NOD2 versus MEFV: Differential diagnosis of Yao syndrome and familial Mediterranean fever

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Abstract

Objectives: Yao syndrome (YAOS, OMIM 617321) was formerly designated as nucleotide-binding oligomerization domain-containing protein-2 (NOD2)-associated autoinflammatory disease (NAID). This disorder shares similar clinical phenotypes with hereditary periodic fever syndromes (HPFS). This study aimed to compare YAOS with familial Mediterranean fever (FMF).

Methods: In this retrospective study, electronic medical records of a case series of YAOS were reviewed and data were analyzed. All patients underwent genetic testing for periodic fever syndrome 6-gene panel.

Results: A total of 6 cases were presented. These patients were initially thought to have FMediterranean FeVer (MEFV)-negative FMF and received treatment with colchicine. They were eventually diagnosed with YAOS. The differences between these diseases were illustrated. In addition, both MEFV and NOD2 mutations were detected in some patients and family members. Patients with carriage of both gene mutations may present with heterogeneous disease expression. A close correlation between phenotypes and genotypes is needed to make a diagnosis.

Conclusions: YAOS may mimic FMF. Molecular analysis should cover NOD2 whole gene sequencing to help distinguish these diseases. Both NOD2 and MEFV mutations may contribute to disease expression in an individual.

Keywords

familial Mediterranean fever • MEFV • NOD2-associated autoinflammatory disease • nucleotide-binding oligomerization domain-containing protein-2 • Yao syndrome

Introduction

Systemic autoinflammatory disease (SAID) is a group of periodic inflammatory diseases without detectable autoantibodies or antigen-specific T lymphocytes.[1] Familial Mediterranean fever (FMF) is the most prototypic of SAIDs,[2] clinically marked by recurrent short episodes of fever and serositis. FMF has been a century-old disease, and its gene MEediterranean FeVer (MEFV) is localized to chromosome 16p13 and encodes for a protein called pyrin or marenas-trin,[3] and MEFV mutations cause the disease.

Nucleotide-binding oligomerization domain-containing protein-2 (NOD2) is a cytosolic NOD-like receptor (NLR).[4] Since its discovery in 2001,[4] NOD2 gene mutations have been associated with Crohn’s disease (CD),[5] Blau syndrome,[6] and Yao syndrome (YASOS), formerly named as NOD2-associated autoinflammatory disease (NAID).[7] Since our initial report in 2011, YAOS is characterized by periodic fever, dermatitis, polyarthritis, gastrointestinal (GI), and sicca symptoms with eyelid swelling and is linked to certain NOD2 variants.[7–10] Both YAOS and FMF share similar clinical phenotypes or resemble each other in some cases, thus posing diagnostic challenges clinically. Moreover, both NOD2 and MEFV genes are mapped at chromosome 16, and their gene products belong to the superfamily of death domain proteins.[11] Both NOD2 and pyrin proteins play important roles in the regulation of apoptosis, cytokine processing, and inflammation.[12] However, a potential relationship between these two disorders has yet to be studied. Herein, we report a case series in conjunction with the literature to illustrate the similarities
and differences between YAOS and FMF on clinical and molecular levels.

**Patients and Methods**

In this retrospective study, electronic medical records of a series of patients with YAOS were reviewed, who fulfilled the diagnostic criteria consisting of characteristic phenotype and the presence of NOD2 variants with the exclusion of other related diseases.[7] The study was approved by the Institutional Review Board of Stony Brook University Hospital with a waiver of informed consent. These patients underwent extensive workup, including genetic testing for periodic fever syndrome 6-gene panel (MEFV, TNFRSF1A, NLRP3, MVK, NLRP12, and NOD2). All patients initially carried the diagnosis of MEFV-negative FMF and received treatment with colchicine. An abstract on this case series was previously presented at the EULAR annual meeting in 2020.[13]

Our prior presentation of a published abstract summarizing 3 patients at the American College of Rheumatology Annual Meeting was also used in this article. In this case series, examination of the NOD2 and MEFV genes for mutations was performed by DNA polymerase chain reaction and DNA sequencing. The phenotypes and genotypes of these patients were individually described.[14]

**Results**

**Working Clinical Diagnosis of FMF before Final Diagnosis of YAOS**

All 3 patients described below presented with autoinflammatory but atypical features for FMF. These patients had therapeutic responses of various degrees and duration to colchicine in the absence of elevated acute phase reactants and MEFV mutations. Further genetic testing identified the presence of characteristic NOD2 variants, and all these patients were diagnosed with YAOS. These cases are individually presented below.

**Patient 1**

A 66-year-old Caucasian female presented with recurrent fever, rash, and arthralgia for the previous 3 years. She had an episodic high fever lasting up to 36 h during each disease flare. Erythematous patches on her face occurred episodically, lasting up to 2 weeks. Arthralgia involved the knee and ankle. She also presented with intermittent abdominal pain and non-bloody diarrhea, but GI workup was negative for inflammatory bowel disease (IBD). She also had sicca- and asthma-like symptoms. There was no family history of periodic fever syndrome. Both erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were normal. Despite the absence of MEFV mutations, the patient was treated with colchicine for presumed FMF with a temporary response. Eventually, the 6-gene periodic fever syndrome panel was only positive and heterozygous for NOD2 IVS8+158 and R702W.

**Patient 2**

A 49-year-old Caucasian female started to have episodic high fever, rash, and arthralgia at age 7. Her rash was described as patchy erythema on her face, chest, abdomen, and limbs, lasting up to 6 weeks with each episode. Arthralgia involved the knees, ankles, and toes. The patient also had intermittent abdominal pain and non-bloody diarrhea lasting up to 4 d with each flare; however, GI workup was negative for IBD. She also had sicca- and asthma-like symptoms. She had multiple first trimester miscarriages. There was a family history of periodic fever syndrome of unclear cause. Acute phase reactants were normal. Despite negative genetic testing for MEFV mutations, the patient was treated with colchicine for a working diagnosis of FMF with a good response. The 6-gene panel was only positive and heterozygous for NOD2 IVS8+158.

**Patient 3**

A 35-year-old Caucasian female started to have an episodic low-grade fever, rash, and arthralgia at age 15. The patient had intermittent patchy erythema on her arms and legs, lasting a few days with each flare. Her rash was described as patchy erythema on her face, chest, abdomen, and limbs, lasting up to 6 weeks with each episode. Arthralgia involved the knees and shoulders. She also had intermittent abdominal pain, nausea, and vomiting without diarrhea lasting up to 4 d with each flare; however, GI workup was negative for IBD. She denied sicca- and asthma-like symptoms. There was no family history of periodic fever. Acute phase reactants were normal. Despite negative genetic testing for MEFV, the patient was treated with colchicine for tentative FMF with minimal response. The 6-gene panel was only positive and heterozygous for NOD2 IVS8+158 and 1007fs.

**Mutations of Both MEFV and NOD2 May Contribute to SAIDs in an Individual or Family**

**Patient 4**

A 25-year-old white woman developed febrile episodes at age 17, with each episode lasting 1.5 d. Her bouts of abdominal pain, nausea, and vomiting lasted up to 7 d during each flare. She also had intermittent erythematous patches on her limbs and bilateral knee pain/swelling. She had fatigue, weight loss, and night sweats but without dry eyes/mouth or serositis. Her aunt, a 37-year-old Caucasian, was diagnosed with FMF in the presence of the heterozygous MEFV mutation
Patient 5

A 27-year-old Caucasian man presented with bouts of fever, abdominal pain, and non-bloody diarrhea, accompanied by poor appetite, nausea, and vomiting since age 17. These symptoms occurred once every 1–2 months. Each febrile episode lasted several hours to 24 h, and GI symptoms often lasted 4–5 d with self-limitation. He complained of mild bilateral shoulder pain at flares but denied rash, chest pain, dry eyes/mouth, or weight loss. His father was Italian and mother was German, and there was no family history of FMF. Physical examination was unremarkable except for flank tenderness. Laboratory work, including CMP, ESR, ANA, and urinalysis, was normal except for mild leukocytosis. Colonoscopic examination showed a few ulcerations and mild inflammation in the distal ileum occasionally. Subsequent biopsy revealed patchy active ileitis. CD was suspected, but his recurrent short episodes of symptoms were also highly suggestive of FMF. Genetic testing identified both heterozygous M694V and NOD2 variant IVS8+158. Due to a lack of good response to colchicine treatment, he was also suspected of having concurrent YAOS given the presence of the NOD2 variant.

Patient 6

A 48-year-old Palestinian American man presented with bouts of bloody diarrhea 15 years ago. The patient was then suspected of having ulcerative colitis and sulfasalazine was initiated with some improvement. Eight years ago, he was found to have colon cancer and underwent total proctocolectomy with ileal pouch construction consequently. He also had an episodic fever lasting 2–3 d each episode since age 15, accompanied by intermittent erythematous patches/papules occurring on his forehead, trunk, and upper extremities, lasting 10–14 d with spontaneous resolution. He developed intermittent pain in the left ankle, wrist, and right knee. He had a dry mouth, oral ulcers, and fatigue also. There was no family history of FMF.

Physical examination was unremarkable. Laboratory work, including CBC, CMP, ESR, ANA, and urinalysis, was all normal. Pouchoscopy was normal on multiple occasions. Genetic testing identified the compound heterozygous MEFV mutation R653H and NOD2 variant IVS8+158. He was suspected to have both FMF and YAOS based upon the phenotypic and genotypic features. Colchicine was initiated with minimal improvement.

Discussion

Our case analysis has indicated that YAOS can partially resemble FMF in clinical phenotypes, thus YAOS should be contemplated as an FMF differential diagnosis, particularly in atypical cases. Molecular analysis of periodic fever syndromes should cover NOD2 whole gene sequencing to help distinguish these two diseases. YAOS and FMF are distinct diseases. Based on the literature data including our prior publications, the demographics, clinical phenotypes, genotypes, and therapies of these two diseases are summarized (Table 1). In addition to different gene mutations, there are distinguishing clinical features between these diseases. For instance, in FMF, the febrile episode is short (<3 d), the erysipelas-like rash tends to occur on the distal lower extremities, and acute abdomen with more constipation than diarrhea is common. A majority of FMF patients have a sustainable response to colchicine. In YAOS, the duration of each disease flare can be several days or longer, erythematous patches often occur on the upper body (face and chest), and mild abdominal cramping and bloating with non-bloody loose stools or diarrhea are common. Intermittent eyelid swelling and distal lower extremity swelling can occur. Most patients do not respond to colchicine or a minority of patients may have a temporary response.

Our study also suggests that both MEFV and NOD2 mutations together may contribute to mixed and heterogeneous autoinflammatory phenotypes for YAOS and FMF in individual patients. The clinical phenotype of patient 4 shared similar phenotypes with her aunt who had FMF with carriage of a heterozygous MEFV mutation. Given her longer duration of disease flares, rash locality, poor response to colchicine, and the absence of MEFV mutations, further genetic testing identified the presence of the NOD2 variants. Thus, YAOS was diagnosed and sulfasalazine was initiated with symptomatic resolution. This case suggests that both MEFV and NOD2 variants may run in a family. Both patients 5 and 6 carried MEFV and concurrent NOD2 variants and had overlapping and mixed phenotypes for both FMF and YAOS. FMF genotype is usually composed of either homozygous or compound heterozygous MEFV mutations. However, a single copy of MEFV gene mutations can be seen in approximately 25% of patients with FMF. YAOS is considered to be a genetically complex disease and is generally sporadic, though 10–15% of cases may have a positive family history. YAOS is associated with the NOD2 variants, IVS8+158 in...
nearly all patients, IVS8+158/R702W in up to 30%, and IVS8+158/1007fs, G908R, or other rarer NOD2 variants in some other patients.\textsuperscript{[9–16]}

CD is another NOD2-associated disease. Approximately 30% of patients with CD harbor NOD2 variants.\textsuperscript{[17]} There have been studies investigating the association between FMF and CD. Some reported a higher prevalence of CD among patients with FMF, and others found an increased rate of FMF among CD patients.\textsuperscript{[18, 19]} Nevertheless, these studies demonstrated no association of the MEFV mutations with CD susceptibility. Instead, the presence of these mutations appears to be associated with a stricter disease pattern and significantly more extra-intestinal manifestations of CD, suggesting that the MEFV gene may be a modifier of CD disease severity.\textsuperscript{[18, 20]} A recent study has demonstrated that the three common NOD2 variants (1007fs, G908R, and R702W) are not associated with increased susceptibility to develop FMF, and the coexistence of these NOD2 variants and MEFV mutations might contribute to higher rates of erysipelas-like erythema, acute scrotum attacks, colchicine resistance, and a more severe disease as compared with FMF patients without mutations.\textsuperscript{[21]} In our study, both patient 5 and 6 carried combined heterozygous NOD2 variant IVS8\textsuperscript{*158} and MEFV mutations, but their clinical phenotypes were atypical for FMF with poor treatment response to colchicine. These data indicate that the NOD2 variant might serve as a modifier gene for FMF or form compound heterozygotes with the MEFV mutation to cause variable phenotypes. With more advanced molecular technology, such as next-generation sequencing, two or more genetic mutations from two or more different genes are occasionally identified in patients with SAIDs.\textsuperscript{[21]} Based on our prior study, a combination of MEFV and NOD2 mutations may be relatively common\textsuperscript{[11]} and they could be synergistic.

Both NOD2 and MEFV genes are located at Chromosome 16, and they are structurally and functionally similar (Figures 1 and 2).\textsuperscript{[22–25]} NOD2 contains 2 N-terminal caspase activation recruitment domains (CARDs), a central NLR (NACHT or nucleotide-binding domain [NBD]), and 6 C-terminal leucine-rich repeats (LRRs) (Figure 1A). The NOD2 proteins are primarily present in the cytosols of monocytes, macrophages, dendritic cells, and intestinal endothelial cells. NOD2 functions through its recognition of bacterial peptidoglycan via RICK or RIP2, thus activating p38 MAP kinases and NF-κB, resulting in inflammation\textsuperscript{[22]} (Figure 2). Pyrin is encoded by MEFV and has five domains, including a PYRIN domain (PYD), a bZIP transcription factor basic domain, a B-box zinc finger domain, an alpha-helical domain, and a B30.2 (PRYSPRY) domain (Figure 1B). Pyrin is expressed in granulocytes, monocytes, dendritic cells, and synovial fibroblasts. Pyrin regulates caspase-1 activation and consequently IL-1b production via the interactions of its N-terminal PYRIN and C-terminal B30.2 domains. Pyrin is cleaved by caspase-1 and the cleaved N-terminal fragment is translocated to the nucleus and enhances NF-κB activation (Figure 2).\textsuperscript{[23]} As both MEFV and NOD2 genes are located...
on the same chromosome, we hypothesize that these two genes might affect each other and modify the phenotypes of FMF and YAOS. Further study would be needed to understand their relationship and combined influence on disease expression.

**Limitations of the Study**

This study is retrospective and its sample size is small. No familial segregation analysis was performed. Further study of larger case series is needed in future research in this regard.

**Conclusion**

YAOS and FMF share similar clinical phenotypes and can masquerade each other, notably in the scenario of atypical cases of FMF, but they are phenotypically and genotypically distinct. Molecular analysis should cover NOD2 whole gene sequencing to help distinguish these diseases. Both MEFV and NOD2 mutations can be simultaneously detected in an individual or family, and the coexistence of both gene mutations may contribute to mixed and heterogeneous phenotypes. Clinical interpretation should base on the correlation between phenotype and genotype data.
Author Contributions
QY drafted the manuscript, and MS designed and created the figures. MS and PG reviewed the manuscript critically for important intellectual content. All authors approved the final version to be published. QY has full access to all of the data in the study and is responsible for the integrity of the data and the accuracy of the data analysis. PG participated in data collection.

Conflicts of Interests
Qingping Yao is an Editorial Board Member of the journal. The article was subject to the journal’s standard procedures, with peer review handled independently of this member and his research groups.

Ethics Approval
The study was approved by the institutional review board of Stony Brook University.

Informed Consents
Informed consents have been obtained. The patients have given their consents for their images and other clinical information to be reported.
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