

BRIEF COMMUNICATION

Allergy to betalactams and nucleotide-binding oligomerization domain (NOD) gene polymorphisms

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Abstract

Background: Polymorphisms of interleukin genes related to IgE production and inflammation are predictors of hypersensitivity to betalactam, but nothing is known on the influence of NOD genes, despite their association with inflammation and atopy.

Objective: To evaluate the association of NOD2 and NOD1 polymorphisms with betalactam allergy.

Method: We genotyped 3 polymorphisms of NOD2 and 1 of NOD1 in 368 Italian and 387 Spanish patients, compared with 368 and 326 controls, respectively.

Results: CT/TT genotypes of rs2066845 of NOD2 predicted a lower risk in Italy ($P = 0.003$), while WT/insC genotype of rs5743293 (also in leucine-rich repeat domain) predicted a higher risk in Spain ($P = 0.007$). G allele of rs2066845 was associated with a higher level of IgE in the Italian population.

Conclusion: The mirrored influence of these NOD2 polymorphisms on betalactam allergy in two populations suggests a link with pathways of inflammation and/or atopy through mechanisms, which need to be clarified.

Betalactam antibiotics (BL) are frequently involved in drug hypersensitivity (HS) through immediate-type HS mediated by IgE or delayed-type reactions mediated by T cells (1). Nucleotide-binding oligomerization domain (NOD) genes are implicated in immunological and inflammatory diseases such as graft-versus-host and Crohn's diseases (2). They are implicated in the recognition of intracellular bacterial small molecules and modulate inflammatory pathways and

T-regulator/T-helper 2 cells' balance (2). NOD2 and NOD1 polymorphisms are associated with atopy and high total IgE in serum (3–5). Moreover, IFN- γ induces NOD2 expression in keratinocytes from patients with atopic dermatitis (6). Recent studies suggested a link between genetic determinants of atopy and immediate-type allergy to betalactams (7–10). Therefore, the objective of this study was to determine whether 3 polymorphisms in NOD2 gene and 1 polymorphism in NOD1 gene were associated with the occurrence of immediate or delayed BL HS.

Methods

We studied two populations with case-control design. The first study was performed in 368 Italian patients with BL allergy and 368 paired controls. It included 210 cases with

Abbreviations

BL, betalactam; BP, penicillin G (benzylpenicillin); HS, hypersensitivity; IgE, immunoglobulin E; IL, interleukin; IL4R, receptor of interleukin 4; LACTB, serine betalactamase-like protein; NOD, nucleotide-binding oligomerization domain; TNF, tumor necrosis factor.

immediate-type reaction (occurring < 1 h after drug administration, with positive skin tests and/or serum-specific IgE assays) and 158 patients with delayed hypersensitivity (occurring more than 1 h after drug administration, delayed positive intradermal tests with or without patch test confirmation). The second study, performed in Southern Spain, included 387 cases with immediate-type allergic reaction compared with 326 controls. The inclusion criteria were similar to those from the Italian study. The allergologic work-up was performed according to the ENDA (European Network for Drug Allergy) guidelines (11, 12). Atopy was defined as total serum IgE >100 kUI/l and a personal history of allergy (i.e., asthma, rhinitis or atopic dermatitis). The age- and gender-paired volunteers were recruited in preventive care consultations. They had no history of allergic disease nor dermatological or respiratory diseases. Subjects with a C-reactive protein blood concentration higher than 5 mg/l were excluded. All patients and controls were of Caucasian origin. The local ethical committees approved the protocols. The study was approved by the institutional board, and informed consent was obtained from participants, as described previously (7, 8). We extracted genomic DNA from serum samples of Italian subjects and from blood buffy coat of Spanish subjects, respectively (Qiagen, Courtaboeuf, France). The polymorphisms in NOD2 (rs2066844 (p.R702W c.C2104T), rs2066845 (p.G908R c.G2722C), rs5743293 (p.L1007 fs c.3020insC)) and in NOD1 (rs2907749, c.T2453 + 16C) were genotyped by real-time polymerase chain reaction (PCR) (Roche Molecular Biochemical, Lyon, France) in the Italian study, as previously described (7, 13). The genotypes of 3 polymorphisms in NOD2, rs2066844, rs2066845, and rs5743293 (Leu1007fsinsC) were genotyped with the ImmunoChip custom array (Illumina® France Holding, Paris, France) in the Spanish population. Statistical analyses were performed as described previously (8) using STATA 12.1 software (College station, Texas, USA). The minimal size of our sample was estimated at 364 patients, with a study power $1-\beta = 0.8$ and $\alpha = 0.05$, assuming a 2.5-fold difference in the less frequent alleles between controls and patients.

Results

The clinical and biological characteristics of the Italian and Spanish populations are summarized in Table 1 and Table 2, respectively. HS patients had a significant higher concentration of total serum IgE than controls. The CT/TT genotype at position rs2066845 of NOD2 predicted a lower risk to develop HS in the Italian population (odds ratio = 0.28, 95% confidence interval: 0.10–0.70, $P = 0.003$, Table 1), while it had no significant influence in the Spanish population ($P = 0.275$, Table 2). This association was significant in Italian patients with immediate HS, but not in those with delayed HS (Table 1). In contrast, the WT/insC genotype at position rs5743293 of NOD2 predicted a higher risk of BL HS in Spanish (odds ratio = 6.08, 95% confidence interval: 1.37–55.40, $P = 0.007$, Table 2) but not in Italian population ($P = 0.738$, Table 1). The G allele at position rs2066845 was

associated with a higher level of IgE >100 IU/ml in the Italian population ($P = 0.047$). No significant association could be identified with rs2907749 of NOD1 regarding HS and total IgE.

Discussion

We report an association of NOD2 polymorphisms in BL HS, in two populations from Italy and Spain. The rs2066845 of NOD2 predicted a lower risk of BL HS in the Italian cohort, in which its frequency was, respectively, 2-fold and 3-fold higher in controls than in our Spanish population and in a German population (4–6) and similar to that reported in another Italian study (Table 1) (14). In contrast, rs5743293 predicted a higher risk of BL HS in Southern Spain, where its frequency was 8-fold lower in controls than in Italian controls (Table 2). Such mirrored associations of both NOD2 polymorphisms with BL HS have been previously observed for the risk of coronary artery diseases (14). They need to be clarified at the mechanistic level, including the respective consequences of the two polymorphisms on protein functionality (2–5) and gene–environment interactions (8, 14, 15). The p.L1007 fs rs5743293 polymorphism produces a protein truncation in the leucine-rich repeat (LRR) domain. Some studies in transgenic mice and human macrophages suggest that this leads to a ‘gain-of-function’, with hyperactivation of NF- κ B and caspase-1 and enhanced release of tumor necrosis factor- α (TNF α). In contrast, the p.G908R missense rs2066845 polymorphism in the LRR domain decreases responsiveness of phagocytic cells to bacterial glycopeptides, with reduced cytokine production (2).

Besides its strong association with inflammatory diseases, NOD2 has been also associated with atopy and asthma in some, but not in all population studies (4–6). Other polymorphisms associated with atopy and/or inflammation have been previously identified as risk factors for BL HS, in Caucasian populations (7–10, 13, 15). They belong to genes encoding interleukin IL-4, IL-4R, IL-10, IL-13, lactamase B (LACTB), and TNF α , which is a target of NOD2. Recently, Cornejo-Garcia et al. (8) suggested that a structural homology between target human proteins of BL haptenization and environmental allergen could contribute to the link between atopy and BL allergy. We hypothesize that NOD2 gene is involved in this puzzle.

Conclusion

We described a mirrored influence of two NOD2 polymorphisms on BL HS, in two populations. These associations may be related to mechanisms, which need to be clarified.

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Table 1 Clinical and biological characteristics of controls and patients with betalactam allergy, in Italy

Characteristics	Controls <i>n</i> = 368 <i>n</i> (%; 95% C.I.)	Betalactam allergy (BLA) <i>n</i> = 368 <i>n</i> (%; 95% C.I.)	Immediate betalactam allergy (IBA) <i>n</i> = 210 <i>n</i> (%; 95% C.I.)	Delayed betalactam allergy (DBA) <i>n</i> = 158 <i>n</i> (%; 95% C.I.)	<i>P</i> -value BLA vs controls	<i>P</i> -value IBA vs controls	<i>P</i> -value DBA vs controls
Age	44 (28–59)	42 (26–55)	40 (24–55)	41 (28–53)	0.258	0.282	0.441
Female gender	242 (65.6, 61.1–70.8), NA	144 (68.6, 62.2–74.9) 12 (3–60)	144 (68.6, 62.2–74.9) 6 (2–18)	115 (72.3, 64.9–8.7) 24 (6–108)	0.248 NA	0.531 NA	0.149 NA
Time interval (months)	NA	12 (3–60)	6 (2–18)	24 (6–108)	NA	NA	NA
Personal history of atopy	0	102 (27.8, 23.1–32.4)	57 (27.1, 21.6–33.5)	47 (29.6, 23.0–37.0)			
Family history of allergy	0	120 (32.8, 202.2 ± 18.4)	69 (33.9, 26.5–39.3) 263.2 ± 341.3	68 (42.7, 59.5–69.9) 82.31 ± 10.5			
Total serum IgE	31.7 ± 2.4	147 (35.5, 35.3–45.7)	109 (49.3, 42.4–56.1)	38 (24.6, 17.8–31.5)	<0.001	<0.001	<0.001
IgE >100 kU/l	16 (8.1, 4.3–11.9)	276 (75.0, 70.5–79.4)	123 (59.0, 52.3–65.5)	153 (91.1, 86.7–95.5)	<0.001	<0.001	<0.001
Allergy to penicillins	0	92 (25.0, 20.5–29.4)	87 (41.0, 34.5–47.7)	5 (3.1, 0.4–5.9)			
Allergy to cephalosporins	0	92 (25.0, 20.5–29.4)	87 (41.0, 34.5–47.7)	5 (3.1, 0.4–5.9)			
Skin tests, positive to							
Culpit drug *	NA	287 (78.2, 73.9–82.4)	144 (78.3, 72.2–84.2)	143 (96.6, 92.3–8.5)	NA	NA	NA
Penicilloyl polylysine	NA	61 (16.6, 12.8–20.4)	55 (26.2, 20.2–32.2)	6 (3.8, 1.8–8.0)	NA	NA	NA
Minor determinant mixture	NA	69 (18.8, 14.8–22.8)	53 (25.2, 19.3–31.2)	16 (10.0, 6.3–15.7)	NA	NA	NA
Benzylpenicillin	NA	131 (35.7, 30.8–40.6)	56 (26.7, 20.6–32.6)	75 (47.2, 39.5–54.9)	NA	NA	NA
Ampicillin	NA	227 (61.9, 56.9–66.8)	76 (36.2, 29.6–42.7)	151 (95.6, 91.1–7.8)	NA	NA	NA
Amoxicillin	NA	227 (61.9, 56.9–66.8)	75 (35.7, 29.2–42.2)	151 (95.6, 91.1–7.8)	NA	NA	NA
NOD2 rs2066844							
CC	349 (96.1, 94.2–98.1), 14 (3.9, 1.9–5.8)	316 (93.7, 91.0–96.3) 21 (6.3, 3.7–9.0)	170 (93.9, 90.4–97.4)) 11 (6.1, 2.6–9.6)	146 (93.6, 89.7–97.4)) 10 (6.4, 2.5–10.2))	0.150	0.244	0.204
NOD2 rs2066845							
GG	343 (93.2, 90.8–96.0)	332 (97.9, 96.4–99.4)	179 (98.8, 97.3–100.0)	153 (92.7, 90.2–95.3)	0.003	0.006	0.150
GC	24 (6.5, 3.8–8.8)	5 (1.5, 0.1–2.8)	1 (0.5, 0.1–1.6)	4 (2.6, 0.1–5.0)			
CC	1 (0.3, –2.8–8.7)	2 (0.6, 0.0–1.4)	1 (0.5, 0.1–1.6)	1 (0.6, 0.1–1.8)			
NOD2 rs5743293							
WT	359 (97.6, 95.9–99.1)	331 (97.9, 96.4–99.4)	177 (97.8, 95.3–99.3)	154 (98.1, 95.9–100.0)	0.738	0.804	0.707
WT/insC	9 (2.4, 0.9–4.0)	7 (2.1, 0.6–3.6)	4 (2.2, 0.01–4.4)	3 (1.9, 0.4–5.0))			
NOD1 rs2907749							
TT	185 (50.3, 45.2–55.4)	162 (46.3, 40.4–51.1)	96 (49.5, 42.4–56.6)	66 (42.3, 34.5–50.1)	0.605	0.984	0.748
TC	155 (42.1, 37.0–47.2)	159 (45.4, 40.3–50.1)	83 (42.8, 35.7–49.8)	76 (48.7, 40.8–56.6)			
CC	28 (7.6, 4.7–10.1)	29 (8.3, 5.4–11.4)	15 (7.7, 3.9–11.5)	14 (9.0, 4.5–13.4)			

NA: not applicable.

*Performed only in 184 patients with immediate betalactam allergy.

Table 2 Clinical and biological characteristics of controls and patients with betalactam allergy, in Spain

Characteristics	Controls <i>n</i> = 326 <i>n</i> (% , 95% C.I.)	Betalactam allergy (BLA) <i>n</i> = 387 <i>n</i> (% , 95% C.I.)	<i>P</i> -value BLA vs controls
Age	43 (33–53)	49 (37–57)	<0.001
Female gender	198 (60.2, 54.8–65.5),	208 (56.9, 51.9–62.1)	0.394
Time interval (months)	NA	5 (2–10)	NA
Personal history of atopy	39 (11.9, 8.3–15.3)	52 (14.3, 10.6–17.9)	0.344
Total serum IgE	123.0 ± 13.9	195.2 ± 21.8	<0.001
IgE >100 kU/l	82 (25.3, 20.4–29.9)	115 (49.8, 43.3–56.2)	<0.001
Allergy to penicillins	0	343 (93.9, 91.5–96.4)	
Allergy to cephalosporins	0	22 (6.1, 3.6–8.4)	
NOD2 rs2066844			
CC	292 (91.0, 87.8–94.1),	348 (90.9, 87.9–93.7)	0.748
CT	27 (8.4, 5.3–11.4)	34 (8.9, 6.2–11.7)	
TT	2 (0.6, –0.2–1.7)	1 (0.2, –0.2–0.7)	
NOD2 rs2066845			
GG	316 (96.9, 95.1–98.8)	380 (98.2, 96.8–99.5)	0.275
GC	10 (3.1, 1.1–4.9)	7 (1.8, 0.4–3.1)	
NOD2 rs5743293			
WT	324 (99.4, 98.5–100.0)	373 (96.4, 94.5–98.2)	0.007
WT/insC	2 (0.6, –0.2–1.4)	14 (3.6, 1.7–5.5)	

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Author contributions

ACB performed genotyping, analyzed the data, did statistical analyses, and wrote the article; RMGR supervised the genotyping analyses, analyzed the data, did the statistical analyses, wrote the article, and had primary responsibility for final content; AR and MB conducted and performed recruitment and subject testing of the Italian and Spanish populations, respectively, revised the article, and had primary responsibility for final content; JAC and FG performed recruitment and subject testing of the Italian population, and revised the article; AO analyzed the data and did the statistical analysis of

the Spanish study; CC and IG supervised the genotyping analyses, and revised the article; AB conducted the research, and wrote the article; JLG designed and conducted the research, analyzed the data, wrote the article, supervised the whole project, and had primary responsibility for final content.

Conflicts of interest

No conflicts of interest are declared.

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