Mediterranean Journal of Hematology and Infectious Diseases

Case Report

Novel Deleterious Sequence Change in the NLRP12 Gene in a Child with the Autoinflammatory Syndrome, Joint Hypermobility and Cutis Laxa from India

Kanjaksha Ghosh[1], Kanchan Mishra[1], Avani Shah[1], Parizad Patel[1] amd Shrimati Shetty[2].

[1] Surat Raktadan Kendra & Research Centre. Udhna Magdalla Road (Nr. Chosath Joganio Mata Temple), Surat 395002 , Gujrat , India.
[2] National Institute of Immunohaematology, 13 the floor KEM hospital MS building, Parel, Mumbai 400012, Maharashtra, India.

Competing interests: The authors have declared that no competing interests exist.

Abstract. An otherwise healthy male child of 9 years presented with paroxysmal fever and diffuse abdominal pain along with the loss of appetite and nausea lasting for 3-4days every 4-6 weeks in the last two years. He also has stretchable skin and hypermobile joints, inherited from his mother who never suffered any paroxysmal attack of the kind. Work up for acute intermittent porphyria, lead poisoning, and familial Mediterranean fever was negative. A novel harmful sequence change in the NLRP12 gene was detected, and a diagnosis of NLRP12 associated autoinflammatory syndrome was made. This sequence change within the NLRP12 gene causing disease has not yet been reported in the literature and is the first such a case reported from India.

Keywords: Autoinflammatory syndrome; Hypermobility; Stretchable skin; Porphyria; New mutation.

Citation: Ghosh K., Mishra K., Shah A., Patel P., Shetty S. Novel deleterious sequence change in the NLRP12 gene in a child with the autoinflammatory syndrome, joint hypermobility and cutis laxa from India. Mediterr J Hematol Infect Dis 2019, 11(1): e2019018, DOI: http://dx.doi.org/10.4084/MJHID.2019.018

Published: March 1, 2019 **Received: September 27, 2018** **Accepted: January 12, 2019**

Correspondence to: Prof Kanjaksha Ghosh MD, FRCP. Director: Surat Raktadan Kendra & Research Centre. E-mail: kanjakshaghosh@hotmail.com

Introduction. Mutations in several genes are known to be involved in autoinflammatory syndromes which present in a paroxysmal fashion.[1,2]

These proteins assemble inside a cell on getting noxious stimulus from the environment. These assembled proteins produce what is called an inflammasome. Once inflammasomes are assembled, they activate secretion of proinflammatory cytokines through various pathways. These cytokines, e.g., IL-1, TNF-alpha, or IL-6 produce inflammation. A large number of proteins that go on create inflammasomes have been variously named, and some of them have multiple domains allowing many proteins to attach and activate other proteins. Detailed nomenclature of these proteins has been described elsewhere.[2] Mutations of some of these proteins lead to the spontaneous assembly of inflammasomes often under natural environmental conditions or in the cold environment etc. leading to the autoinflammatory condition.

Clinical presentations of these conditions vary, i.e., paroxysmal fever, musculoskeletal pain; skin rashes, abdominal pain, etc. These autoinflammatory syndromes are caused by mutations in a number of proteins involved in the initiation and formation of Inflammasomes.[3] NLRP12 is one such protein, and its mutation causes familial cold associated periodic fever syndrome type 2.[3,4]

Only a few such cases have been reported in the world literature.[5-7] We are presenting here another patient with autoinflammatory syndrome due to a novel heterozygous deleterious sequence change in the NLRP12 gene. The case is being reported with the full consent of the parents of the patient as the patient is minor.

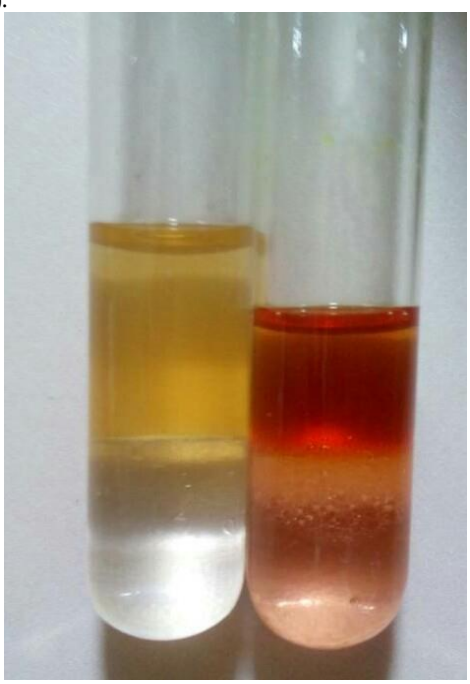
The Case: A 9-year-old boy (45 kg,1.49 mts), full-term normal delivery**,** 2nd child in the family, born of the nonconsanguineous marriage, having good intelligence (std 5, rank holder in the class), completed all the immunization without any complication and was apparently healthy. He presented with recurrent episodes of abdominal pain, moderate grade fever(37.5-38 ^{0}C), complete loss of appetite with nausea but no vomiting for last two years along with a feeling of soreness all over the body. This paroxysmal presentation was coming on once every 4-6 weeks without any apparent relation to food, weather or other environmental or extraneous factors. The paroxysm lasted for 3-4 days, without any skin rash, aphthous ulcers in the mouth, or a sore throat. There were no cold sores. He did not have any skin rash or skin ulcers. There were no sore throat or lymphadenopathy. He was clinically examined during 3 of his attacks when he was found to be withdrawn, apparently suffering continuous pain by his expression. His clinical examination showed a well-built child with hyper stretchable skin, fish mouth scars, and hypermobile joints. There was no iridodonesis, and his vision was normal (6/6 both eyes), there was no other suggestion of Marfan's syndrome on various measurements of the body. Mild diffuse tenderness was found all over the abdomen with normal bowel sounds. There was no organomegaly, and the pain was poorly localized. There were no cardiac murmur, respiratory abnormality, focal neurological abnormality, and the rest of the clinical examination was essentially normal. He recovered automatically after 3-4 days of the ordeal.

He had history neither of any serious illness nor of food or drug allergy.

Elder brother (14 years of age) and parents had no such illness, but the mother has lax joints and skin. There were no pets in the house and no family history of a similar disease in the extended family.

His complete blood count showed neutrophilic leukocytosis (TLC 14-15000/ul with 76 percent polymorphs and no eosinophils during the acute stage) with normal hemoglobin and platelet counts. There were no abnormalities in the morphology of any of the cells seen. His biochemical investigations which involved the liver, renal function tests, electrolytes also showed no abnormality. Blood and urine culture and routine urine examination were unremarkable. Urine for porphobilinogen showed a mild increase (**Figure 1**) however the quantitation of PBG (porphobilinogen) on 24-hour urine during acute stage was normal so also the blood lead levels (<10ugm/L). Serum amylase, lipase, and lipid profile were normal. CRP levels were raised during the acute stage (170mg/L) and was quickly normalized(3mg/L) within 72 hours of resolution of abdominal pain. Upper GI endoscopy and fundoscopy (eye) showed no abnormality. Serum

Figure 1. Urine showing mild increase in porphobilinogen(Hoesch test) and partitioning with chloroform in urine of the patient (ruby red color).



immunoglobulins including IgD levels were normal. Ultrasound examination of abdomen and pelvis, CT scan with contrast and MRI scan of chest and abdomen was essentially normal.

Because of the paroxysmal nature of the attack, a provisional diagnosis of acute intermittent porphyria or one of the autoinflammatory syndromes was made.

Targeted sequencing of exome involving porphyrin metabolism, autoinflammatory conditions, and immunologically important 280 genes was executed following transposase digestion of isolated DNA from the peripheral blood mononuclear cells of the patient on Illumina 2500 Hi seq platform. Except for nine genes (CD55, CFHR1, CORO1A, ITCH1, MAGT1, TNFRS11A, TBX1, FCGR1A, NCF1, 85-98 % coverage), all genes were covered to the tune of 100 % of the exomes including intron-exon boundaries.

Except for common polymorphisms, none of the genes showed any harmful sequence changes or known mutations. However, there was a heterozygous mutation of NLRP12 gene in exon 3 (c 779C>T, p Thr 260> Meth) in the evolutionarily conserved nucleotide sequence on NACHT1 domain of the molecule. This sequence change has not been described previously and was found to be harmful by using Polyphen 2 Sift, Mutation tester-2 software.

This sequence change was confirmed using Sanger sequencing but was not found in any of the parents or his elder brothers DNA sequences. Hence it presented a de novo change. So a final diagnosis of NLRP12 associated autoinflammatory syndrome was made. The patient did not respond to colchicine, and by trial and error with various combinations of anti-inflammatory medicines, Naproxen gave a partial response with a

combination of a short course of corticosteroids (Prednisolone, 15 mg/day, administered when paroxysms started once in the morning after breakfast and was continued for seven days.

Discussion. NLRP12 gene product is a member of the CATERPILLER family of cytoplasmic proteins. This protein contains an N-terminal pyrin domain, a NACHT domain, a NACHT-associated domain, and a C-terminus leucine-rich repeat region(LRR) which functions as an attenuating factor of inflammation by suppressing inflammatory responses in activated monocytes. Mutations in this gene cause familial cold autoinflammatory syndrome type 2.

Alternative splicing of the mRNA of this gene results in multiple transcript variants in different tissues, and which may be responsible for the presentation in different organs with related symptoms.

In the absence or reduced function in different domains of the protein inflammatory cytokine IL1 beta is produced in excess via an increase in NFkbeta activity and is responsible for many of the inflammatory signs and symptoms of the disease.[1,8]

There are only a few cases (forty-four till date) of paroxysmal autoinflammatory syndrome reported with NLRP12 mutation. This condition with autosomal dominant inheritance normally presents with paroxysmal cold-induced periodic fever syndrome with cutaneous, musculoskeletal, lymph node related symptoms.[3,5,6,8] A patient with common variable immunodeficiency associated with this mutation has also been reported.[7]

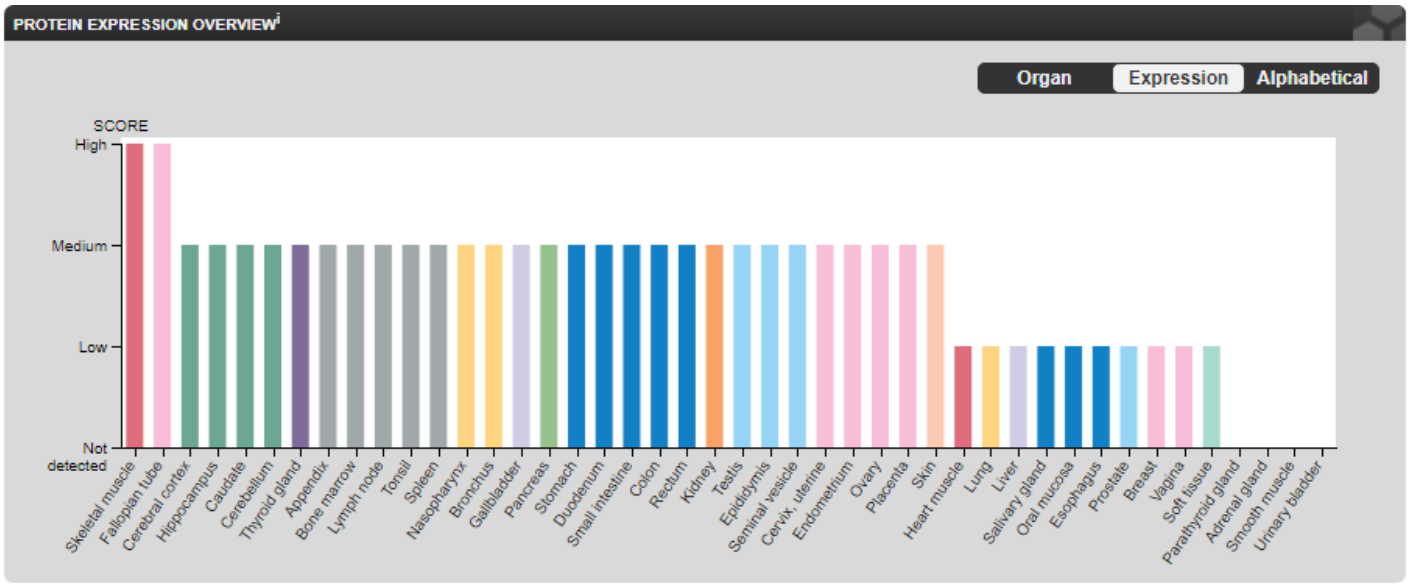
Our patient, however, had several interesting features ie the pathology presented not in early childhood but later. He had no cutaneous symptoms peculiar to NLRP12 mutation, but he had joint laxity, stretchable skin and fishmouth scars like pseudoxanthoma elasticum which may be unrelated to this mutation. He clearly inherited from his mother stretchable skin and hypermobile joints as she also had similar features, but she did not have any symptoms of NLRP12 mutation.

The reason why the patient did not get any cutaneous manifestation, known to occur with the disease may be related to the tropical high ambient temperature in the city of Surat where the boy lived.

Cutis laxa (pseudoxanthoma elasticum) can be caused by mutations in several genes, including *ATP6V0A2*, *ATP7A*, *EFEMP2*, *ELN*, and *FBLN5*. Most of these genes are involved in the formation and function of elastic fibers, which are slender bundles of proteins that provide strength and flexibility to connective tissue throughout the body. There were no mutation or sequence changes in any of these genes in our patient, neither was there any mutation detected in the porphyrin metabolism genes to account for this paroxysmal pain and fever.

The present case is a novel de-novo NLRP12 mutation as it was not found in any of the parents or his elder brother. NLRP12 gene is expressed in many tissues (**Figure 2**). Hence depending on local tissue pathology recurrent inflammation can take place in any tissue and explain the protean nature of manifestation in this disease. Till date in the world literature, only 44 cases of NLRP12 mutation associated autoinflammatory syndrome has been reported. A good number (fifteen) of them has been reported amongst a large cohort of patient with suspected immunodeficiency,[4] when the next-generation sequencing was applied to look for the cause of autoinflammatory syndrome or to rule out a familial Mediterranean fever, a well-known cause of the

Figure 2. Expression of NLRP12 in various tissues.



From: https://www.proteinatlas.org/ENSG00000142405-NLRP12/tissue.

inflammatory syndrome. The authors and the milder presentation have well-described heterogeneity of presentation with this mutation compared to NLRP3 mutation has been emphasized.[4] In 44-50 % of these patients a F402L mutation has been commonly described. Nonsense changes in NLRP12 gene pose no problem in describing it as the genuine pathogenic cause of the disease but a missense mutation needs more attention if it is a single case reported from the world and similar missense mutation from other patients are lacking as has happened in the present case.

In such a situation the change needs to be noted and whether the change is deleterious needs to be confirmed through standard software analysis. The mutation described in the present paper was found to be deleterious using more than one software as already described, and this change is in NACHT domain of the protein which has several important functions like NTP use activity, nucleotide binding activity, etc. to name a few. Several mutations (e.g., D294E, R211H, Y246C, R352C, H304Y) in NACHT domain (aa 211 to aa391) has been reported by other authors either as single or two cases and was pathogenic when analyzed on several softwares as described in this paper.[5,7] Management of this condition has not been standardized, but a good proportion of patients responded to corticosteroids, NSAIDs and anti-allergic drugs given alone or in various combinations. Some of the patients need therapy directed to TNF alpha or Interleukin 1 beta in the form of adalimumab, Canakinumab, infliximab. These antibodies neutralize inflammatory mediators like IL-1, TNF alpha or IL-6 which is increased in this condition and is known to be the final pathogenic mediator in this condition. A few patients have developed amyloidosis or Crohn's disease on follow up.[5,9,10] A recently published review has provided very good information on the management clinical presentation and diagnosis of the systemic autoinflammatory disorder in children.[11]

From India To date, no case of NLRP12 related autoinflammatory disorder has been reported, making this the first such a case from this country.

References:

1. Manthiram K, Zhou Q, Aksentijevich I, Kastner DL. The monogenic autoinflammatory diseases define new pathways in human innate immunity and inflammation. Nature Immunology 2017, 18: 832-42. https://doi.org/10.1038/ni.3777 PMid:28722725
2. Rigante D. A systemic approach to autoinflammatory syndromes: A spelling booklet for the beginner.Expert. Rev.Clin. Immunol. 2017,13:571-97. https://doi.org/10.1080/1744666X.2017.1280396 PMid:28064547
3. Broderick L, De Nardo D, Franklin BS, Hoffman HM, Latz E. The inflammasomes and autoinflammatory syndromes.Annu Rev Pathol. 2015;10:395-424. https://doi.org/10.1146/annurev-pathol-012414-040431 PMid:25423351
4. Jeru I, Le Borgne G, Cochet E, Hayrapetyan H, Duquesnoy P, et al. Identification and functional consequences of a recurrent NLRP12 missense mutation in periodic fever syndromes. Arthritis Rheum. 2011;63:1459–64. https://doi.org/10.1002/art.30241 PMid:21538323
5. Kostik MM, Suspitsin EN, Guseva MN, Levina As, Kazantseva Ay, Sokolenko AP, Imyanitov En. Multigene sequencing reveals heterogeneity of NLRP12 related autoinflammatory disorders. Rheum Internat. 2018. https://doi.org/10.1007/s00296-018-4002-8. Published on line March 2018.
6. Vitale A, Rigante D, Maggio MC, Emmi G, Romano M, Silvestri E, Lucherini OM, Emmi L, Gerloni V, Cantarini L. Rare NLRP12 variants associated with the NLRP12-autoinflammatory disorder phenotype: an Italian case series.Clin Exp Rheumatol. 2013;31(3 Suppl 77):155-6. PMid:24064030
7. Shen M, Tang L, Shi X, Zeng X, Yao Q. NLRP12 autoinflammatory diseases: a Chinese case series and literature review. Clin Rheumatol. 2016, https://doi.org/10.1007/s10067-016-3410-y
8. Lupfer C, Kanneganti T- D. Unsolved mysteries in NLR biology. Front.Immunol. 2013; 4 (art 285). 1-10.
9. Borte S, Celiksoy MH, Menzel V, Ozkaya O , Ozen FZ, Hammarström L, Yildiran A. Novel NLRP12 mutations associated with intestinal amyloidosis in a patient diagnosed with common variable immunodeficiency. Clin Immunol. 2014 ;154:105-11. https://doi.org/10.1016/j.clim.2014.07.003 PMid:25064839
10. Terhar N, Lachmann H, Ozen S, Woo P, Uziel Y, Modesto C et al. Treatment of autoinflammatory diseases: results from Eurofever Registry and review. Ann Rheum Dis. 2013;72:678-85. https://doi.org/10.1136/annrheumdis-2011-201268 PMid:22753383
11. Rigante. D. The broad-ranging panorama of systemic autoinflammatory disorders with specific focus on acute painful symptoms and hematological manifestations in children. Mediterr. J. Hematol. Infect Dis. 2018; 10 (1) :e2018067. DOI: https://doi.org/10.4084/mjhid.2018.067