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Genetic polymorphisms of *TLR1*, *TLR2*, *TLR3* and *TLR4* in patients with recurrent or severe infections

Johanna Teräsjärvi[1] | Leena Kainulainen[2,3] | Ville Peltola[2,4] | Jussi Mertsola[2,4] | Antti Hakanen[4,5] | Qiushui He[1,4]

[1]Institute of Biomedicine, Research Center of Infections and Immunity, University of Turku, Turku, Finland

[2]Department of Pediatrics and Adolescent Medicine, Turku University Hospital, Turku, Finland

[3]Department of Medicine, Turku University Hospital, Turku, Finland

[4]InFLAMES Research Flagship Center, University of Turku, Turku, Finland

[5]Department of Clinical Microbiology, Turku University Hospital, Turku, Finland

Correspondence
Qiushui He, Institute of Biomedicine, Research Center for Infections and Immunity, University of Turku, Turku, Finland.
Email: qiushui.he@utu.fi

Abstract

Toll-like receptors (TLRs) play an important role in innate immunity. Previous studies have shown that single nucleotide polymorphisms (SNPs) in the genes coding for these innate immune molecules can affect susceptibility to and the outcome of certain diseases. The aim of the present study was to examine the clinical relevance of well-studied *TLR1–4* SNPs in individuals who are prone to infections. Four functional SNPs, *TLR1* rs5743618 (1805C > A, Ser602Ile), *TLR2* rs5743708 (2258G > A, Arg753Gln), *TLR3* rs3775291 (1234C > T, Leu412Phe) and *TLR4* rs4986790 (896A > G, Asp299Gly), were analysed in 155 patients with recurrent respiratory infections ($n = 84$), severe infections ($n = 15$) or common variable immunodeficiency ($n = 56$), and in 262 healthy controls, using the High Resolution Melting Analysis method. Polymorphisms of *TLR2* rs5743708 (odds ratio [OR] 3.16; 95% confidence interval [CI] 1.45–6.83, $p = .004$, $ap = .016$) and *TLR4* rs4986790 (OR 1.8; 95% CI 1.05–3.12, $p = .028$, $ap = .112$) were more frequent in patients with recurrent or severe infections than in controls. Interestingly, seven patients were found to carry both variant genotypes of *TLR2* and *TLR4*, whereas none of the control group carried such genotypes ($p \leq .0001$). Moreover, *TLR2* polymorphism was associated with increased risk for acute otitis media episodes (OR, 3.02; 95% CI 1.41–6.47; $p = .012$). This study indicates that children and adults who are more prone to recurrent or severe respiratory infections carry one or both variant types of *TLR2* and *TLR4* more often than control subjects. Genetic variations of *TLR*s help explain why some children are more susceptible to respiratory infections.

KEYWORDS
children, gene polymorphism, recurrent infections, severe infections, TLR2, TLR4

1 | INTRODUCTION

The average child has four to eight respiratory infections per year, and adults around three infections per year (Hernandez-Trujillo, 2015). Especially in childhood, some individuals suffer from recurrent infections which can negatively affect their quality of life (Jiang et al., 2013) and from an increased risk for chronic diseases like asthma (Pelaia et al., 2006; van Meel et al., 2022). Recurrent infections are usually due

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to allergies, anatomical contributions and an unusual burden of exposures (Ruffner et al., 2017). In most cases, individuals with recurrent infections have an intact and functional immune system.

Previous studies have demonstrated the important role of host genetics in susceptibility to respiratory infections such as acute otitis media (AOM) (Teräsjärvi et al., 2023), tuberculosis (TB) (Abel et al., 2014) and respiratory syncytial virus infection (Awomoyi et al., 2007) as well as in an increased severity of certain infections (Faber et al., 2009; Gao et al., 2015). Moreover, these genetic factors have been shown to be associated with microbe colonization (Chen et al., 2010; Vuononvirta et al., 2013), cytokine responses (Vuononvirta et al., 2015) and prognosis of certain respiratory diseases (Haerynck et al., 2013; Koponen et al., 2014; Lauhkonen et al., 2016).

Toll-like receptors (TLRs) are pattern-recognition receptors, and to date, 10 functional TLRs have been identified in humans (TLR1–10). These receptors recognize a wide range of pathogen-associated molecular patterns from microbes and damage-associated molecular patterns (DAMPs) released from damaged tissue (Botos et al., 2011) and are a critical part of innate immunity. TLRs are present either in the plasma membrane of innate immune cells (TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10), or intracellularly in the cytosolic compartment inside vesicles (e.g. in endosomes) (TLR3, TLR7, TLR8 and TLR9), where they recognize microbial genetic material (DNA or RNA) (Frazão et al., 2013).

Studies have shown that TLRs recognize microbial organisms, ranging from bacteria to fungi, protozoa and viruses, via their ligands. For this study, we included four TLRs that sense lipopeptides and peptidoglycans of Gram-positive bacteria (TLR2/TLR1 heterodimer), lipopolysaccharides (LPS) of Gram-negative bacteria (TLR4) and viral double-stranded RNA (dsRNA) (TLR3) (Skevaki et al., 2015).

It is well established that the polymorphisms in *TLR* genes can increase susceptibility to infections (Skevaki et al., 2015) and inflammatory diseases (Raj et al., 2013), as well as certain cancers (Medvedev, 2013). However, so far only slight attention has been paid to how the determination of these SNPs could be utilized in clinical practice. To date, only a few studies have shown that the determination of clinically important SNPs in *TLR*s can help clinicians in predicting the prognosis of diseases such as cystic fibrosis (Haerynck et al., 2013) and colorectal cancer (Messaritakis et al., 2018) or in targeting treatment (Sadik et al., 2015).

In our previous study, we verified the use of High Resolution Melting Analysis (HRMA) when determining the four most studied SNPs of the selected *TLR*s: *TLR1* rs5743618 (1805C > A, Ser602Ile), *TLR2* rs5743708 (2258G > A, Arg753Gln), *TLR3* rs3775291 (1234C > T, Leu412Phe) and *TLR4* rs4986790 (896A > G, Asp299Gly) (Skevaki et al., 2015).

The above-mentioned SNPs cause an amino acid change and are shown to be functional (Schröder & Schumann, 2005). Of these, the most widely studied SNPs include *TLR2* rs5743708 and *TLR4* rs4986790. The *TLR2* SNP is shown to be associated with an increased susceptibility to TB (Chen et al., 2023) and a higher risk to staphylococcal septic shock (Lorenz et al., 2000). In turn, the *TLR4* SNP is found to be associated with an increased susceptibility to Gram-negative bacterial infections (Agnese et al., 2002; Sampath et al., 2013), with an especially increased risk for septic shock from infections by Gram-negative pathogens (Li et al., 2022).

The aim of the present study was to determine the above-mentioned SNPs in *TLR1–4* in subjects prone to recurrent or severe infections and thus identify genetic risk factors for these infections in patients with a physician diagnosed common variable immunodeficiency (CVID) (a type of primary immunodeficiency), to observe what kind of added value the analysis of these polymorphisms brings from the perspective of the patient and the attending physician.

2 | MATERIALS AND METHODS

2.1 | Study subjects and data collection

Blood samples were collected from 234 child and adult patients, who visited the Department of Pediatric and Adolescent Medicine or the Department of Medicine in Turku University Hospital, Finland, between November 2016 and December 2019. All patients were prone to infections, and the selected *TLR* polymorphisms were analysed as a part of other immunological tests. Out of these patients, 155 patients met the inclusion criteria (diagnosis of recurrent otitis, recurrent pneumonia, child's recurrent respiratory tract infection (ICD-10: J06.8)), other recurrent respiratory infections, severe infection (sepsis or/and meningitis) or CVID and were included in the present study. CVID was diagnosed according to IUIS diagnostic criteria (Bousfiha et al., 2022). Patients without an accurate diagnosis of infection (unexplained or prolonged fever, fatigue or autoimmune diseases) were excluded. The medical records of the study subjects ($n = 155$) were reviewed by a clinician, and the diagnoses of infectious diseases were recorded and included in the analyses. Infection-causing microbes were not considered in the statistical analyses, because data on them were only available for severe infections. The patient was considered to have recurrent infections if medical records included two or more severe infections or three or more respiratory infections within 1 year. The control group consists of healthy Finnish infants who participated in a birth cohort study: Steps to the Healthy Development and Well-being of Children (STEPS) (Lagström et al., 2013). The DNA samples were collected from 923 infants at the scheduled 3 months clinic visit when they were 2–3 months of age. From these DNA samples, 262 were randomly selected as controls.

The median age in the study group, for those not diagnosed with CVID, was 7 years (range: 1 month–69 years) and of these 55.6% ($n = 55$) were male and 44.4% ($n = 44$) female. In the CVID group, the median age was 49 years (range: 11–81 years). Among them, 43.4% ($n = 23$) were male and 56.6% ($n = 30$) female. The control group's DNA samples were collected at the first scheduled clinic visit of the STEP study, so there was no notable age variation. Of the 262 controls, 54.2% ($n = 142$) were male, and 45.8% ($n = 120$) were female. Characteristics of the study population are presented in Table 1.

Blood samples obtained from patients were sent for SNP analysis to the processing laboratory at the Institute of Biomedicine, Uni-

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TABLE 1 Characteristics and single nucleotide polymorphisms (SNP) frequencies of the study and control groups.

	Controls[b] (*n* = 262)	Study group, other than CVID (*n* = 99)	*p*-Value (*ap*-value)[d]	OR (95% CI)	CVID (*n* = 53)	*p*-Value	OR (95% CI)
Age[a]	0.25 years[b]	7 years (1 month–69 years)			49 years (11–81 years)		
Gender							
Male	142 (53.8)	55 (55.6)	.817	1.06 (.66–1.68)[c]	23 (43.4)	.175	.65 (.36–1.18)[c]
Female	120 (45.8)	44 (44.4)			30 (56.6)		
SNPs							
TLR1 rs5743618							
CC	166 (63.4)	67 (67.7)	.211		37 (69.8)	.471	
AC	90 (34.6)	27 (27.3)			14 (26.4)		
AA	6 (2.3)	5 (5.1)			2 (3.8)		
A-allele	102 (19.5)	37 (18.7)	.182	.44 (.13–1.48)	18 (17.0)	.552	.85 (.49–1.47)
C-allele	422 (80.5)	161 (81.3)			88 (83.0)		
HWE	.122	.306			.646		
TLR2 rs5743708							
GG	248 (94.7)	84 (84.8)	**.004*** **(.016)**		49 (92.5)	.518	
GA	14 (5.3)	15 (15.2)			4 (7.5)		
AA	0 (0)	0 (0)			0 (0)		
G-allele	510 (97.3)	183 (94.8)	**.004*** **(.016)**	3.16 (1.45–6.83)	102 (96.2)	.537	1.43 (.46–4.43)
A-allele	14 (2.7)	15 (5.2)			4 (3.8)		
HWE	.657	.415			.775		
TLR3 rs3775291							
CC	125 (47.7)	38 (38.4)	.186		25 (47.2)	.450	
CT	108 (41.2)	53 (53.5)			19 (35.8)		
TT	29 (11.1)	8 (8.1)			9 (17.0)		
C-allele	358 (68.3)	129 (65.2)	.096	1.40 (.86–2.25)	69 (65.1)	.517	1.16 (.75–1.80)
T-allele	166 (31.7)	69 (34.8)			37 (34.9)		
HWE	.440	.075			.124		
TLR4 rs4986790							
AA	217 (82.8)	72 (72.7)	.085		42 (79.2)	.200	
AG	43 (16.4)	25 (25.3)			9 (17.0)		
GG	2 (.8)	2 (2.0)			2 (3.8)		
A-allele	477 (91.0)	169 (85.4)	**.028*** **(.112)**	1.74 (1.06 –2.86)	93 (87.7)	.294	1.42 (.74–2.73)
G-allele	47 (9.0)	29 (14.6)			13 (12.3)		
HWE	.935	.921			.125		
Haplotypes							
Variant TLR2 (GA) and TLR4 (AG)	0 (.0)	7 (7.53)	**.000014***				

Note: Data are presented as numbers (*n*) and percentages (%). Differences between genotype frequencies of control and study groups were analysed with χ^2 test.

Abbreviations: CI, confidence interval; CVID, common variable immunodeficiency; OR, odds ratio; TLR, toll-like receptors.

[a]Median age (age range).

[b]The control group, consist of the healthy Finnish infants who were participating in a birth cohort study called Steps to Children's Healthy Development and Wellbeing study (STEPS). The DNA samples were collected at the scheduled 3-month clinic visit when the infants were 2–3 months of age.

[c]Odds ratio for males.

[d]Adjusted *ap*-value was calculated with Bonferroni correction.

*The significance was calculated by the Fisher exact test or $\chi2$ test and two-tailed $p < .05$ considered significant.

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versity of Turku, Turku, Finland. According to the Finnish Medical Research Act, the opinion of the ethics committee is needed only in medical research involving intervention. As the present study was not medical research involving intervention, ethical permission was not needed.

DNA samples of the control group were randomly selected from children participating in the STEPS study, which was approved by the Ministry of Social Affairs and Health (STM 1575/2008, STM 1838/2009) and the Ethics Committee of the Hospital District of Southwest Finland (19.2.2008 §63, 15.4.2008 §134, 19.4.2011 §113). All STEPS study participants or parents of participating children gave their written informed consent.

2.2 | Genotyping

DNA was extracted from 250 μL of whole blood with E.Z.N.A. Blood DNA Mini Kit (VWR/OMEGA Bio-tek, Inc.) according to the manufacturer's protocol. The DNA was eluted with 150 μL of elution buffer. The quality and concentration of DNA were determined with a spectrophotometer (NanoDrop 1000, Thermo Scientific). Prior to further analysis, the DNA was diluted to 8 ng/μL.

Four *TLR* SNPs (*TLR1* rs5743618, *TLR2* rs5743708, *TLR3* rs3775291 and *TLR4* rs4986790) were determined by HRMA (Teräsjärvi et al., 2017). Analyses were performed with LightCycler 480 version 5.1 (Roche) using Roche's original melting master kit (LightCycler 480 High Resolution Melting Master) and 96-well plates (LightCycler 480 Multiwell Plate 96, white). Sequenced controls from each genotype were added to each run of the HRMA analysis. In addition, all samples with *TLR4* rs4986790 GG genotypes and samples with atypical melting curves were confirmed by Sanger sequencing, which was carried out at Institute for Molecular Medicine Finland (FIMM, Helsinki, Finland) or Eurofins Genomics. All control samples were analysed with HRMA and previously with either Sanger sequencing or pyrosequencing (Toivonen et al., 2017; Vuononvirta et al., 2013).

2.3 | Statistical analyses

Data management and analysis were performed using SPSS software, version 28.0 (IBM Corp.). The Hardy–Weinberg equation with χ^2 was used to calculate the genetic variation equilibrium for all studied polymorphisms. Categorical data were compared by the Fisher exact test or χ^2 test. Odds ratio (OR) with 95% confidence interval (CI) was used to interpret association between SNPs and outcome. *p*-Values of less than .05 were considered statistically significant and corrected for multiple testing by Bonferroni.

The linkage disequilibrium (LD) analysis and estimation of haplotype diversity were carried out using Haploview 4.2 software (Barrett et al., 2005). SNP_tools package for MS-Excel was used for the conversion of genotype data (Yang et al., 2020).

3 | RESULTS

3.1 | Characteristics of study subjects and frequencies of studied SNPs

Based on the diagnosis, patients were divided in two groups. The first group (infection group) included all patients with a diagnosis of recurrent or severe infections ($n = 99$), and the second group (CVID group) included those with a physician diagnosed CVID ($n = 53$).

The *TLR2* rs5743708 ($p = .004$, $ap = .016$) and *TLR4* rs4986790 ($p = .028$, $ap = .112$) variant types were more frequent in the infection group than in the control group. In addition, seven subjects in the infection group carried both the above-mentioned variants, whereas none of the control group or the CVID group had such SNP combination ($p \leq .0001$). We also observed that patients in the infection group more often carried three or more variants of studied SNPs, and the frequencies of these haplotypes identified among patients were more irregular than those found in the controls. However, the differences were not statistically significant. To visualize this, LD analysis and estimation of haplotype diversity were carried out and are presented in Figure 1. The chi-square test did not show any significant differences in the frequencies of studied SNPs between patients with CVID and controls.

Characteristics and frequencies of analysed SNPs of the study subjects and controls are presented in Table 1.

Next, we wanted to evaluate if the above differences observed in *TLR2* and *TLR4* among patients with recurrent or severe infections existed in all age groups or only in young children. The infection group was thus divided into three age groups: 0–5, 6–17 and ≥18 years. As shown in Table 2, the first thing of note was the distribution by gender, which was statistically significantly different between groups. In the youngest age group (0–5 years), there were considerably more males (71.1%) than females (28.9%); in the school age group (6–17 years), the gender distribution was almost equal, whereas in the adult group (≥18 years), the female gender (65.2%) was slightly overrepresented.

There were also clear differences in the distribution of diagnoses among different age groups. As assumed, episodes of AOM were most common in the youngest age group (0–5 years), where 80.0% ($n = 36$) had AOM episodes. These episodes were quite common also among 6–17-year olds (61.3%, $n = 19$), but rarer among over 18-year olds (30.4%, $n = 7$) ($p = .001$). In turn, older children (6–17 years) and adults (≥18 years) were more often diagnosed with recurrent acute sinusitis than small children ($p = .005$). When it comes to more severe infections, pneumonia was found equally in all age groups, whereas invasive infections were more common in older age groups.

As shown in Table 2, small children ($p = .007$, $ap = .028$) and adults ($p = .053$) in the infection group had the *TLR2* variant genotype rs5743708 AG more often than the controls. In addition, children at age 6–17 had the *TLR4* rs4986790 (AG or GG) ($p = .024$) or *TLR3* rs3775291 (CT or TT) ($p = .033$, $ap = .132$) variant types more often than the controls. The *TLR2* and *TLR4* SNPs were more common in all

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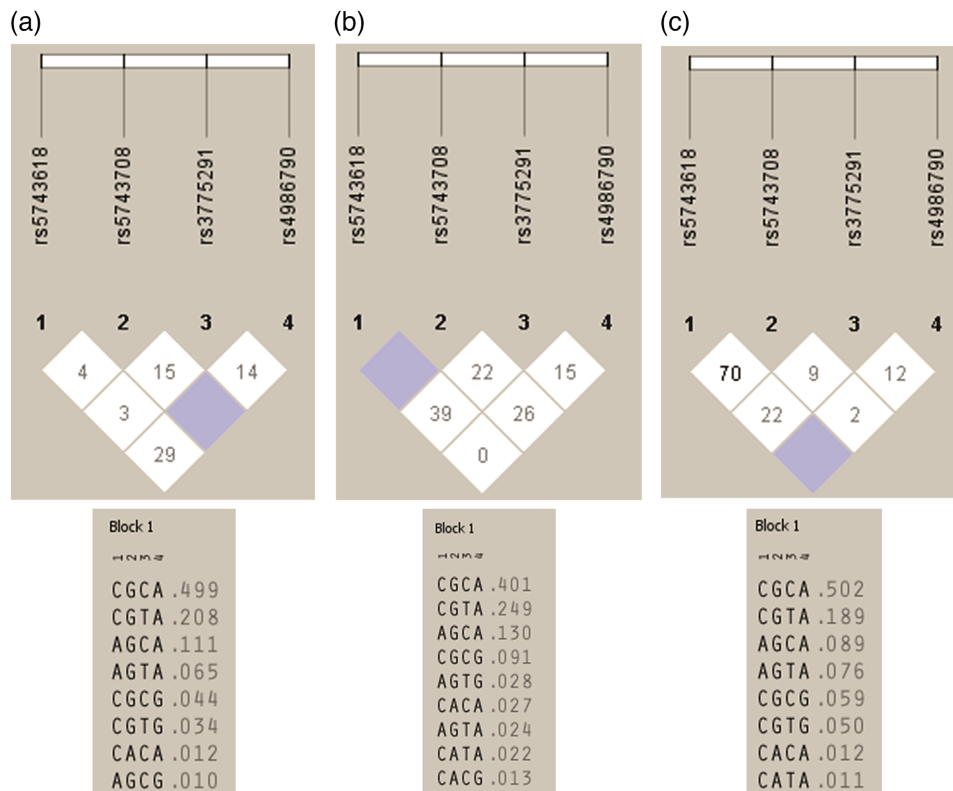

FIGURE 1 Linkage disequilibrium (LD) map of four studied single nucleotide polymorphisms (SNPs) in TLR1–4. The LD is displayed according to following colour schemes, with bright red: LOD > 2, D′ = 1, shades of pink/red: LOD > 2, D′ < 1, blue: LOD < 2, D′ = 1 and white: LOD < 2, D′ < 1. The latter of studied genes TLR1 (4p14), TLR2 (4p31.3), TLR3 (4q35.1) and TLR4 (9q33.1) is located on a different chromosome than the other three, so the distances shown in the LD figure are illustrative. The control population shown in (a), infection group in (b) and the patients with common variable immunodeficiency (CVID) in (c).

age groups in the infection group than in the control group, although the differences were not statistically significant for all age groups.

3.2 | Clinical findings and SNPs

The most frequent diagnosis in the infection group was 'child's recurrent respiratory tract infection' ($n = 48$). The median age in this group was 3.5 years (range: 0.6–16.6 years), and males (68.8%) were more prevalent than females (31.3%). When compared to the control group, it appeared that males were slightly more prone to childhood infections than females (OR 1.86; 95% CI,.96–3.59; $p = .06$). In the study group, the variant GA genotype of *TLR2* was more frequent, and children who carried minor allele A had a threefold higher risk for recurrent respiratory infections in childhood (OR 3.31; 95% CI 1.35–8.13; $p = .012$, $ap = .048$) (Table 3).

Table 4 illustrates the frequencies of *TLR2* rs5743708 and *TLR4* rs4986790 genotypes and their association with different types of respiratory or severe infections. The most common diagnosis was AOM, and 80.1% ($n = 62$) of those belonging to the infection group had at least one AOM episode. Subjects with *TLR2* rs5743708 variant genotype GA had an increased risk of AOM (OR 3.02; 95% CI 1.41–6.47, $p = .012$, $ap = .024$). This genotype also seemed to be associated with an increased risk for pneumonia (OR 3.51; 95% CI 1.12–10.98, $p = .065$) and more severe infections (OR 3.74; 95% CI 1.21–11.62, $p = .055$). However, these findings were not statistically significant (Table 4).

4 | DISCUSSION

All the patients included in this study were prone to infections and, based on the medical records, were divided into two groups. The infection group included patients with a diagnosis of recurrent respiratory infections or severe invasive infections (including meningitis or septicaemia), and the second group included patients diagnosed with CVID. It was clearly seen that *TLR4* and *TLR2* variant genotypes were more frequent in the infection group than in the control group. Furthermore, seven patients with recurrent or severe infections carried mutations in both above-mentioned *TLRs*, whereas no such combinations were detected among the control group. On the contrary, no significant differences were found between patients with CVID and the controls in the frequencies of studied SNPs.

TLR4 is one of the most studied TLRs, which is expressed on the cell surface of hematopoietic and non-hematopoietic cells. TLR4 forms a heterodimer with a myeloid differentiation factor 2 that recognizes LPS molecules (Park et al., 2009). In addition, TLR4 is also capable

TABLE 2 Frequencies of genetic variations in the 3 age groups of 99 patients diagnosed with recurrent or severe infections.

	Controls ($n = 262$)	Age group 0–5 years ($n = 45$)	p-Value (ap-value)[a]	Age group 6–17 years ($n = 31$)	p-Value (ap-value)[a]	Age group >18 years ($n = 23$)	p-Value*
Sex							
Male	142 (54.2)	32 (71.1)		15 (48.4)		8 (34.8)	
Female	120 (45.8)	13 (28.9)		16 (51.6)		15 (65.2)	
SNPs							
TLR1 rs5743618							
CC	166 (63.4)	29 (64.4)	.667	25 (80.6)	.145	13 (56.5)	.277
AC	90 (34.6)	14 (31.1)		5 (16.1)		8 (34.8)	
AA	6 (2.3)	2 (4.4)		1 (3.2)		2 (8.7)	
AC or AA	96 (36.6)	16 (35.6)	.999	6 (20.0)	.104	10 (43.5)	.662
TLR2 rs5743708							
GG	248 (94.7)	37 (82.2)	**.007*** **(.028)**	28 (90.3)	.398	20 (82.6)	.053
GA	14 (5.3)	8 (17.8)		3 (9.7)		4 (17.4)	
AA	0 (0)	0 (.0)		0 (.0)		0 (.0)	
GA or AA	14 (5.3)	8 (17.8)	**.007*** **(.028)**	3 (9.7)	.398	4 (17.4)	.053
TLR3 rs3775291							
CC	125 (47.7)	17 (37.8)	.187	8 (25.8)	.059	14 (60.9)	.453
CT	108 (41.2)	25 (55.6)		20 (64.5)		8 (34.8)	
TT	29 (11.1)	3 (6.7)		3 (9.7)		1 (4.3)	
CT or TT	137 (52.3)	28 (62.2)	.258	23 (74.0)	**.033*** **(.132)**	9 (39.1)	.395
TLR4 rs4986790							
AA	217 (82.8)	35 (77.8)	.545	20 (64.5)	**.027*** **(.132)**	17 (73.9)	.241
AG	43 (16.4)	10 (22.2)		10 (32.3)		5 (21.7)	
GG	2 (0.8)	0 (.0)		1 (3.2)		1 (4.3)	
AG or GG	45 (17.2)	10 (22.2)	.405	11 (35.5)	**.024*** **(.096)**	6 (26.1)	.400
Haplotypes							
Variant TLR2 and variant TLR4	0 (0)	3 (6.7)	**.003***	1 (3.2)	.103	3 (12.5)	**.001***

Note: Data are presented as numbers (n) and percentages (%). Differences between genotype frequencies of control and study groups were analysed using χ^2 test.

Abbreviations: SNPs, single nucleotide polymorphisms; TLR, toll-like receptors.

[a]Adjusted (a)p-value was calculated with Bonferroni correction.

*The significance was calculated by the Fisher exact test or $\chi 2$ test and two-tailed $p < .05$ considered significant.

of recognizing DAMP molecules. These endogenous molecules are released by injured tissues and necrotic cells (Molteni et al., 2016).

Recognition of LPS activates TLR4 via MyD88 or TRIF-mediated signalling and leads to the synthesis of pro-inflammatory cytokines, chemokines and the expression of co-stimulatory molecules (Vaure & Liu, 2014).

In our study, individuals who carried the *TLR4* rs4986790 variant type were more susceptible to infections. It seems possible that these results reflect the role of TLR4 in the recognition of Gram-negative bacteria. In a previous Finnish study by Vuononvirta et al. (2013), individuals who carried the *TLR4* rs4986790 variant type had an increased risk of repeated nasopharyngeal colonization with the Gram-negative bacterium *Moraxella catarrhalis*, which is one of the three most common bacteria causing AOM (Broides et al., 2009). Moreover, the variant type of *TLR4* is also connected to the development of asthma (Carr et al., 2019; Kozik & Huang, 2019; Pérez-Losada et al., 2018) and can be associated with pneumonia in individuals with a weakened immune system (Verduin et al., 2002).

We also noticed that *TLR4* polymorphism was non-significantly associated with severe invasive infections such as meningitis and sepsis. This finding was in agreement with previous studies in which the polymorphism of *TLR4* was associated with an increased risk of invasive infection (Gowin et al., 2017).

TLR2 is located in the plasma membrane and has a critical role in the recognition of peptidoglycan and lipoproteins from a variety of pathogens. TLR2 co-operates with TLR1 and TLR6. The main ligands

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TABLE 3 Frequencies of genetic variations in children diagnosed with recurrent respiratory infections.

	Controls *n* = 262	Recurrent respiratory infections[c] *n* = 48	*p*-Value* (*ap*-value)[d]	OR (95% CI)
Age (years)[a]	0.25	3.5 (0.6–16.6)		
Sex				
Male[b]	142 (54.2)	33 (68.8)	.062	1.86 (.96–3.59)
Female	120 (45.8)	15 (31.3)		
SNPs				
TLR1 rs5743618				
CC	166 (63.4)	32 (66.7)	.057	
AC	90 (34.6)	12 (25.0)		
AA	6 (2.3)	4 (8.3)		
A-allele	102 (19.5)	20 (20.8)	.757	1.09 (.64–1.86)
C-allele	422 (80.5)	76 (79.2)		
TLR2 rs5743708				
GG	248 (94.7)	40 (83.3)	**.005*** **(.02)**	
GA	14 (5.3)	8 (16.7)		
AA	0 (0)	0 (0)		
G-allele	510 (97.3)	88 (91.7)	**.012*** **(.048)**	3.31 (1.35–8.13)
A-allele	14 (2.7)	8 (8.3)		
TLR3 rs3775291				
CC	125 (47.7)	18 (37.5)	.250	
CT	108 (41.2)	26 (54.2)		
TT	29 (11.1)	4 (8.3)		
C-allele	358 (68.3)	62 (64.6)	.720	1.18 (.75–1.87)
T-allele	166 (31.7)	34 (35.4)		
TLR4 rs4986790				
AA	217 (82.8)	35 (72.9)	.234	
AG	43 (16.4)	12 (25.0)		
GG	2 (0.8)	1 (2.1)		
A-allele	477 (91.0)	82 (85.4)	.09	1.73 (.91–3.29)
G-allele	47 (9)	14 (14.6)		
Haplotypes				
Variant TLR2 and variant TLR4	0 (0)	3	**.0035***	

Note: Data are presented as numbers (*n*) and percentages (%). Differences between genotype frequencies of control and study groups were analysed using χ^2 test.

Abbreviations: CI: confidence interval; OR, odds ratio; SNPs, single nucleotide polymorphisms; TLR, toll-like receptors.

[a]Median age (range).

[b]Odds ratio for males.

[c]Diagnose: 'child's recurrent respiratory tract infections'.

[d]Adjusted (a)*p*-value was calculated with Bonferroni correction.

*The significance was calculated by the Fisher exact test or $\chi 2$ test and two-tailed $p < .05$ considered significant.

of the TLR1-TLR2 heterodimer are tri-acylated lipopeptides such as Pam3CSK4 (Botos et al., 2011; Takeda & Akira, 2015). In the present study, we examined two of the most studied polymorphisms of *TLR1* (rs5743618) and *TLR2* (rs5743708). *TLR1* rs5743618 (Ser602Ile) is non-synonymous polymorphism, which affects the transmembrane domain of TLR1 (Skevaki et al., 2015). Unlike in other populations, in European, and especially Caucasian, populations, the serine allele is more common than the isoleucine allele. Hawn et al. (2007) have shown that carriers of the isoleucine allele had an increased cell surface expression of TLR1 on peripheral monocytes, whereas carriers of the serine allele have decreased signalling ability and produce decreased levels of IL-6 after lipopeptide stimulation.

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TABLE 4 Frequencies of *TLR2* rs5743708 and *TLR4* rs4986790 genotypes and their association with different types of respiratory or severe infections.

	TLR2 genotype			*p*-Value* (*ap*-value)[d]	*TLR4* genotype			
	GG (%)	GA (%)	OR (95% CI)		AA (%)	AG/GG (%)	OR (95% CI)	*p*-Value
Control group ($n = 262$)	248 (94.7)	14 (5.3)	Ref.	Ref.	217 (82.8)	45 (17.2)	Ref.	Ref.
AOM ($n = 62$)[a]	52 (83.9)	10 (16.1)	3.02 (1.41–6.47)	**.012*** **(.024)**	46 (74.2)	16 (25.8)	1.50 (.91–2.47)	.147
Pneumonia ($n = 16$)[b]	13 (81.3)	3 (18.8)	3.51 (1.12–10.98)	.065	11 (68.8)	5 (31.3)	1.82 (.84–3.95)	.117
Invasive infections ($n = 15$)[c]	12 (80.0)	3 (20.0)	3.74 (1.21–11.62)	.055	10 (66.7)	5 (33.3)	1.94 (.90–4.16)	.158

Note: Data are presented as numbers (n) and percentages (%). Differences between genotype frequencies of control and study groups were analysed using χ^2 test.

Abbreviations: AOM, acute otitis media; CI, confidence interval; OR, odds ratio; TLR, toll-like receptors.

[a] 4 Subjects with a mutation in both *TLRs* $p = .001$ ($ap = .002$).

[b] 1 Subject with a mutation in both *TLRs* $p = .058$ ($ap = .116$).

[c] 3 Subjects with a mutation in both *TLRs* $p = .003$ ($ap = .006$).

[d] Adjusted (a) *p*-value was calculated with Bonferroni correction. Subjects having both, variant *TLR2* and *TLR4*.

*The significance was calculated by the Fisher exact test or $\chi 2$ test and two-tailed $p < .05$ considered significant.

TLR2 polymorphism rs5743708 causes amino acid substitution from arginine (Arg) to glutamine (Gln) in the C-terminal end of *TLR2* (753) in the position of 753 and has been associated with TB (Patarčić et al., 2015). In a recent study, Toivonen et al. (2017) reported that *TLR2* polymorphism (rs5743708) was associated with recurrent AOM. In the present study, we found that *TLR2* polymorphism was clearly connected with both an increased risk for AOM and severe infections.

TLR3 recognizes dsRNA, which is produced by many viruses during their replication (Takeda & Akira, 2015). In this study, most of the patients were prone to bacterial infections, which might explain why there were no significant differences between control and study groups with the studied polymorphism of *TLR3* rs3775291. Although previous studies have shown that CVID patients are more susceptible to prolonged rhinovirus infections (Kainulainen et al., 2010), in this study, we could not find any association between *TLR3* polymorphism and CVID.

We used a previously developed panel (Teräsjärvi et al., 2017) to determine four clinically important *TLR* SNPs (*TLR1* rs5743618, *TLR2* rs5743708, *TLR3* rs3775291 and *TLR4* rs4986790) in patients who were prone to infections. All selected SNPs have been found in previous studies to be functional and associated with different infections (Skevaki et al., 2015). Here we show that *TLR2* rs5743708 and *TLR4* rs4986790 variant genotypes were especially more frequent in patients with recurrent or severe infections (3.1 and 3.2). Identifying the risk factors for severe disease is the first step towards helping patients. Moreover, by understanding the underlying pathophysiology, one might then search for better treatment options. Our analysis highlights the need to evaluate the impact of these polymorphisms in various clinical conditions. The study was carried out as part of other immunological studies, so the detailed background data of the patients or causative agent of infection were not available, apart from severe infections (sepsis and meningitis). Further studies with a larger number of subjects and detailed data are needed. That would give a more accurate picture of which type of infections the studied polymorphisms actually increase the risk of.

There are certain limitations in this study. The first was the limited number of study subjects, especially when subjects are divided into separate groups. A small sample size leads to insufficient statistical power, and in some cases, a type II error may occur. Second, only one SNP in each TLR studied was included. Therefore, the possible effect of other SNPs in the same TLR was not included. Third, the present study did not include in-depth mechanism research. In addition, we could not exclude the effect of the broad inclusion of patients with different categories of diseases.

5 | CONCLUSIONS

The results of this study indicate that subjects who carry one or both variant types of *TLR2* (rs5743708) and *TLR4* (rs4986790) are prone to recurrent respiratory infections as well as severe infections. The study contributes to our understanding of recurrent infections in subjects who have polymorphisms in the important genes affecting bacterial recognition and signalling among the host cells. Our findings warrant further studies in different ethnic populations with a large number of study subjects.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

Data will be made available on request.

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ORCID

Qiushui He https://orcid.org/0000-0002-1334-6065

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