Serotype-specific pneumococcal disease may be influenced by mannose-binding lectin deficiency

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ABSTRACT: Previous studies of the association between the mannose-binding lectin pathway deficiencies and invasive pneumococcal disease are inconclusive. Invasiveness of *Streptococcus pneumoniae* is dependent on serotype. We aimed to determine the association between invasive pneumococcal disease and *MBL2* and *MASP2* genetic variants, regarding serotype distribution.

A hospital-based case–control study was conducted in children admitted to hospital in rural Mozambique in June 2002–November 2003. The study included children admitted to hospital with invasive pneumococcal disease, in whom *S. pneumoniae* was isolated from blood and subsequently serotyped. Sequence-based typing analysis of amplicons covering the polymorphic regions of *MASP2* (exon 3) and *MBL2* (promoter and exon 1) was performed.

An overall high frequency of *MBL2* genotypes associated with low serum levels of MBL (43%) was found. Carriers of MBL-deficient genotypes were associated with invasive pneumococcal disease produced by low-invasive serotypes (OR 5.55, 95% CI 1.4–21.9; p=0.01).

Our data suggest that susceptibility to pneumococcal disease among MBL-deficient patients may be influenced by serotype invasiveness. Type-specific capsular serotype of *S. pneumoniae* would need to be taken into account in further genetic association studies of invasive pneumococcal disease.

KEYWORDS: Africa, children, invasive pneumococcal disease, mannose-binding lectin, serotype, *Streptococcus pneumoniae*

Invasive pneumococcal disease (IPD) is a leading cause of morbidity and mortality in children worldwide and is responsible for as many as a million deaths each year, mostly in developing countries [1]. Known risk factors for developing IPD are young age, malnutrition, overcrowding and HIV-1 infection, all of which are highly prevalent in sub-Saharan African countries [2]. Furthermore, the ability of pneumococcus to cause invasive disease is highly dependent on capsular serotype [3], and the prevalence of serotypes among IPD cases varies widely according to geographical area and age [4]. Genetic risk factors for IPD focussing on congenital deficiency of mannose-binding lectin (MBL) have been reported in populations other than sub-Saharan Africa with no clear conclusions [5–7].

MBL is one of the recognition modules of the lectin pathway of complement activation. MBL recognises specific carbohydrate moieties found in a wide range of microorganisms [8]. MBL can then promote either direct opsonisation and phagocytosis or complement-mediated lysis through activation of MBL-associated serine proteases (MASPs) [9, 10]. Three nonsynonymous single nucleotide polymorphisms (SNP) located in exon 1 have been described: Arg52→Cys (D variant (rs50307370), Gly54→Asp (B variant (rs1800450)) and Gly57→Glu (C variant (rs1800451)). The wild type variant is considered the A variant and other structural variants will be referred to as 0 in the text. All of them impair MBL oligomerisation, which is essential for association with MASPs and to reach complete functional activity [11]. Furthermore, SNP of the promoter region at positions -550 (G→C, H/L (rs11003125)), -221 (G→C, X/Y (rs7096206)) and the untranslated region +4 (P/Q (rs7095891)) affect *MBL2* transcription, with a dominant downregulatory effect of the

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X allele [12]. The SNP at the promoter and exon 1 are in strong linkage disequilibrium and give rise to a limited number of prevalent haplotypes: LYPA, LYPB, LYQA, LYQC, HYPA, HYPD and LXPA, which can predict MBL serum levels [12]. Distribution of MBL haplotypes differs throughout the world, depending on ethnic groups, and LYQC has been found in high frequencies only among sub-Saharan African populations [12]. MBL deficiency has been associated with a wide range of infectious and noninfectious diseases, specifically in children [13].

MASP-2 is the most relevant serine protease associated with MBL [14]. A frequent nonsynonymous amino acid replacement involving codon 105 (Asp→Gly (rs56392418)) of the CUB-1 domain has been found among Caucasians and has been linked to severe immunodeficiency [15]. We performed a case–control study to investigate the association between *MBL2* and *MASP2* variants, and IPD in children, taking into account serotype distribution. The study was carried out in children <5 yrs of age from a rural area in Southern Mozambique with high incidence of IPD (416 out of 100,000 per child-yr at risk) [16].

MATERIALS AND METHODS

Population and study location

The study was conducted by the Centro de Investigação em Saúde da Manhiça (CISM; Maputo, Mozambique) at Manhiça District Hospital (MDH), which has 110 beds including 26 paediatric beds; the hospital is the referral health facility for Manhiça District, a rural area of Maputo province in Southern Mozambique. Manhiça District had an estimated population of 130,000 inhabitants during the study period, with homogeneous ethnicity (Xironga and Xichangana). Details of the study location have been described elsewhere [17]. Briefly, the climate of the area is subtropical with two distinct seasons: a warm, rainy season between November and April, and a cool, dry season during the rest of the year. Malaria is endemic throughout the year, peaking between December and March. The prevalence of HIV-1 infection among pregnant females during the study period was 19% (unpublished data).

Clinical monitoring and sample collection

Since January 1997, the MDH and the CISM have jointly performed round-the-clock monitoring of all paediatric visits to the outpatient dept and all admissions to the wards [16]. On admission, a trained medical officer complete a detailed clinical questionnaire. A finger-prick blood sample for determination of malaria parasites in thin and thick blood smears were obtained from children with fever (axillary temperature $\geqslant 37.5°C$), and packed-cell volume (PCV) was also measured. As part of routine clinical practice, blood cultures were performed on admission in all children <2 yrs of age and in older children with an axillary temperature >39°C.

The present study was approved by the Mozambican Ethics Review Committee (Ministry of Health, Maputo, Mozambique) and the Ethics Committee of the Hospital Clínic (Barcelona, Spain). Written informed consent was obtained from the parents or tutors of eligible children.

Variable definitions

A child with IPD was defined as a patient admitted to the MDH, in whom *Streptococcus pneumoniae* was isolated from blood.

Increased respiratory rate (IRR) was defined according to age group as a respiratory rate: >60 breaths·min^{-1} for children under 2 months; >50 breaths·min^{-1} for children aged 2–12 months; and >40 breaths·min^{-1} for children aged 1–5 yrs [18]. Following this definition, clinical pneumonia was diagnosed when children were admitted to hospital with cough or breathing difficulty, and IRR or chest indrawing [18]. Anaemia was classified according to levels of PCV as mild (PCV 25–33%), moderate (PCV 15–24%) or severe (PCV <15%). Children were classified as severely malnourished if their weight z-score was $\leqslant$ -3 SD, and moderately malnourished with a weight z-score of -2– -3 SD [19]. *Plasmodium falciparum* infection was defined when one or more asexual parasites were seen in blood after observation of >200 leukocytes. Clinical malaria was defined, according to age, as fever plus any *P. falciparum* parasitaemia (in infants) or >2,500 parasites·mm^{-3} (in other children). The rainy season was considered to be between November and April and the dry season was considered to be the rest of the calendar year. Mortality was defined as the number of deaths from a specific condition occurring in hospital divided by the number of children with known outcome (deaths plus discharges). Children with unknown final outcome, such as those transferred or absconding from hospital were not included in mortality measurements.

Genotypes coding for MBL high (A/A, XA/A), intermediate (A/0, XA/XA), and low (0/0, XA/0) serum levels were defined according to previous reports [12, 20–22]. Individuals with these genotypes will be referred to as MBL-sufficient or MBL-deficient (moderate and severe, respectively).

Highly invasive serotypes of *S. pneumoniae* were considered serotypes with an estimated attack rate of >20, based on a previous report that included serotypes 1, 4, 5, 9V, 12 F and 14 [23].

Study design

We conducted a case–control study in children aged <5 yrs admitted to the MDH between June 2002 and November 2003. Controls were randomly selected, at a ratio of one study case for every two controls, from children admitted to hospital during the same period with no clinical signs or symptoms of pneumonia and with a negative bacterial blood culture on admission. Criteria for matching were as follows: 1) age (frequencies ± 6 months in children >1 yr of age and ± 2 months in children <1 yr of age) and 2) season on admission (dry season or rainy season). Cases were stratified according to serotype invasiveness of *S. pneumoniae* (high-invasive *versus* low-invasive).

Laboratory methods

MBL2 and MASP2 genotyping

DNA was extracted and purified from biological samples using a commercial kit (Qiagen DNA blood kit, Qiagen, Valencia, CA, USA). A 969-bp fragment encompassing the promoter to the end of exon 1 of the *MBL2* gene was obtained by PCR amplification using the sense 5′-GGGGAATTCCTG-CCAGAAAGT-3′ and antisense 5′-CATATCCCCAGGCA-GTTTCCTC-3′ primers and the Expand^{TM} 20kbPLUS PCR System (Roche Diagnostics GmbH, Mannheim, Germany). A 354 bp fragment from exon 3 of the *MASP2* gene was PCR-amplified using the sense 5′-GCGAGTACGACTTCGTCA-AGG-3′ and antisense 5′-CTCGGCTGCATAGAAGGCCTC-3′

TABLE 1	General prevalence of different variables comparing study cases and controls				
Variable	Total	Controls	Cases	OR# (95% CI)	p-value
Age months					
0–12	39 (21.1)	26 (21.5)	13 (20.3)	1.05 (0.7–1.7)	0.8
13–36	98 (53.0)	64 (52.9)	34 (53.1)		
37–60	48 (25.9)	31 (25.6)	17 (26.6)		
Season					
Dry	124 (67.0)	81 (66.9)	43 (67.2)	0.97 (0.5–1.9)	0.9
Rainy	61 (33.0)	40 (33.1)	21 (32.8)		
Sex					
Male	94 (50.8)	62 (51.2)	32 (50.0)	1.01 (0.6–1.9)	0.9
Female	91 (49.2)	59 (48.8)	32 (50.0)		
Parasitaemia density¶					
Negative	62 (34.8)	20 (16.5)	42 (73.7)	0.09 (0.0–0.2)	<0.001
1–99999	90 (50.6)	75 (62.0)	15 (26.3)		
100000–199999	15 (8.4)	15 (12.4)	0 (0.0)		
>200000	11 (6.2)	11 (9.1)	0 (0.0)		
Malaria					
Yes	93 (51.7)	84 (69.4)	8 (14.0)	0.07 (0.0–0.2)	<0.001
No	86 (48.3)	37 (30.6)	49 (86.0)		
Anaemia					
Normal PCV	41 (22.7)	28 (23.1)	13 (21.7)	0.86 (0.7–1.1)	0.3
Mild	69 (38.1)	37 (30.6)	32 (53.3)		
Moderate	68 (37.5)	53 (43.8)	15 (25.0)		
Severe	3 (1.7)	3 (2.5)	0 (0.0)		
Nutrition status					
Normal	118 (67.4)	86 (73.5)	32 (55.2)	1.76 (1.1–2.7)	0.01
Moderate malnutrition	32 (18.3)	19 (16.2)	13 (22.4)		
Severe malnutrition	25 (14.3)	12 (10.3)	13 (22.4)		
Mortality	8 (4.8)	2 (1.8)	6 (11.3)	7.17 (1.4–36.9)	0.02
***MBL2* genotype**					
MBL-sufficient	91 (56.5)	60 (56.6)	31 (56.4)	0.82 (0.5–1.3)	0.4
MBL-moderate deficiency	51 (31.7)	30 (28.3)	21 (38.2)		
MBL-severe deficiency	19 (11.8)	16 (15.1)	3 (5.4)		
***MASP2* variants**					
MASP2 wild type	106 (61.3)	73 (64.6)	33 (55.0)	1.49 (0.8–2.8)	0.2
Any *MASP2* variant	67 (38.7)	40 (35.4)	27 (45.0)		

Data are presented as n (%), unless otherwise stated. PCV: packed-cell volume; MBL: mannose-binding lectin; MASP: MBL-associated serine protease. #: pooled OR for one category of increased adjusted by design variables (season and age) when appropriate; ¶: estimated number of *Plasmodium falciparum* asexual parasites per mm³.

oligonucleotides and the Expand™ High Fidelity PCR System (Roche Diagnostics GmbH).

The cycling conditions used for amplification of both the *MBL2* and *MASP2* genes were 94°C for 8 min, 35 cycles of 94°C for 45 s, 58°C for 30 s, 72°C for 90 s and 72°C for 10 min. 5 µL of the resulting PCR reaction was treated with ExoSAP-IT (USB Corporation, Cleveland, OH, USA) and then subjected to direct sequencing using the sense and antisense gene-specific primers described above and the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were run on an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Warrington, UK).

S. pneumoniae *isolation and serotyping*
Isolation of *S. pneumoniae* and serotyping (Quellung reaction) were performed using previously described standard procedures [16, 24].

Statistical methods and computational analysis
Descriptive results are expressed in proportions or mean±SD, as required. Chi-squared statistics were used to compare 2×2 table associations or a trend in larger tables, and the Fisher exact test was used when numbers were low (<5 in one category). An unpaired t-test was used when comparing two continuous variables. We performed logistic regression for the multivariate analysis and polytomous logistic regression to analyse different outcome groups (cases with highly invasive serotypes and cases with low-invasive serotypes), and to calculate OR and 95% CI. The final logistic regression model included the design variables (age and season) and variables with significant associations after the raw analysis. Allele and genotype frequencies for each variant site and Hardy–Weinberg equilibria were estimated. Statistically significant values were considered when p⩽0.05. Multiple sequence alignments were performed with ClustalW software

TABLE 2	Serotype distribution stratified by *MBL2* genotype			
Serotype	**MBL-deficient**	**MBL-sufficient**	**Attack rate (95% CI)[#]**	**Invasiveness OR (95% CI)[¶]**
Highly-invasive				
1	4	9	∞ (2–∞)	9.6 (1.1–86.5)
4	1	0	75(19–660)	12.1(1.1–104)
5	1	3	∞ (9–∞)	ND
9V	0	3	26 (14–64)	1.5 (0.6–4.0)
12F	2	0	38 (8–347)	0
14	1	5	53 (36–81)	8.8 (5.1–15.4)
Subtotal	9 (43)	20 (77)		
Low-invasive				
6A	4	1	8 (5–14)	0.7 (0.3–1.7)
6B	0	1	5 (4–7)	0.6 (0.3–1.0)
10F	0	1	ND	ND
15A	1	0	0 (0–33)	0
19F	2	0	6 (4–9)	0.6 (0.3–1.1)
22A	0	1	ND	ND
23A	1	0	0 (0–3)	0
23F	3	0	8 (5–13)	0.4 (0.2–0.8)
24F	1	1	ND	0
29	0	1	ND	0
Subtotal	12 (57)	6 (23)		
Total	21	26		

Data are presented as n or n (%), unless otherwise stated. MBL: mannose-binding lectin. [#]: data from SLEEMAN *et al.* [23]. Attack rate is defined as the number of serotype-specific IPD cases by 100,000 nasopharyngeal acquisitions. [¶]: data from BRUEGGEMANN *et al.* [3]. Invasiveness OR is defined as the relative prevalence of specific serotypes among IPD isolates and nasopharyngeal isolates.

(European Bioinformatics Institute, Cambridge, UK). Data were analysed using the STATA 10.0 software package (Stata Corp., College Station, TX, USA).

RESULTS
Descriptive results
185 children (64 cases and 121 controls) were eligible for the study. A total of 161 samples were amplified for *MBL2* and 173 samples were amplified for *MASP2* (recovery rate of 87% for *MBL2* and 94% for *MASP2*; 24 and 12 samples were not amplified, respectively, due to the poor quality of the DNA). A total of 55 cases (86%) and 106 controls (88%) had both *MBL2* and *MASP2* genotyping results. The mean±SD age was 26.4±15.8 months, and about half of the patients were male (51%). Malnutrition and mortality were more frequent among study cases (45 *versus* 27%, p=0.01, and 11 *versus* 2%, p=0.02, respectively). Malaria was predominantly diagnosed among controls (69%) and, hence, parasitaemia was more prevalent among them. Table 1 provides a full description of prevalence of variables among cases and controls.

There were no differences between children with and without genotyping data available for all the study variables described in table 1. Overall, 70 of the 161 children (43%) had a genotype responsible for low MBL deficiency, and 19 out of 161 (12%) had a genotype associated with severe deficiency (10 homozygous for haplotypes involving structural mutations and nine heterozygous for the LXPA haplotype combined with the LYQC haplotype). The Hardy–Weinberg equilibrium was conserved for alleles for the whole sample A/C (p=0.48), H/L (p=0.26), P/Q (p=0.82), and Y/X (p=0.13), as well as the control group (p=0.33, p=0.08, p=0.93 and p=0.28, respectively). 67 children (38.7%) carried a nonsynonymous *MASP2* variant. Full description and prevalence of *MASP2* polymorphisms and *MBL2* genotypes found is reported elsewhere [25].

IPD and MBL pathway variants
Among study children, IPD was neither associated with MBL deficiency (OR 1.44, 95% CI 0.6–3.6; p=0.4) nor *MASP2* variants (OR 1.59, 95% CI 0.6–3.9; p=0.3), even when examining different exposure groups (severely MBL-deficient *versus* normal (p=0.4) or moderately MBL-deficient *versus* normal (p=0.2)) or analysing different *MASP2* variants separately.

IPD and MBL deficiency according to serotype distribution
Analysis according to serotype distribution included 47 out of 64 (73%) cases with available data on serotyping and *MBL2* genotyping. Serotype distribution in terms of MBL deficiency is shown in table 2. Overall, 21 cases were MBL-deficient (18 moderately deficient and three severely deficient) and 29 were infected by a highly invasive serotype. The final logistic regression model showed that MBL deficiency was more prevalent among individuals with IPD caused by low-invasive pneumococcal serotypes than control individuals (OR 3.93, 95% CI 1.1–14.5; p=0.04).

No differences in the prevalence of MBL-deficiency were detected between cases of IPD caused by highly invasive serotypes and controls (OR 0.82, 95% CI 0.3–2.2; p=0.7).

When the dataset was stratified according to MBL deficiency (moderate and severe), results remained significant for individuals with moderate MBL deficiency infected by low-invasive serotypes after univariate and multivariate analysis (OR 3.56, 95% CI 1.1–.11.2, p=0.03, and OR 5.55, 95% CI 1.4–21.9, p=0.01, respectively). Only two cases infected by low-invasive serotypes had severe deficiencies. Furthermore, all infants (children <1 yr of age) infected by low-invasive serotypes (n=8) carried an MBL-deficient genotype (8 out of 8 *versus* 4 out of 10; p=0.001). Tables 3 and 4 shows the univariate and multivariate analysis of data stratified according to highly invasive serotypes and low-invasive serotypes, respectively. To overcome any confounding effect produced by the intermediate serotypes (serotypes with attack ratio >20 but large CI including the cut-off of 20 (4, 9V and 12F; n=6), we carried out an additional analysis excluding or classifying them as low-invasive strains. We did not find any significant change in this additional analysis (p=0.03 for both).

When comparing cases with highly invasive serotypes *versus* low-invasive serotypes, stratified by MBL genotype (sufficient, moderately deficient and severely deficient), we found an almost significant p-value for trend (p=0.054), suggesting increased susceptibility depending on the degree of MBL-deficiency (fig. 1). No association was found when analysing *MASP2* variants *versus MASP2* wild type with IPD and serotype invasiveness (tables 3 and 4).

DISCUSSION

The results of our analysis raise the hypothesis that serotype-specific IPD in children may be influenced by MBL deficiency. Children with MBL deficiency may be more prone to infection by a low-invasive serotype compared with MBL-sufficient children, but not by highly invasive serotypes. The plausibility of this hypothesis is supported by the observation that opsonin activity has been shown to influence intracellular trafficking and killing of *S. pneumoniae* by human alveolar macrophages [26].

TABLE 3	Raw and adjusted analysis of invasive pneumococcal disease infected by highly invasive serotypes (1, 5, 4, 9V, 12F and 14)				
Variable	Cases	Univariate[#] OR (95% CI)	p-value	Adjusted OR[¶] (95% CI)	p-value
Anaemia					
Normal PCV	6 (21.7)	1			
Mild	17 (58.6)	2.13 (0.7–6.2)	0.2		
Moderate	6 (21.7)	0.51 (0.2–1.8)	0.3		
Severe	0 (0)				
Nutrition status					
Normal	16 (59.3)	1			
Moderate malnutrition	7 (25.9)	1.95 (0.7–5.4)	0.2		
Severe malnutrition	4 (14.8)	1.83 (0.6–6.6)	0.4		
Parasitaemia[+,§]					
Negative	21 (75.0)	1			
1–99999	7 (25.0)	0.08 (0.0–0.2)	<0.001		
⩾100000	0 (0)				
Malaria					
No	24 (85.7)	1			
Yes	4 (14.3)	0.01 (0.0–0.3)	<0.001	0.07 (0.0–0.2)	<0.001
***MBL2* genotype**					
Sufficient	20 (69.0)	1			
Moderately deficient	8 (27.6)	0.78 (0.3–2.0)	0.6	1.19 (0.4–3.5)	0.7
Severely deficient	1 (3.5)	0.19 (0.0–1.5)	0.1	0.21 (0.0–2.0)	0.2
Age months					
0–12	3 (10.3)	1			
13–36	19 (65.5)	2.50 (0.7–9.2)	0.2	2.31 (0.5–10.3)	0.3
37–60	7 (24.1)	2.01 (0.5–8.8)	0.4	1.17 (0.2–6.7)	0.9
Season					
Dry	18 (62.1)	1			
Rainy	11 (37.9)	1.30 (0.5–3.1)	0.6	1.30 (0.5–3.7)	0.6
***MASP2*[ƒ]**					
MASP2 wild type	14 (48.3)	1			
Any *MASP2* variant	15 (51.7)	2.34 (1.0–5.4)	0.05	2.17 (0.8–5.9)	0.1

Data are presented as n (%), unless otherwise stated. PCV: packed-cell volume; MBL: mannose-binding lectin; MASP: MBL-associated serine protease. [#]: adjusted by the design variables (when appropriate) of age and season; [¶]: the final logistic model included design variables (age and season), malaria and *MASP2* variants; [+]: all subjects with parasitaemia ⩾100,000 were controls and analysis could not be carried out; [§]: parasitaemia was excluded from final logistic model analysis due to colinearity with clinical malaria; [ƒ]: includes subjects with all data available (*MBL2* and *MASP2* genotyping).

TABLE 4	Raw and adjusted analysis of invasive pneumococcal disease infected by low-invasive serotypes (6A, 6B, 10F, 15A, 19F, 23A, 23F, 24F, 29 and 35F)				
Variable	Cases	Univariate[#] OR (95% CI)	p-value	Adjusted OR[¶] (95% CI)	p-value
Anaemia					
Normal PCV	5 (31.3)	1			
Mild	7 (43.8)	2.53 (0.7–9.1)	0.2		
Moderate	4 (25.0)	0.64 (0.2–2.5)	0.5		
Severe	0 (0)				
Nutrition status					
Normal	8 (50.0)	1			
Moderate malnutrition	5 (31.3)	2.15 (0.7–7.1)	0.2		
Severe malnutrition	3 (18.8)	3.13 (0.9–11.0)	0.08		
Parasitaemia[+,§]					
Negative	12 (75.0)	1			
1–99999	4 (25.0)	0.12 (0.0–0.4)	<0.001		
⩾100000	0 (0)				
Malaria					
No	12 (75.0)	1			
Yes	4 (25.0)	0.10 (0.0–0.2)	<0.001	0.14 (0.0–0.5)	0.004
***MBL2* genotype**					
Sufficient	6 (33.3)	1			
Moderate deficient	10 (55.6)	3.56 (1.1–11.2)	0.03	5.55 (1.4–21.9)	0.01
Severe deficient	2 (11.1)	1.15 (0.2–6.7)	0.9	1.40 (0.2–10.3)	0.7
Age months					
0–12	8 (44.4)	1			
13–36	8 (44.4)	0.44 (0.7–9.2)	0.2	0.42 (0.1–1.6)	0.2
37–60	2 (11.1)	0.35 (0.5–8.8)	0.4	0.10 (0.0–1.1)	0.06
Season					
Dry	17 (94.4)	1			
Rainy	1 (5.6)	0.38 (0.1–1.4)	0.1	0.21 (0.0–1.8)	0.2
***MASP2*[ƒ]**					
MASP2 wild type	10 (55.6)	1			
Any *MASP2* variant	8 (44.4)	0.94 (0.3–2.5)	0.9	1.29 (0.4–4.4)	0.7

Data are presented as n (%), unless otherwise stated. PCV: packed-cell volume; MBL: mannose-binding lectin; MASP: MBL-associated serine protease. [#]: adjusted by the design variables (when appropriate) of age and season; [¶]: the final logistic model included design variables (age and season), malaria and *MASP2* variants; [+]: all subjects with parasitaemia ⩾100,000 were controls and analysis could not be carried out; [§]: parasitaemia was excluded from final logistic model analysis due to colinearity with clinical malaria; [ƒ]: includes subjects with all data available (*MBL2* and *MASP2* genotyping).

The ability of highly invasive serotypes to escape opsonophagocytosis may be the main strategy used. In contrast, low-invasive serotypes would need predisposing host factors (such as MBL deficiency) to cause disease, compared with the truly primary pathogens (*i.e.* highly invasive serotypes), which would be less influenced by any opsonophagocytosis deficiency. It has been shown that highly invasive serotypes act independently of other comorbidities in contrast to the low-invasive or opportunistic serotypes [27].

If the association between MBL deficiency and IPD depends on serotype invasiveness, the chances of observing an association (without taking into account serotype distribution in the final analysis) in a well-conducted study would largely depend on the prevalence of different serotypes. Associations could be diluted and significances would be found in large studies. This would explain the discordant findings in previously published studies where serotype distribution was not taken into account [5–7].

Furthermore, it has been reported that manifestations of MBL deficiency would be more prominent in young children, whose immune system is still immature [28]. This notion is supported by the over-representation of subjects with MBL-deficiency in infants infected by low-invasive serotypes.

Alternatively, HIV infection might explain the observed association between MBL deficiency and low-invasive serotypes. HIV has been established as a strong risk factor for pneumonia and IPD [2, 29]. Recent data from the same hospital show a 26% prevalence of HIV infection among children under 2 years of age admitted to hospital between March 2005–2006 with severe pneumonia [30]. Taking into account that *S. pneumoniae* is the leading cause of severe pneumonia in children, we can assume that the prevalence of HIV infection among cases included in our study would be remarkable. MBL-deficient genotypes have been shown to influence vertical transmission of HIV infection and AIDS progression,
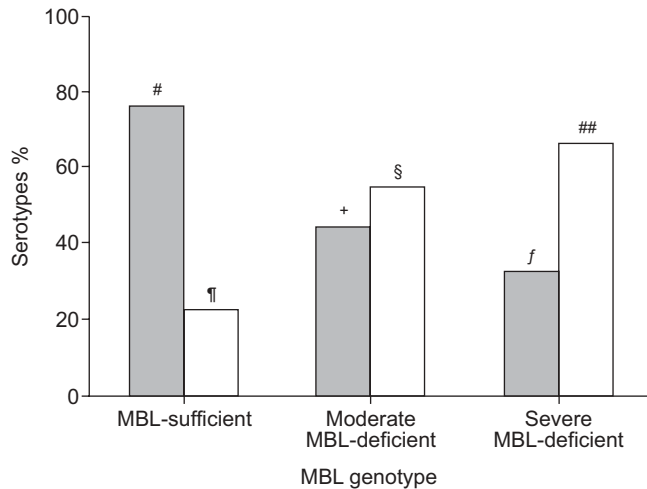
FIGURE 1. *MBL* genotype distribution according to serotype invasiveness among cases. Capsular serotype-specific distribution as follows. #: 1 (n=9), 5 (n=3), 9V (n=3), 14 (n=5); ¶: 6A (n=1), 6B (n=1), 10F (n=1), 22A (n=1), 24F (n=1), 19 (n=1); +: 1 (n=4), 4 (n=1), 5 (n=1), 12F (n=1), 14 (n=1); §: 6A (n=3), 15A (n=1), 19F (n=2), 23A (n=1), 23F (n=2), 24F (n=1); *f*: 12F (n=1); ##: 6A (n=1) and 23F (n=1). ■: high-invasive; □: low-invasive.

as well as AIDS progression among HIV-infected children [31]. HIV-infected individuals may have higher susceptibility to less invasive serotypes than other children. Consequently, the association between MBL deficiency and low-invasive serotypes may be confounded by HIV infection. However, HIV contribution should decrease with age, being insignificant for older cases (5–15 yrs of age), as mortality for HIV-infected children is high when treatment for this age group is scarce. This might explain the over-representation of MBL deficiency among infants (children <1 yr of age) infected by a low-invasive serotype. Further studies are needed to explore this hypothesis, as HIV data were not available from children included this study.

In conclusion, our results suggest the hypothesis that serotype invasiveness in IPD in children may be influenced by MBL deficiency. This observation is in accordance with current knowledge of physiopathology and epidemiology of IPD, may explain the inconclusive results of previous studies [5–7], and can inspire future studies focused on IPD and genetic risk factors. Further studies taking into account the limitations mentioned in our design (such as the small numbers, the lack of HIV data, and the hospital-based source of controls) are necessary to confirm these findings and to determine whether these results can be extrapolated to other geographical areas.

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