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### ► To cite this version:

Farah Diab, Camille Louvrier, Marc Fabre, Mira Rabbaa, Aphrodite Daskalopoulou, et al.. Late-onset expression of an autoinflammatory disease: Identification and functional characterization of a mosaic variant in NLRC4. European Society of Human Genetics, Jun 2024, Berlin (Germany), Germany. inserm-04674678

**HAL Id: inserm-04674678**

**<https://inserm.hal.science/inserm-04674678v1>**

Submitted on 21 Aug 2024

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# Late-onset expression of an autoinflammatory disease: Identification and functional characterization of a mosaic variant in *NLRC4*

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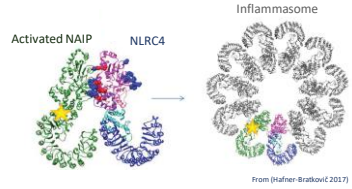
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## Introduction

Autoinflammatory diseases (AIDs) usually emerge in childhood, characterized by fever episodes that resolve spontaneously. They often entail inflammation affecting the skin, serosa (like the peritoneum, pericardium, and meninges), and synovium (joints). The pathophysiology of AIDs is intricate, involving innate immune components such as inflammasomes—complexes of multiple proteins crucial for initiating the inflammatory process.

The *NLRC4*-NAIP inflammasome is composed of *NLRC4*, which acts as an adaptor protein and is recruited by NAIP. Upon the recognition of bacterial elements, this inflammasome utilizes the adaptor protein ASC (apoptotic speck protein containing a CARD) to recruit the caspase-1 protease. Caspase-1 then cleaves the pro-interleukins (pro-IL1 $\beta$  and pro-IL18), resulting in the production of their active forms (IL1 $\beta$  and IL18) and the initiation of the inflammatory response.

Germline variants in *NLRC4* leading to gain-of-function are associated with ***NLRC4*-associated autoinflammatory disease (*NLRC4*-AID)**, an autosomal dominant disorder that may involve severe neonatal enterocolitis or macrophage activation syndrome (MAS). To date, only four mosaic and 15 germline pathogenic variations in the *NLRC4* gene have been reported in *NLRC4*-AID patients.



## Objectives

This study sought to evaluate the functional impact of a *NLRC4* somatic mosaic variation identified in a patient presenting with an autoinflammatory disease and to characterize the related disease phenotype.

## Methods

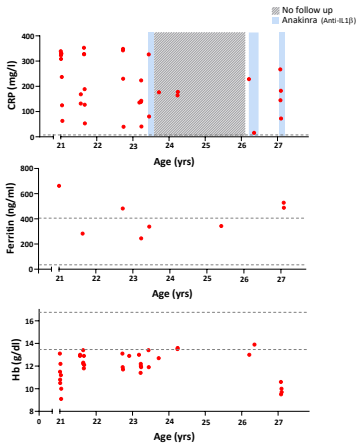
- An autoinflammatory diseases gene panel (1100X) was used to analyze the patient's leukocyte DNA.
- Plasma levels of pro-inflammatory cytokines (IL1 $\beta$ , IL6, TNF $\alpha$  and IL18) were measured by ELISA.
- NLRC4* constructs, whether wild type or mutant, were overexpressed in HEK293T cells co-expressing the inflammasome components ASC and caspase-1.
- Activation of the *NLRC4*-NAIP inflammasome was assessed by measuring the apoptotic speck aggregates (insoluble ASC oligomers) and NF $\kappa$ B pathway activity.

## Results

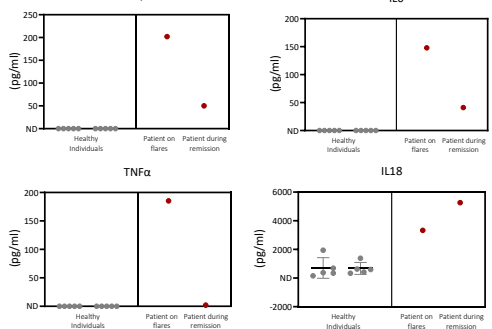
### Clinical Features

The patient is a 28-year-old man with a chronic psychosis since the age of 18 who has been experiencing:

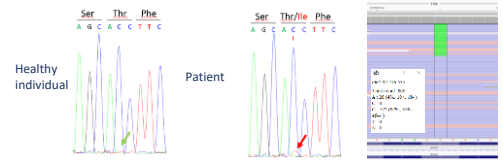
- severe inflammatory crises characterized primarily by fever, arthralgia and abdominal pain
- elevated C-reactive protein levels accompanied by anemia and leukocytosis
- moderate hyperferritinemia



### Increased plasma levels of the pro-inflammatory cytokines IL1 $\beta$ , TNF $\alpha$ , IL6 and IL18 during flares, with sustained elevated levels of IL18 during patient's remission

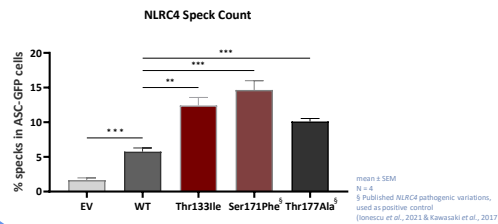


### Identification of a mosaic c.398C>T, p.(Thr133Ile) *NLRC4* variation (variant allele fraction of 4%)

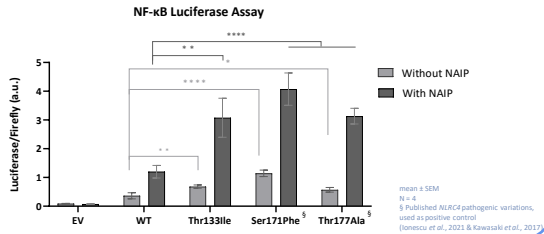


## Functional Analyses

The p.Thr133Ile *NLRC4* variation induced increased inflammasome activation



The p.Thr133Ile *NLRC4* variation activated the NF $\kappa$ B pathway



## Conclusion

This study identified a novel mosaic variation of *NLRC4* in an adult patient exhibiting both an autoinflammatory disease and chronic psychosis. Functional investigations demonstrated that the identified variation had a pathogenic gain-of-function effect on inflammation.

Abbreviations: CARD, caspase recruitment domain; CRP, C-reactive protein; EV, Empty Vector; Hb, hemoglobin; NAIP, nucleotide-binding leucine-rich repeat family apoptosis inhibitory protein; *NLRC4*, nucleotide-binding leucine-rich repeat family CARD domain containing 4; WT, Wild-type.