NOD2-associated autoinflammatory disease: a large cohort study

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Abstract

Objective. The aims of the study were to characterize the genotype profile of nucleotide-binding oligomerization domain containing 2 (NOD2)-associated autoinflammatory disease (NAID) and to report an extended study of the disease.

Methods. A total of 143 adult patients presented with clinical phenotypes suspicious for NAID and all were genotyped for NOD2 sequence variants. The genotype frequencies were compared between our cohort and literature reports. These patients were divided into two groups predicated on the presence or absence of NOD2 variants.

Results. Of the 143 patients, 67 (47%) carry NOD2 variants; the genotype frequency was significantly higher among our cohort than in the historical healthy controls. Fifty-four of the 67 carriers of NOD2 variants had NAID, which has a genotype profile that is somewhat different from Crohn's disease. All NAID patients were non-Jewish whites and 69% were women. The median age at onset was 33.5 years and the median disease duration at diagnosis was 10.7 years. NAID was sporadic in 93% of cases. Patients typically presented with periodic fever, dermatitis and inflammatory arthritis. As compared with the NOD2 variant-negative patients, the skin disease more typically manifested as erythematous patches or plaques on the trunk. Oligopolyarthritis/-arthralgia was common, with characteristic distal lower extremity swelling. Associated NOD2 variants were primarily IVS8+158 or compound IVS8+158 and R702W.

Conclusion. This study underscores the NOD2 genotype association with NAID, which is a genetically complex multisystem disorder. It differs phenotypically from Crohn’s disease with a distinct genotype profile. This disease may be more common than initially thought.

Key words: nucleotide-binding oligomerization domain containing 2 (NOD2), NOD2-associated autoinflammatory disease, Crohn’s disease, IVS8+158.

Introduction

Nucleotide-binding oligomerization domain containing 2 (NOD2), also known as caspase recruitment domain-containing protein 15, is a member of a family of intracellular proteins with N-terminal caspase recruitment domains [1, 2]. Since its discovery in 2001, NOD2 sequence variants have been associated with Crohn’s...
disease (CD) [3], Blau syndrome [4] and most recently NOD2-associated autoinflammatory disease (NAID) [5]. The term autoinflammatory disease was initially proposed and published in the journal Cell in 1999 and was defined as episodes of seemingly unprovoked inflammation without high titre autoantibodies or antigen-specific T cells [6]. This group of clinical disorders is marked by abnormally increased inflammation, mediated predominantly by the cells and molecules of the innate immune system with a significant host predisposition [7].

NAID is an emerging systemic autoinflammatory disease. This disorder is characterized by periodic fever, dermatitis, arthritis and gastrointestinal (GI) and sicca-like symptoms and has a genetic association with NOD2 variants [8]. The prevalence of the common NOD2 variants has been well studied and documented in CD and healthy white populations [9]. Recently there has been increasing interest in the study of the role of NOD2 variants in NAID. In this cohort, we studied the frequency of these variants among patients with clinical phenotypes at risk for NAID and compared the data with those among healthy whites and CD patients in the literature. We also report an extended study of the phenotypic and genotypic features of the disease within the cohort, including 22 previously reported cases [8].

Patients and methods

Setting and participants

A total of 143 adult patients were recruited from our tertiary medical centre between November 2009 and May 2014. These patients were referred to our clinic because of the lack of a clear diagnosis, and they presented with clinical phenotypes suggestive of NAID, that is, periodic disease consisting of fever, rash and arthralgia, among other signs and symptoms. Written consent was obtained from the patients according to the Declaration of Helsinki and this study was approved by the Institutional Review Board of the Cleveland Clinic.

Definitions and data collection

The clinical phenotypes for NAID were considered to be present if patients met the following two criteria: periodic disease occurrence, defined as two or more self-limiting episodes of a few days to several weeks’ duration, and recurrent fever of unknown origin and/or dermatitis. The patients had arthritis/arthralgia, abdominal pain/diarrhoea, sicca-like symptoms or serositis. To fulfil the diagnosis of NAID, the patients also had to carry NOD2 variants, mostly IVS8+158 or compound IVS8+158 and R702W or other variants in the absence or presence of low titres (<1:80 by immunofluorescence) of ANAs. The exclusion criteria were autoimmune diseases (IBD, adult sarcoidosis and SS) and other autoinflammatory diseases.

Blood samples from these patients were collected after informed consent was obtained and the samples were individually shipped the next day to the Centre for Genetic Testing at Saint Francis, Tulsa, OK, USA. Genotyping of NOD2 variants was performed by DNA PCR testing and DNA sequencing of all 12 coding exons. The genetic testing results from all 143 patients were analysed for the presence of NOD2 variants, including IVS8+158, R702W, G908R, 1007fs and rare variants. A periodic fever syndromes panel was tested for those patients who had a clinical suspicion of familial Mediterranean fever, TNF receptor-associated periodic syndrome, cryopyrin-associated periodic fever syndrome or hyper-IgD syndrome (GeneDx, Gaithersburg, MD, USA). Besides routine blood and urine tests, serological markers were tested in central laboratories to target potential systemic autoimmune diseases, including classic CTDs and systemic vasculitis.

All 143 patients were carefully observed, followed and prospectively studied. For the genetic association study, the NOD2 genotype frequencies were computed based upon the data obtained from our study cohort and the gene frequencies were compared with those from published historical studies of healthy controls and CD populations (European and North American) [9–11]. These patients were then classified into two groups according to the presence or absence of NOD2 variants (Fig. 1). The clinical phenotypes and laboratory data were compared between the NAID and NOD2 variant–negative patients.

Statistical analysis

For descriptive statistics, continuous variables are expressed as mean (s.d.) and range and categorical variables are presented as frequency or percentage. Chi-square or Fisher’s exact test was used to compute the difference in the NOD2 genotype frequencies between our study cohort and the historical healthy controls or CD populations and between the NAID and NOD2 variant–negative groups. Student’s t-test was used to compare continuous variables between groups. All tests were two-sided and a P-value <0.05 was considered statistically significant. Odds ratios (ORs) were computed with a 95% CI.

Results

Demographic data

Of the 143 patients, 140 (98%) were white with three Jews and three African Americans, and there were 100 (70%) women. The median age at presentation was 45.0 years (range 20–79), and the median disease duration was 12.1 years (range 2–52). Among 54 patients with NAID, the median age at presentation was 44.5 years (range 20–77) and the median disease duration was 10.7 years (range 2.5–9). All were non-Jewish whites and 39 (69%) were women. Of the 67 NOD2-positive patients, 54 had NAID, including 50 (93%) sporadic and 4 familial cases. In addition, there were seven cases of IBD, three unclear diagnoses, two Blau syndrome and one poorly defined autoimmune disease in the group positive for NOD2 variants. Of the 76 NOD2 variant–negative patients, 28 turned out to have autoimmune diseases, and there were 2 cases of Blau syndrome and 2 cases of familial Mediterranean disease.
fever. The remaining 44 patients were still non-diagnostic (Fig. 1).

The NOD2 genotype frequencies within our study cohort vs historical data

Of the 143 patients who were genotyped for NOD2 variants, 67 (47%) were identified as carrying one or more NOD2 variants. With the exception of one patient, most individuals carried heterozygous NOD2 variants, with 19 (33%) patients being compound heterozygous carriers. Compared with the historical healthy controls, disease-susceptibility allele frequencies were significantly higher among our cohort, including 51 (36%) carriers of IVS8+158, 16 (11%) of R702W and 20 (14%) with rare variants (Table 1). The variant IVS8+158 was significantly more common than among white patients with CD (OR 3.13, P < 0.001). The variant R702W was statistically more prevalent than in the healthy controls (OR 2.76, P = 0.015), but there was no statistical difference between our cohort and the historical CD population. The allele frequencies of 1007fs and G908R were not different from those
in the healthy controls, but the frequency of 1007fs was significantly lower than in the historical CD population (OR 0.18, P = 0.001).

Of the 54 NAID patients carrying NOD2 variants, most carried IVS8+158, including 14 (28%) who were compound heterozygous for IVS8+158 and R702W and two compound heterozygous for IVS8+158 and 1007fs. In addition, there were 12 NAID patients who shared all nine rare variants, and 4 of them were compound heterozygotes with the variant IVS8+158 (Table 2).

Clinical and laboratory features of NAID patients

Fever

Recurrent fever occurred in 61% of NAID patients. Half of the patients had high-grade fever, and typically each episode lasted several days. The fever occurred at varying intervals ranging from several weeks to several months (Table 3).

Cutaneous manifestations

Forty-nine (91%) NAID patients had intermittent skin disease, which more often manifested as erythematous patches and plaques, as compared with the NOD2 variant-negative patients. The disease predominantly affected the trunk, although it occurred on the limbs and face as well (Fig. 2A and B). The genital and perianal areas were unaffected. The skin disease recurred at intervals of several weeks to several months. The duration of the rash ranged from several hours to several weeks, but more typically lasted several days. Of the 20 patients who underwent skin biopsies, 15 had dermatitis, including 7 with spongiotic dermatitis (Fig. 2C), 4 with perivascular dermatitis and 4 with other dermatitis. There were four cases of poorly formed granulomatous changes. There was no vasculitis, erythema nodosum or pyoderma gangrenosum reported.

Musculoskeletal manifestations

Arthritic symptoms were common and mainly manifested as oligo/polyarthritis/-arthralgia. Both large and small joints of the upper and lower extremities were affected, but the upper extremities were significantly less frequently involved, as compared with the NOD2 variant-negative patients. Seventeen (32%) NAID patients had distal lower extremity swelling with more unilateral involvement (Fig. 2D). Two patients had MRI of the ankle, which showed s.c. oedema. Morning joint stiffness was present in varying degrees, and the diseased joints appeared non-erosive or non-destructive radiographically. About one-third of patients presented with non-specific limb or generalized myalgia at flares.

Internal organ involvement

Thirty-nine (72%) patients complained of recurrent and intermittent abdominal pain and/or diarrhea of varying degrees. The pain was usually distributed over the mid-lower abdomen, less commonly the left upper quadrant; it was described as cramp-like. Most of the patients had mild non-bloody diarrhea and some had weight loss, but without malnutrition or progressive anaemia. Of these 39 patients, 27 underwent further work-ups, including CT

Table 1 Genotype and its frequency in the present cohort vs the literature

| NOD2 sequence variants | Our data, patients, n/N (%) | Reported Patients, n/N (%) | Compared with the literature [9-11] |
|------------------------|----------------------------|----------------------------|-----------------------------------|
| IVS8+158               | 51/143 (36)               | Healthy                    |                                 |
|                        |                           | CD                         | 22/143 (15)                      | -0.001                          |
|                        |                           | CD                         | 25/166 (15)                      | 3.05 (1.73, 5.38)               |
| R702W                  | 16/143 (11)               | Healthy                    | 9/206 (4.4)                      | 0.015                           |
|                        |                           | CD                         | 98/906 (10.8)                    | 2.76 (1.18, 6.43)               |
| 1007fs                 | 3/143 (2)                 | Healthy                    | 4/206 (1.9)                      | 0.89                            |
|                        |                           | CD                         | 96/906 (10.6)                    | 1.00 (0.59, 1.82)               |
| G908R                  | 3/143 (2)                 | Healthy                    | 2/206 (1.0)                      | 0.40                            |
|                        |                           | CD                         | 55/906 (6.1)                     | 2.19 (0.36, 13.3)               |
| Rare variants          | 20/143 (14)               | Healthy                    | 12/206 (5.8)                     | 0.009                           |
|                        |                           | CD                         | 118/906 (13.0)                   | 2.63 (1.24, 5.57)               |

Table 2 NOD2 sequence variants in 54 NOD2-associated autoinflammatory disease patients

| NOD2 sequence variants | No. of patients | Compound variants, n |
|------------------------|-----------------|----------------------|
| Common variants        |                 |                      |
| IVS8+158               | 46              | IVS8+158 only (30)    |
| R702W                  | 15              | IVS8+158 + R702W + others (14) |
| 1007fs                 | 2               | IVS8+158 + R702W + 1007fs (1) |
| G908R                  | 1               | IVS8+158 + R702W + G908R (1) |
| Rare variants          |                 |                      |
| N289S                  | 2               | IVS8+158 + R702W + A211A (1) |
| A211A                  | 2               | IVS8+158 + S431L + V793M (1) |
| S431L                  | 2               | IVS8+158 + A885P + R753G (1) |
| V793M                  | 2               | S431L + V793M (1)     |
| R709Q                  | 1               | IVS8+158 + R702W + A885P (1) |
| A885P                  | 1               | IVS8+158 + 1007fs + R709Q (1) |
| R753G                  | 1               | IVS8+158 + A885P + R753G (1) |
| R703C                  | 1               |                      |
| T189M                  | 1               |                      |

in the healthy controls, but the frequency of 1007fs was significantly lower than in the historical CD population (OR 0.18, P = 0.001).

Of the 54 NAID patients carrying NOD2 variants, most carried IVS8+158, including 14 (28%) who were compound heterozygous for IVS8+158 and R702W and two compound heterozygous for IVS8+158 and 1007fs. In addition, there were 12 NAID patients who shared all nine rare variants, and 4 of them were compound heterozygotes with the variant IVS8+158 (Table 2).
enterography, oesophagogastroduodenoscopy and colonoscopy, with unremarkable results in 24 patients. Three patients had non-specific colitis and one had granulomatous changes. However, there was no convincing evidence of IBD among these patients. Nine (14%) patients experienced recurrent chest pain, with five having pleuritis and/or pericarditis. There was one case of bronchitis and one case of non-specific interstitial pneumonitis. Three patients had a transient elevation of liver enzymes. There was no renal or CNS involvement.

Sicca-like symptoms and other manifestations

Thirty (56%) patients complained of dry eyes and/or dry mouth, without cogent evidence of primary SS. One patient had eyelid swelling, eye pain and colour vision loss consistent with ocular myositis. No patients had uveitis. Headache occurred in 56% of patients, recurrent oral ulcers in 26%, lymphadenopathy in 9% and sore throat in 5%.

Laboratory findings

Eight (16%) NAID patients had mild anaemia and seven (14%) had slight leucocytosis. Acute phase reactants were elevated in 21 (43%) patients. Only four patients were found to have low ANA titres (1:80).

Treatment of NAID patients

The therapeutic approach is currently empirical and based on the clinical manifestations of the disease. The medications used to treat the disease in the present cohort included NSAIDs in 20 (35%) patients, glucocorticoids in 21 (37%), SSZ in 18 (32%) and biologic agents. Arthritic symptoms typically did not respond to NSAIDs. Oral prednisone and SSZ were effective. Two patients were treated

### Table 3

Clinical and laboratory features of NAID patients

| Clinical characteristic | NAID       | NOD2 variant-negative | P-value |
|-------------------------|------------|-----------------------|---------|
| Age, mean (s.o.) [range], years | 44.5 (14.2) [20–77] | 45.2 (12.1) [21–79] | 0.78    |
| Female                   | 37 (69)    | 54 (71)               | 0.76    |
| Male                     | 17 (32)    | 22 (29)               |         |
| White                    | 54 (100)   | 73 (96)               | 0.27    |
| Disease duration, mean (s.o.) [range], years | 10.7 (8.5) [2.5–39] | 12.0 (11.4) [2–47] | 0.51    |
| Age at onset, mean (s.o.), years | 33.5 (14.2) | 33.2 (15.4) | 0.91    |
| Weight loss              | 22 (41)    | 20 (26)               | 0.08    |
| Myalgia                  | 19 (35)    | 22 (29)               | 0.45    |
| Family history           | 4 (7)      | 4 (5)                 | 0.72    |
| Fever                    | 33 (61)    | 36 (47)               | 0.12    |
| Interval (weeks to months) | 20 (83) (n = 24) | 8 (47) (n = 17) | 0.014   |
| Rash                     | 49 (91)    | 67 (88)               | 0.64    |
| Lasting several days     | 10 (50) (n = 20) | 3 (15) (n = 20) | 0.018   |
| Patches/plaques          | 40 (82)    | 33 (49)               | <0.001  |
| Trunk                    | 25 (51)    | 13 (19)               | <0.001  |
| Skin biopsy              |            |                       |         |
| Spongiotic dermatitis    | 7 (35) (n = 20) | 7 (32) (n = 22) | 0.83    |
| Granulomatous            | 4 (20) (n = 20) | 3 (14) (n = 22) | 0.69    |
| Perivascular dermatitis  | 4 (20) (n = 20) | 2 (9) (n = 22) | 0.40    |
| Other dermatitis         | 4 (20) (n = 20) | 2 (9) (n = 22) | 0.40    |
| Arthritis/arthralgia     | 47 (87)    | 66 (87)               | 0.97    |
| Oligoα                   | 14 (30)    | 8 (12)                | 0.019   |
| Polyα                    | 32 (68)    | 56 (85)               | 0.034   |
| Upper extremitiesβ       | 26 (55)    | 52 (79)               | 0.008   |
| Lower extremity swellingβ| 17 (32)    | 9 (12)                | 0.006   |
| Sicca-like symptoms      | 30 (56)    | 44 (58)               | 0.79    |
| Oral ulcers              | 14 (26)    | 20 (26)               | 0.96    |
| Gut                      | 39 (72)    | 45 (59)               | 0.13    |
| Chest pain               | 9 (14)     | 16 (21)               | 0.30    |
| Heart                    | 3 (6)      | 6 (8)                 | 0.74    |
| Lungs                    | 4 (7)      | 3 (4)                 | 0.45    |
| Liver                    | 3 (6)      | 5 (7)                 | 1.00    |
| Leucocytosis             | 7 (14)     | 7 (10)                | 0.55    |
| Anaemia                  | 8 (16)     | 7 (10)                | 0.36    |
| Elevated ESR/CRP         | 21 (43)    | 23 (35)               | 0.38    |
| ANA                      | 4 (7)      | 8 (11)                | 0.76    |
| NOD2 sequence variantsβ  | 54 (100)   | 0 (0)                 | <0.001  |

All values are presented as n (%), unless stated otherwise. αCompared with NOD2 variant-negative patients, P < 0.05. βCompared with NOD2 variant-negative patients, P < 0.01. NAID: NOD2-associated autoinflammatory disease.
with infliximab and tocilizumab with modest success. One patient was treated with the anti-IL-1β mAb canakinumab 150 mg s.c. every 8 weeks over 6 months. There was improvement within 1 week and significant improvement in the rash, inflammatory arthritis and overall physical functionality over time.

Discussion

The NOD2 gene is mapped to chromosome 16q 12-21 [1], and 128 NOD2 variants have been reported to date (http://fmf.igh.cnrs.fr/ISSAID/infevers). The structures of the NOD2 gene and protein are schematically shown in Fig. 3 [10]. It is well known that the three main NOD2 variants (R702W, G908R and 1007fs) are linked to CD [3, 11] and the NOD2 variants in the nucleotide-binding domain region are involved in Blau syndrome [4, 12]. We previously reported 22 cases of NAID and linked them to the disease association with NOD2 variants [8]. We hypothesized that the patient population with clinical features at risk of NAID may have a higher prevalence of NOD2 variants than the general population. Since NAID has clinical phenotypes distinct from those of CD, the NOD2 genotype profile may be different between the two diseases. In this large cohort we found that the NOD2 genotype was significantly more prevalent among the patients with clinical suspicion for NAID relative to the historical healthy controls. This study reinforces and extends our prior reports that certain NOD2 variants are associated with NAID distinct from CD.

Previous studies have shown that ~28% of white patients with CD in North America and Europe carry NOD2 variants, with 1007fs being responsible for 11%, R702W for 11% and G908R for 6% [9, 13, 14]. Among our cohort of 143 patients, we identified 2% of patients carrying 1007fs and 2% with G908R. These two allele frequencies were similar to those in the healthy controls (1007fs, 1.6-3.8%; G908R, 1.1-3.0%), but the frequency of 1007fs was significantly lower than among the CD patients. These data suggest that the NOD2 genotype profile is different to some extent between NAID and CD patients. Conversely, the variant R702W rate among our cohort was significantly higher than in the healthy controls (R702W, 3.3-3.7%) with a disease OR of 2.76, but was similar to that among the CD patients. Among the variant R702W carriers in our study, there was no evidence of CD. These data suggest that the variant R702W is linked not only to CD, but also to NAID. Most notably, we found that 36% of the 143 patients...
carried the variant IVS8+158, with a disease OR of 3.05 vs the healthy controls. It is worth mentioning that an initial study by Sugimura et al. [15] identified the frequency of the variant IVS8+158 to be ~15% in both healthy controls and non-Jewish whites with CD. These data persuasively support our prior study results that the NOD2 variant IVS8+158 is associated with NAID.

IVS8+158 is a C>T sequence variant in the palindrome sequence in the intron 8 splicing region of the NOD2 gene [15]. The original report of a potential genotype association with Jewish CD patients was not substantiated later [16, 17]. Instead, it is a common variant identified among NAID patients and interestingly coexists with the mutant R702W in about 28% of NAID patients. One possible explanation is that the haplotype with R702W and IVS8+158 may have arisen through an ancestral recombination or gene conversion event [17]. Of note, there were 28 patients with systemic autoimmune disorders in the group negative for NOD2 variants in our study. Together, these data suggest that the variant IVS8+158, particularly coupled with R702W, could influence susceptibility to NAID. Nevertheless, it presently remains unclear whether these variants may be causative (direct association) or serve as markers in linkage disequilibrium (indirect association) in the disease. Currently NOD2 variants are used as markers for the diagnosis of NAID. In addition, the gene frequency of the rare variants was significantly higher in our cohort than in the historical healthy controls, and 4 of the 12 carriers of the nine rare variants had IVS8+158 concurrently. All 12 patients shared a similar phenotype of NAID without IBD. Further study will be needed to explore the function of the rare variants in this disease.

We previously reported a new category of autoinflammatory disease associated with NOD2 variants in 2011 [5], and this disease has been designated as NAID after further expanding the study and characterizing the disease in 2012 [8]. NAID is a multisystem inflammatory disease. Unlike monogenic autoinflammatory diseases, NAID patients may present with various manifestations. Clinical characteristics of the disease consist of periodic episodes of fever, dermatitis, inflammatory arthritis with pedal swelling, serositis and GI and sicca-like symptoms. Occasionally there may be occurrence of ocular myositis [18]. Acute phase reactants can be elevated, and there are no features of systemic autoimmune diseases. This new entity is invariably associated with certain NOD2 variants. The differential diagnosis of NAID is broad, but it may centre mainly on CD, Blau syndrome and other systemic autoinflammatory diseases. Given our previous detailed summarization of the major differentiating features between NAID, Blau syndrome and other major autoinflammatory diseases [8], we will focus here on the differences between NAID and CD. Dermatitis presenting as erythematous patches/plaques accounts for 90% of patients with NAID, whereas it is extremely rare in CD, in which the most common cutaneous manifestations are erythema nodosum and pyoderma...
gangrenosum [19, 20]. While NAID patients may present with abdominal pain/diarrhoea, these symptoms are much milder and less bloody than CD, based upon our observation. Most notably, NAID patients typically do not have endoscopic or pathological findings of CD, although non-specific colitis is sometimes seen. In addition, febrile episodes and articular manifestations are much more common in NAID than in CD.

In this cohort we also compared the clinical manifestations between the NAID and NOD2 variant-negative patients in order to further characterize the clinical phenotype of NAID. These study results reinforce and extend our prior studies and observations. This disease predominantly affects women, is mostly sporadic and is mostly likely genetically complex. The disease typically occurs at an interval of several weeks to months, with each episode lasting several days. The rash is represented by erythematous patches and plaques primarily occurring on the trunk. Oligopolyarthritis/-arthralgia more often affects the medium to large joints of the lower extremities than the upper extremities. Distal extremity swelling is more common [21]. NAID is a multisystem disease, yet it rarely affects solid internal organs.

In order to roughly estimate the population prevalence of the disease using this large cohort, we calculated the total number of NAID patients seen annually as the numerator and the total number of patients with rheumatic diseases seen in our rheumatology outpatient clinic as the denominator. The frequency of NAID was estimated to be 3–7% among our rheumatology outpatients. By this measure and relative to a yearly census of the patient population with SLE at our clinic, the population prevalence of NAID is estimated to be 1–10/100,000. Future epidemiological studies will be needed to more accurately predict the prevalence of the disease.

The autoinflammation of NAID is unclear and requires investigation. Concerning the potential pathogenic roles of the NOD2 variants, data have been derived primarily from prior studies of CD [22–24]; clues can be obtained from the studies of CD patients. The NOD2 protein resides predominantly in the cytoplasm. As a sensor of bacteria, the NOD2 protein detects a specific component of bacterial cell wall peptidoglycan (muramyl dipeptide), leading to activation of nuclear factor κB [25, 26]. Normally NOD2 serves as a defence mechanism of the gut, whereas disease-associated NOD2 variants may predispose to intestinal inflammation via decreased NOD2 function [27]. Since both CD and NAID involve GI symptoms, we speculated that like CD, a complex interaction of genetics and cofactors (GI infection) may underlie the NAID pathogenesis [28]. Our preliminary study suggested that NAID may involve alterations in the cytokine IL-17, Th17 cells and specific regulatory T cells [29]. Further study of the disease mechanism is warranted.

In this large cohort study, certain NOD2 variant frequencies were significantly higher among patients at risk for NAID, highlighting the NOD2 genetic association with NAID. It represents a genetically complex multisystem disorder and is phenotypically different from CD, with a distinct genotype profile. This disease may be more common than previously thought. We hope that this report will raise awareness of the clinical entity in the medical community.

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