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ORIGINAL ARTICLE

Detection of a novel mutation in *NLRP3/CIAS1* gene in an Indian child with Neonatal-Onset Multisystem Inflammatory Disease (NOMID)

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Abstract

Neonatal-Onset Multisystem Inflammatory Disease (NOMID) or Chronic Infantile Neurologic Cutaneous Articular (CINCA) syndrome is a monogenic autoinflammatory disorder characterized by urticarial skin rash, fever, chronic meningitis and joint manifestations. Here we report a case of an Indian male child who presented at the age of 9 months with fever, respiratory distress, urticarial skin rash, arthritis, and neuroregression. Suspecting NOMID/CINCA syndrome, the child's blood was sent to the Jaslok Hospital and Research Centre for mutation analysis of the *CIAS1/NLRP3* gene. The DNA was screened for mutations in exon 3 of *CIAS1/NLRP3* gene by automated Sanger sequencing. DNA sequencing showed a novel heterozygous c.1813A➔G, p.R605G mutation in exon 3 of *CIAS1/NLRP3* gene (ref no NM_001243133.1). His parents tested negative for this mutation. We therefore identified a novel *de novo* mutation in this family in the *CIAS1/NLRP3* gene responsible for the child's clinical features.

Keywords *de novo* mutation · *NLRP3/CIAS1* gene · NOMID/CINCA · Novel mutation

Introduction

Cryopyrinopathies (CAPS) are dominantly inherited systemic inflammatory conditions caused by mutations in the *CIAS1/NLRP3* gene. CAPS-associated mutations are gain-of-function and they lead to constitutive activation of the *NLRP3* inflammasome and high production of IL-1β cytokine. Patients with CAPS present with a spectrum of inflammatory features that are diagnosed as NOMID/CINCA, Muckle-Wells syndrome (MWS) and familial cold autoinflammatory syndrome (FCAS). NOMID/CINCA was first described by Prieur et al. [1, 2] and represents the most severe phenotype in the context of the clinical spectrum of CAPS. It is a rare disorder and in most cases is caused by a *de novo* mutation in the *NLRP3* gene, while patients with MWS and FCAS have the autosomal dominant pattern of inheritance.

CAPS patients present with fevers and urticarial-like skin rash soon after the birth and with time develop inflammation in multiple organs including CNS. The disease is clinically characterized by the triad of skin rash, severe arthritis/bony overgrowth, and chronic aseptic meningitis leading to neurologic damage and permanent damage of the joints.

The *CIAS1/NLRP3* gene is highly polymorphic, and to date, over 200 missense variants have been reported in the gene. Vast majority of the CAPS-associated mutations are found in exon 3 that encodes the NACHT protein domain. The distribution of the mutations in the *CIAS1* gene as per *in fevers* database is shown in Table 1. Disease causing mutations have been correlated with disease activity only for a subset of mutations [3]. The first patient with NOMID/CINCA in India was reported in 2007 [4]. We report here another novel *de novo* mutation detected in an Indian child with NOMID/CINCA.

Nucleotide sequence data reported are available in the GenBank databases under the accession number MG557564.

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Table 1 Distribution of the variants in the *CIAS1* gene as per *In fevers* database (fmf.igh.cnrs.fr/ISSAID/infevers)

	Location on gene	No of variants
Exons	Exon 1	5
	Exon 3	161
	Exon 4	5
	Exon 5	1
	Exon 6	4
	Exon 7	2
	Exon 8	1
Introns		23
UTR region		3
Total		205

Materials and methods

Clinical background

A 1-year and 3-months-old male born to non-consanguineous marriage presented to the primary pediatrician at 9 months of age with fever, respiratory distress, urticarial skin rash, and generalized anasarca. His workup was suggestive of macrophage activation syndrome, and he was treated with pulse methylprednisolone and IV immunoglobulin after which he was afebrile, asymptomatic and hence was discharged.

Gradually he developed fever, puffy fingers with arthritis of bilateral wrist, right knee, and restriction of neck movements with irritability and neuroregression.

He was diagnosed with refractory systemic Juvenile Idiopathic Arthritis (sJIA) and was referred to us for further management.

On examination he had frontal bossing, saddle back nose with pallor, hepatosplenomegaly, small and large joint arthritis and neuroregression.

His investigations showed anemia, leukocytosis and thrombocytosis with elevated acute phase reactants.

We recognized the atypical fever pattern, the urticarial skin rash and neuroregression, incompatible with the diagnosis of sJIA and chose to investigate him for CAPS mutations.

His brainstem evoked response audiometry (BERA) and ophthalmological examination were normal. MRI brain showed cerebrocortical atrophy. CSF studies were deferred at request of parents pending mutation analysis.

Genetic analysis

After parental consent, genomic DNA was extracted from the blood samples using Qiagen blood DNA extraction kit as per the manufacturer's protocol. PCR was put up to amplify exon 3 of the *CIAS1/NLRP3* gene. Exon 3 was selected for PCR because majority of the mutations reported in literature are present in this exon. Our analysis was restricted to mutations in exon 3 while a wider NLRP3 screen would be considered optimal. After PCR, the product was loaded on a 1% agarose gel to check for the presence of the specific amplified band. The PCR product was then purified using Exo Sap and sequenced using an ABI 310 automated DNA sequencer using the Big Dye terminator kit (Applied Biosystems, Foster City, CA, USA). The sequence output was compared with both In fever database as well as and the NCBI database.

Results

CIAS1/NLRP3 gene (ref no NM_001243133.1) sequencing of the amplified exon 3 in the patient showed a heterozygous single nucleotide substitution (c.1813A➔G, p.R605G). Sequencing of the parents' samples showed that both parents were negative for the substitution. Figure 1 shows the representative electropherograms of the R605G mutation in patient (a), father (b), and mother's (c) sample. The Polyphen-2 and PROVEAN (Protein Variation Effect Analyzer) software tools were used for prediction of functional effects of this mutation. The algorithms showed that the mutation is pathogenic/possibly damaging/deleterious with a score of 0.928 (Polyphen-2) and − 4.940 (PROVEAN). We therefore identified a novel disease-causing mutation in the *CIAS1/NLRP3* gene responsible for our child's clinical features.

Discussion

CINCA/NOMID is the most severe form of CAPS that is characterized by recurrent episodes of inflammation without any infectious or autoimmune cause. In addition to fevers, patients present with various organ manifestation including the central nervous system, bones, joints and skin. The disease often manifest soon after birth and lasts for the entire lifetime [2, 5]. Our patient presented at late infancy rather than neonatal period with typical CAPS like symptoms including joint pain, skin rash, and neuroregression.

NLRP3 inflammasome plays a very important role in innate immunity responses to pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPS). Cryopyrin, the protein product of the NLRP3 gene, regulates the activation and secretion of the interleukin (IL)-1β pro-inflammatory cytokine, which can trigger inflammation and induce fever. Gain-of-function NLRP3 mutations lead to excessive release of interleukin (IL)-1β. The over production of IL-1β causes the symptoms to be present at birth or in infancy and may persist or increase throughout life [6].

NLRP3 belongs to the NOD-like receptor (NLR) family of proteins, and it consists of a nucleotide binding and oligomerization domain (NOD/NBD also called NACHT domain), a

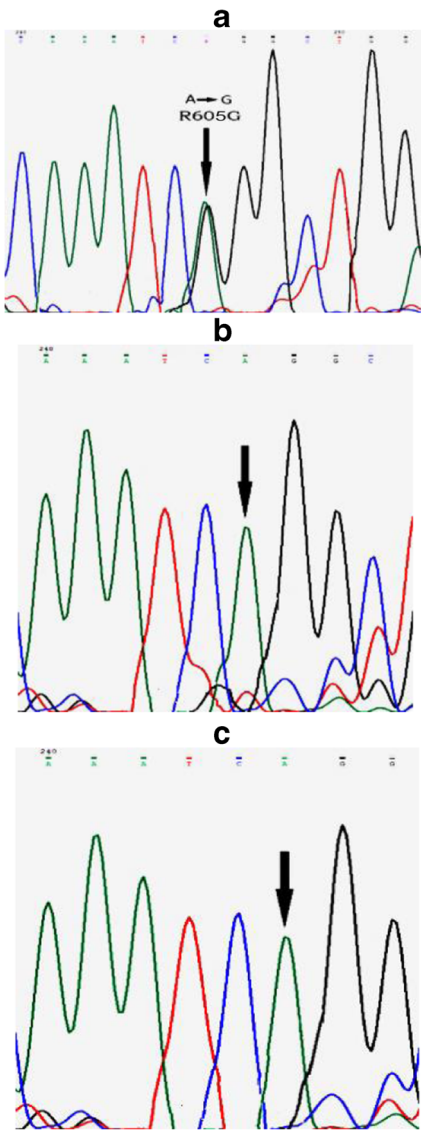

Fig. 1 Representative electropherograms of the R605G mutation in patient's (**a**), father's (**b**), and mother's (**c**) sample

C-terminal leucine rich repeat domain (LRR domain), and either an N-terminal caspase activating and recruitment domain (CARD) or a pyrin domain (PYD), [5, 7]. Mutations in the *NLRP3* gene surrounding the NBD and C-terminal LRR region make the protein constitutively active and cause continuous inflammasome activation [8–10]. The novel mutation detected in our patient is also present in the region between NBS domain and the C-terminal LRR region of the NLRP3 gene suggesting that this mutation may be responsible for the activation of the cryopyrin protein and continuous release of (IL)-1β.

Though NLRP3 mutations have been detected in most of the patients with CAPS, about 40–50% of patients with typical clinical symptoms do not have an identifiable NLRP3 mutation thus also indicating genetic heterogeneity [11]. A subset of patients who lack detectable germline mutations in NLRP3 has been found to have myeloid lineage-specific somatic mutations in NLRP3 [12, 13].

Many cases such as ours are due to spontaneous mutations, and these are distributed among all ethnic groups with an estimated frequency of 1per million [14, 15].

Discovery of the mechanism of the cryopyrin-mediated regulation of caspase − 1 activation and the secretion of IL-1 suggested that IL-1 blockage would be an effective treatment. IL-1 antagonists Anakinra and Canakinumab are now widely used for controlling disease activity and preventing sequelae. Non-steroidal anti-inflammatory drugs and corticosteroids are often used for temporary relief. Various other drugs have been tried including azathioprine, colchicine, cyclosporin, methotrexate, IVIG, etanercept and thalidomide with variable response.

Our patient started on steroids and later methotrexate and thalidomide were added since Anakinra and Canakinumab are not available in our country.

As this mutation is present only in our index child and not in his parents, parents have thus been counseled about the negligible risk of recurrence in subsequent pregnancies.

Data availability statements All data generated and analyzed during this study are included in this published article.

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Compliance with ethical standards

Disclosure None.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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