



LETTER TO THE EDITOR

Common *NOD2* mutations are rare in patients with inverse acne

KEYWORDS

Inverse acne; Hidradenitis suppurativa; *CARD15*; *NOD2*

Inverse acne (IA) is a chronic inflammatory skin disease affecting the hair follicles mainly in the axillary, perianal and inguinal area. Its manifestations are cutaneous and subcutaneous nodular inflammation, fistulae and production of a malodorous secretion. Healing occurs with substantial scarring. Both men and women can be affected by the disease. The incidence rates vary between 1% and 4% with a peak in the second and third decade of life. The exact etiology and pathogenesis of IA are unknown. Epidemiological studies have suggested that various factors, such as cigarette smoking and obesity, as well as a positive family history of IA may contribute to the risk of being affected with IA.

Several authors have described a pathogenetic relationship of IA with Crohn's disease (CD) due to a remarkable coincidence of the two diseases and similar histopathological features.

Detailed genome-wide linkage analyses in families with CD have identified several susceptibility loci for the disease. The strongest association is attributable to the *NOD2* gene [1–3]. Besides a number of rare mutations, three single nucleotide polymorphisms in the *NOD2* gene (R702W, G908R and 1007fs) are associated with susceptibility to CD. The risk of CD is increased two to four times in the presence of a single disease-associated polymorphism. It is increased 20–40 times if two variant alleles are present [4]. In healthy individuals, the overall frequency of these three polymorphisms is 7.0–9.0% [1,3,5].

The *NOD2* gene is located on chromosome 16q12 and encodes a cytoplasmatic protein. *NOD2* is impli-

cated in the recognition of muramyl dipeptide (MDP), a component of peptidoglycan present in cell walls of gram-positive and gram-negative bacteria. Binding of MDP activates the transcription factor nuclear factor κ B (NF κ B), leading to transcription of genes for proinflammatory cytokines. *NOD2* expression has been demonstrated in monocytes, cultured intestinal epithelial cells and, recently, in keratinocytes. There is evidence that *NOD2* serves as a pattern recognition receptor in cutaneous innate immunity by sensing pathogenic invading bacteria in keratinocytes and augmenting the production of antimicrobial peptides [6]. The role of *NOD2* in the development of IA has not been investigated yet.

The three main mutations in the *NOD2* gene occur in the leucine-rich-repeats domain or in its neighborhood, suggesting that they alter the recognition of bacterial lipopolysaccharides [5]. Functional experiments performed by Ogura et al., demonstrating that the 1007fs mutation decreased the NF κ B activation by bacterial lipopolysaccharides, support this hypothesis [3]. Mutations within the nucleotide-binding domain of the *NOD2* gene have been associated with two other granulomatous diseases: Blau's syndrome and early-onset sarcoidosis [7].

In this study, we investigated the frequency of the three described *NOD2* variants in a population of patients with IA, hypothesizing that patients with *NOD2* mutations might be more likely to develop chronic inflammatory diseases due to a decreased recognition of bacterial lipopolysaccharides and altered immune response.

DNA samples were obtained from 51 patients with IA and 20 healthy controls. All patients were treated and monitored in the Charité University Hospital, Department of Dermatology and Allergy. A written informed consent was received from all patients. Nucleated cells (peripheral blood mononuclear cells, PBMC) were obtained of each blood sample by density gradient centrifugation, genomic DNA was isolated from PBMC by a standard procedure. Then, PCR for the exons harbouring the three main

Abbreviations: IA, inverse acne; CD, Crohn's disease.

NOD2 variants, i.e. R702W in exon 4, G908R in exon 8 and 1007fs in exon 11, were performed. The peptide change P268S in exon 4, another mutation occurring frequently in patients with CD [5], was not amplified because of its tight linkage disequilibrium with other variants. Previous studies had shown that it occurs in phase with the R702W, G908R, and 1007fs mutations [1].

The primers were used as described by Lesage et al. [5]. Of the five primer sets designed for exon 4 the set 4e was chosen. PCR conditions were applied as originally described for the corresponding primer sets (see above). For direct bidirectional sequencing of all PCR products the BigDye[®] Terminator v1.1. Cycle Sequencing Kit has been used according to the manufacturer's protocol (Applied Biosystems, Weiterstadt, Germany). Automated DNA sequencing was done on the ABI PRISM[®] 310 Genetic Analyser (Applied Biosystems). The sequencing data received were evaluated and aligned by the Sequence Navigator or SeqScape-vs 3.70 software (Applied Biosystems).

The investigators analyzing the genotypes were unaware of the clinical characteristics of the patients. Patients categorized as carrying variants exhibited at least one variant of the *NOD2* gene.

The study cohort included 32 women (62%) and 19 men (38%) of German origin with a median age of 37 years (range, 15–70 years). The median body-mass-index was 27.7 kg/m² (range, 19.8–46.9 kg/m²). Five patients (9.8%) had a positive family history for IA. No patient had CD. On admission, 23 patients (46.9%) had elevated levels of C-reactive protein (>0.5 mg/dl) and 9 patients (18.4%) had elevated white blood cell count (>11.000/nl). The control group included 20 healthy individuals of German origin.

Analysis of the exons 4, 8 and 11 bearing the three main *NOD2* variants identified sequence changes in 6

out of 51 patients (11.8%). Two of these patients were homozygous for the mutation, corresponding to an overall allele frequency of 7.8%. The *NOD2* variants found are displayed in Table 1. None of the shown sequence changes had an allele frequency of >5%. One mutation in exon 8, a 2735 G → C nucleotide change affecting the leucin-rich-repeats domain of the protein, had not been described previously (see Fig. 1). This newly described variant was not analyzed functionally.

Analysis of the healthy controls revealed sequence changes in 3 out of 20 patients (15%, see Table 1, allele frequency 7.5%).

The high frequency of the *NOD2* mutations previously described for CD was not found in this group of patients with IA, but the allele frequencies were similar to the healthy controls (Table 1). Our results are consistent with a recent pilot study, analyzing *NOD2* mutations in 10 patients with IA (allele frequency 5%) [8].

There was no correlation between the presence of *NOD2* mutations and any of the following clinical parameters: sex, body-mass-index, positive family history for IA, smoking, number of body regions effected by IA, levels of C-reactive protein or white blood cell count on admission (data not shown).

There are limitations to our study. In particular, most of the non-coding *NOD2* gene segments were not screened even though they may also harbour pathogenic mutations. Moreover, heterozygous large intragenic *NOD2* deletions may have been missed likewise, since they are not revealed by DNA sequencing methods. Finally, the newly described variant of exon 8 was not analyzed functionally.

In conclusion, our data indicate a rather minor role of *NOD2* mutations in the pathogenesis of IA in patients of German origin.

Table 1 *NOD2* variants in patients with inverse acne

Location and nucleotide change	Peptide change	Variant alleles frequencies				
		IA (number) (n = 51 ptx)	HC (number) (n = 20 ptx)	CD (literature)	HC (literature)	
Exon 4	2104 C → T	R702W	1.9% (2)	10% (2)	11–14% [5,9]	4% [5,10]
	2107 C → T	R703C	2.9% (3)	0	1.2% [5]	0
	2264 C → T	A755V	1.0% (1)	0	1.2% [5]	0
Exon 8	2722 G → C	G908R	1.0% (1)	5% (1)	6–8% [5,9]	1% [5,10]
	2735 ^a G → C	G912A	1.0% (1)	0	0	0
Exon 11	3020insC	1007fs	0	0	2–14% [3,5,9]	2–4% [3,5,10]

IA, inverse acne; CD, Crohn's disease, HC, healthy controls. The main *CARD15* variants are indicated in bold letters.

^a New variant, not previously described.

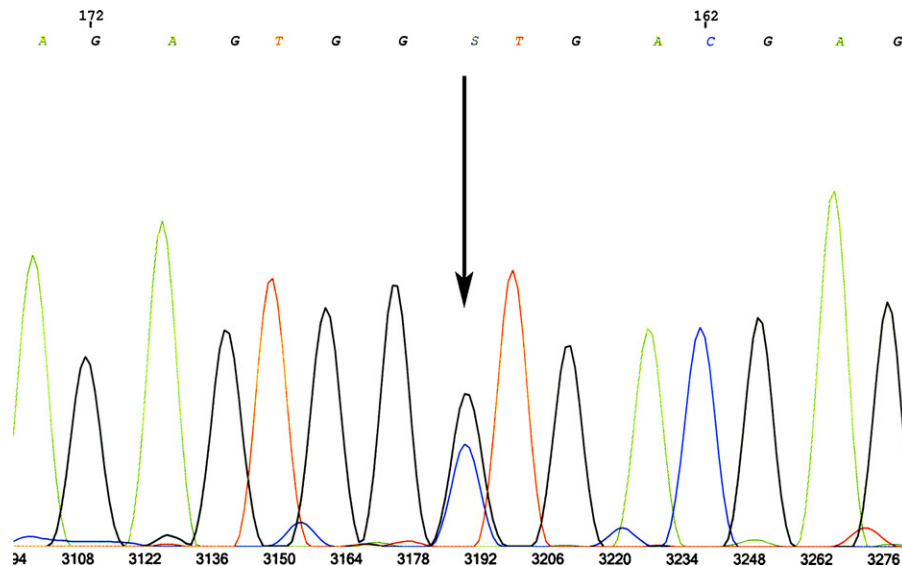


Fig. 1 Electropherogram showing the novel mutation: a heterozygous 2735 G → C exchange (arrow).

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Sylke Schneider-Burrus*

Daniela Meixner

Wolfram Sterry

Ansgar Lukowsky

*Department of Dermatology and Allergy,
Charité, Universitätsmedizin-Berlin,
Berlin, Germany*

*Corresponding author at:

Department of Dermatology,

Venerology and Allergy,

Charité-Universitätsmedizin Berlin,

Charitéplatz 1, 10117 Berlin, Germany.

Tel.: +49 30 54714015;

fax: +49 30 54714016

E-mail address: sylke.schneider@charite.de

(S. Schneider-Burrus)

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