI were calculated.

All analyses were performed with SPSS statistical software version 12.0.2 (SPSS Inc., Chicago, IL, USA). A *post hoc* power calculation was performed using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/) [22].

Results

Among the 900 children of our final study population with complete information on RTI, according to our definition 55 (6%) had frequent RTI during the first 4 years of life (group 1), 715 children (79%) had a low to moderate reported frequency of RTI (group 2) and 130 (14%) did not have any reported RTI during this period (group 3).

Single locus analyses

All determined SNPs in *FCN2* and *FCN3* were common with the MAF > 12% (Table 2). The MAF of the nine SNPs in *FCN2* were similar to those reported previously in Caucasians [2,4,5,23]. The MAF of the two SNPs in *FCN3* were comparable to reported frequencies in a population of northern and western European ancestry (http://hapmap.org/hapmappopulations.html). All SNPs adhered to Hardy–Weinberg expectations ($P > 0{\cdot}05$). Data of both parents were available for 193 children. Analyses for inheritance patterns on these data by FBAT showed inheritance errors (caused supposedly by genotyping errors) in < 1% of the cases.

No significant difference in genotype frequencies of *FCN2* and *FCN3* was observed between the children with frequent RTI and those without RTI reported ($P = 0{\cdot}25$), nor with the group with intermediate number of infections reported (Table 3).

Haplotype analyses

A total of 36 *FCN2* haplotypes was constructed from the genotype data. Linkage disequilibrium led to six haplotypes with a frequency of > 1% based on the five well-known functionally relevant SNPs of *FCN2* (−986A > G, −602G > A, −4A > G, Thr236Met and Ala258Ser) (Table 4). Haplotype frequencies are in agreement with previous reported findings in Caucasian studies [2,23]. No significant difference in haplotype frequencies was observed between the groups, i.e. children with frequent RTI and those without RTI reported or the intermediate group. Percentage differences in prevalence of specific haplotypes between children with frequent RTI and without RTI varied between −2 (95% CI: −13; 9) for GGACG and 2 (95% CI: −1; 5) for AGACG (Table 4). The absence of an association between haplotypes of *FCN2* and percentages of children in all categories of frequencies of RTI reported in the annual questionnaires at age 1–4 years is visualized in Fig. 3. The presence of the GGACT haplotype, known to result in low serum levels of Ficolin-2, was not related to a larger percentage of children in the higher categories of reported frequency of RTI (Fig. 3).

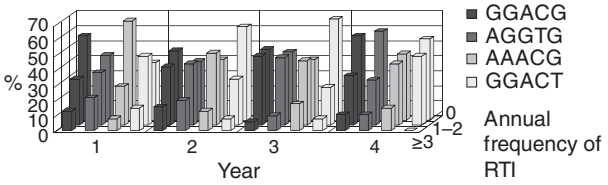

Fig. 3. Percentage of children according to annual reported frequency of respiratory tract infections (RTI) (0, 1–2, ≥ 3) as derived from questionnaires in years 1, 2, 3 and 4 and stratified for estimated Ficolin-2 (*FCN2*) haplotype. Only children homozygous for haplotypes with estimated frequency > 5% are included in this analysis. The haplotypes are constructed from the five functionally relevant polymorphisms in FCN2: −986A > G, −602G > A, −4A > G, Thr236Met and Ala258Ser.

Four *FCN3* haplotypes were found; the frequency of the homozygous wild-type haplotype was 47·5% in contrast to the homozygous variant haplotype, which was rare (1·8%) (Table 5). No significant difference in frequencies of haplotypes was observed between the children with frequent RTI and those without RTI reported or the intermediate group. Percentage differences in the prevalence of the specific haplotypes varied between −7 (95% CI: −17; 2) for the heterozygous haplotype AC and 8 (95% CI: −3:19) for the homozygous wild-type haplotype TC (Table 5).

Atopic children

We performed a subgroup analysis in the 237 atopic children from our cohort. Results of these analyses were similar to those obtained in the group as a whole, i.e. *FCN2* and *FCN3* genotypes and haplotypes were not associated with susceptibility to RTI (not shown).

Discussion

This is the first large prospective population-based birth cohort study on the clinical significance of *FCN2* and

Table 3. Genotype frequencies of single nucleotide polymorphisms (SNPs) of Ficolin-2 (*FCN2*) and Ficolin-3 (*FCN3*) according to frequency of reported respiratory tract infections (RTI) in 1–4 years.

		No RTI ($n = 130$)		Moderate frequency of RTI ($n = 715$)		Frequent RTI* ($n = 55$)				
	Genotype	*n*	%	*n*	%	*n*	%	PD†	95% CI‡	*P*-value§
FCN2 SNP										
−986A > G	AA	35	28	157	22	12	23	5	−9; 19	0·25
	AG	60	49	347	50	33	62	−13	−28; 2	
	GG	28	23	195	28	8	15	8	−4; 20	
−602G > A	GG	78	64	437	62	31	61	3	−12; 18	0·89
	GA	41	34	229	33	19	37	−3	−18; 12	
	AA	3	2	37	5	1	2	0	−4; 4	
−4A > G	AA	71	58	371	54	30	57	1	−15; 17	0·94
	AG	43	35	266	39	20	38	−3	−18; 12	
	GG	8	7	46	7	3	6	1	−2; 6	
Thr236Met	CC	54	53	286	52	24	51	2	−14; 18	0·81
	CT	39	38	227	41	20	43	−5	−21; 11	
	TT	9	9	41	7	3	6	3	−5; 11	
Ala258Ser	GG	101	79	537	77	43	81	−2	−15; 11	0·92
	GT	25	20	152	22	9	17	3	−9; 15	
	TT	2	2	10	1	1	2	0	−4; 4	
FCN3 SNP										
1861A > T	AA	72	60	388	56	27	49	11	−5; 27	0·30
	AT	42	35	270	39	26	47	−12	−28; 4	
	TT	6	5	30	4	2	4	1	−5; 7	
4473A > C	AA	58	49	327	48	24	47	2	−14; 18	0·98
	AC	48	40	291	43	21	41	−1	−16; 14	
	CC	13	11	66	10	6	12	−1	−11; 9	

*Frequent RTI, three or more RTI per year in 3 or 4 years during first 4 years of life. †PD, percentage difference between children with frequent RTI and children without RTI; ‡95% confidence interval (CI) of percentage difference; §*P*-values derived from 3×2 χ^2 comparisons.

FCN3 polymorphisms with respect to susceptibility to RTI. We found that neither *FCN2* nor *FCN3* polymorphisms or their haplotypes were associated with reported frequency of RTI in Dutch Caucasian children during preschool years in the general population.

A previous study by Atkinson *et al.* suggested that Ficolin-2 levels might be lower in children with recurrent RTI compared with control children attending the hospital for reasons unconnected with infections or respiratory disease [16]. The association appeared to be most outspoken

Table 4. Estimated percentage of children with different haplotypes of Ficolin-2 (*FCN2*) based on five functionally relevant single nucleotide polymorphisms (SNPs)* within the groups of questionnaire reported frequency of respiratory tract infections (RTI) in 1–4 years.

		No RTI ($n = 130/260$)‡		Moderate frequency of RTI ($n = 715/1430$)		Frequent RTI§ ($n = 55/110$)			
Haplotype	Ficolin-2 serum level prediction†	*n*	%	*n*	%	*n*	%	PD¶	95% CI††
AGGTG	High	59	22·7	370	25·9	27	24·5	−1·8	−11; 8
AAACG	High	51	19·6	306	21·4	23	20·9	−1·3	−10; 8
GGACG	Medium	98	37·7	473	33·1	44	40·0	−2·3	−13; 9
AGACG	Medium	8	3·1	59	4·1	1	0·9	2·2	−1; 5
GGATG	Medium	8	3·1	21	1·5	3	2·7	0·4	−3; 4
GGACT	Low	30	11·5	177	12·4	12	10·9	0·6	−6; 8
Total‡‡		254	97·7	1406	98·3	110	100		

*Functionally relevant SNPs, −986A > G, −602G > A, −4A > G, Thr236Met and Ala258Ser; †Ficolin-2 serum concentration estimated on the basis of the paper by Munthe-Fog [2]; high ~ > 6 μg/ml, medium ~ 3–6 μg/ml, low ~ < 3 μg/ml. ‡Number of patients/number of haplotypes; §Frequent RTI, three or more RTI per year in 3 or 4 years during first 4 years of life; ¶PD, percentage difference between children with frequent RTI and children without RTI; ††95% CI, 95% confidence interval (CI) of percentage difference; ‡‡total, number of haplotypes, only haplotypes with estimated frequency > 1% are shown.

Table 5. Estimated percentage of children with different haplotypes of Ficolin-3 (*FCN3*) based on two haplotype tagging single nucleotide polymorphisms (SNPs) within the groups of questionnaire reported frequency of respiratory tract infections (RTI) in 1–4 years.

	No RTI (n = 130/260)*		Moderate frequency of RTI (n = 715/1430)		Frequent RTI† (n = 55/110)			
Haplotype	n	%	n	%	n	%	PD‡	95% CI§
TC	126	48·5	686	48·0	45	40·9	7·6	−3; 19
TA	80	30·8	414	29·0	35	31·8	−1·0	−11; 9
AC	50	19·2	300	21·0	29	26·4	−7·2	−17; 2
AA	4	1·5	30	2·1	1	0·9	0·6	−2; 3
Total	260	100	1430	100	110	100		

*Number of patients/number of haplotypes; †frequent RTI, three or more RTI per year in 3 or 4 years during first 4 years of life; ‡PD, percentage difference between children with frequent RTI and children with no RTI; §95% confidence interval (CI) of percentage difference.

in a relatively small group of 90 children with allergic signs or symptoms (mainly asthma or allergic rhinitis and a few with elevated immunoglobulin E not further specified). Based on the results from Atkinson *et al.*, we performed a subgroup analysis in the atopic children from our cohort. In these analyses *FCN2* and *FCN3* genotypes and haplotypes were also not associated with susceptibility to RTI. This contrasts with the findings by Atkinson *et al.*, but reinforces our results.

More recently, Chapman *et al.* showed that functionally relevant polymorphisms in the *FCN2* gene are not associated with invasive pneumococcal disease [23]. The results of this study are in line with ours, as *S. pneumoniae* is one of the most important bacterial pathogens in infection of the respiratory tract. The role of ficolins in innate immune defence against viral infections is still unknown, and the relevance of polymorphisms of *FCN2* or *FCN3* in susceptibility to other diseases has not yet been studied.

To our knowledge, we are the first to analyse SNPs in *FCN3* and find no association with RTI in childhood. These findings need replication in other cohorts, but our *FCN3* results point in the same direction as our observations on *FCN2*. Recently, we showed in the same birth cohort that *MBL2* polymorphisms, known to result in low serum levels of MBL, do not seem to pose an increased risk of RTI during the first 4 years of life as based on parental questionnaires [24].

The major strengths of our study are its large sample size, prospective follow-up, population-based character and homogeneous background of the study population. We studied all children next to studying only the extreme groups; both analyses showed similar results. Moreover, *FCN2* and *FCN3* genotyping was performed according to the highest standards and haplotypes were created based on functionally relevant or haplotype-tagging polymorphisms.

To appreciate our results, some potential limitations should also be considered. First, the PIAMA study was designed originally to study atopy and asthma. Exact measures of numbers of RTI episodes, as was conducted for the MBL study by Koch *et al.* [15], were not recorded. Instead, we grouped the children according to the frequency of RTI, as reported in annual questionnaires. Moreover, we addressed RTI as a whole. The spectrum of infections in our study was wide: from otitis to pneumonia and bronchitis. The effect of *FCN* mutations may differ when observing different clinical manifestations or aetiological agents. Strictly speaking, in our study RTI means 'respiratory symptoms' which the parents considered to be more serious than a common cold. This may lead to misclassification of reported frequency of RTI, but the misclassification can be supposed to be non-differential (independent of *FCN* genotype status). To study the validity of our definitions, we compared the questionnaire data on reported frequency of RTI with data on prescription of antibiotics and adenoidectomy and tonsillectomy. Antibiotic prescription was associated highly ($P < 0{\cdot}01$) with frequent RTI as defined by the questionnaires, and a similar association was seen with adenoidectomy and tonsillectomy ($P < 0{\cdot}01$). Furthermore, similar to the frequency of RTI, no association of *FCN* genotypes status with ear, nose and throat surgery or antibiotic prescription rates was observed in our study.

Secondly, we did not determine serum levels of Ficolin-2 and Ficolin-3 but used instead polymorphisms considered to be associated with serum levels [2]. This approach is used commonly in association studies, especially in studies with relatively large study populations [23–25]. Thirdly, selection bias may have occurred if the association between *FCN2* and *FCN3* polymorphisms and frequent RTI was different for the included children and those who were excluded from the analyses because of missing information. Among the children who were not included, there were higher percentages of children from non-allergic mothers (Table 1). This difference is caused by the original design of the PIAMA study, not by selective dropout [19]. Nevertheless, we separated the children by allergy and confirmed the lack of association between *FCN2* and *FCN3* polymorphisms and frequent RTI in both groups (results not shown). Moreover, selection bias is unlikely, as the genotype distribution of the ficolin genes we found is similar to other studies involving Dutch Caucasians and conforms to the Hardy–Weinberg equilibrium.

Finally, we performed a *post hoc* power analysis to strengthen our negative findings. In this calculation we assumed an allele frequency of 0·208 for the high-risk allele (−602G > A) and a prevalence of 6·1% for frequent RTI

based on our own data. The Genetic Power Calculator showed that the number of cases ($n = 55$) is sufficient to detect genotype relative risks of, respectively, 2 and 2·5 for heterozygotes and homozygotes for the high-risk allele (power > 80% to reject the null hypothesis with an alpha of 0·050) [22]. Genotypic risks of this size were found for MBL by Koch *et al.* [15]. Moreover, we consider relative risks of this size to be clinically relevant. Therefore, our study population seems to be sufficient to elucidate clinically relevant effects of ficolin polymorphisms on susceptibility to RTI.

To our knowledge, we have explored for the first time the impact of *FCN2* and *FCN3* polymorphisms on susceptibility to RTI in a large birth cohort of Caucasian children. Knowledge on the genetic control of serum levels and functionality of the ficolins is still emerging, and we expect more disease association studies to appear in the future [10]. We do not exclude that *FCN2* or *FCN3* polymorphisms may contribute, in specific circumstances of impaired immunity, to the complex genetic control of protective immunity to infection. Nevertheless, our results suggest that in healthy children susceptibility to RTI is not influenced by polymorphisms in the ficolin genes.

In conclusion, this population-based birth cohort study shows that Caucasian children with *FCN2* polymorphisms, presumed to result in low serum levels of Ficolin-2, do not seem to have an increased risk of RTI during the first 4 years of life. Moreover, *FCN3* polymorphisms were also not associated with risk of RTI during these years. The results suggest that Ficolin-2 and Ficolin-3 may not be critical for host defence against pathogens causing RTI during preschool years in Caucasian children at a population level.

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