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Unusual phenotype in Muckle-Wells Syndrome: Atypical NLRP3 variant and its role in inflammasome activation

Carla Maria Correia Serrão

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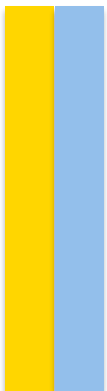

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Author:

- Carla Maria Correia Serrão
- carla22serrao@hotmail.com
- Mestrado Integrado em Medicina
- Instituto Ciências Biomédicas Abel Salazar- Universidade do Porto

Supervisor:

- Dr.ª Lúcia Raquel Moreira Faria
- raquelfaria.uic@chporto.min-saude.pt
- Assistente Graduada - Unidade de Imunologia Clínica, Centro Hospitalar Universitário do Porto
- Colaboradora Externa do Instituto Ciências Biomédicas Abel Salazar- Universidade do Porto

Co- Supervisor:

- Professor Doutor Carlos Alberto da Silva Vasconcelos
- cvcarlosvasconcelos@gmail.com
- Chefe de Serviço de Medicina Interna do Centro Hospitalar Universitário do Porto
- Unidade de Imunologia Clínica, Centro Hospitalar Universitário do Porto
- Prof. Catedrático Convidado do Instituto Ciências Biomédicas Abel Salazar- Universidade do Porto

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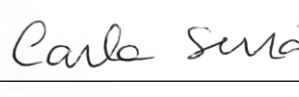

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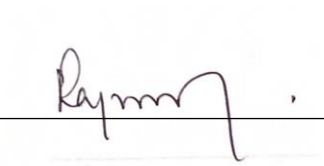

Supervisor | Dra. Lúcia Raquel Moreira Faria

Co- Supervisor | Professor Doutor Carlos Alberto da Silva Vasconcelos

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Resumo

A síndrome de Muckle-Wells (MWS) pertence às síndromes periódicas associadas à criopirina (CAPS), um grupo de distúrbios causados por uma mutação no gene NLRP3 que leva à sobreprodução de IL-1β/IL-18. O exantema urticariforme parece ser a manifestação clínica mais proeminente nas CAPS, porém foram descritos alguns casos sem envolvimento cutâneo.

O objetivo deste trabalho é apresentar os casos clínicos de duas irmãs portadoras de uma mutação *missense* rara (Tyr861Cys) no exão 6 do NLRP3 e de uma variante mitocondrial MT-TS2 (m.12236G>A) de significado desconhecido (VOUS) e discutir a possível relação entre essas variantes, o fenótipo atípico (não urticariforme) de Muckle-Wells e o papel da mitocôndria na ativação do inflamassoma NLRP3.

Ambas as doentes apresentavam surdez neurossensorial, conjuntivite recorrente, cefaleia crónica, meningite asséptica, episódios *stroke-like*, anemia ferropriva e artropatia. A pseudo-urticária estava ausente. Estudos laboratoriais mostraram níveis elevados de amiloide A, velocidade de sedimentação e proteína C-reativa. Níveis elevados de proteína e pleocitose foram ainda detetados no líquido cefalorraquidiano e a RMN cerebral de ambas mostrou lesões confluentes da substância branca e focos glióticos. A biópsia de gordura abdominal não mostrou evidência de deposição de amiloide. Destacou-se história de surdez neurossensorial de início na adolescência na família. Uma sobrinha e outra irmã foram diagnosticadas com MWS e apresentaram a mutação Tyr861Cys, mas não a VOUS mitocondrial. Ambas as doentes cumpriram os critérios diagnósticos para CAPS. Foi excluída miopatia mitocondrial e nenhuma das doentes preenche critérios de diagnóstico de síndrome de MELAS. O tratamento com anakinra levou a baixa atividade clínica e laboratorial da doença com tempo de seguimento de 3 anos.

Mutações em locais-chave do domínio rico em repetições de leucina (LRR) do NLRP3 demonstraram promover a ativação espontânea do inflamassoma e estar associadas ao fenótipo CAPS não urticariforme e ao fenótipo de sobreposição MWS-CINCA. A disfunção mitocondrial leva à produção e libertação de espécies reativas de oxigénio, DNA mitocondrial oxidado e cardiolipina, todos os quais parecem ativar o inflamassoma NLRP3.

Em suma, esta rara mutação no exão 6 do NLRP3 e a VOUS mitocondrial parecem atuar sinergicamente na ativação do inflamassoma NLRP3 e, consequentemente, contribuir para o fenótipo atípico de CAPS apresentado.

Palavras-chave: Síndrome Muckle-Wells; Síndrome CINCA; Síndromes periódicas associadas à criopirina (CAPS); Fenótipo não urticariforme; Mutação atípica; Domínio LRR; Inflamassoma NLRP3; Síndrome MELAS; Mitocôndria.

Abstract

Muckle-Wells syndrome (MWS) belongs to the cryopyrin-associated periodic syndromes (CAPS), a group of disorders caused by a NOD-like receptor family pyrin domain containing 3 (NLRP3) mutation that leads to IL-1β/IL-18 overproduction. Urticariform rash seems to be the prominent clinical manifestation in CAPS, however some cases have been reported without cutaneous involvement.

The aim of this paper is to present the clinical and laboratory features of two sisters carrying a rare missense NLRP3 mutation (Tyr861Cys) in exon 6 who have additionally a MT-TS2 mitochondrial variant (m.12236G>A) of unknown significance (VOUS) and discuss the potential adding effect of these variants on the atypical (non-urticarial) Muckle-Wells phenotype presented and the role of mitochondria in NLRP3 inflammasome activation.

Both patients presented early-onset sensorineural deafness, recurrent conjunctivitis, chronic headaches, aseptic meningitis, stroke-like episodes, iron deficiency anaemia and arthropathy. Pseudo-urticaria was absent. Laboratory studies showed markedly elevated serum amyloid A, erythrocyte sedimentation rate and C-reactive protein. Elevated protein levels and pleocytosis were detected in cerebrospinal fluid and periventricular white matter confluent lesions and focal gliotic lesions were present in brain MRI. Abdominal fat biopsy showed no evidence of amyloid deposition. Adolescence-onset sensorineural deafness runs in the family. A niece and another sister were previously diagnosed with MWS with the Tyr861Cys mutation, but not the mitochondrial VOUS. Both our patients met the diagnostic criteria for CAPS. Mitochondrial myopathy was ruled out and neither patient met the diagnostic criteria for mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome. Treatment with anakinra led to clinical and laboratory low disease activity with a follow-up time of 3 years.

Mutations in key sites in leucine-rich repeat (LRR) domain of NLRP3 were proved to promote spontaneous inflammasome activation and to be associated with atypical non-urticarial CAPS phenotype and MWS-CINCA syndrome overlapping phenotype. Reactive oxygen species, oxidized mitochondrial DNA and cardiolipin released by dysfunctional mitochondria seem to activate NLRP3 inflammasome.

Overall, this rare mutation in exon 6 of NLRP3 plus a mitochondrial VOUS seem to be acting synergically in NLRP3 inflammasome activation and contributing for an atypical CAPS phenotype.

Keywords: Muckle-Wells syndrome; CINCA syndrome; Cryopyrin-associated periodic syndromes (CAPS); Non-urticarial phenotype; LRR domain; NLRP3 inflammasome; MELAS syndrome; Mitochondria.

Abbreviation List

CAPS- Cryopyrin-associated periodic syndromes

CINCA- Chronic infantile neurological, cutaneous and articular syndrome

FCAS- Familial cold auto-inflammatory syndrome

IL-1β- Interleukin 1β

IL-18- Interleukin 18

LRR- Leucine-rich repeat

MELAS- Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes syndrome

MRI- Magnetic resonance imaging

mtDNA- Mitochondrial deoxyribonucleic acid

MT-TS2- Mitochondrially encoded TRNA-Serine 2

MWS- Muckle-Wells syndrome

NALP3- Nacht leucine-rich repeat protein 3

NEK7- NIMA-related kinase 7

NLRP3- NOD-like receptor family pyrin domain containing 3

NOD- Nucleotide-binding oligomerization

NOMID- Neonatal onset multi-system inflammatory disease

PYD- Pyrin domain

SND- Sensorineural deafness

TRNA- Transfer Ribonucleic acid

VOUS- Variant of uncertain significance

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Clinical implications

Rare mutation in exon 6 of NLRP3 plus a mitochondrial VOUS may be acting synergically in NLRP3 inflammasome activation and contributing for an atypical (non-urticarial) CAPS phenotype.

Introduction

Muckle-Wells syndrome (MWS) is a rare autoinflammatory disease inherited in an autosomal-dominant fashion.[1] It belongs to the spectrum of cryopyrin-associated periodic syndromes (CAPS) also known as cryopyrinopathies.[2] Beside MWS, CAPS encompass familial cold autoinflammatory syndrome (FCAS) and neonatal onset multi-system inflammatory disease (NOMID) also designated chronic infantile neurological, cutaneous and articular (CINCA) syndrome.[3] FCAS represents the mildest form and, as the name implies, is characterized by cold-induced symptoms. The most severe form, CINCA, typically presents itself in neonatal period, with severe neurological manifestations, bone deformities and urticarial rash.[3,5] MWS, the intermediate phenotype, has progressive hearing loss and secondary amyloidosis as distinctive clinical features. Early-onset recurrent urticaria-like rash seems to be the prominent feature in all phenotypes, although some cases have been reported without cutaneous involvement.[6-9]

Cryopyrinopathies are caused by gain-of-function mutations in NLRP3 (NOD-like receptor family pyrin domain containing 3) gene[2,3,6] located on chromosome 1q44,[1] that codes for the protein cryopyrin (NALP3 or NLRP3),[6] that lead to hyperactivation of NLRP3 inflammasome and overproduction of proinflammatory cytokines (IL-1β and IL-18) responsible for the systemic inflammation and clinical manifestations.[1,9]

NLRP3 inflammasome is an intracellular multiprotein complex essential in the regulation of innate immune system.[3,5] It can be activated by several stimuli[5,10] in a complex not fully understood process in which mitochondria seems to be involved.[11]

Objectives

To present the case of two Portuguese sisters carrying a heterozygous NLRP3 mutation [c.2582A>G p.(Tyr861Cys)] in exon 6 who have additionally a homoplasmic mitochondrial variant of uncertain significance (VOUS) in mitochondrially encoded TRNA-Serine 2 (MT-TS2) gene (m.12236G>A) and discuss the potential adding effect of these variants on the non-urticarial MWS phenotype presented and the mitochondria's role in NLRP3 inflammasome activation.

Clinical Cases report

Patient A: a 55-year-old female with early-onset sensorineural deafness (SND) (at age 13) and chronic headaches associated with fever, nausea, and vomiting. History of recurrent aseptic meningitis and several stroke-like episodes. In one of these episodes, a brain MRI showed no acute lesions, several chronic changes in periventricular and subcortical white matter compatible with demyelization, micro haemorrhagic foci, and multiple chronic lacunar infarctions. She also had dyschromatopsia, recurrent conjunctivitis, polyarthritis, iron deficiency anaemia, myocardial infarction at age 43 and arterial hypertension since the age of 18. No history of urticarial rash or other cutaneous involvement.

Patient B: patient's A younger sister is a 48-year-old woman with onset of SND at age 10, chronic headaches with concurrent nausea, vomiting and fever initiated at age 15, several aseptic meningitis episodes and stroke-like episodes. Brain MRI showed atrophy, syringomyelia, bilateral hyperintense lesions in periventricular, temporal, and cerebellar hemispheres white matter. She also had since at least 30 years old: polyarthritis, synovitis, enthesopathy, clinodactyly, frontal bossing, recurrent eye inflammation, optic nerve alterations, dyschromatopsia, chronic disease anaemia and arterial hypertension. Like in patient A urticarial rash was absent.

Patients A and B had blood analysis showing markedly elevated serum amyloid A, erythrocyte sedimentation rate and C-reactive protein. Elevated protein levels and pleocytosis were detected in cerebrospinal fluid. Abdominal fat biopsy was performed and showed no evidence of amyloid deposition at the age of 55 and 48 years old, respectively. Serum lactate levels were normal. Electromyography and muscle biopsy were performed to rule out mitochondrial myopathy.

These two sisters (from a sibship of 4 females and 1 male) belong to a family with history of adolescence-onset SND. A nice and another sister were diagnosed with MWS in an other centre.

Genetic tests based on polymerase chain reaction and Sanger sequencing confirmed heterozygous NLRP3 mutation (Tyr861Cys) previously diagnosed in the other family members. Due to the previous suspicion of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome, a mitochondrial VOUS (m.12236G>A) was identified, which was not carried by the other family members.

Both patients met the diagnostic criteria for CAPS proposed by Kuemmerle-Deschner et al.[4] Treatment with IL-1 receptor antagonist (anakinra) led to clinical and laboratory low disease activity with a follow-up time of 3 years.

Discussion

The role of NLRP3 mutations in CAPS pathophysiology is widely established.[2] NLRP3 is a cytosolic sensor composed of leucine-rich repeat (LRR) domain, nucleotide-binding oligomerization (NOD) domain and pyrin domain (PYD).[8] The majority of mutations known to cause CAPS are missense mutations in NOD domain encoded by exon 3.[8] The patients we described present a rare missense mutation, Y861C or previous described as Y859C, in exon 6 which encodes LRR domain.[12] The role of this domain in inflammasome activation remains unclear, however it seems to be involved in interactions between NLRP3 and its endogenous inhibitor, NIMA-related kinase 7 (NEK7).[8] Mutations in this domain lead to increased NLRP3 inflammasome activity by lowering the activation threshold.[8] Moreover, mutations that lead to dephosphorylation in key sites in LRR domain, as Y861C, were proved to promote spontaneous inflammasome activation.[3]

The first symptom to be commonly noticed in MWS[2] - pseudo-urticaria or neutrophilic urticarial dermatosis [1]- was absent. Several neurological manifestations, frontal bossing, and optic nerve involvement, classically associated with CINCA[3,5,9] syndrome, were present, like in previous reports in which Y861C mutation was associated with atypical non-urticarial CAPS phenotype and MWS-CINCA syndrome overlapping phenotype.[6,7]

In parallel, the m.12236G>A mitochondrial variant was previously associated with non-syndromic SND,[13] MELAS syndrome[14] and respiratory chain deficiency (complexes I, III and IV).[15] The presence of stroke-like episodes before the age 40, headaches, vomiting and hearing impairment made previous physicians suspect MELAS, however neither patient met the diagnostic criteria[16] or MELAS would explain the inflammatory phenotype. Mitochondrial dysfunction caused by NLRP3 stimuli[11] and characterized by decrease or loss of membrane potential, leads to reactive oxygen species, oxidized mitochondrial deoxyribonucleic acid (mtDNA) and cardiolipin release, all of which seem to activate NLRP3 inflammasome,[10,11] by acting as danger signals.[17] This makes us wonder if the mitochondrial variant they carry is acting synergically in NLRP3 activation and contributing to the atypical phenotype observed or is this a more severe MWS-CINCA syndrome overlapping phenotype only due to the NLRP3 LRR domain mutation?

Conclusion

These cases show how a rare mutation in exon 6 of NLRP3 is responsible for an atypical clinical phenotype, emphasizes the role of mitochondria in inflammasome activation and its possible contribution to the phenotype observed.

Conflict of interest

The authors declare no conflicts of interest.

Declaration of patients consent

The authors certify that they obtained all appropriate patient consent forms. In the form the patients consent for their clinical information to be reported in the journal.

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