

Properdin in childhood and its association with wheezing and atopy

Staley KG, Kuehni CE, Strippoli MPF, McNally T, Silverman M, Stover C. Properdin in childhood and its association with wheezing and atopy.

Pediatr Allergy Immunol 2010.

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Properdin, a serum glycoprotein, is an important component of innate immunity, the only known positive regulator of complement, acting as an initiation point for alternative pathway activation. As an X-linked protein, we hypothesized that properdin may play a modulatory role in the pathogenesis of viral wheeze in children, which tends to be more common and more severe in boys. We aimed to determine properdin levels in a community-based paediatric sample, and to assess whether levels of properdin were associated with childhood wheeze phenotypes and atopy. We studied 137 school-children aged 8–12 yrs, a nested sample from a cohort study. Properdin was measured by a commercial enzyme-linked immunoabsorbant assay. We assessed wheeze by questionnaire, validated it by a nurse-led interview and performed skin prick tests and a methacholine challenge in all children. Forty children (29%) reported current wheeze. Serum properdin levels ranged between 18 and 40 µg/ml. Properdin was not associated with age, gender, atopy, bronchial responsiveness, current wheeze (neither the viral wheeze nor multiple-trigger wheeze phenotype) or severity of wheeze, but was slightly lower in south Asian (median 21.8 µg/ml) compared with white children (23.3 µg/ml; $p = 0.006$). Our data make it unlikely that properdin deficiency is common in healthy children or that levels of properdin are a major risk factor for wheeze or atopy.

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Key words: properdin; children; wheeze; asthma; phenotype; complement; X-chromosome

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Accepted 13 November 2009

Properdin, a serum glycoprotein, is the only known positive regulator of complement activation. It causes an amplification of the complement cascade by stabilizing the C3 and C5 convertases of the alternative pathway (C3bBb and C3bBbC3b) and has been shown to bind directly to a variety of non-self antigens, thus also acting as an 'initiation point' for complement activation (1). Properdin deficiency in humans is associated with severe infections and variant meningococcal disease (W135, X, Y and Z serogroups) (2–4). There are few published data on properdin levels in normal unselected children, although it is speculated, that deficiency may be relatively common in Caucasians (5).

There is increasing interest in the role of complement in respiratory disease, especially

childhood infection and asthma (6–11). The basis for this interest is the potential modulatory role of complement in asthma, whether by a specific complement protein such as mannan-binding lectin (MBL) (6, 8–10), or by generic factors common to all three arms of complement activation, such as the anaphylatoxins C3a and C5a (7, 12). In contrast to MBL, properdin levels and their potential relationship to asthma and atopy have received little interest in recent years (13). This is despite the fact that components of the alternative pathway of complement activation have been found in respiratory secretions in humans (14); and that properdin has been shown to be produced by mast cells in mice models (15) and as mRNA in a human mast cell line (A Dupont, unpublished thesis). We believe,

therefore, there is a need to assess the role of properdin in atopy and wheeze, especially in light of recent evidence to suggest that the MBL-mediated-route of complement activation depends on the 'properdin-driven' alternative pathway amplification loop (16, 17).

We have already published data from the same study population, which showed an association between levels of MBL and severity of asthma in children (10). Based on these findings, and considering that properdin is located on the X-chromosome and viral wheeze tends to be more common and more severe in boys (18), we hypothesized that properdin deficiency might be involved in the pathogenesis or modulation of wheezing illness in young children, particularly viral wheeze. In view of a potentially wider role for complement in allergy and asthma, we also assessed whether levels of properdin were associated with atopy. Specifically, the aims of this study were to describe properdin values in unselected healthy children, and to assess whether these are associated with atopy and with childhood wheeze, considering both the viral wheeze and the multiple-trigger wheeze phenotype.

Methods

Study design

From an ongoing population-based cohort study of 3300 children born between 1993 and 1996 in Leicestershire, UK (19, 20), all children aged ≥ 9 yrs whose parents had answered two or more previous questionnaires ($n = 2021$) were invited for a lab visit in 2004–2006. The cohort, which includes a large proportion of children of south Asian ethnic origin, had been recruited in 1998 and parents received postal questionnaires in 1998, 2001 and 2003. Properdin levels were assessed during an 8-month period between July 2005 and February 2006. The study was approved by the Leicestershire Research Ethics Committee.

Questionnaires

During their lab visits, parents completed a standard respiratory questionnaire (21). Current wheeze was assessed with a validated question from the International Study of Asthma and Allergies in Childhood (21–23). We grouped children into those with viral wheeze if attacks were triggered exclusively by viral infections (wheeze only during or shortly after a cold) and those with multiple-trigger wheeze (asthma) if

they also wheezed with other triggers (wheeze occurring in the absence of colds, whether or not they also reported attacks with colds). In addition, we grouped wheezers by severity based on the treatment steps recommended by the British Thoracic Society as untreated, using only bronchodilators and using inhaled corticosteroids (10, 24). Reported current wheeze, and its triggers (defining the phenotype) were validated in a nurse- or doctor-led interview. The questionnaire included also detailed questions on frequency, severity and triggers of wheeze, inhaler treatment, and frequency and duration of colds and ear infections during the previous 12 months. Self-reported ethnic origin (white or south Asian) and socio-economic status (parental education, crowding and Townsend score) were also assessed (25).

Measurements

Serum properdin concentrations were measured using a commercially available enzyme-linked assay (Antibody Shop, Gentofte, Denmark). The manufacturer's protocol was followed, except we found that our samples required further dilution (1/1000) than recommended. The inter- and intra-assay coefficients of variation were 9.0% ($n = 4$) and 3.8% ($n = 4$) respectively. This enzyme-linked-immunosorbent serologic assay (ELISA) cannot identify a type III properdin deficient subject, as it does not distinguish between a dysfunctional and functional properdin protein. However, type III properdin deficiency is very rare, and has only been reported in one family to date (other properdin deficiencies are: type I properdin deficiency, categorized by absence of protein, and type II, categorized by only $\sim 10\%$ of normal circulating levels). MBL level was measured using a mannan-coated commercial ELISA kit with an anti-MBL antibody, recognizing only functional oligomers (HK323; Hycult Biotechnology, Uden, The Netherlands) (10).

Skin prick tests were performed with four common antigens: dog, cat, grass pollen and house dust mite (*Dermatophagoides pteronyssinus*) (ALK Abello, Hungerford, UK). A positive result was classed as a weal > 3 mm mean in diameter. Bronchial responsiveness (BR) to methacholine was measured by a tidal breathing technique as previously described (Cockcroft protocol). On the basis of the provocative concentrations of methacholine causing a 20% fall in forced expiratory volume in 1 s (FEV_1) (PC_{20}), subjects were classified as having bronchial hyper-responsiveness if PC_{20} was < 8 g/l.

Pre- and post-bronchodilator spirometry (Vitalograph Pneumotrac Vitalograph Ltd, Maids Moreton, Buckingham, UK), was also performed using standard techniques.

Analysis

Analyses were performed with STATA, version 10.0 (Stata Corporation, Austin, TX, USA). Because of skewness (Fig. 1), we reported properdin levels as medians and interquartile ranges (IQR) and used nonparametric tests (Mann–Whitney and Kruskal–Wallis) to compare groups. Correlations between properdin levels and lung function measurements and MBL levels were evaluated using Spearman's rank correlation coefficient. For analysis by multivariable linear regression, data were log-transformed to correct for skewness and results presented as estimated effects of predictors in term of deviation from mean properdin levels in children with baseline characteristics.

Results

During the period of data collection 350 children attended the laboratory. Of these 137 (39%) consented to venepuncture (109 white children and 28 south Asians). Forty of them (29%) reported current wheeze, 13 (9%) viral wheeze and 27 (20%) multiple-trigger wheeze. Properdin levels ranged from 18.5 to 39.6 µg/ml with a positively skewed distribution (Fig. 1). The median (IQR) was 23.2 µg/ml (18.8–32.4). None of the children was deficient in properdin (type I deficiency characterized as <0.1 µg/ml and type II deficiency ~2 µg/ml).

Properdin levels were not associated with gender, age, atopy, BR, wheeze phenotype or severity of wheeze either in unadjusted analysis (Table 1) or in a multivariable regression model (Table 2). Median (IQR) properdin was

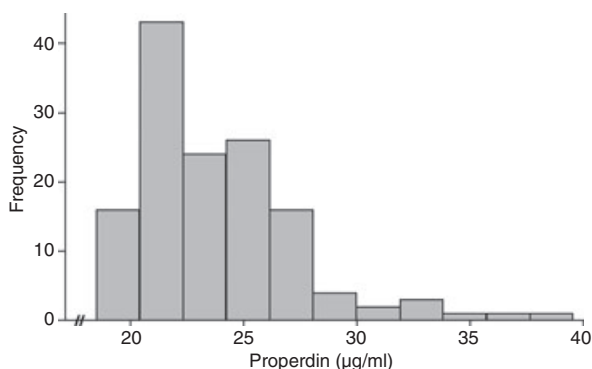


Fig. 1. Distribution of serum properdin levels in the study sample (n = 137).

Table 1. Association between serum properdin levels and biological characteristics of the subjects (unadjusted, n = 137)

	n	Properdin level		p-Value
		Median (µg/ml)	IQR (µg/ml)	
Ethnicity				
White	109	23.3	21.8–26.1	0.006
South Asian	28	21.8	20.7–23.4	
Gender				
Girls	65	22.5	21.1–24.8	0.074
Boys	72	23.4	21.8–26.1	
Age (yrs)				
8–9	27	23.3	21.8–25.4	0.754
9–10	50	23.3	21.7–26.2	
>10	60	22.8	21.4–25.0	
Atopy*				
Yes	90	23.3	21.7–26.2	0.234
No	47	22.4	21.3–24.8	
Type of wheeze†				
No wheeze	97	22.9	21.3–25.4	0.206
Viral wheeze	13	22.4	21.9–23.4	
Multiple-trigger wheeze	27	24.8	22.1–26.7	
Severity of wheeze‡				
No wheeze	97	22.9	21.3–25.4	0.299
Very mild wheeze (untreated)	12	22.4	21.9–25.1	
Mild wheeze (only bronchodilators)	12	22.9	21.4–24.8	
Moderate wheeze (ICS)	16	25.0	22.4–27.1	
BR§				
Normal/borderline	87	22.7	21.4–25.9	0.494
Hyper-responsiveness	34	23.3	21.9–26.1	

*Defined as a positive skin prick test to at least one allergen.

†During the past 12 months.

‡Severity measured by the step-wise use of inhaler treatment recommended by the British Thoracic Society (24).

§Bronchial responsiveness (BR): normal/borderline BR = PC₂₀ ≥ 8 g/l; hyper-responsiveness BR = PC₂₀ < 8 g/l.

IQR, interquartile range; ICS, inhaled corticosteroids; BR, bronchial responsiveness; PC₂₀, see text for definition.

Table 2. Association between serum properdin levels and biological characteristics of the subjects (multivariable linear regression, n = 137)

	Change in properdin level (µg/ml)	p-Value
Constant*	23.6	
Ethnicity – South Asian vs. Whites	–1.47	0.042
Gender – male vs. female	+0.34	0.549
Wheeze phenotype		0.233
Viral wheeze vs. no wheeze	–0.05	0.958
Multiple-trigger wheeze vs. no wheeze	+1.22	0.096
Atopy†	–0.41	0.511

*According to the model, children with baseline characteristics (white, female, non atopic and no current wheeze) have a mean of properdin of 23.6 µg/ml.

†Defined as a positive skin prick test to at least one allergen.

23.4 µg/ml (22.0–26.0) in the 40 children reporting current wheeze and 22.9 µg/ml (21.3–25.4) in the 97 non-wheezers (Fig. 2; p = 0.213). South

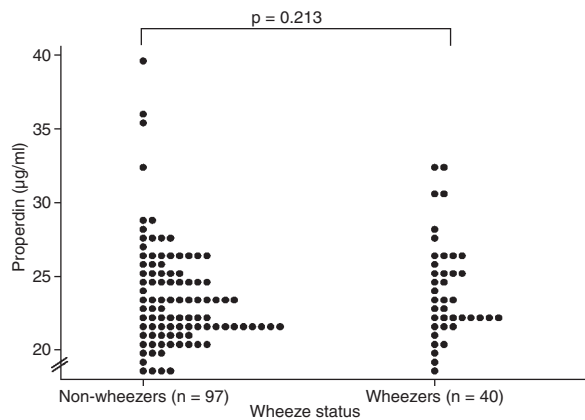


Fig. 2. Serum properdin levels in the study sample, by wheeze status ($n = 137$).

Asian children had slightly lower levels of properdin than white children ($21.8 \mu\text{g/ml}$ vs. $23.3 \mu\text{g/ml}$; $p = 0.006$). This finding was confirmed after adjustment for other factors in the multivariable analysis ($22.1 \mu\text{g/ml}$ vs. $23.6 \mu\text{g/ml}$; $p = 0.042$). Properdin levels were not associated with frequency of wheeze or frequency of upper respiratory infection (data not shown). Using Spearman's rank correlation coefficient, properdin levels were not correlated with MBL levels ($r = 0.12$, $p = 0.157$) or FEV_1 ($r = -0.05$, $p = 0.587$).

Discussion

In this group of healthy unselected school-children, serum properdin levels ranged between 18 and $40 \mu\text{g/ml}$ and were marginally lower in south Asian than in white children. Properdin levels were not associated with atopy or with wheeze phenotype.

This is one of the largest samples of healthy children where properdin levels were reported. We found no case of properdin deficiency (type I and II) which highlights the fact that amongst healthy persons, properdin deficiency is rare. Our outcome, parent-reported wheeze, was confirmed in an interview with a medical doctor or respiratory nurse during the lab visit. Unfortunately, our sample contained relatively few children with viral and multiple-trigger wheeze. A power calculation suggests that with this sample size, we have 80% power to detect (at the 0.05 level) a difference in properdin levels of $2 \mu\text{g/ml}$ between current wheezers and non-wheezers and of $2 \mu\text{g/ml}$ between current viral wheezers and non-wheezers. We think that smaller differences are unlikely to be clinically relevant.

Comparisons with other studies and interpretation of our findings

To our knowledge, no study has reported ethnic differences in properdin levels. Previous studies have reported a maturation effect of properdin, whereby levels in infants were lower than in adults (26–29). This is similar to other complement proteins such as MBL and factor B. In our population, we found no association between properdin levels and age, perhaps because of the relatively narrow age-range (8–12 yrs). As in our study, Prellnar et al. (28) also reported a positively skewed distribution of properdin levels. We could not confirm any association between gender and properdin levels in our population. Although there has been no previously published analysis of an association between properdin levels and viral wheeze, we confirm the findings of a study showing that levels of properdin were not related to atopy or asthma (13), neither were they related to severity of wheeze. This is in contrast to levels of MBL in our population, which have been documented previously as being higher in children with more severe wheeze (10).

In conclusion, in this largest study to describe levels of properdin from a community-based paediatric sample, we found no association with wheeze in general or wheeze phenotypes in particular, but found lower properdin levels in south Asian compared with white children, which might warrant further investigation.

Acknowledgments

The authors thank the parents and children of Leicestershire for participating and Tony Davis, Specialist Community Child Health Services, Leicester City Primary Care Trust for his assistance with the Leicestershire Child Health Database. The authors also thank Dawn Jotham for her work in the clinical laboratory.

Funding

This study was funded by Asthma UK (Grant N°07/048), Swiss National Science Foundation (PROSPER grant 3233-069348, 3200-069349, SNF grant 823B-046481), Swiss Society of Paediatrics (Glaxo-Smithkline Scholarship for Paediatric Pulmonology 2001), Deutsche Forschungsgemeinschaft (STO430-1/2) and MRC (G0400300). Initial data collection was supported by a research grant from Trent NHS Executive, UK (Trent Research Scheme, RBF # 98XX3).

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