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Research paper

Genetic susceptibility to invasive pneumococcal disease

Anna Sangil[a,b], María J. Arranz[c,*], Roberto Güerri-Fernández[d], Maite Pérez[e], Helena Monzón[f], Antoni Payeras[g], Marta Andrés[h], Jorge Torviso[h], Laura Ibañez[c], Javier Garau[i], Esther Calbo[a,b,**]

[a] Hospital Universitari Mutua Terrassa, Terrassa, Spain
[b] Universitat Internacional de Catalunya, Barcelona, Spain
[c] Fundació Docència i Recerca Mútua Terrassa, Terrassa, Spain
[d] Hospital del Mar, Barcelona, Spain
[e] Xerencia de Gestion Integrada de Vigo, Vigo, Spain
[f] Hospital San Joan de Deu de Martorell, Martorell, Spain
[g] Hospital Universitari Son Llatzer, Palma de Mallorca, Spain
[h] Consorci Sanitari de Terrassa, Terrassa, Spain
[i] Clínica Rotger QuirónSalud, Palma de Mallorca, Spain

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ABSTRACT

Background: The pathogenesis of IPD remains unknown, especially among middle-aged individuals without risk factors (WRF).
Objectives: The aim of the present study was to investigate the role of single nucleotide polymorphisms (SNP) within key genes involved in innate immune response on IPD susceptibility.
Methods: Forty-three SNPs within 10 immunological genes were investigated in a cohort of 144 Caucasian IPD patients and 280 ethnically matched controls.
Results: The allele distribution of the NFKBIA rs1050851 and NFKBIE rs2282151 variants were associated with IPD susceptibility ($\chi^2 = 4.23$, $p = 0.04$ and $\chi^2 = 5.13$, $p = 0.02$, respectively). Additionally, the genotype distribution of NFKBIZ rs645781 ($\chi^2 = 8.25$, $p = 0.02$) and IL1R1 rs3917254 ($\chi^2 = 6.70$, $p = 0.04$) were also associated with IPD risk. When only IPD-WRF patients were considered; the allele distribution of IL1R1 rs2160227 ($\chi^2 = 5.62$, $p = 0.03$), rs13020778 ($\chi^2 = 5.73$, $p = 0.02$), rs3917267 ($\chi^2 = 3.72$, $p = 0.05$) and IL4 rs2227284 ($\chi^2 = 3.76$, $p = 0.05$) and the genotype distribution of IL10 rs3024509 ($\chi^2 = 7.70$, $p = 0.02$), IL1R1 rs3917254 ($\chi^2 = 13.40$, $p = 0.001$), NFKBIZ rs645781 ($\chi^2 = 13.86$, $p = 0.001$) and rs677011 ($\chi^2 = 9.06$, $p = 0.01$) variants were associated with IPD risk.
Conclusions: We found several associations between variants in the IL1R1, IL4, IL10, NFKBIE, NFKBIA, and NFKBIZ genes and risk of IPD. If validated, these biomarkers may help to identify people with higher risk of IPD.

1. Introduction

Streptococcus pneumoniae is the leading cause of a broad spectrum of diseases including pneumonia, meningitis, bacteremia, and otitis media (Kyaw et al., 2003). Invasive pneumococcal disease (IPD) has a high mortality rate. It accounts for over a million deaths worldwide annually and mortality is highest in children younger than 5 years of age in developing countries (O'Brien et al., 2009).The incidence of IPD declined from 24.4 to 13.5/100,000 in the US, after pneumococcal conjugate vaccine introduction (Pilishvili et al., 2010). We have recently reported similar values in Spain (Sangil et al., 2015).

IPD risk has been associated with pathogen virulence, host susceptibility and epidemiologic factors. Several primary and acquired immunodeficiencies have been associated with IPD susceptibility (Gaschignard et al., 2014). However, the pathogenesis of IPD remains unknown in most patients, especially among middle-aged individuals without known risk factors. Sporadic IPD may be caused by unknown underlying host factors that might confer a selective predisposition to IPD.

Single nucleotide polymorphisms (SNP) of several genes encoding for key molecules in pathways of the innate and adaptive host immunity, complement, cytokines and other inflammatory mediator

Abbreviations: COPD, Chronic Obstructive Pulmonary Disease; HIV, Human Immunodeficiency Virus; Iκb, inhibitors of the NFκb; IL-1R, interleukin-1 receptor type 1; IPD, Invasive pneumococcal disease; NFκb, Nuclear factor-kappaB; SNP, Single nucleotide polymorphisms; WRF, Without risk factors

* Correspondence to: M. J Arranz, Unitat Laboratori Recerca, Fundació Docència i Recerca Mútua Terrassa, 08221 Terrassa, Barcelona, Spain.
** Correspondence to: E. Calbo, Infectious Disease Unit, Service of Internal Medicine, Hospital Universitari Mútua Terrassa, Plaza Dr Robert 5, 08221 Terrassa, Barcelona, Spain.
E-mail address: ecalbo@mutuaterrassa.es (E. Calbo).

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pathways have been associated with susceptibility to IPD (Lingappa et al., 2011; Chapman et al., 2007; Chapman et al., 2010). Other genes including IL10 and TNF alpha have been identified as regulators of the intensity of the inflammatory response and thus associated with the severity and outcome of pneumococcal diseases (Schaaf et al., 2003; Waterer et al., 2001; Wunderink et al., 2002). Genetic variants in five different cytokines (IL1b, IL1R1, Il4, Il10, IL12B) were found associated with IPD risk in a population based study of European-American and African-American children (Lingappa et al., 2011). There is increasing evidence supporting the critical role of the nuclear factor-kappaB (NFκb) pathway in the host immune response to pneumococcal infection (Schaaf et al., 2003). NFκb is a major regulator of innate immunity. Its activation results in the expression of genes encoding both pro and anti-inflammatory cytokines (Barichello et al., 2013). Previous studies have reported associations between genetic variants of the inhibitors of the NFκb (Iκb) protein family and increased risk of IPD (Chapman et al., 2007; Chapman et al., 2010). However, these findings need confirmation in independent studies. We hypothesized that genetic factors in these crucial pathways may contribute to IPD risk and may explain, at least in part, the presence of IPD in middle aged adult patients that do not present known risk factors.

The aim of the present study was to investigate the impact of genetic polymorphisms in ten candidate genes (IL10, IL12B, IL1A, IL1B, ILR1, IL4, NFKBIA, NFKBIE, NFKBIL2 & NFKBIZ) involved in the innate immunologic response on the susceptibility to IPD in a population of middle-aged Caucasian patients and to assess their value as IPD susceptibility biomarkers.

2. Material and methods

2.1. Study sample

2.1.1. Inclusion criteria and clinical variables

Participants were identified through the Microbiology Department database of six acute care centers in Spain. The search was restricted to individuals who suffered an episode of IPD between 1990 and 2013 and were aged 18 to 50 years during the IPD episode. Participants were contacted by phone and blood samples for DNA extraction were obtained at the time of recruitment. Data including the clinical syndrome, severity of disease, outcome, and site of *Streptococcus pneumoniae* isolation were retrospectively collected from the medical notes. Additionally, demographics, comorbidities and presence of IPD risk factors were prospectively recorded via personal interviews. Finally, one hundred and forty-four individuals were recruited for the study (mean age; 38 years ± 7.2, 57% males). The most frequent IPD clinical syndrome was pneumonia as anticipated. Population characteristics and risk factors distribution are summarized in Tables 1 and 2. Risk factors included smoking, COPD or asthma, liver cirrhosis, splenectomy or asplenia, autoimmune disease, hypogammaglobulinemia or hypocomplementemia, active solid or hematologic tumors, immunosuppressive or biological therapies, chronic systemic steroid use (> 20 mg prednisone/day > 15 days), neutropenia, and HIV infection. One third of the recruited patient did not present known risk factors. In addition, 280 ethnically matched healthy adult blood donors were collected from Hospital Universitari Mutua Terrassa. This sample has a statistical power of 95% to detect associations (odds ratios ≥ 2.5) with genetic variants with a minor allele frequency ≥ 0.05. Only Caucasian subjects were analysed to reduce genetic heterogeneity. All participants gave informed consent and the study was approved by the respective Ethics Committees.

Table 1
Characteristics of the study population.

Study population	Controls 280	Cases whole cohort 144	Cases with risk factors 100	Cases without risk factors 44
Mean age, years (SD)	60 (17) [18–91]	38 (7.2)	38.5 (7.2)	37 (7.3)
Male (%)	108 (38.6%)	82 (56.9%)	61 (61%)	21 (47.7%)
Pneumonia	NA	136 (94.4%)	94 (94%)	42 (95.5%)
Meningitis	NA	7 (4.9%)	5 (5%)	2 (4.5%)
Bacteraemia only	NA	1 (0.7%)	1 (1%)	0
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian

Table 2
Description of risk factors in IPD patients (N = 100).

Risk factors[a]	Cases (N = 100)
Smoking	84
COPD or asthma	26
Hepatic cirrhosis	5
Splenectomy or asplenia	1
Autoimmune disease	4
Hypogammaglobulinemia	1
Active solid tumor or hematologic malignancy	2
Immunosuppressive therapy or Biological therapy	4
Chronic systemic steroid use[b]	7
HIV infection	16

[a] Some cases have > 1 risk factor.
[b] > 20 mg prednisone/day > 15 days.

2.2. Genetic characterisation of the sample

DNA was extracted from whole blood using a commercial kit and following the manufacturer recommendations (EZNA SQ blood DNA Kit II, VWR-Omega). DNA samples were stored at −20 °C until required for study. Forty-three informative single nucleotide polymorphisms (SNPs) within the 10 candidate genes (IL10, IL12B, IL1A, IL1B, ILR1, IL4, NFKBIA, NFKBIE, NFKBIL2 & NFKBIZ) were selected for study using the tagSNP option in the hapmap programme (www.hapmap.org, selection criteria: r2 = 0.8 and minor allele frequency = 0.05, see Table 1 for complete list). Two of the selected SNPs (ILR1 rs2160227 & rs2287047) had been associated with IPD risk in a previous study (Lingappa et al., 2011). Genotyping was performed using the Sequenom MassARRAY platform and iPLEX Gold reaction assays (CEGEN, Santiago de Compostela, Spain). Five percent of the samples were retested for confirmation. A genotyping success rate higher than 95% was obtained for all SNPs and samples.

3. Statistical analyses

Statistical analyses were performed using the G*Power Calculator, SPSS (version 21, IBM, USA), PLINK (version 1.07, Purcell et al. 2007) and EpiInfo (Centers for disease control and prevention, version 7.1.4.0) statistical packages. Single marker analyses were performed using chi-square tests to compare the allele and genotype distributions between patients and control subjects. A second analysis was performed including only the subgroup of patients who did not present known IPD risk factors (IPD-WRF). Phased haplotype analyses (using the *E*-M algorithm) including all SNPs within a gene were performed.

4. Results

Tables 3 and 4 summarize the results of the genetic comparisons between IPD patients and controls, and between IPD patients without known risk factors (IPD-WRF) and controls, respectively. All SNPs were in Hardy-Weinberg equilibrium, except IL1A rs2856838 (p = 0.04).

Single marker analyses revealed significant differences in the allele distribution of the NFKBIA rs1050851 ($\chi^2 = 4.23$, p = 0.04) and