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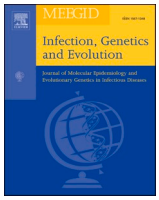

Gene polymorphisms of *IL-17A* and bacterial meningitis in Angolan children

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ABSTRACT

Interleukin (IL)-17 A plays a crucial role in protecting hosts from invading bacterial pathogens. In this study, we investigated if single nucleotide polymorphisms (SNPs) in *IL-17A* are associated with susceptibility and outcome of bacterial meningitis (BM) in Angolan children. The study sample comprised 241 confirmed BM patients and 265 controls, which were matched for age and ethnicity. Three *IL-17A* SNPs – rs2275913 (−197G > A), rs8193036 (-737C > T) and rs4711998 (−877 A > G) – were determined by high-resolution melting analysis (HRMA). The frequency of variant genotype rs4711998 was significantly higher in patients with BM caused by *Haemophilus influenzae* (odds ratio [OR] 3.5; 95% confidence interval [CI] 1.49–8.23; $P = 0.0025$) than in controls. Also, patients with BM caused by Gram-negative bacteria and who carried the variant genotype rs2275913 had a lower glucose level ($P = 0.0051$) in cerebrospinal fluid (CSF). Patients with BM caused by *Streptococcus pneumoniae* who carried the variant type rs8193036 had a reduced risk for severe neurological sequelae (OR: 0.14; 95% CI: 0.029–0.68; $P = 0.0079$), blindness (OR: 0.012; 95% CI: 0.012–0.87; $P = 0.017$) and ataxia (OR: 0.28; 95% CI: 0.091–0.83; $P = 0.023$). This study suggests an association of *IL-17A* genetic variations with susceptibility and outcome of bacterial meningitis in Angolan children.

1. Introduction

Bacterial meningitis (BM) is a life-threatening infectious disease with a high fatality rate, and may lead to long-term complications, especially in low- and middle-income countries. The disease progresses quickly, and without treatment the patient usually dies within 24–48 h after the onset of symptoms. Typical symptoms of bacterial meningitis are stiff neck with limited range of motion, high fever, sensitivity to light, confusion or sleepy feeling, headaches, vomiting and a rash on the skin (Nakamura et al., 2021). Survivors of BM have a high risk of developing permanent neurological sequelae, learning and behaviour disorders, focal neurological deficits, severe psychomotor retardation (Sanders et al., 2011), as well as hearing loss (Roine et al., 2013).

The most common bacterial pathogens that cause BM are *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae*. The introduction of vaccines has changed the etiology of BM in the past 30 years. Across all ages, *N. meningitis* and *S. pneumoniae* are the most prevalent pathogens causing BM worldwide (Oordt-Speets et al., 2018). In non-industrialized countries, *H. influenzae* still remains a common causative bacteria of BM. This is mostly due to the lack of effective vaccinations that have otherwise dramatically decreased invasive *H. influenzae* type b (Hib) disease in industrialized countries (Pelkonen et al., 2009) (Oordt-Speets et al., 2018). *H. influenzae* is predominantly carried by young children in the nasopharynx as a part of the normal nasopharyngeal microbiota, typically causing BM in children under five years of age (Rodrigues and Maiden, 2018) (Saikia et al., 2012). *S. pneumoniae* is the most prevalent pathogen causing BM after the neonatal period, and in adults of all ages (Mańdziuk and Kuchar, 2023). After the introduction of the Hib vaccine against *H. influenzae* type b and conjugate vaccines against *S. pneumoniae*, the BM cases have been

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reduced significantly worldwide. Group B *Streptococcus* (GBS) is the most common cause of BM in neonates (Mańdziuk and Kuchar, 2023), while *N. meningitidis* causes BM mainly in young adults; it spreads sporadically and causes local epidemics (Sanders et al., 2011) (Biller and Ferro, 2014). In sub-Saharan Africa, *N. meningitis* type A has been causing major outbreaks of meningococcal meningitis, affecting up to 1% of the population. Over the last decade, large-scale vaccination campaigns with a MenA conjugate vaccine decreased the disease burden, but since then, other serogroups such as serotypes C and W have been emerging (Mustapha and Harrison, 2018) (Pizza et al., 2020).

IL-17 is a pro-inflammatory cytokine, and acts as a central player in the adaptive immune response, particularly against extracellular bacteria and fungi (Isailovic et al., 2015). *IL-17A* is mainly produced by $CD4^+$ T-helper 17 (Th17) cells, along with γδ T-cells, natural killer T-cells and astrocytes (Luo et al., 2019) (Isailovic et al., 2015). *IL-17A* mediates its immune regulatory function by generating pro-inflammatory cytokines and chemokines, which attracts neutrophils and macrophages to the inflammation area. Elevated levels of several cytokines including IL-17, IL-6 and TNF-α are found in CSF of children with BM, and IL-17 had been suggested to play a key role in neutrophil infiltration into CSF and neuronal protection in BM (Asano et al., 2010). Indeed, these inflammatory factors have been also reported to mediate blood-brain barrier breakdown, which can ultimately lead to the infiltration of peripheral leukocytes and brain injury (Foroshani et al., 2018) (Siqueira and Stipursky, 2022) (Galea, 2021). Recent in vitro study has also demonstrated that *IL-17A* can increase the permeability of blood-brain barrier by down-regulating the expression level of tight junction proteins, which contributes to pathogenesis and development of BM (Xu et al., 2022). In addition, the receptor of *IL-17A* is widely expressed on fibroblasts, epithelial cells, macrophages, neutrophils, neurons, and astrocytes (Luo et al., 2019) (Isailovic et al., 2015) (Jin and Dong, 2013) (Miossec and Kolls, 2012) (Roark et al., 2008).

Genetic factors are one of major determinants for susceptibility to infectious diseases. Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation that occur throughout a person's DNA; these can cause differences in disease susceptibility (Shastry, 2007). SNPs in the gene encoding *IL-17A* have been shown to be involved in the susceptibility, severity, and outcome of severe infections like BM and other invasive pneumococcal and meningococcal diseases (Sanders et al., 2011).

Here, we aimed to investigate if susceptibility and outcome of BM in Angolan children are associated with SNPs in *IL-17A*. All three analysed SNPs – rs2275913 (−197G > A), rs8193036 (-737C > T) and rs4711998 (−877 A > G) – are located in the promoter region of the *IL-17A* gene. It is known that polymorphisms in the regulatory regions of cytokine-encoding genes affect the amount of cytokines produced. The *IL-17A* rs2275913 has previously been shown to be associated with impaired or increased production of *IL-17A* (Vuononvirta et al., 2015) (Abdellah et al., 2023). The same polymorphism is associated with increased colonization rate of *S. pneumoniae* in healthy Finnish children (Vuononvirta et al., 2015). In a previous study, Zheng et al. showed that the haplotype A-C-A of the three abovementioned *IL-17A* SNPs were more frequent within Chinese children diagnosed with pneumococcal meningitis, and were also associated with an increased expression of *IL-17A*. In addition, they showed that the patients who carried the haplotype had low level of CSF glucose and high level of CSF protein (Zheng et al., 2019).

2. Materials and methods

2.1. Study subjects, laboratory tests and clinical findings

The DNA samples for this study are from two prospective randomized clinical trials of children with BM in the Luanda Children's Hospital (Hospital Pediátrico David Bernardino), Luanda, Angola (Tenhu et al., 2022) (Tenhu et al., 2020). Control samples were collected after a Sunday service, or at the Luanda Children's Hospital in pediatric wards, surgery ward, surgery outpatient clinic, and vaccination clinic. The studies were approved by the Luanda Children's Hospital's ethics committee, and the patients were included in the study after their guardian's informed consent (Pelkonen et al., 2011). The guardians of controls provided verbal informed consent.

The diagnosis of BM was made by the attending physician, and the diagnostic criteria have been described previously (Pelkonen et al., 2008). The study cohort consisted of 241 confirmed BM cases, of which 114 (47%) were female and 127 (53%) were male; the median age was 15 months (range 1 month–13 years, 5 months). The control group consisted of 265 Angolan children with no history of BM. The patients were recruited from 2005 to 2008, and from 2012 to 2017. The control group was recruited from 2008 to 2017. The patients' detailed background and clinical and laboratory results, as well as the controls' background have been previously described (Tenhu et al., 2020) (Tenhu et al., 2022).

In the present study, we compared the genotype frequencies of *IL-17A* with prognostic factors for poor outcome in BM, which have been previously identified (Pelkonen et al., 2009). The analysed laboratory variables were glucose, protein, leukocyte count, and MMP-8 in CSF at admission. C-reactive protein (CRP) and leukocyte levels in the blood were also analysed during the hospital stay. The assessed clinical features were prior and present convulsions, poor general condition, level of consciousness, Glasgow and Blantyre coma scores at admission, and possible other causes of infection during hospital stay. The at-discharge assessed outcome measures were neurological sequelae (excluding ataxia), deafness, blindness, and survival. The severe neurological sequelae were defined as blindness, quadriplegia and/or paresis, hydrocephalus requiring a shunt, or severe psychomotor retardation (Pelkonen et al., 2009). Ataxia is a minor disability, defined as impaired coordination of voluntary muscle movement (Ashizawa and Xia, 2016) (Roine et al., 2013).

Microscopic examination, leukocyte count, as well as glucose, matrix metalloproteinase-8 (MMP-8) and protein concentration measurements were performed for CSF specimens taken at admission. CSF was cultured on blood and chocolate agar plates, and bacteria were identified by Gram stain and standard bacteriological phenotypic methods. When available, the Pastorex™ Meningitis agglutination test (Bio-Rad Laboratories Inc., Marne-La-Coquette, France) was conducted if >100 leukocytes/mm^3 were present and the bacterial culture was negative. Whenever possible, the remaining CSF sample was stored at −80 °C and shipped for PCR identification to the National Institute of Health, Lisbon, Portugal, or later to the Centre for Respiratory Diseases and Meningitis (CRDM), National Institute for Communicable Diseases (NICD), Johannesburg, South Africa. Concentration of MMP-8 was determined with a time-resolved immunofluorometric assay (Medix Biochemica, Espoo, Finland).

2.2. DNA isolation and genotyping

The BM patients' blood samples (n = 241) and first set of control samples (n = 74) were collected with a dried blood spot (DBP) collection card (PerkinElmer 226 Sample Collection Device, PerkinElmer, Waltham, Massachusetts, USA). The second control set of DNA samples (n = 191) was collected with SK-1S DNA buccal swabs (Isohelix, Harrietsham, Kent, UK). Genomic DNA was extracted with the QIAamp® mini DNA extraction kit (Qiagen, Hilden, Germany).

The analysed SNPs *IL-17A* rs2275913 (−197G > A), rs8193036 (-737C > T) and rs4711998 (−877 A > G) were determined by High resolution melting analysis (HRMA) (Roche Diagnostics Light Cycler 480, Basel, Switzerland) as described previously (Teräsjärvi et al., 2017) (Korppi et al., 2017). Additionally, the SNP *IL-17A* rs188151182 (-753C > A) was determined by Sanger sequencing. This SNP is a rare mutation reported in the African/African American population (minor allele frequency T = 0.001). Since it occurs near the *IL-17A* rs8193036 SNP, all

samples with an atypical HRMA difference curve or melting peak were additionally analysed by Sanger sequencing.

The primers and parameters used in the HRMA are described previously (Korppi et al., 2017). Primers used for PCRs were designed using the Primer-BLAST (NCBI, Bethesda MD, USA), and purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). PCR reactions with selected primer pairs (forward: 5′-TTAGAGTACAGGAAAAGAACCGCT-3′and reverse:5′- TGCATGCTACCAAGCAACTT-3′) were carried out in a volume of 30 μL, containing 3 μL genomic DNA, 1× PCR buffer (including 1.5 mM $MgCl_2$), 200 μM dNTP, 1 μM of each primer, and 1 U DyNAzyme II DNA Polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA). PCR products were amplified under the following conditions: initial denaturation 94 °C 10 min, followed by 40 cycles of 94 °C for 60 s, 50 °C for 40 s, 72 °C for 60 s; and final elongation at 72 °C for 5 min. Sanger sequencing was carried out at FIMM Institute for Molecular Medicine Finland (Helsinki, Finland), and DNA sequencing data were analysed with Applied Biosystems Sequence Scanner Software version 2.0 (Applied Biosystems/ Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.3. Statistical and genetic analyses

Statistical analyses were carried out using the JMP Pro software for Windows, version 14 (SAS Camous Drive, Cary NC, USA) and IBM SPSS Statistics for Windows, version 28 (IBM Corp., Armonk, N.Y., USA). Categorical variables were described by numbers (n) and percentages (%), and bivariate analyses were performed with Fisher's exact test. Continuous data were described using means (SD) or medians (IQR) as appropriate. SNP data and continuous data were compared using a Mann-Whitney *U* test, since the data were not normally distributed. A *P*-value <0.05 was considered significant.

A Hardy-Weinberg Equilibrium (HWE) test was used to calculate the observed genotype distribution in the control population. Differences in the numbers of patients arose because of missing or non-determined data.

The frequency distribution of genotypes were analysed with two different models, i.e. dominant and recessive models. In the dominant model, wild-type was compared with heterozygous and homozygote variant genotypes, whereas in the recessive model, homozygote variant genotype was compared with heterozygous and wild genotype combinations.

Haplotype analysis was performed for three polymorphisms in the *IL-17A*, *TLR4* and *TLR9* genes, these analyses of selected genes based on their role in microbial infections and biologic relevance in the pathogenesis of meningitis. Table 1 includes a description of the selected genes and SNPs and their reported functions. The linkage disequilibrium (LD) analysis and estimation of haplotype diversity was carried out using Haploview 4.2 software (Barrett et al., 2005). SNP_tools tool package for MS-Excel was used for conversion of genotype data (Chen et al., 2009).

3. Results

Of the 241 patients with confirmed BM, 56% (n = 136) had BM

Table 1
IL-17A gene polymorphism of rs2275913, rs4711998 and rs8193036 in bacterial meningitis patients and their African ancestor controls.

	All BM patients	*H. influenzae*	*N. meningitidis*	*S. pneumoniae*	Control group
rs2275913 (%)					
GG (%)	216 (89.6)	34 (87.2)	39 (90.7)	99 (91.7)	236 (89.4)
AG (%)	19 (7.9)	5 (12.8)	3 (7.0)	7 (6.5)	23 (8.7)
AA (%)	6 (2.5)	0	1 (2.1)	2 (1.9)	5 (1.9)
Total n	241	39	43	108	264
HWE	0.00003*	0.679	0.017	0.0006	0.00003*
P-value [1]	0.898	0.587	1	0.894	Ref.
GG vs AG/AA[2]					
P-value [1]	1	0.782	1	0.57	Ref.
OR (95% CI)	1.03 (0.58–1.82)	1.24 (0.45–3.43)	0.86 (0.29–2.60)	0.77 (0.35–1.68)	Ref.
AA vs AG/GG[3]					
P-value [1]	0.764	1	0.600	1	Ref.
OR (95% CI)	0.76 (0.23–2.52)	–	0.81 (0.09–7.11)	1.02 (0.20–5.36)	Ref.
rs4711998					
AA (%)	82 (34.2)	8 (20.5)	19 (44.2)	36 (33.3)	117 (43.8)
AG (%)	119 (49.6)	24 (61.5)	15 (34.9)	56 (51.9)	120 (44.9)
GG (%)	39 (16.3)	7 (17.9)	9 (20.9)	16 (14.8)	30 (11.2)
Total n	240	39	43	108	267
HWE	0.704	0.148	0.085	0.44	0.93
P-value [1]	0.061	**0.015**	0.164	0.148	
AA vs AG/GG[2]					
P-value [1]	**0.037***	**0.008***	1	0.063	Ref.
OR (95% CI)	1.47 (1.03–2.11)	3.02 (1.34–6.82)	0.99 (0.51–1.88)	1.58 (0.99–2.52)	Ref.
GG vs AG/AA[3]					
P-value [1]	0.120	0.290	0.086	0.386	Ref.
OR (95% CI)	1.52 (0.91–2.53)	1.72 (0.70–4.24)	2.08 (0.91–4.76)	1.37 (0.71–2.63)	Ref.
rs8193036					
TT (%)	140 (58.1)	23 (59.0)	24 (55.8)	60 (55.6)	157 (59.0)
CT (%)	97 (40.2)	16 (41.0)	17 (39.5)	46 (42.6)	90 (33.8)
CC (%)	4 (1.7)	0	2 (4.7)	2 (1.9)	19 (7.1)
Total n	241	39	43	108	266
HWE	0.0049*	0.107	0.641	0.041*	0.21
P-value [1]	0.0068*	0.192	0.775	0.054	
TT vs CT/CC[2]					
P-value [1]	0.787	1	0.740	0.56	Ref.
OR (95% CI)	0.944 (0.66–1.35)	1.00 (0.51–1.98)	1.14 (0.60–2.18)	1.16 (0.74–1.82)	Ref.
CC vs CT/TT[3]					
P-value [1]	0.0026*	0.147	0.750	0.048*	Ref.
OR (95% CI)	4.60 (1.54–13.71)	–	1.58 (0.36–7.05)	4.09 (0.94–17.89)	Ref.

Data are presented as numbers (n) and valid percentages (%), without missing observations.[1] Bivariate analysis was calculated by Fisher exact test between BM and control group (Ref.). [2]Dominant model, [3] Recessive model. *P*-values < 0.05 were considered significant (*).

caused by Gram-positive bacteria and 44% ($n = 105$) by Gram-negative bacteria. The most common causative pathogen was *S. pneumoniae*, (44.8%, $n = 108$), followed by *N. meningitidis* (17.8%, $n = 43$) and *H. influenzae* (15.8%, $n = 38$). The overall mortality rate was 34% ($n = 63$). The highest mortality rate was in pneumococcal meningitis (34%, $n = 37$), and the lowest in meningococcal meningitis (5%, $n = 8$). The detailed pathogen distribution has been published previously (Tenhu et al., 2020).

We studied three SNPs, rs2275913 (−197G > A), rs8193036 (-737C > T) and rs4711998 (−877 A > G), in the promoter region of the *IL-17A* gene in relation to the causative pathogens and clinical variables. The frequencies of the studied genotypes in relation to BM and the causative pathogens are presented in Table 1. An association between *IL-17A* rs4711998 (−877 A > G) and BM was observed. In dominant model the frequency of variant genotypes AG and GG was significantly higher in patients with BM (OR: 1.47; 95 % Cl 1.03–2.11) than in controls. The association seemed to be particularly related to BM caused by *H. influenzae* (OR: 3.02; 95% CI: 1.34–6.82; $P = 0.008$). No associations were observed between BM caused by *S. pneumoniae* or *N. meningitidis* and the other analysed *IL-17A* SNPs.

In our previous study (Tenhu et al., 2020), we found an association between polymorphism of *TLR4* and *TLR9* and *H. influenzae* meningitis in the same study group. We thus analysed haplotypes between *TLR4* rs4986790 (896 A > G), *TLR9* rs187084 (−1486 T > C) and *IL-17A* rs4711998 (−877 A > G). Further analyses showed an association between patients who carry variants of *TLR4* and *IL17 A* and wildtype of *TLR9* with *H. influenzae* meningitis (OR: 6.4; 95% CI: 1.8–23.2; $P = 0.0039$) (Table 2). Altogether, there were nine (23.7%) patients with *H. influenzae* meningitis who had the above-mentioned haplotype combination, whereas in the control group this haplotype combination was found only in 19 children (7.2%). The LD analysis confirmed above mentioned finding (Fig. 1). There were as six different haplotypes, and the GTG haplotype was more frequent (0.111) in patients with *H.influenzae* causing BM (Fig. 1 B) than in the control group (0.031) (Fig. 1 A). Each box provides estimated statistics of the coefficient of determination (r2), with darker shades representing stronger LD.

The severity of BM can be characterised by clinical features and laboratory variables. In the present study, patients with BM caused by Gram-negative bacteria and variant genotypes of *IL-17A* rs2275913 (−197G > A) had a lower CSF glucose (GA and AA: 4.8 mg/dL (1.4–17.0); $P = 0.0051$) (Table 3). We also observed an association between patients with pneumococcal meningitis and polymorphism of *IL-17A* rs8193036 (-737C > T). Patients with the variant genotype (CT/CC) of *IL-17A* rs8193036 had reduced risk of severe neurological sequelae (OR: 0.14; 95% CI: 0.029–0.68; $P = 0.0079$), blindness (OR: 0.10; 95% CI: 0.012–0.87; $P = 0.017$) and ataxia (OR: 0.28; 95% CI: 0.091–0.83; $P = 0.023$) (Table 3).

Table 2
The haplotype analysis of TLR4 rs4986790 (wildtype AA, variants AG and GG), TLR9 rs187084 (wildtype TT, variants CT and CC) and IL-17A rs4711998 (wildtype AA, variants AG and GG) in children with meningitis caused by *Haemophilus influenzae* versus control children.

TLR4	*TLR9*	*IL-17A*	*H. influenzae* n = 38 (%)	Controls *n* = 263 (%)	P-value	OR (95%CI)
AA	TT	GG	4 (10.5)	54 (20.5)	Ref.	Ref.
AA	TT	GA / AA	13 (34.2)	58 (22.1)	0.069	3.0 (0.93–9.85)
AA	TC/ CC	GG	1 (2.6)	50 (19.0)	0.37	0.27 (0.029–2.5)
AA	TC/ CC	GA / AA	8 (21.1)	61 (23.2)	0.54	1.8 (0.51–6.21)
AG/ GG	TT	GG	1 (2.6)	5 (1.9)	0.4	2.7 (0.25–29.0)
AG/ GG	TT	GA/ AA	9 (23.7)	19 (7.2)	**0.0039**[*1]	6.4 (1.8–23.2)
AG/ GG	TC/ CC	GG	1 (2.6)	6 (2.3)	1	2.3 (0.22–23.5)
AG/ GG	TC/ CC	GA/ AA	1 (2.6)	10 (3.8)	1	1.4 (0.14–13.4)

Data are presented as numbers (n) and valid percentages (%) without missing observations. Bivariate analysis was calculated by Fisher's exact test by comparing to the reference genotype "AA-TT-GG" and control group.

[*] P-values < 0.05 were considered significant.

[1] $P = 0.027$ after Bonferroni correction (multiplying the *P*-value by the number of paired comparisons).

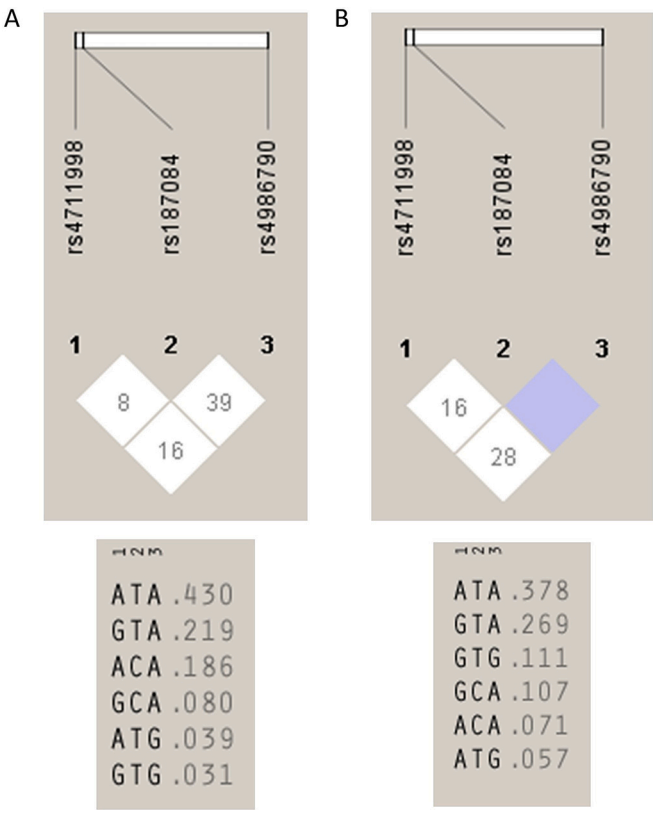

Fig. 1. LD SNP plot for *IL-17A* rs4711998, *TLR9* rs187084 and *TLR4* rs4986790.
The LD is displayed according to following colour schemes, with bright red: LOD > 2, D′ = 1, light red: LOD > 2, D′ < 1, blue: LOD < 2, D′ = 1 and white: LOD < 2, D′ < 1. The genes *IL-17A* (6p12.2), *TLR9* (3p21.2) and *TLR4* (9q33.1) are located on different chromosomes, so the distances shown in the figure are illustrative. The control population shown in Fig. 1A and the patients with *H. influenzae* meningitis in Fig. 1B. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

In this study, we analysed the frequencies of three *IL-17A* promoter area polymorphisms – rs4711998 (−877 A > G), rs8193036 (-737C > T) and rs2275913 (−197G > A) – in Angolan children with BM, and compared the genotype frequencies to age- and ethnically-matched controls. We observed an association between patients with BM caused by *H. influenzae* and the gene polymorphism of *IL-17A* rs4711998 (−877 A > G). Patients with variant genotypes AG and GG of rs4711998 (−877 A > G) had an increased susceptibility to BM caused by *H. influenzae*. In our previous study, we found an association between *H. influenzae* and the gene polymorphisms of *TLR4* rs4986790 (896 A > G) and *TLR9* rs187084 (−1486 T > C) (Tenhu et al., 2020). For that reason, we performed haplotype analyses between the above genotypes

Table 3
Summary of all significant findings between IL-17A SNPs and clinical features and causative pathogens.

Clinical features	IL-17A rs2275913		OR (95 % Cl)	*p*-value	Causative pathogen
	GG	GA / AA			
Other focus of infection	24 (70.6)	1 (20.0)	0.10 (0.01–1.05)	0.0469 [1]	*H. influenzae*
Any neurological sequelae	16 (61.5)	0	0	0.0365 [1]	*H. influenzae*
Any neurological sequelae	1 (2.94)	2 (50.0)	33 (2.02–538.76)	0.0247 [1]	*N. meningitidis*
CSF glucose (mg/dL)	11.7 (6.0–26.9) $n = 249$	8.2 (3.1–16.8) $n = 32$		0.0219 [2]	All meningitis
CSF glucose (mg/dL)	12.6 (5.4–24.5) $n = 85$	4.8 (2.6–10.2) $n = 13$		0.0050 [2]	Gram-negative
CSF glucose (mg/dL)	16.5 (8.6–29.5) $n = 36$	3.85 (1.8–10.7) $n = 4$		0.0228 [2]	*N. meningitidis*
Blood leucocyte count (10^9/L)	15.4 (10.1–21.4) $n = 89$	9.7 (4.5–15.7) $n = 9$		0.418 [2]	Gram-positive
	IL-17A rs4711998				
	AA	**AG / GG**			
Any neurological sequelae	2 (7.4)	20 (37.7)	7.6 (1.6–35.5)	0.0037 [1]	Gram-negative
Ataxia	2 (7.4)	17 (32.1)	5.9 (1.3–27.9)	0.0241 [1]	Gram-negative
CSF glucose (mg/dL)	5.2 (1.7–7.2) $n = 7$	9.3 (4.6–19.6) $n = 29$		0.0435 [2]	*H. influenzae*
Blood CRP (mg/L)	161 (161–181) $n = 27$	161 (108.5–161) $n = 62$		0.0385 [2]	Gram-negative
	***IL-17A* rs8193036**				
	TT	**CT and CC**			
Severe neurological sequelae	24 (19.5)	6 (6.5)	0.28 (0.1–0.7)	0.0089 [1]	All meningitis
Severe neurological sequelae	14 (25.5)	3 (7.5)	0.24 (0.06–0.9)	0.0303 [1]	Gram-positive
Severe neurological sequelae	12 (31.6)	2 (6.1)	0.14 (0.03–0.7)	0.0079 [1]	*S. pneumoniae*
Blindness	17 (13.9)	3 (3.3)	0.2 (0.06–0.8)	0.0155 [1]	All meningitis
Blindness	10 (18.2)	1 (2.6)	0.1 (0.01–1.0)	0.0233 [1]	Gram-positive
Blindness	9 (23.7)	1 (3.1)	0.1 (0.01–0.9)	0.0172 [1]	*S. pneumoniae*
Ataxia	18 (48.7)	6 (20.7)	0.3 (0.09–0.8)	0.0227 [1]	*S. pneumoniae*
CSF glucose (mg/dL)	19.6 (13.7–33) $n = 20$	16.8 (10.2–18.5) $n = 13$		0.0297 [2]	*H. influenzae*

[1] Calculated by Fisher's exact test.
[2] Calculated by Wilcoxon / Kruskal-Wallis Tests.

and BM caused by *H. influenzae*. The patients with the haplotype combination of *TLR4* variants, *TLR9* wildtype and *IL-17A* variants had a higher probability to contract BM caused by *H. influenzae*, when compared to the other haplotype combinations.

CSF analysis is an important diagnostic tool of neurological diseases, and the levels of glucose, leucocytes and protein in the CSF are used to discriminate BM from viral meningitis. Specifically, low glucose and elevated protein and leucocyte levels in CSF may indicate severe BM. Children with BM typically have low levels of glucose in CSF because glycolysis converts glucose into other compounds in the white cells and pathogens (Nigrovic et al., 2012). In the present study, we found that patients with BM caused by Gram-negative bacteria and variant genotypes of *IL-17A* rs2275913 (−197G > A) had a lower CSF glucose (GA and AA: 4.8 mg/dL (1.4–17.0); $P = 0.0051$). This observation is in line with the study performed by Zheng et al., who also studied the same *IL-17A* SNPs (rs4711998, rs8193036 and rs2275913) in patients diagnosed with BM. In that study, the authors found that the haplotype combination A-C-A of the three abovementioned *IL-17A* SNPs was associated with a low level of cerebrospinal fluid (CSF) glucose and high level of CSF protein (Zheng et al., 2019). In addition, they found that the haplotype combination A-C-A was more frequent within patients diagnosed with pneumococcal meningitis and increased expression of *IL-17A* (Zheng et al., 2019). We had previously studied the association of the *IL-17A* rs2275913 (−197G > A) SNP with nasopharyngeal bacterial colonization and serum *IL-17A* levels in healthy Finnish children, and found that subjects with variant genotypes (AG/AA) of *IL-17A* rs2275913 had an increased risk of *S. pneumoniae* colonization in the nasopharynx (Vuononvirta et al., 2015). However, the present study did not find such association between *IL-17A* rs2275913 genotypes and pneumococcal BM.

It is known that patients with BM are at a high risk of developing different neurological complications (Sanders et al., 2011). Here we found that patients with pneumococcal meningitis who carried the variant type of *IL-17A* rs8193036 (-737C > T) had a reduced risk for severe neurological sequelae, blindness, and ataxia. It has been shown that gene polymorphisms in the regulatory regions of cytokine-encoding genes like *IL-17A* affect the amounts of cytokines produced, and it has been shown that the AA genotype of the *IL-17A* SNP rs2275913 alone (Vuononvirta et al., 2015) (Abdellah et al., 2023) and CC genotype of the *IL-17A* rs8193036 SNP together with *IL-17A* rs2275913 AA-genotype and rs4711998 AA-genotype (Zheng et al., 2019) are associated with *IL-17A* levels in serum and plasma. High levels of plasma *IL-17A* are associated with severe neurological sequelae in Langerhans cell histiocytosis (Ismail et al., 2020), as well as in CSF in BM (Asano et al., 2010). In this study, the *IL-17A* levels of serum or plasma samples were not determined in patients with BM, which is clearly one limitation of the study.

We acknowledge that there were also some other limitations in the study. Even though the total number of confirmed BM patients is relatively large, the number of individual causative pathogens, e.g., *H. influenzae* and *N. meningitidis* is limited. Therefore, we could have missed some weak associations. Secondly, only three SNPs of *IL-17A* were tested, and the effects of other SNPs in the gene on susceptibility and outcome of BM should also be kept in mind.

5. Conclusion

This study found that *IL-17A* rs4711998 (−877 A > G) was associated with susceptibility to *H. influenzae* meningitis in Angolan children. We also observed an association between polymorphism of *IL-17A* rs2275913 (−197G > A) and low CSF glucose in children with meningitis caused by Gram-negative bacteria. Furthermore, the variant type of *IL-17A* rs8193036 (-737C > T) may reduce the risk of neurological sequelae after pneumococcal meningitis. However, it should be kept in mind that currently these results only apply to children in Angola.

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CRediT authorship contribution statement

Johanna Teräsjärvi: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Elina Tenhu:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Manuel Leite Cruzeiro:** Conceptualization, Data curation, Investigation, Writing – review & editing. **Okko Savonius:** Conceptualization, Data curation, Writing – review & editing. **Emilie Rugemalira:** Conceptualization, Data curation, Writing – review & editing. **Qiushui He:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Tuula Pelkonen:** Conceptualization, Data curation, Funding acquisition, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

We have no conflict of interest to declare.

Data availability

Data will be made available on request.

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