ASTHMA

Gene Variants, mRNA and NOD1/2 Protein Levels in Tunisian Childhood Asthma

Rafik Belhaj[1,2] · Wajih Kaabachi[1,2] · Ikbel Khalfallah[2,3] · Basma Hamdi[2,3] · Kamel Hamzaoui[1,2,3] · Agnes Hamzaoui[2,3,4]

Abstract

Introduction Asthma is a common respiratory childhood disease that results from an interaction between genetic, environmental and immunologic factors. The implication of nucleotide-binding and oligomerization domain 1 and 2 (NOD1/CARD4, NOD2/CARD15) was highlighted in many inflammatory diseases.

Methods In this case-control study, we analyzed the association of three NOD2 polymorphisms and one NOD1 variant, in 338 Tunisian asthmatic children and 425 healthy Controls, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. We also assessed NOD1 and NOD2 mRNA and protein levels by qRT-PCR and ELISA techniques.

Results The homozygous AA genotype of rs2075820 was a risk factor for asthma (OR 2.39). The influence of the E266K variant in the presence of the heterozygous AG genotype was higher in male than female groups. The homozygous AA genotype was a risk factor associated with asthma, for patients aged between 6 and 18 years OR 2.39, IC95% (1.04–5.49) $p < 0.01$. The mRNA expression of NOD1, but not NOD2, was enhanced in asthma patients compared to Controls. We noted a significant difference between asthmatics and healthy controls in NOD1 protein expression (asthma patients : 31.18 ± 10.9 pg/ml, Controls: 20.10 ± 2.58 pg/ml; $p < 0.001$).

Conclusions The NOD1 rs2075820 variant was associated with a higher childhood asthma risk and the NOD1 expression at mRNA and protein levels was significantly increased in asthma patients.

Keywords Childhood asthma · NOD1/2 · SNP · mRNA · PCR RFLP · E266K

Introduction

Asthma is a common respiratory childhood disease that results from an interaction between genetic and environmental factors [1]. This disorder is characterized by airway

✉ Rafik Belhaj
rafik_belhaj@yahoo.fr

Wajih Kaabachi
Kaabachi.wajih@gmail.com

Ikbel Khalfallah
ikbel.khalfallh@gmail.com

Basma Hamdi
basma.hamdi@gmail.com

Kamel Hamzaoui
kamel.hamzaoui@gmail.com

Agnes Hamzaoui
agnes.hamzaoui@gmail.com

[1] University of Sciences Tunis, Tunis El Manar University, Tunis, Tunisia

[2] Expression Moléculaire des Interactions Cellulaires et de leurs modes de Communication dans le Poumon, Medical Faculty of Tunis, UR/12-SP15, Tunis El Manar University, 15 Rue Djebel Lakdar 1007, Tunis, Tunisia

[3] Department of Respiratory Diseases, Hospital A. Mami, Pavillon B, Ariana, Tunisia

[4] Unit Research Homeostasis and Cell dysfunction, Medicine Faculty of Tunis, 15 Rue Djebel Lakdar 1007, Tunisia, Tunisia

inflammation, increased bronchoconstriction and airway hyperresponsiveness [2]. The innate immune system represents the first mechanism for protection against infection recognizing structures present in many different microorganisms [3].

As a response to allergen exposure, airway lung epithelial cells produce cytokines which initiate an immune response [4]. The pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) or peptidoglycan (PG) are recognized through a set of pattern recognition receptors (PRRs) [5]. The nucleotide-binding and oligomerization domain 1 and 2 (NOD1, NOD2) has an N-terminal caspase-recruitment domain (CARD), intermediate Nod, and C-terminal leucine-rich repeats (LRRs) are responsible for binding/detecting PAMPs [6]. NOD1 is expressed ubiquitously, while NOD2 is only expressed on monocytes, macrophages, dendritic cells and Paneth cells [7]. NOD1 and NOD2 induce NF-kB activation, with subsequent inflammatory cytokine production and co-stimulatory molecules on antigen presenting cells expression [8, 9].

The NOD1 gene (CARD4) is localized on chromosome 7p14–p15, which is an atopy susceptibility locus [10, 11]. Recently, it was shown that single nucleotide polymorphism (SNP) in CARD4 was associated with increased risk of asthma and inflammatory bowel diseases [12, 13]. CARD15 (NOD2) variants were described to be associated with Crohn's disease, ulcerative colitis, early onset sarcoidosis and atopic phenotypes [14–16].

The rs2075820 SNP is located in the coding region of the CARD4 gene, exon 6, which suggest a possible functional effect of the mutation [17]. Three CARD15 polymorphisms were extensively studied for their association with many diseases [18–22]. Two of this polymorphisms are missense mutations (rs2066844 (R702W) and rs2066845 (G908R)) [23], and one is a frameshift mutation (rs2066847 (1007fs)) giving a premature stop codon and the synthesis of truncated protein [24].

In the present study, we have attempted to study SNPs in CARD4 (E266K) and CARD15 (R702W, G908R and 1007fs) genes in asthma childhood patients in order to determine the nature of association between these variants and childhood asthma. We also assessed mRNA and protein level of NOD1 and NOD2 in PBMCs of patients and healthy Controls.

Materials and Methods

Population

The study population included 763 subjects. A total of 338 asthmatic children were recruited from the department of Pediatric and Respiratory Diseases, Abderrahmane MAMI

Hospital of Chest Diseases, Ariana, Tunisia. Asthma diagnosis was established according to GINA recommendations. We measured atopic status according to the prick test. We defined atopy by a positive skin test reaction characterized by a weal of at least 3 mm in diameter, to one or more allergens in the presence of a positive histamine control and a negative uncoated control. The classification of asthma severity was determined due to clinical symptoms and lung function (GINA guidelines). The asthmatic population includes 183 patients diagnosed with atopic asthma and 155 without atopy. As indicated in Table 1, patients were classified according to severity in three groups: mild persistent ($n = 193$), moderate persistent ($n = 118$) and severe persistent asthma ($n = 27$).

Table 1 Clinical characteristics of the asthmatic children population

Characteristic	Asmathic patients n (%)	Healthy controls n (%)
Number of subjects	338	425
Age		
Mean (SD)	7.9	11.4
Range	2-18	5-16
Origin		
Urban	100	–
Rural	13	–
House		
Modern	96	–
Old	16	–
Sex		
Males	196 (57.98)	228 (53.64)
Females	141 (41.71)	197 (46.35)
Atopy		
Yes	183 (62.9)	–
No	108 (37.1)	–
Family history (%)	42 (37.2)	–
Passive smoking		
Yes	75 (34.8)	–
No	140 (65.1)	–
Severity		
Mild persistant	193 (57.1)	–
Moderate persistant	118 (34.9)	–
Severe persistant	27 (7.9)	–
Phenotypes associated		
Rhinitis	23 (22.3)	
Conjunctivitis	7 (8)	
Dermatitis	7 (8)	
RGO	21 (20.6)	
Total IgE (IU/ml)		
> 200 IU/ml	50 (82)	
≤ 200 IU/ml	11 (18)	

SD standard deviation, *RGO* gastro-oesophageal reflux

In addition, 425 age-matched healthy children acted as controls. They were recruited from the emergency department of Tunis children hospital for acute pathologies related to accidents of daily life such as fractures and without chronic, respiratory or allergic manifestations. All controls were free of any infectious symptoms. The written consents were obtained from parents of participating children. The local ethics committee approved all data and sample collections from this study.

CARD4 and CARD15 Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the Salting-out procedure as previously described [25]. The NOD2 rs2066844, rs2066845 and rs2066847 polymorphisms and NOD1 rs2075820 polymorphisms were genotyped using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP)-based method.

The PCR-RFLP conditions are summarized in Table 2.

The digested fragments were visualized on an ultraviolet illuminator after separation on 3% agarose gel stained with 0.1% ethidium bromide.

NOD1 and NOD2 Protein Expression Levels

Nucleotide-binding oligomerization domain containing proteins (NOD1 and NOD2) kit were provided from CUSABIO (ABIN1145824 and ABIN846423, respectively). The detection methods for NOD1 and NOD2 were as reported by the manufacturer's instruction. Detection range for NOD1 and NOD2 was 15.6–1000 and 25–1600 pg/ml, respectively. Sensitivity was 3.9 pg/ml for NOD1 and 25 pg/ml for NOD2.

Total RNA Quantification

Peripheral blood mononuclear cells (PBMCs) were separated on a Percoll density gradient. RNA was extracted by Trizol method and quantified by absorbance at 260 nm, approximately 1 µg of each sample was used to obtain cDNA using the Improm II Reverse Transcription System (Promega, Madison, WI, USA). The qRT-PCR was performed with the Power SYBR® Green Master Mix kit (Life Sciences). GAPDH was used to normalize messenger RNA (mRNA), and probes and primers were taken from Pre-Developed TaqMan Assay Reagents (Applied Biosystems) Forward (5′-ATC ACC ATC TTC CAG GAG-3′) and Reverse (5′-ATG GAC TGT GGT CAT GAG-3′). The primer sequences were Forward (5′-CAG CAC TTT CCC ATG TAT TGA T-3′) and Reverse (5′-TCA AAT CCC ACA CTG CAC A-3′) for NOD1, and Forward (5′-GGT TGA TGC CTG TGA ACT GAA-3′) and Reverse (5′-AAA TGA AAT GGA ACT GCC TCT T-3′) for NOD2. The delta Ct method was used to determine the relative expression of real-time PCR

products, as previously described by Bloch et al. [26]. A comparative threshold cycle (Ct) was used to determine the gene expression relative to a normal control (calibrator). Each set of samples was normalized with the housekeeping gene (GAPDH) using the formula $\Delta Ct = Ct\ BAFF - Ct\ GAPDH$. One of the control samples was then chosen as a calibrator, and relative mRNA levels were calculated using the term $2^{-\Delta\Delta C}t$, where $\Delta\Delta\ Ct = \Delta\ Ct\ sample - \Delta Ct\ calibrator$. Hence, NOD1/2 mRNA levels were expressed as an n-fold difference relative to the calibrator. Each reaction was performed at least in triplicate.

Statistical Analysis

The Hardy–Weinberg's equilibrium (HWE) was explored for all samples by the Chi-square test (χ^2) on a contingency table. All statistical tests were two-sided. Data were analyzed using Epi info version 7 software and SPSS V18. The frequencies of the NOD1/2 polymorphisms were tested and compared between cases and Control by Pearson's χ^2 test or Fisher's exact test, when appropriate. For all of the tests, the level of significance was 0.05. The odd ratios (OR) and the 95% confidence intervals (95% CI) were also calculated to estimate the association of genetic polymorphisms with disease risk. For continuous variables, we used T and Mann–Whitney U tests.

Results

NOD1 and NOD2 Genotyping

We observed a higher frequency of the genotypes AG and AA of NOD1 rs2075820 (E266K) polymorphism in patients than Controls (Table 3). The heterozygous AG genotype and homozygous AA genotype were associated with a higher asthma risk, OR 2.15, IC95% (1.53–3.01), $p < 0.001$ for AG and OR 4.6, IC95% (2.89–7.31), $p < 0.01$ for AA. The frequency of the mutate allele A was higher than the wild allele G and was associated with an increased risk of asthma (OR 1.99, IC 95% (1.62–2.45), $p < 0.01$).

CARD15 rs2066844, rs2066845, rs2066847 SNPs were not polymorphic in our population. The distribution of the three CARD15 variants did not show significant differences between cases and Control groups (Table 3).

The distribution of E266K variant was different according to gender (Table 4). A higher frequency of the genotypes AG ($p < 0.05$) and AA ($p < 0.001$) was observed for patients than Controls for male group (OR 5.99, IC95% (3.13–11.4); OR 2.74, IC95% (1.79–4.2), respectively). Also, in females, higher frequencies were observed for the genotypes AG and AA in patients than Controls groups (OR 2.08, IC95% (1.23–3.52), $p = 0.005$; OR 3.58, IC% (1.81–7.09), $p < 0.001$,

Table 2 PCR-RFLP characteristics

SNPs	Amplification conditions	Exon	Annealing T°	Restriction enzyme	Digestion products	Amorces
1007fs (rs2066847)	94 °C for 5 min 35 cycles of (94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s) 72 °C for 7 min	Exon 13 (NOD2)	60 °C/151pb	ApaI	Wt/Wt 151pb Wt/mt151pb, 130pb, 21pb mt/mt 130pb, 21pb	R 5'-CCT CAA AAT TCT GCC ATT CC-3' F 5'-GGC AGA AGC CCT CCT GCA GGG CC-3'
G908R (rs2066845)	94 °C for 10 min 30 cycles of(94 °C for 30 s,55 °C for 30 s, 72 °C for 30 s) 72 °C for 10 min	Exon 12 (NOD2)	55 °C/380pb	HhaI	GG 380bp GC 380pb, 242pb, 138pb CC 242pb, 138pb	R 5'-AAG TCT GTA ATG TAA AGC CAC-3' F 5'-CCC AGC TCC TCC CTC TTC-3'
R702W (rs2066844)	95 °C for 12 min 30 cycles of (94 °C for 30 s, 66 °C for 30 s, 72 °C for 30 s) 72 °C for 7 min	Exon 8 (NOD2)	66 °C/185pb	MspI	CC 76pb, 54pb, 35pb, 20pb CT 130pb, 76pb, 54pb, 35pb, 20pb TT 130pb, 35pb, 20pb	R 5'-CAC GCT CTT GGC CTC TCA CC-3' F 5'-AGA TCA CAG CAG CCT TCC TG-3'
E266K (rs2075820)	95 °C for 4 min 39 cycles of (95 °C for 30 s, 61 °C for 20 s, 72 °C for 20 s) 72 °C for 10 min	Exon 3 (NOD1)	58 °C/379pb	AvaI	GG 379pb GA 379pb, 209pb, 170pb AA 209pb, 170pb	R 5'-CTT CCC ACT GAG CAG GTT G-3' F 5'-TGA GAC CAT CTT CAT CCT GG-3'

Table 3 Distribution of genotypes and alleles frequencies of NOD1 and NOD2 polymorphisms in asthmatics and controls subjects

Gene	SNPs	Position	Genotype	Allele	Cases	Controls	OR (CI95%)	p value
NOD1	E266k	rs2075820						
			GG		72 (21.30%)	174 (40.9%)	1[a]	
			AG		186 (55%)	209 (49.1%)	2.15 (1.53–3.01)	**0.0000078**
			AA		80 (23,6%)	42 (9.8%)	4.6 (2.89–7.31)	**0.0001**
			AA + AG		266 (78,6%)	251 (58.9%)	2.56 (1.85–3.54)	**0.0001**
				G	330 (48.8%)	557 (65.5%)	1[a]	
				A	346 (51.2%)	293 (34.4%)	1.99 (1.62–2.45)	**0.00001**
NOD2	R702W	rs2066844						
			CC		337 (99.7%)	425 (100%)	1[a]	
			CT		2 (0.3%)	0	ID (0.23–ID)	0.19
			TT		0	0	0	0
				C	675 (99.8%)	850 (100%)	1[a]	
				T	1 (0.2%)	0	ID (0.03–ID)	0.44
	G908R	rs2066845						
			GG		338 (100%)	424 (99.8%)	1[a]	
			GC		0	3 (0.2%)	0.00 (0.00–3.05)	0.25
			CC		0	0	0	0
				G	676 (100%)	849 (99.8%)	1[a]	
				C	0	1 (0.2%)	0.00 (0.00–23.89)	1.00
	3020InsC	rs2066847						
	1007fs	INS C	GG		337 (99.7%)	425 (100%)	1[a]	
		WT G	GC		1 (0.3%)	0	ID (0.032–ID)	0.44
			CC		0	0	0	0
				G	675 (99.8%)	850 (100%)	1[a]	
				C	1 (0.2%)	0	ID (0.032–ID)	0.44

Bold number indicates significant association

ID undetermined value

[a]Reference group

respectively). The influence of the E266K variant in the presence of the heterozygous AG genotype was observed in both gender groups and was higher in male than female group.

Between 6 and 11 years of age, higher frequencies of the genotypes AG and AA were observed in patients than in Controls (OR 2.54, IC95% (1.58–4.07), $p < 0.001$; OR 5.83, IC95% (3.02–11.23), $p < 0.01$, respectively). In the 12–18 year-population, a significant association was observed between asthma and the AA genotype (OR 2.39, IC95% (1.04–5.49), $p < 0.05$) but not the AG genotype (OR 1.41, IC95% (0.76–2.6), $p = 0.26$). There was no association between E266K variant and asthma (OR 3.5, IC95% (0.63–19.29) $p = 0.13$) in subjects less than 5 years (Table 4). The homozygous AA genotype was a risk factor associated with asthma, for patients aged between 6 and 18 years OR 2.39, IC95% (1.04–5.49) $p < 0.01$.

We analyzed our results according to the atopic status. The association of E266K to atopy was similar in atopic but also in non-atopic group (OR 3.43 and 2.64, respectively).

A similar result of E266K was also observed for the investigation with rhinitis. Then, the presence of E266K was not associated nor with atopy neither with rhinitis (Table 5). Stratification based on asthma severity did not show any significant association of E266K SNP and disease severity (Supplementary Table 1). Similarly, stratification according to clinical features and environmental factors (body mass index, gastro oesophageal reflux, conjunctivitis, atopic dermatitis, familial history of atopy, geographical origin, type of house and second hand smoking exposure) indicated no significant association between E266K genotypes variants and asthma risk (Supplementary Table 2).

NOD1 and NOD2 Quantification

We evaluated the expression of NOD1 and NOD2 in peripheral blood immune cells from asthmatic patients. The mRNA

Table 4 Distribution of genotypes frequencies of NOD1 polymorphism according to the sex and age

E266k	Gender	Cases	Controls	OR(CI95%)	p value
rs2075820					
	Men				
	GG	44	101	1	
	AG	105	109	2.21 (1.41–3.44)	**0.0004**
	AA	47	18	5.99 (3.13–11.4)	**0**
	AA + AG	152	127	2.74 (1.79–4.2)	**0.00000226**
	Women				
	GG	28	73	1	
	AG	80	100	2.08 (1.23–3.52)	**0.00569**
	AA	33	24	3.58 (1.81–7.09)	**0.00018**
	AA + AG	113	124	2.37 (1.43–3.93)	**0.00065**
	Age				
	2y − 5				
	GG	10	3	1	
	AG	21	3	2.1 (0.35–12.31)	0.4
	AA	8	0		0.14
	AA + AG	29	3	2.9 (0.5–16.76)	0.22
	6y − 11				
	GG	41	85	1	
	AG	128	104	2.55 (1.62–4.01)	**0.000041**
	AA	58	19	6.32 (3.34–11.98)	**0**
	AA + AG	186	123	3.13 (2.02–4.85)	**0.00000016**
	12y − 18				
	GG	21	89	1	
	AG	35	105	1.41 (0.76–2.6)	0.26
	AA	13	23	2.39 (1.04–5.49)	**0.03**
	AA + AG	48	128	1.58 (0.89–2.83)	0.11

Bold number indicates significant association

OR odds ratio

Table 5 Distribution of genotypes and alleles frequencies of NOD1 polymorphisms according to atopic and rhinitis status

rs2075820	Cases	Controls	p	OR (95% CI)
Atopy				
GG	32	174		1
AG	91	209	**0.0001**	2.36 (1.51–3.71)
AA	31	42	**0.000003**	4.01 (2.21–7.29)
AA + AG	122	251	**0.000008**	2.64 (1.71–4.08)
No atopy				
GG	20	174		1
AG	65	209	**0.0002**	2.7 (1.57–4.64)
AA	34	42	**0**	7.04 (3.68–13.45)
AA + AG	99	251	**0.000002**	3.43 (2.04–5.76)
Rhinitis				
GG	3	174		1
AG	17	209	**0.007**	4.71 (1.36–16.36)
AA	3	42	0.06	4.14 (0.8–21.25)
AA + AG	20	251	**0.007**	4.62 (1.35–15.79)
No rhinitis				
GG	21	174		1
AG	39	209	0,1	1.54 (0.87–2.72)
AA	25	42	**0.000002**	4.93 (2.52–9.64)
AA + AG	64	251	**0.004**	2.11 (1.24–3.58)

Bold number indicates significant association

OR odds ratio

healthy Control for NOD2 expression (Asthma patients: 29.62 ± 7.41 pg/ml; Controls: 31.66 ± 6.59 pg/ml, $p = 0.227$).

Discussion

This is the first study based on the association of NOD1/2 SNPs, mRNA and proteins levels with pediatric asthma in Tunisian population. The results of this study showed a significant association between NOD1 rs2075820 variant and childhood asthma but not for NOD2 (G908R, 1007FS and R702W) SNPs. In the Tunisian population, there is not different ethnic groups.

Several SNPs located in the gene encoding NBD were studied, and different associations with atopy and others clinical manifestations were observed; Reijmerink et al. [27] found that other NOD1 variants, rather than rs2075820, were associated with asthma. Ege et al. [28] showed the interaction of NOD1 SNP rs2075817 with specific IgE, hay fever and wheeze. Hysi et al. [12] found a significant correlation between NOD1 SNPs and elevated IgE levels in the presence of asthma. Weidinger et al. [11] showed an association of NOD1 SNPs with susceptibility to atopic disorders. Tanabe et al. [29] showed that E266K mutant was failed in the recognition of *Propionibacterium acne*. This impaired
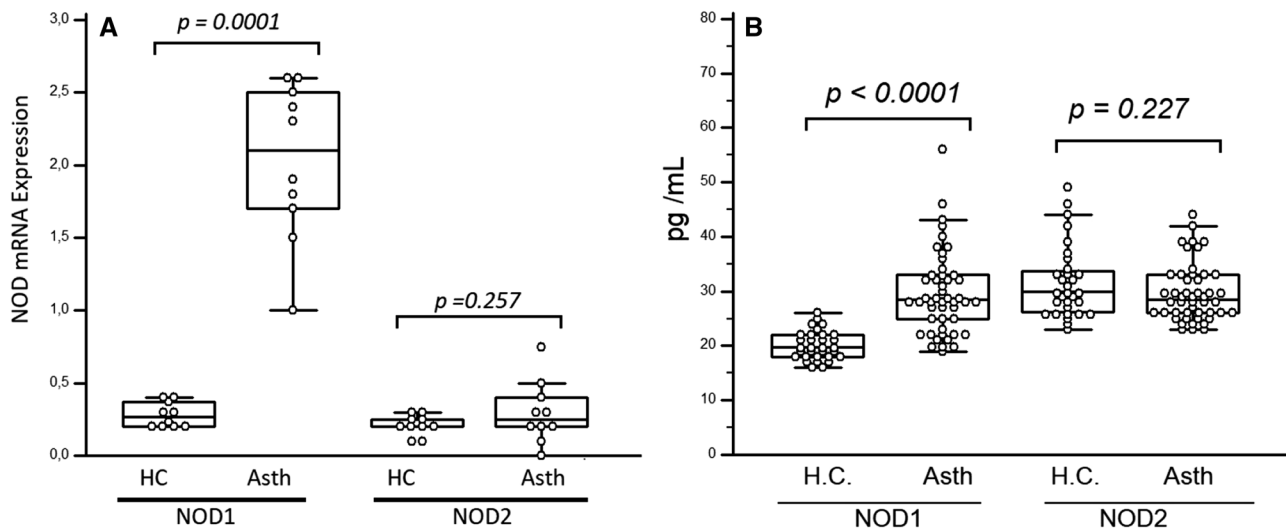
expression of NOD1, but not NOD2, was enhanced in asthma patients compared to Controls (Fig. 1a). Then, we tested NOD1 and NOD2 protein levels (Fig. 1b). NOD1 was significantly expressed for asthmatic patients contrasting with levels in healthy Controls (asthma patients : 31.18 ± 10.9 pg/ml; Controls : 20.10 ± 2.58 pg/ml, $p < 0.001$). In contrast, no significant difference was noted between asthmatics and

Fig. 1 NOD1 and NOD2 expression in asthmatic patients. **a** mRNA expression of NOD1 and NOD2, in asthmatic mononuclear cells isolated from peripheral blood mono nuclear cells (PBMCs). Gene expression was assessed by quantitative real-time polymerase chain reaction (qPCR) and calculated relative to the house-keeping gene as described in the Method. Data are representative of ten independent experiments and are expressed as the means ± SD. **b** ELISA NOD1 and NOD2 expression in serum from 50 asthmatic patients compared to 30 healthy controls. Analysis was performed by the Student's t test (qPCR) or a one-way ANOVA (ELISA)

response was due to the downregulation of the mutant protein. Consequently, given the important role related to NBD, we can suppose that the presence of rs2075820 mutation might affect different signaling pathways and then asthma phenotype risk of development.

Mekki et al. [30] observed a lower frequency of the mutants NOD2 alleles in Tunisian when compared to European and American populations. They did not discern a significant association between Crohn's disease and CARD15 variants in the Tunisian population [30]. In the same population, Feki et al. [31] found that NOD2 (G908R, 1007FS and R702W) variants were not polymorphic for inflammatory bowel disease (IBD) patients. In the Japanese population, Yamazaki et al. [32] showed similar findings considering NOD2 variants.

Therefore, considering NOD1 SNPs, we observed a significant association between rs2075820 variant and asthma risk. Similar results were reported through several studies in colorectal cancer, Crohn's disease, Guillain–Barré syndrome and sarcoidosis [33–36]. In fact, the rs2075820, G796A variation encodes a non-conservative peptide change (E266K) in the Nucleotide Binding Domain of NOD1. The corresponding glutamic acid residue appears to be conserved in CARD15 [34]. This domain is crucial in the oligomerization of the NOD1 and so in the signal transduction particularly for MAPK and NF-κB pathway [35].

Our findings suggest also that the NOD1 at mRNA and protein levels is up-regulated in immune cells of asthmatic patients. Unlike us, other researchers found that only mRNA expression of NOD2 was down-regulated in asthma [37, 38].

A higher mRNA NOD2 level was observed in Behçet disease and rheumatoid arthritis [39, 40]. Bogefors et al. [41] found similar results in NOD1 mRNA expression in allergic rhinitis. However, Kinose et al. [42] did not record an correlation between NOD1/2 expression and COPD exacerbation.

In fact, NOD1 was found to be expressed in the human nose and its expression was down-regulated during pollen season among patients with allergic rhinitis [43]. Kvarnhammar et al. [43] and Wong et al. [44] results suggested that γ-D-glutamyl-meso-diaminopimelic acid (iE-DAP)-mediated NOD1 and NOD2 activation and asthma exacerbation. It was demonstrated that bronchial epithelial cells secrete the chemokine CXCL8 after activation by the NOD1 ligand (iE-DAP) [44]. The activation of NOD1 and NOD2 enhanced the serum concentration of total IgE, chemokine CCL5 and IL-13 in bronchoalveolar lavage fluid of sensitized mice [44]. In a mouse model, AitYahia et al. [45] demonstrated that NOD1 exacerbated lung allergic response through the induction of CCL17 by dendritic cells. NOD1 also suppressed induction of regulatory T cells in a model of allergic rhinitis [46].

Mercier et al. [47] showed that NOD1 can act as a costimulatory receptor for CD8 T cells depending on the adaptor molecule RIP2. Park et al. [48] revealed that NOD1 stimulation on mesothelial peritoneal cells induces secretion of the chemokines CXCL1 and CCL2 which allow neutrophils recruitment to the peritoneal cavity. Neutrophilic infiltration is an important player of inflammation on asthma and was associated with the up-regulation of NOD1, this cell maintain disease's manifestations. In addition, Kvarnhammar

et al. [43] noted that human eosinophils could be activated by NOD1 and NOD2 agonists. IL-5 and GM-CSF enhanced this activation and induced IL-8 production and adhesion molecules expression (CD11b and CD62L) [43]. NOD2 mRNA expression levels from sputum were lower in asthmatic patients compared to healthy volunteers, whereas expression of NOD1 did not vary significantly between groups [37]. Duan et al. observed that intranasal delivery of Nod2 ligand in a mice model induced lung expression of the thymic stromal lymphopoietin (TSLP), IL-25 and OX40 ligand [49]. These 3 molecules blocked the generation of regulatory T cells, the tolerance was blocked [49]. In the same way, patients suffering from allergic rhinitis exhibited lower Nod1 mRNA levels than both controls and patients during pollen season [41].

These findings suggest that NOD family is a major player in asthma participating to eosinophilic airway inflammation, disease manifestations and exacerbations. Moreover, it seems that there is an agonist of NOD1 for asthmatic patients responsible of the up-regulation of this PRR. This constitutive activation could maintain the airway inflammation. Therefore, another limitation of the study is no control for bacterial colonization who seems to be a major player in inflammatory disorders.

Our results suggest a role of NOD1 SNPs and NOD1 expression in childhood asthma. Further studies with a larger sample of patients and other genes will improve our understanding of the contribution of NOD1 and NOD2 to asthma status and atopy.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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