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Authors

Jacob, Noam
Dasharathy, Sonya S
Bui, Viet
et al.

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Generalized Cytokine Increase in the Setting of a Multisystem Clinical Disorder and Carcinoid Syndrome Associated with a Novel *NLRP12* Variant

Noam Jacob[1,3], **Sonya S. Dasharathy**[1], **Viet Bui**[1,2], **Jihane N. Benhammou**[1,3], **Wayne W. Grody**[4,7], **Ram Raj Singh**[1,2,5,6,7], **Joseph R. Pisegna**[3,4]

[1]UCLA Vatche and Tamar Manoukian Division of Digestive Diseases, Department of Medicine, David Geffen School of Medicine at University of California at Los Angeles (UCLA), Los Angeles, CA 90095, USA

[2]Autoimmunity and Tolerance Laboratory, Division of Rheumatology, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

[3]Division of Gastroenterology, Hepatology and Parenteral Nutrition, Department of Veterans Affairs, David Geffen School of Medicine at UCLA, VA Greater Los Angeles Healthcare System (691/111C), 11301 Wilshire Blvd., Los Angeles, CA 90073, USA

[4]Departments of Human Genetics and Pediatrics, and the UCLA Clinical Genomics Center, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

[5]Molecular Toxicology Interdepartmental Program, UCLA, Los Angeles, CA 90095, USA

[6]Jonsson Comprehensive Cancer Center, UCLA, Los Angeles, CA 90095, USA

[7]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

Abstract

Background—Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are a group of cytoplasmic sensors that survey danger signals released by invading pathogens or damaged tissue. Mutations in the *NLRP* subfamily affect pro-inflammatory mediators and cause nonspecific systemic symptoms.

Aims—We sought to identify a potential genetic etiology of an inflammatory syndrome in a patient that presented with an atypical multisystem illness with carcinoid syndrome as well as atopic and autoimmune features.

Methods—Exome sequencing was performed using the Agilent SureSelect Clinical Research Exome XT kit on an Illumina HiSeq 2500. Longitudinal monitoring of pro-inflammatory cytokines was performed.

Joseph R. Pisegna, jpisegna@ucla.edu.

Conflicts of interest The authors have declared that no conflicts of interests exist.

Results—We identified a novel variant (heterozygous c.536C > T [p.Thr179Ile]) in the *NLRP12* gene in a 63-year-old woman and her daughter, who presented with an unusual clinical syndrome that differs from autoinflammatory disorders previously reported in association with the *NLRP* subfamily gene mutations. This *NLRP12* variant was predicted to be pathogenic by functional analysis through Hidden Markov Models (FATHMM). Both the mother and the daughter had episodes of abdominal pain, fever, diarrhea, skin rash, hypothyroidism, and elevated urine 5-hydroxyindoleacetic acid (5-HIAA) levels. The proband also had elevated serum levels of pro-inflammatory (IL-1β, IL-6, IL-12, and TNF-α), Th1 (IL-2, IFN-γ), and Th2 (IL-4, IL-5, IL-13) cytokines, but not of Th17 (IL-17) and IL-10.

Conclusion—This report adds to the expanding spectrum of clinical manifestations attributed to the *NLRP* subfamily gene variants and suggests a role of *NLRP12* in the regulation of multiple cytokines.

Keywords

Autoimmune; Autoinflammatory; Cytokines; Genetic syndrome; Gastrointestinal

Introduction

Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are a group of cytoplasmic sensors that survey danger signals released by invading pathogens or damaged tissue [1]. Mutations in the *NLRP* subfamily affect pro-inflammatory mediators and are associated with autoinflammatory disorders known as hereditary periodic fevers (HPFs) [2]. These disorders cause nonspecific systemic symptoms, which make them a diagnostic challenge and often overlooked. We describe a previously unreported variant c.536C > T (p.Thr179Ile) in *NLRP12* in association with an atypical multisystem illness with carcinoid syndrome as well as atopic and autoimmune features, the latter potentially related to the role of *NLRP12* in regulating inflammatory cytokine production.

Clinical and Laboratory Data

A 63-year-old female patient of northern and central European descent presented to the gastrointestinal clinic for symptoms of abdominal pain and non-bloody diarrhea. She also described a history of chest pressure, tachycardia, shortness of breath, flushing, and hypoglycemia. At the time of presentation, the patient's symptoms were partially improved with subcutaneous octreotide; however, she continued to have episodic abdominal pain and intermittent elevations in hepatic transaminases. In addition, she reported a long-standing history of symptoms including fevers, oral ulcers, Raynaud's phenomenon, polyarthritis, and a mild malar rash. At one point, she was diagnosed with undifferentiated connective tissue disorder with a positive antinuclear antibody at 1:160 and low titer IgM anti-cardiolipin antibody. She was previously treated with prednisone, methotrexate, and azathioprine and eventually transitioned to leflunomide with some improvement in symptoms. None of these symptoms were affected by exposure to environmental temperature changes.

In addition, the patient's history was significant for reported numbness and tingling in the base of the neck and right upper extremity, and she was diagnosed with thoracic outlet

syndrome (TOS) with a positive nerve conduction study. She underwent two surgeries with subtotal resection of multiple muscles, and biopsy of the right scalene muscle revealed fibrosis and chronic inflammation.

The past medical history is also significant for hypothyroidism, asthma, bilateral avascular necrosis of the shoulders requiring decompression surgeries, endometriosis, IgG deficiency requiring intravenous immunoglobulin, and gastroesophageal reflux. Her social history is negative for smoking or alcohol, and additional family history is significant for colon cancer in her mother. Her physical exam was largely normal except for hepatomegaly. Laboratory studies demonstrated a normal white blood cell count, but with a differential significant for an eosinophil count as high as 240 cells per microliter of blood. Additionally, she had intermittently abnormal liver tests with elevated transaminases and alkaline phosphatase and elevated lipase levels ranging from 66 to 132 units per liter. On subsequent evaluation when the patient was receiving immunosuppressive treatment, autoantibodies, including repeat antinuclear antibody, were negative. Although her 24-h urine 5-hydroxyindoleacetic acid (5-HIAA) was elevated, serum insulin, C-peptide, gastrin, serotonin, and chromogranin-A levels were all within normal range. Transglutaminase IgA, endomysial IgA, and gliadin antibodies were negative. Hepatitis serologies and EBV IgG antibody were non-reactive. Liver biopsy showed periportal inflammation.

Given her elevated urine 5-HIAA and symptoms concerning for carcinoid syndrome, the patient underwent extensive gastrointestinal workup including octreotide and ^{68}Ga-DOTATATE PET/CT scans, small bowel enteroscopy, and endoscopic ultrasound which showed elevated DOTATATE activity localized to a small focus in the left hepatic lobe, along the lesser curvature of the stomach, tail of the pancreas, and small bowel. Her abdominal symptoms improved after treatment with octreotide. MRCP revealed mild prominence of the central biliary tree and hepatomegaly, but no pancreatic tumor.

Genome-level sequencing was requested due to concern for one of the multiple endocrine neoplasias or autoinflammatory disorders, given her constellation of symptoms. Exome sequencing was performed using the Agilent SureSelect Clinical Research Exome XT kit on an Illumina HiSeq 2500 and identified a heterozygous c.536C > T (p.Thr179Ile) variant in the *NLRP12* gene (Fig. 1, 2); no mutation in any of the familial cancer syndrome genes or in any of the other known periodic fever genes was identified.

Analysis of serum cytokines revealed elevated levels of pro-inflammatory (interleukin (IL)-1β, IL-6, IL-12, and TNF-α), Th1 (IL-2, IFN-γ), and Th2 (IL-4, IL-5, IL-13) cytokines, but not of Th17 (IL-17) and IL-10 (Table 1). No mutations in any cytokine genes were detected. Levels of pro-inflammatory cytokines, including IL-1β, TNF-α, and IL-6, were persistently elevated over several months (Fig. 3). Treatment with a single dose of canakinumab, a human monoclonal antibody targeting IL-1β, resulted in some improvement in the diarrhea and abdominal pain.

The patient's daughter was seen at UCLA in consultation shortly after we identified the novel genomic variant. The daughter also expressed similar symptoms of diarrhea, abdominal pain, and flushing, with the notable addition of urticaria. Her medical history was

also significant for hypothyroidism and TOS, and her laboratory studies revealed an elevated 24-h urine 5-HIAA. Whole exome sequencing was positive for the identical *NLRP12* variant.

Discussion

HPFs or systemic autoinflammatory diseases are a group of disorders characterized by recurrent fevers and systemic inflammation and are categorized into at least six disorders based on clinical criteria. Among the *NLRP* subfamily, the *NLRP3* autosomal-dominant mutations are known to be associated with three of these disorders, including familial cold autoinflammatory syndrome (FCAS) and Muckle-Wells syndrome (MWS), as it encodes the dysfunctional protein, cryopyrin, which is involved in the processing and secretion of IL-1β, eventually leading to systemic inflammation [3]. FCAS is usually mild with generalized inflammatory attacks including skin urticaria, fevers, or joint pains triggered by cold, while MWS is more severe, causing progressive hearing loss and renal amyloidosis [4]. New autoinflammatory disorders with their associated genes and mutations are still being identified and reported regularly in the literature.

Over the last 10 years, other mutations in the *NLRP12* gene have been shown to be associated with HPFs, as its nucleotide-binding site, sequence, and effects on IL-1β are similar to that of *NLRP3*. In the first reported study of disease-causing mutations in *NLRP12, Jeru* et al. sought to determine the molecular basis of a disorder in twin brothers who presented with symptoms overlapping FCAS and MWS—namely sensorineural hearing loss and the triggering effect of cold—without mutations in the known HPF genes. Sequencing of *NLRP12* revealed a heterozygous nonsense mutation (c.850C > T, p.Arg284X) in both brothers, as well as their father, who displayed a milder phenotype. A second *NRLP12* mutation (c.2072 + 3insT) was identified in a young girl in a different family presenting with periodic fevers, abdominal pain, and arthralgia associated with exposure to cold, similar to FCAS. Her father was found to have the same mutation and had similar symptoms as a child. This study elucidated the autosomal-dominant mode of inheritance of *NLRP12*-associated syndromes and designated these syndromes as FCAS-2 or *NLRP12*-AD [2].

At least 46 cases have been reported thus far of *NLRP12* variants in patients [3–6]. Among the reported *NRLP12* variants, p. F402L (c.1206 C > G) is the most frequent NLRP12 mutation (16/30, 53%) [3]. The other reported *NLRP12* gene variants are p. R284X (c.850 C > T) and c.2072 + 3insT (as above), p. D294E (c.882 C > G), p.R352C (c.1054 C > T), p. G448A (c.1343 G > C), and p.H304Y (c.910 C > T). Although the disorder is more prominent among Caucasians, there are now multiple reported cases of Chinese patients with *NLRP12*-AD [3]. About 70% of reported patients have had a childhood onset with overall male-to-female ratio of 7:5 [3]. Our patient does not have features typical of FCAS, other than abdominal pain, diarrhea, arthralgia, and arthritis, which are relatively nonspecific symptoms, and she has a previously unreported novel *NLRP12* variant (heterozygous g. 54314377G > A, c.536C > T, p.Thr179Ile).

NLRP12-AD patients typically present with periodic fever (28/30, 93%), cold exposure as a trigger (20/30, 66%), urticaria-like rash (19/30, 63%), arthralgia/arthritis (18/30, 60%), myalgia (18/30, 60%), abdominal pain/diarrhea (13/30, 43%), headache (11/30, 36%), and lymphadenopathy (9/30, 30%), and elevated acute phase reactants (9/30, 30%) [2, 7–11]. Splenomegaly, sensorineural deafness, and thoracic pain have been reported in less than 10% patients.

Our patient and her daughter lack most of these typical features and instead present with a multisystem illness and autoimmune diathesis, including positive ANA, polyarthritis, muscle inflammation and fibrosis, and hypothyroidism. Their particular *NLRP12* variant, not previously reported in the literature or databases, may therefore suggest a new hereditary syndrome. Although no biological model (either cellular or animal) exists for this specific variant, Functional Analysis through Hidden Markov Models (FATHMM) predicts that this mutation may be damaging with a score of − 2.26 (VarSome database: NM_001277126.1:c.536C > T) [12, 13]. The clinical spectrum associated with *NLRP12* gene variants in 48 cases, including 2 cases in this report, is summarized in Fig. 4.

A similar case report recently described a 20-year-old female *postmortem* who expressed two heterozygous non-synonymous amino acid substitutions in the *NLRP12* gene, encoding the NALP12 protein (p.H304Y and p.A629D) [14]. Her phenotype consisted of progressive B cell lymphopenia/antibody deficiency diagnosed as common variable immunodeficiency, juvenile idiopathic arthritis, and development of intestinal inflammatory amyloidosis. No cold-induced symptoms, skin rashes, urticaria, or periodic fever could be observed or elucidated in the index patient or related family members. Transcriptome analyses revealed steady-state normal IL-1β expression, but significantly increased baseline transcription of *XCL2, CXCL9, CXCL10*, and IFN-γ. Our patient presents with features that resemble the above reported case, specifically in relation to low IgG levels, polyarthritis, diarrhea, and lack of cold-induced symptoms, though obviously she has survived much longer.

Furthermore, our patient's diarrhea, atopic symptoms, and eosinophilia could be secondary to an increase in various cytokines associated with *NLRP12* mutation. In experimental studies of mice receiving *Nlrp12*$^{-/-}$ T cells, excessive levels of Th2 cytokines (such as IL-4, IL-5, and IL-13) were detected, promoting atopic dermatitis of the skin and ears as well as colitis, colitis-associated colon cancer, and atypical autoimmune encephalomyelitis [15]. Consistently, our patient had increased circulating IL-4, IL-5, and IL-13 and gave a history of bronchial asthma, eosinophilia, allergies, and recurrent skin rash. Her daughter with the same *NLRP12* variant has had recurrent urticaria. Similarly, high amounts of Th2 cytokines have been implicated in the development of fibrosis and could contribute to the presence of muscle fibrosis in our patient [16]. Recently, *NLRP12* has been shown to have a protective role on intestinal inflammation by inhibiting the NF-kB pathway (Fig. 5) and through alterations of the intestinal microbiome [17].

Nlrp12 deficiency in mice predisposes to tumorigenesis [18, 19]. In humans, the expression levels of *NLRP12* gene and protein are dysregulated in malignant prostate tumor tissues [20]. Consistently, our patient and her daughter had features suggestive of carcinoid syndrome (though without a tissue diagnosis). Further studies are needed to establish

association between our newly discovered *NLRP12* variant and the development of carcinoid syndrome and other malignancies.

In terms of treatment, only one-third of patients respond to glucocorticoids and anti-histamine drugs, and thus, IL-1 inhibitors, such as anakinra, may be beneficial given the role of IL-1β in the pathogenesis of *NLRP12*-AD [3]. However, another study reported recurrence of symptoms on anakinra within a few months of treatment, which was concerning for an acquired resistance to the medication [9]. Canakinumab, a human monoclonal antibody targeting IL-1β, could be efficacious in patients who are resistant to anakinra, as has been shown in other autoinflammatory disorders; however, there have been no reported cases of treatment with canakinumab in *NLRP12*-AD. After a single administration of canakinumab, our patient reported some improvement in diarrhea and abdominal pain. Encouraged by this observation, we have initiated long-term treatment with canakinumab in this patient.

In summary, we present a previously unreported variant in *NLRP12* in a patient and her daughter with a multisystem illness without classic symptoms of *NLRP12-AD*, but with persistently elevated levels of IL1β and several other cytokines. They presented with chronic nonspecific symptoms including flushing, diarrhea, abdominal pain, atopy, and arthralgia, which were severe enough in our patient to require treatment with several immunosuppressive drugs. Our observations warrant the search for mutations in *NLRP12* and related genes in patients with such nonspecific and severe symptoms, within the context of the ever-expanding constellation of autoinflammatory disorders.

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References

1. Schroder K, Tschopp J. The inflammasomes. Cell 2010;140:821–832. [PubMed: 20303873]
2. Jeru I, Duquesnoy P, Fernandes-Alnemri T, et al. Mutations in NALP12 cause hereditary periodic fever syndromes. Proc Natl Acad Sci USA 2008;105:1614–1619. [PubMed: 18230725]
3. Shen M, Tang L, Shi X, Zeng X, Yao Q. NLRP12 autoinflammatory disease: a Chinese case series and literature review. Clin Rheumatol 2017;36:1661–1667. [PubMed: 27633793]
4. Kostik MM, Suspitsin EN, Guseva MN, et al. Multigene sequencing reveals heterogeneity of NLRP12-related autoinflammatory disorders. Rheumatol Int 2018;38:887–893. [PubMed: 29500522]
5. De Pauli S, Lega S, Pastore S, et al. Neither hereditary periodic fever nor periodic fever, aphthae, pharingitis, adenitis: undifferentiated periodic fever in a tertiary pediatric center. World J Clin Pediatr 2018;7:49–55. [PubMed: 29456932]
6. Cetinkaya PG, Ayvaz DC, Karaatmaca B, et al. A young girl with severe cerebral fungal infection due to card 9 deficiency. Clin Immunol 2018;191:21–26. [PubMed: 29307770]
7. Borghini S, Tassi S, Chiesa S, et al. Clinical presentation and pathogenesis of cold-induced autoinflammatory disease in a family with recurrence of an NLRP12 mutation. Arthritis Rheumatism 2011;63:830–839. [PubMed: 21360512]

8. De Pieri C, Vuch J, Athanasakis E, et al. F402L variant in NLRP12 in subjects with undiagnosed periodic fevers and in healthy controls. Clin Exp Rheumatol 2014;32:993–994. [PubMed: 25327218]
9. Jeru I, Hentgen V, Normand S, et al. Role of interleukin-1beta in NLRP12-associated autoinflammatory disorders and resistance to anti-interleukin-1 therapy. Arthritis Rheum 2011;63:2142–2148. [PubMed: 21480187]
10. Jeru I, Le Borgne G, Cochet E, et al. Identification and functional consequences of a recurrent NLRP12 missense mutation in periodic fever syndromes. Arthritis Rheum 2011;63:1459–1464. [PubMed: 21538323]
11. Vitale A, Rigante D, Maggio MC, et al. Rare NLRP12 variants associated with the NLRP12-autoinflammatory disorder phenotype: an Italian case series. Clin Exp Rheumatol 2013;31:155–156.
12. Shihab HA, Gough J, Cooper DN, et al. Predicting the functional, molecular and phenotypic consequences of amino acid substitutions using hidden Markov models. Hum Mutat 2013;34:57–65. [PubMed: 23033316]
13. Chengliang D, Peng W, Xueqiu J, et al. Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies. Hum Molec Genet 2015;24:2125–2137. [PubMed: 25552646]
14. Borte S, Celiksoy MH, Menzel V, et al. Novel NLRP12 mutations associated with intestinal amyloidosis in a patient diagnosed with common variable immunodeficiency. Clin Immunol (Orlando, Fla) 2014;154:105–111.
15. Lukens JR, Gurung P, Shaw PJ, et al. NLRP12 negatively regulates autoinflammatory disease by modulating interleukin-4 production in T cells. Immunity 2015;42:654–664. [PubMed: 25888258]
16. Hams E, Bermingham R, Fallon PG. Macrophage and innate lymphoid cell interplay in the genesis of fibrosis. Front Immunol 2015;6:597. [PubMed: 26635811]
17. Chen L, Wilson JE, Loenigsknecht MJ, et al. NLRP12 attenuates colon inflammation by maintaining colonic microbial diversity and promoting protective commensal bacterial growth. Nat Immunol 2017;18:541–554. [PubMed: 28288099]
18. Zaki MH, Vogel P, Malireddi RK, et al. The NOD-like receptor NLRP12 attenuates colon inflammation and tumorigenesis. Cancer Cell 2011;15:649–660.
19. Allen IC, Wilson JE, Schneider M, et al. NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-κB signaling. Immunity 2012;25:742–754.
20. Karan D, Tawfik O, Dubey S. Expression analysis of inflammasome sensors and implication of NLRP12 inflammasome in prostate cancer. Sci Rep 2017;7:4378. [PubMed: 28663562]

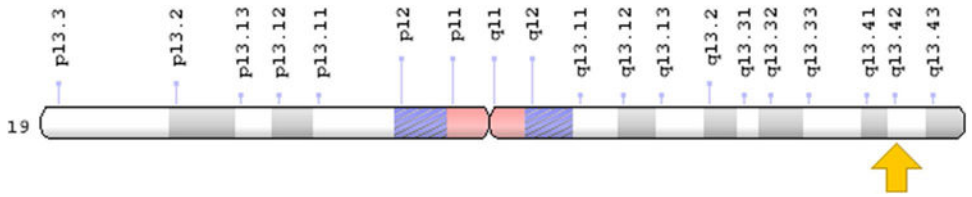

Fig. 1.

Cytogenetic location of NLRP12: 19q13.42 on the long arm of chromosome 19 at position 13.42

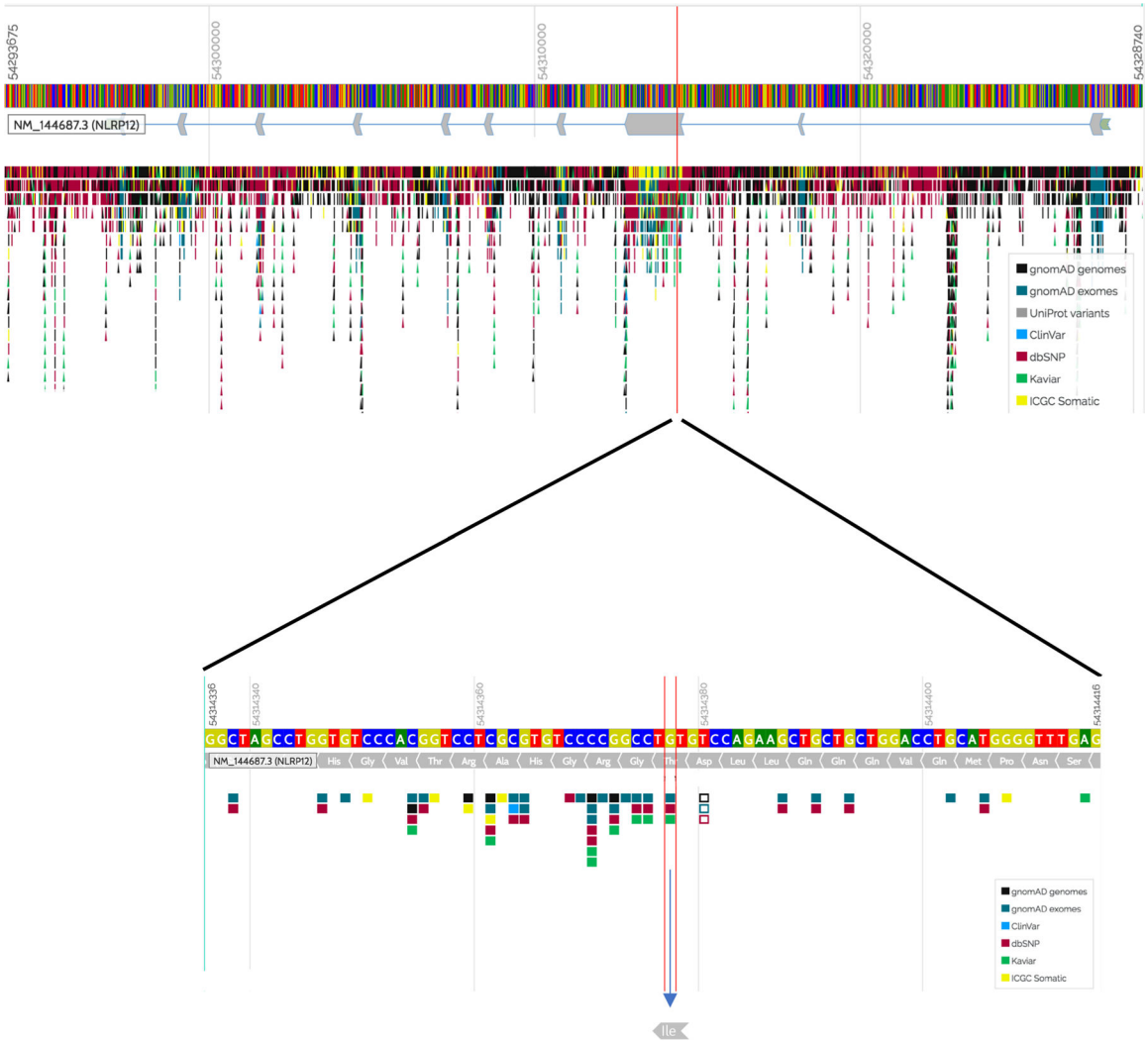

Fig. 2.

Sequence map of c.536C > T (p.Thr179Ile) variant in Exon 3 of *NLRP12*

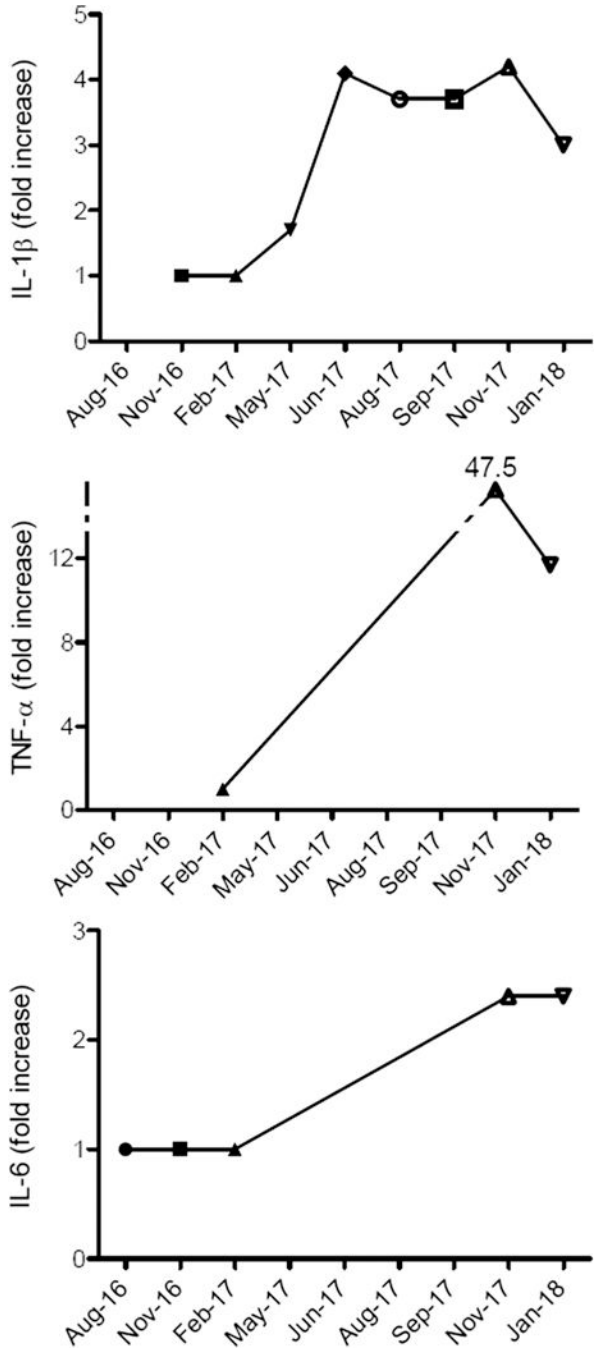

Fig. 3.

Longitudinal monitoring of pro-inflammatory cytokines in a patient with *NLRP12* T179I variant. Cytokines in the serum of the patient were tested at Crescendo Bioscience Clinical Laboratory, San Francisco, CA (August 2016), Quest Diagnostics Nichols Institute, San Juan Capistrano, CA (November 2016 to September 2017), and ARUP Laboratories, Salt Lake City, UT (November 2017 and January 2018). Fold-increase was calculated relative to the upper limit of the normal reference range

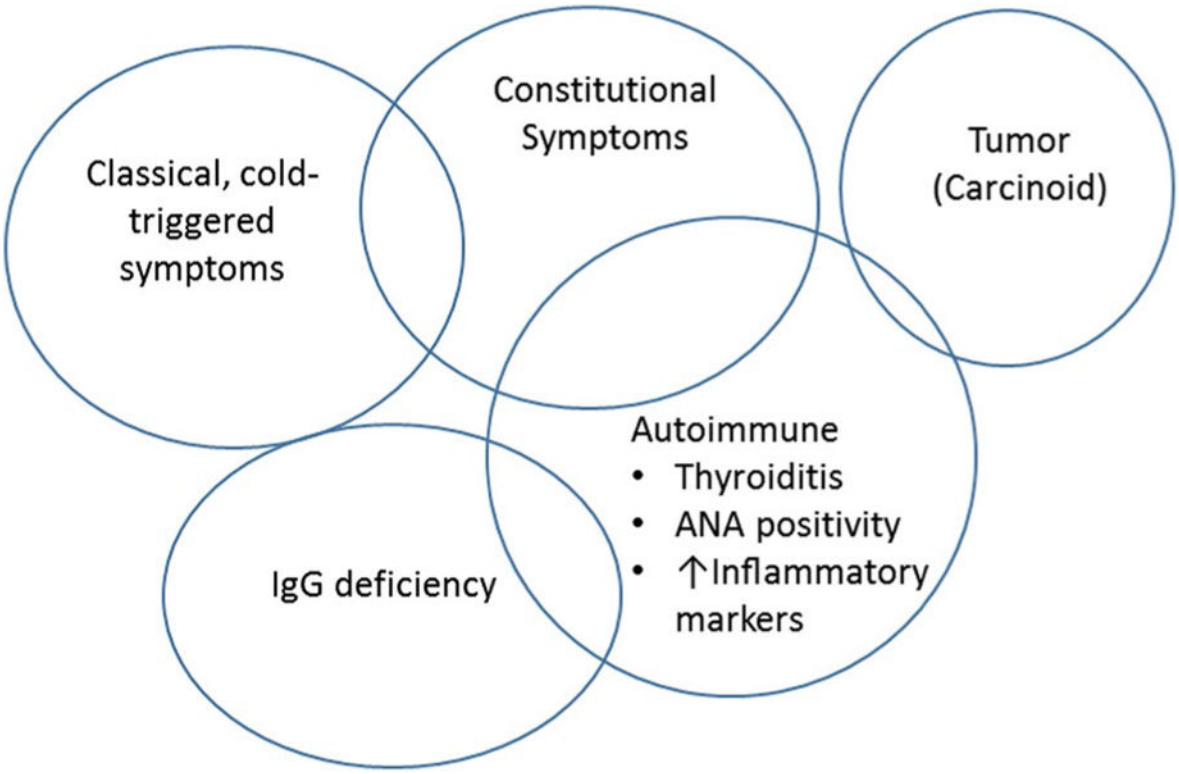

Fig. 4.

Clinical spectrum associated with NLRP12 gene variants in 48 cases, including 2 cases in this report. On a review of the literature, we identified 46 previously reported cases; ref: [3–6]

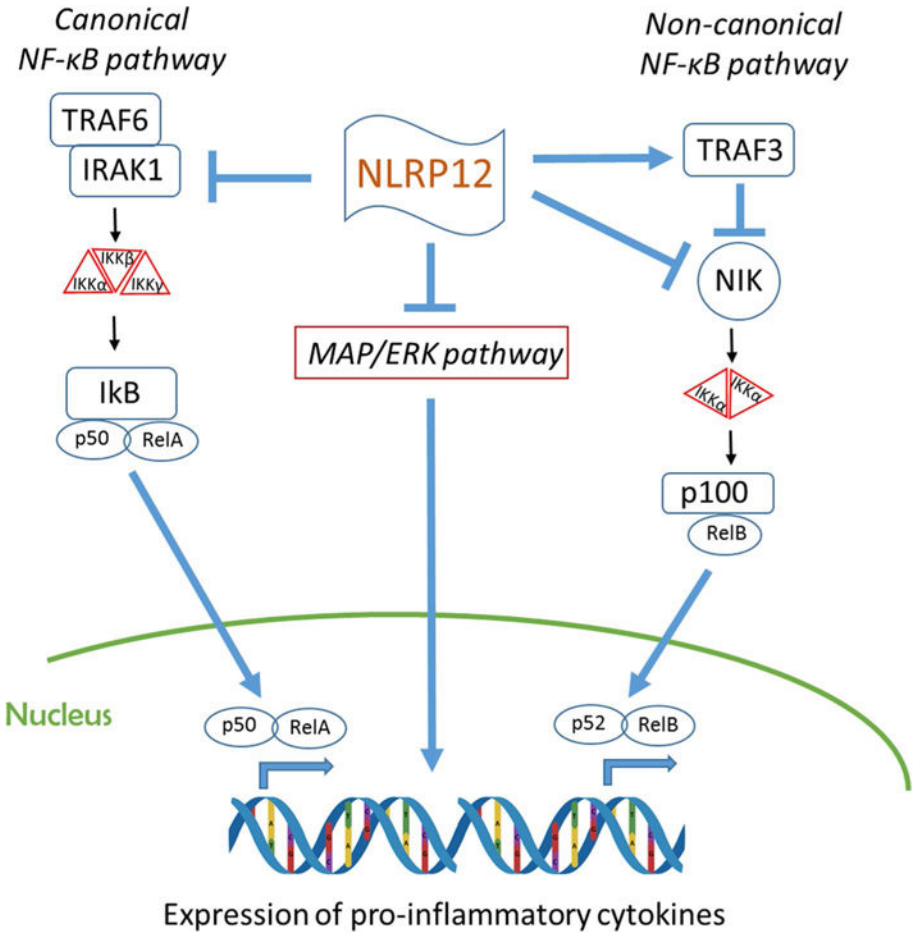

Fig. 5.

Schematic of NLRP12 inhibition of inflammatory pathways. NLRP12 inhibits expression of pro-inflammatory cytokines via both canonical NF-kB and non-canonical NF-kB pathways. NLRP12 antagonizes TRAF6, NIK, and the MAP/ERK pathways. Additionally, NLRP12 promotes TRAF3, which also inhibits NIK signaling

Table 1

Cytokine abnormalities associated with NLRP12 mutations

Cytokine	Index Patient (Serum)				Previous human reports		Mouse studies	
	Ref. range[a]	November 2017	January 2018	July 2018	Jeru, et al. (PBMC)[b]	Borte, et al. (mRNA)[c]	*Nlrp12−/−*[d]	*Nlrp12−/−* T cells[e]
IFN-γ	≤ 5	**87**	**14**	**38**		↑ > 5-fold		↑ 2-fold
IL-1ß	≤ 36	**150**	**107**	**69**	↑ 75–180-fold	N	↑ > 2.5-fold	
IL-2	≤ 12	**418**	**508**	**526**		N		
sIL-2RS	≤ 1033	892	1011	575				
IL-4	≤ 5	**43**	**7**	**32**		N		↑ > 3-fold
IL-5	≤ 5	**8**	**20**	**24**		N		↑ > 4-fold
IL-6	≤ 5	**12**	**12**	**12**	↑ 43–110-fold	N	↑ > 1.5-fold	↑ > 2-fold
IL-8	≤ 5	< 5	< 5	< 5		N		
IL-10	≤ 18	< 5	< 5	< 5		N		↑ 3-fold
IL-12	≤ 6	**309**	**201**	**244**		N	↑ 3-fold	
IL-13	≤ 5	**34**	<5	< 5		N		↑ > 2-fold
IL-17	≤ 13	5	<5	5		N		↑ > 2-fold
TNF-α	≤ 22	**1046**	**257**	**561**		N	↑ 2-fold	↑ > 2-fold

Serum levels of VCAM-1, EGF, VEGF-A, TNF-RI, MMP-1, MMP-3, and leptin were found to be within the normal range at Crescendo Bioscience Laboratory, San Francisco, CA, in August 2016

Values that are greater than the standard reference range are given in bold

IFN interferon; *IL* interleukin; *N* within the normal range; *sIL-2R* soluble interleukin-2 receptor (CD25); *Ref* reference; *TNF* tumor necrosis factor

[a] All in pg/ml; cytokine panel was performed (ARUP Laboratories, Salt Lake City, UT)

[b] Cytokines measured in PBMCs cultured without or with LPS in two patients, which were reduced after treatment with anakinra

[c] mRNA levels of cytokines in 1 patient. Levels of *XCL2, CXCL9,* and *CXCL10* were also increased

[d] *Nlrp12*-deficiency increased susceptibility to experimental colitis, inflammation-induced tumorigenesis, and atypical autoimmune encephalomyelitis with ataxia. The latter was reversed by anti-IL-4 antibody treatment

[e] Transfer of these cells in mice caused severe inflammation in skin and ears