Genetic polymorphisms and the development of invasive bacterial infections in children

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Abstract

To evaluate the associations between single nucleotide polymorphisms (SNPs) of factors involved in the development of invasive bacterial disease (IBD) in children, 47 SNPs of 18 candidate genes were analysed in 49 children with IBD and 100 controls. The G/T genotype of TLR2 rs2149356 and the C genotype of LTA rs2229094 were associated with significantly reduced risk of developing IBD ($P = 0.04$ and $P = 0.05$, respectively), whereas the C/T genotype of RFP175 rs1585110 was associated with a significantly higher risk of developing IBD ($P = 0.02$). These results support the evidence that some genetic variants of factors involved in innate immunity may influence IBD risk in children.

Keywords

adaptive immunity, genetic polymorphisms, innate immunity, invasive bacterial disease, *Neisseria meningitidis*, sepsis, *Streptococcus pneumoniae*

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Introduction

Susceptibility to infections varies significantly among humans. A number of studies have suggested that heritable factors play a dominant role in conditioning inter-individual variability.¹ Some genetic polymorphisms appear to be associated with specific diseases, and it has been shown that individuals with rare monogenic defects have increased susceptibility to a narrow range of pathogens.¹ Toll-like receptors (TLRs) are a group of trans-membrane proteins, present in monocytes, macrophages and dendritic cells that play a crucial role in the innate immune system. This is by differentially recognising pathogen-associated molecular patterns through their extracellular receptor modules and initiating inflammatory signalling pathways through an intracellular domain.² Several studies have demonstrated that TLRs are involved in the recognition of pathogens ligands and that they elicit a complex cascade of signalling events.

These culminate in trans-activation of a repertoire of pro-inflammatory cytokines that should favour the elimination of the infectious agents.^{3–6} It has been reported that excessive serum levels of pro-inflammatory cytokines can be frequently found in severe infectious diseases.^{7–9}

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However, studies specifically planned to evaluate the importance of genetics in conditioning susceptibility to and clinical manifestations of infectious diseases have reported conflicting results for all these genetic variants. Understanding the relationships between genetic variations and susceptibility to infectious diseases provides useful insight into the pathogenesis of infectious diseases and allows for the elucidation of host-pathogen interactions, improving preventive strategies and providing novel therapeutic approaches. Despite great breakthroughs in human genomics, current knowledge remains poor in this regard, and further studies are needed to better define the roles of genetic variations in conditioning the development of these different infections. This study describes the associations between single-nucleotide polymorphisms (SNPs) of some factors of innate immunity and the development of invasive bacterial disease (IBD) in children.

Material and methods

Study population

The prospective study was carried out in the Paediatric Highly Intensive Care Unit of the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy, between 1 January 2014 and 31 December 2014. The protocol was approved by the Ethics Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, and written informed consent was obtained from the parents or legal guardian of each patient before enrolment.

All otherwise healthy children admitted to the hospital with a microbiologically documented IBD, i.e. sepsis associated with the culture of a bacterial pathogen from a normally sterile body site,¹⁰ were considered eligible for the study. From each of these patients, a blood sample for genetic study was drawn. During the study period, a control group of twice the number of healthy children, of a similar age and gender distribution, who had never suffered from IBD, was selected from patients admitted to the outpatient clinic for minor surgical interventions. A blood sample for genetic analysis was also collected from each member of the control group.

During the study period, 50 patients with IBD and 100 controls were enrolled. The parents of one

IBD patient refused permission to use genetic data from their child, and this case was not included in the analysis.

Genetic analysis

A total of 47 SNPs of 18 candidate genes, all involved in immune regulation and the pathogenesis of inflammation and sepsis as previously reported,^{11,12} were considered for analysis.^{11,12} These genes encode pattern-recognition receptors (CD14, TLR2 and TLR4), intracellular signalling proteins (IRAK1), pro-inflammatory cytokines (interleukin [IL1] α , IL1 β , IL6 and lymphotoxin-alpha [LTA]), anti-inflammatory cytokines (IL10), chemokines (IL8, CXCL10), bactericidal-permeability increasing protein (BPI), mannose binding lectin-2 (MBL2), beta-defensin1 (DEF β 1), matrix metalloproteinase-16 (MMP-16), serpin1, heat shock protein12A (HSPA12A) and ring finger protein 175 (RFP175). All are located on autosomes, except IRAK1, which is located on the X chromosome. The selected SNPs were the following: TLR2 rs11938228, rs4696480, rs5743708, rs3804099 and rs3804100; TLR4 rs1927911, rs2149356, rs4986790, rs4986791 and rs1554973; CD14 rs2569190; RFP175 rs1585110; IRAK1 rs1059703 and rs3027898; IL1 α rs1800587; IL1 β rs1143643, rs1143633, rs1143627 and rs16944; IL6 rs1800797 and rs1554606; IL8 rs4073; IL10 rs1800872, rs1800896 and rs1800871; CXCL10 rs8878, rs3921, rs4859587 and rs4859588; LTA rs1800629, rs1799964, rs2229094 and rs1041981; MBL2 rs5030737, rs7096206, rs1800451 and rs1800450; BPI rs4358188, rs1341023, rs5743507 and rs2232578; serpin1 rs7242; DEF β 1 rs11362, rs1799946 and rs2741136; MMP16 rs2664349; HSPA-12A rs740598. Most of these SNPs are functional variants or tagging SNPs characterised by the International HapMap Project. Some are known to be involved in the onset, severity or outcome of sepsis in experimental animals or humans,¹¹ and the others have been previously found to be associated with an increased risk of developing specific infections or an abnormal immune response.^{12,13}

DNA was extracted according to the manufacturer's instructions, and a 50- μ L final elution volume was used after purification. The DNA extracted was quantified using the Picogreen reagent (Life Technologies, Monza, Italy) and an Infinite M200

Table 1. Characteristics of the study population.

Characteristic	Patients with invasive bacterial disease (n = 49)	Otherwise healthy controls (n = 100)
Gender, boys (n, (%))	34 (69.4)	68 (68.0)
Age, mean $\pm$ SD (years)	6.5 $\pm$ 5.74	6.3 $\pm$ 5.91
Diagnosis (n (%))		
Pneumococcal empyema	21 (42.8)	—
Pneumococcal meningitis	10 (20.4)	—
Pneumococcal arthritis	9 (18.4)	—
Meningococcal meningitis	9 (18.4)	—
Outcome (n (%))		
Cure	44 (89.8)	—
Hearing loss	5 (10.2)	—
Previous hospitalisation (median (range))	2 (0–4)*	1 (0–2)
Previous use of antibiotics in the preceding 6 months (median (range))	3 (1–6)*	1 (0–3)

* $P < 0.05$ vs. controls; no other significance.

PRO fluorimeter (Tecan Italia, Cernusco sul Naviglio, Italy). Following nucleic acid purification procedures, samples were stored at -20°C until use. Forty-seven SNPs of the 33 genes were genotyped using the Custom TaqMan® Array Microfluidic Cards genotyping system on an ABI 7900HT (Applied Biosystems, Foster City, CA, USA), as described previously.¹² After PCR amplification, the alleles were detected by means of end-point analysis using SDS and TaqMan Genotyper software (Applied Biosystems, Foster City, CA, USA). The data were entered into a Progeny database (Progeny Software, LLC, South Bend, IN, USA) for the generation of datasets for analysis.

Statistical analysis

Genotype frequencies were determined by means of direct counting. In order to investigate Hardy-Weinberg equilibrium (HWE), the expected number of each genotype was compared with the observed number, and potential deviations were assessed using Fisher's exact test. Univariate odds ratios (OR), their 95% confidence intervals (CI) and pertinent P values obtained by Fisher's exact test were calculated in order to measure the associations between selected SNPs and susceptibility to sepsis by comparing all children with sepsis and controls. The data were controlled for multiple testing using the false discovery rate method (with the Benjamini-Hochberg procedure). All statistical analyses were conducted using R software, version 3.1.1, with additional packages genetics and exact2x2.

Results

Table 1 shows characteristics of the study population. Patients with IBD and otherwise healthy controls were similar in gender and age distribution, whereas previous hospitalisations and the use of antibiotics in the preceding 6 months were significantly more frequent in patients than in controls ($P < 0.05$). IBD in 40 (81.6%) cases was due to *Streptococcus pneumoniae* and in nine (18.4%) cases was due to *Neisseria meningitidis* (all due to serogroup B). All the patients with IBD had evidence of sepsis in absence of severe sepsis or septic shock according to established criteria.¹⁰ None of the patients with IBD had significant previous infection history or chronic disease that could predispose them to sepsis and none of them showed sequelae.

In Table 2, genotype frequencies with significant differences in the selected SNPs between controls and children with IBD are reported. The G/T genotype of TLR2 rs2149356 and the C genotype of LTA rs2229094 were significantly more frequent in the controls being associated with a lower risk of developing IBD (OR 0.43, 95% CI 0.19–0.97, $P = 0.04$, and OR 0, 95% CI 0–0.9, $P = 0.05$, respectively), whereas the C/T genotype of RFP175 rs1585110 was associated with a significantly elevated risk of developing IBD (OR 3.85, 95% CI 1.19–11.59, $P = 0.02$). No other significant nor relevant differences were observed in genotype frequencies in the selected SNPs between children with sepsis and otherwise healthy controls.

Table 2. Genotype frequencies with significant differences in the selected SNPs between controls and children with invasive bacterial disease (IBD).

Gene and polymorphic alleles	Control group (n = 100)		Children with IBD (n = 49)		HWE, χ^2 Controls	HWE, χ^2 IBD	Outcome		
	n	%	n	%	P value	P value	OR	95% CI	P value*
TLR4 rs2149356									
G	32	32	25	51	0.83	0.1	1	(reference)	
G/T	48	48	16	32.7			0.43	0.19–0.97	0.04
T	15	15	8	16.3			0.69	0.24–1.88	0.62
NA	5	5	0	0					
RFP175 rs1585110									
C	91	91	39	79.6	0.02	1	1	(reference)	
C/T	6	6	10	20.4			3.85	1.19–11.59	0.02
T	2	2	0	0			0	0–8.31	1
NA	1	1	0	0					
LTA rs2229094									
C	8	8	0	0	1	0.17	0	0–0.9	0.05
C/T	41	41	19	38.8			0.76	0.36–1.56	0.48
T	49	49	30	61.2			1	(reference)	
NA	2	2	0	0					

CI, confidence interval; HWE, Hardy–Weinberg equilibrium; OR, odds ratio.

*P values from univariate analysis, not adjusted for multiple testing. None of the P values were significant after adjusting for multiple testing.

Discussion

This study seems to confirm that some genetic variants of factors involved in innate immunity influence IBD risk in children. In particular, the evidence for a protective role was shown for TLR4 rs2149356 G/T and LTA rs2229094 C, whereas RFP175 rs1585110 C/T appeared to be associated with an increase IBD risk. The finding regarding TLR4 is not surprising considering its roles in the recognition of invading microorganisms and the initiation of the immune response.¹⁴ However, in this study no association was shown between IBD and polymorphisms TLR4 rs4986790 and TLR4 rs4986791, which have been previously reported to be related to a higher susceptibility to a great number of infectious diseases, including meningococcal infections.¹⁴ A possible explanation for these conflicting results is the relatively low number of meningococcal cases among the enrolled patients in comparison to some of the previous studies, which have demonstrated the potential relevance of these TLR4 SNPs in conditioning susceptibility to severe bacterial infections. It is known that meningococcal disease is associated with exceedingly high lipopolysaccharide concentrations and that the bacterial component is a strong TLR4 ligand.¹⁴ Consequently, a high number of meningococcal cases could more easily

evidence the influence of non-functional TLR4. On the contrary, in this study, a protective effect of TLR4 rs2149356 on IBD was found. This seems in line with the data reported by Agúndez et al., who documented a protective effect of this genetic variant for the risk of hepatitis C virus-induced liver carcinoma,¹⁵ but this finding is in contrast with results from Castaño-Rodríguez et al., who described an increased incidence of *Helicobacter pylori* infection in patients with this SNP.¹⁶

In this study, an LTA SNP was significantly less common in children with IBD than in healthy controls. This suggests that genetic variations of this factor, which is a mediator of the sepsis cascade, may play a significant role in protecting the development of severe bacterial infections. The importance of LTA polymorphisms in this regard has been previously reported. It was found that LTA rs13192469 and rs36221459 were associated with leprosy,¹⁷ and LTA rs1800629 was more common in neonates suffering from sepsis due to Gram-negative rods than in age-matched healthy controls.¹¹ However, in this study, no association with IBD was found for LTA rs1800629, whereas LTA rs2229094 was more common among otherwise healthy controls than among patients with IBD. These conflicting findings are difficult to explain

and warrant further study in a larger cohort to evaluate the role of single SNPs in conditioning gene expression.

Data regarding RFP175 rs1585110 C/T have never been previously published. The RFP gene was included among candidate genes because zinc finger domains are protein motifs with various functions, including DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly, and lipid binding.¹⁸ Considering these functions and the importance of zinc in the host defence system,¹⁹ it was hypothesised that polymorphisms of this gene could lead to an increased risk of infection. This supposition seems to be confirmed by the data collected in this study, suggesting a role for RFP175 in controlling susceptibility to severe bacterial infections. Although these results are interesting and encouraging, further studies with a high statistical power on RFP175 mechanism of action are needed.

A limitation of this study is the fact that the study population included a relatively low number of patients. These patients' IBD was mainly due to *Streptococcus pneumoniae* and, in a minority of cases, to *Neisseria meningitidis* serogroup B. The study was a candidate-gene study. Different pathogens may evoke different responses from the immune system, and candidate gene studies are debated because of the poor degree of replication when carried out in a reduced number of individuals. Consequently, caution must be paid in the evaluation of these results. However, considering that these data indicate a potential role for some genetic variations in the protection or development of severe bacterial infections, they justify further studies specifically planned to obtain confirmation of the results. This seems particularly important for the RFP175 gene, as this new information, if confirmed, could significantly improve our knowledge of the mechanisms of host defence against severe bacterial infection.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Chapman SJ and Hill AV (2012) Human genetic susceptibility to infectious disease. *Nature Reviews Genetics* 13: 175–188.
2. Ishii KJ, Uematsu S and Akira S (2006) 'Toll' gates for future immunotherapy. *Current Pharmaceutical Design* 12: 4135–4142.
3. Esposito S, Marchisio P, Orenti A, et al. (2015) Genetic polymorphisms of functional candidate genes and recurrent acute otitis media with or without tympanic membrane perforation. *Medicine (Baltimore)* 94: e1860.
4. Esposito S, Ierardi V, Daleno C, et al. (2014) Genetic polymorphisms and risk of recurrent wheezing in pediatric age. *BMC Pulmonary Medicine* 14: 162.
5. Esposito S, Molteni CG, Giliani S, et al. (2012) Toll-like receptor 3 gene polymorphisms and severity of pandemic A/H1N1/2009 influenza in otherwise healthy children. *Virology Journal* 9: 270.
6. Esposito S, Molteni CG, Zampiero A, et al. (2012) Role of polymorphisms of toll-like receptor (TLR) 4, TLR9, toll-interleukin 1 receptor domain containing adaptor protein (TIRAP) and FCGR2A genes in malaria susceptibility and severity in Burundian children. *Malaria Journal* 11: 196.
7. Khor CC, Chapman SJ, Vannberg FO, et al. (2007) A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteraemia, malaria and tuberculosis. *Nature Genetics* 39: 523–528.
8. Hamann L, Kumpf O, Schuring RP, et al. (2009) Low frequency of the TIRAP S180L polymorphism in Africa, and its potential role in malaria, sepsis, and leprosy. *BMC Medical Genetics* 10: 65.
9. Esposito S, Patria MF, Spena S, et al. (2015) Impact of genetic polymorphisms on paediatric atopic dermatitis. *International Journal of Immunopathology and Pharmacology* 28:286–295.
10. Carcillo JA and Fields AI; American College of Critical Care Medicine Task Force Committee Members (2002) Clinical Practice parameters for hemodynamic support of pediatric and neonatal patient in septic shock. *Critical Care Medicine* 30: 1365–1378.
11. Namath A and Patterson AJ (2009) Genetic polymorphisms in sepsis. *Critical Care Clinics* 25: 835–856.
12. Esposito S, Zampiero A, Pugni L, et al. (2014) Genetic polymorphisms and sepsis in premature neonates. *PLoS One* 9: e101248.
13. Jabandziev P, Smerek M, Michalek J Sr, et al. (2014) Multiple gene-to-gene interactions in children with sepsis: A combination of five gene variants predicts outcome of life-threatening sepsis. *Critical Care* 18: R1.
14. Schröder NW and Schumann RR (2005) Single nucleotide polymorphisms of Toll-like receptors and

- susceptibility to infectious disease. *Lancet Infectious Diseases* 5: 156–164.
15. Agúndez JA, García-Martín E, Devesa MJ, et al. (2012) Polymorphism of the TLR4 gene reduces the risk of hepatitis C virus-induced hepatocellular carcinoma. *Oncology* 82: 35–40.
 16. Castaño-Rodríguez N, Kaakoush NO, Pardo AL, et al. (2014) Genetic polymorphisms in the Toll-like receptor signalling pathway in *Helicobacter pylori* infection and related gastric cancer. *Human Immunology* 75: 808–815.
 17. Ali S, Chopra R, Aggarwal S, et al. (2012) Association of variants in BAT1-LTA-TNF-BTNL2 genes within 6p21.3 region show graded risk to leprosy in unrelated cohorts of Indian population. *Human Genetics* 131: 703–716.
 18. Laity JH, Lee BM and Wright PE (2001) Zinc finger proteins: New insights into structural and functional diversity. *Current Opinion in Structural Biology* 11: 39–46.
 19. Rink L and Gabriel P (2000) Zinc and the immune system. *Proceedings of the Nutrition Society* 59: 541–552.