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Identification et caractérisation de gènes impliqués dans les maladies auto-inflammatoires

Guillaume Sarrabay

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**THÈSE POUR OBTENIR LE GRADE DE DOCTEUR
DE L'UNIVERSITÉ DE MONTPELLIER**

En Biologie-Santé

École doctorale Sciences Chimiques et Biologiques pour la santé (CBS2)

Unité de recherche INSERM U1183

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impliqués dans les maladies auto-
inflammatoires**

Présentée par Guillaume SARRABAY

Le 16 décembre 2019

Sous la direction de Isabelle TOUITOU

Devant le jury composé de

Michaël HOFER, Professeur associé, Université de Lausanne
Capucine PICARD, PU-PH, Université Paris-V - René Descartes
Michel KOENIG, PU-PH, Université de Montpellier
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Rapporteur
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Résumé :

Les maladies autoinflammatoires monogéniques sont des pathologies mendéliennes causées par des mutations de gènes impliqués dans l'immunité innée. Elles se traduisent chez les patients par des poussées inflammatoires systémiques, des atteintes tissulaires, sans production d'autoanticorps. La première partie des résultats rapporte une étude comparative entre le séquençage Sanger et le séquençage nouvelle génération (NGS). Les 297 patients étaient inclus prospectivement et randomisés en 2 bras : Sanger et NGS. Nous avons conclu que l'approche NGS doublait le rendement diagnostique (10.1%) par rapport au Sanger (4.1%). Nous avons également montré que les 4 fièvres récurrentes héréditaires historiques n'étaient pas les plus fréquentes dans notre série. La deuxième étude rapporte la première duplication entière du gène *MEFV* chez une patiente atteinte de FMF. Nous avons montré qu'il s'agit d'une duplication en tandem et avons pu séquencer le point de cassure. L'implication de la duplication dans l'apparition de la maladie n'est pas claire et des études d'expression seraient nécessaires pour pouvoir conclure. Le troisième volet traite du phénomène de mosaïcisme dans les MAI, notamment les CAPS, par une revue de la littérature, enrichie par de nouveaux cas identifiés à Montpellier. Nous n'avons pas mis en évidence d'impact du taux de mosaïcisme sur les manifestations cliniques ou sur l'âge de début des symptômes. La quatrième étude rapporte une série de cas de patients atteints de déficit en adénosine déaminase de type 2, nous permettant d'enrichir le spectre des mutations connues. Nous avons également proposé un arbre décisionnel afin de proposer un diagnostic génétique plus rapide. Enfin, la cinquième étude est l'identification d'un nouveau gène responsable de syndrome autoinflammatoire associé au protéasome (PRAAS) chez une patiente consanguine. Nous avons montré l'implication du gène *PSMB10* dans la physiopathologie de la patiente, en conduisant des études *in vitro* et *ex vivo*. Les résultats *in vitro* montraient, dans une lignée cellulaire transfectée, une altération des fonctions enzymatiques du protéasome et une anomalie du clivage normal de la protéine, empêchant probablement l'assemblage correct de l'immunoprotéasome. Les études *ex vivo* sur cellules nucléées de la patiente montraient également des anomalies enzymatiques, ainsi qu'une accumulation de protéine ubiquitinylées, témoignant d'un dysfonctionnement de l'immunoprotéasome.

Abstract

Monogenic systemic autoinflammatory diseases (SAIDs) are Mendelian pathologies caused by gene mutations involved in innate immunity. Autoinflammatory patients exhibit inflammatory flares, tissue damage, without production of autoantibodies. The first part of the thesis is a comparative study between Sanger sequencing and Next Generation Sequencing (NGS). The 297 patients were included prospectively and randomized into 2 arms: Sanger and NGS. We concluded that the NGS approach doubled the diagnostic yield (10.1%) compared to the Sanger (4.1%). We also showed that the 4 historical hereditary recurrent fevers were not the most frequent in our series. The second study reports the first duplication of the *MEFV* gene in a FMF patient. We have shown that this is a tandem duplication and have been able to sequence the breakpoint. The implication of this duplication in the onset of the disease is unclear and studies of expression would be necessary. The third part deals with the phenomenon of mosaicism in SAIDs (cryopyrin associated periodic syndrome (CAPS) in particular), by a literature review, enriched by new cases identified in Montpellier. We did not show any impact of the mosaicism rate on clinical manifestations or age of onset of symptoms. The fourth study reports a series of cases of patients with type 2 adenosine deaminase deficiency, allowing us to expand the mutation spectrum. We also proposed a decision tree to fasten the genetic diagnosis. Finally, the fifth study is the identification of a new gene responsible for proteasome-associated autoinflammatory syndrome (PRAAS) in a consanguineous patient. We have shown the involvement of the *PSMB10* gene in the pathophysiology of the patient, conducting *in vitro* and *ex vivo* studies. The *in vitro* results showed, in a transfected cell line, an alteration of the enzyme functions of the immunoproteasome, and defects of the normal cleavage of the protein, probably preventing the correct assembly of the immunoproteasome. The *ex vivo* cell studies of the patient also showed enzymatic abnormalities, as well as ubiquitinated protein accumulation, indicating immunoproteasome dysfunction.

INTRODUCTION

1

Avant-propos

Les maladies autoinflammatoires (MAI) sont caractérisées par des poussées inflammatoires systémiques survenant chez les patients, et conjuguées à des atteintes tissulaires, sans production d'auto-anticorps. Le terme « autoinflammatoire » est d'apparition récente, utilisé la première fois par Michael F McDermott en 1999, pour les distinguer des maladies auto-immunes. Au sein des MAI, on retrouve des MAI multifactorielles, telles que la maladie de Crohn, la maladie de Behçet ou la polyarthrite rhumatoïde, qui sont seulement évoquées dans ce manuscrit, et des MAI monogéniques, qui sont le sujet principal de cette thèse.

Les MAI monogéniques forment un vaste groupe de maladies héréditaires rares. Le premier gène, identifié en 1997, est *MEFV*, responsable de la fièvre méditerranéenne familiale (FMF), la plus fréquente des MAI monogéniques. Avec l'avènement du séquençage haut débit (NGS) et notamment des études de whole exome sequencing (WES), la liste des gènes impliqués dans les MAI s'est allongée ces dernières années, permettant de mieux comprendre leur physiopathologie, et de proposer des traitements plus ciblés. Néanmoins, une grande proportion de patients souffrant de MAI reste sans diagnostic génétique, et ne peut bénéficier d'une prise en charge thérapeutique rapide et efficace, soit parce qu'il s'agit de forme multifactorielle, soit parce que le gène causal n'a pas encore été découvert. La génétique des MAI est un domaine d'étude récent, qui a éclot à la fin des années 90, et qui connaît une accélération exponentielle depuis les nouvelles techniques de séquençage.

Ainsi, mon travail de thèse a été de mener une étude génétique des MAI, du diagnostic à la recherche translationnelle, d'une part en caractérisant des mutations ou des gènes déjà connus dans les MAI, et d'autre part en cherchant des gènes candidats par l'utilisation de WES en trio.

La partie introductive de ce manuscrit situe sur un plan médical le contexte des MAI. La revue « Panorama des maladies autoinflammatoires » détaille ainsi l'approche diagnostique permettant d'évoquer une MAI, puis les informations à collecter pour s'orienter vers une MAI

en particulier. Les différentes MAI monogéniques y sont décrites en fonction de leurs signes cliniques respectifs et de leur classification physiopathologique.

Cette introduction est complétée par deux revues portant plus spécifiquement sur deux MAI. La revue sur les syndrome périodiques associés à la cryopyrine (CAPS) permet de décrire plus précisément ce spectre clinique, qui s'étend de la forme bénigne appelée urticaire familiale au froid (FCAS) à la forme grave et multisystémique CINCA/NOMID (Chronic Infantile Neurological Cutaneous Articular Syndrome/Neonatal Onset Multisystem Autoinflammatory syndrome) et introduit une étude sur le mosaïcisme somatique, dans CAPS principalement, présentée dans les résultats.

Enfin, j'inclus dans cette introduction une revue sur le déficit en adénosine déaminase de type 2 (DADA2), une vascularite autoinflammatoire qui fait également l'objet d'un travail expérimental présenté en seconde partie.

La partie expérimentale décrit les différents projets menés lors de cette thèse portant sur l'étude génétique des maladies autoinflammatoires.

Un premier axe de ce projet de thèse s'attachait à caractériser de gènes ou variants déjà connus comme responsables de MAI.

En premier lieu je présente la première étude comparative et randomisée entre le séquençage classique Sanger et le séquençage nouvelle génération (NGS) de panels de gènes, dans les MAI, qui permet de documenter plus précisément l'apport diagnostique de l'approche par panel phénotypique.

La deuxième étude décrit ensuite la première duplication entière du gène *MEFV*, impliqué dans la FMF. Cette découverte est intéressante car le gène *MEFV* n'est que très rarement le siège de remaniements. La physiopathologie de la FMF n'étant pas complètement élucidée, cela rend son interprétation d'autant plus complexe, ce qui est discuté dans l'étude.

Dans une troisième étude, j'étudie le phénomène de mosaïcisme dans les gènes responsables de MAI, et dans les CAPS en particulier, dont on sait que beaucoup de formes peuvent être dûes à des mutations somatiques.

La dernière étude de cet axe 1 est une série de nouvelles mutations du gène *ADA2*, responsable du DADA2. J'ai pu proposer un algorithme décisionnel visant à accélérer la prise en charge de cette pathologie dont le pronostic peut être impacté par l'errance diagnostique.

Le deuxième axe de ce travail, probablement le plus important, consistait à découvrir de nouveaux gènes responsables de MAI.

Cette dernière partie traite donc de la découverte de *PSMB10*, un nouveau gène codant une sous-unité de l'immunoprotéasome, non décrit en pathologie humaine, responsable de syndrome autoinflammatoire associé au protéasome (PRAAS). J'y décris le cas pédiatrique d'une patiente atteinte d'une forme de PRAAS, dont les explorations génétiques classiques étaient non contributives, et qui porte une mutation homozygote dans *PSMB10*. Plusieurs études fonctionnelles, *in vitro* et *ex vivo* permettent de prouver le lien causal entre la mutation de ce gène, et la symptomatologie de la patiente.

2

Panorama des maladies auto-inflammatoires

Clinical overview of autoinflammatory diseases

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Résumé

Les maladies auto-inflammatoires sont caractérisées par des anomalies génétiques codant des protéines intervenant dans l'immunité innée. Ces maladies ont été définies en miroir par rapport aux maladies auto-immunes en raison de l'absence d'anticorps circulants. Leur caractéristique est de comporter un syndrome inflammatoire biologique en phase de poussée clinique et en l'absence d'infection. Le groupe le mieux connu comporte 4 fièvres récurrentes ou encore appelées « historiques » car elles ont été décrites les premières : la fièvre méditerranéenne familiale, les cryopyrinopathies, la fièvre intermittente liée au récepteur de type 1A du Tumor necrosis factor et le déficit partiel en mévalonate kinase. Depuis la description de ces 4 maladies, de très nombreuses autres ont été découvertes en 10 ans grâce aux progrès de la génétique. Cette revue dresse un panorama en 2017 des principales maladies autoinflammatoires monogéniques connues à ce jour et classées en dehors des 4 « historiques » et des interféronopathies qui sont détaillées dans d'autres chapitres de ce numéro spécial. Certaines se comportent comme des fièvres récurrentes, d'autres n'évoluent pas selon le mode poussée/rémission, enfin certaines sont associées à des déficits immunitaires. Comme diagnostic différentiel trois pathologies seront abordées : la maladie de Still, le syndrome de Schnitzler et le syndrome PFAPA, ce dernier étant très fréquent chez l'enfant mais pouvant perdurer à l'âge adulte. Le diagnostic de ces maladies repose en grand partie sur l'anamnèse, la présence d'une inflammation en crise et sur des analyses génétiques de plus en plus performantes.

Mots clefs: autoinflammatory diseases, monogenic recurrent fevers, PSTPIP1, NOD2, PFAPA

Abstract

Autoinflammatory diseases are characterized by genetic abnormalities involving innate immunity. They were initially described in mirror with autoimmune diseases because of the absence of circulating autoantibodies. In autoinflammatory diseases (AID), inflammatory blood biomarkers are elevated in crisis without infection. The four best known AID consist of the historical monogenic AI diseases that were first described: familial Mediterranean fever (FMF), cryopyrinopathies, mevalonate kinase deficiency and TNFRSF1A related intermittent fever. Since the initial description of these disease in the late 1990's, more than 30 different diseases have been discovered, especially thanks to the progress in genetics. In this review, we propose a panorama of the main new AID; the 4 historical and several others are described as a full chapter in this special issue of this journal focused on autoinflammation. Some of these new AID feature recurrent fevers, whereas others are characterized by predominant cutaneous symptoms or immune deficiency. As differential diagnosis, three pathologies will be shortly described: Still disease, Schnitzler syndrome and the PFAPA or Marshall syndrome; this latter is very frequent among children and as some adults can develop it, it seems important to describe it. The diagnosis of each AID is largely based on anamnesis, the presence of peripheral inflammation in crisis is crucial. Overall, continuous progress in genetics brings a range of innovative techniques which seem especially efficient in detecting rare entities and discovering new.

Key words: autoinflammatory diseases, monogenic recurrent fevers, PSTPIP1, NOD2, PFAPA

1. Abréviations

APLAID	auto-inflammation and PLCg2-associated antibody-deficiency and immune dysregulation
CAMPS	CARD14-mediated psoriasis
CANDLE	chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature
CAPS	cryopyrin-associated periodic syndrome
CINCA	syndrome chronique infantile neurologique, cutané et articulaire
DIRA	déficit du récepteur antagoniste de l'IL-1
DITRA	déficit du récepteur antagoniste de l'IL-36
DUB	déubiquitinase
FCAS	urticaire familial au froid (forme bénigne de cryopyrinopathie)
FCAS2	urticaire familiale au froid par mutation de NLRP12
FMF	fièvre méditerranéenne familiale
HA20	haplo-insuffisance de la protéine A20
LUBAC	complexe d'assemblage linéaire de la chaîne d'ubiquitine linéaire
MKD	déficit en mévalonate kinase
MWS	syndrome de Muckle Wells
NAIAD	NLRP1-associated auto-inflammation with arthritis and dyskeratosis
NAID	NOD2-associated auto-inflammatory diseases
NLRC4-MAS	NLRC4-associated macrophage activation syndrome
ORAS	Otulin-related auto-inflammatory syndrome
PAAND	Pyrin-associated auto-inflammatory syndrome with neutrophilic dermatosis
PAID	PSTPIP1-associated auto-inflammatory diseases
PAMI	PSTPiP1-associated myeloid-related-proteinemia inflammatory syndrome
PAPA	syndrome arthrite pyogénique, pyoderma gangrenosum, acné
PAPASH	PSTPiP1-associated Pyoderma gangrenosum, Acne and Hidradenitis Suppurativa
PAC	Pyoderma gangrenosum, Acne and ulcerative Colitis
PFAPA	periodic fever aphthous stomatitis pharyngitis adenitis
PFIT	periodic fever with immunodeficiency and thrombocytopenia

PLAID	PLCg2-associated antibody-deficiency and immune dysregulation
SAVI	STING-associated vasculopathy with onset in infancy
SIFD	sideroblastic anemia with B-cell immunodeficiency, periodic fevers and developmental delay
TNFRSF11A	syndrome périodique associé au récepteur du TNF11 α
TRAPS	syndrome périodique associé au récepteur du TNF α

2. Introduction

Depuis la création du terme d'auto-inflammation, plusieurs définitions et en conséquence classifications des maladies auto-inflammatoires (MAI) ont été proposées (1). Nous retiendrons ici comme auto-inflammatoires des maladies qui ont une composante clinique et biologique inflammatoire et qui sont caractérisées par une anomalie de l'immunité innée. Les immenses progrès de la génétique des dernières années ont permis de découvrir chaque année plusieurs nouvelles MAI monogéniques (figure 1). Le sous-groupe le mieux connu car décrit en premier est celui des “ fièvres récurrentes héréditaires ”, comportant 4 maladies historiques : la fièvre méditerranéenne familiale (FMF), la plus fréquente des MAI, les cryopyrinopathies qui forme un spectre de maladies liées aux mutations de *NLRP3*, le déficit partiel en mévalonate kinase et le syndrome TRAPS (fièvre intermittente liée au récepteur de type 1A du Tumor necrosis factor) (1,2). Parmi les nouvelles MAI monogéniques décrites certaines sont responsables de fièvres récurrentes également, d'autres d'épisodes inflammatoires permanents ; certaines ont des signes cutanés au premier plan et certaines autres sont associées à un déficit immunitaire. De façon intéressante, les découvertes de ces dernières années ont permis de découvrir que certaines maladies ont une expression clinique différente suivant la mutation du gène. Ainsi le spectre clinique peut être étendu en fonction de l'anomalie génétique comme dans les maladies liées aux mutations de *MEFV*, de *NLRC4*, de *NOD2*, de *PSTPIP1*(3–7). Une autre avancée de la génétique est d'avoir mis en évidence la présence chez certains patients de mutations somatiques de gènes associés à des MAI soit lorsque la maladie survient tard dans la vie, soit si elle survient tôt mais que seule une fraction de cellules est mutée (8–13). Cet article a pour objectif de détailler la démarche diagnostique devant une suspicion de MAI, puis de détailler les principales nouvelles MAI et leur diagnostic différentiel enfin de résumer la physiopathologie des MAI et les principes du traitement.

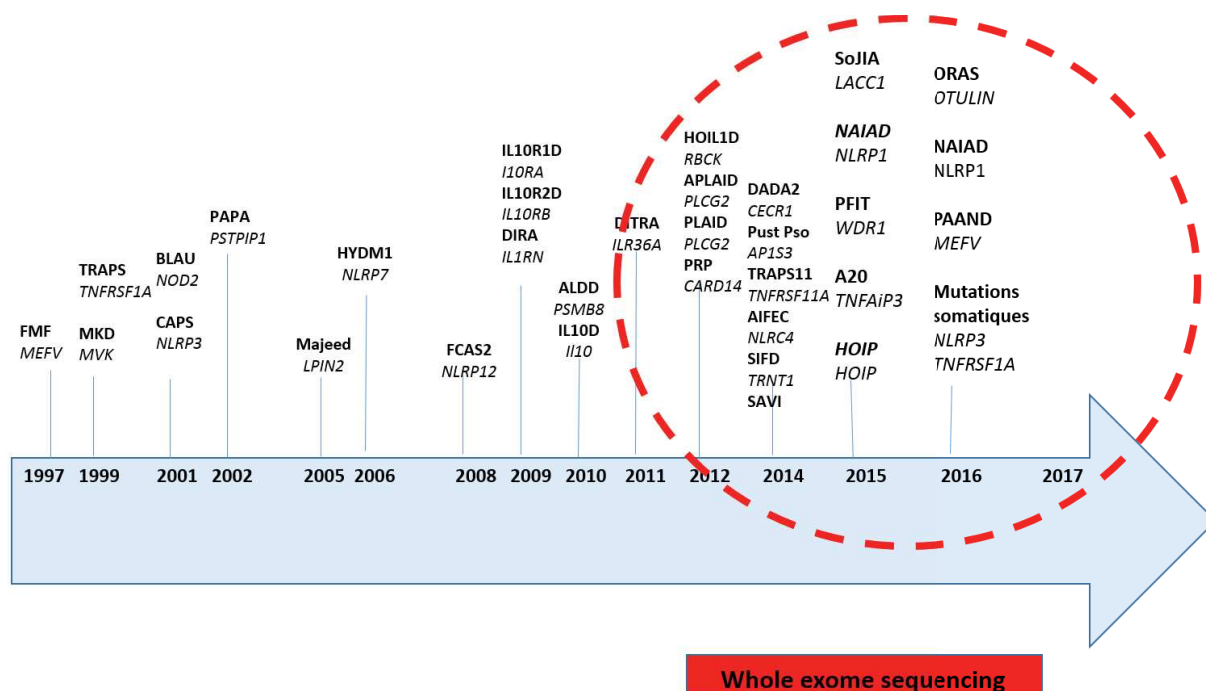


Figure 1. Représentation schématique de la découverte des gènes responsables des principales MAI au cours du temps

Découverte des principales MAI monogéniques au cours du temps : les 5 dernières années, de nombreux gènes ont été découverts grâce aux progrès de la génétique notamment aux techniques de séquençage de tous les exons (whole exome sequencing).

3. Démarche diagnostique

La table 1 résume le plan de l'analyse sémiologique d'une MAI afin de s'orienter vers telle ou telle catégorie et guider une éventuelle analyse génétique.

3.1. Prérequis : il faut impérativement objectiver la présence d'un syndrome inflammatoire en crise par la mesure de la CRP et la recherche d'une hyperleucocytose à prédominance de polynucléaires neutrophiles sur l'hémogramme. On peut remettre au patient une ordonnance de prise de sang à réaliser lors des crises et il faut recueillir 3 preuves de l'élévation de la CRP (3 crises différentes) depuis au moins 6 mois pour considérer une MAI comme possible.

Table 1. Plan d'analyse sémiologique d'une suspicion de maladie autoinflammatoire

QUESTION	REPONSE
CRP élevée en crise?	OUI
Age au début des signes	A préciser au besoin avec les parents
Durée des crises	A préciser en jours
Fréquence des épisodes	A préciser par an ou par mois
Origine méditerranéenne?	Oui/non (remonter aux grand parents)
Mode de transmission?	Arbre généalogique : récessif ou dominant ?
Consanguinité?	Oui ou non
Signes associés fréquents	- Cutanés : aphtes, érythème, urticaire, pustules, dermatose neutrophilique, livedo - Digestifs : douleurs, diarrhée - Articulaires : arthromyalgies, arthrites
Signes associés rares	Neurologiques : AVC, méningites aseptique ORL : surdité Ophtalmologiques : conjonctivite, uvéite, épisclérite Adénopathies : cervicales ? profondes ? Hépatosplénomégalie Déficit immunitaire associé (infections à répétition)

3.2. *Recueil de l'âge de début et de la durée des crises* (figures 2 et 3) : L'âge où les premiers symptômes ont commencé est très important pour évoquer une MAI génétique, en fonction de l'âge de début, on peut évoquer certaines maladies, par exemples dans les cryopyrinopathies, l'urticaire est présente dès les premiers jours de vie, dans le déficit partiel en mévalonate kinase, les troubles digestifs comment dans la première année de vie. On évoquera davantage une MAI avec mutation somatique si les symptômes commencent après 40 ans. De même la durée des poussées permet d'évoquer certaines pathologies en priorité, lorsque la crise dure plus de 7 jours, on pense au syndrome TRAPS, lorsqu'elle dure plus de 4 semaines, on évoque la maladie de Still.

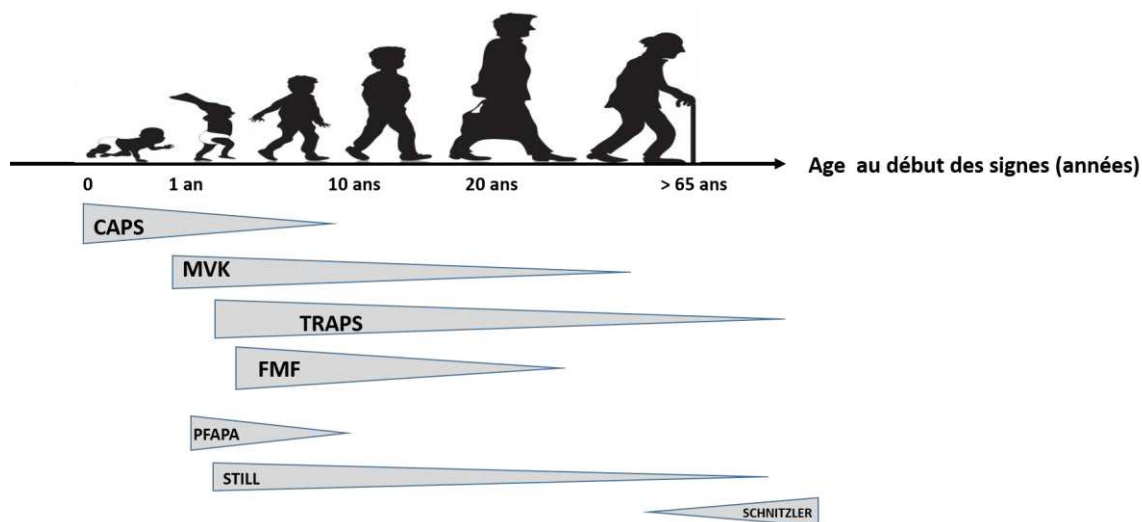


Figure 2. Orientation diagnostique vers une MAI en fonction de l'âge moyen de début des signes.

Il est très important de rechercher l'âge de début des signes et des poussées de fièvre, si besoin en interrogeant les parents du patient et en consultant son carnet de santé. Les cryopyrinopathies et le déficit en mévalonate kinase commencent très tôt dans la vie (souvent la première année de vie). La fièvre méditerranéenne familiale peut débuter dans l'enfance, entre 3 et 8 ans ; le TRAPS peut débuter entre 3 ans et 20 ans, voir après si les premières poussées sont frustrées ou passent inaperçues. Les deux maladies suivantes ont de très rares causes monogéniques : la maladie de Still est dite à début pédiatrique si elle débute avant 16 ans (cas le plus fréquent) et dite à début adulte si elle démarre après 16 ans ; la maladie qui débute le plus tard dans la vie est le syndrome de Schnitzler classiquement vers 65 ans.

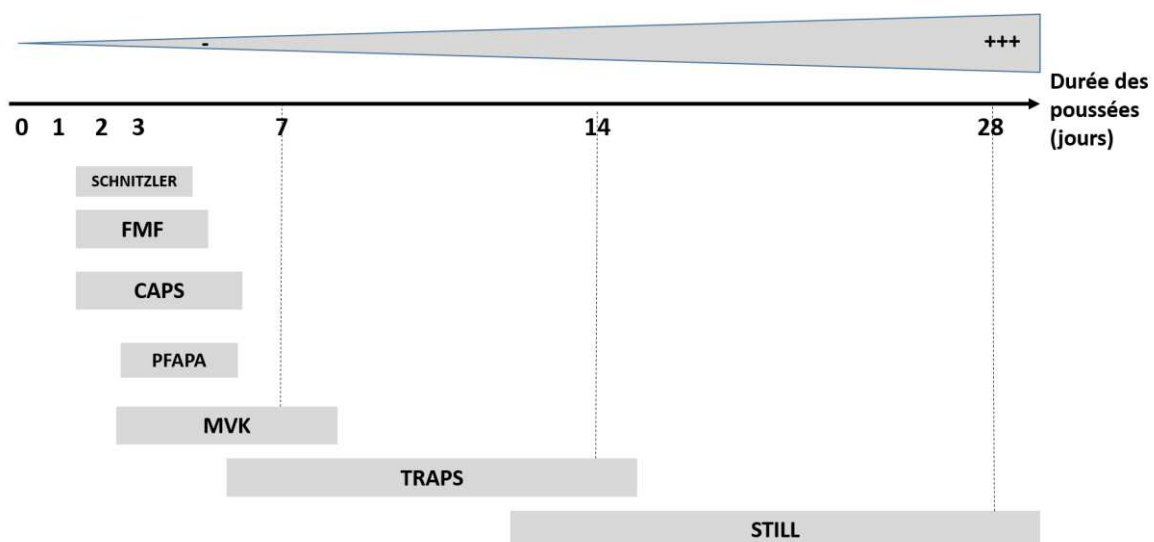


Figure 3. Orientation diagnostique vers une MAI en fonction de la durée moyenne des crises.

La durée de la crise permet de s'orienter vers une maladie combinée à l'âge de début, aux signes cliniques associés et aux antécédents familiaux. Les poussées de fièvre méditerranéenne familiale et de cryopyrinopathie sont volontiers courtes ; au cours du déficit en mévalonate kinase, la crise peut durer

jusqu'à 1 semaine. Le syndrome TRAPS a la particularité d'avoir des poussées longues d'au moins 5 jours, en moyenne 10-15 jours. La maladie de Still a classiquement des poussées minimum 2 semaines, souvent 4 semaines.

3.3. *Dessin de l'arbre généalogique et recueil des origines du patient.* L'arbre généalogique doit remonter jusqu'aux arrière grand parents du patient et ses descendant s'il en a. L'objectif est de voir si chaque génération est touchée ce qui évoque une transmission autosomique dominante ou pas (plus en faveur d'une transmission récessive). On note les mariages consanguins en faveur d'un trait récessif. On recherchera également des cas de décès par insuffisance rénale (patients dialysés). Enfin les origines des arrière grand parents sont notées afin en priorité d'éliminer une FMF, mais aussi pour rechercher certaines maladies décrites dans certaines populations (comme pour les mutations de *LACCI* par exemple (14,15)).

3.4. *Analyses des signes cliniques principaux ou cardinaux (table 2).*

La fièvre récurrente s'accompagne habituellement de signes cliniques, les 3 appareils les plus souvent impliqués sont la peau, le tube digestif et le système musculo-squelettique. Il faut rechercher ces atteintes en priorité puis celles moins fréquentes. La table 2 résume les atteintes principales qui doivent faire évoquer en priorité telle ou telle MAI.

Table 2. Orientation vers une maladie autoinflammatoire en fonction de certains signes cliniques, biologiques ou histologiques prédominants.

Signe clinique, biologique ou histologique prédominant	Présence dans les quatre fièvres récurrentes classiques	Présence dans les autres maladies auto-inflammatoires
Signes cutanés prédominants		
Urticaire	<i>NLRP3</i> (urticaire au froid) <i>MVK</i>	<i>NLRP12</i> <i>NLRC4</i> PLAID (<i>PLC-gamma2</i>) Syndrome de Schnitzler
Dermatose neutrophilique	FMF (<i>MEFV</i>) TRAPS (<i>TNFRSF1A</i>)	PAAND (<i>MEFV</i>) DIRA (<i>IL-1 RN</i>) DITRA (<i>IL-36 RN</i>) Syndrome PAPA (<i>PSTPI1</i>)

		<p>Syndrome ORAS (<i>OTULIN</i>)</p> <p>Syndrome de Majeed (<i>LPIN2</i>)</p> <p>Syndromes associés aux anomalies du protéasome = CANDLE (<i>PSMB8</i>)</p> <p>Syndrome des abcès aseptiques</p>
Aphtes	<p>CAPS (<i>NLRP3</i>)</p> <p>MKD (<i>MVK</i>)</p>	<p>Fièvre liée à NLRP12 (<i>NLRP12</i>)</p> <p>Syndrome de Majeed (<i>LPN2</i>)</p> <p>Déficit en A20 (<i>TNFAIP3</i>)</p> <p>NAID (<i>NOD2</i>)</p> <p>Syndrome de Marshall ou PFAPA</p> <p>PAPA-like (<i>PSTPiP1</i>)</p>
Erythème	<p>FMF (<i>MEFV</i>)</p> <p>TRAPS (<i>TNFRSF1A</i>)</p> <p>Fièvre liée à NLRP12 (<i>NLRP12</i>)</p>	<p>NLRC4-MAS (<i>NLRC4</i>)</p> <p>Fièvre liée à TNFRF11A (<i>TNFR11A</i>)</p> <p>Arthrite juvénile systémique (<i>LACCI</i>)</p> <p>Maladie de Still</p>
Pustules et/ou psoriasis		<p>PAAND (<i>MEFV</i> exon 2)</p> <p>DIRA (<i>IL-1 RN</i>)</p> <p>DITRA (<i>IL-36 RN</i>)</p> <p>PAPA et PAMI (<i>PSTPiP1</i>)</p>
Pityriasis rubra pilaire		<p>CAMPS (<i>CARD14</i>)</p>
Acné, abcès, Pyoderma gangrenosum, et/ou hidradénite suppurée		<p>PAAND (<i>MEFV</i> exon 2)</p> <p>PAID (<i>PSTPiP1</i>)</p> <p>Maladies liées à des mutations de la nicastrine (<i>NCSTN</i>)</p>

		Syndrome des abcès aseptiques
Eczéma, éruption micropapuloerythémateuse, « atopie-like »		NAID (<i>NOD2</i>) Syndrome de Blau(<i>NOD2</i>)
Livedo	0	Interféronopathies ADA2 (<i>CECR1</i>)
Lipodystrophie		Syndrome CANDLE (<i>PSMB8</i>)
Signes digestifs		
	FMF (<i>MEFV</i>) <i>MVK</i> <i>NLRP3</i> TRAPS (<i>TNFRSF1A</i>)	NLRC4-MAS (<i>NRLC4</i>) Ubiquitinopathie (<i>A20</i> , <i>Otulin</i>)
Signes neurologiques		
Méningite chronique aseptique	CAPS (CINCA, MWS) <i>NLRP3</i> MKD (<i>MVK</i>)	<i>NLRC4</i>
AVC		Interféronopathies SFID (<i>TRNT1</i>) Déficit en ADA2 (<i>CECR1</i>)
Anomalies hématologiques ou autoimmunes		
Déficit immunitaire	0	PLAID (<i>PLC-gamma2</i>) Ubiquitinopathie (<i>HOIL-1</i>) SFID (<i>TRNT1</i>) ADA2 (<i>CECR1</i>) PFIT (<i>WDRI</i>)
Cytopénies	0	PFIT (<i>WDRI</i>) SFID (<i>TRNT1</i>) NLRC4-MAS Majeed (<i>LPIN2</i>)

Auto-anticorps	0	Ubiquitinopathies (<i>A20</i> , <i>ORAS</i>) NAIAD (<i>NLRP1</i>) PLAID (<i>PLC-gamma2</i>) Interféronopathies ADA2 (<i>CECR1</i>)
Immunoglobuline monoclonale	0	Syndrome de Schnitzler
Anomalies histologiques		
Granulome	0	Syndrome de Blau (<i>NOD2</i>) Déficit en ADA2 (<i>CECR1</i>) PLAID et APLAID (<i>PLC-gamma2</i>) Syndrome ORAS (<i>OTULIN</i>)
Vascularites	FMF (<i>MEFV</i>) TRAPS (<i>TNFRSF1A</i>) CAPS (<i>NLRP3</i>)	PAAND (<i>MEFV</i>) Déficit en A20 (<i>TNFAIP3</i>) ORAS (<i>OTULIN</i>) PAPA (<i>PSTPIP1</i>) Déficit en ADA2 (<i>CECR1</i>) Syndrome SAVI (<i>TMEM173</i>)

- Signes cutanés (figure 4) : il faut rechercher une urticaire, une dermatose neutrophilique, des aphtes buccaux et ou bipolaires, un érythème, des pustules, des lésions de psoriasis, de pityriasis rubra pilaire, des lésions d'acné banale ou kystique sévère, du pyoderma gangrenosum, une hidradénite suppurée, des lésions eczématiformes ou mimant une atopie, du livédo, une lipodystrophie du visage ou des membres. Toute lésion suspecte doit être biopsiée à la recherche d'une lésion spécifique (infiltrat neutrophilique, vascularite, granulomes...). Toute lésion doit faire l'objet d'une photographie et il faut demander au patient de photographier toute lésion dermatologique au cas où une poussée survienne au domicile, il vous enverra la photo par mail ou l'apportera à la consultation.



Figure 4. Signes cutanés principaux rencontrés au cours de MAI les plus fréquentes.

Panel A : aspect de pseudoérysipèle de cheville au cours d'une fièvre méditerranéenne familiale. Panel B : aspect d'urticaire au froid au cours d'une cryopyrinopathie de type urticaire familial au froid. Panel C : Eruption érythémato-maculeuse du tronc chez un patient avec un syndrome TRAPS. Panel D : Aphte buccale chez un patient avec déficit en mévalonate kinase. Panel E : lésions urticariennes au cours d'un syndrome de Schnitzler. Panel F : lésion rosée fugace du tronc chez un patient avec une maladie de Still de l'adulte.

-Articulaires : l'interrogatoire recherche des myalgies, des douleurs articulaires et précisera leur siège. L'examen recherchera des arthrites, des synovites, des arguments pour une sacroïlite, une spondyloarthrite.

-Digestifs : douleurs abdominales, ballonnements, régurgitations acides, diarrhée. Il faut tenter de déterminer si la douleur abdominale fébrile est une péritonite, et en cas d'intervention chirurgicale, récupérer le compte-rendu opératoire.

-Neurologiques : céphalées, méningite chronique aseptique, accidents vasculaires cérébraux

-Ophtalmiques : uvéites, conjonctivite, sclérote, épisclérite

-ORL : surdité de perception, chondrite de l'oreille, adénopathies cervicales

-Immuno/hématologiques : hépatosplénomégalie, adénopathies périphériques, infections à répétition témoignant un déficit immunitaire, signes évocateurs de cytopénies (thrombopénie, anémie notamment)

3.5. Critères diagnostiques

Certaines maladies bénéficient de jeux de critères qu'il convient de connaître comme la FMF, les maladies associées aux mutations de NOD2 ou NAID, le syndrome de Schnitzler, la maladie de Still et les cryopyrinopathies (16–21). Pour les 4 fièvres récurrentes historiques (FMF, TRAPS, CAPS, MKD), il existe un site internet qui permet d'aider à orienter vers l'une des 4 maladies où un algorithme en ligne permet d'aider le clinicien (lien: <http://printo.it/eurofever/scoreCriteria.asp>) développé par les pédiatres du groupe de travail Eurofever (17). Il peut être utile en pratique quotidienne pour s'orienter.

3.6. Place de la génétique

Jusqu'à très récemment, le diagnostic génétique des MAI en France était effectué par la méthode de Sanger, technique de séquençage classique et de référence ; cette technique permet d'analyser les gènes en ciblant un exon du gène d'intérêt, elle est fiable, lente, ne rendant pas possible l'exploration simultanée de plusieurs gènes (22). L'avènement des techniques de séquençage de nouvelle génération (NGS en anglais « Next Generation Sequencing »), depuis quelques années a permis la découverte de nombreux gènes impliqués en MAI. Né des progrès de la bioinformatique et des biotechnologies, le NGS repose sur une fragmentation de l'ADN du patient, puis une ligation d'adaptateurs ; elle permet une augmentation très importante du nombre de gènes analysés et permet d'étudier des pathologies cliniquement similaires mais génétiquement différentes comme les urticaires au froid ; et également d'étudier, sous forme de panels, des gènes responsables de formes chevauchantes, ou de diagnostics différentiels notamment les MAI avec déficit immunitaire ou les interféronopathies (22). La technique de NGS permet également de détecter les mosaïques ou mutations somatique (figure 5), qui sont le reflet de la coexistence de deux populations cellulaires chez un individu, différant sur un variant de séquence apparue *de novo* au cours de l'embryogénèse. Ces mutations ne sont en général pas détectées par l'approche classique de type Sanger qui manque de profondeur de séquençage. En fonction du stade d'apparition des mutations somatiques, elles peuvent être présentes dans des proportions variables selon les tissus. Ce phénomène est a été rapporté dans les cryopyrinopathies, le syndrome de Blau, le syndrome de Schnitzler, la maladie avec mutation de *NLRC4* et le TRAPS (6,9–13,23–25). Les résultats de l'analyse par NGS nécessitent un lourd investissement bioinformatique. Enfin en cas de famille multiplex, voire consanguine ou syndromique on peut envisager une étude génétique de type exome (22).

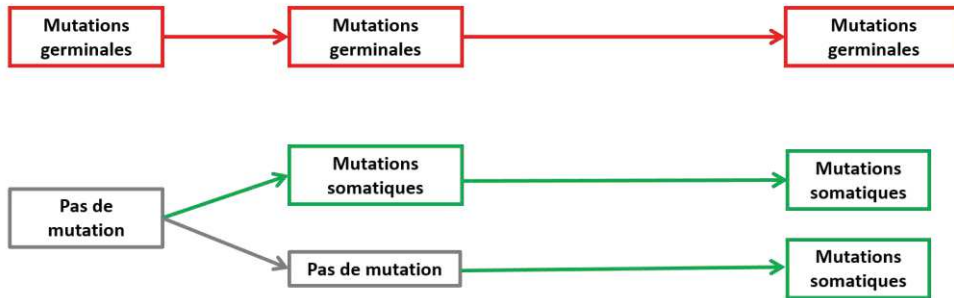
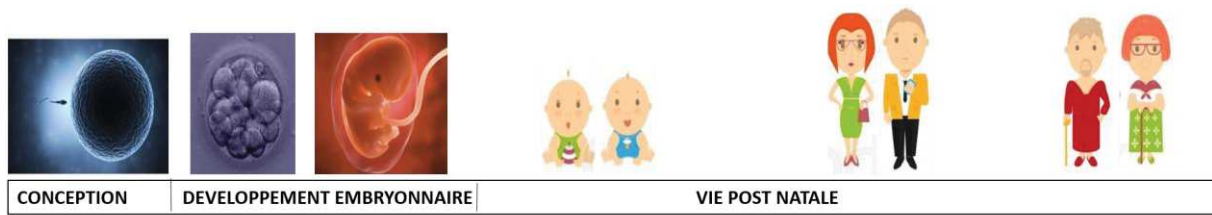


Figure 5. Schéma synthétisant la différence entre une mutation germinale et somatique.

Les mutations germinales sont présentes dès la conception et tout au long de la vie alors que les mutations somatiques ne sont pas présentes dès la conception et peuvent survenir pendant le développement embryonnaire ou tout au long de la vie post natale.

4. Synthèse physiopathologique (Figures 6 et 7) (2,26)

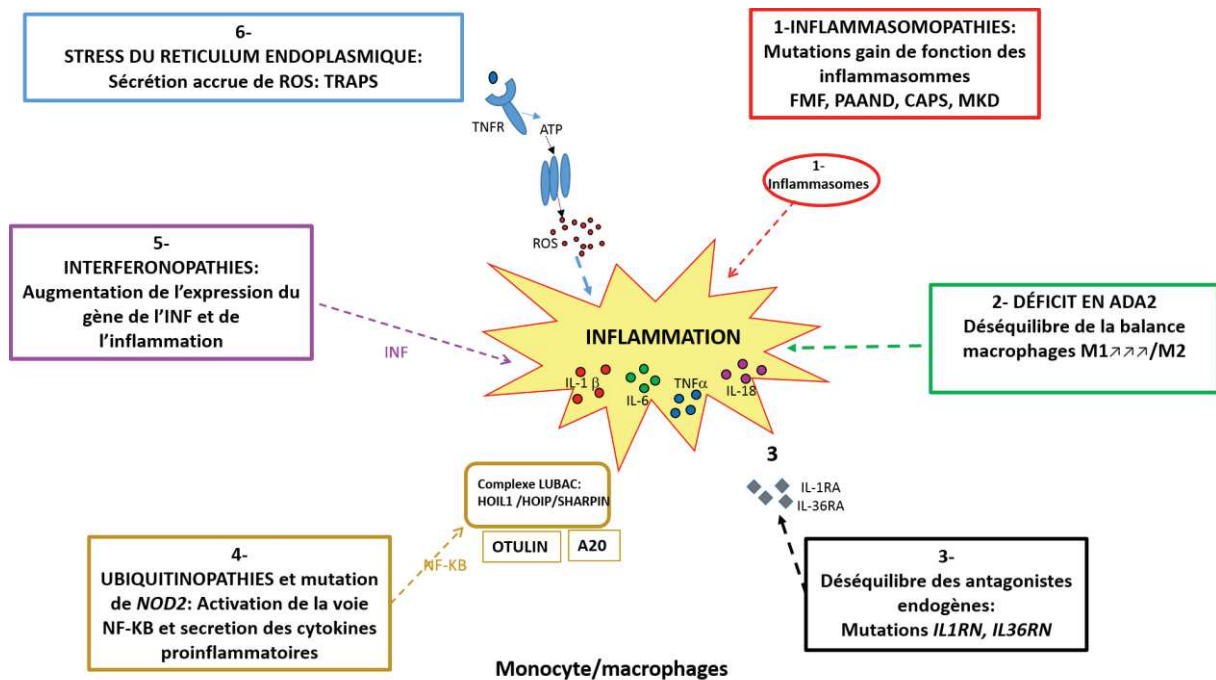


Figure 6 : Principaux mécanismes physiopathologies des MAI monogéniques.

1- Les principales maladies autoinflammatoires médiées par l'interleukine 1 font partie des inflammasomopathies où les mutations portent sur des gènes codant des protéines agissant ou constituant des inflammasomes comme la pyrine, NLRP3, NLRP12, NLRC4 ; le déficit en mévalonate kinase aboutit également à une stimulation de la pyrine. 2- Dans le déficit en ADA2, il y a un déséquilibre de la balance des macrophages M1/M2 avec excès de macrophages proinflammatoires et vascularites. 3- Le signal de danger peut provenir d'un déséquilibre des antagonistes endogènes dans les rares mutations de l'IL1RN (syndrome. DIRA) ou IL36RN (syndrome DITRA). 4- Les maladies qui aboutissent à la stimulation de la voie NFκB passent par NOD2 ou le système des ubiquitines. 5- Les interféronopathies comportent plusieurs entités où des mutations induisent une surexpression du gène de l'interféron. 6- Le signal de danger peut également venir d'un défaut de repliement des protéines comme dans les mutations du TNFRSF1A (syndrome TRAPS) qui entraîne la sécrétion accrue de radicaux oxygénés (ROS). Les mécanismes 1, 2, 3, 6 induisent l'excès des cytokines proinflammatoires, essentiellement IL1, IL6, IL18 et TNFα. Les mécanismes 4 et 5 aboutissent à un excès d'interféron et de cytokines proinflammatoires. Ce schéma permet de comprendre les cibles thérapeutiques les plus appropriées en fonction des maladies.

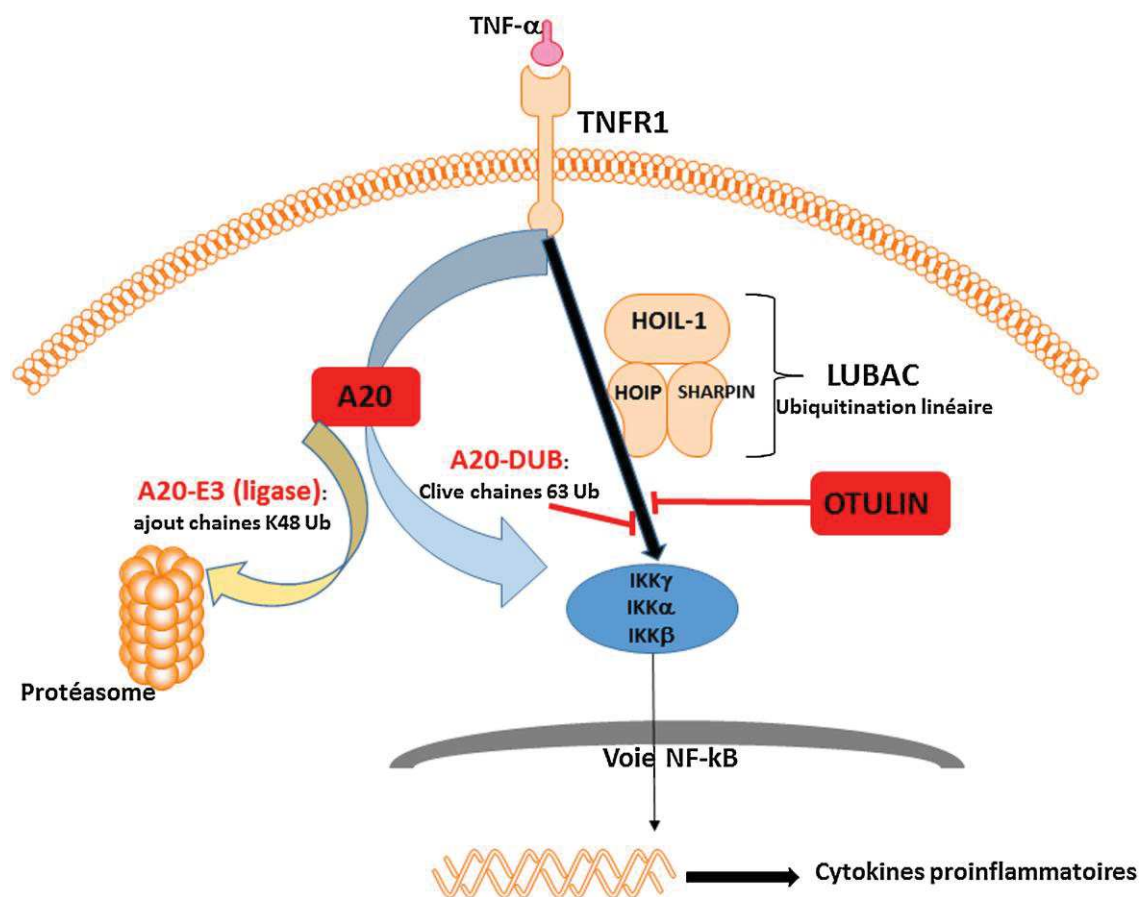


Figure 7. Mécanismes proposés de la pathogenèse de l'haplo-insuffisance de l'A20 (HA20), de l'otulipénie et du syndrome auto-inflammatoire lié à l'otuline (ORAS) et les déficiences linéaires du complexe d'assemblage de chaînes d'ubiquitine (LUBAC).

La voie canonique NF-κB est régulée à la fois par K63 (Lys63) et la chaîne linéaire d'ubiquitine (Ub). En aval du récepteur TNFR1 se situent des protéines cibles prédominantes pour l'ubiquitination par K63 et les chaînes linéaires Ub. Ces protéines recrutent le complexe IKK qui est également cible pour l'ubiquitination. Le complexe IKK ubiquitinylé est moins sensible à sa dégradation par le protéasome. Les chaînes Ub linéaires sont ajoutées par LUBAC. A20 et OTULIN régulent négativement la voie NF-

κ B en clivant les chaînes K63 et Ub linéaires des molécules cibles, dont IKK γ . En outre, A20 à travers son E3 L'activité ligase ajoute des chaînes K48 Ub à IKK γ en les ciblant pour la dégradation du protéasome. En cas de diminution de l'expression d'A20, chez les patients avec HA20, ou d'OTULIN chez les patients présentant une otulipénie/ORAS cela conduira à l'activation de la voie NF- κ B, une augmentation de l'expression des transcrits pro-inflammatoires dans les cellules immunitaires et l'inflammation systémique. La diminution de l'expression des sous-unités du complexe LUBAC, chez les patients porteurs de mutations HOIP ou HOIL-1, entraîne une inhibition de la voie NF- κ B dans les fibroblastes et les lymphocytes B causant l'immunodéficience. Cependant, leurs monocytes hyperproduisent les cytokines pro-inflammatoires. TNFR1, Le récepteur du TNF 1 ; IKK γ /NEMO, inhibiteur du facteur nucléaire kappa B kinase sous-unité gamma.

4.1. L'immunité innée

La réponse immunitaire innée a évolué pour protéger les organismes contre les dangers exogènes et endogènes qui menacent, endommagent ou détruisent l'organisme (Figure 6, Figure 7, Figure 8 et Table 3)(2,26). Le but ultime de la réponse immunitaire est d'éliminer ou de séquestrer la source de la perturbation et de rétablir la fonctionnalité et l'homéostasie tissulaire. Pour ce faire, le système immunitaire inné utilise un nombre limité de récepteurs (PRR) qui reconnaissent les motifs moléculaires exogènes et endogènes : les motifs moléculaires associés aux agents pathogènes exogènes (PAMP) et les modèles moléculaires associés aux lésions cytoplasmiques endogènes (DAMP). Si un danger moléculaire est détecté par un PRR, une cascade de voies moléculaires est induite qui conduira à la production de cytokines et de chimiokines dans le but de recruter des cellules immunitaires dans les tissus et de coordonner une défense tissulaire. Pour éviter des réponses inappropriées et préjudiciables, il existe de nombreux mécanismes régulateurs. Les maladies auto-inflammatoires sont dues à des anomalies qui peuvent survenir à chaque étape de la réponse immunitaire et de sa régulation.

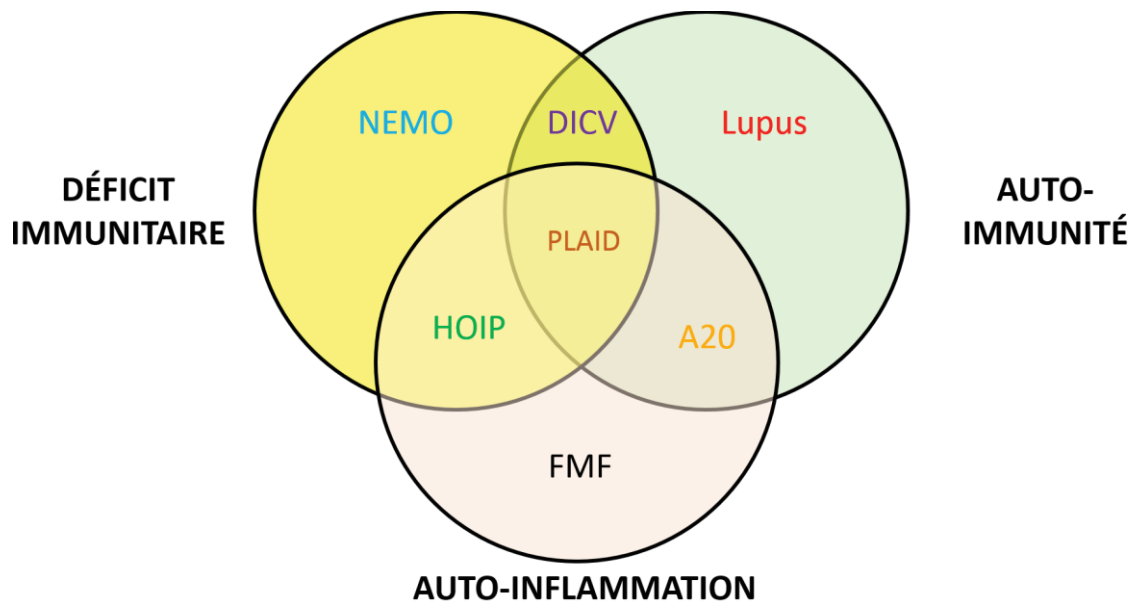


Figure 8. Interactions entre les maladies autoinflammatoires, autoimmunes et les déficits immunitaires.

Les MAI avec déficit immunitaire parfois associé à la présence d'auto-anticorps ont permis de mettre en évidence des chevauchements possibles entre ces 3 entités ce qui signifie que certaines mutations peuvent toucher à la fois l'immunité innée et l'immunité adaptative.

Table 3. Principaux éléments qui différencient les maladies auto-inflammatoires médiées par l'interleukine-1 à celles médiées par l'interféron

Maladie auto-inflammatoire	Médiées par l'interleukine-1	Médiées par l'interféron
<i>Syndrome inflammatoire</i>		
CRP	Élevée en poussée	Élevée seulement si crises sévères
Formule leucocytaire	Polynucléose neutrophile en poussée	Lymphopénie ou leucopénie en poussée
<i>Atteinte du système nerveux central</i>		
Méningite	Méningite aseptique à neutrophiles	Méningite lymphocytaire discrète, augmentation des protéines dans le LCR
Imagerie	Parfois : adhésions, inflammation de la cochlée	Calcification des ganglions basaux, anomalies de la substance blanche
<i>Autres organes atteints</i>		
Peau	Urticaire avec infiltrats neutrophiliques	Engelures, Panniculite avec neutrophiles immatures Lipodystrophie
Musculo-squelette	Arthrite aseptique à prédominance polynucléaires neutrophiles Ostéomyélite, hyperostose	Myosite
Cardiovasculaire	Rare, péricardite	Hypertension, HTAP, vascularite, calcification aortique Occlusion vasculaire
Yeux	Conjonctivite, uvéite antérieure, épisclérite	Kératoconjonctivite, Glaucome

Poumon	Sérite incluant pleurésie, péricardite	Pneumopathie interstitielle, fibrose pulmonaire
Abdomen	Péritonite++	Fréquents+++
<i>Présence d'auto-anticorps ?</i>	Rare, ANCA	+ FAN

4.2. Les inflammasomopathies

Certaines des maladies auto-inflammatoires sont dues à une détection exagérée des signaux de danger extracellulaires et intracellulaires. Les meilleurs exemples en sont les inflammasomopathies dus à une anomalie génétique d'un composant d'un PRR cytosolique responsable d'une production facilitée voire permanente de cytokines pro-inflammatoires. Les inflammasomopathies sont les maladies associées à des mutations des inflammasomes : pyrine (FMF, PAAND), *NLRP3* (CAPS), *NLRC4*, *NLRP12*. Dans ces maladies, le rôle de l'interleukine-1 est au premier plan. En bref, les inflammasomes sont des plateformes protéiques, qui en réponse à des signaux de danger, vont recruter la caspase-1, capable de cliver les pro-IL1 β et la pro-IL18 et aboutir à la sécrétion des cytokines pro-inflammatoires IL1 et IL18. Le gain de fonction d'autres PRR cytosoliques, tels que *CARD15* et *CARD14*, conduit à une sur-activation de la voie NF- κ b. Une réaction exagérée similaire pourrait impliquer la réponse antivirale dans laquelle les interférons de type I jouent un rôle crucial et être responsable des pathologies dénommées interféronopathies.

4.3. L'ubiquitination et les maladies liées aux anomalies de ce processus

L'ubiquitine est un peptide de 76 acides aminés appelée ainsi car elle est localisée dans tous les compartiments subcellulaires de toutes les cellules des organismes, elle est ainsi dite ubiquitaire. L'ubiquitination est le processus qui entraîne la fixation spécifique et régulée d'une ou plusieurs molécules d'ubiquitines sur une protéine cible (on parle de monomère ou de chaînes d'ubiquitine). Cette modification post-traductionnelle a pour principale fonction la reconnaissance puis la destruction de la protéine ainsi marquée, par le complexe protéolytique du protéasome, les chaînes d'ubiquitine sont dans ce cas insérées par l'intermédiaire de la lysine en position 48. La formation de polymère d'ubiquitine par l'intermédiaire de lysine située à un autre endroit de la chaîne d'ubiquitine conduit à d'autres destins la protéine ubiquitinée. Ainsi l'usage de la lysine 63 n'engage pas la protéine ubiquitinée vers la dégradation mais vers

d'autres fonctions dont l'inflammation. L'ubiquitination fait intervenir des enzymes ligases appelées E1, E2 et E3. Ce processus hautement dynamique est réversible : ainsi on appelle déubiquitination le processus par lequel les chaînes Ub sont retirées des substrats modifiés par une classe d'enzymes connues sous forme de déubiquitinases (DUB)(27). Il existe une centaine de protéases avec activité DUB dont les protéines suivantes : A20, OTULIN, qui fonctionnent comme des régulateurs négatifs de la signalisation NF- κ B. Le complexe d'assemblage linéaire de la chaîne d'ubiquitine linéaire (LUBAC) – est un ensemble de protéines : HOIP, HOIL-1 et SHARPIN qui, avec la ligase E3 participe au maintien de la stabilité de récepteurs membranaires dont le récepteur TNF 1 (TNFR1), des TLR, l'IL-1R. Après stimulation avec des signaux pro-inflammatoires, LUBAC est recruté pour attacher des chaînes Ub linéaires à des substrats cibles tels que : IKK (NEMO), MyD88 et ASC. Ainsi, en cas d'anomalie d'une des protéines constitutives du LUBAC, cela réduit sa stabilité dans les cellules. Comme les réponses immunitaires doivent être régulées pour éviter l'inflammation chronique, l'activité LUBAC est contre-réglée par les comme l'OTULIN et l'A20. L'OTULIN hydrolyse les chaînes Ub linéaires ce qui réduit l'activation de la voie NF- κ B et A20 est capable d'adresser les protéines vers le protéasome. Récemment, les dérégulations dans la voie des ubiquitines ont été rapportées chez des patients présentant des maladies auto-inflammatoires : les anomalies associées au défaut de l'assemblage linéaire de la chaîne d'ubiquitine (LUBAC), et des anomalies liées à des défauts de la déubiquitination des protéines : le déficit en A20 (HA20) et l'otulinopénie (ORAS).

4.4. Autres mécanismes

D'autres maladies auto-inflammatoires sont dues à une clairance insuffisante des protéines du système immunitaire qui ne sont plus nécessaires (maladies liées au protéasome) ou à une accumulation de protéines mal pliées dans le réticulum endoplasmique responsable d'une sécrétion accrue de cytokines pro-inflammatoires. Un exemple d'un tel mécanisme est le TRAPS et le déficit en mévalonate kinase.

Enfin, certaines maladies auto-inflammatoires sont liées à l'incapacité de réguler la signalisation des cytokines ce qui conduira au développement de boucles anormales de cytokines qui amplifient et perpétuent les réponses inflammatoires. Un bon exemple de ce mécanisme est illustré par DIRA et DITRA, dans lesquels les signaux IL-1 et IL-36 de cytokine antagonistes sont absents.

Dans le déficit en ADA2, il existe un déséquilibre de la balance des macrophages pro- et anti-inflammatoire au profit des pro-inflammatoires, qui est à l'origine de l'inflammation de la paroi des vaisseaux et du relargage de cytokines pro-inflammatoires, dont le TNF α .

4.5. Déficit immunitaire et MAI

La découverte récente de MAI comportant un certain degré de déficit immunitaire et/ou d'auto-immunité montre la complexité de ces maladies dont certaines se situent aux confins de l'auto-inflammation, de l'auto-immunité et du déficit immunitaire le plus souvent de type DICV (Figure 8). L'avenir nous permettra peut-être de mieux comprendre les voies physiopathologiques impliquées dans ces désordres rares associant une atteinte de l'immunité innée et de l'immunité adaptative.

5. Description des principales maladies autoinflammatoires

Table 4. Classification des MAI selon la physiopathologie des MAI.

Mécanisme physiopathologique	Maladie	Gène/protéine	Transmission	Mécanismes impliqués
Inflammasomopathies	CAPS	<i>NLRP3/NLRP3</i>	AD	<p>Mutations gains de fonction avec activation constitutionnelle de NLRP3 et surproduction d'IL1β</p> <p>Activation de NLRP3 par les CARs (<i>calcium sensing receptor</i>) via l'augmentation du Ca²⁺ intracellulaire</p> <p>Théorie perte de fonction : la pyrine mutée perd son activité inhibitrice sur NLRP3, menant à une activation de la voie IL1β</p>
	FMF	<i>MEFV/PYRIN</i>	AR	<p>Théorie gain de fonction : la pyrine active directement la caspase-1 (inflammasome de la pyrine), avec un effet gène-dose</p> <p>Blocage des sites de phosphorylation de PKN1/2 kinases via RhoA GTPase → activation de l'inflammasome de la pyrine</p>
	PAPA syndrome	<i>PSTPIP1/PSTPIP1</i>	AD	<p>La PSTPIP1 mutée activerait directement la caspase-1 en interagissant avec ASC et l'inflammasome de la pyrine, débouchant sur la sécrétion d'IL-1β</p> <p>Formation de podosomes/filopodes aberrants et altération de la matrice extracellulaire</p>

	FCAS2	<i>NLRP12/NLRP12</i>	AD	Régulation aberrante de l'activation de NF- κ B Altérations oxydatives et sécrétion d'IL1 β
	NLRC4-MAS	<i>NLRC4/NLRC4</i>	AD	Gain de fonction avec activation de caspase-1 et surproduction d'IL-18 > IL-1 β
	PAAND	<i>MEFV/PYRIN</i>	AD	Activation constitutive de l'inflammasome pyrine et surproduction d'IL1 β
	NAIAD	<i>NLRP1/NLRP1</i>	AR	Activation constitutive de l'inflammasome NLRP1 et surproduction d'IL1 β
	PFIT	<i>WDR1/WDR1</i>	AR	Dérégulation des filaments d'actine du cytosquelette entraînant une activation de l'inflammasome pyrine et une surproduction d'IL1 β
				Mécanismes de clivages du TNFR1 défectueux, apoptose induite par le TNF et activation NF- κ B
				Troubles du trafic intracellulaire du TNFR1A et défauts de conformation spatiale aboutissant à la rétention intracellulaire de protéine mutante \rightarrow activation aberrante de c-Jun N-terminal kinase (JNK) et de p38 <i>mitogen-activated protein kinase</i> (MAPK)
Stress intracellulaire menant à un état pro-inflammatoire	TRAPS	<i>TNFRSF1A/TNFR1</i>	AD	Stress du réticulum endoplasmique, majoration de la production de radicaux libres de l'oxygène et défaut d'autophagie \rightarrow surproduction d'IL1 β

	MKD/HIDS	<i>MVK/MK</i>	AR	<p>Perte de fonction, raccourcissement des produits isoprenoïdes non stéroïdes et défaut de prénylation protéique menant à l'activation de la caspase-1 et au stress réticulaire endoplasmique</p> <p>Déficit de l'autophagie et surproduction d'IL1β</p>
Déficit de mécanismes régulateurs	Syndrome de Majeed	<i>LPIN2/LPIN2</i>	AR	<p>Perte de fonction de l'inhibiteur de la phosphatidate phosphatase, qui exerce normalement une fonction anti-inflammatoire</p>
	Syndrome DIRA	<i>IL1RN/ILR1A</i>	AR	<p>Mutation perte de fonction de IL-1Ra</p> <p>Déséquilibre de régulation d'IL-1β</p>
	Syndrome DITRA	<i>IL36RN/IL36RA</i>	AR	<p>Mutation perte de fonction de IL-36Ra, le récepteur antagoniste de IL-36α, IL-36β et IL-36γ (IL-1F6, IL-1F8 et IL-1F9) – qui appartiennent à la famille IL-1 et sont abondamment exprimés dans la peau</p>
Sur-activation de la voie NF-κB	Syndrome de Blau	<i>CARD15/CARD15</i> <i>(NOD2/NOD2)</i>	AD	<p>Au moment d'interaction ligand-récepteur, CARD15/NOD2 subit un changement conformationnel aboutissant au recrutement d'une sérine thréonine kinases (RIP2) requise pour l'activation de NF-κB</p>
	CAMPS	<i>CARD14/CARD14</i>	AD	<p>Mutations gain de fonction de CARD14, principalement exprimés par les kératinocytes épidermiques</p> <p>Induction de l'activité NF-κB, avec</p>

				transcription de cytokines/chimiokines associées au psoriasis
	HA20	<i>TNFAIP3/A20</i>	AD	Perte de fonction d'A20, un régulateur de l'activation de NF-κB
	Otulinopénies (ORAS)	<i>OTULIN/OTULIN</i>	AR	Perte de fonction de l'OTULIN, défaut de l'hydrolyse des chaînes linéaires d'ubiquitines des protéines, induction de l'activité NF-κB
	Déficit en HOIL-1	<i>RBCK/HOIL-1</i>	AR	Perte de fonction de HOIL-1 → déséquilibre du complexe LUBAC, défaut de l'ubiquitines linéaire des protéines, hyper réponse à l'IL1 et au TNF des cellules, activation de la voie NF-κB
	Déficit en HOIP	<i>RNF31/HOIP</i>	AR	Perte de fonction de HOIP → déséquilibre du complexe LUBAC, défaut de l'ubiquitines linéaire des protéines, hyper réponse à l'IL1 et au TNF des cellules, activation de la voie NF-κB
		<i>TREX1/TREX1</i>	AR/AD (DN)AR	Protéines impliquées dans la clairance des débris d'acides nucléiques
		<i>RNASEH2A/RNAS EH2A</i>	AR	Perte de fonction : activation immune normalement induite par de l'acide nucléique viral
Interferonopathies	Aicardi-Goutières	<i>RNASEH2B/RNAS EH2B</i>	AR	Gain de fonction : activation constitutive du récepteur d'ARN double brin viral MDA5
		<i>RNASEH2C/RNAS EH2C</i>	AR	
		<i>SAMHD1/SAMHD1</i>	AR/AD	
		<i>ADAR1/ADAR1</i>	AR/AD (DN)	

		<i>IFIH1/IFIH1</i> ou <i>MDA5</i>	AD	
		<i>PSMB8/PSMB8</i>		Perte de fonction : défaut d'expression → activité protéasome défectueuse et accumulation de protéines ubiquitinées
	CANDLE/PR AAS	<i>PSMA3/PSMα7</i> , <i>PSMB4/PSMβ7</i> , <i>PSMB9/PSMβ1i</i>	AR	Renforcement du signal interféron de type I Signes d'hyper-activité du système immunitaire adaptatif
	SAVI	<i>TMEM173/STING</i>	AR	Gain de fonction. Activation constitutive de STING → activation de TBK1 et IRF3 → surproduction d'interféron de type I
Déficit en ADA2	DADA2	<i>CECR1/ADA2</i>	AR	Perte de fonction. Défaut de différentiation en macrophage M2. Dysfonction endothéliale
Autres	PLAID et APLAID	<i>PLCG2/PLCG2</i>	AD	Augmentation de la production d'inositol triphosphate, de diacylglycérol et de calcium ; surproduction d'IL-1 et d'autres cytokines
	SIFD	<i>TRNT1/TRNT1</i>	AR	Défaut de maturation d'ARNt, de synthèse de protéines et surexpression de cytokines pro- inflammatoires

5.1. Fièvres récurrentes « historiques »

Les fièvres récurrentes sont caractérisées par des poussées récurrentes d'inflammation systémique se manifestant par des épisodes soudains de fièvre associés à une élévation des protéines de la phase aiguë et à un certain nombre de manifestations cliniques, telles que des éruptions cutanées urticariennes, des inflammations des séreuses (péritonite, pleurésie, péricardite), des adénopathies cervicales et de l'arthrite (Table 4). Les poussées de maladie sont

généralement séparées par des intervalles sans symptômes et de durée variable, caractérisés par un bien-être complet, une croissance normale et une normalisation marqueurs biologiques de l'inflammation.

5.1.1. La fièvre méditerranéenne familiale (FMF)

La fièvre méditerranéenne familiale (FMF) est la première maladie auto-inflammatoire génétique décrite et la plus fréquente des MAI génétique ; elle est associée à des mutations dans l'exon 10 du gène *MEFV* pour MEditerranean FeVer qui code la protéine pyrine et dont l'activation entraîne une hypersécrétion d'interleukine-1(28–30). La FMF débute entre les âges de 3 et 10 ans et se manifeste par des accès d'inflammation aiguë des séreuses : péritonite, pleurite, péricardite, vaginalite testiculaire et synoviales. Elle touche principalement les populations méditerranéennes orientales et est autosomique récessive. Les poussées de FMF durent de 2 à 3 jours avec fièvre et douleurs abdominales dans 95 % des cas. Les patients peuvent présenter un pseudo-érysipèle en regard de la malléole externe de cheville. Le diagnostic repose sur les signes cliniques et sur l'analyse génétique montrant deux mutations dans *MEFV* et le traitement repose sur la colchicine quotidienne à vie qui vise à prévenir les crises et l'apparition de l'amylose inflammatoire(29–32).

5.1.2. Le déficit en mévalonate kinase (MKD)

Le déficit en mévalonate kinase (MKD pour *mevalonate kinase deficiency*), initialement appelé syndrome d'hyperimmunoglobulinémie D, a été appelé ainsi initialement du fait de son association à une élévation des IgD sériques dans les premières descriptions qui n'est en fait pas spécifique car retrouvé dans nombre d'autres maladies auto-inflammatoire et dont la recherche n'est plus conseillée de nos jours (33). Sa transmission est autosomique récessive ; la maladie commence au cours de la première année de vie avec des accès inflammatoires d'une semaine récurrents tous les un ou deux mois ; la fièvre est élevée accompagnée de douleurs abdominales, diarrhée, vomissement, arthralgies ou arthrites, céphalées, aphtes buccaux et adénopathies cervicales douloureuses (33,34). Le traitement n'est pas totalement codifié (33–35).

5.1.3. La fièvre héréditaire intermittente liée au récepteur de type 1A du TNF (TRAPS)

Le syndrome TRAPS est un syndrome très rare de transmission autosomique dominante et est caractérisé par des longs accès de fièvre durant 5 à 20 jours qui peut s'accompagner de douleurs abdominales, myalgies, raideur musculaire, conjonctivite, arthralgies, douleurs thoraciques et

de deux signes qui sont assez spécifiques, bien que rares : l'œdème périorbitaire et l'atteinte cutanée à type de pseudocellulite très douloureuse pouvant toucher les 4 membres (35). L'âge de début est souvent avant 20 ans mais il peut y avoir une longue errance diagnostique. Le traitement n'est pas totalement codifié mais peut comporter les corticoïdes en crise et les anti-IL-1 en crise ou au long cours (35,36).

5.1.4. Les cryopyrinopathies (CAPS)

Les cryopyrinopathies ou CAPS pour cryopyrin-associated periodic syndrome sont un spectre de maladies autosomiques dominantes associant des crises caractérisées par une urticaire au froid, de la fièvre, des arthromyalgies en rapport avec des mutations du gène *NLRP3* codant pour l'inflammasome NLRP3 (37). La forme la plus grave est le syndrome CINCA (chronique infantile neurologique cutané et articulaire) débutant dans les premiers jours de vie avec en plus de l'urticaire : arthrites, méningite aseptique, surdité ; la forme intermédiaire, anciennement appelée syndrome de Muckle et Wells comporte une surdité neurosensorielle, et parfois de l'amylose inflammatoire en absence de traitement ; l'urticaire au froid familiale est caractérisée par une urticaire déclenchée par l'exposition environnementale au froid ; les poussées d'urticaire y sont associées à d'autres signes : fièvre, arthralgies et conjonctivite(18,37). Les cryopyrinopathies débutent en général très tôt dans la vie et les crises durent quelques jours. Le traitement est basé sur les anti-IL-1(37).

5.2. Les autres fièvres récurrentes héréditaires monogéniques

5.2.1. Le syndrome auto-inflammatoire avec dermatose neutrophilique associé à des mutations de l'exon 2 de la pyrine (PAAND=periodic auto-inflammation with neutrophilic dermatosis).

Il s'agit d'un syndrome de transmission autosomique dominante découvert initialement en 2016 dans une grande famille belge qui présentait des épisodes récidivants de fièvre avec arthromyalgies et dermatose neutrophilique débutant tôt dans la vie (3). Des mutations de la pyrine (gène *MEFV*) en position S242R ont été découvertes dans un domaine différent de celui où sont habituellement retrouvées les mutations de la FMF. Il a ensuite été confirmé chez deux autres patients un français et un libanais (3). Une publication très récente a retrouvé des mutations de cet exon sur le codon E244S dans une famille d'origine espagnole avec 3 membres atteints (4). Ils présentaient une acné pustuleuse, une hydrosadénite suppurée, du pyoderma gangrenosum et une panniculite neutrophilique. Ces deux publications montrent que le spectre des maladies liées aux mutations de *MEFV* n'est pas limité à la classique fièvre méditerranéenne familiale de transmission récessive puisqu'en cas de mutation de l'exon2, des patients d'origine

non méditerranéenne présentent une maladie de transmission dominante avec des dermatoses neutrophiliques au premier plan et pouvant même mimer une maladie de Verneuil !

5.2.2. La fièvre périodique associée à des mutations de NLRP12

Des mutations de *NLRP12* pathogènes ont été identifiées chez 18 patients à ce jour. La première famille était Guadeloupéenne, mais d'autres cas ont ensuite été décrits chez des italiens, un arménien, un turque et un chinois (38–41). Les signes cliniques principaux sont des épisodes de fièvre récurrente avec arthromyalgies et une urticaire au froid qui mime une cryopyrinopathie de type urticaire familiale au froid; le traitement n'est pas codifié mais repose sur les antalgiques, les corticoïdes et parfois les inhibiteurs de l'interleukine 1 ; la maladie semble être moins active après l'adolescence (39–42).

5.2.3. Les maladies liées à des mutations de NLRC4.

Un nouveau gène a été découvert et associé en fonction des mutations à des maladies autoinflammatoires de phénotype différent ; il s'agit du gène *NLRC4* qui code un inflammasome NLRC4. Les familles décrites présentaient soit une entérocolite inflammatoire très grave (43), soit une urticaire au froid (44), soit un syndrome d'activation macrophagique récurrent (6,45). Ainsi, comme pour les cryopyrinopathies avec mutation de *NLRP3*, il existe un spectre large de manifestations cliniques pour des mutations dans le gène *NLRC4* (6). Bien que ce syndrome ait été décrit majoritairement dans des formes graves chez des enfants, il a été décrit chez le père de l'un d'entre eux avec une fièvre récurrente depuis l'enfance, une entérocolite, des conjonctivites à répétition, des lésions cutanées à type de nodules érythémateux des membres inférieurs et des lésions urticariennes des 4 membres de la face et du tronc et des douleurs abdominales récidivantes (43). Une mutation somatique de *NLRC4* a été décrite chez un jeune japonais qui avait un phénotype clinique évocateur de cryopyrinopathie sans mutation de *NLRP3* (46). Le traitement n'est pas totalement codifié à ce jour mais est basé sur les biothérapies anti-IL1 voir tout récemment anti-IL18 (6,47).

5.2.4. Le syndrome autoinflammatoire avec arthrite et dyskératose associé à des mutations de NLRP1 (NAIAD).

La nouvelle entité décrite en 2017 dans 2 familles est de transmission autosomique récessive ou dominante selon les mutations (48). Le début est précoce (6 premiers mois de vie). La clinique est riche avec une atteinte cutané-muqueuse polymorphe (hyperkératose papillaire ou filiforme, pseudo-phrynoderma, condylomes HPV négatifs, candidose étendue), une polyarthrite prédominant aux genoux, chevilles, poignets et mains, avec anomalies de la croissance osseuse, et une fièvre récurrente inexplicée souvent associée à une dysimmunité (facteurs anti-nucléaires positifs, anémie hémolytique, thyroïdite, uvéite)(48). Les mutations activatrices de l'inflammasome NLRP1 sont à l'origine d'une hypersecrétion d'interleukine-1 et d'une diminution des cellules B mémoires CD27+ et les traitements par anti-TNF ou anti-IL-1 ont montré leur efficacité (48).

5.2.5 La fièvre récurrente associés à des mutations de TNFRSF11A

Une équipe française a rapporté 3 patients avec fièvre récurrente héréditaire proche sur le plan clinique d'un syndrome TRAPS, qui avait une mutation du gène *TNFRSF11A*, un gène connu pour réguler la fièvre chez les rongeurs (49). Cette mutation était associée à une sécrétion accrue de plusieurs cytokines inflammatoires et modifiait les effets biologiques du récepteur sur signalisation NF- κ B. Cette entité était cliniquement très proche du syndrome TRAPS, avec des fièvres longues, et est associée à des mutations d'un gène de la même famille (*TNFRSF11A*)(49).

5.2.6. Les ubiquitinopathies

Il s'agit d'une nouvelle famille de maladies autoinflammatoires dont les mutations touchent des gènes codant des protéines impliquées dans la voie de l'ubiquitination des protéines et aboutissant à la stimulation de la voie NF κ B (27).

5.2.6.1. Le déficit en protéine HA20

Une nouvelle maladie auto-inflammatoire de transmission autosomique dominante a été décrite fin 2015, liée à une mutation du gène *TNFAIP3* qui code une protéine régulatrice du facteur transcriptionnel NF- κ B, la protéine A20 (50). Elle simule une maladie de Behçet familiale avec des aphtes buccaux récidivants, des abcès dermiques axillaires, des cicatrices chorioretiniennes au fond d'œil et un test de pathergie positif. Les patients sont d'origine européenne et /ou turques. Les auteurs ont également montré que la protéine A20 est également impliquée dans

l'activation de l'inflammasome NLRP3. Plusieurs patients ont développé des auto-anticorps au cours de leur maladie en particulier des anticoagulants lupiques et des anticorps anti-ADN. Les anti-interleukine 1 ont été utilisés chez un patient avec succès(50).

5.2.6.2. L'otulinopénie ou ORAS (OTULIN related autoinflammatory syndrome) par mutations de l'OTULIN.

Très récemment une nouvelle fièvre récurrente héréditaire a été découverte en rapport avec des mutations d'un gène appelé *OTULIN* qui code une déubiquitinase (51,52). Au total deux équipes ont rapporté quatre mutations de ce gène touchant 8 patients issus de 4 familles consanguines d'origine variées (anglaise, turque et pakistanaise). Il s'agit d'une maladie de transmission autosomale récessive caractérisée par une fièvre récurrente débutant très tôt dans la vie (des premiers jours aux premiers mois) associée à une panniculite nodulaire à neutrophiles, des arthromyalgies et une diarrhée. La fièvre dure généralement une quinzaine de jours. Les immunoglobulines totales sont discrètement élevées et parfois il existe des anticorps anti muscles lisses. Au plan thérapeutique, les anti-TNF sont très efficaces et même davantage que les anti interleukine-1 (27,51,52).

5.2.6.3. Les mutations de HOIL1

Des mutations de *HOIL-1* (ou *RBCK1*), une sous unité du complexe LUBAC qui ubiquitine les protéines intracytoplasmiques avant de les adresser au protéasome, ont été décrites par une équipe française. Ce syndrome, associe une fièvre récurrente, une colite inflammatoire, une hépatosplénomégalie, des adénopathies, des infections récurrentes à pyogènes et une diminution des lymphocytes B mémoires switchés ; Il n'y a pas d'autoimmunité mais une amylopectinose (53,54).

5.2.6.4. Les mutations de HOIP

Des mutations de *HOIP* (ou *RNF31*), une autre sous unité du complexe LUBAC qui ubiquitine les protéines intracytoplasmiques avant de les adresser au protéasome, ont été décrites par la même équipe française (55). Ce syndrome, associe une fièvre récurrente avec arthralgies,

diarrhée, splénomégalie, sans autoimmunité, hypogammaglobulinémie et anomalies des lymphocytes T ; une amylopectinose infraclinique et une lymphangiectasie (55).

5.3- Les maladies auto-inflammatoires monogéniques sans déficit immunitaire

Dans ce groupe sont décrites des maladies génétiques autoinflammatoires mais qui ne sont pas toujours caractérisées par des épisodes récurrents de fièvre. En effet les patients présentent de la fièvre sur une durée plus ou moins longue associée à des signes d'atteinte d'organes comme la peau les articulations au premier plan, le tout dans un contexte de syndrome inflammatoire

5.3.1. Les maladies associées aux mutations de NOD2

Le syndrome de Blau : il s'agit d'une maladie autosomique dominante liée à des mutations gains de fonction du gène *CARD15/NOD2* se présentant volontiers sous forme d'une triade caractéristiques avec atteinte rhumatologique, cutanée et oculaire (56). Au plan rhumatologique, on observe chez plus de 90 % des patients une polyarthrite symétrique avec synovites et ténosynovite touchant préférentiellement les extrémités : les pieds et les mains (articulations interphalangiennes proximales), les poignets et chevilles et les genoux et pouvant aboutir à une déformation appelée « camptodactylie ». Au niveau cutané ; il s'agit d'une éruption maculo-micro-papuleuse érythémateuse, eczématiforme, débutant dès la première année de vie, pouvant mimer une atopie ou une ichtyose vulgaire ; une biopsie cutanée montrera des granulomes non caséux. Au plan ophtalmologique, on observe une uvéite granulomateuse chez 80% des patients. D'autres atteintes plus rares comportent : un érythème noueux, des atteintes des nerfs crâniens (56). De façon intéressante, un cas de syndrome de Blau avec une mutation somatique gain de fonction de NOD2 a récemment été publié (13).

Les autres maladies (NAID : NOD2-associated autoinflammatory diseases) : en 2011, Yao *et al* ont décrit une nouvelle catégorie de maladie autoinflammatoire appelée NAID pour NOD2-associated autoinflammatory diseases ou plus récemment syndrome de Yao associée à des variants de *NOD2* IV58⁺¹⁵⁸ ou R702W (19,57,58). Il s'agit d'une entité sporadique touchant les caucasiens avec une prédominance féminine (70%) et diagnostiquée en moyenne vers 35 ans ; les patients présentent des épisodes récurrents associant des plaques érythémato-maculeuses du tronc (>90% cas) avec une oligo ou polyarthrite non érosive dans près de 80 % des cas avec gonflement assez caractéristiques des extrémités distales chez 1/3 des patients, des symptômes digestifs à type de douleurs et diarrhée et une fièvre récurrente (>65% des cas) avec asthénie et

perte de poids, un syndrome sec dans plus de la moitié des cas et des aphtes buccaux chez 1/3 des patients ; parfois on note des sérites (19,57).

5.3.2. Le syndrome de Majeed (mutations de LPIN2)

Il s'agit d'une maladie autosomique récessive, décrite dans quelques familles majoritairement bédouines, qui associe une ostéomyélite chronique multifocale récurrente, des fièvres récurrentes toutes les 2 à 4 semaines durant 3 à 4 jours ou plus, une dermatose neutrophilique en particulier un syndrome de Sweet ou une pustulose cutanée voire un psoriasis et une anémie congénitale dysérythropoïétique microcytaire de sévérité variable. Le plus souvent le diagnostic est porté dans l'enfance avec un retard de croissance associé, mais quelques cas ont été décrits chez des adolescents /adultes jeunes. Des mutations du gène *LPIN2* ont été rapportées chez ces malades (59,60).

5.3.3. La maladie inflammatoire associée à des mutations de l'antagoniste du récepteur de l'IL-1 ou DIRA (deficiency of the interleukin one receptor antagonist).

Des mutations récessives de l'antagoniste du récepteur de l'IL-1 ont été trouvées chez quelques enfants atteints d'une maladie néonatale très inflammatoire associant une pustulose ressemblant à un psoriasis pustuleux, une ostéomyélite aseptique multifocale avec périostite. Les patients sont apyrétiques ou peu fébriles mais présentent une élévation importante des marqueurs biologiques de l'inflammation. La maladie est autosomique récessive et très sensible à l'inhibition de l'interleukine 11(61).

5.3.4. La maladie inflammatoire associée à des mutations de l'antagoniste du récepteur de l'IL-36 ou DITRA (deficiency of the interleukine thirty-six receptor antagonist).

Il s'agit d'une maladie autosomique récessive décrite en 2011 dans des familles tunisiennes. Les patients souffrant d'un psoriasis pustuleux généralisé ayant débuté pour la plupart dans l'enfance. Tous avaient une éruption diffuse érythémateuse plus ou moins squameuse, rapidement pustuleuse, évoluant par poussées et accompagné de fièvre élevée associée à un syndrome inflammatoire. Ces patients sont à risque de sepsis et l'anakinra a été efficace chez certains patients (62).

5.3.5. Pytiriasis rubra pilaire ou psoriasis associé aux mutations de CARD14

Cette maladie autosomique dominante a été récemment décrite dans des familles avec pityriasis rubra pilaire (63,64). La maladie débute avant l'âge de 3 ans et est caractérisée par des placards étendus érythémateux parfois squameux coalescents contenant des îlots de peau saine associées à des papules folliculaires et une kératodermie palmoplantaire sans atteinte unguéale psoriasiforme (63,64). Les mutations du gène *CARD14* codent une protéine principalement exprimée dans les kératinocytes et capable d'activer la voie NFκB ; en histologie, on voit de la parakératose, de l'orthokératose, de l'acanthose et un infiltrat dermique mononucléé(64).

5.3.6. Les syndromes autoinflammatoires associées aux mutations de PSTPIP1 (table 5).

Table 5. Maladies inflammatoires associées aux mutations de *PSTPiP1* et signes cliniques principaux.

Acronyme	Nom complet	Nombre de cas rapportés	Atteinte articulaire	Atteinte cutanée	Atteinte hématologique	Autres atteintes
PAPA	<i>Pyogenic arthritis, pyoderma gangrenosum and acne syndrome</i>	> 50	Arthrites > 75 %	Acné > 50 %		
			Arthralgies > 10 %	PG > 25 %	Anémie	Rares : otites ; néphropathies ; diabète (%)
				Abcès > 15 %		
			Ulcères > 10 %			
			Pustulose > 10 %			
			Arthrites > 50 %	PG > 40 %	Hépatosplénomégalie (88 % =)	Hypercalprotectinémie et hyperzincémie (88 %)
			Arthralgies > 10 %	Ulcères nécrotiques > 30 %	Lyphadénopathie (38 %)	Retard de croissance (56 %)
PAMI	<i>PSTPiP1-associated myeloid-related-proteinemia inflammatory syndrome</i>	20		Acné 25 %	Neutropénie 94 %	Néphropathie (25 %)
				Pustulose 20 %	Anémie 88 %	Hépatopathie (19 %)
				Abcès > 10 %	Thrombopénie 44 %	Retard mental (19 %)
				Érythème > 10 %		Sepsis (19 %)
						Ostéomyélite (13 %)
				colite (6 %)	Vasculite cérébrale (6 %)	
PAPASH	<i>Pyogenic arthritis, pyoderma gangrenosum, acne and hidradenitis suppurativa syndrome</i>	< 5	Arthrite aseptique	Acne modérée		
				Rare : abèses, PG HS		(%)
PAPA-like	<i>Pyogenic arthritis, pyoderma gangrenosum and acne-like syndrome</i>	< 5	Arthralgies	Acne	Anémie	Aphthose buccale
				PG		
PAC		< 5		Acné		Colite

*Pyoderma
gangrenosum,
acne and
ulcerative
colitis
syndrome*

PG

Rash récalcitrant

Les maladies liées aux mutations de PSTPiP1 sont appelées PAID (PSTPiP1-associated autoinflammatory diseases) (5,65). A ce jour plus de 27 mutations différentes ont été décrites dans le gène *PSTPIP1* qui code une protéine appelée CD2-BP1 (CD2-binding protéine 1), protéine adaptatrice qui peut se lier à l'inflammasome pyrine ; cette protéine est impliquée dans l'organisation des structures du cytosquelette et a un impact sur la dynamique cellulaire des cellules de l'immunité innée, la libération d'IL1 β et la modulation de l'activation lymphocytaire T (5). Les patients présentent des poussées inflammatoires récurrentes avec des signes systémiques comme de la fièvre, des signes cutanés inflammatoires locaux et articulaires ; en fait, comme dans les cryopyrinopathies, il s'agit d'un spectre de maladies, la plus fréquente et initialement décrite est le syndrome PAPA (Pyogenic arthritis, Pyoderma gangrenosum and Acne syndrome) mais depuis, plusieurs entités cliniques différentes associées à des mutations différentes du gène ont été décrites, la plus grave étant le syndrome PAMI (PSTPiP1-Associated Myeloid-related-proteinemia Inflammatory syndrome) (5). Dans le PAPA et le PAMI, il y a des taux élevés sériques d'une alarmine proinflammatoire : la protéine MRP8/14 (myeloïd-related-protein) ; il semble intéressant de doser cette alarmine dans le sang des patients avec un phénotype clinique de PAID afin d'orienter le séquençage de *PSTPIP1* si son taux est élevé (5).

Le syndrome PAPA (Pyogenic arthritis, Pyoderma gangrenosum and Acne syndrome). Ce syndrome est le plus fréquent des maladies liées aux mutations de PSTPiP1 associe des arthrites récurrentes à polynucléaires neutrophiles dans plus de 75% des cas et deux types d'atteintes dermatologiques: une acné kystique grave (plus de 50% des cas), un pyoderma gangrenosum (1/3 des cas), des aphtes buccaux, des abcès aseptiques, des pustules, parfois du psoriasis voire de l'hidradénite suppurée (66).

Le syndrome PAMI (PSTPiP1-Associated Myeloid-related-proteinemia Inflammatory syndrome). Il associe au plan clinique une des arthrites aseptiques dans plus de 55% des cas, un pyoderma gangrenosum dans près de la moitié des cas, des lésions ulcérées et/ou nécrotiques dans 30% des cas, une acné et/ou un rash pustuleux dans 20-25% des cas (65). Parmi les signes spécifiques à cette entité, on note une hépatosplénomégalie dans près de 90% des cas et une polyadénopathie dans 40% des cas, une pancytopenie (neutropénie 95%>anémie 90%>

thrombopénie 50%) (5,65). Une néphropathie peut survenir chez ¼ des patients et une atteinte rénale dans 20% ; on peut également observer un retard de croissance ou un retard mental et rarement des vasculites cérébrales(5). Au plan biologique, la caractéristique principale (près de 90% des cas) est des taux sériques extrêmement élevés de calprotectine (MRP8/14) et une hyperzincémie également ; un moyen simple et plus rapide que la génétique de faire le diagnostic est donc de doser dans le sang la calprotectine sérique et le zinc (5,65). Ces patients présentent une bonne réponse aux anti-interleukines 1 (anakinra).

Le syndrome PAPASH (pyoderma gangrenosum, acne and hidradenitis suppurativa) a été donné pour l'association d'arthrite aseptique, de pyoderma gangrenosum, d'acné et d'hidradénite suppurée. C'est proche des atteintes associées aux mutations de la nicastrine (*NCSTN*) et de l'exon 2 de *MEFV* (67).

Le syndrome PAPA-like (Pyogenic arthritis, Pyoderma gangrenosum and Acne-like syndrome) associe des arthralgies de l'acné et du pyoderma gangrenosum, des aphtes buccaux et de l'anémie (68).

Le syndrome PAC (pyoderma gangrenosum, acne and ulcerative colitis) associe une colite ulcérée et une acné sévère, des ulcérations cutanées récidivantes, une éruption pustuleuse récalcitrante avec parfois du pyoderma gangrenosum (69).

5.3.7. Les hidradénites suppurées associées à des mutations de la nicastrine (*NCSTN*)

En 2011 Liu décrivait des cas d'hidradénites suppurées associées à des mutations du gène de la nicastrine (70). La nicastrine est une glycoprotéine de type protéase transmembranaire agissant comme une gamma-sécrétase (70). Depuis plusieurs équipes ont rapportés des familles avec une transmission autosomique dominante d'hidradénite suppurée de toutes les régions du globe : chine, France Iran notamment (71–73). Suivant les familles les atteintes peuvent être uniquement des abcès aseptiques récurrents des grands plis (axillaires, inguinaux, de la région périnéale ou anorectale) ou associés à du pyoderma gangrenosum et de l'acné ; cette maladie est donc un diagnostic différentiel des maladies associées aux mutations de PSTPiP1 (PAID) et aux mutations touchant l'exon 2 du gène *MEFV* (PAAND) (4,73,74).

5.3.8. La maladie de Still à début pédiatrique monogénique par mutation de *LACCI/FAMIN*

Une mutation du gène *LACCI/FAMIN* a été retrouvée à ce jour chez 15 patients, tous issus de mariages consanguins souffrant de forme systémique d'arthrite juvénile idiopathique provenant d'Arabie Saoudite (n=13) ou du Liban (n=2) ; il s'agissait d'une transmission autosomique récessive (14,15). Ce gène code pour l'enzyme laccase, qui appartient à la famille des oxydases multicuivres qui comporte d'autres enzymes comme la céruléoplasmine. Les patients présentaient tous une polyarthrite symétrique touchant les petites et grosses articulations accompagnée de fièvre et d'un syndrome inflammatoire ayant débuté entre 12 et 36 mois de vie. Parmi les autres signes cliniques, étaient retrouvés un rash maculopapuleux (n=13), une organomégalie (n=7), des adénopathies profondes (n=2) ; aucun patient n'a développé de syndrome d'activation macrophagique (14,15).

5.3.9 Les interféronopathies (sauf syndrome d'Aicardi-Goutières)

5.3.9.2. STING-associated vasculopathy with onset infancy

Une maladie appelée SAVI, pour STING-associated vasculopathy with onset in infancy, et appartenant aux interféronopathies, a été décrite chez des patients présentant une vascularite cutanée sévère (pouvant engendrer des pertes de substance) avec une pneumopathie interstitielle pouvant évoluer vers une fibrose pulmonaire précoce et une inflammation systémique ; cette maladie autosomique dominante est associée à des mutations gains de fonction de *TMEM173* codant une protéine appelée STING (75,76). L'atteinte pulmonaire est sévère et représente la principale cause de décès et peut nécessiter une greffe pulmonaire (77).

5.3.9.2. Les PRAAS (proteasome associated autoinflammatory syndrome)

Le CANDLE (*chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature*) est une maladie autosomique récessive liée à des mutations de l'immunoprotéasome qui associe lipodystrophie, atrophie musculaire, lésions cutanées neutrophiliques et une fièvre périodique (78). Il est également appelé PRAAS (*proteasome-associated auto-inflammatory syndrome*) et est en lien avec une perte d'activité de la sous-unité β de type 8 du protéasome, $\alpha 7$, $\beta 7$, $\beta 1i$, et la protéine de maturation du protéasome, encodées respectivement par le gène *PSMB8*, *PSMA3*, *PSMB4*, *PSMB9* et *POMP* (79). Peu de traitements sont efficaces et les inhibiteurs de JAK1/2 ont été utilisés avec des résultats encourageants (77).

5.4. Les maladies auto-inflammatoires associées à un déficit immunitaire

5.4.1. Les maladies associées aux mutations de la *PLCG2* (*PLAID* et *APLAID*)

Des maladies autosomiques dominantes avec mutations de la *PLCgamma* responsables d'auto-inflammation associée à un déficit immunitaire et une dérégulation immunitaire.

Le *PLAID* (mutations de type délétions qui signifie en anglais « *PLCG2 associated antibody deficiency and immune dysregulation* ») qui a été décrit chez 27 patients issus de 3 familles américaines : tous présentaient une urticaire au froid environnemental (100%), un déficit immunitaire proche d'un déficit immunitaire commun variable (*DICV*) avec infections ORL et pulmonaires récidivantes (75%), la présence d'anticorps anti-nucléaires ou d'une maladie auto-immunes (thyroïde notamment) dans 56% ; 7 patients avaient des granulomes sur des histologies (80).

L'*APLAID* (mutations de type faux sens avec gain de fonction), décrit initialement dans une famille est donc plus rare Il signifie en anglais « *Autoinflammatory PLCG2 associated antibody deficiency and immune dysregulation* ». Il associe la présence de lésions cutanées bulleuse et/ou suintantes, une entérocolite, une inflammation oculaire (ulcération/érosion, pouvant entraîner une hypertension intra-oculaire et une cataracte), une pneumopathie interstitielle non spécifique, des arthralgies et un *DICV* modéré avec infections sinopulmonaires récurrentes par diminution des IgM et IgA. (81). Ces anomalies de la *PLCgamma* modifient le métabolisme intracellulaire du calcium, ce qui active l'inflammasome *NLRP3* (82).

5.4.2. Certaines ubiquitinopathies (dont *HOIL1*) (53)

5.4.3. Les mutations de *TRNT1*

Des mutations du gène *TRNT1* associées à un syndrome associant : une anémie sidéroblastique avec déficit immunitaire, une fièvre récurrente et un retard du développement (*SIFD* pour *Sideroblastic anemia with B-cell Immunodeficiency, periodic Fevers and Developmental delay*, mutations du gène *TRNT1*) (83). Les cas touchent surtout des jeunes enfants de toutes origines ethniques qui présentent une anémie sévère (7g/dL) microcytaire (VGM moyen à 62), fièvre récurrente avec troubles digestifs, panhypogammaglobulinémie avec diminution des lymphocytes B totaux et des signes variés neurologiques (épilepsie, surdité, retard mental), néphrocalcinose, rétinite pigmentaire et cardiomyopathie dilatée conduisant souvent au décès du patient. Un seul adulte a été rapporté à ce jour du fait de la morbi/mortalité importante. Le gène *TRNT1* code une ARN polymérase nécessaire à la maturation cytosolique et au transfert des ARNt vers les mitochondries (83,84).

5.4.4. Les mutations de ADA2 (déficit en ADA2)

Le déficit en Adénosine Deaminase 2 (ADA2) est une maladie auto-inflammatoire rare de description récente associée à des mutations autosomiques récessives du gène *ADA2*, codant pour l'enzyme ADA2. A ce jour, une centaine de patients sont décrits dans la littérature et une vingtaine ont été identifiés en France. Les patients sont un peu plus souvent des hommes (sex ratio F/H = 0,73) et dans 15% des cas issus d'une famille consanguine. Dans plus de la moitié des cas il existe au moins un frère/une sœur atteint(e) dans la famille. Les premiers symptômes commencent habituellement tôt dans la vie (en moyenne à 5,7 ans) mais il y a fréquemment une longue errance diagnostique. Les principales manifestations sont : cutanées (78% - livedo : 56%, nécrose/ulcères 17%) ; neurologiques (51% - AVC ischémiques 42%); digestives (37% - douleurs abdominales et troubles du transit 19%), oculaire (16%), infections ORL et respiratoires à répétition (17%), hypogammaglobulinémie (28%) lymphopénie (19%). On retrouve de façon inconstante des facteurs antinucléaires. Les biothérapies anti-TNF sont très efficaces dans cette maladie. Cette maladie fait partie des maladies auto-inflammatoires pouvant se présenter comme une vascularite type péri-artérite noueuse (85).

5.4.5. Les mutation de *WDR1* (syndrome PFIT pour *periodic fever with immunodeficiency and thrombocytopenia*).

Il s'agit d'une maladie autosomique récessive décrite pour la première fois en 2017 chez deux jumelles pakistanaïses issues de parents consanguins(86). Elle associe dès la naissance des fièvres récurrentes, des infections opportunistes sévères à germes encapsulés ou champignons et une thrombocytopénie à petites plaquettes (86). La maladie est liée à des mutations du gène *WDR1*, dont la perte de fonction conduit à l'accumulation de molécules d'actine qui activent la pyrine, responsable d'une hypersecrétion d'interleukine-18. La perturbation du cytosquelette aboutit à la dysfonction des lymphocytes T, des cellules présentatrices d'antigènes et des mégacaryocytes, à l'instar du syndrome de Wiskott-Aldrich (86). La seule thérapeutique efficace à ce jour semble être l'allogreffe de moelle osseuse, les corticoïdes et les biothérapies n'ayant qu'une efficacité partielle et favorisant les infections (86).

5.5 MAI inclassées et diagnostics différentiels constitués de maladies multifactorielles ont des origines génétiques.

On parle de MAI dites « inclassées » lorsqu'elles ne remplissent pas les critères des maladies précédemment citées mais possèdent les critères généraux clinico-biologiques de maladies autoinflammatoires et une réponse positive aux traitements utilisés dans les MAI classiques comme la colchicine, les inhibiteurs de l'IL-1. Ces maladies sont caractérisées par des épisodes de fièvre récurrente avec syndrome inflammatoire biologique et des caractéristiques cliniques de type auto-inflammatoire, sans qu'aucune cause génétique ne soit être identifiée à ce jour.

Certaines maladies inflammatoires non-mendéliennes offrent des similitudes cliniques, histologiques, physiopathologiques ou génétiques avec les syndromes auto-inflammatoires héréditaires mais semblent avoir une origine multifactorielle.

5.5.1. Le syndrome de Schnitzler défini par l'association d'une urticaire acquise à l'âge adulte et d'une IgG ou IgM monoclonale par L. Schnitzler en 1972 partage avec les cryopyrinopathies une éruption très similaire sur le plan clinique et histologique (urticaire neutrophilique) (20). Une équipe néerlandaise a rapporté la présence de mutations somatiques de *NLRP3* restreintes à la lignée myéloïde chez des patients avec un syndrome de Schnitzler mais ils n'ont pas été confirmés sur une plus grande population (23).

5.5.2. La maladie de Still de l'adulte est une entité sans doute hétérogène caractérisée par l'association de 4 éléments clinico-biologiques cardinaux : fièvre marquée, éruption cutanée évanescence, arthralgies ou arthrites et une hyperleucocytose à polynucléaires neutrophiles en l'absence de facteur rhumatoïde ou d'anticorps antinucléaires (21). Ainsi définie, la maladie de Still était une maladie auto-inflammatoire avant l'heure, même si les mécanismes moléculaires et cellulaires qui la sous-tendent n'ont pas encore été totalement élucidés.

5.5.3. Le syndrome de Marshall ou PFAPA

Le syndrome de Marshall ou PFAPA est une des causes les plus fréquentes de fièvre récurrente en pédiatrie ; l'acronyme PFAPA signifie « periodic fever aphtous stomatitis pharyngitis adenitis » (87). Sa cause est à ce jour inconnue ; il associe des poussées de fièvre récurrentes de périodicité régulière environ une fois par mois avec pharyngite, aphtes buccaux, adénopathies cervicales et parfois céphalées, et douleurs abdominales pendant 3 à 6 jours. Les corticoïdes en cure courte sont très efficaces sur les poussées et constituent la pierre angulaire

du traitement. Le pronostic est bon : habituellement les signes disparaissent à l'adolescence, mais des cas chez l'adulte ont déjà été décrits (88).

5.5.4. Le syndrome des abcès aseptiques

Il est défini par l'existence d'abcès tissulaires diffus à polynucléaires neutrophiles majoritairement dans des organes intra-abdominaux, et plus rarement extra-abdominaux (poumon, cerveau, peau) ; il est souvent associé à la maladie de Crohn ou à la polychondrite atrophiante, voire à un pyoderma gangrenosum, mais il peut être isolé ; il y a parfois des antécédents familiaux de maladie inflammatoire du tube digestif, de sarcoïdose, mais pas d'abcès aseptique (89,90).

Principe de la prise en charge thérapeutique

La colchicine reste le traitement de base puisque la FMF est la maladie auto-inflammatoire la plus fréquente. Son observance doit être vérifiée en cas d'inefficacité. Pour les CAPS, les anti-IL-1 sont le traitement de référence. Dans les MAIx on commence souvent par de la colchicine et on adapte en fonction de la présentation clinique qui nous semble la plus proche d'une MAI connue. Dans les autres MAI monogéniques rares, le traitement n'est pas toujours codifié. Globalement dans les inflammasomopathies, les anti-IL1 sont tentés en premier, puis si échec les anti-IL-6 (26). Les anti-TNF α sont très efficaces dans le déficit en ADA2. Dans les ubiquitinopathies, des anti-IL1 ont été utilisés, parfois avec succès et en cas d'échec des anti-TNF. Le traitement des interféronopathies est détaillé dans un chapitre dédié de ce numéro spécial. Concernant les rares maladies associant déficit immunitaire et autoinflammation, leur traitement n'est pas codifié, il faut agir en priorité sur la partie la plus parlante autoinflammatoire par une biothérapie et discuter une supplémentation par immunoglobuline polyvalente d'autre part. De façon général, le traitement de ces maladies très rares doit être discuté en réunion de concertation pluridisciplinaire avec des référents qui ont l'habitude de prendre en charge ces patients.

Conclusions

L'analyse de l'interrogatoire et de signes en poussées est très importante pour orienter vers le diagnostic précis d'une maladie auto-inflammatoire. De plus en plus de traitements efficaces sont disponibles et permettent de soulager les patients et de prévenir l'apparition de l'amylose inflammatoire, la complication chronique de ces maladies inflammatoires. Depuis la découverte

du gène de la fièvre méditerranéenne familiale il y a 20 ans, de très grand progrès en génétique ont été faits afin de mieux démembrer ces maladies rares de l'immunité innée et pour certaines de montrer qu'il s'agit de spectres cliniques en fonction du siège des mutations dans les gènes. Il s'agit d'un nouveau champ de maladies systémiques en médecine interne qui laisse des opportunités de découvertes cliniques, physiopathologiques et génétiques très intéressantes pour les plus jeunes générations.

3

Les multiples facettes du déficit en ADA2, vascularite, maladie auto-inflammatoire et immunodéficit : mise au point à partir des 135 cas de la littérature.

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Résumé

Le déficit en adénosine déaminase 2 (DADA2) est une maladie auto-inflammatoire rare de description récente. Elle est de transmission génétique autosomique récessive, liée des mutations du gène *ADA2* (ou *CECRI*) codant pour l'enzyme ADA2. Outre son rôle dans le métabolisme des purines, ADA2 agirait comme facteur de croissance pour les cellules endothéliales et dans la différenciation des monocytes ; ainsi ce serait la déviation en macrophages M1 pro-inflammatoire et la survenue de lésions endothéliales secondaires à l'absence d'ADA2 qui seraient à l'origine des manifestations du DADA2. Ses manifestations se regroupent en trois grands types : vasculaire inflammatoire, hématologique et immunologique pouvant remplir les critères diagnostiques d'une périartérite noueuse, d'une aplasie médullaire ou d'un déficit immunitaire commun variable respectivement. La présentation vasculaire inflammatoire associe dans la plupart des cas une fièvre intermittente avec syndrome inflammatoire biologique, des manifestations vasculaires cutanées, telles qu'un *livedo racemoso* qui précède volontiers les autres symptômes, et une atteinte du système nerveux central (accidents vasculaires cérébraux ischémiques et hémorragiques), périphérique (multinévrite), ainsi que des arthralgies ou des atteintes digestives .. La forme hématologique se caractérise par des cytopénies, lymphopénie et anémie surtout, pouvant être sévères et nécessiter allogreffe de cellules souches hématopoïétiques. Le déficit immunitaire se rapproche d'un déficit immunitaire commun variable ; il est secondaire à l'hypogammaglobulinémie et une lymphopénie, à l'origine d'infections ORL ou pulmonaires récidivantes. Les corticoïdes et les immunosuppresseurs sont peu efficaces et le traitement repose sur les anti-TNF pour les formes inflammatoires, sans que le mécanisme expliquant leur efficacité ne soit parfaitement élucidé. Ces derniers doivent être introduits en première intention. La place des antithrombotiques est débattue en raison du sur-risque d'hémorragie cérébrale.

Mots clefs : ADA2, autoinflammation, vascularite monogénique, AVC, livedo

Abstract

Deficiency of adenosine deaminase 2 (DADA2) is a recently described auto-inflammatory disorder. It is an autosomal recessive inherited disease, caused by mutations in the *ADA2* gene (formerly known as *CECRI*) encoding ADA2 enzyme. Besides its role in the purine metabolism, it has been postulated that ADA2 may act as a growth factor for endothelial cells and in the differentiation of monocytes. Thus, deficiency of ADA2 would lead to endothelial damage and a skewing of monocytes into M1 pro-inflammatory macrophage, causing DADA2 manifestations. Three core clinical features have been described: inflammatory-vascular signs, hematologic abnormalities and immunodeficiency. Inflammatory-vascular phenotype is characterized by inconstant association of intermittent fever, cutaneous vascular manifestations, such as *livedo*, ischemic strokes, arthralgia and abdominal pain crisis. The hematologic phenotype consists of cytopenia, mainly anemia or lymphopenia, of variable severity, which may require heterologous hematopoietic stem cell transplantation. Immunodeficiency in DADA2 patients is secondary to hypogammaglobulinemia, inducing recurrent ENT or pulmonary infections. Corticosteroids and immunosuppressive agents (*i.e.* cyclophosphamide, azathioprin, ciclosporin, methotrexate) appear to be poorly effective. Although the mechanism has not been elucidated, anti-TNF agents have been proven efficient in DADA2 and should therefore be used as first line therapy for vasculitis. Role of anti-platelet and anticoagulant therapies in stroke-prophylaxis remains to be discussed, as those patients display a high risk of intracranial bleeding.

Key words: ADA2, autoinflammation, monogenic vascularitis, *livedo*, ischemic strokes

Introduction:

Le déficit en Adénosine Déaminase 2 (DADA2) est une vasculopathie auto-inflammatoire autosomique récessive de découverte récente. En 2014, deux équipes indépendantes identifiaient cette nouvelle entité chez des patients présentant soit une péri-artérite noueuse soit une vascularite cérébrale avec infarctus cérébraux précoces(91,92). L'hypothèse d'une origine génétique a été émise devant le regroupement des cas au sein de mêmes familles. L'analyse par whole exome sequencing (WES) a permis d'identifier des mutations autosomiques récessives du gène *CECRI* (Cat Eye syndrome Chromosome Region, candidate 1), aujourd'hui renommé *ADA2*, codant pour l'enzyme Adénosine Deaminase 2 (ADA2).

Depuis, de nombreux cas ont enrichi la littérature, et on voit se dessiner les contours d'une pathologie polymorphe, caractérisée par 3 grands types de présentation, non exclusives les unes des autres :

- Une présentation vasculaire / inflammatoire mimant une péri-artérite noueuse (PAN)
- Une présentation hématologique dominée par les cytopénies de la lignée érythrocytaire
- Une présentation dysimmunitaire en lien avec une hypogammaglobulinémie et une lymphopénie

1. Données démographiques

La littérature rapporte 135 patients porteurs de DADA2. Cinquante-quatre d'entre eux sont des femmes (Sexe ratio F/H = 0,72). La maladie a initialement été décrite chez des patients Géorgiens juifs (n=19), des Turcs (n=10) et des blancs-américains (n=5)(91,92). Depuis de nombreux patients caucasiens européens ont été rapportés (n=54) ainsi que quelques patients originaires du moyen Orient (n=16) d'Asie (n=7) ou d'Afrique du Nord (n=5). Il est à noter qu'aucun patient originaire d'Afrique subsaharienne n'a été rapporté à ce jour. Dix-huit (13, 3%) patients ont une histoire familiale de consanguinité et 74 (54,8%) ont au moins un frère / sœur atteint(e).

2. Présentation clinique (figures 1 et 2)

La maladie est, dans la grande majorité des cas, de début pédiatrique : les premiers symptômes surviennent presque toujours avant 10 ans (87,4% n=118). Seuls 5 cas débutant à l'âge adulte

sont rapportés, avec un début à 59 ans pour le plus tardif. Le tableau 1 présente l'ensemble des articles rapportant des patients DADA2 à ce jour ainsi que leurs caractéristiques cliniques et génétiques.

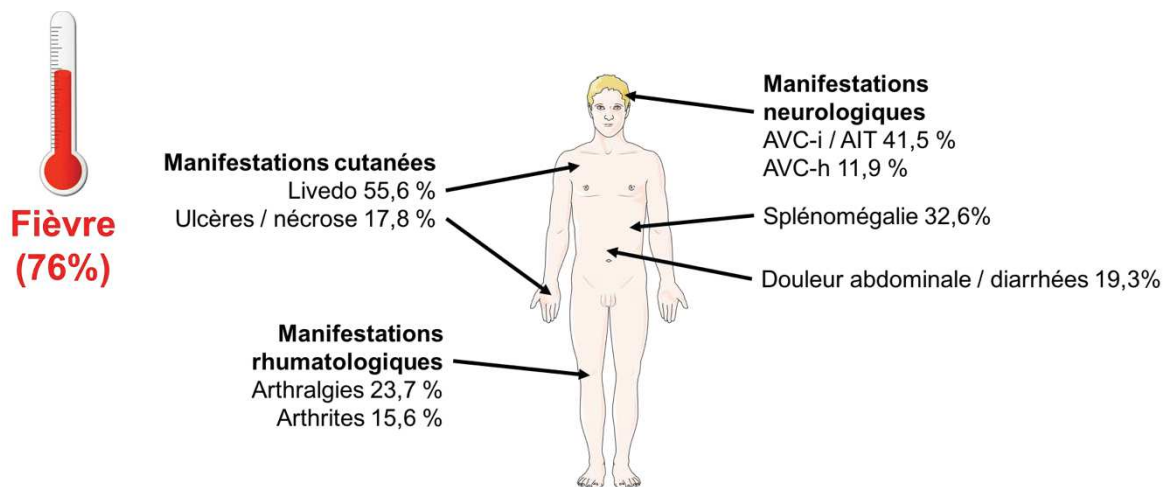


Figure 1 : Les principales manifestations cliniques du DADA2

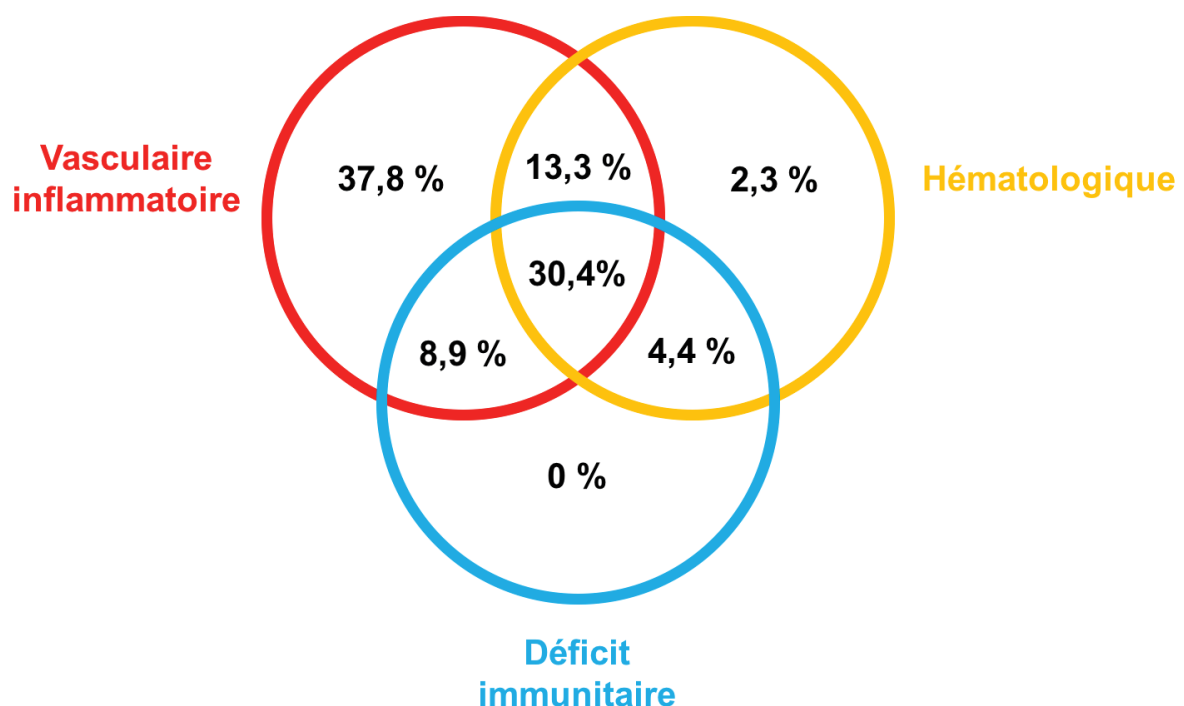


Figure 2 : Les trois principales présentations de DADA2 et leur répartition les unes par rapport aux autres

Quatre-vingt-dix pourcent des patients présentent des manifestations vasculaires et/ou inflammatoires, isolées pour près de 40% d'entre eux. Les manifestations anomalies hématologiques concernent la moitié des malades. Elles sont le plus souvent associées à un seul ou aux deux autres types de manifestations. Cependant les atteintes hématologiques isolés sont habituellement sévères (type érythroblastopénie). Le déficit immunitaire, présent chez 43,7% des DADA2, n'est quant à lui jamais isolé.

Table 1 : Cas de DADA2 rapportés dans la littérature:

Réf.	Année	Effectif	Ethnie	Mutation	Fièvre (n)	Symptômes cutanés	SNC	HTA	Déficit immunitaire	Atteinte hématologique
(92)	2014	24	Juif géorgien (19) Caucasien européen (4) Turc	G47R/G47R (17) R169Q/P251L (4) G47V/W264S	13	<i>Livedo</i> (16) Ulcère / nécrose (7) Autre (14)	AVC-i (4) Transformation hémorragique	7	-	-
(91)	2014	9	Blanc américain (5) Turc (3) Caucasien européen	A109D/Y453 G47A/Y453C R169Q/délétion G47A/H112Q R169Q/Y453C M1T/I93T G47R/G47R (3)	9	<i>Livedo</i> (9) Ulcère / nécrose	AVC-i (8) Transformation hémorragique (3)	1	Hypogamma Déficit en sous type d'Ig (4)	Splénomégalie (7) Cytopénie (5) SAM
(93)	2014	2	Caucasien européen Pakistanaï	R169Q/P193L G47R/G47R	2	<i>Livedo</i> Ulcère / nécrose	AVC-i (2) AVC-h	1	Hypogamma	-
(94)	2014	1	Turc	G47R/R306*	1	Autre	AVC-i AVC-h	-	Hypogamma	Lymphopénie
(95)	2014	4	Caucasien européen (4)	T119A/G142S (3)	4	<i>Livedo</i> (4) Ulcère / nécrose (4)	AVC-i (4)	-	-	-
(96)	2014	1	-	G47R/G47R (1)	1	-	-	-	-	PolyADP Splénomégalie (1) Anémie
(97)	2015	2	Caucasien européen (2)	K13del/N328K (2)	1	<i>Livedo</i> Autre	-	-	Hypogamma (2) Infections récurrentes	-
(98)	2015	6	Turc (6)	G47R/G47R (6)	6	<i>Livedo</i> / nécrose (6)	AVC-i (3)	-	Déficit en sous type d'Ig (2)	Myélofibrose SAM
(99)	2015	2	Caucasien européen (2)	R169Q/R169Q (2)	-	Autre (2)	AVC-i (2)	-	-	Splénomégalie Anémie
(100–103)	2014 2015 2016	9	Caucasien européen (7) Caucasien européen / marocain (2)	R169Q/R169Q (9)	3	<i>Livedo</i> (3) Ulcère / nécrose Autre (7)	AVC-i (6) AVC-h	1	Hypogamma (8) Déficit en sous type d'Ig (1) Infections récurrentes (4)	PolyADP (4) Splénomégalie (8) Pancytopenie (2) Cytopénie AI (2) LAM
(104)	2016	2	-	R169Q/M243R (2)	1	Autre	AVC-h	-	Hypogamma (2) Infections récurrentes (2)	Splénomégalie (2) Lymphopénie

(105)	2016	2	-	Y453C/délétion exon7 (2)	2	Autre	AVC-i (2)	1	Déficit en sous type d'Ig (2)	Lymphopénie (2) Anémie
(106)	2016	1	Brésilienne	G47R/Y453C	-	<i>Livedo</i> Ulcère / nécrose Autre	AVC-i	-	-	-
(107)	2016	15	Irakien (6) Indien (2) Caucasien européen (7)	P251L/P251L (6) G47R/G47R (2) P251L/c.114delC (2) R169Q/c.2T>C c.2T>C/c.144delG R169Q/Y453C R169Q/R169Q (2)	-	<i>Livedo</i> (11) Ulcère / nécrose (3) Autre (2)	AVC-i (4) AVC-h (3)	3	Hypogamma (3) Déficit en sous type d'Ig (3)	Lymphopénie (6)
(108)	2016	1	Caucasien européen	G47A/R169Q	1	<i>Livedo</i> Autre	AVC-i	-	-	-
(109)	2016	2	Sri-Lanka (2)	Délétion homozygote de 770 kb (2)	-	Autre (2)	-	-	Infections récurrentes (2)	Splénomégalie (2) Myélofibrose
(110)	2016	1	Singapour	F355L/E328K	1	<i>Livedo</i> Autre	-	1	-	-
(111)	2016	5	Caucasien maghrébin (5)	F178S/F178S L451F/L451F R49Afs*13/R49Afs*13 (2) D261Pfs*2/D261Pfs*2	2	-	-	2	Infections récurrentes	Splénomégalie (3) PolyADP EBP (2) AHAI Pancytopenie Neutropénie Anémie
(103,112)	2016	1	Latino-américain	Q265*/Q265*	-	-	-	-	Infections récurrentes Déficit en sous type d'Ig	-
(113)	2017	7	Turc	G47R/G47R (6) G47R/R306*	5	<i>Livedo</i> (5) Ulcère / nécrose	AVC-i (2) AVC-h (2)	4	Hypogamma (2)	Lymphopénie (2) Pancytopenie
(114)	2017	15	Caucasien européen (15)	R312*/E328D (2) T360A/T360A (5) G47A/P251L c.138/144delG/T360A L249P/T360A G47R/T360A Y453C/Y453C (2) L249P/P344L (2) N370K/G358R	11	<i>Livedo</i> (11) Ulcère / nécrose (3) Autre (6)	AVC-i (7) AVC-h	8	Hypogamma (2) Infections récurrentes (2)	Splénomégalie (3) PolyADP
(103,115)	2017	1	-	N370K/G358R	1	Autre	-	-	Déficit en sous type d'Ig Infections récurrentes	Hépatosplénomégalie EBP
(116)	2017	11	-	V458D/V458D C408Y/c.542+1G>A R169Q/M243R (2) G47A/G47A c.973-1G>A/c.973-1G>A R169Q/R169Q (2) H112Q/R169Q H112Q/H112Q	7	<i>Livedo</i> (3) Ulcère / nécrose	AVC-i (3)	3	Hypogamma (11) Infections récurrentes (5)	Anémie (4) Lymphopénie (5) Thrombopénie (3) Neutropénie (1) Splénomégalie (8) PolyADP

				c.972+3A>G/c.973-2A>G							
(117)	2017	2	Caucasien (2)	G47R/C408Y (2)	2	<i>Livedo</i> (2) Ulçère / nécrose Autre	AVC-i (2)	-	-	-	-
(118)	2017	1	Japonais	E328D/Y236del	1	Autre	-	-	-	-	PolyADP
(103)	2017	9 (et 5 autres précédemment rapportés)	Caucasien (8) Latino-américain	Y220*/Y220* R49FS/R169Q (2) R169Q/R169Q (2) R169Q/M1T G47V/H112Q R49fs/c.47+2T>C R169Q/G358R	-	<i>Livedo</i> (2)	AVC-h (3)	1	Hypogamma (4) Déficit en sous type d'Ig Infections récurrentes (3)	PolyADP (3) Splénomégalie (7) EBP (5) PTI Pancytopenie Lymphopénie	

Table 2 : Résumé des caractéristiques cliniques des 135 patients de la littérature

a) Phénomènes vasculaires

Sexe F/H	54/75	Atteinte neurologique	
Âge au début (médiane, [Q1-Q3]) - années	3,0 [1,0–7,0]	AVC-i / AIT	56 (41,5)
Fièvre	76 (56,3)	AVC-h / transformation h	16 (11,9)
Retard de croissance	10 (7,4)	Déficit d'un nerf crânien	24 (17,8)
HTA	33 (24,4)	Surdité neurosensorielle	8 (5,9)
CRP (médiane, [Q1-Q3]) – mg/L	48 [15-65]	Vertige / nystagmus	6 (4,4)
Atteinte cutanée / muqueuse		Retard mental	4 (3,0)
Livedo	75 (55,6)	NRP périphérique	13 (9,6)
Ulcères / nécrose	24 (17,8)	Atteinte hématologique	
Apthes buccaux	15 (11,1)	SMG	44 (32,6)
Autre	60 (40,4)	PolyADP	13 (9,6)
Atteinte digestive		Anémie	32 (23,7)
Douleurs / troubles du transit	26 (19,3)	Lymphopénie	19 (14,1)
Pseudo MICI	9 (6,7)	Neutropénie	19 (14,1)
Perforation / sténose	4 (3,0)	Thrombopénie	16 (11,9)
HMG	28 (20,7)	EBP	8 (6,0)
Insuffisance hépatique	5 (3,7)	Déficit immunitaire	
Atteinte oculaire		Hypogamma	38 (28,2)
Vasculaire	3 (2,2)	Infections récurrentes	23 (17,0)
Neuropathie optique	8 (5,9)	Atteinte articulaire	
Uvéite	1 (0,7)	Arthralgies	32 (23,7)
		Arthrites	21 (15,6)

Rapportés depuis la description initiale, ils constituent le type de manifestation le plus fréquent du DADA2 : dans la littérature, ils existent chez 79,3% (n=107) des patients. Ils concernent principalement la peau, le système nerveux central et les reins.

- L'atteinte cutanée est souvent au premier plan : un *livedo* est rapporté chez 55,6% (n=75) des patients. Dans certains cas un phénomène de Raynaud peut également exister, revêtant quelques fois un caractère sévère : 17,8% (n=24) des patients rapportés ont présenté au moins un épisode de nécrose des extrémités (digitales surtout, auriculaires parfois) ou d'ulcère.
- L'atteinte neurologique vasculaire ischémique est également classique. Elle existe chez 41,5% (n=56) des patients rapportés à ce jour. Elle peut toutefois être sous-estimée puisque 17,8% (n=24) des patients ont présenté une atteinte de nerf crânien rapidement résolutive, compatible avec un accident ischémique transitoire. L'IRM cérébrale peut montrer un, voire plusieurs infarctus (39,3%, n=53) ou, plus rarement, un saignement (14,8%, n=20). Les infarctus sont volontiers lacunaires, profonds et il existe parfois des anomalies de signal de la substance blanche non ischémique (91,101,113). Ces éléments sont alors évocateurs d'une maladie des petites artères

cérébrales. L'atteinte des gros vaisseaux intracrâniens est plus rare (3 patients rapportés avaient une disparité de calibre ou une sténose, un seul avait un anévrisme).

- L'atteinte rénale se limite souvent à une hypertension artérielle (24,4%, n=33). Toutefois des formes cliniques plus sévères peuvent exister : insuffisance rénale chronique, protéinurie, syndrome néphrotique. Elles représentent 4,4% (n=6) des patients rapportés. Devant une forme vasculaire de DADA2, des imageries rénales peuvent être réalisées. Elles sont souvent normales mais peuvent parfois montrer un infarctus rénal (n=4), des sténoses / irrégularités artérielles (n=4) ou bien des anévrismes artériels (n=11).
- D'autres atteintes vasculaires moins fréquentes peuvent exister. Les explorations angiographiques peuvent montrer des anévrismes artériels céliaques / mésentériques (5,9%, n=8) ou hépatiques (2,2%, n=3). 3 patients avec anomalies vasculaires rétiniennes ont été rapportés : une occlusion de l'artère centrale de la rétine, une occlusion de la veine centrale de la rétine et une sténose artérielle rétinienne.

b) Phénomènes inflammatoires

Ils sont fréquents (77,8%, n=105) et coexistent souvent avec les phénomènes vasculaires, ce qui explique que près d'un tiers des patients remplissent les critères de périartérite noueuse (critères PReS ou ACR). Ces symptômes évoluent volontiers selon un schéma poussée-rémission, comme la plupart des maladies auto-inflammatoires.

- Plus de la moitié des patients présentent une fièvre récurrente (56,3%, n=76)
- Lors des poussées un syndrome inflammatoire biologique apparaît et la CRP est élevée (48 mg/L en moyenne - IQR : [15 – 65])
- Diverses atteintes cutanées inflammatoires sont rapportées chez un peu moins de la moitié des DADA2 (44,0%, n=59) : surtout des exanthèmes maculeux / papuleux, des urticaires ou des érythèmes noueux.
- Les arthralgies sont fréquentes (23,7%, n=32) mais seulement 15,6% (n=21) des patients actuellement rapportés présentent d'authentiques arthrites. Lorsqu'elles existent elles atteignent plutôt les petites articulations, sont bilatérales et symétriques.
- Des douleurs abdominales et une diarrhée sont rapportées dans près 20% des cas (n=26). Quelques cas plus graves, mimant des maladies inflammatoires chroniques intestinales, ont été décrits, ainsi que d'authentique vascularite digestive avec sténose ou perforation.
- Les autres atteintes inflammatoires moins fréquentes comprennent, entre autres, névrite optique, uvéite, pharyngite et hépatite

c) Phénomènes hématologiques

De description plus récente dans le DADA2, des symptômes hématologiques sont néanmoins présents chez la moitié des patients (50,4% n=68). Ils sont rarement au premier plan mais lorsque c'est le cas ils constituent un défi dans la prise en charge thérapeutique. L'examen physique du patient DADA2 peut révéler une splénomégalie (32,6%, n=44) ou plus rarement une polyadénopathie (9,6%, n=13). Les ganglions ne présentent alors pas de signe de malignité. L'hémogramme montre souvent des cytopénies de sévérité variable. Par ordre de fréquence on observe des anémies (23,7%, n=32), des lymphopénies (18,5%, n=25), des neutropénies (14,1%, n=19) et des thrombopénies (11,9%, n=16). Des atteintes hématologiques plus sévères de type aplasie médullaire, de description récente et nécessitant des traitements spécifiques sont rapportées. Elles sont heureusement rares. La mieux décrite est l'érythroblastopénie (6,0%, n=8).

d) Déficit immunitaire

Cet aspect du DADA2 est exceptionnellement isolé. Le mécanisme principal est une hypogammaglobulinémie, présente chez un peu plus d'un quart des patients (28,2%, n=38), ou un déficit spécifique d'un sous type d'immunoglobuline comme dans un déficit immunitaire commun variable. Ces anomalies sont à l'origine d'infections récidivantes. Il s'agit alors principalement d'infections du tractus respiratoire ou ORL. Il peut également exister une lymphopénie globale en lien avec l'atteinte hématologique.

e) Autres

En plus de ces manifestations cardinales du DADA2, certains patients peuvent présenter des signes d'auto-immunité. Ces patients sont cependant rares, il s'agit surtout de cytopénies auto-immunes (3,7%, n=5). La présence d'anticorps anti nucléaires (ACAN) est inconstante. Un patient diagnostiqué avec une maladie de Castleman HHV8 négatif a également été décrit (NEJM cf lettre). Récemment, un DADA2 a été rapporté chez deux frères présentant un phénotype qui avait initialement évoqué un syndrome d'Aicardi Goutières aux auteurs ainsi que des lésions cutanées discoïdes proches de celles observées dans le lupus. Ces patients présentaient tous deux des ACAN, dont le titre évoluait parallèlement à la sévérité des manifestations cliniques (117). Chez ces mêmes malades était retrouvée une signature interféron de type 1 (IFN I), qui avait déjà été décrite dans le DADA2 en 2014 chez deux autres patients aux phénotypes proches (93). À ce jour, 13 signatures IFN I ont été rapportées pour 7 patients différents, parmi lesquels 2 patients ont

présenté une signature négative à une seule reprise (93,117,119). Ainsi, un lien entre l'activation de la voie de l'IFN I et le déficit en ADA2 a été suggéré, mais mérite d'être confirmé sur un plus grand nombre de patient.

Le tableau 2 récapitule les caractéristiques cliniques du DADA2 organe par organe.

3. Aspects biologiques

Outre les anomalies biologiques sus-citées, un examen utile au diagnostic de DADA2 est le dosage de l'activité ADA2 plasmatique. Elle est en règle inférieure à 50% de celle d'un témoin sain, souvent effondrée <1 mU/mL. Dans de rares cas l'activité peut être normale. La mesure de l'activité doit être interprétée en fonction de l'âge car celle-ci diminue progressivement au cours de la vie si bien qu'il est crucial d'avoir des contrôles appariés pour l'âge. Ce dosage n'est cependant pas disponible dans tous les centres et fait l'objet d'investigations de recherche. Les individus hétérozygotes ont une activité intermédiaire, entre celle des patients homozygotes et des contrôles sains.

4. Aspects histologiques

Lorsque le DADA2 se présente sous sa forme vasculaire inflammatoire, une biopsie d'organe atteint peut contribuer au diagnostic. Dans deux tiers des cas (33 sur 49 biopsies rapportées dans la littérature) il existe une vascularite. Il s'agit d'une vascularite granulomateuse et/ou nécrosante de type péri-artérite noueuse (2/3 cas – n=21) ou d'une vascularite leucocytoclasique (1/3 des cas – n=10).

5. Génétique et physiopathologie (figure 3)

Une approche par WES, menée en parallèle par 2 équipes indépendantes, a permis d'identifier l'anomalie génétique en cause dans le DADA2. Il s'agit de mutations autosomiques récessives du gène *ADA2* (auparavant *CECRI* - Cat Eye syndrome Chromosome Region, candidate 1), composé de 13 exons et localisé sur le bras long du chromosome 22, en 22q11.1. Ce gène code pour l'enzyme Adénosine déaminase 2 (ADA2). Depuis février 2014, plus de 50 mutations différentes ont été identifiées. Certaines semblent associées à une origine géographique en particulier. Ainsi la mutation G47R à l'état homozygote ou hétérozygote composite a été détectée chez presque tous les patients juifs géorgiens et turcs. De la même manière la mutation R169Q se retrouve chez les caucasiens européens, en particulier les hollandais (fréquence de la mutation à l'état hétérozygote :

1:500 contre 1:1500 dans le reste de l'Europe) et la mutation T360A n'est décrite que chez des italiens.

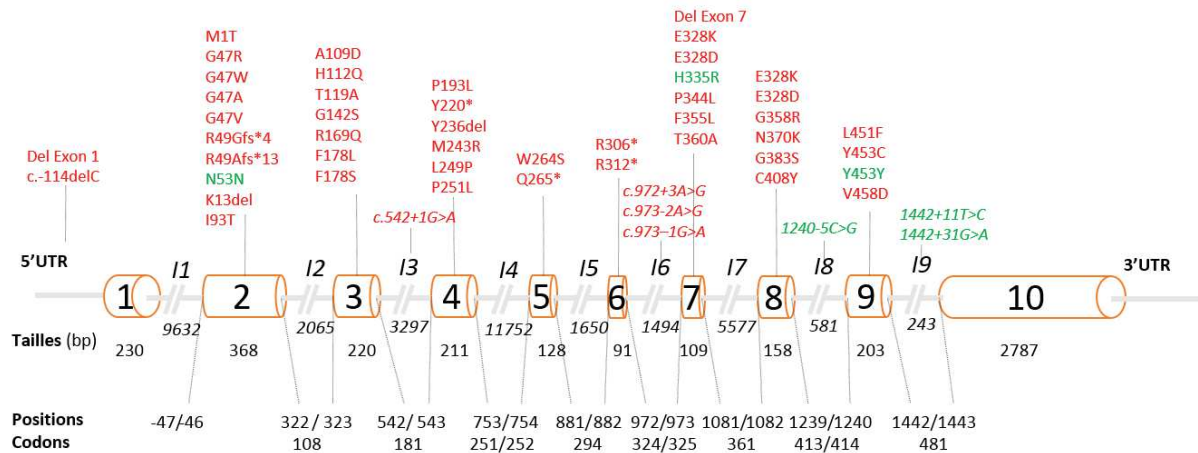


Figure 3 : Représentation du gène et des principales mutations décrites à ce jour.

Les principaux variants identifiés dans le gène *ADA2* (NM_001282225.1) en noms usuels. Les polymorphismes sont représentés en vert, et les mutations pathogènes en rouge. Les variants introniques sont en italique.

ADA2 est une protéine dimérique, principalement exprimée par les cellules myéloïdes, et sécrétée dans l'environnement extracellulaire. Sa concentration plasmatique et son activité peuvent ainsi être dosées. ADA2 se compose de 4 domaines : la séquence signal, le domaine de dimérisation, le domaine de liaison à l'adénosine et le domaine catalytique. Tous ces domaines peuvent être affectés par une mutation. Elle ne doit pas être confondue avec la protéine ADA1, autre isoforme d'ADA, qui est connue pour être impliquée dans une forme de déficit immunitaire combiné sévère en cas de déficit. Les enzymes ADA interviennent dans le métabolisme des purines. Leur rôle est la transformation de l'adénosine en inosine (et de la 2'-deoxyadénosine to 2'-deoxyinosine). ADA1 se distingue d'ADA2 par sa localisation intracellulaire, son expression ubiquitaire et son affinité pour le substrat près de 100 fois supérieure à celle de l'ADA2.

La fonction de l'ADA2 dans la physiopathologie du DADA2 est encore mal comprise et l'absence d'orthologue chez la souris rend les explorations difficiles. Deux hypothèses sont actuellement discutées.

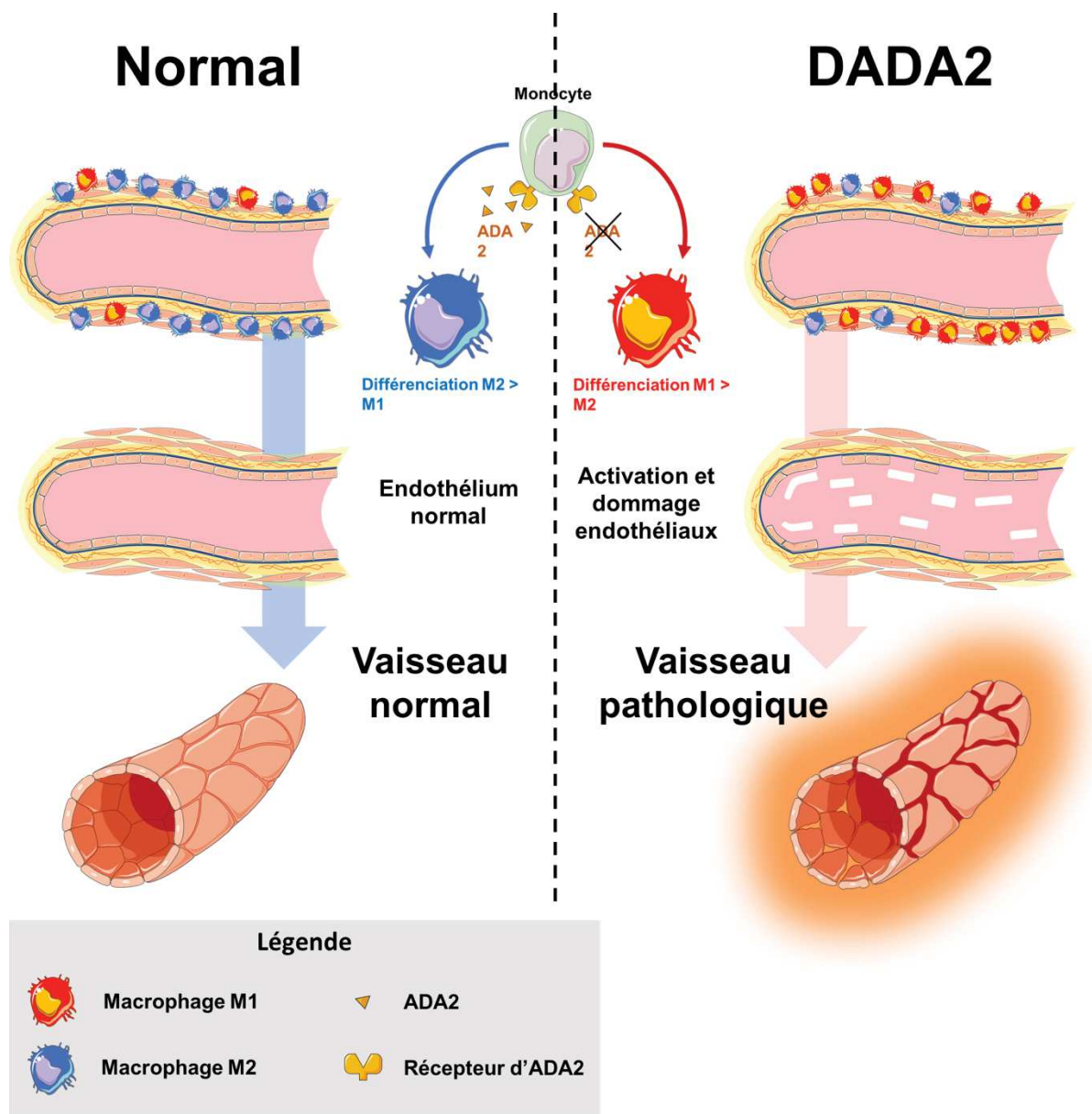


Figure 4 Physiopathologie du DADA2

En situation physiologique l'ADA2 agit comme facteur de croissance monocyttaire et promeut la différenciation en macrophage M2 plutôt que M1. En l'absence d'ADA2 la différenciation se fait préférentiellement vers un phénotype M1 pro-inflammatoire. On suppose que l'ADA2 a une activité facteur de croissance semblable sur la cellule endothéliale : ainsi, en son absence on observe une activation endothéliale à l'origine de dommages vasculaires. Ces deux mécanismes associés aboutissent aux manifestations vasculaires et/ou inflammatoires du DADA2 telles que le *livedo*, la fièvre récurrente ou les accidents vasculaires cérébraux.

La première soutient que l'accumulation d'adénosine non dégradée par l'ADA2 déficiente dans l'espace intercellulaire induit une activation excessive de récepteurs à l'adénosine couplés aux protéines G et ainsi une activité pro-inflammatoire. Il faut cependant noter qu'à concentration physiologique l'ADA2 a une affinité pour l'adénosine inférieure à celle des transporteurs membranaires et que la concentration en adénosine libre intracellulaire reste presque nulle du fait

de la forte affinité d'ADA1. De plus certains patients DADA2 rapportés ont un dosage d'adénosine sérique normal (100). Ces éléments sont en faveur d'une régulation de l'adénosine extracellulaire non pas par l'ADA2 mais plutôt par l'ADA1, intracellulaire, via les transporteurs membranaires d'adénosine. Ainsi il n'est pas certain que le défaut catalytique joue un rôle important dans la physiopathologie du DADA2.

La seconde hypothèse est celle d'une activité type facteur de croissance de l'ADA2, dont les cibles seraient les cellules immunitaires (monocytes principalement) et les cellules endothéliales. Cette hypothèse est soutenue par des expériences *in vitro* montrant une expansion de lymphocytes T humains en présence d'ADA2 ou d'ADA, et la différenciation de monocytes en macrophages en présence d'ADA2 indépendamment de l'activité enzymatique(120). Par ce biais il est possible que l'absence d'ADA2 soit associée à une différenciation préférentielle des monocytes en macrophages M1 pro-inflammatoire(91). L'activation des neutrophiles a été évoquée également avec l'expression d'une signature neutrophilique dans le sang total de patients et des anomalies phénotypiques des PNN en cytométrie qui présente des signes d'activation (Belot et al). Il a également été montrée *in vitro* que l'ADA2 se lie aux principales cellules impliquées dans l'inflammation et l'immunité, sans que l'effet de cette liaison ne soit expliqué(121,122). Cette hypothèse reste à tester *in vivo*, ce qui ne semble pas encore possible en l'absence de modèle animal convenable. Concernant l'activité facteur de croissance sur les cellules endothéliales, plusieurs observations *in vitro* et *in vivo* sont en faveur de cette hypothèse(91) :

- Chez les patients DADA2 on observe des lésions endothéliales vasculaires et une surexpression des marqueurs d'activation endothéliale
- Le poisson zèbre KO pour *cecr1a* (modèle animal de DADA2) présente un tableau hémorragique du système nerveux central en l'absence d'anomalie morphologique vasculaire. Ce tableau est résolutif avec la transfection d'ARN-m ADA2 non muté humain.
- *In vitro*, les monocytes issus de patients DADA2 cultivés avec des cellules endothéliales humaines induisent la destruction de ces dernières.

6. Prise en charge thérapeutique

Le DADA2 étant un syndrome auto-inflammatoire rare de découverte récente. Aucun traitement n'a encore été clairement validée dans cette indication. En outre, le recul sur ces patients est insuffisant et dans ces conditions le dialogue étroit entre les acteurs de proximité et le centre de référence / de compétence apparaît essentiel à la prise en charge optimale.

Néanmoins les données actuelles de la littérature semblent converger vers une efficacité des anti-TNF alpha dans les formes vasculaires et inflammatoires. En effet, dès la description initiale de la pathologie, Navon *et al.* rapportaient 10 patients traités par anti-TNF dont 8 avaient une rémission complète de leurs symptômes. Plus récemment 12 patients étaient traités pendant une médiane de 10 mois par anti-TNF : il n'était pas noté de nouvel événement cliniquement significatif et les marqueurs inflammatoires ainsi que l'anémie s'amendaient progressivement(123). Dernièrement Caorsi *et al.* rapportaient 15 DADA2 de présentation PAN-like dont 9/10 traités par anti-TNF seuls ou en association avaient une réponse complète(114). Ainsi 43,0% (n=58) des patients rapportés à ce jour reçoivent ou ont reçu des anti-TNF, parfois en première ligne. Etanercept, infliximab et adalimumab sont utilisés indistinctement. L'efficacité des anti-TNF dans cette pathologie n'est pas bien comprise, mais pourrait s'expliquer par l'inhibition du TNFa par les macrophages M1.

La corticothérapie à forte dose, comme dans la plupart des maladies auto-inflammatoire, permet d'obtenir une réponse rapide. Cependant la majorité des patients traités par corticoïdes seuls deviennent cortico-dépendants et nécessite l'adjonction d'autres traitements(91–94,98,101,124,125).

Les traitements immunosuppresseurs usuels (Cyclophosphamide, Azathioprine, Methotrexate, Rituximab) sont décevants et permettent une réponse seulement transitoire, souvent incomplète(92,93,98,104,114,124,125). Le plasma frais congelé s'il apporte l'enzyme déficitaire est probablement peu efficace en raison de la demi vie courte de l'enzyme ADA2.

La place des traitements anti-thrombotiques reste à définir. En effet, bien qu'ils semblent intéressants pour la prophylaxie des événements neurologiques ischémiques, leur bénéfice est à mettre en balance avec le risque hémorragique chez ces patients parmi lesquels environ 10% (n=16) ont présenté un saignement intracrânien (transformation hémorragique d'un infarctus ou AVC hémorragique).

Le déficit immunitaire lié à l'hypogammaglobulinémie est souvent peu symptomatique et tous les patients ne relèvent pas d'un traitement spécifique. En revanche en cas d'infections récurrentes on peut proposer une supplémentation par immunoglobulines polyvalentes intraveineuses (11 patients dans la littérature – pas de recul sur l'efficacité).

Dans les formes hématologiques sévères les traitements immunosuppresseurs, y compris les anti-TNF, semblent peu efficace. L'ADA2 étant produite par les cellules de la lignée myéloïde (principalement monocytes et macrophages) l'allogreffe a rapidement été envisagée dans ces

formes cliniques. A ce jour 15 patients ayant bénéficié d'une allogreffe dans le cadre d'un DADA2 ont été rapportés. Les indications retenues étaient : une érythroblastopénie (n=7), une neutropénie sévère (n=1), une pancytopénie profonde avec lymphoprolifération (n=1), une pancytopénie profonde (n=2), une lymphopénie profonde (n=1), une leucémie aigüe myéloïde (n=1), des accidents ischémiques transitoires récidivants (n=1) et la coexistence de cytopénies auto-immunes avec un déficit immunitaire profond à l'origine d'infections virales sévères (n=1) (100–103,112,115). L'allogreffe a été un succès chez l'ensemble de ces patients. Elle corrige le taux et l'activité sérique d'ADA2. Elle permet également la disparition des symptômes de DADA2, qu'ils soient vasculaires, inflammatoires ou hématologiques, au prix des effets indésirables spécifiques qui lui sont bien connus. Certains patients ont été allogreffés avant d'avoir été diagnostiqués DADA2 (100,101,112). C'est pour eux que le recul est le meilleur, jusqu'à 10 ans, et on observe un bénéfice persistant avec l'absence totale de manifestation de la maladie (n=2). Le taux de GVH apparaît néanmoins élevé et des cas d'autoimmunité post-greffe sont reportés. Par analogie avec le déficit en ADA1, certaines équipes envisage de mettre au point des stratégies de thérapie génique.

7. Complications

a) L'amylose inflammatoire

Comme toute maladie auto-inflammatoire non diagnostiquée ou mal contrôlée, le DADA2 peut se compliquer d'une amylose inflammatoire (ou Amylose AA). Cette complication reste rare puisque seulement 2 cas ont été rapportés (91,98). Dans les deux cas il s'agissait de Turcs porteurs de la mutation G47A à l'état homozygote (la mutation la plus fréquente dans cette population). On notait alors des atteintes rénales et digestives. On peut par conséquent ajouter le DADA2 à la liste des maladies génétiques rares à rechercher devant une amylose AA inexpliquée.

b) Les complications neurologiques

Il existe une importante morbi-mortalité neurovasculaire chez les patients atteints de DADA2. La survenue d'accidents vasculaires multiples récidivants à un âge précoce est pourvoyeuse de lourdes séquelles neurologiques à l'origine de handicap, ainsi que de décès. Il est également à noter qu'il peut exister un retard mental, rapporté dans 4 cas à ce jour. Ces séquelles, si elles ne peuvent être évitées, doivent être évaluées et prises en charge de manière optimale, idéalement au sein d'une équipe pluridisciplinaire faisant intervenir autant des médecins (neurologues, pédiatres, psychiatres) que des professionnels paramédicaux tels que des kinésithérapeutes, des psychomotriciens, des orthophonistes ou encore des ergothérapeutes. Des actions sociales, financières (Allocation d'Education de l'Enfant Handicapé AEEH) et éducatives peut être mis en

place autour de l'enfant en situation de handicap. Pour ce faire, il est impératif de collaborer étroitement avec le CDAPH et la MDPH ainsi qu'avec l'assistante sociale de secteur.

c) Les complications infectieuses

Leur origine est double : soit en lien avec le déficit immunitaire propre à la maladie elle-même, soit du fait des traitements immunosuppresseurs ou des corticoïdes utilisés. Un tiers de décès rapportés chez les patients DADA2 (n=4 /11 décès rapportés) est secondaire à une complication infectieuse sévère. La morbidité liée au déficit immunitaire propre au DADA2 peut être réduite en supplémentant systématiquement les patients hypogammaglobulinémiques en immunoglobulines polyvalentes en cas d'infections récidivantes. Celle liée aux corticoïdes et aux immunosuppresseurs est bien connue de l'interniste : il est indispensable de dépister +/- traiter les infections chroniques latentes avant d'initier un tel traitement, de réaliser un déparasitage digestif par ivermectine avant corticoïdes et parfois de mettre en place une prophylaxie anti-infectieuse primaire (cotrimoxazole, valaciclovir).

Conclusion

Bien que d'individualisation récente, le DADA2 n'a eu de cesse de voir son spectre clinique s'étoffer durant ces trois dernières années, de sorte qu'aujourd'hui on distingue des formes vasculaires, inflammatoires (souvent associées), immunitaires ou hématologiques. Le diagnostic passe par la mise en évidence d'une mutation du gène *ADA2* à l'état homozygote ou hétérozygote composite, par technique de séquençage haut débit (NGS) ou Sanger. Un dosage de l'activité ADA2 plasmatique peut aider à trancher en cas de génétique équivoque. Dans la forme vasculaire inflammatoire un diagnostic précoce est nécessaire puisque la mise en place rapide d'un traitement par anti-TNF semble être efficace, d'une part pour contrôler les manifestations de la maladie et d'autre part pour prévenir la survenue d'un événement vasculaire. En cas de formes sévères, hématologique dans la plupart des cas, l'allogreffe de cellules souches hématopoïétiques semble un traitement prometteur. Des recherches centrées sur la physiopathologie du DADA2 sont nécessaires et pourraient aboutir à la compréhension des bons résultats des anti-TNF ainsi qu'à l'émergence de nouvelles stratégies thérapeutiques. La place des anti-thrombotiques chez ces patients à haut risque neurovasculaire reste également à définir.

4

Diagnosis of cryopyrin associated periodic syndrome: challenges, recommendations and emerging concepts

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Summary

Cryopyrin associated periodic syndrome (CAPS) are rare autosomal dominantly inherited diseases. They include 3 overlapping phenotypes: Familial Cold Autoinflammatory Syndrome (FCAS), Muckle-Wells Syndrome (MWS), and Chronic Infantile Neurological Cutaneous Articular Syndrome/Neonatal Onset Multisystem Autoinflammatory syndrome (NOMID/CINCA). Recurrent fevers, joint pain, and urticarial skin-rash are the main clinical features of these conditions. Renal amyloidosis and sensorineural complications may occur. Gain-of-function mutations in *NLRP3* gene are responsible for the over-activation of the NLRP3 inflammasome, a multimolecular complex involved in the inflammatory process. Missense mutations are almost always encountered, particularly in exon 3 which encodes the nucleotide binding domain (NBD). Mosaicism is not rare, especially in CINCA/NOMID. Next generation sequencing will grant access to new insights about *NLRP3* implication in oligogenic and multifactorial diseases.

Keywords

CAPS, cryopyrin, inflammasome, NLRP3, FCAS, MWS, CINCA, autoinflammatory syndrome, mosaicism

Introduction

Cryopyrinopathies encompass a spectrum of diseases caused by a defect of NLRP3 (NOD-like receptor family, pyrin domain containing protein 3) (or cryopyrin) functions. The generated autoinflammation is characterized by seemingly unprovoked episodes of systemic and local inflammation, without auto-activated T-cells and high titer of auto-antibodies. Cryopyrinopathies also called CAPS (cryopyrin associated periodic syndrome) include 3 phenotypes, FCAS (Familial Cold Autoinflammatory syndrome), MWS (Muckle-Wells syndrome), and CINCA/NOMID (Chronic Infantile Neurological Cutaneous Articular Syndrome/Neonatal Onset Multisystem Autoinflammatory syndrome) and are dominantly inherited diseases.

In this review, we will discuss the most recent knowledge allowing CAPS diagnosis, and the ongoing challenges related to these disorders.

Physiopathology

The term cryopyrin is the initial name of this protein proposed by the discoverer of the gene responsible for FCAS and MWS in 2001(126). Since then, different names have been suggested, based on the progressive elucidation of its role as PYPAF1 (PYRIN-containing APAF1-like protein 1)(127), or the characterization of protein families, i.e. NLRP3 and NALP3 (NACHT, LRR and PYD domains-containing protein 3)(128); currently, the proper name is NLRP3.

This protein belongs to the NLRP family, which contains 14 members in human. This group of innate intracellular pattern recognition receptors (PRRs) plays a crucial role in the recognition of a wide spectrum of DAMPS (danger-associated molecular patterns) and PAMPS (pathogen-associated molecular patterns). All of them share an N-terminal pyrin domain (PYD), a nucleotide-binding domain (NBD) domain, and a C-terminal leucine-rich repeat (LRR) motif(129).

The PYD domain belongs to the “death domain-fold” family, known to be involved in many apoptosis and inflammation processes. This domain allows the homotypic interactions with the PYD of other proteins such as ASC (Apoptosis-associated Speck-like protein containing a CARD) for NLRP3.

The NBD domain binds and hydrolyzes ATP, allowing the protein oligomerization.

The LRR motif recognizes PAMPs and DAMPs, leading to the activation of the inflammation process.

Inflammation is a tightly regulated response of the innate immune system to infection and tissue damages that aims to restore tissue homeostasis. NLRP3 is an essential player in the innate inflammatory pathways as it binds to several protein partners to form a multimolecular complex named the NLRP3 inflammasome. The protein NLRP3 sensor, the ASC adaptor, and the caspase-1 effector define the NLRP3 inflammasome.

NLRP3 is thought to be in an autoinhibited form in the resting state. Different PAMPs and DAMPs activate the formation of the NLRP3 inflammasome such as bacterial, viral and fungal pathogens, ATP, contact allergens and products of dying cells (e.g. uric acid crystals)(130,131). The molecular mechanisms underlying the assembly of the NLRP3 inflammasome are still debated, but it is formed when activated NLRP3 recruits the adaptor molecule ASC, which is required for the procaspase-1's participation in this complex (Figure 1).

Once procaspase-1 is recruited into the inflammasome, the active complex further increases in size. It allows procaspase-1 autoproteolysis into active caspase-1. This effector molecule induces cleavage of the interleukin IL-1 β and IL-18 proforms, and leading to an inflammatory type of cell death called pyroptosis, by unsolved mechanisms.

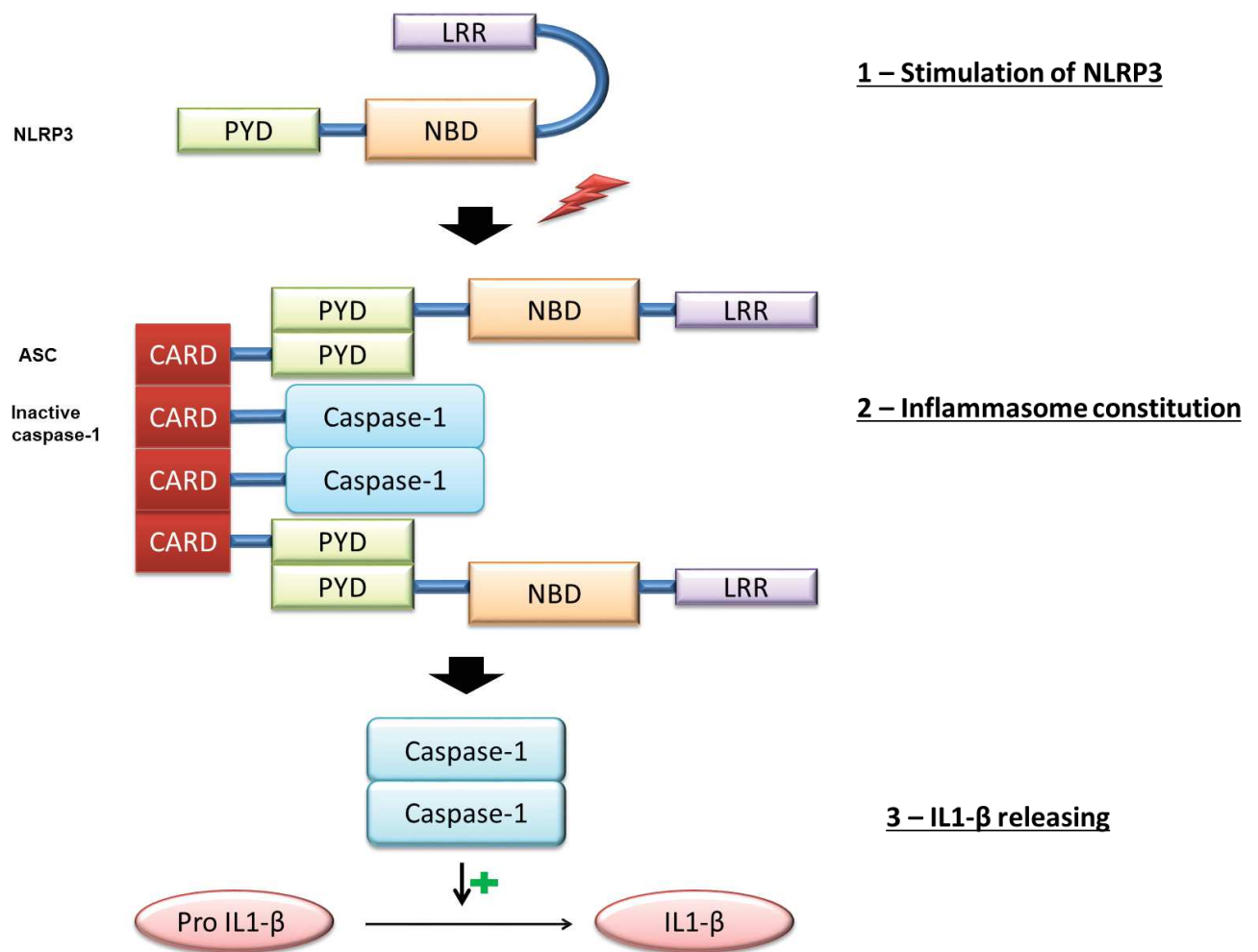


Figure 1: The NLRP3 inflammasome

It consists of 3 components: the sensor molecule NLRP3, the adaptator molecule ASC (Apoptosis-associated Speck-like protein containing a CARD), and the effector molecule caspase-1. The NLRP3 activation is triggered by exogenous and/or endogenous signals. Homotypic interactions allow the recruitment of ASC and inactive caspase-1. Caspase-1, when active, can cleave pro-IL1-β into IL1-β, allowing its releasing.

IL-1β is known to be a major cytokine of the inflammatory response(132). It has multiple effects such as fever induction, endothelial cell stimulation responsible for cutaneous rash, and bone marrow cell stimulation leading to hyperleukocytosis and thrombocytosis. Moreover, IL-1β activates adaptive T helper (Th) 1 and Th17 differentiation, and humoral immunity(133).

Mostly all *NLRP3* pathogenic mutations are substitutions affecting the NBD domain. Monocytes isolated from a MWS patient with p.Arg760Trp, a clearly pathogenic mutation, showed increased levels of IL-1β secretion, suggesting a constitutive activation of the NLRP3 inflammasome(134).

This result has been also observed by Janseen et al., who showed increased IL-1 β secretion by CINCA/NOMID patient's monocytes (135). The spectacular efficiency of IL-1 β targeting drugs in CAPS patients proves the central role of this cytokine in cryopyrinopathies(136–139). Safety of IL1 inhibitors is now well recognized(140). Their efficacy has also been demonstrated in patients who have already developed reactive amyloidosis(141).

Epidemiology, mode of transmission

CAPS are rare monogenic diseases. Current epidemiological data suggest an incidence in the U.S. of 1 in 1000000 people(142). According to Cuisset et al., the estimated prevalence in France is equal to 1/360000(143). Since they are quite recently recognized diseases, we hypothesize that this prevalence is underestimated. CAPS affect all ethnic groups equally; however a shared haplotype in four large North American FCAS families has been reported, demonstrating a founder effect. The authors identified a missense mutation (p.Leu353Pro), in exon 3 of *NLRP3*, responsible for the pathology in all four families (144). CAPS are dominantly inherited in most cases. Familial history is often positive, although *de novo* mutations are regularly reported(145,146). Mosaicism has also been reported, especially in severe NOMID/CINCA form(147–150). Low-level somatic and germinal mosaicism, barely detectable with Sanger classic method, could be the mechanism responsible for some of the *de novo* transmission previously reported(151) and false negative CAPS-like patients.

Clinical diagnosis

The CAPS spectrum

The term “spectrum” is used because the clinical manifestations of CAPS are a continuum with overlapping phenotypic features. Indeed, CAPS patients share clinical features such as recurrent fever, joint pain, and skin lesions (urticaria-like rash).

The disease usually occurs during pediatric ages (mostly before the 2nd year of life). The biological markers such as CRP (C-reactive protein), SAA (serum amyloid A) are raised, revealing an inflammatory process. Some of the patients do not exhibit fever during flares, a recent study on 136 European CAPS patients showed that the fever occurs in 84% of them(152).

In this series, 86% of the 136 patients suffered from musculoskeletal involvement such as arthralgia or myalgia(152).

The urticarial lesions are not pruritic, but patients describe a warmth sensation, and appear on the trunk and limbs. The lesions include erythema, papules and plaques, and rash severity tends to

increase in the evening(153). They usually are migratory and last less than 24 hours(154). The urticarial rash associated with CAPS differs from conventional urticaria. Histological examination shows a neutrophilic infiltrate in the eccrine adventice. Therefore, "neutrophilic urticaria dermatosis" (NUD) is a more relevant wording for these manifestations(155).

Apart from these common features, the three CAPS main forms: FCAS, MWS and CINCA/NOMID can be differentiated on the basis of their organ manifestations.

Familial cold autoinflammatory syndrome

FCAS is the mildest CAPS phenotype, and was first reported in 1940(156). This entity is defined by recurrent, cold-induced episodes of inflammation with low grade fever, urticaria-like rash, arthralgia, and conjunctivitis. The patients may also present with nausea, drowsiness, extreme thirst, sweating and headaches. Other clinical features such as lymphadenopathies, periorbital oedema and serositis have not been reported(157). The flares are short, they can last 12 to 24h, and appear 1 or 2 hours after cold exposure(158,159). Family history is usually positive, and the onset can be variable, although the majority of the patients develop their symptoms before 6 months of age(160). The complications encountered in other CAPS syndromes are rare in FCAS patients. Especially, very few cases of amyloidosis (less than 2%) or neurological involvement have been reported.

Muckle-Wells syndrome

MWS is the intermediate phenotype and was first described in 1962 by Muckle and Wells(161). It is characterized by flares with urticaria-like rash, fever, joint pain, headaches, and conjunctivitis. They are cold-induced and can last 1-3 days. Fatigue, exercise or stress can also trigger the flares. MWS onset is variable, although the first symptoms usually occur in the first year of age. Sensorineural impairments such as hearing-loss(162) can develop, due to chronic inflammation in the cochlea(163). Bilateral sensorineural hearing loss has been described in up to 85% of patients with MWS. Although it is progressive, deafness as early as the age of 10 is not rare(164). Secondary amyloidosis is the main complication of this pathology, which can develop in 25% of the patients(165), and can result in chronic renal insufficiency. Ocular manifestations such as conjunctivitis, uveitis, episcleritis, papillary oedema or visual loss can be encountered in these patients(166,167).

Chronic infantile neurological cutaneous articular syndrome/Neonatal onset multisystem autoinflammatory syndrome

CINCA/NOMID was first reported in 1981(168) and is characterized by neonatal onset of cutaneous symptoms along with end-organ damage (169). These include the “triad” of arthropathy, chronic urticaria, and central nervous system (CNS) involvement. CNS manifestations range from aseptic meningitis to increased intracranial pressure and can result in cognitive impairment. Headaches, seizures and strokes have also been described(169–171). Most of the cases present with no family history (50% to 60% of the patients(146,172)). *De novo* mutations are responsible for a large subset of CINCA/NOMID patients. These patients have sensorial impairment and can develop progressive hearing and vision loss. Perceptive deafness of various degrees is found in 75% of the patients with CINCA/NOMID and occurs sooner than in MWS patients, being usually clinically detectable in the first decade of age(169). Bone and joint deformities, leading to short stature, can be found in patients, although the real frequency of this complication seems to be lower than previously described(173,174). Renal amyloidosis appears to be rare in these patients, although some cases have been described in European patients(175). CINCA/NOMID patients tend to have increased mortality compared to other CAPS phenotypes(169).

Genetic diagnosis

Structure of the *NLRP3* gene

The *NLRP3* gene is located on chromosome 1 (1q44). Its implication with FCAS and MWS was discovered in 2001 by Hoffman et al.(126).

This gene is expressed in monocytes and macrophages, as in monocytes derived cells (chondrocytes, synoviocytes and osteocytes), but not in lymphocytes (176). Multiple alternatively spliced transcript variants encoding distinct isoforms have been identified, but the *NLRP3* transcript used as reference is NM_001243133.1.

The spectrum of *NLRP3* mutations in CAPS

To date, almost all the disease-causing mutations (Figure 2) have been found in the region encoding the NBD domain (in exon 3) and its flanking structures. They are collected in a dedicated online database, Infevers (<http://fmf.igh.cnrs.fr/ISSAID/infevers/>) (177–179). Neven et al. demonstrated through tridimensional modeling that several mutations located in exon 3 could affect nucleotide binding, nucleotide hydrolysis or protein oligomerization, leading to NLRP3

inflammasome dysfunction(175). A few mutations have been described in other exons, such as exon 4 or 6, encoding the leucine-rich repeat, but their consequences remain unclear(180,181).

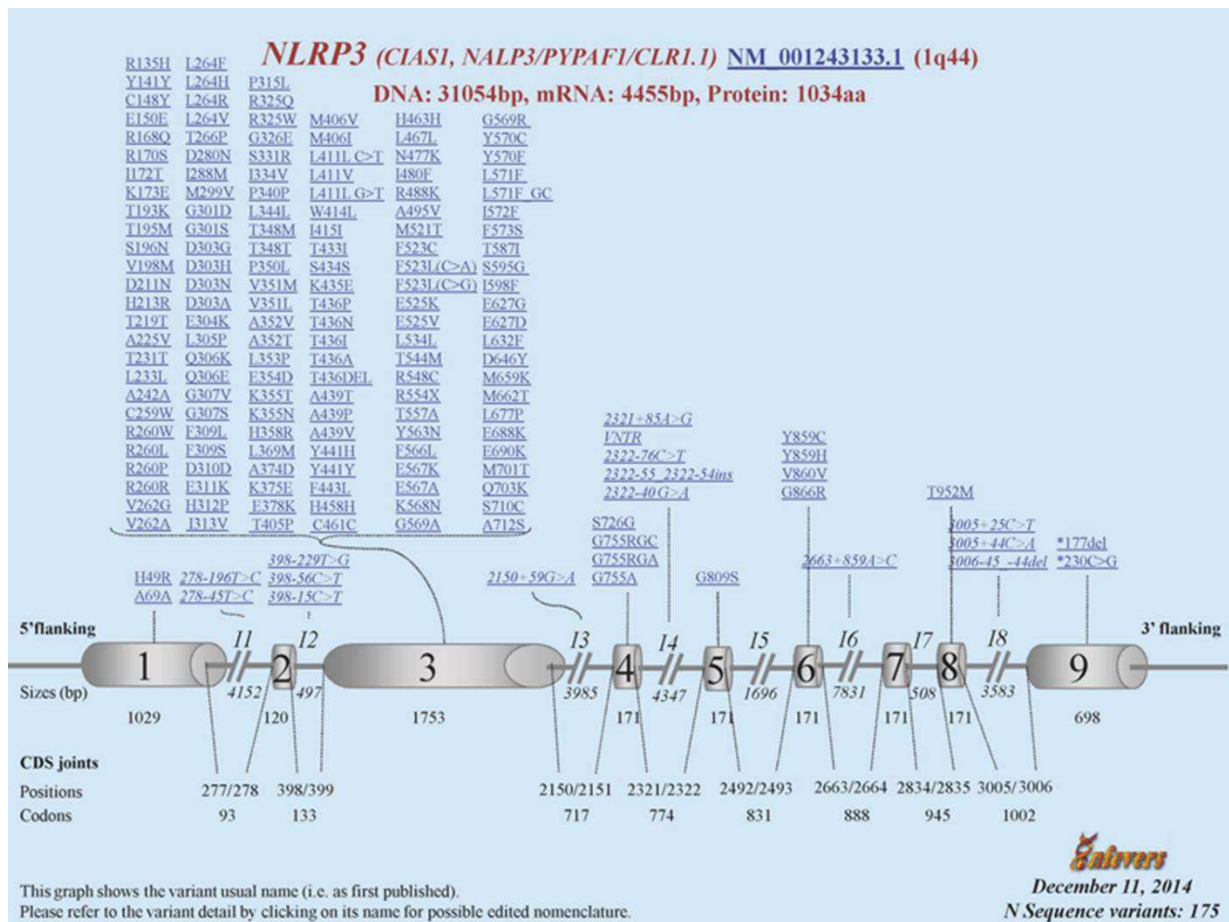


Figure 2: NLRP3 mutation spectrum

The *NLRP3* gene with all the mutations as reported in *InFever: an online database for autoinflammatory mutations*. Copyright. Available at <http://fmf.igh.cnrs.fr/ISSAID/infevers/> (accessed December 11th 2015).

Mostly all of these variants result in amino-acid change. One point deletion (p.Thr436del), and one nonsense mutation (p.Arg554*) have also been reported. Interestingly, the latter was associated with an unusual FMF-like phenotype, but triggered by cold-exposure(182). Since missense mutations impact is more difficult to assess than the nonsense mutations or the frameshifts, *in vitro* studies should be performed to prove their pathogenicity. Nevertheless, *in silico* analysis and familial segregation may help for the clinical interpretation.

Clearly pathogenic variants

The best known clearly pathogenic mutations have been listed in recent guidelines (183). The variants p.Arg260Trp, p.Asp303Asn, p.Leu305Pro, p.Asp311Lys, p.Thr348Met, p.Leu353Pro, and p.Ala439Val are all located in exon 3, and their link with CAPS phenotype is well established.

Controversial variants

Patients with CAPS phenotype often exhibit low-penetrance, controversial mutations, such as p.Val198Met or p.Gln703Lys. These VUS (variants of unknown significance) have been considered either causative variants or benign polymorphisms, depending on the studies.

In 2012, Vitale et al. followed a cohort of seven patients carrying the p.Gln703Lys variant, among 71 CAPS patients(184). They could show that all of the p.Gln703Lys carriers exhibited FCAS-like symptoms, as compared to the others, suggesting that this mutation was involved in the phenotype. They did not exclude however possible genetic and/or environmental modulator factors. The same year, *in vitro* assays could confirm a gain-of function effect of p.Gln703Lys on the NLRP3 inflammasome, leading to increased production of IL-1 β (185). However, given its high frequency (ranging from 1.16 to 4.67% according to Exome Server Project), p.Gln703Lys cannot be solely responsible for a CAPS phenotype.

The variant p.Val198Met has a frequency ranging from 0.16 to 0.95%, which approaches the classical definition of polymorphism. However, some studies established that this mutation was associated with classical CAPS phenotypes, atypical autoinflammatory disease, and even Schnitzler syndrome(186).

Some of the most common controversial *NLRP3* variants have been also associated to the adult-onset of the mildest CAPS phenotype, FCAS. In these controversial cases the presence of a family history positive for FCAS symptoms may support the diagnosis(187). In this paper, a Caucasian mother and her daughter with FCAS both carried the low-penetrance p.Val198Met mutation and displayed the onset of symptoms during adulthood, with clinical manifestations triggered by cold exposure). Low-penetrance mutations are also associated with oligosymptomatic disease presentation(188)

In a recent study, the release of mature IL-1 β , IL-18, and caspase-1 in monocytes was compared between CAPS patients carrying pathogenic (n=17) or controversial mutations (p.Val198Met or p.Gln703Lys) (n=11), and from healthy controls(189). While the patients with pathogenic mutations exhibited increased production of IL-1 β , IL-18 and caspase-1, the patients with either

one of the two VUS showed similar levels than the healthy controls. These results suggest alternative pathophysiological mechanisms for the two low-penetrance variants.

Mosaicism

A first evidence of pathogenic somatic mosaicism in *NLRP3* was revealed in 2005 by Saito et al. (190). In this study, the authors described a CINCA/NOMID patient who exhibited a somatic mutation p.Tyr570Cys found by Sanger analysis. Using subcloning of exon 3, they confirmed that this variant occurred at low frequency (16.7% in whole blood). Functional analysis revealed that the patient's PBMCs (peripheral blood mononuclear cells) produced increased levels of IL-1 β in the absence of LPS stimulation.

Soon after, this unexpected result was confirmed by a international case-control study in CINCA/NOMID patients who had no mutation through conventional sequencing (they represented about 40% of the series)(150). In this work, the authors could show mosaicism in up to 62% of these patients, ranging from 4.2 to 35.8% in whole blood.

In another study, the authors performed whole-exome sequencing on DNA from a CINCA/NOMID patient, showing a somatic mutation with a frequency of 17.7%, confirmed by another massive parallel sequencing method, with a 14.5% frequency(191).

A more recent paper reported for the first time a vertical transmission of a somatic *NLRP3* mutation(151). The asymptomatic mother of a CAPS patient exhibited a variant with a very low frequency in different tissues (ranging from 2.4 to 8.2%), disproving a possible *de novo* event.

Finally, a recent paper showed the implication of a somatic mutation in MWS, suggesting that mosaicism could be involved with all CAPS phenotypes(148). Somatic *NLRP3 mutations* were detected in 12.5% of the patients with variable degree of mosaicism (5.5 to 34.9%). These variants were furthermore confirmed by *in silico* and functional analysis.

All together, these results highlight the growing impact of NGS (Next Generation Sequencing) strategy over conventional sequencing, especially in “mutation-negative” CINCA/NOMID patients.

Expert commentary

Genotype/phenotype correlations?

The discrepant literature data on genotype/phenotype correlations in CAPS underlies marked phenotypic variability(145). Variants found in patients with mild forms of CAPS have not been identified in severely affected patients and vice versa. Aksentijevich et al. showed that the variants p.Leu353Pro and p.Leu305Pro are often associated with FCAS, while p.Phe309Ser and p.Tyr570Cys tend to be associated with more severe phenotypes. Conversely, mutations implicating amino acid p.Ala439, p.Arg260 and p.Asp303 result in CAPS phenotype of variable severity, suggesting that genetic or environmental factors may modulate the phenotypic expression of the disease(175,192).

Recommendations

A committee gathering recognized geneticist and clinician experts has worked on guidelines for the interpretation of hereditary recurrent fever gene variants. An agreed set of practice guidelines was proposed by Shinar et al. for genetic diagnosis, reporting of results, and their clinical interpretation(183). Cuisset et al. suggested that genetic diagnosis of CAPS should be first aimed at patients with recurrent flares, with biologically proven inflammation, occurring before the age of 20(143).

Overdiagnosis and overtreatment are increasingly observed, and are often due to misinterpretation of genetic analysis. A good response to IL-1 inhibition (184) may support the pathogenic role of the variant.

Differential diagnosis

Familial cold autoinflammatory syndrome 2

Patients with CAPS like symptoms may present variant in a gene other than *NLRP3*. In particular, Jeru et al. described in 2007 two families with periodic fever syndromes, lasting 2-10 days, with urticaria, arthralgia and myalgia(193). The episodes were triggered by cold exposure, and neurosensorial loss was even observed in two patients further mimicking CAPS. No causative mutations were found in *NLRP3*, neither in the other known autoinflammatory genes. The authors analyzed the other NLRP family genes, and brought out pathological variants in *NLRP12*. Further studies showed other mutations in this gene, responsible for FCAS2(194). However, *NLRP12* variants such as p.Phe402Leu should be interpreted with caution(194–196). Treatment is based on corticosteroids and antihistamines, but IL1- β blockade becomes inefficient after a few weeks of use(197).

Schnitzler syndrome

The Schnitzler syndrome is a rare, autoinflammatory disease described in 1977 by a French dermatologist, Liliane Schnitzler(198). This is a late-onset pathology (51 years of age), with a slight male predominance(199). It is characterized by an urticaria-like skin rash and production of monoclonal IgM. Other signs can be associated, such as lymphadenopathies, spleen and/or liver enlargement, arthralgia and myalgia. Inflammatory biological syndrome is often seen. The skin-rash is actually a neutrophilic urticaria dermatosis, the same as encountered in CAPS phenotype. The complications are inflammatory anemia, renal amyloidosis, and 20% of the patients can present with lymphoproliferative disorder, such as Waldenström disease, or lymphoma. IL-1 blockade is very efficient on Schnitzler patients. The pivotal role of IL-1 and the similarities between Schnitzler syndrome and CAPS have led de Koning et al. to analyze *NLRP3* implication(200). Using NGS, the authors found somatic mutations in this gene in two patients with Schnitzler syndrome, only in the myeloid lineage cells(200). These preliminary results need to be reproduced but the authors suggest that the patients with Schnitzler syndrome and *NLRP3* somatic mutation should be added to the CAPS spectrum.

CAPS and NLRC4

In 2014, Kitamura et al. performed exome analysis in patients from a Japanese family, who exhibited cold-induced urticaria and arthralgia, but without any causative mutation in *NLRP3* or *NLRP12*(201). Interestingly, they found a *NLRC4* variant, with strong evidences for its pathological involvement in the phenotype observed. Thus the authors suggest performing *NLRC4* analysis for CAPS patients without causative mutation in *NLRP3*.

Five-year view

Evolution of CAPS diagnosis

Classical Sanger sequencing approach remains the most used technique, because of the mutational hot-spot in exon 3. The benefits are the reliability, as it is considered to be the gold standard approach, and the cheapest; though, it is time consuming. However, low-level mosaicism can be difficult to detect by Sanger sequencing, which can be quite a limitation because of the growing evidence of this molecular mechanism in CAPS patients, especially CINCA/NOMID.

NGS has allowed a drastic increase in available sequence data. Targeted gene panels or full-exome can thus be performed. An informal international network demonstrated successful use of these novel strategies for the genetic diagnosis of autoinflammatory diseases in general. Targeted

approaches (amplicon or exon-capture based) are well fitted for routine settings as they allow parallel screening of multiple genes, making easier the identification of the causal mutation in atypical patients with overlapping phenotypes. On the other side, exomes are less suitable for routine (to date), but they will grant better knowledge of autoinflammatory genes and will permit to identify other genes involved in CAPS-like phenotypes. Moreover, both of these approaches remain interesting for CAPS patients as deep sequencing is particularly suitable for low-mosaicism detection.

NLRP3 in other multifactorial diseases

Many DAMPs, such as ATP, monosodium urate, asbestos, amyloid, silica, alum, cholesterol, and glucose can activate the NLRP3 inflammasome. Since these molecules are extensively implicated in many multifactorial diseases, it has been suggested that the NLRP3 inflammasome has a potential role in the progression of these diseases (202).

Recent studies showed many associations between *NLRP3* variants and multifactorial diseases. Some authors suggest a link between NLRP3 activation and Alzheimer disease(203,204), or Behçet disease(205).

Crystalline arthropathies, such as acute gouty arthritis and pseudo gout are induced by deposits in joints of monosodium urate crystals, and calcium pyrophosphate crystals respectively. Some studies demonstrated that these crystals could activate NLRP3 inflammasome(206). Anti IL-1 therapies were successfully used to treat these patients, validating this pathophysiological mechanism.

NLRP3 inflammasome could also be linked to type 2 diabetes mellitus, another very common metabolic disorder: anakinra (an IL-1 blockade) treatment in these patients showed improvement of β -cells function and a better glycemic control(207).

A study showed a refractory autoinflammatory syndrome caused by a digenic process implicating low-penetrance variants in *TNFRSF1A* and *NLRP3*(208).

All these associations between NLRP3 inflammasome and very frequent diseases highlight the pivotal role of NLRP3 in the pathophysiology of inflammatory diseases. It underscores the growing importance of better understanding the NLRP3 gene and function, to develop new targeted therapeutics.

Key issues:

NLRP3 (NOD-like receptor family, pyrin domain containing protein 3) inflammasome is a multimolecular complex involved in PAMPS (pathogen-associated molecular patterns) and DAMPS (danger-associated molecular patterns) recognition and leading to IL-1 β secretion, and pyroptosis.

The CAPS (Cryopyrin associated periodic syndrome) spectrum encompasses 3 overlapping phenotypes with common features such as urticarial skin-rash, arthralgia: FCAS (Familial cold autoinflammatory syndrome), MWS (Muckle-Wells syndrome) and CINCA/NOMID (Chronic Infantile Neurological Cutaneous Articular Syndrome/Neonatal Onset Multisystem Autoinflammatory syndrome).

MWS major risk is renal amyloidosis, while CINCA/NOMID patients have a high mortality rate due to severe neurologic impairment.

To date, IL-1 blockade is the most efficient treatment for CAPS patients.

Mostly all *NLRP3* mutations are substitutions located in exon 3, encoding the NBD (nucleotide binding domain).

No *NLRP3* mutation is found in 40% of the CINCA/NOMID patients. Up to 60% of the latter patients present with pathological somatic *NLRP3* mosaicism.

FCAS2 is a CAPS-like phenotype caused by *NLRP12* mutations, but IL-1 blockade is ineffective.

Schnitzler syndrome patients present with somatic *NLRP3* mosaicism.

Several studies show implication of NLRP3 in multifactorial diseases (crystalline arthropathies, Alzheimer disease, Type 2 diabetes mellitus...).

RESULTATS

5

Evaluation of targeted next-generation versus Sanger sequencing in autoinflammatory diseases: diagnostic contribution and clinical impact

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ABSTRACT

The aim of this study was to compare the effectiveness of the gene-panel next-generation sequencing (NGS) strategy versus the gold-standard Sanger sequencing for the genetic diagnosis of autoinflammatory diseases (AIDs). Secondary goals were to describe the gene and mutation distribution in AID patients and to evaluate the impact of the genetic report on the patient's medical care and treatment. Patients with AID symptoms were enrolled prospectively and randomized to 2 arms, NGS (n=99) and Sanger sequencing (n=197). Genotypes were classified as "consistent/confirmatory", "uncertain significance" or "non-contributory". The proportion of patients with pathogenic genotypes concordant with the AID phenotype (consistent/confirmatory) was significantly higher with NGS than Sanger sequencing (10/99 [10.1%] vs 8/197 [4.1%]). *MEFV*, *ADA2* and *MVK* were the most represented genes with a consistent/confirmed genotype, whereas *MEFV*, *NLRP3*, *NOD2* and *TNFRSF1A* were found in the "uncertain significance" genotypes. Six months after the genetic report was sent, 54/128 (42.2%) patients had received effective treatment for their symptoms; 13/128 (10.2%) had started treatment after the genetic study. For 59/128 (46%) patients, the results had an impact on their overall care, independent of sequencing group and AID diagnosis. Targeted NGS improved the diagnosis and global care of patients with AIDs.

KEYWORDS

Hereditary autoinflammatory diseases, targeted next-generation sequencing, Sanger sequencing, genetic testing.

INTRODUCTION

Systemic autoinflammatory diseases (AIDs) are a group of disorders characterized by deregulation of the innate immune system resulting in excessive inflammation. The inflammatory attacks are mediated by neutrophils and monocytes and by cellular signalling pathways involving mostly interleukin 1 (IL-1) and IL-6 but also tumour necrosis factor alpha (TNF- α), interferon type 1 (IFN-1) or the NF- κ b transcription factor.

AIDs are commonly distinguished from autoimmune diseases because they lack pathogenic autoantibodies, autoreactive T lymphocytes. The disease course depends on the gene and the diagnosis: patients affected by AIDs present continuous or recurrent elevated acute-phase reactants. Manifestations usually begin in early infancy or adolescence. Typical AID patients manifest fever, arthritis, skin rash and serositis (pleura, peritoneum, pericardium). Clinical signs and severity are extremely variable. These variations probably reflect the large range of possible causative AID genes and the signalling pathway. The large clinical spectrum contributes to misdiagnoses. Monogenic hereditary AIDs are considered rare diseases when it affects less than 1 person per 2000 (http://www.orpha.net/consor/cgi-bin/Education_AboutRareDiseases.php?lng=FR).

The year 1997 was a milestone in our knowledge of the AID pathophysiology. The first gene involved in AIDs was discovered by 2 consortia 21 years ago (209). *Mediterranean fever (MEFV)* was identified in families who shared the same familial Mediterranean fever (FMF) phenotype. FMF is the most common AID and affects almost exclusively people with Mediterranean ancestries (Armenian, Turkish, Sephardic Jew, Maghrebian etc.). The disease usually starts during childhood and is characterized by recurrent fever, arthritis, cutaneous rash and biological inflammation during attacks. Patients with FMF respond favourably to colchicine. However, lack of anti-inflammatory treatment for several years may be life threatening because of the risk of amyloid A amyloidosis, resulting in chronic end-stage renal failure.

Three other hereditary recurrent fevers are well described. Cryopyrin-associated periodic syndromes (CAPSs) are caused by dominant mutations in *NLR family pyrin domain containing 3 (NLRP3)* and characterized by deregulation of IL-1 activity. CAPSs include familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and chronic infantile neurologic, cutaneous, articular (CINCA) syndrome, also known as neonatal-onset multisystem inflammatory disease (NOMID). The three diseases have in common recurrent fever, joint

involvement, urticaria and neurosensory impairment, with a wide range of symptoms, from mild (FCAS) to most severe (CINCA).

Dominant mutations in *TNF receptor superfamily member 1A (TNFRSF1A)* are responsible for TNF receptor-associated syndrome (TRAPS). Patients manifest febrile episodes lasting from a few days to a few weeks, joint involvement, myalgia, skin lesions and serositis.

Finally, mevalonate kinase deficiency (MKD) is caused by recessive mutations in *mevalonate kinase (MVK)* and results in fever, vomiting, diarrhoea, and skin rash, most often with a neonatal onset. In its most severe form, mevalonic aciduria (MA), neurological complications are frequently observed.

Since 1997, the list of genes involved in AIDs has been growing significantly (210). More than 40 genes are now identified as responsible for AIDs (211,212).

Autoinflammatory medical advances are due in part to progress in genetic research and diagnosis. The discovery of new genes has highlighted new cellular signalling pathways involved in the inflammatory reaction (e.g., inflammasome, NF- κ B, IFN pathways). Understanding the physiology of AIDs leads to the development of new therapies. Genetic testing is essential for AID diagnosis and then initiating appropriate treatment. Today, a large number of undiagnosed patients receive non-specific anti-inflammatory therapies such as corticosteroids or immunomodulation therapy. In addition, mutational screening may not be exhaustive if it focuses on a limited number of known genes and/or mutational hotspots. Indeed, regardless of the sequencing method used, genetic tests are inconclusive for 50% to 75% of patients with clinical criteria of AIDs (213,214).

A positive diagnosis depends on the patient's phenotype, the gene-sequencing strategy and the sensitivity of the test (allowing or not the detection of low-level mosaicism, for example), and the classification used for variant pathogenicity. The development of new sequencing approaches, such as next-generation sequencing (NGS), has allowed for investigating many different genes simultaneously.

To improve the performance of molecular diagnoses of AIDs, our laboratory has elaborated a strategy to simultaneously screen a panel of AID genes. The main objective of this work was to formally evaluate, in a large cohort of patients with clinical suspicion of an AID, the potential added value of systematic NGS of a panel of genes over Sanger sequencing for genes requested on the basis of clinical criteria by the prescribing physician. Therefore, patients with initial NGS request were not included in this study because testing for gene panels is generally requested when the patient's clinical picture does not point to a specific gene. Our 2 secondary analyses were 2) to

evaluate the distribution of genes and mutations in these patients and 3) to compare, for both sequencing approaches, the impact of the genetic report on the patient's medical care and treatment.

MATERIALS AND METHODS

Consent

This prospective study received full ethical approval from the regional committee for the protection of persons (no. ID-RCB: 2015-A00362-47) and was registered at ClinicalTrials.gov (ID: NCT02976948). Each patient (or legal guardian if relevant) was fully informed and gave written consent for DNA analysis and participation in the study. With clinicians of reference centres for AIDs, we had developed a clinical form common for all AIDs to be provided with genetic diagnosis requests. This form collects epidemiological data and includes a list of clinical symptoms and biological markers frequently encountered in AIDs (Figure S1).

Study design

We prospectively recruited consecutive patients referred to our laboratories for genetic diagnosis of AID. We did not select them for further specific clinical criteria and only checked whether they fulfilled the following inclusion criteria: symptoms of AIDs (more than 3 attacks, elevated C-reactive protein level, age of onset before 30 years old [French reference centre's criteria], request for routine Sanger genetic diagnosis (1 to 4 genes), clinical form correctly completed, and signed informed consent form to participate in the study. A reference centre physician approved each inclusion. We excluded patients with an inflammatory disease other than AIDs (neoplasia, autoimmune disease, infection, etc.), an initial request for targeted AID gene panel testing (NGS method), and ethnicity Armenian, Turkish, Sephardic, and Arabic when mentioned (to limit bias toward FMF patients).

The patients fulfilling the inclusion criteria were randomized to 2 groups by using Clinsight (Ennov, Paris, France). Randomization was performed using a 1:2 ratio, blocks of variable size, and stratified on the age of onset and sex of the patients. The random allocation sequences were computer-generated and were implemented by a data-manager who was not otherwise involved in the trial. Patients of the first group had a genetic test as requested by the clinician, and patients of

the second group benefited from NGS sequencing with a high-throughput–targeted AID gene panel including the genes requested.

Clinicians were contacted by email 6 months after we sent the genetic report for their patient(s). They had to complete an online form to evaluate the patient’s treatment and its efficiency before and after the genetic test, the effect of the genetic report on the medical care of their patient, and the clinician’s perception of the genetic diagnosis (Figure S2). We sent reminder e-mails the next month if necessary. The study design is summarized in Figure 1.

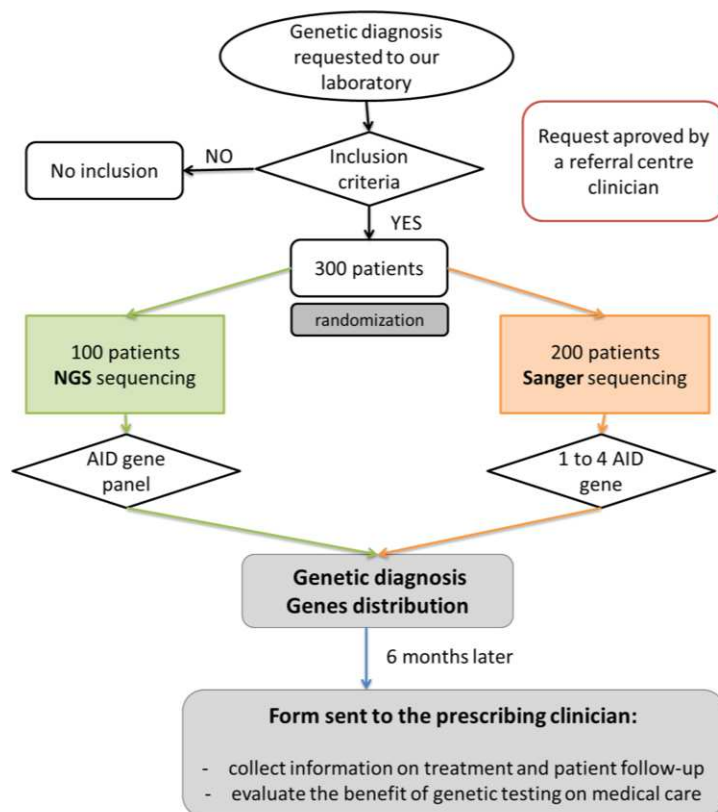


Figure 1. Design of the study.

Genetic analysis

Sanger sequencing

Two different amplicons were generated for each exon to circumvent the risk of allele dropout. Coding exons and exon–intron junctions were sequenced in both directions by using ABI PRISM Big Dye Terminator v3.1, the Ready-Reaction Cycle-Sequencing kit and ABI 3130 XL (Applied Biosystems). Primer sequences used for Sanger sequencing are available on request.

Targeted AID gene panel NGS

Our targeted panel contains genes involved in autoinflammatory syndromes. Illumina Design Studio was used to design a panel targeting 32 genes (<https://www.illumina.com/informatics/sample-experiment-management/custom-assay-design.html>). An updated version was available on in June 28, 2016, because novel genes were described, which resulted in a final list of 55 AID genes (Table S1). For this second design, we used the Agilent Online Sure Design tool (<https://earray.chem.agilent.com/suredesign/>). Coding regions, plus 25 bp flanking each exon, were included in each design. Libraries were prepared by using SureSelect Target Enrichment Capture custom kits (Agilent). Sequencing reactions were run on MiSeq or NextSeq500 equipment (Illumina). Point mutations were systematically confirmed by Sanger sequencing and copy number variations were investigated by quantitative PCR.

Quantitative PCR (qPCR)

When relevant (e.g., when a single clearly pathogenic variant was detected in recessive disease or when we suspected a large deletion/duplication by NGS), each coding exon was analysed by real-time qPCR with a LightCycler 480 thermocycler (Roche).

Segregation analysis:

Phasing was performed when DNA from family members was available, after obtaining informed consent.

Bioinformatics analysis and pathogenicity assessment of identified variants

We paid close attention to the coverage data: for each sample, we checked the Phred quality score, total number of reads, percentage of reads mapped to the target region, percentage of bases in a specific region, thresholds for coverage, and distribution of forward and reverse strand reads, by using SeqNext (JSI medical system <http://www.jsi-medisys.de/products/seqnext>). Coverage was assessed for each sample. The cut-off for data re-analysis was coverage < 90% at 40X.

Variants were analysed by using SeqScape (Applied Biosystem), SeqNext (JSI) and standard *in silico* tools. These latter tools included the Alamut software pipeline (Interactive Biosoftware) for missense mutations (GVGD, SIFT, Polyphen2, MutationTaster, Grantham score) and

MaxEntScan (MES), Human splice finder (HSF), and neural network splice (NNSplice) for splice variant. Minor allele frequency was obtained by using the allele frequency database browsers 1000 Genomes Project and GnomAD/ ExAC.

The identified variants were individually assessed and classified into pathogenicity groups by American College of Molecular Genetics guidelines (215): Class 1, clearly not pathogenic; Class 2, unlikely to be pathogenic; Class 3, uncertain significance; Class 4, likely to be pathogenic; Class 5, clearly pathogenic.

When possible, pathogenicity was retrieved from a reputable source (e.g., guidelines for hereditary recurrent fevers(216)) or from the autoinflammatory-specific locus database Infevers (217). The description of variants conforms to Human Genome Variant Society recommendations (218).

The genetic diagnosis was considered “confirmatory” or “consistent” if we detected one class 4 or 5 variant for dominant monogenic hereditary diseases; two paired class 4 or 5 variants for recessive monogenic hereditary diseases; or genotype/phenotype concordance. It was considered “uncertain” with detection of single or multiple sequence VUS only or heterozygous mutations for autosomal recessive disorders. Otherwise, it was considered “non-contributory” in all other cases.

Statistical tests

Sample size calculation

We expected to identify a mutation for 25% of patients with our new genetic diagnostic strategy and for 10% with the current strategy. The minimal number of participants required to demonstrate such a difference with power of 90%, a bilateral alpha risk of 5% and randomization performed according to a ratio of 1/2 (1 NGS/2 conventional Sanger method) was 300.

Comparison of the 2 sequencing strategies

We used chi-square test or Fisher’s exact test when expected cell frequencies was less than 5 to compare the 2 sequencing strategies in terms of age classes, sex ratio, percentage of genetic diagnosis and patient care after genetic results were received. $P < 0.05$ was considered statistically significant. We used SAS version 9.1 (SAS Institute, Cary, North Carolina) for statistical analysis.

RESULTS

A total of 300 patients were enrolled in the study from September 29, 2015 to December 28, 2017. Four patients were excluded after randomization (genetic testing cancelled by the ordering physician or exclusion criteria met after randomization). Among the remaining 296 participants, 197 were randomized to the Sanger sequencing strategy and 99 to the NGS panel strategy (Figure 2).

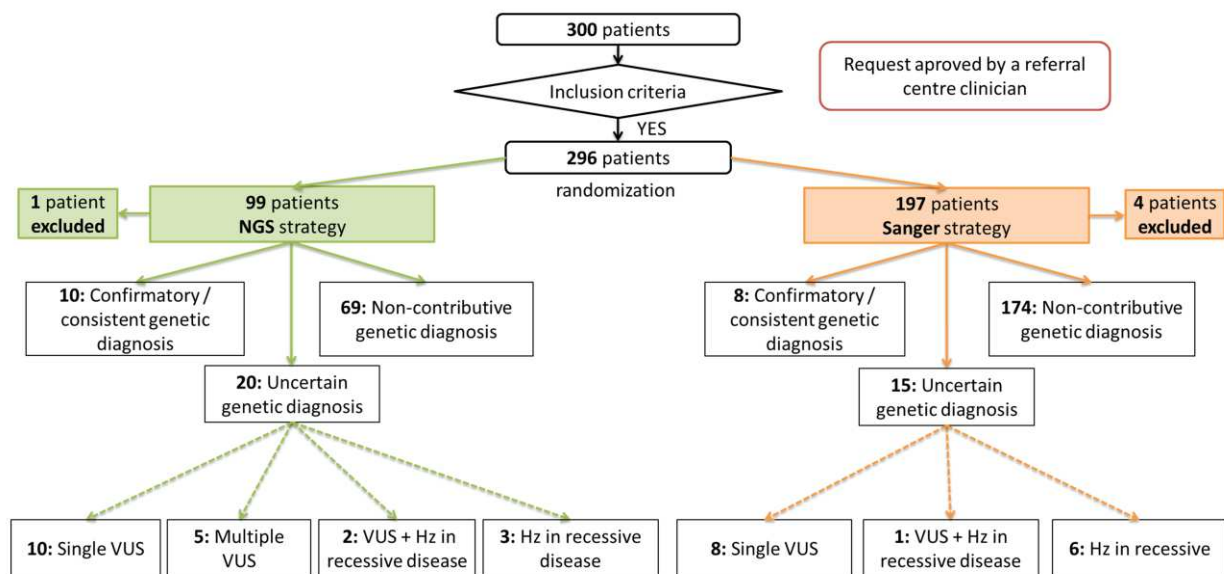


Figure 2. Flow chart of participants in the study.

NGS, next generation sequencing; VUS, variant of uncertain significance; Hz: heterozygous variant.

Diagnostic yield: NGS versus Sanger sequencing for a given clinical suspicion

The genotypes were confirmatory or consistent in 18/296 (6%) patients overall (Table 1). With the Sanger strategy, 8/197 (4.1%) patients had a confirmatory or consistent genotype (Figure 3A). With the NGS strategy, 10/99 (10.1%) had a confirmatory or consistent genotype (Figure 3B). The initial Sanger prescription was consistent with the final diagnosis in 6/10 NGS strategy patients with a confirmatory or consistent genotype. These diagnostic yields significantly differed between the 2 groups ($p=0.040$). Overall, 35/296 (11.8%) patients had an “uncertain significance” genotype, 15/197 (7.6%) with the Sanger strategy and 20/99 (20.2%) with the NGS strategy (Figure 2, Table 2). As well, 53/296 (17.9%) patients had at least one variant identified in the AID genes, more patients with the NGS than Sanger strategy: 30/99 (30.3%) and 23/197 (11.6%) ($p\leq$

0.001). Clinical signs of patients with a confirmatory or consistent genotype and an uncertain significance genotype are available in supplemental tables (Tables S2 and S3).

Table 1: Variants and genotypes of patients with consistent or confirmatory genotype of autoinflammatory diseases (AIDs) by sequencing strategy: Sanger or next-generation sequencing (NGS) (n=18).

ID	Gene	NM refseq	Mutation c.	Mutation p.	rs ID	MAF (gnomAD)	Genotype p.
Patients							
Sanger strategy							
18	<i>NLRP3</i>	NM_001243133.1	c.2107C>A	p.(Gln703Lys)	rs35829419	0.03861	p.[(Gln703Lys)]; [Gln703=]
	<i>TNFRSF1A</i>	NM_001065.3	c.361C>T	p.(Arg121Trp)	rs104895276	NA	p.[(Arg121Trp)]; [Arg121=]
20	<i>MVK</i>	NM_000431.3	c.709A>T	p.(Thr237Ser)	rs104895366	1.804e-5	p.[(Thr237Ser)];[(Val377Ile)]
	<i>MVK</i>	NM_000431.3	c.1129G>A	p.(Val377Ile)	rs28934897	0.001591	
23	<i>MEFV</i>	NM_000243.2	c.442G>C	p.(Glu148Gln)	rs3743930	0.06564	p.[(Glu148Gln)];[(Met694Ile)]
	<i>MEFV</i>	NM_000243.2	c.2082G>A	p.(Met694Ile)	rs28940578	0.0001263	
27	<i>ADA2</i>	NM_001282225.1	c.144del	p.(Arg49Glyfs*4)	rs199614299	3.25e-5	p.[(Arg49Glyfs*4)];[(Gly450Cys)]
	<i>ADA2</i>	NM_001282225.1	c.1348G>T	p.(Gly450Cys)	NA	1.218e-5	
50	<i>MVK</i>	NM_000431.3	c.709A>T	p.(Thr237Ser)	rs104895366	1.804e-5	p.[(Thr237Ser)];[(Val377Ile)]
	<i>MVK</i>	NM_000431.3	c.1129G>A	p.(Val377Ile)	rs28934897	0.001591	
59	<i>MEFV</i>	NM_000243.2	c.1772T>C	p.(Ile591Thr)	rs11466045	0.01082	p.[(Ile591Thr)];[(Met694Val)]
	<i>MEFV</i>	NM_000243.2	c.2080A>G	p.(Met694Val)	rs61752717	0.0002669	
242	<i>MEFV</i>	NM_000243.2	c.2040G>C	p.(Met680Ile)	rs28940580	0.0001056	p.[(Met680Ile)];[(Glu148Gln)]

	<i>MEFV</i>	NM_000243.2	c.442G>C	p.(Glu148Gln)	rs3743930	0.06564	
288	<i>NOD2</i>	NM_022162.1	c.1759C>T	p.(Arg587Cys)	rs104895479	3.231e-5	p.[(Arg587Cys)]; [Arg587=]
NGS strategy							
Patients	Gene	NM RefSeq	mutation c.	mutation p.	rs ID	MAF (gnomAD)	Genotype p.
54	<i>NLRC4</i>	NM_021209.4	c.188_189insT	p.(Glu64Argfs*4)	NA	NA	p.[(Glu64Argfs*4)]; [Glu64=]
63	<i>MVK</i>	NM_000431.3	c.37A>C	p.(Lys13Gln)	NA	NA	p.[(Lys13Gln)]; [(Val377Ile)]
	<i>MVK</i>	NM_000431.3	c.1129G>A	p.(Val377Ile)	rs28934897	0.001591	
66	<i>MEFV</i>	NM_000243.2	c.1105C>T	p.(Pro369Ser)	rs11466023	0.01471	p.[(Pro369Ser)]; [(Pro369Ser)]
	<i>MEFV</i>	NM_000243.2	c.1105C>T	p.(Pro369Ser)	rs11466023	0.01471	
83	<i>MEFV</i>	NM_000243.2	c.442G>C	p.(Glu148Gln)	rs3743930	0.06564	p.[(Glu148Gln)]; [(Met694Ile)]
	<i>MEFV</i>	NM_000243.2	c.2082G>A	p.(Met694Ile)	rs28940578	0.0001263	
108	<i>NLRC4</i>	NM_021209.4	c.530C>G	p.(Thr177Ser)	NA	NA	p.[(Thr177Ser)]; [Thr177=]
112	<i>DDX58</i>	NM_014314.3	c.1723C>T	p.(Arg575*)	rs371404578	1.627e-5	p.[(Arg575*)]; [Arg575=]
141	<i>ADA2</i>	NM_001282225.1	c.427delA	p.(Ile143Serfs*41)	NA	NA	p.[(Ile143Serfs*41)]; [?]
	<i>ADA2</i>	NM_001282225.1	c.973-2A>G	SAV	rs139750129	0.0001266	
164	<i>PSTPIP1</i>	NM_003978.3	c.748G>C	p.(Glu250Gln)	rs28939089	NA	p.[(Glu250Gln)]; [Glu250=]
166	<i>TNFAIP3</i>	NM_006290.3	c.(?- 1)_(*1_?)del	HTZ deletion	NA	NA	p.[?]; [=]
203	<i>ADA2</i>	NM_001282225.1	c.476G>A	p.(Cys159Tyr)	rs774636844	4.061e-6	p.[(Cys159Tyr)]; [(Tyr453Cys)]
	<i>ADA2</i>	NM_001282225.1	c.1358A>G	p.(Tyr453Cys)	rs376785840	8.658e-5	

HTZ-MAR: Heterozygous mutation associated with autosomal recessive disorders; SAV: splice acceptor variant; NA: not available; MAF, minor allele frequency

Table 2: Variants and genotypes of patients with uncertain significance genotype (n=35).

ID patients	Gene	NM RefSeq	mutation c.	mutation p.	rs ID	MAF (gnomAD)	Genotype p.	Interpretation
Sanger strategy								
9	<i>NLRP3</i>	NM_001243133.1	c.592G>A	p.(Val198Met)	rs121908147	0.008460	p.[(Val198Met);Val198=]	VUS
70	<i>NLRP3</i>	NM_001243133.1	c.592G>A	p.(Val198Met)	rs121908147	0.008460	p.[(Val198Met);Val198=]	VUS
81	<i>TNFRSF1A</i>	NM_001065.3	c.362G>A	p.(Arg121Gln)	rs4149584	0.01290	p.[(Arg121Gln);Arg121=]	VUS
109	<i>ADA2</i>	NM_001282225.1	c.139G>A	p.(Gly47Arg)	rs202134424	2.437e-5	p.[(Gly47Arg);Gly47=]	HTZ-MAR
113	<i>MEFV</i>	NM_000243.2	c.2230G>T	p.(Ala744Ser)	rs61732874	0.001753	p.[(Ala744Ser);Ala744=]	HTZ-MAR
121	<i>NOD2</i>	NM_022162.1	c.1177C>T	p.(Arg393Cys)	rs140716236	0.0001479	p.[(Arg393Cys);Arg393=]	VUS
122	<i>TNFRSF1A</i>	NM_001065.3	c.362G>A	p.(Arg121Gln)	rs4149584	0.01290	p.[(Arg121Gln);Arg121=]	VUS
127	<i>MEFV</i>	NM_000243.2	c.1772T>C	p.(Ile591Thr)	rs11466045	0.01082	p.[(Ile591Thr);Ile591=]	VUS
136	<i>NOD2</i>	NM_022162.1	c.2883-2A>G	SAV	rs564226539	2.442e-5	NA	VUS
164	<i>MEFV</i>	NM_000243.2	c.2080A>G	p.(Met694Val)	rs61752717	0.0002669	p.[(Met694Val);Met694=]	HTZ-MAR
206	<i>MEFV</i>	NM_000243.2	c.1772T>C	p.(Ile591Thr)	rs11466045	0.01082	p.[(Ile591Thr);Ile591=]	VUS
232	<i>MEFV</i>	NM_000243.2	c.2084A>G	p.(Lys695Arg)	rs104895094	0.005894	p.[(Lys695Arg);Lys695=]	HTZ-MAR
	<i>NLRP3</i>	NM_001243133.1	c.2107C>A	p.(Gln703Lys)	rs35829419	0.03861	p.[(Gln703Lys);Gln703=]	VUS
233	<i>MEFV</i>	NM_000243.2	c.2084A>G	p.(Lys695Arg)	rs104895094	0.005894	p.[(Lys695Arg);Lys695=]	HTZ-MAR

237	<i>MEFV</i>	NM_000243.2	c.2080A>G	p.(Met694Val)	rs61752717	0.0002669	p.[(Met694Val);Met694=]	HTZ-MAR
275	<i>MEFV</i>	NM_000243.2	c.2080A>G	p.(Met694Val)	rs61752717	0.0002669	p.[(Met694Val);Met694=]	HTZ-MAR
NGS strategy								
5	<i>MEFV</i>	NM_000243.2	c.1105C>T	p.(Pro369Ser)	rs11466023	0.01471	p.[(Pro369Ser);Pro369=]	VUS
19	<i>NLRP3</i>	NM_001243133.1	c.592G>A	p.(Val198Met)	rs121908147	0.008460	p.[(Val198Met);Val198=]	VUS
31	<i>IL10RA</i>	NM_001558.3	c.883C>T	p.(Pro295Ser)	rs201777547	0.0001912	p.[(Pro295Ser);Pro295=]	HTZ-MAR
99	<i>NLRP3</i>	NM_001243133.1	c.592G>A	p.(Val198Met)	rs121908147	0.008460	p.[(Val198Met);Val198=]	VUS
105	<i>APIS3</i>	NM_001039569.1	c.318T>A	p.(Asn106Lys)	NA	NA	p.[(Asn106Lys);Asn106=]	VUS
	<i>NOD2</i>	NM_022162.1	c.2507T>C	p.(Ile836Thr)	rs763192145	4.062e-5	p.[(Ile836Thr);Ile836=]	VUS
113	<i>TNFRSF1A</i>	NM_001065.3	c.362G>A	p.(Arg121Gln)	rs4149584	0.01290	p.[(Arg121Gln);Arg121=]	VUS
129	<i>NLRP3</i>	NM_001243133.1	c.404G>A	p.(Arg135His)	NA	NA	p.[(Arg135His);Arg135=]	VUS
133	<i>MEFV</i>	NM_000243.2	c.442G>C	p.(Glu148Gln)	rs3743930	0.06564	p.[(Glu148Gln);Glu148=]	VUS
	<i>NLRP3</i>	NM_001243133.1	c.2107C>A	p.(Gln703Lys)	rs35829419	0.03861	p.[(Gln703Lys);Gln703=]	VUS
135	<i>MEFV</i>	NM_000243.2	c.442G>C	p.(Glu148Gln)	rs3743930	0.06564	p.[(Glu148Gln);Glu148=]	VUS
	<i>MEFV</i>	NM_000243.2	c.1105C>T	p.(Pro369Ser)	rs11466023	0.01471	p.[(Pro369Ser);Pro369=]	VUS
138	<i>NOD2</i>	NM_022162.1	c.2883-2A>G	SAV	rs564226539	2.442e-5	NA	VUS
139	<i>NLRP3</i>	NM_001243133.1	c.1556T>C	p.(Ile519Thr)	NA	NA	p.[(Ile519Thr);Ile519=]	VUS
151	<i>NOD2</i>	NM_022162.1	c.2050C>T	p.(Arg684Trp)	rs5743276	0.0003952	p.[(Arg684Trp);Arg684=]	VUS
	<i>NOD2</i>	NM_022162.1	c.2722G>C	p.(Gly908Arg)	rs2066845	0.01085	p.[(Gly908Arg);Gly908=]	VUS
	<i>NOD2</i>	NM_022162.1	c.3056G>T	p.(Arg1019Leu)	rs5743295)	3.972e-5	p.[(Arg1019Leu);Arg1019=]	VUS

	<i>IL10RA</i>	NM_001558.3	c.698T>G	p.(Val233Gly)	rs138929400	0.001378	p.[(Val233Gly);Val233=]	HTZ-MAR
183	<i>CARD14</i>	NM_024110.4	c.2870_2872delAGG	p.(Glu957del)	NA	3.23e-5	p.[(Glu957del);Glu957=]	VUS
187	<i>PSTPIP1</i>	NM_003978.3	c.203C>T	p.(Thr68Met)	rs201872851	0.001518	p.[(Thr68Met);Thr68=]	VUS
225	<i>PSTPIP1</i>	NM_003978.3	c.1213C>T	p.(Arg405Cys)	rs201253322	0.0005487	p.[(Arg405Cys);Arg405=]	VUS
231	<i>ADAR</i>	NM_001111.5	c.3401A>G	p.(Tyr1134Cys)	rs150284449	9.74e-5	p.[(Tyr1134Cys);Tyr1134=]	HTZ-MAR
	<i>MEFV</i>	NM_000243.2	c.442G>C	p.(Glu148Gln)	rs3743930	0.06564	p.[(Glu148Gln);Glu148=]	VUS
	<i>PSTPIP1</i>	NM_003978.3	c.1213C>T	p.(Arg405Cys)	rs201253322	0.0005487	p.[(Arg405Cys);Arg405=]	VUS
232	<i>NLRP3</i>	NM_001243133.1	c.2107C>A	p.(Gln703Lys)	rs35829419	0.03861	p.[(Gln703Lys);Gln703=]	VUS
	<i>TNFRSF1A</i>	NM_001065.3	c.362G>A	p.(Arg121Gln)	rs4149584	0.01290	p.[(Arg121Gln);Arg121=]	VUS
235	<i>MEFV</i>	NM_000243.2	c.442G>C	p.(Glu148Gln)	rs3743930	0.06564	p.[(Glu148Gln);Glu148=]	VUS
	<i>NLRP12</i>	NM_144687.3	c.1054C>T	p.(Arg352Cys)	rs199881207	0.0003863	p.[(Arg352Cys);Arg352=]	VUS
236	<i>MEFV</i>	NM_000243.2	c.2080A>G	p.(Met694Val)	rs61752717	0.0002669	p.[(Met694Val);Met694=]	HTZ-MAR
241	<i>RNASEH2B</i>	NM_001142279.2	c.529G>A	p.(Ala177Thr)	rs75184679	0.001361	p.[(Ala177Thr);Ala177=]	HTZ-MAR

HTZ-MAR: Heterozygous mutation associated with autosomal recessive disorders; SAV: splice acceptor variant; NA: not available; VUS, variants of uncertain significance

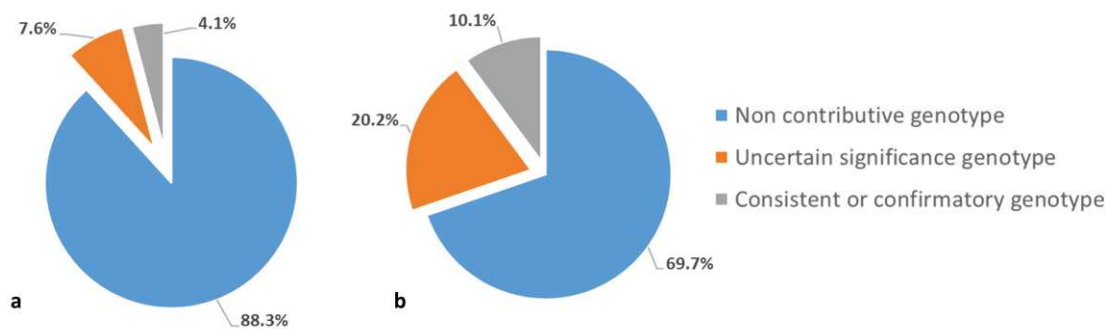


Figure 3. Diagnostic yields in the 297 patients

(A) Diagnostic yield for the 99 patients with analysis by the Sanger sequencing strategy. (B) Diagnostic yield for the 197 patients with analysis by the NGS strategy.

Distribution of genes and mutations identified in AIDs

Nine different genes were identified in confirmatory or consistent genotypes. Their distribution is in Figure 4A. The most commonly mutated genes were *MEFV*, *ADA2* and *MVK*. For patients with an uncertain significance genotype, variants in 12 genes were identified (Figure 4B). Genes most represented in these patients were *MEFV*, *NLRP3*, *NOD2* and *TNFRSF1A*. All the identified mutations were submitted to Infevers, the international registry of hereditary auto-inflammatory disorder mutations (<https://infevers.umai-montpellier.fr/web/>).

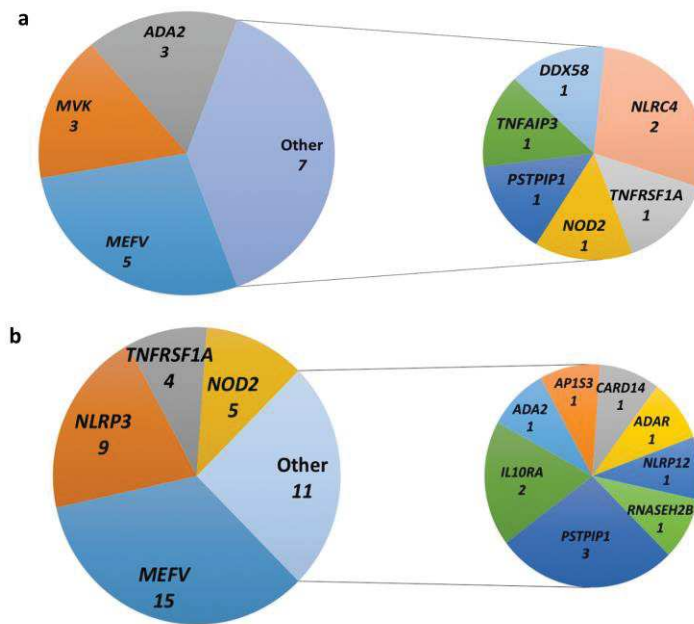


Figure 4. Distribution of mutated genes in SAID patients

(A) Autoinflammatory disease (AID) genes identified in 18 patients with confirmatory or consistent genotype. (B) AID genes identified in 35 patients with uncertain significance genotypes.

Impact of the genetic report on patients' medical care and treatment

We collected responses from 134/300 (44.7%) prescribing doctors. Six clinicians responded that their patients were lost to follow-up or had died (Sanger strategy, n=4; NGS strategy, n=2).

Treatment was fully effective for 54/128 (42.2%) patients and did not differ by sequencing strategy — Sanger, 35/87 (40.2%) patients; NGS, 19/41 (46.3%) patients ($p=0.513$) — nor when patients were analysed by their genetic status.

Receipt of the genetic report resulted in the initiation of effective treatment for only 13/128 (10.2%) patients and did not differ by sequencing strategy — Sanger, 8/87 (9.2%) patients; NGS, 5/41 (12.2%) patients ($p=0.755$) — nor by genetic status. Physicians acknowledged that the genetic report resulted in improved medical care for 59/128 (46.1%) patients, with no significant difference by sequencing strategy ($p=0.732$).

DISCUSSION

The aim of this study was to compare the effectiveness of the gene-panel NGS strategy versus the gold-standard Sanger sequencing for the genetic diagnosis of AIDs. Secondary goals were to describe the gene and mutation distribution in AID patients and to evaluate the impact of the genetic report on the patient's medical care and treatment. The proportion of patients with pathogenic genotypes concordant with the AID phenotype was significantly higher after NGS than Sanger sequencing, and targeted NGS improved the diagnosis and global care of patients with AIDs.

NGS sequencing is known to perform better than conventional Sanger sequencing (i.e., one or a few genes), but this has never been demonstrated formally in patients with clinical suspicion of an AID. One clear exception is *MEFV* exon 10 sequencing in AID patients with recurrent fever and parents from an at-risk ethnicity, which results in more than 70% genetic confirmation and is time- and cost-efficient (219). Therefore, we excluded Mediterranean patients with a clinical FMF and patients with undefined AID and with an initial NGS prescription from the study.

As expected, we demonstrated a clear enhancement of the diagnostic yield when switching from Sanger sequencing to NGS (4% vs 10%, $p=0.04$). For the time being, the Sanger technique remains cheaper and faster in expert laboratories, which are generally small- and medium-sized. A way to improve the rate of confirmatory genetic diagnosis in AIDs could be a wider use of existing diagnostic criteria or discussions within multidisciplinary staff meetings. Preliminary evidence-based classifications have been proposed for periodic fevers (FMF, MKD, CAPS and TRAPS) (220) and recently deficiency of adenosine deaminase 2 (DADA2) (221).

However, the performance of NGS seems to be lower than in the literature. The diagnosis rate approached 30% in 2 available studies (214,222). The design and inclusion criteria of these studies likely explain this apparent discrepancy. These studies considered variants of uncertain significance (VUS) such as R92Q for *TNFRSF1A* or V198M for *NLRP3* as confirmatory results (222). If we consider uncertain significance genotypes, our results are very similar to the literature. However, in a more recent report, the yield was similar to ours (223).

A second objective of this study was to evaluate the distribution of genes and mutations in this population. We identified 16 different AID genes among the 53 patients harbouring at least one mutation (class ≥ 3). The most frequent were, in decreasing order of frequency, *MEFV*, *NLRP3*, *NOD2*, *TNFRSF1A*, *ADA2* and *MVK*, *PSTPIP1* and *NLRC4*. FMF, MKD, CAPS and TRAPS are

often coined "the most common recurrent hereditary fevers" because these are the ones whose implicated gene has been known for the longest time. In this study (Figures 3A and 3B), we provide probably more accurate figures of the relative involvement of the genes present in our panel in unselected AID patients. Our results corroborate the rarity of monogenic AIDs in adult and paediatric populations. Among the 4 best-known recurrent-fever genes, *MEFV* was the most frequently mutated, even despite efforts to limit recruitment bias. *ADA2* was the second most frequent (7.5%). Studies over time will tell whether this is a true score or a possible cohort effect, because *DADA2* was discovered as a new AID during the time of this study. The 3 other recurrent fevers were very rare, as in previous reports. We did not identify any patient with CAPS, although 62% of the patients we included were under age 15 years at disease onset. This finding likely represents a cohort effect rather than defective technique sensitivity because we had identified patients with as low as 6% *NLRP3* mosaicism using the same NGS panel in previous analyses (unpublished personal data).

The third aim of this study was to collect information on treatment and patient follow-up at least 6 months after receipt of the genetic results by the clinician to evaluate the benefit of genetic testing on medical care. Clinicians declared a benefit of this genetic test on global medical care for 59/128 (46.1%) patients, and more specifically the initiation of treatment (13/128 [10.2%]), but with no difference by strategy or final diagnosis. This medical care included initiation of targeted therapies, improved disease prognosis, and better genetic counselling.

The limitation of this study is the low contribution of the clinicians to the survey and the results must be considered preliminary. Aside from expected lack of time of the clinicians, one explanation is possibly that therapeutic management is not clearly established for AIDs caused by the most recently identified genes.

In conclusion, we formally compared the diagnosis rate with conventional Sanger sequencing and NGS with a targeted gene panel in rare monogenic AIDs. We improved our confirmatory diagnoses from 4% to 10% (150%) with NGS high-throughput sequencing. The gene distribution in our cohort confirms the genetic heterogeneity and rarity of AIDs but provides an initial refinement of their respective frequencies. Clinicians acknowledged a general beneficial effect of genetic diagnosis on patient care.

SUPPLEMENTARY FIGURES

Patient identity

4 – CLINICAL FORM

ASYMPTOMATIC individual

- Last name, first name of index case:
- Family relationship with index case:

SYMPTOMATIC patient
(Box the symptoms presented by the patient before the start of the treatment
+++ differentiate No and ND (No determined))

ATTACK CHARACTERISTICS:							
Triggering factor	Cold	Hot	Vaccination	Stress	Fatigue	No	ND
Other:							
Attack frequency	< 1/month	1 à 2 /month	> 2/month	chronic			
Number of unexplained attacks: ...							
Age at first symptoms month years					
Attack duration hours days					

BIOLOGY:	
CRP value during an attack: mg/L
Other auto-inflammatory genes already analyzed:	
Mevalonaturia during attacks:	
Autoantibodies:	Anemia:

TREATMENT:	Nom	Dose	Age début	Effet (N nul, P partiel, T total)			
Colchicin	—fj	N	P	T	ND
Corticoids	—fj	N	P	T	ND
NSAID	—fj	N	P	T	ND
Biotherapy IL-1	—fj	N	P	T	ND
Biotherapy TNFα	—fj	N	P	T	ND
Autre	—fj	N	P	T	ND

Inflammation				
Fever	Yes	No	ND	
CRP ↑	Yes	No	ND	
Thorax				
Pain	Yes	No	ND	
Pleuritis-Pericarditis	Yes	No	ND	
Pneumopathy	Yes	No	ND	
Neuro-sensoriel				
Stroke	Yes	No	ND	
Calcifications	Yes	No	ND	
Headache	Yes	No	ND	
Conjunctivitis	Yes	No	ND	
Encephalitis	Yes	No	ND	
Epilepsy	Yes	No	ND	
Meningitis	Yes	No	ND	
Papillitis	Yes	No	ND	
Uveitis	Yes	No	ND	
Deafness	Yes	No	ND	
Mental retardation	Yes	No	ND	
Skeleton				
Arthralgia	Yes	No	ND	
Arthritis	Yes	No	ND	
Myalgia	Yes	No	ND	
Degenerative arthropathy	Yes	No	ND	
Osteitis	Yes	No	ND	
Kidney				
Amyloidosis	Yes	No	ND	
Renal failure	Yes	No	ND	
Proteinuria	Yes	No	ND	
Gastro-intestinal				
Abscess	Yes	No	ND	
Hepatic cytolysis	Yes	No	ND	
Diarrhea	Yes	No	ND	
Abdominal pain	Yes	No	ND	
Bleeding	Yes	No	ND	
Vomiting	Yes	No	ND	
Muco-cuteaneous				
Oral aphthosis	Yes	No	ND	
Genital aphthosis	Yes	No	ND	
Erythema nodosa	Yes	No	ND	
Folliculitis	Yes	No	ND	
Lipodystrophy	Yes	No	ND	
Maculopapula	Yes	No	ND	
Necrosis	Yes	No	ND	
Oedema	Yes	No	ND	
Pseudoerysipelas	Yes	No	ND	
Psoriasis	Yes	No	ND	
Pyoderma	Yes	No	ND	
Tenosynovitis	Yes	No	ND	
Urticaria	Yes	No	ND	
Vasculitis	Yes	No	ND	
Miscellaneous				
Growth retardation	Yes	No	ND	
Lymphadenopathy	Yes	No	ND	
Allergy	Yes	No	ND	
Hépatomegaly	Yes	No	ND	
Immune deficiency	Yes	No	ND	
Recurrent infections	Yes	No	ND	
Pharyngitis	Yes	No	ND	
Splenomegaly	Yes	No	ND	
Hemophagocytic syndromes	Yes	No	ND	
Other? (precise)				

Figure S1. Clinical form for requesting genetic diagnosis.

Questionnaire

1/ Patient's identification:

.....

2/ Treatment **BEFORE** the genetic report:

	Zero effect	Partial effect	Total effect	Effectiveness not available	Medication not prescribed
Colchicine					
Corticoïdes					
NSAI					
Anti-Il1					
Anti-TNF					
Other					

3/ Treatment **AFTER** the genetic report:

	Zero effect	Partial effect	Total effect	Effectiveness not available	Medication not prescribed
Colchicine					
Corticoïdes					
NSAI					
Anti-Il1					
Anti-TNF					
Other					

4/ In your opinion, has this genetic result (contributory or not) had an impact on the patient's overall care?

Yes No

5/ Comments – free text:

.....

Figure S2. Questionnaire sent to the clinicians 6 months after the genetic report.

Abbreviations

AIDs: autoinflammatory diseases

CAPS: cryopyrin associated periodic syndrome

CINCA: chronic neurologic cutaneous and articular syndrome

FCAS: familial cold autoinflammatory syndrome

FMF: familial Mediterranean fever

IFN-I: interferon type I

IL-1: interleukin-1

IL-6: interleukin-6

MKD: mevalonate kinase deficiency

MWS: Muckle-Wells syndrome

NOMID: neonatal-onset multisystem inflammatory disease

TNF- α : tumour necrosis factor alpha

TRAPS: tumour necrosis factor receptor-associated periodic syndrome

Table S1 Details of autoinflammatory disease genes included in the next-generation sequencing panel version 1 and version 2.

Gene	Pathology	OMIM number	Transmission	NM
<i>ACP5</i>	SPENCD	# 607944	AR	NM_001111035.2
<i>ADAM17</i>	NISBD1	# 614328	AR	NM_003183
<i>ADAR</i>	Aicardi-Goutieres	# 225750	AR	NM_001111
<i>APIS3</i>	Psoriasis susceptibility to	# 616106	AD	NM_001039569.1
<i>CARD14</i>	PRP/PSORS2	# 173200/ # 173200	AD	NM_024110.4
<i>CASP1</i>	N/A		N/A	NM_001257118
<i>CECR1</i>	DADA2	# 615688	AR	NM_001282225.1
<i>COPA</i>	AILJK	# 616414	AD	NM_001098398.1
<i>DDX58</i>	Singleton-Merten	# 616298	AD	NM_014314
<i>FAM105B</i>	AIPDS	# 617099	AR	NM_138348
<i>FAS</i>	ALPS	# 601859	AD	NM_000043.5
<i>FASLG</i>	ALPS	# 601859	AD	NM_000639.2
<i>IFIH1</i>	Aicardi-Goutieres	# 225750	AD	NM_022168.3
<i>IL10</i>	IL10 deficiency		AR	NM_000572.2
<i>IL10RA</i>	IL10 R1 deficiency	# 613148	AR	NM_001558.3
<i>IL10RB</i>	IL10 R2 deficiency	# 612567	AR	NM_000628.4
<i>IL1RN</i>	DIRA	# 612852	AR	NM_173841.2
<i>IL36RN</i>	DITRA	# 614204	AR	NM_173170.1
<i>LACCI1</i>	SJIA-like	# 604302	AR	NM_001128303.1
<i>LPIN2</i>	Majeed	# 609628	AR	NM_014646.2
<i>MDFIC</i>	N/A		N/A	NM_199072.4
<i>MEFV</i>	FMF	# 249100	AR	NM_000243.2
<i>MVK</i>	MKD	# 260920/ # 610377	AR	NM_000431.3
<i>NCSTN</i>	PASH		N/A	NM_015331.2
<i>NLRC4</i>	AIFEC/ FCAS4	# 616050/ # 616115	AD	NM_021209.4
<i>NLRP1</i>	NAIAD	# 617388	AD/AR	NM_033004.3
<i>NLRP12</i>	NAPS12	# 611762	AD	NM_144687.3
<i>NLRP3</i>	CAPS	# 120100	AD	NM_001243133.1
<i>NOD2</i>	Blau	# 186580	AD	NM_022162.2
<i>PLCG2</i>	APLAID	# 614878	AD	NM_002661.4
<i>POMP</i>	PRAAS/CANDLE	# 601952	AD	NM_015932.5
<i>PSENFEN</i>	N/A		N/A	NM_172341.2

<i>PSMA3</i>	PRAAS/CANDLE		AR	NM_002788.3
<i>PSMB4</i>	PRAAS/CANDLE		AR	NM_002796.2
<i>PSMB8</i>	PRAAS/CANDLE	# 256040	AR	NM_148919.3
<i>PSMB9</i>	PRAAS/CANDLE		AR	NM_002800.4
<i>PSMG2</i>	PRAAS/CANDLE		AR	NM_020232.4
<i>PSTPIP1</i>	PAPA	# 604416	AD	NM_003978.4
<i>PYCARD</i>	N/A		N/A	NM_013258.4
<i>RBCK1</i>	PBMEI	# 615895	AR	NM_031229.3
<i>RNASEH2A</i>	Aicardi-Goutieres	# 610333	AR	NM_006397.2
<i>RNASEH2B</i>	Aicardi-Goutieres	# 610181	AR	NM_024570.3
<i>RNASEH2C</i>	Aicardi-Goutieres	# 610329	AR	NM_032193.3
<i>RNF31</i>	N/A		N/A	NM_017999.4
<i>SAMHD1</i>	Aicardi-Goutieres	# 612952	AR	NM_015474.3
<i>SERPING1</i>	HAE1	# 106100	AD	NM_000062.2
<i>SH3BP2</i>	Cherubism	# 118400	AD	NM_003023.4
<i>SLC29A3</i>	H syndrom	# 602782	AR	NM_018344.5
<i>TMEM173</i>	SAVI	# 615934	AD	NM_198282.3
<i>TNFAIP3</i>	AISBL	# 616744	AD	NM_001270508.1
<i>TNFRSF11A</i>	TRAPS11		AD	NM_003839.3
<i>TNFRSF1A</i>	TRAPS	# 142680	AD	NM_001065.3
<i>TNFRSF9</i>	N/A		N/A	NM_001561
<i>TREX1</i>	Aicardi-Goutieres	# 225750	AD/AR	NM_016381.5
<i>TRNT1</i>	SIFD	# 616084	AR	NM_182916.2

Legend:

IN NORMAL LETTERS Version 1 : 32 genes MAI panel

IN BOLD Version 2 : 55 genes MAI panel

N/A Candidate genes

AD Autosomal dominant

AR Autosomal recessive

AIFEC Autoinflammation with infantile enterocolitis

AILJK Autoimmune interstitial lung, joint, and kidney disease

AIPDS Autoinflammation, panniculitis, and dermatosis syndrome

AISBL Autoinflammatory syndrome, familial, Behçet-like

ALPS	Autoimmune lymphoproliferative syndrome
APLAID	Autoinflammation, antibody deficiency, and immune dysregulation, PLCG2-associated
CAPS	Cryopyrin associated periodic syndrome
DADA2	Adenosine deaminase 2 deficiency
DIRA	Interleukin 1 receptor Antagonist deficiency
DITRA	Interleukin 36 receptor antagonsit deficiency
FCAS4	Familial cold autoinflammatory syndrome 4
FMF	Familial mediterranean fever
HAE1	Angioedema hereditary type 1
MKD	Mevalonate Kinase deficiency
NAIAD	NLRP1-associated autinflammation with arthritis
NAPS12	NLRP12 associated periodic syndrome
NISBD1	Inflammatory skin and bowel disease, neonatal, 1
PAPA	Pyogenic Arthritis, Pyoderma gangrenosum, and Acne
PASH	Pyoderma gangrenosum, acne and suppurative hidradenitis
PBMEI	Polyglucosan body myopathy 1 with or without immunodeficiency
PRAAS/CANDLE	Proteasome associated autoinflammatory syndrome / Chronic Atypical Neutrophilic Dermatosi s with Lipodystrophy and Elevated Temperature
PRP/PSORS2	Pytiriasis rubra pilaris / Psoriasis 2
SAVI	STING-associated vasculopathy with onset in infancy
SIFD	Sideroblastic anemia, B-cell immunodeficiency, periodic fevers, and developmental delay
SJIA-like	Systemic juvenile idiopathic arthritis - like
SPENCD	Spondyloenchondrodysplasia with immune dysregulation
TRAPS	TNF receptor associated periodic syndrome
TRAPS11	TNFRSF11A-associated hereditary fever disease

Table S2: Clinical characteristics of patients with confirmatory or consistent genotype of autoinflammatory diseases by sequencing strategy (n=18).

Id patient/sex	Gene (s)	Age (Y)	Age onset (Y)	E	CRP (mg/L)	No. of attacks/month	Duration of Access (days)	Fever	Thorax Involvement	Neuro-skeletal disorders	Peripheral and central nervous system involvement	Cutaneous involvement	Gastro-intestinal involvement	Other
Sanger strategy														
18/M	<i>TNFRSF1A</i>	28	15	C	NA	<1	3-7	NA	NA	NA	NA	oral ulcers; Abscess	abdominal pain; liver cytolysis	NA
20/M	<i>MVK</i>	3	1	C	NC	>2	3-4	yes	NA	NA	NA	NA	diarrhoea	ulcers
23/M	<i>MEFV</i>	31	NC	A	NC	1-2	NA	yes	NA	NA	NA	NA	abdominal pain; liver cytolysis	NA
27/M	<i>ADA2</i>	22	14	NA	>5	<1	NA	yes	pleurisy; pericarditis	NA	stroke attack; nerve palsy; meningitis; headache	PAN; vasculopathy; maculo-papular rash; necrosis	abdominal pain	NA
50/M	<i>MVK</i>	13	<1	C	21	1-2	3-7	yes	NA	NA	headache; conjunctivitis	oral ulcers	diarrhoea; abdominal pain; emesis	Pharyngitis; adenopathy
59/M	<i>MEFV</i>	4	<1	C	NA	1-2	3-7	yes	NA	myalgia	conjunctivitis	NA	NA	NA
242/M	<i>MEFV</i>	25	23	C	>5	<1	NA	yes	NA	NA	NA	NA	diarrhoea; abdominal pain	NA

288/F	<i>NOD2</i>	57	25	C	50	NA	NA	NA	NA	NA	headache; uveitis	NA	NA	renal granulo- matosis
NGS strategy														
54/F	<i>NLRC4</i>	17	14	C	61	1-2	1-3	yes	pain	arthralgia myalgia	headache	acne; folliculitis	diarrhoea; abdominal pain; liver cytolysis	macrophage activation syndrome
63/M	<i>MVK</i>	4	<1	C	NA	1-2	1-3	yes	pain; pericarditis	NA	NA	NA	diarrhoea; abdominal pain	pharyngitis; pneumopathy; adenopathy; recurrent infections
66/F	<i>MEFV</i>	20	2	M	237	<1	3-7	yes	NA	myalgia	NA	recurrent pseudo erysipelas	NA	recurrent infections; elevated IgD
83/M	<i>MEFV</i>	42	30	M	NA	>2	1-3	yes	pain; pericarditis	NA	NA	urticaria; folliculitis;	diarrhoea; abdominal pain	NA
108/F	<i>NLRC4</i>	15	3	C	353	<1	NA	yes	pain; pericarditis; pleurisy	arthralgia	headache; conjunctivitis	œdema; urticaria	liver cytolysis; abdominal pain; emesis	pharyngitis; adenopathy
112/F	<i>DDX58</i>	17	16	C	122	chronic	chronic	yes	pain; pericarditis	NA	arthralgia; myalgia; arthritis	Maculo-papular rash; tenosynovitis	emesis; liver cytolysis; hepatomegaly- splenomegaly	NA

141/M	<i>ADA2</i>	18	2	C	NA	NA	NA	NA	NA	NA	stroke attack; headache; meningitis; vertigo; nystagmus	vasculitis; oral ulcers	emesis;	NA
164/M	<i>PSTPIP1</i>	6	NC	C	>5	NA	NA	NA	NA	myalgia; arthralgia deforming arthro- pathy	NA	urticaria	NA	NA
166/M	<i>TNFAIP3</i>	27	7	C	100	NA	NA	yes	<i>situs inversus;</i> tetralogy of Fallot	arthralgia myalgia	palpebral ptosis	PAN; oral ulcers	abdominal pain; pseudo- membranous colitis	acute renal failure; haematuria; haemolytic anemia; thrombocyte- penia; anti-DNA autoanti-bodies
203/F	<i>ADA2</i>	20	4	C	>5	chronic	chronic	yes	NA	arthritis	stroke attack	livedo racemosa; extremities necrosis; PAN	NA	Hypogamma- globulinemia; recurrent infections

NA: not available; F: female; M: male; E: ethnicity; C: caucasian; M: Maghreb; A: African; I: Indian; Y: years; CRP: C-reactive protein; PAN: periarteritis nodosa

Table S3: Clinical characteristics of patients with uncertain genotype by sequencing strategy (n=35).

Id patient/sex	Gene (s)	Age (Y)	Age onset (Y)	E	CRP (mg/L)	No. of attacks/month	Duration Access (days)	Fever	Thorax involvement	Neuro-skeletal disorders	Peripheral and central nervous system involvement	Cutaneous involvement	Gastro-intestinal involvement	Other
Sanger strategy														
9/F	<i>NLRP3</i>	39	15	C	NA	< 1	NA	yes	pain	arthralgia	NA	NA	NA	recurrent infections; adenopathy
70/M	<i>NLRP3</i>	8	6	M	> 5	chronic	chronic	yes	NA	arthritis	NA	urticaria; maculopapular rash	NA	activated macrophage syndrome; adenopathy
81/M	<i>TNFRSF1A</i>	52	40	C	NA	< 1	1-3	yes	NA	arthralgia; myalgia	headache	NA	abdominal pain	NA
109/M	<i>ADA2</i>	10	5	C	> 5	< 1	NA	yes	NA	arthralgia; myalgia	headache	œdema	diarrhoea; abdominal pain; emesis	recurrent infections
113/F	<i>MEFV</i>	51	20	C	> 5	< 1	NA	yes	NA	myalgia	headache	NA	liver cytolysis	NA

121/F	<i>NOD2</i>	6	4	M	NA	NA	NA	No	NA	NA	NA	NA	NA	NA	uveitis
122/F	<i>TNFRSF1A</i>	10	<1	C	60	>2	NA	yes	pain	arthralgia; myalgia	headache	urticaria; maculopapular	diarrhoea; abdominal pain; emesis; liver cytolysis	pharyngitis	
127/M	<i>MEFV</i>	5	1	C	186	<1	NA	yes	NA	arthralgia; myalgia	headache	NA	abdominal pain; emesis	pharyngitis; adenopathy	
136/M	<i>NOD2</i>	13	1	M	63	1-2	NA	yes	pain	arthralgia; myalgia; osteitis; deforming arthropathy	headache; conjunctivitis	pseudo erysipelas	diarrhoea; abdominal pain;	insensitivity to pain	
164/M	<i>MEFV</i>	7	1,5	C	38	1-2	NA	yes	NA	arthralgia; myalgia	headache	NA	emesis	pharyngitis	
206/F	<i>MEFV</i>	5	3	C	83	1-2	NA	NA	NA	NA	papillitis	NA	abdominal pain	pharyngitis; recurrent infections; adenopathy	
232/F	<i>MEFV, NLRP3</i>	53	NC	C	>5	NA	NA	yes	NA	arthralgia	stroke attack; cephalagia; deafness	oral ulcers; pseudo erysipelas; urticaria	Spleno- megaly	NA	

233/F	<i>MEFV</i>	53	30	C	> 5	NA	NA	yes	NA	arthralgia; myalgia	NA	oral ulcers	diarrhoea; abdominal pain	NA
237/F	<i>MEFV</i>	5	2	NA	> 5	1-2	NA	yes	NA	arthralgia	NA	NA	abdominal pain	failure to thrive
275/M	<i>MEFV</i>	30	17	C	no	< 1	NA	NA	NA	NA	NA	NA	abdominal pain	NA

NGS strategy

5/M	<i>MEFV</i>	11	1.5	M	> 5	1-2	1-3	yes	NA	arthralgia	headache	NA	diarrhoea; abdominal pain	adenopathy
19/M	<i>NLRP3</i>	28	24	C	NA	< 1	3-7	yes	NA	arthralgia; myalgia; arthritis	headache; conjunctivitis	urticaria; oral ulcers	NA	NA
31/M	<i>IL10RA</i>	9	<1	C	NA	1-2	5-30	yes	NA	arthralgia; myalgia; arthritis	headache	urticaria	abdominal pain	NA
99/F	<i>NLRP3</i>	24	11	C	NA	< 1	< 1	yes	NA	myalgia	headache	NA	diarrhoea; abdominal pain; emesis	pharyngitis
105/M	<i>AP1S3, NOD2</i>	12	10	M	NA	NA	NA	yes	NA	arthritis; arthralgia	NA	urticaria	Hepato- megaly spleno-megaly	NA

113/F	<i>TNFRSF1A</i>	34	21	C	29	< 1	NA	yes	NA	arthralgia	headache	Maculo-papular rash	emesis	NA
129/M	<i>NLRP3</i>	50	50	C	no	< 1	NA	no	NA	arthralgia; myalgia	deafness; meningitis	Maculo-papular rash; vasculitis; urticaria	abdominal pain	NA
133/F	<i>MEFV, NLRP3</i>	7	1	I	200	1-2	NA	yes	NA	arthralgia	NA	urticaria; oral ulcers	diarrhoea; abdominal pain	pharyngitis; failure to thrive
135/F	<i>MEFV</i>	56	Child-hood	Ma	193	< 1	< 7	yes	NA	arthralgia; myalgia; polyarthritis; deforming arthropathy	NA	oedema; cutaneous rash; urticaria; pseudo erysipelas; palpebral oedema	NA	recurrent infections; proteinuria
138/M	<i>NOD2</i>	10	<1	M	NA	< 1	NA	yes	NA	arthralgia; myalgia; osteitis; deforming arthropathy	headache	pseudo erysipelas	abdominal pain	NA

139/F	<i>NLRP3</i>	3	1,5	M	90	1-2	NA	yes	NA	NA	NA	NA	NA	pharyngitis; adenopathy
151/M	<i>NOD2 X3, IL10RA</i>	32	26	C	80	1-2	NA	yes	NA	arthralgia	headache	folliculitis	diarrhoea; abdominal pain; emesis	NA
183/F	<i>CARD14</i>	7	1	C	> 5	> 2	NA	yes	NA	arthralgia	headache; conjunctivitis	oral ulcers; maculo- papular rash; urticaria	abdominal pain	
187/M	<i>PSTPIP1</i>	13	9	C	50	1-2	NA	no	NA	arthralgia; myalgia; arthritis	NA	oral ulcers; pseudo erysipelas	NA	NA
225/F	<i>PSTPIP1</i>	10	<1	C	47	> 2	1-3	yes	NA	arthralgia	headache; conjunctivitis	oral ulcers; maculo- papular rash; lipodystrophy	diarrhoea; abdominal pain	recurrent infections; pharyngitis
231/F	<i>ADAR, MEFV, PSTPIP1</i>	6	6	C	no	NA	NA	NA	NA	NA	uveitis; papillitis	Maculo- papular rash; folliculitis	NA	NA

232/M	<i>NLRP3</i> , <i>TNFRSF1A</i>	31	30	C	100	< 1	NA	yes	pain	arthralgia	headache	NA	abdominal pain; liver cytolysis	pharyngitis
235/F	<i>MEFV</i> , <i>NLRP12</i>	3	<1	N A	251	chronic	chronic	yes	pericarditi s; pleurisy	NA	papillitis; meningitis	oral ulcers; oedema; vasculitis	diarrhoea; abdominal pain; haemorrhage	pharyngitis; adenopathy
236/F	<i>MEFV</i>	46	30	N A	> 5	< 1	NA	yes	NA	arthralgia	NA	NA	NA	NA
241/F	<i>RNASEH2B</i>	23	1	M	100	1-2	NA	NA	NA	arthralgia	headache; meningitis	oral ulcers	diarrhoea	recurrent infections

NA: not available; F: female; M: male; E: ethnicity; C: caucasian; M: Maghreb; A: African; I: Indian; Ma: Malagasy; Y: years; CRP: C-reactive protein; PAN: periarteritis nodosa

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First report of *MEFV* gene duplication in a patient suffering from Familial Mediterranean fever

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ABSTRACT

Background

Familial Mediterranean fever (FMF) is a rare autoinflammatory disease caused by recessive substitutions in the *MEFV* gene. Anecdotal point deletions and duplications have been described in FMF patients.

Methods

We used Sanger sequencing, quantitative polymerase chain reaction (Q-PCR), and targeted next generation sequencing (NGS) to explore the patient and her family, and characterize the copy number variation and breakpoint in her genome.

Results

We describe a 21-year-old woman who presented with classical FMF phenotype including recurrent fever, arthralgia, and abdominal pain with vomiting. Attacks lasted three days and biological inflammation was documented. Colchicine treatment was efficient on her symptoms. Her father was Armenian and her mother Malagasy, and both were asymptomatic. Sanger sequencing and NGS revealed the clearly pathogenic p.Met694Val and the variant of uncertain significance p.Glu148Gln, both at ratio < to the expected 50%. Mosaicism was excluded because all other polymorphisms in the *MEFV* gene displayed the unbalanced (2/3 or 1/3) ratio. DNA contamination was ruled out through microsatellite analysis. We thus suspected a gene rearrangement. Q-PCR confirmed a heterozygous duplication of the entire wild *MEFV* gene inherited from the mother. Breakpoint sequencing demonstrated that the rearrangement was a tandem duplication.

Conclusion

We report here the first duplication of a normal *MEFV* allele in a FMF patient who therefore expressed only 1/3 dose of pathogenic mutation, 1/3 dose of VUS and 1/3 dose of wild-type allele. This duplication could participate to the disease.

Key words: autoinflammatory disease, FMF, *MEFV*, duplication

INTRODUCTION

Familial Mediterranean fever (FMF [MIM249100]) is the first described and most frequent monogenic autoinflammatory disease (AID). The causative gene, *MEFV* (*Mediterranean FeVer* [MIM 608107]), is located on the short arm of chromosome 16 and was identified in 1997 by positional cloning.(224,225) FMF frequently affects people of Mediterranean descent, particularly Turkish, Sefardi-Jewish, Arab and Armenian individuals. In these populations, the carrier frequency ranges from 1:3 to 1:10, making this disease very prevalent in Armenia and Turkey notably.(226) Its diagnosis is mainly clinical, and genetic testing is often used as a confirmation. FMF is characterized by recurrent episodes of fever - usually of acute onset and variable frequency - abdominal pain due to peritonitis and pleuritis. Repeated bouts of inflammation in untreated patients may lead to renal amyloidosis and terminal renal failure. Major criteria were initially proposed in Israel, including isolated fever, generalized peritonitis, unilateral pleurisy or pericarditis and monoarthritis (hip, knee, and ankle).(227) Family history and response to colchicine are also important information to collect. As in most AIDs, increased acute phase reactants during flares is an important diagnostic criterion. More recently, new classification criteria for FMF, taking into account the *MEFV* genotype, have been proposed.(228)

FMF is classically a recessively inherited disease. However, a growing number of publications describe patients with only one *MEFV* mutation fulfilling clinical criteria. A previous study suggested a possible gene dosage-effect in the patient phenotype.(229) Mutations in *MEFV*, encoding pyrin, lead to interleukin-1 β (IL-1 β) mediated autoinflammation. Pyrin can form an inflammasome in response to bacterial stimuli, through RhoA GTPase inactivation.(230) A recent study confirmed that monocytes from patients bearing two *MEFV* exon 10 pathogenic variants displayed an increased Pyrin inflammasome response compared with monocytes from patients with a single exon 10 pathogenic variant. FMF-associated *MEFV* mutations do not lead to a constitutive activation of Pyrin. Instead, they act as hypermorphic mutations which specifically decrease the activation threshold of the Pyrin inflammasome.(231)

MEFV comprises 10 exons and 781 codons. Thus far, more than 300 sequence variants have been recorded in Infevers (<https://infevers.umai-montpellier.fr/web/>), a dedicated registry of autoinflammatory mutations.(232) Most variants associated with FMF are missense mutations. There are very few nonsense substitutions or small in frame intragenic rearrangements. Large rearrangements such as exonic deletions or duplications are not detected with classical Sanger

method. Van Gjin et al have conducted an exhaustive study to determine whether copy number variations (CNV) could be responsible for the FMF phenotype. (233) Multiplex ligation probe amplification analysis in more than 200 FMF patients did not show large duplications or deletions. Therefore, rearrangements are not routinely searched for in FMF patients.

We report and characterize here the first duplication of the *MEFV* gene in a typical FMF patient.

METHODS

Patient and family

EDTA blood samples from the proband and her parents were sent to our laboratory for *MEFV* molecular analysis. Informed consents for genetic testing and research were obtained from all participants. DNA was extracted using MaxiPrep kit (Thermofisher) according to manufacturer's protocol.

***MEFV* mutations identification and familial analysis**

We amplified *MEFV* (NM_000243.2) exons 2, 3, 5, 9 and 10 by using M13 tailed oligonucleotides (sequences available upon request). We designed two different amplicons for each exon to circumvent the risk of allele dropout. We used MasterMix (Promega) to amplify exons 3, 4, 5, 9 and 10, and QbioTaq (Qbiogene) with 10% DMSO addition to amplify exon 2. Amplicons from all family members were Sanger sequenced in both directions by using ABI PRISM Big Dye Terminator V3.1 and the Ready-Reaction Cycle-Sequencing kit on ABI 3130 XL analyser (Applied Biosystems). DNA sequences were analysed by using Seqscape software (Applied Biosystem). Microsatellites analysis was performed to exclude exogenous DNA contamination by using Qiagen multiplex PCR kit (Qiagen) on ABI 3130 XL instrument and analysed with GeneMapper software (Thermofisher).

Rearrangement studies

We conducted quantitative real-time polymerase chain reaction (Q-PCR) assays in *MEFV* exons 4,10, 3'UTR and 5'UTR, in adjacent genes (*ZNF200*, *OR1F1*, *ZNF263*) and intergenic regions for the patient, her parents, and 2 healthy controls. We used LightCycler SYBRGreen I Master kit (Roche) on a LightCycler 480 thermocycler (Roche), according to manufacturer's protocol. Breakpoint was searched for, by using Sanger sequencing, as previously described, using a forward primer (5'-AGAATGGGCTTTGACAAGTTGA-3') in the 5' intergenic *MEFV*

region (3' on the forward strand), and a reverse primer (5'-CGAACAGGGAGTAGGTCACA-3') in the 3' intergenic *ZNF200* region.

Next Generation sequencing (NGS)

We performed sequencing of a 32 AID genes (supplementary table S1) panel on a Miseq platform (Illumina) to exclude differential diagnosis and to determine the allele balance. Library was prepared using a Nextera Rapid Capture Custom Enrichment kit (Illumina). Bioinformatic analysis (single nucleotide variation and copy number variation detection) was performed using SeqNext software (JSI medical system) and Alamut (Interactive Biosoftware). Coverage was greater than 80X in 99% of *MEFV* coding nucleotides.

RESULTS

Phenotype of the patient

The index case was a 21-year-old woman, non-consanguineous, with history of recurrent fever. Her mother was Malagasy, her father was Armenian, and both were asymptomatic. The patient presented with classical FMF features according to Livneh's criteria, starting from the age of 6 years.(227) The fever attacks lasted 2 to 3 days, occurred 1 to 2 times a month, and were associated with arthralgia, abdominal pain and vomiting. She had elevated C-Reactive Protein level (102 mg/L). Her symptoms were efficiently alleviated by colchicine.

FMF genetic diagnosis

Sanger sequencing of the *MEFV* gene detected the known pathogenic p.Met694Val mutation inherited from her father, and the variant of uncertain significance (VUS) p.Glu148Gln inherited from her mother. According to current guidelines, this genotype could be consistent with clinical diagnosis of FMF.(216,234) NGS sequencing of a panel of 32 AID genes including all known recurrent fever genes did not reveal any other pathogenic variant, further supporting FMF diagnosis.

Identification of a *MEFV* duplication

Interestingly, Sanger electrophoregrams showed apparent decreased variant peaks for the p.Met694Val (figure 1A) and the p.Glu148Gln variants in the proband and her mother suggesting a possible duplication of the wild-type allele. Thus, we examined other common polymorphisms in exon 2 (p.Asp102Asp, p.Gly138Gly, p.Ala165Ala, p.Arg202Gln), exon 3 (p.Arg314Arg), exon 5 (p.Glu474Glu, p.Gln476Gln, p.Asp510Asp) and exon 9 (p.Pro588Pro).

All had apparent unbalanced ratio as well. Microsatellite analysis did not detect exogenous DNA, ruling out DNA contamination (supplementary figure S2).

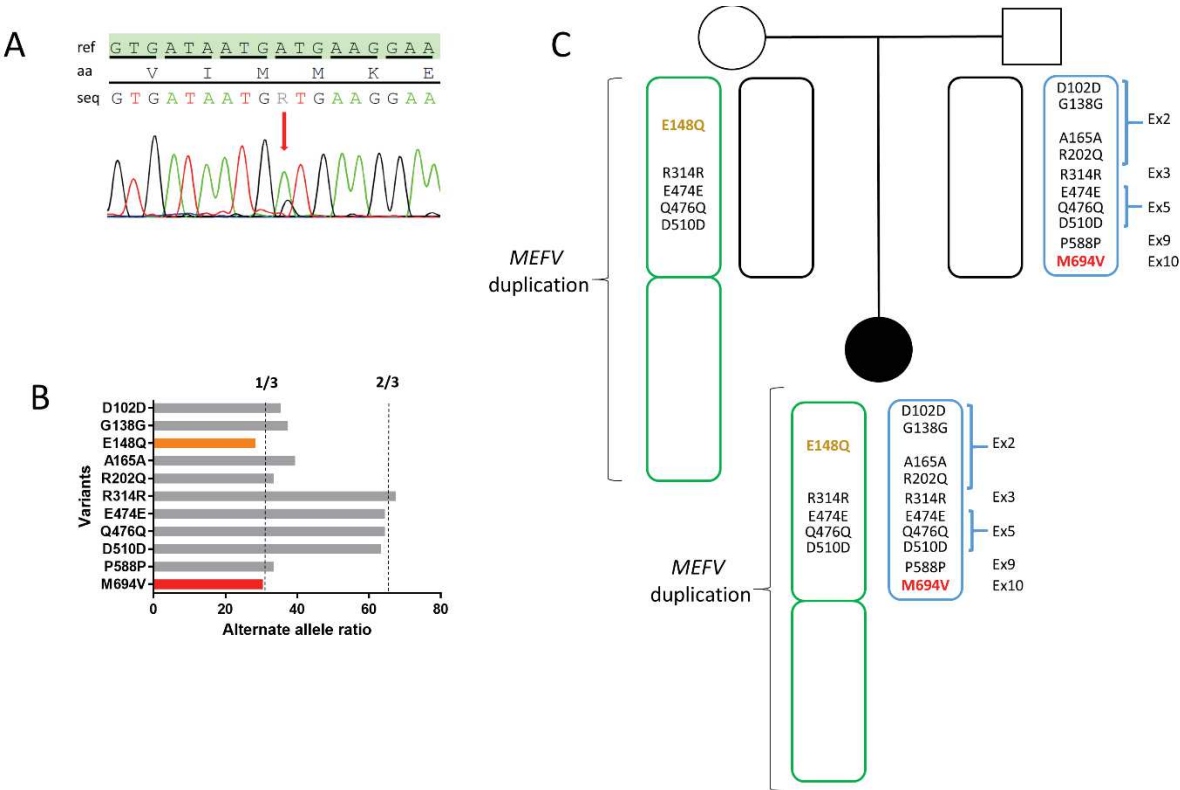


Figure 1: Allele unbalancing and familial variant segregation.

(A) *MEFV* Sanger electropherograms showing the unbalanced allele ratio in the proband for the p.Met694Val mutation. The red arrows indicate the mutations. ref: reference nucleotide sequence; aa: reference amino acid sequence; seq: patient nucleotide sequence. (B) Quantitative evaluation of proband alleles by NGS, showing 1/3 or 2/3 ratio. For reasons of clarity, we used in tables a short usual protein denomination as M694V for p.Met694Val. (C) Pedigree with the pathogenic mutation (in bold red), the VUS (in bold orange) and the synonymous variants (in black)

Just as Sanger sequencing, NGS demonstrated genetic unbalance (either 1/3 or 2/3) for all *MEFV* variants (figure 1B). Considering the variant allele fraction (1/3) of the p.Glu148Gln variants and others polymorphisms inherited from the mother (2/3), we deduced that the duplicated *MEFV* region was inherited from the mother and was wild-type (figure 1C).

Q-PCR analysis of multiple amplicons spanning from *ZNF200* (the 5' most adjacent gene) to the 3' end of *MEFV* confirmed the presence of 3 copies of this region in the proband versus 2 copies in healthy controls, supporting a heterozygous duplication encompassing *MEFV* and *ZNF200* (figure 2A). The adjacent genes *ORIF1* and *ZNF263* did not exhibit a supernumerary

copy. Q-PCR in the two asymptomatic parents confirmed that the patient's mother carried the duplication but not her father.

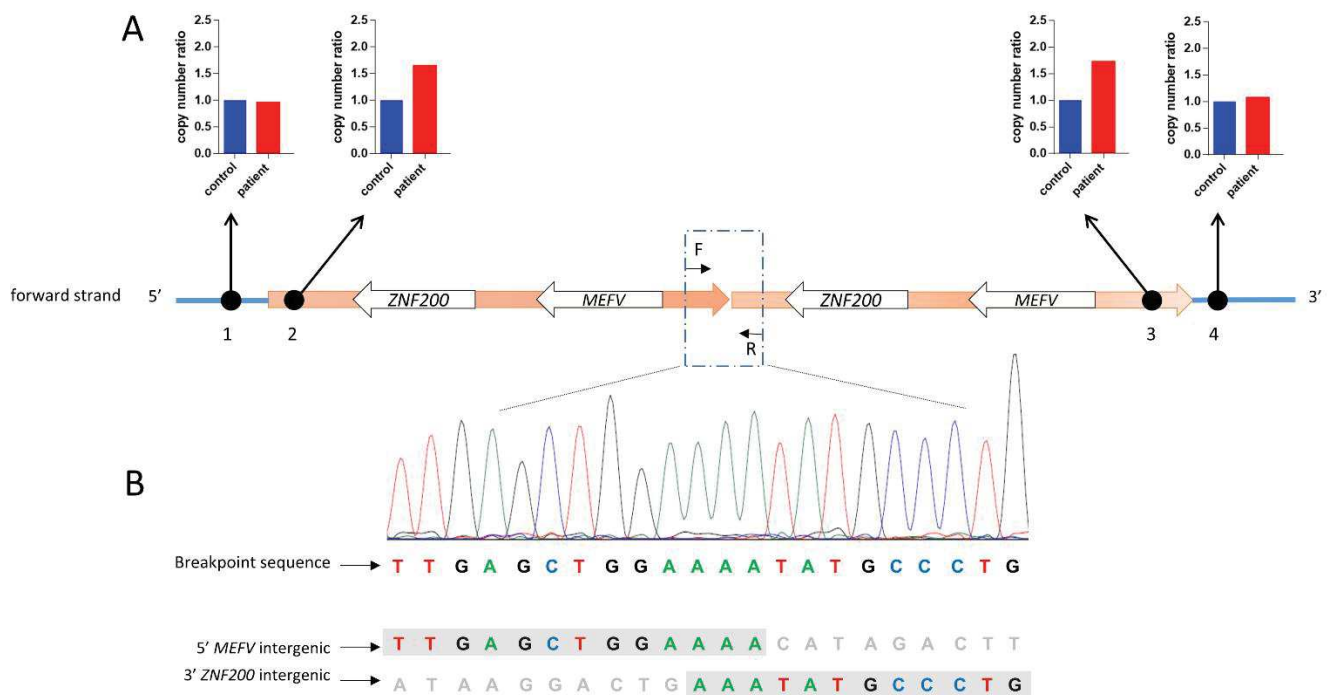


Figure 2: Tandem duplication and breakpoint sequencing.

(A) The orange arrows symbolize the tandem duplication and the white arrows represent the *MEFV* and *ZNF200* genes (both located on the reverse strand). The black circles indicate the regions studied by Q-PCR assays: 1 and 4 are located outside the duplication and show a patient's copy number ratio of 1 (2 copies); 2 and 3 are located inside the duplication and show a patient's copy number ratio of 1,5 (3 copies) (B) Zoom at the breakpoint on the Sanger sequence obtained with primers F and R. Alignment of the wild-type 5' *MEFV* and the 3' *ZNF200* intergenic sequences reveals an overlap at a polyA site.

Duplication characterization

The breakpoint of the duplication was successfully identified: the sequence of the 3' intergenic *ZNF200* region followed the sequence of the 5' intergenic *MEFV* region (figure 2B). We could therefore conclude that the rearrangement was a tandem duplication in direct orientation (i.e. the duplicated region was inserted into the genome next to the original sequence), and that its length was 64 179 pb. This genomic alteration was named chr16:g.3256171_3320350dup (hg19/GRCh37) according to the official nomenclature (<http://varnomen.hgvs.org/>) and was not found in the healthy controls database DGV (<http://dgv.tcag.ca>).

DISCUSSION

This is the first report of a *MEFV* duplication identified in a FMF patient. Several lines of evidence support this gene as responsible for her phenotype. Clinically, she exhibited autoinflammatory features including elevated acute phase reactants during flares. Other causes of inflammation such as infection or cancers have been eliminated. She fulfilled Livneh's criteria as she had typical attacks (fever $>38^{\circ}\text{C}$, more than 3 attacks, lasts 12 hours to 3 days) associated with incomplete abdominal pain (no peritonitis).(227) On the genetic side, she inherited one mutation clearly pathogenic and one non allelic VUS, a genotype classified as consistent with FMF diagnosis according to established consensus guidelines for the diagnosis of hereditary recurrent fevers.(216) She exhibited no alternative relevant pathogenic mutations when we sequenced a panel of 32 AID genes. Finally, the patient met the Eurfever/PRINTO classification criteria for FMF, with episodes lasting from 1 to 3 days, abdominal pain, and presence of a non-confirmatory – but consistent – genotype.(228) Altogether, our patient's ethnic, clinical and genetic data are consistent with a diagnosis of FMF.

This report highlights the necessity of analysing every gene variant when allelic unbalance is found by Sanger sequencing, and to conduct familial analysis. Indeed, *MEFV* exon 10 sequencing alone in our patient could have led to a false diagnosis of somatic mosaicism. Combined Sanger sequencing, panel sequencing and CNV analysis has allowed the proper diagnosis of *MEFV* duplication.

Tandem duplications are the most frequent (83%) CNVs in humans.(235) However, a previous multicentre study searching for CNVs in hereditary recurrent fevers did not highlight any *MEFV* rearrangements in 216 patients, demonstrating that such molecular events are more than rare in FMF.(233) In healthy controls, only 2 complete and one partial heterozygous *MEFV* duplications were identified and never at homozygous state (<https://gnomad.broadinstitute.org/>). This rearrangement has never been described with the same breakpoint in the database DGV. These data do not rule out a possible pathogenic effect of this CNV although duplications are particularly complex to interpret in absence of specific functional study.

Complex alleles and heterozygous severe mutations have been sometimes associated with FMF.(236,237) However, neither the patient's mother harbouring the heterozygous

p.Glu148Gln VUS and the *MEFV* duplication nor her father heterozygous for the p.Met694Val mutation exhibited FMF symptoms. Two hypotheses can be done regarding the impact of the genotype on the phenotype of our patient:

Based on recessive inheritance pattern and familial segregation, the inherited maternal chromosome should bear one pathogenic *MEFV* variant. The p.Glu148Gln VUS is frequent in the general population and controversial as whether it is a benign polymorphism or may have a non specific proinflammatory effect. (238) Therefore, the implication of the *MEFV* duplication, alone or in combination with p.Glu148Gln, as the second pathogenic allele in trans of p.Met694Val, cannot be ruled out. The duplicated region includes the *MEFV* promotor (located 1kb upstream of the start codon), and all exons, supporting that the additional copy encodes a normal protein. However, whether this duplication could lead to *MEFV* deregulation remains unclear. The familial segregation and the rarity of the duplication advocate for this hypothesis.

On the other hand, the duplication could be an epiphenomenon not related to the pathogenesis in our patient. FMF patients bearing only 50% pathogenic mutation such as p.Met694Val have been largely described. However, our patient would be the first with an FMF disease caused by only 1/3 of pathogenic mutation and, if assumed as a susceptibility factor, 1/3 of the p.Glu148Gln. The reason why individuals with less than two pathogenic variants do express the disease, whereas most carriers such as her father do not, is largely not understood and likely involves other genetic or environmental factors.

This report highlights the complexity of the genetic pathophysiology in FMF, which does not always follow the “pure” monogenic pattern. The involvement of the p.M694V is clearly associated with the disease, while the duplication could play a role in our patient phenotype through gene dosage effect or *MEFV* deregulation.

In conclusion, we pinpointed a novel and original molecular mechanism involved in a patient with FMF, yet unsolved in this case. Other case reports of *MEFV* CNVs and functional studies are necessary to further evaluate such rearrangement consequences on the patients.

SUPPLEMENTARY FIGURE

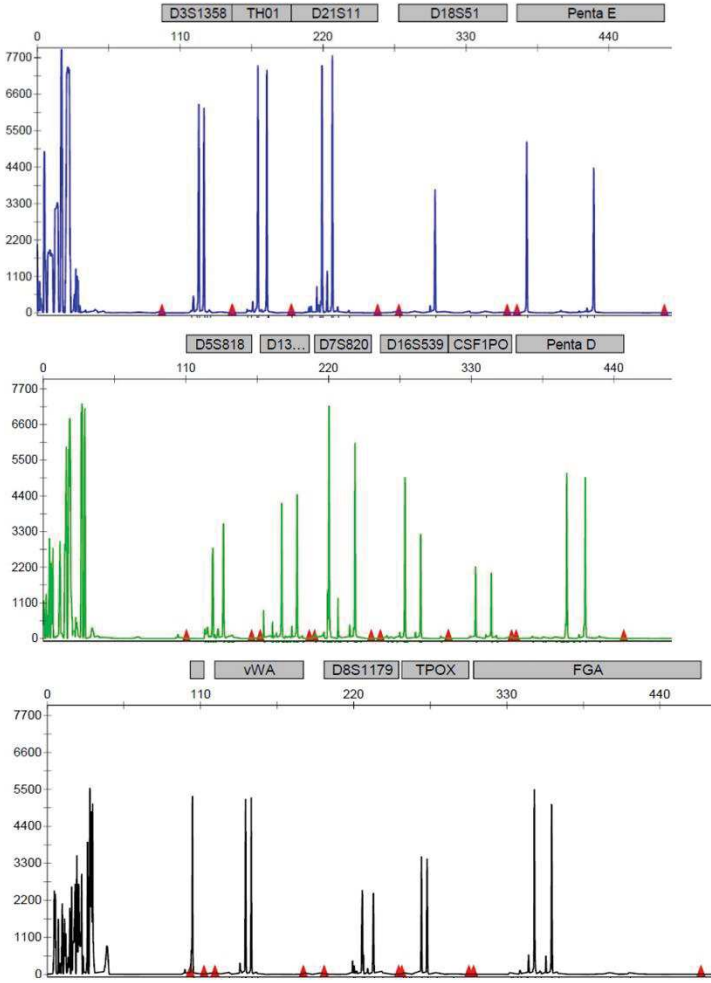


Figure S1: Analysis of 16 microsatellite loci in the proband
 They are split in 3 panels (blue, green and black). For each locus (upper grey squares) there is either one (homozygous) or two peaks (heterozygous).

Table S1 Details of the 32 SAID genes included in the NGS panel

Gene	Pathology	OMIM number	Transmission	NM
<i>CARD14</i>	PRP/PSORS2	# 173200/ # 173200	AD	NM_024110.4
<i>CASP1</i>	N/A		N/A	NM_001257118
<i>CECR1</i>	DADA2	# 615688	AR	NM_001282225.1
<i>IL10</i>	IL10 deficiency		AR	NM_000572.2
<i>IL10RA</i>	IL10 R1 deficiency	# 613148	AR	NM_001558.3
<i>IL10RB</i>	IL10 R2 deficiency	# 612567	AR	NM_000628.4
<i>IL1RN</i>	DIRA	# 612852	AR	NM_173841.2
<i>IL36RN</i>	DITRA	# 614204	AR	NM_173170.1
<i>LPIN2</i>	Majeed	# 609628	AR	NM_014646.2
<i>MDFIC</i>	N/A		N/A	NM_199072.4
<i>MEFV</i>	FMF	# 249100	AR	NM_000243.2
<i>MVK</i>	MKD	# 260920/ # 610377	AR	NM_000431.3
<i>NCSTN</i>	PASH		N/A	NM_015331.2
<i>NLRC4</i>	AIFEC/ FCAS4	# 616050/ # 616115	AD	NM_021209.4
<i>NLRP1</i>	NAIAD	# 617388	AD/AR	NM_033004.3
<i>NLRP12</i>	NAPS12	# 611762	AD	NM_144687.3
<i>NLRP3</i>	CAPS	# 120100	AD	NM_001243133.1
<i>NOD2</i>	Blau	# 186580	AD	NM_022162.2
<i>PLCG2</i>	APLAID	# 614878	AD	NM_002661.4
<i>POMP</i>	PRAAS/CANDLE	# 601952	AD	NM_015932.5
<i>PSENNEN</i>	N/A		N/A	NM_172341.2
<i>PSMA3</i>	PRAAS/CANDLE		AR/digenic	NM_002788.3
<i>PSMB4</i>	PRAAS/CANDLE		AR/digenic	NM_002796.2
<i>PSMB8</i>	PRAAS/CANDLE	# 256040	AR/digenic	NM_148919.3
<i>PSMB9</i>	PRAAS/CANDLE		AR/digenic	NM_002800.4
<i>PSTPIP1</i>	PAPA	# 604416	AD	NM_003978.4
<i>RBCK1</i>	PBMEI	# 615895	AR	NM_031229.3
<i>SH3BP2</i>	Cherubism	# 118400	AD	NM_003023.4
<i>SLC29A3</i>	H syndrom	# 602782	AR	NM_018344.5
<i>TNFRSF11A</i>	TRAPS11		AD	NM_003839.3
<i>TNFRSF1A</i>	TRAPS	# 142680	AD	NM_001065.3
<i>TNFRSF9</i>	N/A		N/A	NM_001561

Legend:

N/A	Candidate genes
AD	Autosomal dominant
AR	Autosomal recessive
AIFEC	Autoinflammation with infantile enterocolitis
APLAID associated	Autoinflammation, antibody deficiency, and immune dysregulation, PLCG2-
CAPS	Cryopyrin associated periodic syndrome
DADA2	Adenosine deaminase 2 deficiency
DIRA	Interleukin 1 receptor Antagonist deficiency
DITRA	Interleukin 36 receptor antagonsit deficiency
FCAS4	Familial cold autoinflammatory syndrome 4
FMF	Familial mediterranean fever
MKD	Mevalonate Kinase deficiency
NAIAD	NLRP1-associated autinflammation with arthritis
NAPS12	NLRP12 associated periodic syndrome
PAPA	Pyogenic Arthritis, Pyoderma gangrenosum, and Acne
PASH	Pyoderma gangrenosum, acne and suppurative hidradenitis
PBMEI	Polyglucosan body myopathy 1 with or without immunodeficiency
PRAAS/CANDLE Neutrophilic Dermatitis with Lipodystrophy and Elevated Temperature	Proteasome associated autoinflammatory syndrome / Chronic Atypical
PRP/PSORS2	Pytiriasis rubra pilaris / Psoriasis 2
TRAPS	TNF receptor associated periodic syndrome
TRAPS11	TNFRSF11A-associated hereditary fever disease

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Mosaicism in autoinflammatory diseases: cryopyrin-associated periodic syndromes (CAPS) and beyond. A systematic review.

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Abstract

Purpose: Autoinflammatory diseases (AIDs) are conditions related to defective regulation of the innate immune system. Sanger sequencing of the causative genes has long been the reference for confirming the diagnosis. However, for many patients with a typical AID phenotype, the genetic cause remains unknown. A pioneering study in 2005 demonstrated mosaicism in patients with cryopyrin-associated periodic syndromes (CAPS). The authors found somatic mosaicism in 69% of their cohort of Sanger-negative CAPS patients. We aimed to address the extent to which mosaicism contributes to the etiology of AIDs and its impact on phenotype.

Methods: We retrieved English-language publications reporting mosaicism in AIDs by querying PubMed with no restriction on date, and surveyed French reference centres.

Results: We provide a comprehensive clinical and genetic picture of mosaicism in AIDs. We estimated that the proportion of CAPS-like patients presenting mosaicism ranged from 0.5% to 19%. We also discuss the possible links between the proportion of mutated alleles and various clinical features.

Conclusions: This review re-evaluates the contribution of mosaic DNA variants in CAPS. Mosaicism might be more common than anticipated in other AIDs. No significant difference was demonstrated between variant allele frequency and clinical phenotype. Such knowledge has implications for the development of guidelines for genetic diagnosis, genetic counseling of affected families, and effective patient care.

Key Words: CAPS; Mosaicism; Autoinflammatory disease; Systematic review

Introduction

Autoinflammatory diseases (AIDs) are hereditary or non-hereditary conditions related to defective regulation of the innate immune system [1]. They mainly result from increased inflammasome-mediated production of the cytokine interleukin 1 β and from abnormal NF- κ B signalling, ubiquitination or type I interferon production [2]. The symptoms presented by patients with AIDs include recurrent fever, urticarial rash, arthritis and severe neurologic complications and are often overlapping. Hence, early and correct diagnosis is sometimes difficult. In addition, there is a continuum among AIDs, primary immunodeficiency and autoimmune diseases, and patients with more or less prominent autoinflammatory, autoimmune, and/or immunodeficiency signs have been described [3, 4]. Patients with AIDs have long been empirically treated with non-specific anti-inflammatory drugs. The identification of mutations in the causative genes provides a pathophysiological basis for the initiation of relevant and targeted therapy.[5] Interest in AIDs has increased over the years, as many novel genes have been identified with the advances in next-generation sequencing (NGS) approaches [6].

As a result of these discoveries, clinicians invested much hope in genetic diagnosis, which gradually cooled because its performance did not improve as expected. Indeed, for many patients with a typical AID phenotype, the genetic cause remained unsolved, which suggests that other genes remain to be discovered [7, 8, 9, 10, 11]. As an example, a subset of patients with inflammatory symptoms and urticarial rash resembling cryopyrin-associated periodic syndromes (CAPS) were found to harbor no pathogenic mutation in NLR family, pyrin domain containing 3 (*NLRP3*), the CAPS causative gene [12]. NLRP3 is the sensor component of the NLRP3 inflammasome, a polymeric complex formed following the assembly of several effector and adapter proteins in response to pathogens and other damage-associated signals [13, 14, 15, 16].

Exome sequencing performed in these “CAPS-like” patients revealed mutations in three genes not previously known to be responsible for AIDs (i.e., NLR family, pyrin domain-containing 12 [*NLRP12*], phospholipase C gamma 2 [*PLCG2*], and NLR family, caspase recruitment domain-containing 4 [*NLRC4*]) [17, 18, 19, 20]. These new diseases were called familial cold autoinflammatory syndrome (FCAS) 2, FCAS3 and FCAS4, respectively, in the Online Mendelian Inheritance in Man registry (OMIM, omim.org) to mimic the name of the mildest CAPS phenotype, FCAS or FCAS1 [21]. However, only a handful of patients with mutations

in these novel genes have been reported, which supports that genetic heterogeneity accounts for only a few patients with a CAPS-like phenotype.

In a pioneering study in 2005, Saito et al. identified a c.1709A>G (p.Y570C) mutation located in exon 3 of the *NLRP3* gene in a patient with neonatal urticarial rash, arthritis of the knee, chronic meningitis, and papilloedema [22]. This result supported the diagnosis of chronic infantile neurologic, cutaneous, articular syndrome (CINCA), the most severe CAPS phenotype. The unaffected parents and sister did not carry this mutation, which was thus considered *de novo*. In 2005, only conventional Sanger sequencing was available for routine genetic diagnosis. The investigators noted that the intensity of the mutated G at position 1709 was considerably and reproducibly lower than that of the A at this position. They postulated that this low frequency, as compared with the expected 50%, might result from somatic mosaicism. To test this hypothesis, the authors subcloned exon 3 of *NLRP3* from the patient's whole blood and found a 16.7% allele frequency of the c.1709A>G mutation. This discovery revealed that only a few affected cells were sufficient to generate a *bona fide* CAPS disease with neonatal onset. Therefore, geneticists hypothesized that these "Sanger-negative" patients actually had a reduced frequency of mutated alleles.

This inaugural article by Saito et al. was followed by many others demonstrating the involvement of somatic mosaicism in CAPS-like patients [22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35]. Tanaka et al. found that this molecular mechanism could explain up to 69% of CINCA patients in whom no germline mutation in *NLRP3* can be identified by Sanger sequencing. These patients may actually harbor low amounts of *NLRP3* mutations undetectable by this technique. Time-consuming subcloning strategies are not amenable to routine diagnosis. Therefore, deep or massively parallel sequencing quickly replaced conventional sequencing to speed up the genetic diagnosis of such patients [26].

First proven in young CINCA patients, somatic mosaicism was then identified in patients with the Muckle-Wells syndrome (MWS), an intermediary CAPS-type syndrome [29], then in older patients including two with a clinical diagnosis of Schnitzler syndrome [28]. Of interest, somatic mosaicism has since been found in other forms of AIDs such as Blau syndrome [36]. Rare cases of combined germline and somatic mosaicism have also been reported [37, 38]. Chimerism was described only once, in a patient with idiopathic aplastic anemia who underwent allogenic bone-marrow transplantation from his FMF brother [39].

In this article, we aim to provide a comprehensive clinical and genetic picture of mosaicism in AIDs. We systematically reviewed all published cases of mosaicism reported to date and added newly diagnosed cases from French reference centers. We also address the possible correlations between the proportion of mutated alleles and various clinical features and discuss the impact of mosaicism on patient care.

Patients and methods

2.1 Identification of patients with mosaicism

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations to conduct this systematic review [40, 41]. We retrieved English-language publications reporting mosaicism in AIDs by querying MEDLINE via PubMed (<https://www.ncbi.nlm.nih.gov/pubmed>) with the following algorithm: (mosaic* OR somatic) AND (autoinfl* OR NLRP3 OR CIAS1 OR NOD2 OR MVK OR MEFV OR TNFRSF1A OR NLRC4 OR TMEM173 OR TNFAIP3), with no restriction on date.

Exclusion criteria were papers that were off-target, editorials or reviews, studies that were essentially experimental, and those already describing published cases (redundant article). We also screened the references mentioned in the selected articles. In addition, we surveyed the French national rare disease network on autoimmune and autoinflammatory diseases (FAI2R) in April 2017 to identify unreported cases of mosaicism in AID patients. Comprehensive demographic, clinical and genetic data were extracted from the selected publications and patient records and were blindly double-checked by one author (IT). Investigators were directly contacted if necessary.

Figure 1 describes the 22 initial publications resulting from the search of PubMed (CAPS, N=15; other AIDs, N=7). We identified three more published cases of mosaicism from references in these articles: one patient with a *TMEM173* mutation, and two asymptomatic parents with a *TNFAIP3* mutation. Finally, we retrieved five additional cases (CAPS, N=4; HA20, N=1) from the French consultations [42].

2.2 Statistics

Mean allele frequencies according to the different symptoms were analysed by non-parametric Wilcoxon test. Correlation of mosaic rates with age at onset was analysed by Spearman's correlation coefficient. Comparison of the rate of symptoms by existence of a p.E567K or Q mutation involved the chi-square or Fisher exact test as appropriate. Analyses involved using

SAS v9.4 (SAS Institute, Cary, NC, USA), with two-sided $p < 0.05$ considered statistically significant.

RESULTS

3.1 Description of patients

We retrieved reports of 64 individuals carrying somatic or germline mosaicism in one AID gene (Figure 1). We addressed the *NLRP3* cases first because these patients are the most numerous and described patients harbouring the other genes separately. Table 1S shows extensive demographic, clinical and genetic data for all known individuals with *NLRP3* mosaicism: 49 patients and one asymptomatic carrier mother described in the literature and four unpublished cases recruited in France. We sorted the patients by phenotype when the information was available. Indeed, the clinical boundaries between the three classical phenotypes FCAS, MWS and CINCA are blurred, and geneticists often prefer the global definition of CAPS, which echoes the pathophysiology of the disease rather than the phenotype. We found no records for FCAS. Table 1S also describes two patients diagnosed with Schnitzler syndrome, a rare systemic AID characterized by chronic neutrophilic urticaria and monoclonal IgM component. De Koning et al. suggested that these two patients actually had a variant of CAPS [28].

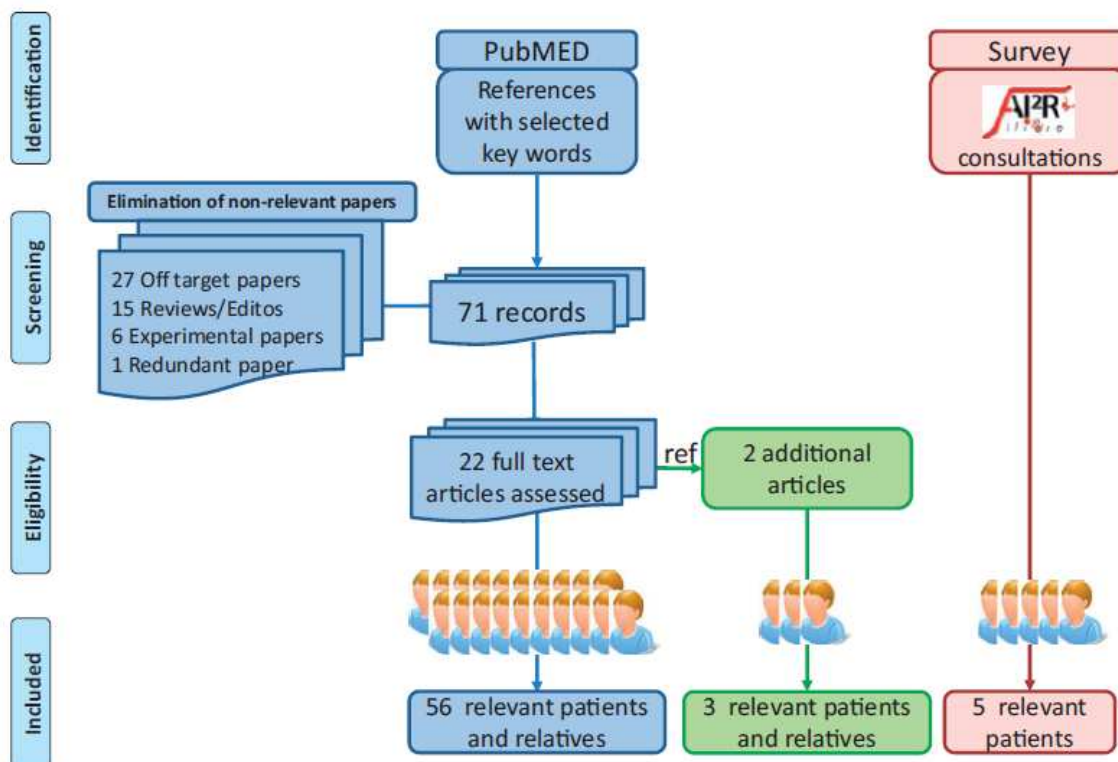


Figure 1: Flow diagram of the identification of patients with mosaicism in autoinflammatory disease (AID) genes.

We used the PRISMA recommendations. Two different sources were used: references from the PubMed database and a survey of colleagues from French consultations for cases.

This compilation naturally led us to investigate a possible link between the proportion of mutated alleles and the different clinical characteristics of patients (Table 2S). We found no convincing phenotype–genotype correlations. The median age at disease onset was 0.5 years (interquartile range: 0–38), and the mean age was 16. Papilloedema was the only qualitative factor significantly correlated with mosaicism ($p=0.02$) but surprisingly showed an inverse relation with the proportion of mutated alleles. Epilepsy, urticaria, typical facies, headache and arthritis were associated but not significantly, the latter two items also showing an inverse relation. These results are contradictory and need to be reassessed with a specific study. Figure 2 illustrates the mean proportion of mutated alleles by presence or absence of the main CAPS symptoms in patients with *NLRP3* mosaicism.

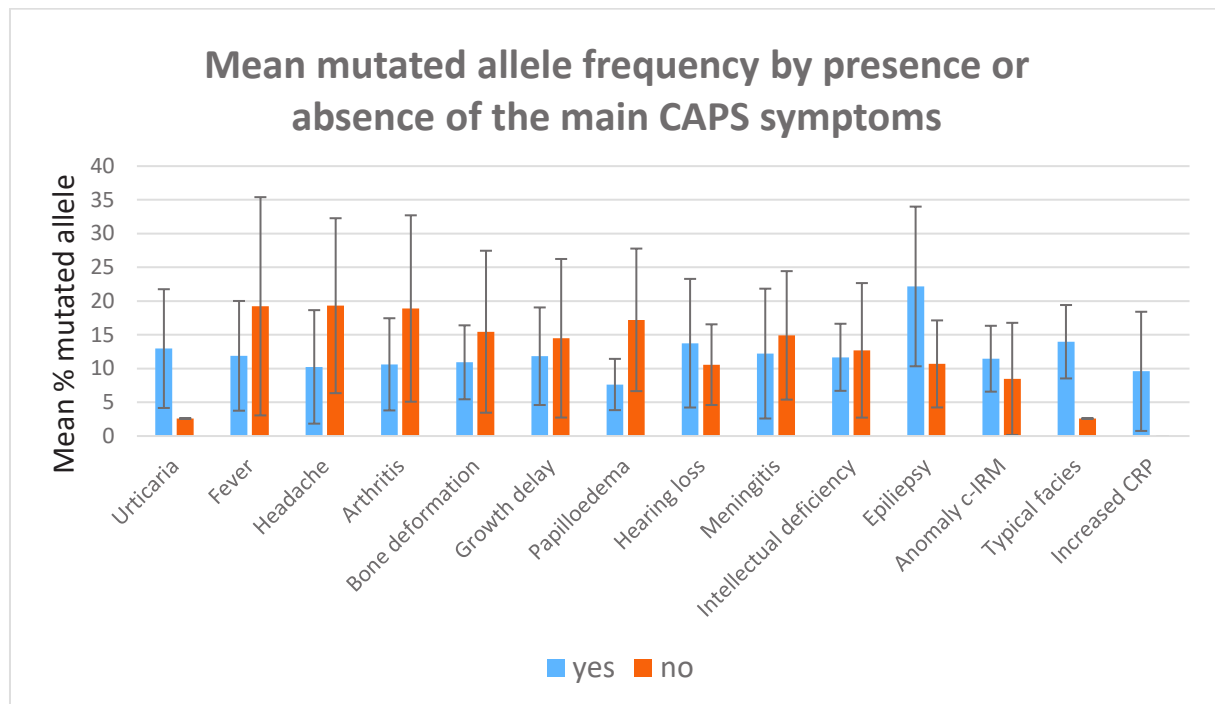


Figure 2: Mean frequency of mutated alleles by presence or absence of cryopyrin-associated periodic syndromes (CAPS).

Proportion of presence (blue bars) or absence (orange bars) of each investigated symptom found in the patients described in table 1S (whole blood). Data are mean±SD. Crude data are provided in table 2S.

Mosaicism is well documented in *NLRP3*, but much less known in other AIDs. Tables 2 and 3S describe cases of non-*NLRP3* mosaicism in eight non-CAPS patients (*MEFV*: N=1 [43], *NLRC4*: N=2 [44, 45], *NOD2*: N=2 [36, 46], *TMEM173*: N=1 [47], *TNFAIP3*: N=1 [37]), *TNFRSF1A*: N=1 [48]. The clinical manifestations were without peculiarities, except for late onset and absence of tenosynovitis and joint deformities for the two Blau patients. Moreover, germline mosaicism was demonstrated in two families with A20 haploinsufficiency because low levels of *TNFAIP3* mutations were detected in asymptomatic parents [37].

3.2 Spectrum of *NLRP3* mosaic mutations

We examined the spectrum and distribution of the mutations associated with *NLRP3* mosaicism (Table 1). Thirty-five different mutations were identified; 23 were novel (66%). All mutations but p.G755R were located on exon 3. We highlighted two putative mutational hotspots. Six mutations lay between codons 302 and 307 in the NOD domain, and 11 were confined to a short segment (codons 563-570) between the NOD and LRR domains. The most common mutation was p.E567K (c.1699G>A), responsible for one case of late-onset undefined CAPS, three cases of MWS, and three cases of CINCA. We found no association between the presence of

mutations at this position and phenotype (data not shown). The frequency of the p.E567K allele did not seem to significantly affect the clinical characteristics of these patients because the proportion of the mutated allele in whole blood did not differ substantially among patients (range: 5.4–16.8%). We lack enough data from isolated cell types to investigate the impact of the cell subtype containing the mutation.

Tanaka et al. found that up to 69% of CINCA patients may actually harbour low amounts of *NLRP3* mutations undetectable by Sanger sequencing [25]. To estimate this rate in all available studies, we counted the total number of patients investigated in series describing patients with a CAPS phenotype and apparently negative Sanger-sequencing results (Table 2). We excluded case reports because they might introduce a selection bias. The proportion of reported "Sanger-negative" CAPS-like patients who were found to carry a mosaic *NLRP3* mutation was re-evaluated at 19% (44/231). In two specific laboratories, the actual rate of detection of *NLRP3* mosaicism in patients referred for genetic diagnosis of CAPS is much lower, about 0.5%. In one lab (DR, personal communication), mosaic variants were found in 10 of the 2238 patients tested, including the 8 late onset described in reference [35], and two patients with early-onset CAPS carrying the p.T347I and the p.E526V *NLRP3* variants (not shown). In the second lab, the two patients with mosaic variants (Fam5565 and Fam6354, table 1S) were detected among 438 patients tested (GS, personal communication). However, some of these referred patients may have atypical symptoms or have non-AID conditions.

3.3 Paper highlights

Interesting points were revealed during the review (Table 2).

“First times”

Saito et al., in 2005, were the first to report *NLRP3* somatic mosaicism in a patient with CINCA syndrome [22]. The first description of the implementation of whole-exome sequencing for detecting *NLRP3* somatic mosaicism was by Omoyinmi et al., in 2014, in a CINCA “mutation-negative” patient [27]. De Koning et al., in 2015, demonstrated that somatic mosaicism was confined to the myeloid lineage in two patients with a non-malignant disorder [28]. Jiménez-Treviño et al., in 2013, first revealed vertical transmission of a somatic *NLRP3* mutation (p.T348M) in CAPS [38]. Moreover, the authors hypothesized that the mutational event probably occurred early during embryogenesis because the somatic mutation was present in the mother’s cells from different embryonic origins. Broadening the spectrum of autoinflammatory

diseases caused by somatic mosaicism (table 2), Liu et al. in 2014, De Inocencio et al. and Shinar et al., in 2015, Rowczenio et al. in 2016, Kawasaki and Liang et al. in 2017, and Kadowaki et al. in 2017 provided the first demonstration of somatic *TMEM173* [47], *NOD2* [46] and *MEFV* [43], *NLRC4* [44, 45], and *TNFAIP3* [37] mutations. Mensa-Vilaro and Rowczenio, in 2016, were the first to describe germline mosaicism in a gene other than *NLRP3* [36, 48].

Phenotype–genotype correlation

Several authors searched for possible specific characteristics of patients with mosaic somatic mutations by comparison with patients with germline mutations.

Onset of the disease

The patients described by Zhou [31], Mensa-Vilaro [33], and Rowczenio [35] (5/8 of the series) and our Fam6354 patient were older than 50 years when the first symptoms occurred. Late onset was also described in the Schnitzler (De Koning et al. [28]) and Blau syndromes (de Inocencio et al. [46]), which supports several late-onset sporadic diseases possibly sharing a genetic basis.

Clinical constellation

Tanaka et al. addressed the important question of how *NLRP3* somatic mosaicism modifies the clinical presentation of CINCA [25]. Overall, the authors observed a milder neurologic involvement in patients with somatic mosaicism. However, they noted no difference in other factors such as age at disease onset, skin symptoms, joint involvement, or response to interleukin 1 blockade.

Nakagawa et al. detected no significant difference in the main clinical features: fever, urticaria-like rash, joint, neurological and ocular involvements, and deafness in CAPS patients with *NLRP3* somatic mosaicism versus patients with germline mutations [29].

Table 1. Mutations associated with NLRP3 mosaicism in patients with a cryopyrin-associated periodic syndrome (CAPS)-like phenotype.

Nucleotide change	Amino-acid change	Asymptomatic	Schnitzler	CAPS	MWS	CINCA	TOTAL
c.779G>C	p.R260P				1	1	2
c.790C>T	p.L264F					1	1
c.791T>C	p.L264P				1		1
c.906C>A	p.F302L					1	1
c.907G>C	p.D303H					4	4
c.908A>C	p.D303A				1		1
c.919G>A	p.G307S					1	1
c.920G>A	p.G307D			1			1
c.920G>T	p.G307V					1	1
c.1000A>G	p.I334V				1		1
c.1043C>T	p.T348M	1					1
c.1054G>A	p.A352T			1			1
c.1064A>C	p.K355T				1		1
c.1065A>T	p.K355N					1	1
c.1216A>G	p.M406V					1	1
c.[1231C>T;1233G>T]	p.L411F				1		1
c.1298C>T	p.T433I					3	3
c.1303A>G	p.K435E		1				1
c.1315G>C	p.A439P					1	1
c.1569C>A	p.F523L				1		1
c.1569C>G	p.F523L		1				1
c.1688A>G	p.Y563C			3		1	4
c.1690G>A	p.G564S					1	1
c.1691G>A	p.G564D			1			1
c.1698C>A	p.F566L					3	3
c.1699G>A	p.E567K			1	3	3	7
c.1700G>C	p.E567Q			1			1
c.1704G>C	p.K568N					1	1
c.1706G>T	p.G569V			1			1
c.1708T>A	p.Y570N				1		1
c.1708T>C	p.Y570H			1			1
c.1709A>G	p.Y570C				1	1	2
c.1792A>T	p.I598F					1	1
c.1906C>G	p.Q636E				1		1
c.2263G>A	p.G755R					2	2

TOTAL	1	2	10	13	28	54
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The most frequent mutation and the mutational hot spots are framed.

Novel mutation are in bold letters.

Mutated allele frequency over time

Rowczenio et al. pointed to a unique genetic evolution of the mutated allele in a CAPS patient with *NLRP3* mosaicism [35]. The patient had been healthy until age 46 years. Her symptoms became progressively more severe over 12 years. The authors performed a time-course study using blood samples obtained at the initial assessment in 2004 and then in 2013 and 2016, which revealed an increase in the mutated allele frequency, from a baseline of 5.1% (2004) to 27% (2013) and 45% (2016). They did not observe this phenomenon in their other patients followed up for a median of only 3 years. A possible increase in mosaicism rate was addressed in two other reports. Jiménez-Treviño et al. noted that the frequency of the mutated allele remained identical in blood samples obtained over a 9-year period in an asymptomatic mother carrying *NLRP3* mosaicism [38]. Shinar et al. tested a DNA sample preceding by 4 years the onset of fever in a patient with late FMF symptoms [43]. In the early sample, the mutated *MEFV* variant was repeatedly observed at a negligible level but became clearly visible with a Sanger approach at the time of the fever.

Table 2. Key information from papers on mosaicism in AIDs

Reference		Gene	Type	Patients with mosaicism			Original content of the publication				
#	First author	Year		N	Total examined in the series	%	First description of	Functionnal studies	Evidence of gonosomal mosaicism	Phenotype genotype correlation	Increase % mosaicism overtime
22	Saito	2005	<i>NLRP3</i>	Case report CINCA, Sanger small peak	1	1	100%	<i>NLRP3</i> mosaicism	Cell death and NFKB activation		
23	Saito	2008	<i>NLRP3</i>	Case reports CAPS, Sanger negative	3	3	100%	<i>NLRP3</i> mutations cause cell death	Cell death and NFKB activation		
24	Arostegui	2010	<i>NLRP3</i>	Case report CINCA, Sanger negative	1	1	100%				
25	Tanaka	2011	<i>NLRP3</i>	Series CINCA, Sanger negative	18	26	69%		Cell death and NFKB activation	Lower incidence of mental retardation in patients with mosaicism	
26	Izawa	2012	<i>NLRP3</i>	Series CAPS, Sanger negative	4	10	40%	Routine pipeline to detect low level <i>NLRP3</i> mosaicism	Cell death and NFKB activation		
38	Jimenez-Trevino	2013	<i>NLRP3</i>	Familial study of apparently de novo CAPS cases	1	4	25%	Gonosomal mosaicism <i>NLRP3</i>		Asymptomatic mother with mosaicism	No
27	Omoyinmi	2014	<i>NLRP3</i>	Case report CINCA, Sanger small peak	1	1	100%	Use of WES			
28	De Koning	2015	<i>NLRP3</i>	Series Schnitzler, candidate gene screening	2	11	18%		Spontaneous production of IL6 and IL1B		
29	Nakagawa	2015	<i>NLRP3</i>	Series MWS, Sanger negative	6	56	11%		ASC activation, THP1 cell death	No difference between germline and somatic	

30	Paloni	2015	<i>NLRP3</i>	Case report CINCA, Sanger negative	1	1	100%	Delayed relapse after the withdrawal of anti-IL1 β therapy		
31	Zhou	2015	<i>NLRP3</i>	Case report, late onset CAPS Sanger negative	1	1	100%			
32	Eroglu	2016	<i>NLRP3</i>	Series CAPS, Sanger negative	2	14	14%		ASC activation, THP1 cell death	
33	Mensa-Vilaro	2016	<i>NLRP3</i>	Case report	1	1	100%	Late onset CAPS	ASC activation, THP1 cell death	
34	Lasiglie	2017	<i>NLRP3</i>	Series CAPS, Sanger negative	4	14	29%			
35	Rowczenio	2017	<i>NLRP3</i>	Series late onset CAPS, Sanger negative	8	100	8%		ASC activation, Cytokine profile 5.1% (2004), 27% (2013), 45% (2016)	
Total series <i>NLRP3</i>					44	231	19%			
43	Shinar	2015	<i>MEFV</i>	Case report postpolycythemia myelofibrosis	1	1	100%	Acquired <i>MEFV</i> mosaicism	Expansion over 4 years	
44	Kawasaki	2017	<i>NLRC4</i>	Case report CINCA like, Sanger negative	1	1	100%	<i>NLRC4</i> mosaicism Use of iPSC	Cytokine profile in iPSC	
45	Liang	2017	<i>NLRC4</i>	Case report pregnant woman with ultrasound abnormalities	1	1	100%	Foetus	Protein conformational analysis	
46	De Inocencio	2015	<i>NOD2</i>	Case report Blau, Sanger small peak	1	1	100%	<i>NOD2</i> mosaicism		
36	Mensa-Vilaro	2016	<i>NOD2</i>	Family report	1	3	33%	Gonosomal mosaicism <i>NOD2</i> Late onset Blau	Vertical transmission to two sons	
	Liu	2014	<i>TMEM173</i>	Series of undifferentiated	1	6	17%			

47				AID patients				<i>TMEM173</i> as a cause of AID	Investigation of the IFN pathway
37	Kadowaki	2017	<i>TNFAIP3</i>	Series HA20	2	30	7%	Gonosomal mosaicism <i>TNFAIP3</i>	Asymptomatic mother and father with mosaicism
48	Rowczenio	2016	<i>TNFRSF1A</i>	Case report TRAPS	1	1	100%	Gonosomal mosaicism <i>TNFRSF1A</i>	Sperm positive

WES: Whole exome sequencing; iPSC: Induced pluripotent stem cells; AID: Autoinflammatory disease; MAF: Minor allele frequency

In bold letters: series included in the evaluation of the rate of *NLRP3* mosaicism in CAPS-like patients "Sanger negative"

Discussion

Rare AIDs have long been considered inherited diseases with a classical mode of inheritance (e.g., recessive in FMF or dominant in CAPS). The first discovery of a patient with a somatic mosaic mutation was about 13 years ago [22]. Since then, mutations with allelic frequency less than 50% have also been identified in other AID genes, mainly in the myeloid lineage.

Although not suspected for years, mosaicism was found to account for up to 69% of CINCA patients who were negative for mutations on Sanger sequencing [25]. In a study of MWS, this mutational mechanism was detected in only 12% of the patients [29]. In this review, we provide a more general estimate for *NLRP3* mosaicism, about 19%. We believe that this rate is probably overestimated because by essence, the publications we reviewed concerned only series including at least one patient with *NLRP3* mosaicism. This hypothesis is further supported by the low yield of patients with mosaicism detected in laboratories with long-term experience in deep sequencing such ours. A prospective study is warranted to properly assess the true involvement of this molecular mechanism in AID patients. Systematic approaches using NGS will provide this response shortly as this sequencing technique progressively replaces Sanger sequencing in most genetic laboratories. Nevertheless, because *NLRP3* mosaicism is an important molecular mechanism responsible for CAPS, deep sequencing is recommended for the genetic diagnosis of all patients with a CAPS phenotype, which could also be valid for all other AIDs.

Two thirds of the sequence variants we describe were novel. The pathogenicity of these variants was not systematically assessed in familial and/or functional studies, and some might actually be simple polymorphisms. Nevertheless, considering the best-known example of CINCA, how only a low frequency of true gain-of-function mutations can result in neonatal disease is puzzling and remains to be understood. The type, rate or cellular location of the mutation are among the hypotheses. As in classical CAPS, almost all mutations were located in exon 3. However, the regions surrounding codon 303 and 567 may be more sensitive to mosaicism. The most common mutation was p.E567K (Table 1). Nakagawa et al. suggested that with germline status, mutations in hot-spot regions of the gene might be incompatible with life [29].

We hypothesized a possible relation between the rate of mosaicism and the clinical expression of the disease. We reveal an apparent inverse association between mutation frequency and presence of papilloedema. This link remains to be confirmed and if so, the underlying pathophysiological mechanism remains to be elucidated. We could not demonstrate a

significant association with any of the other clinical features examined. This is relatively consistent with most individual studies. The interesting hypothesis raised by Tanaka et al., that the difference in the severity of neurologic manifestations depends on the level of mosaicism, is unlikely considering our findings. One of the potential limitations of this study is its lack of power. Given the small sample size of documented items, concluding with certainty on the lack of association between the studied items and the mean mutated allele frequency is difficult.

Another hypothesis is that phenotypes may be also shaped by the specific tissue location of the mutation. De Koning et al. demonstrated myeloid lineage-restricted somatic mosaicism of *NLRP3* mutations in patients with variant Schnitzler syndrome [28]. Rowczenio et al. also showed the same trend, finding that the frequency of mutated allele was highest among neutrophils and monocytes (median 12.3% and 11%, respectively) as compared with other DNA sources (median 4.9%) [35]. In contrast, Tanaka et al. demonstrated no significant difference in mutation frequency between neutrophils, T cells, B cells, monocytes, and buccal cells [25]. The authors suggested that the mutational event leading to mosaicism probably arose during early embryogenesis, before the pluripotent stem cells committed to hematopoietic progenitor stem cells or ectoderm-derived non-hematopoietic cells. None of the other reports demonstrated a clear difference of mutation rate by cell origin (Table 1S).

These new discoveries have a significant impact on the indication of the genetic test and its interpretation. Early age at onset has long been an important criterion before posing the diagnosis of hereditary AID because genes with a strong effect are expected to be expressed at birth or soon after. Indeed, in a national epidemiological study conducted in 2012 [49], we found that 86% of genetically confirmed CAPS patients showed symptoms before age 10, which prompted us to establish early age at disease onset as one of the prerequisites for ordering genetic analysis of the *NLRP3* gene. This study was based on Sanger sequencing, and mosaicism was not investigated at that time. In the present review, focused on mosaicism, a significant number of patients were over age 30 years. Although we cannot rule out that this observation may be due to the fluctuation of the cellular subset carrying the mutation in whole blood, an increase in the mutated allele frequency over time was detected in two studies. For these reasons, we are now reconsidering the prerequisite "early age at onset" in patients with a convincing AID clinical and biological phenotype, even if no association between the age of the symptoms and frequency of the mutated allele was demonstrated.

The interpretation of the family trees and the sequencing results are two essential steps for assessing the risk of transmission and penetrance of the disease. The search for mosaicism in asymptomatic parents of patients with apparent *de novo* mutations is fundamental for proper genetic counseling of the family because of the risk of recurrence in siblings. The risk is very low in the case of genuine somatic mosaicism in the proband. However, it is ethically and technically difficult to exclude the existence of mutation in gonadal tissues. Detection of mosaicism also has implications for patient care, treatment and monitoring.

Conclusion

This is the first review providing a complete picture of mosaicism in AIDs to date. This genetic mechanism was first detected in a CINCA patient. Mosaicism was subsequently confirmed in a proportion of "Sanger-negative" CAPS-like patients, that we are now reevaluating to range between 0.5% and 19%. Mosaicism might also be more common than anticipated in other AIDs. Because of the increasing awareness among geneticists and clinicians, it has been looked for more often and found in several AIDs. A correct appraisal will undoubtedly be obtained by systematic NGS approaches, which should now represent the gold-standard technique for genetic diagnosis of most patients with an AID phenotype. The spectrum of mutations as well as the associated patient's phenotype will certainly be revisited, and we will learn much about the real impact of non-classical modes of inheritance in AIDs such as somatic versus germline mosaicism, digenism, and incomplete penetrance. Such knowledge also has implications for the development of guidelines and decision trees for genetic diagnosis, genetic counseling of affected families, and patient care.

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Supplemental data

Supplemental Table 1 Epidemiological, clinical, and genetic data from published and unpublished reports of individuals with *NLRP3* mosaicism

Ref	Patient	Demographics	Phenotype														Genetics		% mosaicism																	
			Disease	Agc ons et (y)	Urtic an like rash	Fev er	Heada che	Arthr itis	Bone dyspla sia	Gro wth delay	Papil lo- edem a	Heari ng loss	Meni ngitis	Intellec tual deficie ncy	Epile psy	Brain abnormal ities (MRI)	Typi cal facie s	Increa sed CRP	Nucleotide change	Amino -acid chang e	Wh ole blood	Monoc ytes	Neutrop hils	T cell s	B cell s	N K cell s	Myeloi d progeni tors	Lymph oid progeni tors	Fibroblast	Mucosal swab	Sali va	Na il	Uri ne			
38	Mother	Female, ND	Asymptomatic	NA	No	No	No	No	No	No	No	No	No	No	No	No	c.1043C>T	p.T348M	2.6																6.9	8.2
32	6	ND, Turkish	CAPS		Yes	Yes		Yes	Yes		Yes	Yes			Yes	Yes	c.1708T>C	p.Y570H	11.9																	
32	7	ND, Turkish	CAPS		Yes	Yes		Yes	Yes							Yes	c.920G>A	p.G307D	4.5																	
35	1	Female, English	CAPS	50	Yes	Yes	Yes				Yes	Yes					c.1688A>G	p.Y563C	5.1*	44.8	36.2	11.4	15.4									20.5	0.6			
35	2	Female, English	CAPS	51	Yes	Yes	Yes	Yes					Yes				c.1688A>G	p.Y563C	3.3	6.6	7.4	0.4	0.6	**	**						6.1		**			
35	3	Male, English	CAPS	54	Yes	Yes					Yes	Yes					c.1688A>G	p.Y563C	11.1	10.6	10.7	1.6	6.6									12.3				
35	4	Female, English	CAPS	46	Yes	Yes						Yes					c.1054G>A	p.A352T	14.6	14.9	16.2	4.8	12.3									14.9				
35	5	Male, English	CAPS	71	Yes	Yes						Yes					c.1706G>T	p.G569V	21.2	23.2	21.8	1.5	17									14.3				
35	6	Male, English	CAPS	41	Yes	Yes	Yes	Yes			Yes	Yes	Yes				c.1699G>A	p.E567K	5.4	11	12.3	1.6	7.1								3.1	0.4	**			
35	7	Female, Irish	CAPS	31	Yes	Yes	Yes	Yes				Yes					c.1700G>C	p.E567Q	15																	
35	8	Female, Irish	CAPS	50	Yes	Yes	Yes					Yes					c.1691G>A	p.G564D	5																	
22	Index	Male, Japanese	CINCA	0	Yes	Yes		Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	c.1709A>G	p.Y570C	14.3	12.3	19.6	17.5							34.3	17.5						
23	8	Female, Japanese	CINCA	0	Yes	Yes		No	No	No	No	Yes	No	No			c.790C>T	p.L264F	4.3	10.9																
23	9	Male, Japanese	CINCA	0.5	Yes	Yes		Yes	Yes	Yes		Yes	No	No	No		c.919G>A	p.G307S	7.83	6.2	9.1	6.9	10.6							9						
24	Index	Male, Spanish	CINCA	0	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes	c.907G>C	p.D303H	19.1	0	15.5	16.9	17							17.5		15.5				
25	J5	ND, Japanese	CINCA	0	Yes	Yes		Yes	Yes	Yes		Yes	Yes	No	No		c.907G>C	p.D303H	11.9	8.7	14.4	7.7	8.5							13.5						
25	F1	ND, French	CINCA	0		Yes		Yes	Yes	Yes		No	Yes	No	No		c.1298C>T	p.T433I	5.2																	
25	F2	ND, French	CINCA	0		Yes		Yes	Yes	Yes		Yes	Yes	No	No		c.907G>C	p.D303H	4.2																	
25	F3	ND, French	CINCA	0	Yes	Yes		Yes	Yes	Yes		No	No	No	No		c.1315G>C	p.A439P	21.9																	
25	F4	ND, French	CINCA	0		Yes		Yes	Yes	Yes		Yes	Yes	Yes	No		c.1216A>G	p.M406V	9.2																	
25	F5	ND, French	CINCA									No	Yes		No		c.1698C>A	p.F566L	11.5																	
25	N1	ND, Dutch	CINCA	0	Yes	Yes		Yes	No	No		No	No	No	No		c.1699G>A	p.E567K	6.3																	
25	N2	ND, Dutch	CINCA	0	Yes	Yes		Yes	No	No		Yes	Yes	No	No		c.2263G>A	p.G755R	6.3																	
25	S1	ND, Spanish	CINCA	2	Yes	Yes		Yes	Yes	Yes		No	Yes	No	No		c.920G>T	p.G307V	9.6																	
25	A1	ND, American	CINCA	0	Yes	Yes		Yes	Yes	Yes		Yes	Yes	Yes	No		c.1065A>T	p.K355N	18.8																	
25	A2	ND, American	CINCA	0	Yes	Yes		Yes	Yes	Yes		Yes	Yes	Yes	Yes		c.1698C>A	p.F566L	14.6																	
25	A3	ND, American	CINCA	0	Yes	Yes		No	Yes	Yes		No	Yes	Yes	No		c.1704G>C	p.K568N	9.4																	
25	A4	ND, American	CINCA	0	Yes	Yes		No	No	No		Yes	Yes	No	Yes		c.2263G>A	p.G755R	35.8																	
26	P1		CINCA														c.907G>C	p.D303H	9																	
26	P2		CINCA														c.1699G>A	p.E567K	5.8																	
26	P3		CINCA														c.1699G>A	p.E567K	16.8																	

26	P4		CINCA																c.906C>A	p.F30 2L	9.7																																
27	Index	Male, Scottish	CINCA	0	Yes	Yes	Yes	Yes	Yes	Yes	No	No			Yes	Yes	Yes	Yes	c.1698C>A	p.F566 L	16.1																																
34	2	Male, Italy	CINCA	0	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes					c.1690G>A	p.G56 4S	6.37	7.1	7.3	14.7	3.6	6.1								6.7	4.7	7.8																	
34	6	Female, Italian	CINCA	0	Yes	Yes	Yes	Yes		Yes									c.1688A>G	p.Y56 3C	2.44	0.79	1.68	4.9	2.0	1.3						4.27	1.87										2.9										
34	7	Female, Italian	CINCA	0	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes							c.1298C>T	p.T43 3I	5.38	2.87	5.13	7.2	6.1	8.3						0.6	1.73																				
30	Index		CINCA	2	Yes	Yes	Yes	Yes				Yes							c.1298C>T	p.T43 3I	3.2																																
In preparation	Fam4 981	Male, Indian	CINCA	2	Yes	Yes	No	No	No	Yes	No	Yes	No	No					c.1792A>T	p.I598 F	30																																
This study	1	Female, French	CINCA	0	Yes	Yes		No											c.779G>C	p.R26 0P	10.18																																
23	10	Female, Japanese	MWS	0	Yes	Yes		Yes	Yes	Yes		Yes		No					c.1699G>A	p.E56 7K	6.5	10.6	3.5	3.7	3.4							9.8	2.2																				
29	1		MWS	18	Yes	Yes	No	Yes	No	No	No	Yes	No					c.908A>C	p.D30 3A	31.3																																	
29	2	Male, Spain	MWS	2	Yes	No	Yes	No	No	No	No	Yes	Yes						c.1000A>G	p.I334 V	34.9																																
29	3	Male, Japan	MWS	0	Yes	No	Yes	Yes	No	No	No	Yes	No						c.1064A>C	p.K35 5T	20.2																																
29	4	Female, Japan	MWS	14	Yes	Yes	Yes	Yes	No	No	No	No	No						c.[1231C>T;123 3G>T]	p.L41 1F	15																																
29	5	Female, Spain	MWS	4	Yes	Yes	No	Yes	No	No	No	Yes	No					c.1569C>A	p.F523 L	8.7																																	
29	6	Male, Spain	MWS	4	Yes	Yes	Yes	Yes	No	No	No	No	No						c.1699G>A	p.E56 7K	5.6																																
29	7	Female, Japan	MWS	0.5	Yes	Yes	Yes	Yes	No	No	No	Yes	No						c.1699G>A	p.E56 7K	5.5																																
31	Index	Male, Japan	MWS	52	Yes	Yes	Yes		No	No	No	No	No						c.1709A>G	p.Y57 0C	10.9	16.8	18	0	5.6**						0																						
33	Index	Female, ND	MWS	56	Yes	Yes		Yes					Yes						c.1906C>G	p.Q63 6E	18.4	31.8	24.6	0	0		11.2	0																									
34	1	Male, Spanish	MWS	0	Yes	Yes													c.779G>C	p.R26 0P	10.4	1.23	11.97	14.7	9.6	9.4																											
This study	Fam5 565	Female, Italian	MWS	13	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	No	No				c.791T>C	p.L26 4P	6																																
This study	Fam6 354	Female, Hungarian	MWS	53	Yes	Yes	No	No	No	No	No	Yes	No	No	No				c.1708T>A	p.Y57 0N	24																																
28	7		Schnitzler	38	Yes	Yes													c.1569C>G	p.F523 L	8	6.5	13	0	0																												
28	8		Schnitzler	50	Yes	Yes													c.1303A>G	p.K43 5E	27	29	32	0	0																												

Blank cells denote absence of information. Novel mutation are in bold letters; *Increased over time. ** Possible contamination; CINCA: Chronic infantile neurologic cutaneous articular syndrome; MWS: Muckle-Wells syndrome; CAPS: Cryopyrin associated syndrome.

Supplemental Table 2 Association between frequency of mutated alleles and presence or absence of CAPS symptoms

Symptom	Presence of the symptom	N patients	Mean mutated allele frequency (\pm sd)	Median (Q1;Q3)	p
Papilloedema	No	13	17.22 (\pm 10.56)	16.10 (8.70;24.00)	0.0212
	Yes	9	7.64 (\pm 3.80)	6.00 (5.38;11.10)	
Epilepsy	No	18	10.69 (\pm 6.45)	9.30 (6.00;14.35)	0.0535
	Yes	3	22.17 (\pm 11.83)	16.10 (14.60;35.80)	
Urticaria	No	1	2.60 (\pm .)	2.60 (2.60;2.60)	0.1060
	Yes	45	12.96 (\pm 8.80)	10.43 (6.00;18.40)	
Arthritis	No	8	18.90 (\pm 13.81)	17.09 (6.85;32.45)	0.1794
	Yes	32	10.63 (\pm 6.83)	8.26 (5.45;15.00)	
Typical facies	No	1	2.60 (\pm .)	2.60 (2.60;2.60)	0.1817
	Yes	4	13.98 (\pm 5.44)	15.22 (10.36;17.60)	
Headache	No	5	19.32 (\pm 12.96)	24.00 (8.70;30.00)	0.1857
	Yes	18	10.25 (\pm 8.41)	5.80 (5.10;15.00)	
Meningitis	No	14	14.92 (\pm 9.52)	12.95 (6.30;21.90)	0.3807
	Yes	17	12.22 (\pm 9.62)	9.40 (6.00;14.35)	
Hearing loss	No	13	10.58 (\pm 5.98)	9.60 (5.60;15.00)	0.4818
	Yes	30	13.76 (\pm 9.53)	11.50 (6.00;18.80)	
Fever	No	3	19.23 (\pm 16.17)	20.20 (2.60;34.90)	0.5103
	Yes	46	11.89 (\pm 8.13)	9.50 (5.50;15.00)	
Bone deformation	No	16	15.46 (\pm 12.00)	9.80 (5.80;27.00)	0.6123
	Yes	18	10.93 (\pm 5.48)	9.50 (6.37;14.60)	
Intellectual deficiency	No	17	12.70 (\pm 9.97)	7.83 (6.00;19.10)	0.6224
	Yes	5	11.67 (\pm 4.97)	9.40 (9.20;14.60)	
Growth delay	No	15	14.49 (\pm 11.75)	8.70 (5.60;24.00)	0.8599
	Yes	18	11.83 (\pm 7.23)	9.50 (6.37;16.10)	

Supplemental Table 3 Epidemiological, clinical, and genetic data from published and unpublished reports of individuals with mosaicism in AID genes other than *NLRP3*

Ref	Patient	Demographics	Phenotype																Genetics			% Mosaicism													
			Disease	Aggravation (y)	Fever	Increase CRP	Abdominal signs	Musculoskeletal signs	Thoracic signs	Skin signs	Hepatosplenomegaly	Vasculopathy	Eye signs	Headaches	Meningitis	Intellectual deficiency	Brain abnormalities (MRI)	Other	Gene	Nucleotide change	Amino-acid predicted change	White blood cell count	Monocytes	Neutrophils	T cells	B cells	Fibrinogen	Mucocutaneous	Skin	Nails	Hair	Uterus	Splenomegaly		
45	Index	Female, ND	AIFEC	0	Yes		Ascites Necrotizing enterocolitis	Respiratory distress syndrome Focal heart infarction	Yes	Fetal thrombotic vasculopathy						Congenital anemia Hemophagocytic lymphohistiocytosis Macrophage activation syndrome Adenopathies	<i>NLR C4</i>	c.512C>T	p.Ser171Phe	25															
46	Index	Female, ND	Blau syndrome	36	No		Oligoarthritis of the large joints No tenosynovitis	No heart involvement No pericarditis	No		Bilateral uveitis		No					<i>NO D2</i>	c.1001 G>A	p.Arg334Gln	7.7	11	7.3	8.3	8.2		4.9	7					7.4		
36	I.1	Male, Malaysian	Blau syndrome	22	No	No	No				Bilateral anterior uveitis							<i>NO D2</i>	c.1001 G>A	p.Arg334Gln	12.9						8.5							0.9	
44	Index	Male, Japanese	CINCA-like	0	Yes	Yes	Arthritis	Periodic erythema Urticaria		Yes		Yes	Yes	Yes	Typical facies of CINCA Growth delay		<i>NLR C4</i>	c.529A>G	p.Thr177Ala	30.3						34.1									
43	Index	Female, Israeli	FMF	52	Yes	Yes	Yes	Myalgia										<i>ME FV</i>	c.1955 G>A	p.Arg652His	46	46	27	0		0									

38	Mot herP3	Female, ND	Asymptomatic														<i>TNF AIP3</i>	c.2088+5	NA		10								
38	FatherP21	Male, ND	Asymptomatic														<i>TNF AIP3</i>	c.1245_1248del	p.Lys417Serfs*4	ND									
In preparation	Fam 2838	Male, French	HA20	2	Yes	Yes	Arthralgia	Thoracic pain	Yes	Erythema nodosum	Papillite	Yes	Encephalitis	Growth delay			<i>TNF AIP3</i>	c.1151A>G	p.Asp384Gly	7									
47	6	Male, American	SAVI	0	No	Yes	No	Interstitial lung disease	Acral violaceous plaques and nodules	Generalized pustules	Live do reticulularis	Amp utation extremities	No	membranoproliferative glomerulonephritis			<i>TME M173</i>	c.461A>G	p.Asn154Ser	Yes	Yes	Yes	Yes						
39	Index	Male, British	TRAPS	12	Yes	Yes	Severe abdominal pain	Arthralgia, Myalgia	Pleuritic chest pain	Generalized erythema	No periorbital edema	Recurrent eye	Yes	Night sweats	Late onset of puberty	Unilateral cervical lymphadenopathy		<i>TNF RSF1A</i>	c.255_278	p.Ser86_Glu93del	21	19	3	2	0	0	1	4	16

In bold letters: novel mutation. TRAPS: TNF Receptor associated periodic syndrome, CINCA: Chronic infantile neurologic cutaneous and articular, AIFEC: autoinflammation with infantile enterocolitis; SAVI: STING-associated vasculopathy with onset in infancy; HA20: Haploinsufficiency of A20; NA: Not applicable.

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A decision tree for the genetic diagnosis of deficiency of adenosine deaminase 2 (DADA2): a French reference centres experience

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ABSTRACT

Deficiency of adenosine deaminase 2 (DADA2) is a recently described autoinflammatory disorder. Genetic analysis is required to confirm the diagnosis. We aimed to describe the identifying symptoms and genotypes of patients referred to our reference centres and to improve the indications for genetic testing. DNA from 66 patients with clinically suspected DADA2 were sequenced by Sanger or next-generation sequencing. Detailed epidemiological, clinical and biological features were collected by use of a questionnaire and were compared between patients with and without genetic confirmation of DADA2. We identified 13 patients (19.6%) carrying recessively inherited mutations in *ADA2* that were predicted to be deleterious. Eight patients were compound heterozygous for mutations. Seven mutations were novel (4 missense variants, 2 predicted to affect mRNA splicing and 1 frameshift). The mean age of the 13 patients with genetic confirmation was 12.7 years at disease onset and 20.8 years at diagnosis. Phenotypic manifestations included fever (85%), vasculitis (85%) and neurological disorders (54%). Features best associated with a confirmatory genotype included fever with neurologic or cutaneous attacks (odds ratio [OR] 10.71, $p=0.003$ and OR 10.9, $p<0.001$), fever alone (OR 8.1, $p=0.01$), and elevated C-reactive protein (CRP) level with neurologic involvement (OR 6.63, $p=0.017$). Our proposed decision tree may help improve obtaining genetic confirmation of DADA2 in the context of autoinflammatory symptoms. Prerequisites for quick and low-cost Sanger analysis include one typical cutaneous or neurological sign, one marker of inflammation (fever or elevated CRP level), and recurrent or chronic attacks in adults.

Keywords: Autoinflammatory disease, DADA2, genetic diagnosis, decision tree, vasculopathy

INTRODUCTION

Deficiency of adenosine deaminase type 2 (DADA2) is an autosomal recessive systemic autoinflammatory disorder (SAID) described for the first time in 2014(239,240). Both homozygous or compound heterozygous genotypes have been detected(241). Although one mutation c.139G>A;p.(Gly47Arg) is frequent, notably in the Georgian population, due to a founder effect, the disease seems to occur ubiquitously; indeed, patients with DADA2 have been identified in several countries(242).

The phenotype and outcome observed in DADA2 are quite heterogeneous(243). Age at disease onset is usually before the second decade of life. The clinical spectrum ranges from single cutaneous lesions to severe systemic inflammatory disease with cerebral complications(244). Clinical and histopathological features of polyarteritis nodosa (PAN), vasculopathy-related manifestations (myalgia, hypertension and gastrointestinal symptoms), and ischaemic and haemorrhagic strokes are the most frequent DADA2 manifestations(241). Other clinical presentations include those resulting from immune deficiency and haematological presentations(245–248).

The *ADA2* gene, previously named cat eye syndrome chromosome region 1 (*CECRI*), is located on chromosome 22q11.1 and has 10 exons. It encodes the adenosine deaminase 2 (ADA2) enzyme. ADA2 is 25% identical to the ADA1 protein according to a BLAST search (<https://blast.ncbi.nlm.nih.gov/>). ADA1 is a ubiquitous intracellular enzyme that acts as a monomer(249–251). It catalyses the irreversible deamination of adenosine and deoxyadenosine in the purine catabolic pathway. ADA1 deficiency is associated with a severe combined immunodeficiency autosomal recessive disease.

ADA2 contains 4 domains: signal peptide and dimerization, putative receptor binding, and catalytic domains(241). The protein is expressed in cells of the myeloid lineage: promonocyte cell lines and monocytes differentiated into macrophages and dendritic cells(249,250). ADA2 acts in the extracellular space as an endothelial growth factor(249–251). ADA2 deficiency may upregulate neutrophil-expressed genes and increase the secretion of pro-inflammatory cytokines(252). The role of ADA2 in the adaptive immune response remains unclear.

Our autoinflammatory diseases unit in Montpellier University Hospital has been designated as a reference laboratory and is a team of the French reference center for autoinflammatory diseases. The aim of this study was to describe the clinical characteristics and genotype of a series of patients referred to our laboratory for genetic diagnosis of DADA2 in the context of

autoinflammatory symptoms. The objective was to retrospectively identify the symptoms predicting a positive genetic test result, and propose a decision tree to improve DADA2 diagnosis.

PATIENTS AND METHODS

Study design

Patients were enrolled when a routine genetic diagnosis was requested. With clinicians of reference centres, we had developed a common clinical form for all SAIDs to be provided with all genetic diagnosis requests. This form collects epidemiological data and includes a list of clinical symptoms and biological markers frequently encountered in SAIDs (see supplementary Figure S1). We retrospectively reviewed these forms for all patients who were screened for *ADA2* mutations.

Consent and genetic analysis

Each participant (or legal guardian if relevant) was fully informed and gave written consent for DNA analysis. Symptomatic cases and, if necessary, some of their relatives were screened for *ADA2* mutations (NM_001282225.1). Sanger sequencing (n=59) or Next-generation sequencing (n=7) was performed, given the two techniques have proved 100% concordance when NGS was implemented in the laboratory.

Sanger sequencing: Two different amplicons were amplified for each exon to circumvent the risk of allele drop-out. Exons 2 to 10 and exon–intron junctions were sequenced in both directions by using ABI PRISM Big Dye Terminator V3.1, the Ready-Reaction Cycle-Sequencing kit and ABI 3130 XL (Applied Biosystems).

Next-generation sequencing (NGS): We performed NGS for 7 patients (panel of 55 autoinflammatory genes including *ADA2*; list available upon request, exons 2 to 10 and exon–intron junctions were sequenced). Libraries were prepared by using SureSelect Target Enrichment Capture custom kits (Agilent). Sequencing reactions involved MiSeq or NextSeq500 equipment (Illumina).

Quantitative PCR (qPCR): When relevant (eg, when a single clearly pathogenic variant was detected by sequencing or with apparent homozygosity), real-time qPCR was performed for each coding exon (2 to 10) by using a LightCycler 480 thermocycler (Roche) to search for a possible small rearrangement of the second allele.

Interpretation: Variants were analysed by using Seqscape (Applied Biosystem), Seqnext (JSI) and standard *in silico* tools. These latter tools included the Alamut pipeline (Interactive Biosoftware) for missense mutations (GVGD, SIFT, Polyphen2, MutationTaster) and MaxEntScan (MES), Human splice finder (HSF), and neural network splice (NNSplice) for splice mutations.

Statistical analysis

Data on clinical symptoms and biological markers were extracted from our standard clinical form for genetic requests related to autoinflammatory diseases (supplementary Figure S1). The association between these potential diagnostic items and genetic confirmation of the disease was estimated by odds ratios (ORs), 95% confidence intervals (CIs), and Fisher exact test. Then, we estimated the percentage of correctly classified cases (a classical measure of accuracy) and the sensitivity and specificity of each of the diagnostic elements. This methodology was repeated to examine the association and diagnostic values of various combinations of these items. Highly associated combinations of DADA2 clinical symptoms and biological markers were chosen as potential prerequisites for the decision tree. As a preliminary validation procedure, we applied these prerequisite items to an external sample of all published cases of genetically confirmed DADA2 whenever our items were available in reports and to an internal series of all patients with a genetically confirmed AID other than DADA2, referred since the implementation of the present version of our clinical form (supplementary Figure S1).

RESULTS

Demographic data

Requests for genetic diagnosis of DADA2 have greatly increased since 2014. Our series includes all patients (n=66) who were referred to our laboratory for clinical suspicion of DADA2. The referring clinicians were from various medical specialties: 33 paediatricians [paediatric rheumatology (n=13), generalist paediatrics (n=11), paediatric neurology (n=6) and paediatric haematology (n=3)] and 33 clinicians for adults [internal medicine (n=20), dermatology (n=9) genetics (n=3), and nephrology (n=1)]. Patients were of European Caucasian (n=35), Maghrebian (n=19), Middle East (n=5), African (n=3), Jewish (n=3), or Asian ancestries (n=1). Only two families had more than one symptomatic member (Families A and B, Figure S2 in supplementary file). Consanguinity was reported in 2 families (B and F).

The male to female ratio was 0.91. The mean age at disease onset was 14.0 years (median 10 years, min–max: 4 months–69 years, standard deviation (SD): 14,4 years).

***ADA2* mutations**

DADA2 was confirmed in 13 (19.6%) of the 66 patients from 11 unrelated families (Table 1). We found 8 missense and 5 non-sense different mutations. In all families but family J, DNA from relatives was available and the variants could be confirmed to be located in trans. Eight patients were compound heterozygous and 5 were homozygous for mutations c.73G>T;p.(Gly25Cys), c.506G>A;p.(Arg169Gln) or c.1358A>G;p.(Tyr453Cys). Six variants had previously been associated with DADA2: c.144del;p.(Arg49Glyfs*4), c.139G>A;p.(Gly47Arg), c.506G>A;p.(Arg169Gln), c.1358A>G;p.(Tyr453Cys), c.1078A>G;p.(Thr360Ala) and deletion of exon 7 (245,253–256). Seven novel mutations were found in families B, E, G, H and K (Figure 1). *In silico* tools predicted that two novel variants, c.973-2A>G and c.753G>A, may affect mRNA splicing (Figure 1a). Mutation c.973-2A>G is a rare canonical splicing variant absent in the ExAC (<http://exac.broadinstitute.org>) and dbSNP databases (<https://www.ncbi.nlm.nih.gov/projects/SNP/>). It is predicted to alter the wild-type acceptor site (> 30% impact according to HSF and 58% according to MES). The second variant, c.753G>A, is a substitution, which apparently does not change codon 251. However, this guanine is the last nucleotide of exon 4 and is located within a donor splicing consensus site. Hence, this mutation is predicted to result in a truncated protein.

a) *In Silico* tools predictions for the newly identified variants

gDNA position	Ref	Alt	cDNA position	Ref	Alt	Protein position	Ref	Alt	ExAC	PolyPhen	PolyPhen Value	SIFT	SIFT Value	CADD (PHRED)	MaxEnt	HSF	NNSplice
17662804	C	A	1348	G	T	450	Gly	Cys	0.0012%	probably damaging	1	deleterious	0	27.8	NA	NA	NA
17669339	T	C	973-2	A	G	NA	NA	NA	0.012%	NA	NA	NA	NA	24.3	-100%	-100%	-100%
17672582	G	A	872	C	T	291	Ser	Leu	0.00041%	probably damaging	0.974	deleterious	0	25.8	NA	NA	NA
17684453	C	T	753	G	A	251	Pro	Pro	0.0024%	NA	NA	NA	NA	9.957	-48.9 %	-11.5 %	-60%
17684494	C	T	712	G	A	238	Asp	Asn	NA	probably damaging	1	deleterious	0	27.5	NA	NA	NA
17690495	C	A	73	G	T	25	Gly	Cys	0.0016%	benign	0.003	tolerated	0.13	8.138	NA	NA	NA
17688076	T	-	427	A	-	143	Ile	Serfs*41	NA	NA	NA	NA	17.36	NA	NA	NA	
17687992	G	A	511	C	T	171	Arg	Trp	0.12%	benign	0.067	deleterious	0	19.56	NA	NA	NA

b) Adenosine deaminase type 2 gene (*ADA2*, NM_001282225.1) with already published and newly identified variants

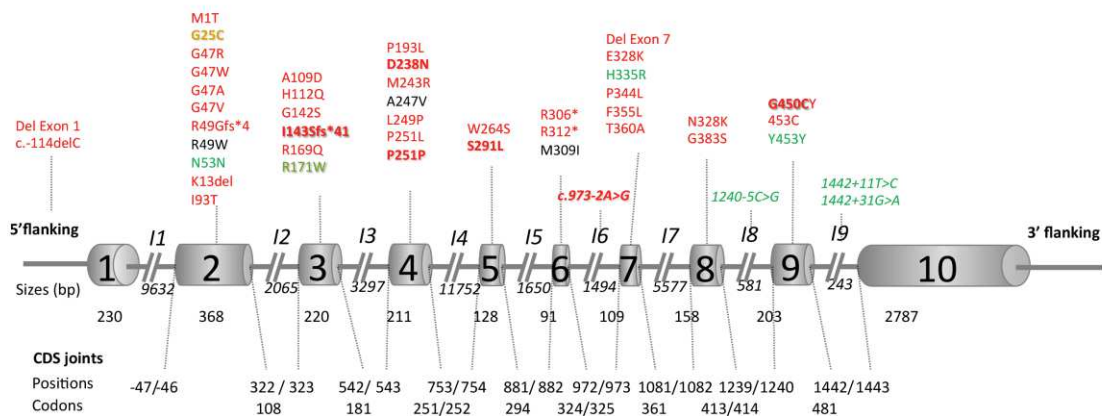


Figure 1 The adenosine deaminase type 2 (*ADA2*) genes and novel sequence variants

a- In silico tool predictions for the newly identified variants

gDNA: genomic position on human genome HG 37, Ref: reference allele, Alt: new allele, cDNA: coding sequence position, prediction using the Combined Annotation Dependent Depletion (CADD) *in silico* tool cadd.gs.washington.edu, HSF: Human Splicing Finder, MES: MaxEntScan, NNS: Neuronal Network Slipce, NA: Not applicable.

b- Schematic representation of the adenosine deaminase type 2 (*ADA2*) gene (adapted from Infevers).

In bold newly identified *ADA2* variants, in red: pathogenic *ADA2* variants, in orange: variant of unknown clinical significance (VUS), in green: common polymorphism, in black: Behçet susceptibility factors. This figure shows the variant usual name (i.e., as first published). Bp: Base pair; CDS: CoDing Sequence.

We identified one new frameshift mutation, c.427delA;p.(Ile143Serfs*41), and 4 novel missense variants: c.73G>T;p.(Gly25Cys), c.1348G>T;p.(Gly450Cys), c.712G>A;p.(Asp238Asn) and c.872C>T;p.(Ser291Leu). Two consanguineous siblings, B1 and B2, were homozygous for p.(Gly25Cys) and presented the same phenotype. They had severe pleomorphic vasculitis features (PAN, livedo, renal microaneurysms) with inflammation. Their parents were asymptomatic and heterozygous for this variant, which moreover was absent from general databases. For this reason and although the pathogenicity score obtained for this variant was conflicting, we considered it a disease-causing mutation. In

family E, p.(Gly450Cys) was predicted to have deleterious effects on the protein function. The glycine located at position 450 in the catalytic domain of the *ADA2* protein is well conserved across species, and this variant is absent from ExAC or 1000Genome databases. A young female of Maghrebian origin (family H1) had a history of recurrent livedo racemosa and folliculitic rashes accompanied by fever since age 14. At age 16, she had musculoskeletal involvement with inflammatory arthralgia and biological hyperandrogenism. DADA2 was suspected despite the absence of neurological attacks or increased C-reactive protein (CRP) level. *ADA2* sequencing revealed two novel variants: p.(Asp238Asn) and p.(Ser291Leu). Both are located in the catalytic domain of the protein in exons 4 and 5, respectively, and are highly conserved across species. They were both predicted to be probably damaging to the protein function.

Identifying symptoms in genetically confirmed patients

Demographic features and clinical presentations of the 13 patients with genetically confirmed DADA2 are in Table 1. The mean age at disease onset was 12.0 years (min–max: 1–33, SD: 9,1) and mean age at diagnosis 20.8 years (min–max: 7–39, SD: 10,4) (Table 2). A few patients had a particular disease course. One patient (J1) had late clinical manifestations at age 33, with a cutaneous phenotype and an immunological disorder. Patient D1 was first diagnosed with juvenile idiopathic arthritis; typical DADA2 manifestations, such as ischemic stroke, occurred secondarily.

Table 1 Clinical characteristics, genotype and treatment for 13 patients with *ADA2* mutations

Patient/ sex	E	Age at disease onset (years)	Age at genetic diagnosis (years)	Fever	CRP level mg/L	Cutaneous involvement	Musculo- skeletal disorders	Peripheral and central nervous system involvement	Immunologic/hematologic involvement	Other	Current treatment	<i>ADA2</i> genotypes and predicted protein alterations
A1/F	C	3,5	9	Yes	140	Cutaneous vasculitis	Inflammatory myositis	Ischemic stroke	Low IgM and IgA level; lymphopenia	Internuclear ophthalmoplaegia; hypertension; inflammatory anemia	TNF alpha blockade	c.[1358A>G];[(972+1_973- 1)_(1081+1_1082-1)del] p.[(Tyr453Cys)];[(?)]
A2/F	C	1	7	Yes	> 5	No	No	Paralysis of the right extrinsic third cranial nerve; cephalalgia; meningitis	Low IgM and IgA level; lymphopenia	Inflammatory anemia	TNF alpha blockade	c.[1358A>G];[(972+1_973- 1)_(1081+1_1082-1)del] p.[(Tyr453Cys)];[(?)]
B1/F	M	19	32	No	100	Livedo racemosa; urticaria; Erythema nodosum	No	Convulsive attacks	ND	Uveitis; papillitis; Abdominal pain; micro renal and mesenteric aneurysms	IVIg; Endoxan; MTX; Imurel	c.[73G>T];[73G>T] p.[(Gly25Cys)];[(Gly25Cys)]
B2/M	M	20	27	Yes	> 5	Livedo racemosa; urticaria; necrosis	Arthritis; arthralgia	No	ND	Pericarditis; abdominal pain; hepatic aneurysms	IVIg; Endoxan; MTX; Imurel	c.[73G>T];[73G>T] p.[(Gly25Cys)];[(Gly25Cys)]

C1/F	C	3	12	Yes	65	Livedo racemosa; urticaria	Arthralgia	No	ND	Abdominal pain	TNF alpha blockade	c.[144del];[1078A>G] p.[(Arg49Glyfs*4)];[(Thr360Ala)]
D1/M	C	13	24	Yes	100	No	Arthralgia; myalgia	Protuberant ischemic strokes	Low IgM level; lymphopenia	No	TNF alpha blockade	c.[506G>A];[506G>A] p.[(Arg169Gln)];[(Arg169Gln)]
E1/M	C	14	20	Yes	> 5	Cutaneous vasculitis; necrosis; maculo- papular rash	Arthralgia; myalgia	Ischemic strokes; meningitis; headaches; peripheral neuropathy	ND	Abdominal pain; pleuropericarditis	Steroids	c.[144del];[1348G>T] p.[(Arg49Glyfs*4)];[(Gly450Cys)]
F1/F	M	10	30	Yes	> 5	Livedo racemosa; PAN; necrosis	Arthralgia	No	ND	No	ND	c.[1358A>G];[1358A>G] p.[(Tyr453Cys)];[(Tyr453Cys)]
G1/M	C	2	17	Yes	> 5	Cutaneous vasculitis; early onset PAN; aphthosis;	No	Brainstem ischemic stroke; left optic neuropathy; 3th cranial nerve palsy; vertigo; nystagmus	ND	Persistent left sided visual loss	Cyclophosphamid; Rituximab; Steroids	c.[427del];[973-2A>G] p.[(Ile143Serfs*41)];[(p?)]
H1/F	M	14	16	Yes	< 5	Livedo racemosa; folliculitis	Arthralgia	No	ND	Hyper-androgenism	NSAIDs	c.[712G>A];[872C>T] p.[(Asp238Asn)];[(Ser291Leu)]

I1/F	C	17	30	Yes	ND	PAN; erythema nodosum	Arthralgia; arthritis	Ischemic strokes	ND	Hematuria; buccal aphthosis	ND	c.[139G>A];[(972+1_973-1)_(1081+1_1082-1)del] p.[(Gly47Arg)];[(p?)]
J1/M	M	33	39	Yes	60	Livedo racemosa; PAN; necrosis; erythema nodosum	Arthralgia; myalgia	No	HypoIg	Abdominal pain	Steroids	c.[1358A>G];[1358A>G] p.[(Tyr453Cys)];[(Tyr453Cys)]
K1/M	C	6	7	No	< 5	Livedo racemosa	No	Ischemic strokes; 3th cranial nerve palsy	Low IgM level; immunodeficiency	Splenomegaly; hepatomegaly	TNF alpha blockade	c.[506G>A];[753G>A] p.[(Arg169Gln)];[(p ?)]

E: ethnicity; C: Caucasian, M: Maghrebian, CRP: C-reactive protein, M: male, F: female, ND: not determined, HypoIg: hypogammaglobulinemia, PAN: polyarteritis nodosa, NSAID: non-steroidal anti-inflammatory drug, in **bold**: new mutations identified, TNF: tumor necrosis factor, IVIg: intravenous immunoglobulin, MTX: methotrexate

Table 2 Clinical characteristics of the patients with and without genetically confirmed DADA2

	Unconfirmed DADA2		Confirmed DADA2	
Disease course	Age, years (mean/median)		Age, years (mean/median age)	
Onset	14.0	(9)	12.0	(13)
Diagnosis	-		20.8	(20)
Clinical signs	n (N)	%	n (N)	%
CRP level \geq 5 mg/dL	25 (44)	56.8%	10 (12)	83.4%
Fever \geq 38°C	16 (45)	35.5%	11 (13)	84.6%
Neurologic involvement	17 (50)	34%	7 (13)	53.8%
Stroke	15		6	
Intracranial haemorrhagia	1		0	
PNS involvement	3		4	
Other	6		2	
Ophthalmologic involvement	2 (50)	4%	3 (13)	23.1%
Hepato-splenomegaly	15 (50)	30%	1 (13)	7.7%
Musculoskeletal disorder	24 (50)	48%	9 (13)	69.2%
Cutaneous involvement	37 (50)	74%	11 (13)	84.6%
Livedo racemosa	9		7	
Ulcerations of extremities	3		4	
Vasculitis	28		2	
Maculopapular rash	15		1	
Immunodeficiency	7 (50)	14%	5 (13)	38%
Low immunoglobulin level	5		5	
Lymphopenia	3		3	
Recurrent infections	2 (50)	4%	0	0%

CRP: C-reactive protein, PNS: peripheral neurological system, Other: meningoencephalitis or meningitis or epilepsy. n= number of affected patients, N= number of patients with complete clinical form.

Among the 13 patients with confirmed DADA2, fever was present in 11 (85%) and elevated CRP level in 10 (Table 2). Eleven patients showed cutaneous involvement, including livedo racemosa, nodular rash, vasculitis (PAN), erythema nodosum or peripheral necrosis. Musculoskeletal manifestations concerned 9 patients (69%). Seven patients (54%) presented peripheral and/or central nervous system involvement such as ischemic and/or haemorrhagic stroke or peripheral nerve palsy. Five patients (38%) had a history of recurrent infection, immunodeficiency and/or hypo-gammaglobulinemia. Immunologic deficiency was always associated with other symptoms in families A, D, J and K.

Clinical characteristics of patients with no confirmatory genotype

Three patients without a family history of DADA2 were heterozygous for an *ADA2* variant (supplementary Table S3). One presented a variant of uncertain significance (VUS) with discordant *in silico* predictions, and one presented a benign missense variation. Both presented few DADA2 clinical features. Patient M1 carried the known p.(Ala247Val) variant(257); symptoms occurred at age 1 year. Raynaud's syndrome was the only clinical sign indicated by the clinician requesting *ADA2* sequencing. There were no other DADA2 characteristics such as immunologic deficiency or cutaneous involvement or clinical inflammation during episodes or increased CRP level. Patient N1, from Algeria, had a missense variant, c.511C>T;p.(Arg171Trp), that we considered a polymorphism because of high minor allele frequency of 1.5% in individuals of African origin according to ExAC (Figure 1a). The symptoms had begun at age 5 years and included oral aphthosis, myalgia and increased CRP level during flares. No neurological episode was reported. The third patient (L1) had symptoms more consistent with DADA2. Disease began at age 5 with a discrete inflammatory syndrome including fever and CRP level increased to 27 mg/dL. The accompanying signs were cephalalgia, arthralgia and myalgia, papular rash with pruritis and erratic gastrointestinal manifestations (especially diarrhea). Only one variant, p.(Gly47Arg), was found on conventional sequencing analysis. This variant was known to be clearly pathogenic(241,254). Although the hypothesis of a copy-number variation was ruled out on qPCR, a second disease-causing variant affecting the gene's promoter or non-coding regulatory sequences may exist. However, *ADA2* activity measurement (not shown) revealed an intermediate profile, consistent with the phenotype.

We detected no disease-causing mutation in *ADA2* in the remaining 50 patients (Table 2). The mean age at disease onset was 14.0 years (min–max: 4 months–69 years, SD: 15.3). Fever and

elevated CRP level were observed in 35.5% and 56.8% of the patients, respectively. Cutaneous involvement was also a predominant clinical feature, but neurologic symptoms were less frequent. Fifteen patients presented stroke, 1 patient intracranial haemorrhagia and 3 patients peripheral neuropathy.

Comparison of patients with and without genetically confirmed DADA2

Phenotypes of patients with and without genetically confirmed DADA2 were compared (Table 3). Fever was more frequent in patients with than without genetic confirmation (OR=8.1, $p=0.01$). As well, cutaneous and neurological signs were significantly more frequent when associated to fever. Elevated CRP level was the biological sign with the best sensitivity (83%) and specificity (46%). The other characteristics taken alone were not contributive. We then evaluated the performance of combined symptoms. The association of a marker of inflammation (fever or CRP level) with skin or neurological manifestations enhanced the odds of a confirmatory genotype, for example, elevated CRP level combined with central ischemic and haemorrhagic involvement, or peripheral neuropathy (OR 6.63, $p=0.017$). The association of 3 clinical characteristics further increased this performance, which was the best for fever and neurological and cutaneous disorders (OR 17.72, $p=0.008$), and for inflammation markers (fever and CRP) and either of the DADA2 typical features such as ischemic stroke or livedo racemosa (OR >6 , $p \leq 0.01$). Figure 2 highlights that more than 65% of the patients were misclassified when considering CRP, musculoskeletal, neurological, cutaneous signs or fever individually. Altogether, the association of 2 or 3 clinical signs improved the proportion of cases correctly classified ($> 80\%$).

Table 3 Sensitivity and specificity of biological and clinical characteristics in 13 patients with and without genetically confirmed DADA2

Clinical disease	Se [95% CI]	Sp [95% CI]	OR [95% CI]	p-value
Fever + CRP + NI	0.42 [0.14; 0.70]	0.95 [0.89; 1.01]	13.9 [1.86 ; 172.87]	0.003 **
Fever + CRP + CI	0.58 [0.30; 0.86]	0.82 [0.71; 0.93]	6.04 [1.28 ; 31.44]	0.01 **
Fever + NI + CI	0.31 [0.05; 0.57]	0.98 [0.94; 1.02]	17.72 [1.53 ; 955.70]	0.008 **
Fever + NI	0.46 [0.18; 0.74]	0.93 [0.86; 1.00]	10.71 [1.81 ; 82.45]	0.003 **
Fever + CI	0.69 [0.43; 0.95]	0.84 [0.73; 0.95]	10.9 [2.37 ; 61.47]	<0.001 ***
CRP + NI + CI	0.25 [0.01; 0.50]	0.93 [0.86; 1.00]	4.29 [0.49 ; 37.64]	0.11
CRP + NI	0.42 [0.14; 0.70]	0.91 [0.83; 0.99]	6.63 [1.13 ; 43.07]	0.017 *
CRP + CI	0.67 [0.40; 0.94]	0.63 [0.49; 0.77]	3.26 [0.75 ; 17.02]	0.104
CI + NI	0.31 [0.05; 0.57]	0.77 [0.65; 0.90]	1.45 [0.27 ; 6.57]	0.719
Fever	0.85 [0.65; 1.05]	0.6 [0.46; 0.74]	8.1 [1.50 ; 84.40]	0.01 **
CRP level \geq 5 mg/dL	0.83 [0.62; 1.04]	0.46 [0.31; 0.61]	4.21 [0.76 ; 44.27]	0.095
NI	0.54 [0.26; 0.82]	0.33 [0.19; 0.47]	0.59 [0.14 ; 2.53]	0.515
CI	0.69 [0.43; 0.95]	0.44 [0.29; 0.59]	1.78 [0.42 ; 9.12]	0.526
Musculoskeletal disorders	0.69 [0.43; 0.95]	0.61 [0.47; 0.75]	3.49 [0.82 ; 18.05]	0.064

Se: Sensitivity; Sp: Specificity; OR: odds ratio; 95% CI: confidence interval; *: p=0.05–0.005; **: p=0.005–0.001; ***: p < 0.001; CRP: C-reactive protein; NI: neurologic involvement such as ischemic or haemorrhagic stroke or peripheral palsy; CI: cutaneous involvement such as livedo racemosa, nodular rash, erythema nodosum, vasculitis and necrosis.

Figure 2 Percentage of cases correctly classified (CCR)

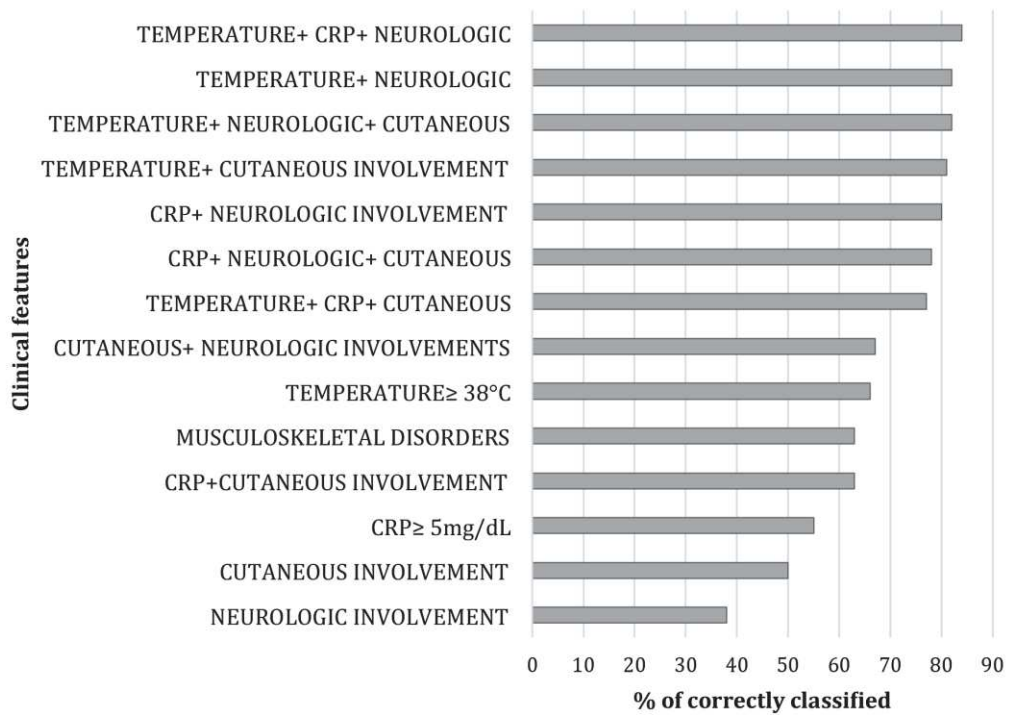


Figure 2: Percentage of cases correctly classified (CCR)

The combined or isolated items are classified from highest to lowest according to their likelihood of being associated with a confirmatory genetic diagnosis of DADA2. CRP: C-reactive protein level increased up to 5 mg/dL during an episode.

A proposed decision tree for genetic diagnosis of DADA2

As shown previously, a number of cutaneous or neurological signs and inflammation (fever or elevated CRP level) were the identifying symptoms that when combined were best associated with genetic confirmation of the DADA2 diagnosis. All of our 13 patients with genetic confirmation had more than 3 episodes of systemic inflammation. To better rule out a non-hereditary origin of the phenotype, we suggest observing at least one recurrence or chronic evolution in adults before requesting molecular investigation. In children, the evolution may be dramatic, and a relevant diagnosis may be an emergency.

To validate the items described as possible prerequisites for gene-targeted (Sanger) genetic diagnosis, we tested them in all published cases of genetically confirmed DADA2 with enough data (n=52)(241,254,258). Two paediatric cases did not fulfil the prerequisites. One boy presented at age 5 with recurrent fever, splenomegaly, generalized lymphadenopathy, increasing levels of acute-phase reactants, anaemia, thrombocytosis, and polyclonal hyperimmunoglobulinemia(259). The other boy was diagnosed at age 6 with fever, hypogammaglobulinemia, arthralgia and hepatosplenomegaly(258). However, our NGS panel would have identified both patients.

We also tested these prerequisites in a series of 53 patients with other SAIDs that we genetically confirmed in our lab, notably, familial mediterranean fever (FMF) (n= 32), mevalonate kinase deficiency (n=5), A20 haploinsufficiency (n=3), tumor necrosis factor receptor-associated periodic syndrome (n=3), and cryopyrin-associated periodic syndrome (n=1). Only one patient met the prerequisites and would have been eligible for ADA2 testing. He was homozygous for c.2080A>G;p.(Met694Val) and had severe FMF and PAN, a well-known complication of this disease.

These studies led to the identification of a minimal common clinical set of symptoms in positive patients. We propose a provisional decision tree (Figure 3) that should help define optimized conditions predicting a positive genetic analysis.

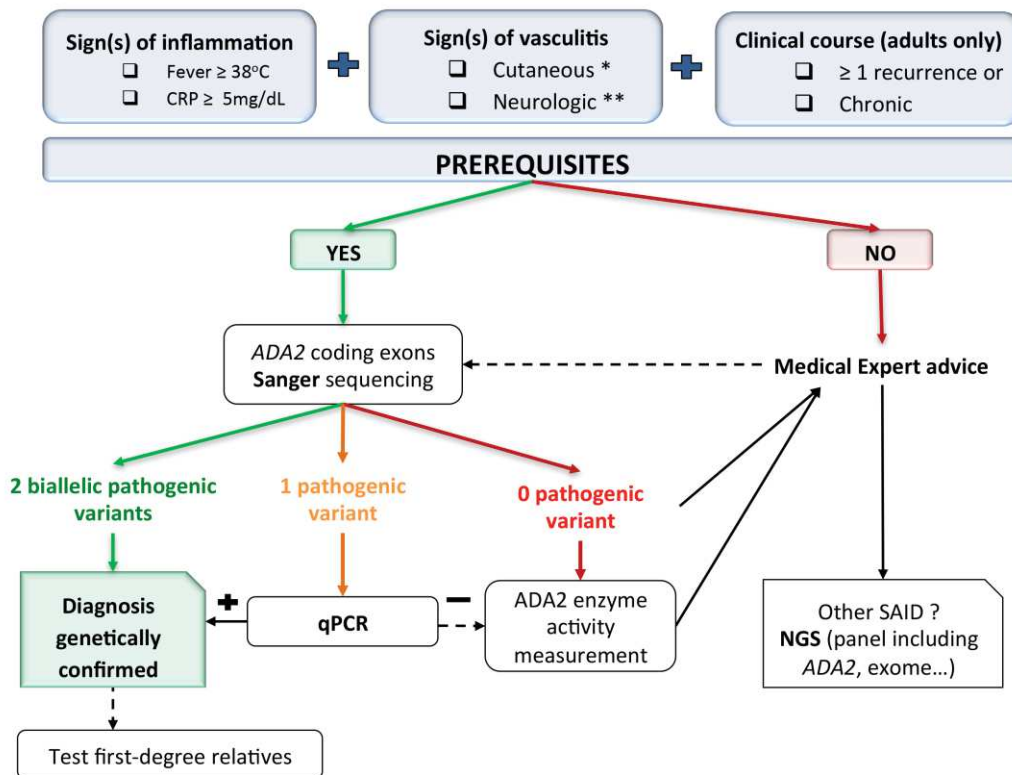


Figure 3 Decision tree for genetic diagnosis of DADA2

At the top of the figure are the selected prerequisites for a genetic diagnosis. At least one item of each of inflammation, vasculitis and clinical course must be present for Sanger sequencing. Bold lines depict advised steps. Dotted lines show optional decisions. CRP: C-reactive protein, *ADA2*: adenosine deaminase type 2, SAID: systemic autoinflammatory disorder, NGS: next-generation sequencing.

*: Livedoid skin rash, vasculitis, periarteritis nodosa, erythema nodosum, necrosis of extremity

** : central or peripheral neurologic involvement, ischemic, hemorrhagic or palsy

DISCUSSION

We report a large series of patients referred to us for genetic diagnosis of DADA2. We used information provided by the ordering clinicians to 1) describe the population with suspected DADA2, 2) compare our patients to those previously reported and 3) try to delineate prerequisites for a positive genetic diagnosis. We identified 13 patients carrying recessively inherited mutations in *ADA2* that were predicted to be deleterious. Eight patients were compound heterozygous for mutations. Seven mutations were novel (4 missense variants, 2 predicted to affect mRNA splicing and 1 frameshift). Phenotypic manifestations included fever, vasculitis and neurological disorders. Prerequisites for quick and low-cost Sanger analysis

included one typical cutaneous or neurological sign, one marker of inflammation (fever or elevated CRP level), and recurrent or chronic attacks in adults.

We describe a large spectrum of disease expression and severity, ranging from limited cutaneous vasculitis to severe cerebral vasculitis, in agreement with previous reports. Our SAID clinical form revealed novel symptoms at DADA2 presentation. For example, patient D1 had neither vasculitis nor neurologic involvement at first. His initial symptoms were arthritis symptoms. Musculoskeletal disorders (arthritis, arthralgia or myalgia) accompanied more specific symptoms in 9 patients (69% of patients with genetic confirmation of DADA2) and were not necessarily associated with vasculitis. The importance of rheumatologic involvement was not highlighted in previous series and suggests that patients with undiagnosed DADA2 may consult in rheumatologic departments. Caorsi et al. also hypothesized that DADA2 might represent an unrecognised condition in adult patients consulting rheumatologists(258). The age at disease onset in our study group was twice later than in published paediatric series, (mean 12.7 vs 5.3 years; median 13.5 vs 3 years). Seven of our patients were indeed recruited in adult departments. One patient (J1) had a very late and severe dermatological disease, with inaugural necrosis at age 33. These symptoms could account for the apparent later disease onset of our patients.

Our study expands the spectrum of known DADA2-associated mutations recorded in the Infevers registry of hereditary autoinflammatory-disorder mutations (257). Indeed, we identified 7 novel mutations: 4 missense, 1 frameshift and 2 splicing variants associated with typical DADA2 symptoms. Figure 1b highlights that DADA2 mutations are distributed all along the gene, with 2 mutational hot spots at codon 47 (4 different mutations) and codon 251 (2 mutations). It also shows that exonic deletions may occur, thereby justifying the use of qPCR when only one pathogenic mutation is identified in a patient with a clear DADA2 phenotype(256,260).

We found 2 allelic pathogenic ADA2 mutations in one-fifth of our patients, thus confirming the DADA2 diagnosis. Three patients had heterozygous mutations (Table 1S). Two presented one VUS: c.740C>T; p.(Ala247Val) and c.511C>T; p.(Arg171Trp), respectively. Because the clinical features of these patients included none of the DADA2 features of vasculitis, systemic inflammation, immunodeficiency or neurological manifestations, the suspicion of this diagnosis was considered too weak to extend the ADA2 analysis, and the physicians considered that their patients had another, still undefined, SAID. The genotype p.(Gly47Arg);(Gly47=) found in the third heterozygous patient was probably responsible for the mild phenotype, as supported by

the reduced but not null enzyme activity. A recent study showed that ADA2 heterozygote patients exhibit mild symptoms such as livedo, arthromyalgia, and recurrent infections(258). This work and ours support the hypothesis of a gene dosage effect accounting for the variable clinical expression observed in patients with DADA2-like disease, as previously demonstrated in other autoinflammatory diseases(220).

Phenotypic variability is common in DADA2(241,247). Our series is too small to detect a definitive or novel genotype–phenotype correlation. However, we could confirm some trends. Our 2 patients who were homozygous for the pathogenic p.(Tyr453Cys) variant (patients F1 and J1) had a cutaneous presentation and were referred in the third decade of life by a dermatologist. Two other patients who were heterozygous for this variant (patients A1 and A2) had also cutaneous signs. All reported patients carrying this mutation had livedoid skin rash(241). Two patients (D1 and K1) presenting the p.(Arg169Gln) variant, one homozygous and 1 heterozygous, had hypogammaglobulinemia, a defect frequently associated with this variant (62%)(241). While preparing this manuscript, Schepp et al. published data for a cohort of 181 adult patients with immunodeficiency or hypogammaglobulinemia as a common failure. The authors' NGS analysis (large panel or exome) highlighted 2 *ADA2* pathogenic variants in 11 patients(246). Vascular manifestations and non-infectious fever were present in 64% of his patients, demonstrating 2 clinical presentations, which might overlap in some patients. It also confirms that immunodeficiency seems a more common trait of the disease in adults than previously anticipated. Of note, 5 of 11 patients carried the p.(Arg169Gln) variant.

At least 6 other large DADA2 series have been reported (239–241,247,254,261). The inclusion criteria and study design were variable, according to the goal of the study. The 2 initial papers described the identification of the gene in patients with recurrent stroke(239) or PAN(240). Two studies preferred clinical criteria and analysed the prevalence of ADA2 mutations in patients with a typical DADA2 phenotype(241,261). Two opted for a genetic criterion and discussed presymptomatic diagnosis and variable expression, respectively (247,254). We took a different strategy. The aim of our study was to compare patients with a clearly pathogenic genotype to those with no genetic confirmation in order to identify the clinical constellation most likely to lead to genetic confirmation. We imposed no clinical selection or criteria before testing but collected uniform clinical data for each patient. Therefore, this series exactly reflects the context of requests for sequencing the ADA2 gene we receive in our laboratory.

In our series, the best performance resulted from the combination of biological and clinical signs (Table 3). We propose the decision tree illustrated in Figure 3. The first mandatory

prerequisite we suggest is fever (or at least elevated CRP level) because this clinical sign, alone or in combination with other symptoms, was a significant marker of genetic confirmation. We also advise associating any one of the following signs of vasculitis: PAN, livedoid skin rash, or systemic vasculitis such as that involving the cerebral or peripheral neurologic system because the clinical symptoms may differ among patients. In addition, we estimate that a chronic or recurrent clinical course is an important criterion to decrease the risk of sporadic causes of inflammation in adults. All patients with genetically confirmed DADA2 had at least 3 flares; therefore, we consider it reasonable to require at least one recurrence as a condition for genetic analysis.

We include 2 additional items in this decision tree that we did not evaluate formally. We do not require reduced enzymatic activity as a condition for genetic analysis. However, measurement of ADA2 activity likely represents an added value to the diagnosis, because serum ADA2 enzyme activity was significantly lower in all confirmed DADA2 cases than in healthy controls, even in the absence of ADA2 mutation(241). Nanthapaisal et al. strongly recommended screening first-degree relatives because presymptomatic molecular diagnosis of DADA2 may allow for early treatment in the event of an acute presentation, so we retain this suggestion. We do test symptomatic relatives and plan to test asymptomatic relatives on request also. Finally, we could not evaluate cytopenia and hypogammaglobulinemia as possible prerequisites, because these items are not present in our clinical form. However, these data could be extracted in 5 of 13 (38%) of our confirmed patients for whom the space “other symptom” was used. This finding is consistent with previous data (33-55%)(258). Moreover, Caorsi et al. observed no difference in incidence of hypogammaglobulinaemia by mutation status of patients(241). Therefore, this item is probably optional in our proposed decision tree.

Our model performed well retrospectively. Two paediatric patients would have been missed by using only the proposed prerequisites for Sanger sequencing (258,259). Their outcome is unknown. They could show a complete phenotype in later ages. Diagnosis in childhood may be an urgent matter, and delaying molecular investigation in children not fulfilling our prerequisites seems not advisable. On the other hand, our decision tree encompasses this risk by clearly suggesting medical expert advice, with possible NGS including this gene. However, our decision tree would not have resulted in too much testing either. Indeed, a simulation showed that unnecessary genetic analysis of ADA2 would have been performed for only 1.9% of SAID patients without DADA2.

In summary, this work demonstrated that our patients with DADA2 in France present clinical features similar to those of patients from different countries. We also contribute to expanding the mutational spectrum associated with the DADA2 phenotype. Finally, we used systematic and homogeneous clinical forms along with genetic testing orders to highlight the items most frequently encountered in patients with a confirmed genotype. We propose a preliminary decision tree for genetic diagnosis of DADA2 in the context of SAIDs and plan to confirm this algorithm in prospective cohorts. We believe that it may already help physicians prioritize molecular screening among SAID patients with possible DADA2 disease.

Supplementary files

Figure S1 Clinical form required for genetic analysis

Etiquette patient

PATIENT :
FAMILY NAME
First name
Date of birth --/--/----
Sex F M
Address

MANDATORY PRE-REQUISITES :

1. **Medical prescription**
2. **Sampler identity**
3. **Consent (or certificate)** signed by the patient or his/her guardian
4. **Clinical form** filled by the prescriber
5. **Genealogic tree**
6. **Number of unexplained attacks** ≥ 3 : Yes No
7. **CRP value during an attack** : -----mg/L or ----- μ mol/L

Triggering factor	Cold Hot Vaccination Stress Fatigue Other ----	No	ND
Attack frequency	< 1/month 1 à 2 / month > 2 / month chronic		ND
Age at first symptoms	---- month ---- year		ND
Attack duration	---- hours ---- days		ND
Consanguinity		Yes No	ND

BIOLOGY already performed to be documented if known

Other auto-inflammatory genes already analyzed ----
Mevalonaturia during attacks----- Anemia----- Autoantibodies -----

TREATMENT	Name	Dose	Start Age	Effect (N null, P partial, T total)	
Colchicin	-----	----/j	-----	N P T	ND
Corticoids	-----	----/j	-----	N P T	ND
Biotherapies	-----	----/j	-----	N P T	ND
Other -----	-----	----/j	-----	N P T	ND

Box the symptoms presented by the patient before the start of the treatment
+++ differentiate No and ND (Not determined)

Inflammation	Yes	No	ND	Gastro-Intestinal	Yes	No	ND
Fever				Abcess			
CRP↑				Hepatic cytolysis			
Thorax				Diarrhea			
Pain				Abdominal pain			
Pleuritis-Pericarditis				Bleeding			
Pneumopathy				Vomiting			
Neuro-sensoriel				Mucocutaneous			
Stroke				Oral aphthosis			
Calcifications				Genital aphthosis			
Headache				Erythema nodosa			
Conjunctivitis				Folliculitis			
Encephalitis				Lipodystrophy			
Epilepsy				Maculopapula			
Meningitis				Necrosis			
Papillitis				Edema			
Mental retardation				Pseudoerysipelas			
Deafness				Psoriasis			
Uveitis				Pyoderma			
Skeleton				Tenosynovitis			
Arthralgia				Urticaria			
Arthritis				Vasculitis			
Myalgia				Divers			
Arthropathy deformans				Growth retardation			
Osteitis				Lymphadenopathy			
Kidney				Allergy			
Amyloidosis				Hepatomégalie			
Renal failure				Recurrent infections			
Proteinuria				Pharyngitis			
				Splenomégalie			
				Other? (define)			

Figure S1 Clinical form required for genetic analysis

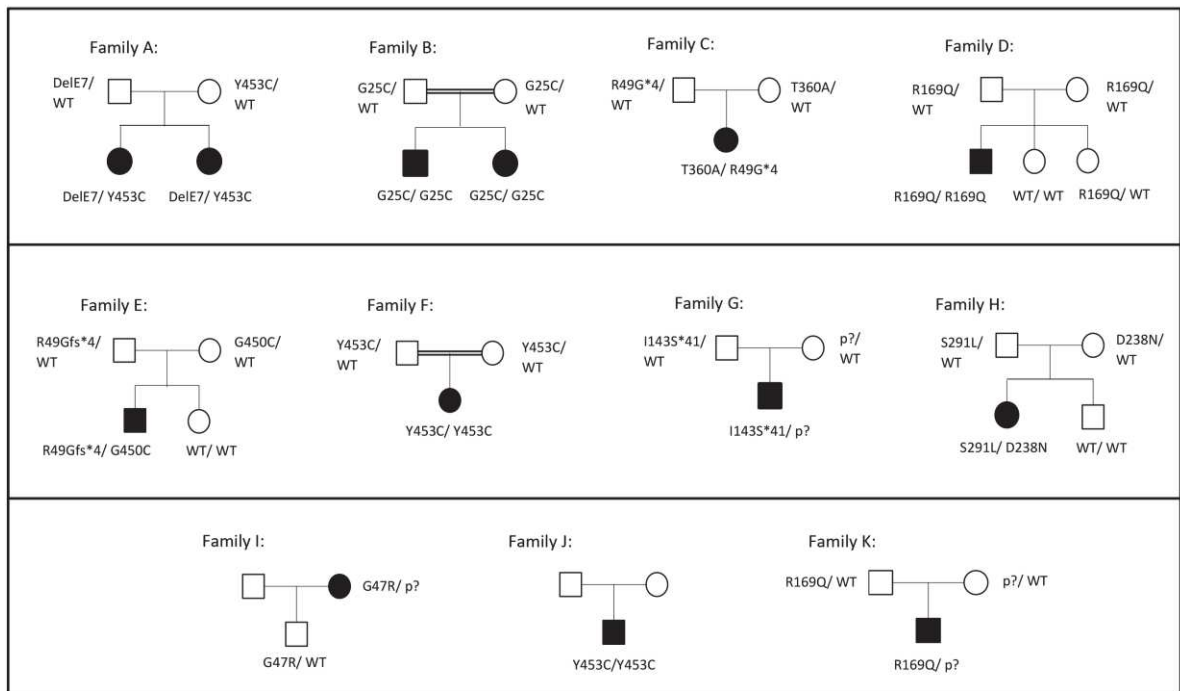


Figure S2 Genealogy trees for the 13 positive patients

Supplementary File Table S3 Clinical characteristics of 3 heterozygous patients:

Subject /Sex	E	Age at disease onset (years)	Fever	CRP level mg/L	Peripheral and central nervous system involvement	Cutaneous involvement	Immunologic /Hematologic involvement	Musculo-skeletal disorders	Other	Current treatment	ADA2 genotypes and protein alleles
L1/M	C	5	Yes	27	Cephalalgia	Edema	NA	Arthralgia; Myalgia	Gastrointestinal disorders; Elevated CPK	Colchicine	c.[139G>A];[139G=] p.[(Gly47Arg)];[Gly47=]
M1/F	M	1	No	<5	No	Raynaud's syndrome	NA	No	No	NA	c.[740C>T];[740C=] p.[(Ala247Val)];[Ala247=]
N1/F	C/M	5	No	200	No	Oral aphtosis	NA	Myalgia	No	NA	c.[511C>T];[511C=] p.[(Arg171Trp)];[Arg171=]

E: Ethnicity; C: Caucasian, M: Maghrebian, C/M: mixed Caucasian and Maghreb, M: male, F: female, NA: Not available, CPK: creatine phosphokinase, CRP : C-reactive protein,.

9

PSMB10, the last immunoproteasome gene missing for PRAAS

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Capsule summary

We describe a patient exhibiting clinical features of PRAAS due to a homozygous mutation in *PSMB10*. This gene encodes the only immunoproteasome subunit in which mutations have not yet been associated with PRAAS.

Key words

SAID, PRAAS, CANDLE, Nakajo-Nishimura syndrome, JMP, proteasome, immunoproteasome, interferonopathy, PSMB10

Abbreviations

APRIL:	A proliferation-inducing ligand
CANDLE:	chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature
CRP:	C-reactive protein
ELISA:	enzyme-linked immuno assay
ENU :	N-ethyl-N-nitrosourea
Hb:	haemoglobin
IFN α :	interferon alpha
IFN β :	interferon beta
IFN γ :	interferon gamma
IL-18:	interleukin 18
IL-1 β :	interleukin-1 beta
IL-6:	interleukin 6
iP:	immunoproteasome
ISG:	IFN stimulated genes
JMP:	joint contractures, muscle atrophy, microcytic anaemia and panniculitis-induced lipodystrophy
MCP-3:	monocyte-chemotactic protein 3
MCV:	mean corpuscular volume
MIG:	monokine induced by gamma interferon
NFKB:	nuclear factor-kappa B
NNS:	Nakajo-Nishimura syndrome
PBMC:	peripheral blood mononuclear cell
PRAAS:	proteasome associated autoinflammatory syndrome
SAID:	systemic autoinflammatory disease
SCID:	severe combined immunodeficiency
smMIP:	single-molecule molecular inversion probes
TNF α :	tumour necrosis factor alpha

To the Editor:

Recently, de Jesus et al described proteasome assembly chaperone mutations in PSMG2/PAC2 as causing a novel proteasome-associated autoinflammatory syndrome (PRAAS) called PRAAS4(262). Monogenic systemic autoinflammatory diseases (SAIDs) are characterized by systemic and organ specific inflammatory damage resulting from defective regulation of the innate immune system, with no evidence of other causes such as infections(263). The term “PRAAS” encompasses several phenotypes namely PRAAS1 [MIM 256040], PRAAS2 [MIM 618048], PRAAS3 [MIM256040] and PRAAS4, with overlapping clinical features including joint contractures, muscle atrophy, microcytic anaemia, chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature. Because interferon (IFN) type I dysregulation has been documented in PRAAS, these diseases are considered both SAIDs and interferonopathies(264). Known PRAAS mutations occur in genes encoding constitutive proteasome subunits (*PSMB4*, *PSMA3*), chaperone proteins (*POMP*, *PSMG2*), or immunoproteasome (iP) subunits (*PSMB8*, *PSMB9*)(78,79,265–267). The third iP specific subunit is PSMB10.

Here, we describe a 4-year-old girl with clinical features evoking of PRAAS, and who harboured a novel homozygous mutation in *PSMB10*, not previously known to be associated with PRAAS (FIG 1A). The patient was born to related Algerian parents and had 5 siblings. All living relatives were asymptomatic. One brother died from respiratory distress with jaundice at age 1 month. The patient showed a cutaneous rash on day 7 of life and became febrile at age 1 year. Several episodes of cutaneous disease occurred, characterised by a polymorphic rash being predominantly annular shaped and periorbital (FIG 1B). Cutaneous biopsy revealed a nonspecific lymphocytic infiltration. The child failed to thrive (she was in the 1st percentile for height and weight) and had persistent hepatosplenomegaly. She had an emaciated face and long and slender fingers. Routine biological examinations revealed elevated acute phase reactant levels, microcytic anaemia in the context of a minor thalassemia trait, and hypertriglyceridemia (Table E1). She exhibited partial response to steroids and methotrexate, but relapsed when doses were reduced. A diagnosis of PRAAS was postulated on the basis of the cutaneous phenotype.

Sequencing of a panel of 62 genes for SAIDs including known PRAAS genes did not reveal any pathogenic mutation (Table E2). Low-level mosaicism was not seen by smMIPS (single-molecule molecular inversion probes) analysis (data not shown). However, trio-based (patient and her parents) whole exome sequencing revealed a homozygous c.41T>C (p.Phe14Ser)

variant in *PSMB10* (NM_002801.3), which was confirmed by Sanger analysis. Several lines of evidence supported the pathogenicity of this mutation. Familial segregation of this mutation was consistent with an autosomal recessive inheritance (FIG 1A). The variant, absent from 70728 alleles in the gnomAD cohort, was predicted to be pathogenic according to *in silico* bioinformatics tools (GVGD, SIFT, Polyphen2, Mutation Taster) (Table E3). The phenylalanine at position 14 is conserved across evolution (FIG 1C).

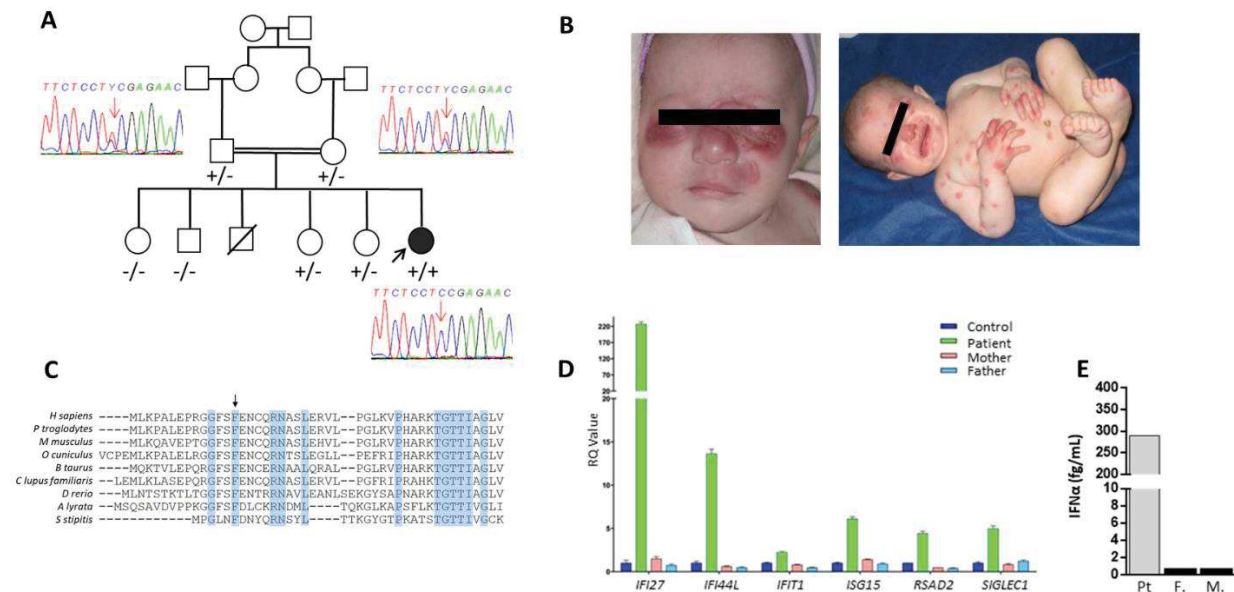


FIG 1: Clinical presentation and genetic findings in *PSMB10*-PRAAS

(A) Pedigree and Sanger sequencing of the family. The red arrow shows the *PSMB10* c.41T>C, p.Phe14Ser mutation. Homozygous mutated (+/+), heterozygous (+/-), and homozygous wild-type (-/-) genotypes. (B) Pictures showing annular cutaneous rashes (C) *PSMB10* protein alignment showing conservation of Phe14 across species (D) Targeted interferon stimulated gene RNA expression in total blood in one patient's sample compared to her parents and 29 healthy controls. RQ: (relative quantification) is the normalized fold change relative to the control data. (E) Serum from the patient and her parents were analyzed by digital ELISA on a Simoa platform (control values < 10 fg/ml).

Proteasomes are hetero multimeric proteins found in all eukaryotic cells. Their major role is to cleave ubiquitylated proteins, resulting in the release of peptides which are bound by the major histocompatibility complex (MHC) class I molecules. This ubiquitin-proteasome system contributes to intracellular proteostasis. The 20S proteasome is a barrel-shaped complex consisting of two outer heptameric rings (subunits α 1 to α 7) and two inner heptameric rings (subunits β 1 to β 7). The trypsin-, chymotrypsin- and caspase-like proteolytic sites are located in the β 2, β 5 and β 1 subunits respectively. The iP is a proteasome subtype assembled spontaneously in hematopoietic cells, or other cell types after induction of its 3 inducible

subunits: PSMB8 ($\beta 5i$), PSMB9 ($\beta 1i$), and PSMB10 ($\beta 2i$) by IFN γ or tumour necrosis factor α (TNF α).

To investigate type I interferon (IFN) signalling status in our patient, we performed transcriptional analyses, demonstrating increased expression of six type I IFN stimulated genes (ISGs) compared to healthy controls (FIG 1D). Digital ELISA showed elevated amounts of the IFN α protein in the serum of the index case (290 fg/mL, normal < 10 fg/mL (FIG 1E). ISG expression and IFN α protein levels in her heterozygous parents were normal. Multiplex ELISA showed that interleukin 6 (IL-6), monocyte-chemotactic protein 3 (MCP-3), TNF α and monokine induced by gamma IFN protein (MIG) levels were elevated in the patient compared to paediatric controls whereas interleukin 18 (IL-18), and A proliferation-inducing ligand (APRIL) were not (FIG E1). IFN β , IFN γ and IL-1 β were undetectable. These data suggest a positive feedback-loop involving type I IFN pathway.

The p.Phe14Ser mutation is located at the N-terminus, which is auto-cleaved into its mature form (FIG 2A)(268).

To assess whether this mutation would prevent cleavage, we conducted immunoblot assays of HeLa cells transfected with wild-type (WT) or mutant PSMB10 protein (FIG 2B). Without induction, WT and mutant PSMB10 protein exhibited two upper bands (~33 and ~30kDa) corresponding to uncleaved, immature PSMB10 proforms. On IFN γ induction, the WT protein had a lower band (~27kDa) corresponding to the mature auto cleaved protein, which is absent with mutant protein, supporting our hypothesis. Considering its location in the immunoproteasome complex, the propeptide retention probably disturbs the assembly of 20S proteasome (FIG 2C)

To measure *in vitro* the impact of the mutation on trypsin-like, chymotrypsin-like and caspase-like enzymatic activities, HEK293T cells were transfected with WT or mutant PSMB10 constructs and induced with IFN γ (FIG 2D). Only trypsin-like activity was significantly decreased in mutant cell extracts compared to WT ($p < 0.0001$).

To confirm immunoproteasome dysfunction in the patient's cells, we measured *ex vivo* enzymatic activities. Trypsin-like activity was lower in PBMCs from our patient versus healthy controls (FIG 2E). No fresh blood was available to replicate this experiment. In addition, we confirmed the accumulation of ubiquitinated proteins in PBMCs lysates from the patient, compared to healthy controls, with or without MG132 inhibition. (FIG 2F).

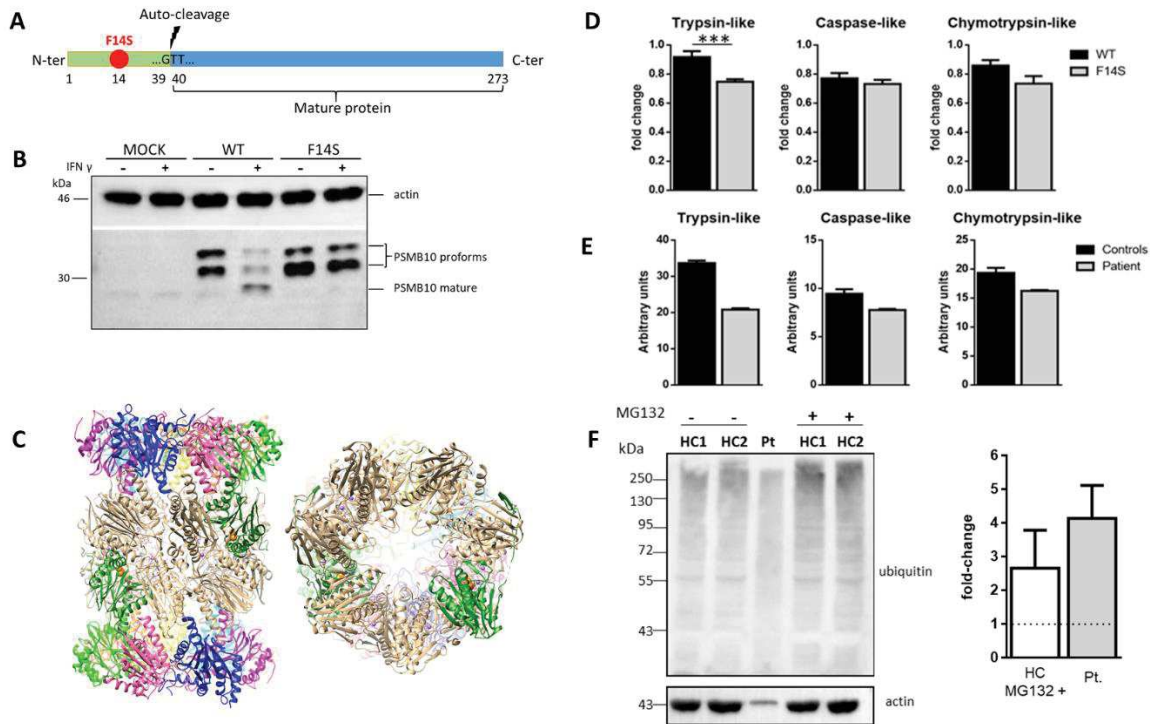


FIG 2: Functional studies of PSMB10-PRAAS

(A) PSMB10 secondary structure showing the mutation (in red) located in the auto-cleavable propeptide (in green). (B) PSMB10-flag immunoblot from transfected HeLa cells, with or without IFN γ induction. MOCK: empty vector, WT: wild-type vector, F14S: mutant vector (C) Left panel: human 20S immunoproteasome structure with PSMB10 subunit in green, N-ter atoms in orange, all α subunit in various colors. Right panel: upside view with hidden upper coloured α subunit with better view of PSMB10. (D) *In vitro* enzymatic activities measured in lysates from IFN γ induced WT (black bars) or mutant (grey bars) transfected HEK293T cells, and normalized with empty vector (triplicates, three independent experiments). (E) *Ex vivo* enzymatic activity measured in patient's PBMCs (grey bars) compared to three healthy controls (3 technical replicates). (F) Left panel: Representative ubiquitin immunoblot from healthy controls (HCs), with or without MG132 proteasome inhibitor, and the patient PBMCs lysates. Right panel: Fold-change of immunoblot lane intensities, normalized with actin intensities, from 2 HCs inhibited with MG132 and the patient, compared to untreated HCs. Data are from two experiments.

Further support *PSMB10* as the gene involved in the phenotype of our patient is from Treise and colleagues, who recently described a *psmb10* mutant mouse line, called TUB6(269). The mice harboured a homozygous p.Gly170Trp mutation responsible for severe combined immunodeficiency, systemic autoinflammation, and symptoms mimicking PRAAS. The authors stated that TUB6 was the first mouse model of human PRAAS and suggested *PSMB10* as a candidate gene for screening in humans. Our patient shared many features with the TUB6 model, except immunodeficiency. The phenotype discrepancy between mouse and human has been described in the SAVI mouse models as well, since they exhibit a SCID phenotype, not reported so far in SAVI patients.

To conclude, we describe a patient exhibiting a PRAAS phenotype due to a homozygous mutation in *PSMB10*, that led to IFN type I dysregulation, PSMB10 maturation defect, and enzymatic impairment. This is the third and last immunoproteasome gene reported to be responsible for PRAAS.

Material and methods

Patient recruitment

The patient was enrolled in the framework of the European INSAID consortium project (Grant n° 9003037603). Written informed consent was obtained from study participants or their legal representative.

Genetic analysis

We first performed next generation sequencing of a panel of 62 genes. The library was prepared with a SureSelectQXT target enrichment capture custom kit (Agilent) and sequenced by using NextSeq500 equipment (Illumina). Bioinformatic analysis involved using SeqNext (JSI). Gene mosaicism screening was performed using single-molecule molecular inversion probes (smMIP). The assay was adapted from previously published protocols (270,271) for screening of SAID patients, targeting 5 genes associated with dominantly inherited SAID. Probe sequences are available upon request. Sequencing was performed on a NextSeq500 sequencer (Illumina), analysis was performed with SeqNext software (JSI). We performed a trio based (patients and the 2 parents) whole exome analysis. The library was generated by using the Clinical Research Exome V2 kit (Agilent) and sequenced by using the NextSeq500 sequencer. Bioinformatic analysis involved using SeqOne (www.seq.one). After quality and coverage filtering, our strategy was based on the autosomal recessive model, and excluded frequent (>1% according to ExAC), synonymous and intronic mutations. Variant confirmation and the family study were performed with Sanger sequencing.

Protein alignment

We aligned PSMB10 protein sequences by using Uniprot, from *H sapiens* (human) *P troglodytes* (chimpanzee) *M musculus* (mouse), *O cuniculus* (rabbit), *B taurus* (bovine), *C lupus familiaris* (dog), *D rerio* (zebrafish), *A lyrata* (arabidopsis) and *S stipitis* (yeast).

Protein 3D model

We use the recent (released 9th September 2019) crystallographic structure, PDB Number 6E5B, to illustrate the position of PSMB10 subunit in human immunoproteasome. We use Chimera software to design pictures.

Cloning and transfection of expression vectors

A pCMV6 with wild-type (WT) *PSMB10* Myc-DDK-tagged in the 3' end (Origene technologies) was used as a template. The p.Phe14Ser mutant vector was obtained using directed mutagenesis primers (forward (5'-P-CCCGAGGGGGCTTCTCCTCCGAGAAGTCCCAAAG-3', reverse 5'-P-CTTTGGCAGTTCTCGGAGGAGAAGCCCCCTCGGG-3') and high-fidelity Pfu DNA polymerase (Promega) following a DpnI digestion. Integrity of WT and mutant plasmid sequences was monitored by Sanger sequencing.

SDS-PAGE and western-blot analysis

HeLa cells (human epithelial carcinoma non immune cell line) were cultivated with DMEM/F-12 medium with 10% foetal bovine serum, penicillin (100 U/mL), streptomycin (100 U/mL) and 0.5% glutamine. Cells were plated in 6-well plates ($4 \cdot 10^5$ cells/mL) 24h prior transfection in medium without antibiotic, and were transfected with 7.5 μ L Lipofectamine (Invitrogen) according to the manufacturer's protocol with 4 μ g empty vector, WT vector or p.Phe14Ser mutant p.CMV6-PSMB10 vector. Five hours later, the transfection medium was replaced by a culture medium supplemented with IFN γ (200U/mL) during 48h. Cell lysates were prepared using CellLytic M Cell Lysis Reagent (Sigma) with the addition of anti-protease. Equal amounts of protein were separated on SDS-PAGE (15%) and western-blot analysis was performed with the primary anti-Myc-DDK antibody (1/1000 $^\circ$) (Sigma) followed by the secondary anti-mouse antibody (1/1000 $^\circ$) (LifeTechnologies), and the primary anti-actin antibody (1/200 $^\circ$) (Santa Cruz) followed by the secondary antibody anti-goat (1/5000 $^\circ$) (Santa Cruz).

Ubiquitin blots were performed with cell lysates from PBMC, which were purified from heparin blood samples from the patient and 2 healthy controls, and seeded for 6 hours in RPMI-1640 GlutaMAX with penicillin (100 U/mL), streptomycin (100 U/mL) and 20% foetal bovine serum. Cell lysates were prepared using CellLytic M Cell Lysis Reagent (Sigma) with the addition of anti-protease. Equal amounts of protein were separated on SDS-PAGE (10%) and western-blot analysis was performed with the primary anti-ubiquitin antibody (1/1000 $^\circ$) (Abcam) followed by the secondary anti-rabbit antibody (1/10000 $^\circ$) (Pierce), and the primary anti-actin antibody (1/200 $^\circ$) (Santa Cruz) followed by the secondary antibody anti-goat

(1/5000°) (Santa Cruz). Due to haemolysis in the patient sample, we normalized the results with actin bands intensities. Data on histogram represented the fold-change of the means of lane intensities from the patient and 2 HCs treated with MG-132 (Sigma), a proteasome inhibitor, compared to untreated HCs (two experiments). Detection and imaging were performed by using ChemiDoc (BioRad) and ImageLab (BioRad) respectively.

Enzymatic assays

Ex vivo studies: PBMC were purified from heparin blood samples from the patient and 3 healthy controls, and were seeded for 6 hours in RPMI-1640 GlutaMAX with penicillin (100 U/mL), streptomycin (100 U/mL) and 20% foetal bovine serum.

In vitro studies: HEK293T cells (embryonic kidney non immune cell line) were cultivated with DMEM/F-12, 10% foetal bovine serum, penicillin (100 U/mL), streptomycin (100 U/mL) and 0.5% glutamine. Cells were plated in 6-well plates ($4 \cdot 10^5$ cells/ml) and were transfected with Lipofectamin (Invitrogen), according to the manufacturer's procedure, with empty vector, WT or p.Phe14Ser mutant p.CMV6-PSMB10 vector. Five hours later, HEK293 cells were induced with IFN γ (200U/mL) for 48h.

PBMCs and HEK293T cells were lysed with home-made lysis buffer (25mM Tris-HCl pH 7.5, 1mM EDTA, 1mM ATP, 0.5mM DTT and 15% glycerol). Supernatants were collected and protein concentration was measured by BCA protein assay (Pierce). Briefly, 5 μ g total protein was used for detecting caspase-like activity and 10 μ g were used for the chymotrypsin-like and trypsin-like activity. The same amount of total protein has been loaded for each sample. Cell lysates were incubated, in the dark at room temperature, with 50 μ M 7-amino-4-methylcoumarin (AMC)-coupled peptides: Suc-LLVY-AMC, Z-LLE-AMC and Z-ARR-AMC for chymotrypsin, caspase and trypsin-like activity respectively. MG-132 (Sigma), 20 μ M, a proteasome inhibitor, was used to determine the background noise to be subtracted. Fluorescence was measured after 60 min with a Varioskan reader (Thermo Scientific).

In vitro results were normalized to empty vector, each analysis was performed in triplicates. Data are mean \pm SEM and were calculated from 3 independent transfections (statistical analysis: t-test, Mann-Whitney, unpaired, two-tailed. ***p<0,0001).

Ex vivo results were calculated from one sample from the patient or the 3 healthy controls. Each analysis was performed in triplicates. Data are mean \pm SEM.

Cytokine analysis

All cytokines were measured in serum from the patient, her parents, 4 healthy pediatric controls and one healthy adult control. A custom Human Premixed Multiplex Luminex (catalog no. PPX-09-MXU627U; ThermoFisher Scientific, Waltham, MA) was used to measure 9 markers with 1:2 sample dilution: IFN- α , IFN- β , IFN- γ , IL-1 β , IL-6, IFN- γ -induced protein 10 (IP10), monocyte-chemotactic protein 3 (MCP3), monokine induced by gamma interferon (MIG) and tumour necrosis factor-alpha (TNF- α). A Human IL-18 Simplex Kit (catalog no. EPX01A-10267-901; ThermoFisher Scientific, Waltham, MA) was used to measure IL-18 with 1:2 sample dilution. Samples were diluted by using the calibrator diluent provided in the kits, processed according to the manufacturer's specification, and measured on a MAGPIX (Luminex Corp., Austin, TX) with xPONENT software. Instrument settings were set according to assay protocol. Data were analyzed by using ProcartaPlex Analyst. For the calibration and verification of the MAGPIX, a MAGPIX Performance Verification Kit and a Calibration Kit (catalog no. MPX-PVER-K25 and MPX-CAL-K25; Luminex Corp., Austin, TX) were used.

Targeted ISGs RNA expression in total blood

Whole blood from the patient and the parents was collected into PAXgene tubes, total RNA was extracted by using a PreAnalytix RNA isolation kit and RNA concentration assessed by using a spectrophotometer (FLUOstar Omega, Labtech). Quantitative reverse transcription polymerase chain reaction (qPCR) analysis was performed using the TaqMan Universal PCR Master Mix (Applied Biosystems), and cDNA derived from 40 ng total RNA. To generate a standard 6 probe interferon score, using TaqMan probes for IFI27 (Hs01086370_m1), IFI44L (Hs00199115_m1), IFIT1 (Hs00356631_g1), ISG15 (Hs00192713_m1), RSAD2 (Hs01057264_m1), and SIGLEC1 (Hs00988063_m1), the relative abundance of each target transcript was normalized to the expression level of HPRT1 (Hs03929096_g1) and 18S (Hs999999001_s1), and assessed with the Applied Biosystems StepOne Software v2.1 and DataAssist Software v.3.01. For each of the 6 probes, individual data were expressed relative to a single calibrator. RQ (relative quantification) is equal to $2^{-\Delta\Delta Ct}$ i.e. the normalized fold change relative to the control data. The median fold change of the 6 genes compared to the median of 29 previously collected healthy controls is used to create an interferon score for each individual, with an abnormal interferon score being defined as greater than +2 standard deviations above the mean of the control group i.e. 2.466.

Quantification of interferon alpha (IFN- α) in serum by Simoa assay

As reported by Rodero et al. (E5), a Simoa IFN- α assay was developed by using a Quanterix.Homebrew Simoa assay and two autoantibodies specific for IFN- α isolated and cloned from two APS1/APECED patients recently described. The 8H1 antibody clone was used as a capture antibody after coating on paramagnetic beads (0.3mg/mL), and the 12H5 was

biotinylated (biotin/Ab ratio = 30/1) and used as the detector. Recombinant IFN- α 17/ α I (PBL Assay Science) was used to generate a calibration curve after crossreactivity testing. The limit of detection was calculated by the mean value of all blank runs + 3SDs and was 0.64 fg/mL.

Supplementary datas

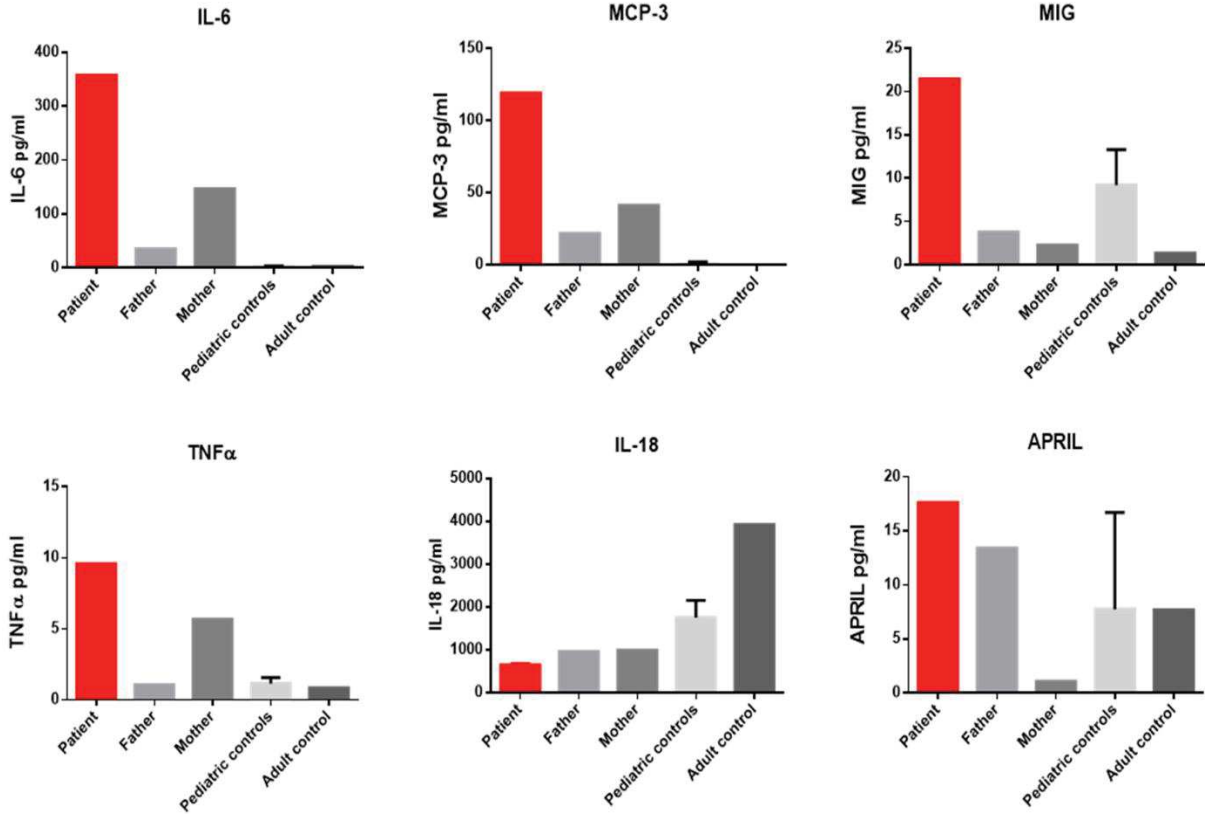


FIG E1: Cytokine panel dosage

Sera levels of IL-6, MCP-3, MIG, TNF α , IL-18 and APRIL from the patient, the 2 asymptomatic parents, age-matched paediatric controls (n=4) and an adult control were analyzed. Data were obtained from one sample.

Table E1: Routine biological findings in the patient

	21/02/2017	12/03/2017	17/03/2017	23/05/2017	03/07/2018	Reference values
Complete blood count						
White blood cells (G/L)	11,18	10,41	20,86	11,32		6,00-15,00
neutrophils (G/L)	4,73	3,05	5,65	5,21		1,50-8,70
lymphocytes (G/L)	5,96	6,84	11,43	5,77		0,03-13,50
monocytes (G/L)	0,42	0,48	3,3	0,28		0,00-2,00
eosinophils (G/L)	0,04	0,02	0,38	0,02		0,00-1,00
basophils (G/L)	0,02	0,02	0,1	0,03		0,00-0,10
Red blood cells (T/L)	3,53	3,73	5,23	3,37		3,70-5,15
Hemoglobin (g/dL)	5,6	6,1	12,1	5,6		10,2-13,4
Hematocrit (%)	22,3	24,1	35,6	21,9		31,5-40,5
Mean corpuscular volume (fL)	63,2	64,6	68,1	65		72,0-93,0
Mean corpuscular hemoglobin (pg)	15,9	16,4	23,1	16,6		23,5-31,0
Mean corpuscular hemoglobin concentration (g/dL)	25,1	25,3	34	25,6		30,0-35,0
Platelets (G/L)	311	408	576	514		220-490
Biochemistry						
Total cholesterol (g/L)	1,48	1,29		1,15		<1,80
Triglycerides (g/L)	2,95	2,25		2,82		<2,00
C-Reactive Protein (mg/L)	106,4	123		85,6		<10,00
Immunology						
Immunoglobulin G (g/L)					16,85	8,00-18,00
Immunoglobulin M (g/L)					1,73	0,6-2,80
Immunoglobulin A (g/L)					2,12	N/A

Table E2: Panel of 62 genes associated with autoinflammatory diseases

Gene	Pathology	NM
<i>ACP5</i>	SPENCD	NM_001111035.2
<i>ADAMI7</i>	NISBD1	NM_003183
<i>ADAR</i>	Aicardi-Goutieres	NM_001111
<i>AP1S3</i>	Psoriasis susceptibility to	NM_001039569.1
<i>CARD14</i>	PRP/PSORS2	NM_024110.4
<i>CASP1</i>	N/A	NM_001257118
<i>CECR1</i>	DADA2	NM_001282225.1
<i>COPA</i>	AILJK	NM_001098398.1
<i>DDX58</i>	Singleton-Merten	NM_014314
<i>FAM105B</i>	AIPDS	NM_138348
<i>FAS</i>	ALPS	NM_000043.5
<i>FASLG</i>	ALPS	NM_000639.2
<i>IFIH1</i>	Aicardi-Goutieres	NM_022168.3
<i>IL10</i>	IL10 deficiency	NM_000572.2
<i>IL10RA</i>	IL10 R1 deficiency	NM_001558.3
<i>IL10RB</i>	IL10 R2 deficiency	NM_000628.4
<i>IL1RN</i>	DIRA	NM_173841.2
<i>IL36RN</i>	DITRA	NM_173170.1
<i>LACCI</i>	SJIA	NM_001128303.1
<i>LPIN2</i>	Majeed	NM_014646.2
<i>MDFIC</i>	N/A	NM_199072.4
<i>MEFV</i>	FMF	NM_000243.2
<i>MVK</i>	MKD	NM_000431.3
<i>NCSTN</i>	PASH	NM_015331.2
<i>NLRC4</i>	AIFEC/ FCAS4	NM_021209.4
<i>NLRP1</i>	NAIAD	NM_033004.3
<i>NLRP12</i>	NAPS12	NM_144687.3
<i>NLRP3</i>	CAPS	NM_001243133.1
<i>NOD2</i>	Blau	NM_022162.2
<i>PLCG2</i>	APLAID	NM_002661.4

<i>POMP</i>	PRAAS/CANDLE	NM_015932.5
<i>PSENFEN</i>	N/A	NM_172341.2
<i>PSMA3</i>	PRAAS/CANDLE	NM_002788.3
<i>PSMB4</i>	PRAAS/CANDLE	NM_002796.2
<i>PSMB8</i>	PRAAS/CANDLE	NM_148919.3
<i>PSMB9</i>	PRAAS/CANDLE	NM_002800.4
<i>PSMG2</i>	PRAAS/CANDLE	NM_020232.4
<i>PSTPIP1</i>	PAPA	NM_003978.4
<i>PYCARD</i>	N/A	NM_013258.4
<i>RBCK1</i>	PBMEI	NM_031229.3
<i>RNASEH2A</i>	Aicardi-Goutieres	NM_006397.2
<i>RNASEH2B</i>	Aicardi-Goutieres	NM_024570.3
<i>RNASEH2C</i>	Aicardi-Goutieres	NM_032193.3
<i>RNF31</i>	N/A	NM_017999.4
<i>SAMHD1</i>	Aicardi-Goutieres	NM_015474.3
<i>SERPING1</i>	HAE1	NM_000062.2
<i>SH3BP2</i>	Cherubism	NM_003023.4
<i>SLC29A3</i>	H syndrom	NM_018344.5
<i>TMEM173</i>	SAVI	NM_198282.3
<i>TNFAIP3</i>	AISBL	NM_001270508.1
<i>TNFRSF11A</i>	TRAPS11	NM_003839.3
<i>TNFRSF1A</i>	TRAPS	NM_001065.3
<i>TNFRSF9</i>	N/A	NM_001561
<i>TREX1</i>	Aicardi-Goutieres	NM_016381.5
<i>TRNT1</i>	SIFD	NM_182916.2

Legend:

N/A	Candidate genes
AIFEC	Autoinflammation with infantile enterocolitis
AILJK	Autoimmune interstitial lung, joint, and kidney disease
AIPDS	Autoinflammation, panniculitis, and dermatosis syndrome
AISBL	Autoinflammatory syndrome, familial, Behçet-like
ALPS	Autoimmune lymphoproliferative syndrome

APLAID	Autoinflammation, antibody deficiency, and immune dysregulation, PLCG2-associated
CAPS	Cryopyrin associated periodic syndrome
DADA2	Adenosine deaminase 2 deficiency
DIRA	Interleukin 1 receptor Antagonist deficiency
DITRA	Interleukin 36 receptor antagonsit deficiency
FCAS4	Familial cold autoinflammatory syndrome 4
FMF	Familial mediterranean fever
HAE1	Angioedema hereditary type 1
MKD	Mevalonate Kinase deficiency
NAIAD	NLRP1-associated autinflammation with arthritis
NAPS12	NLRP12 associated periodic syndrome
NISBD1	Inflammatory skin and bowel disease, neonatal, 1
PAPA	Pyogenic Arthritis, Pyoderma gangrenosum, and Acne
PASH	Pyoderma gangrenosum, acne and suppurative hidradenitis
PBMEI	Polyglucosan body myopathy 1 with or without immunodeficiency
PRAAS/CANDLE	Proteasome associated autoinflammatory syndrome / Chronic Atypical Neutrophilic Dermatitis with Lipodystrophy and Elevated Temperature
PRP/PSORS2	Pytoriasis rubra pilaris / Psoriasis 2
SAVI	STING-associated vasculopathy with onset in infancy
SIFD	Sideroblastic anemia, B-cell immunodeficiency, periodic fevers, and developmental delay
SJIA	Systemic juvenile idiopathic arthritis
SPENCD	Spondyloenchondrodysplasia with immune dysregulation
TRAPS	TNF receptor associated periodic syndrome
TRAPS11	TNFRSF11A-associated hereditary fever disease

Table E3: Patient's mutation *in silico* predictions

Genomic	cDNA	Protein	GnomAD	MAF	GERP	Polyphen2	SIFT	Mutation Taster	PROVEAN	CADD PHRED
chr16:67970612A>G	NM_002801.3:c.41T>C	NP_002792.1:p.Phe14Ser		0	5,499	Probably damaging	Deleterious	Disease causing	Damaging	33

DISCUSSION

Les MAI monogéniques représentent à ce jour un vaste champ de découvertes potentielles. Plus de 40 de gènes sont associés à ce jour aux MAI(272). En effet, bien que des progrès considérables aient été faits, une proportion majoritaire de patients ne sont pas diagnostiqués au plan génétique(273,274). Une part de ces patients présente probablement des formes multifactorielles, voire oligogéniques, qui représentent les défis de demain pour les généticiens. De nombreuses MAI monogéniques sont encore à découvrir, et les études pan génomiques comme le WES représentent un outil précieux pour y parvenir.

Etude comparative entre séquençage classique et nouvelle génération

L'étude BIOSAID m'a permis de confirmer l'apport diagnostique du NGS dans les MAI. L'utilisation de panels phénotypiques a fait ses preuves dans les MAI(275,276). En effet, les formes cliniques des MAI peuvent être chevauchantes, et le diagnostic génétique final peut parfois être surprenant chez un patient.

Mais l'augmentation des informations génétiques récoltées s'accompagne mécaniquement d'une l'augmentation de variants de signification incertaine, dont l'interprétation se heurte aux limites des outils dont nous disposons. Les données combinées des études de ségrégation familiale, d'outils de prédiction *in silico*, et de consultation de bases de données ne permettent pas toujours de statuer sur la pathogénicité des mutations.

Il existe également le risque de « surdiagnostic », lui aussi mécaniquement augmenté par le grand nombre de variants rares identifiés chez les patients.

Au total, la génétique en France est en mutation, le goulot d'étranglement ne se situe plus à l'étape de génération de données génétiques, mais bien à leur interprétation, qui nécessite des explorations fonctionnelles. Ces études, quelquefois considérées comme appartenant au champ de la recherche, devraient être considérées comme un prolongement du diagnostic.

Caractérisation de gènes associés aux MAI

Duplication du gène MEFV chez une patiente atteinte de FMF

Grâce à ce travail, j'ai pu identifier la première duplication entière du gène *MEFV* chez une patiente atteinte de FMF. Les mutations impliquées dans la FMF sont de type gain de fonction et diminuent le seul d'activation de la pyrine/marénostriine (230,231,277). Une précédente étude n'avait pas détecté de remaniement dans ce gène(233)).

Si la détection de la duplication a été relativement aisée, le séquençage du point de cassure a été un processus long et laborieux, par succession de PCR quantitatives dans des zones

intergéniques parfois difficiles à amplifier. Les techniques de séquençage de longs fragments et sans amplification qui ont vu le jour récemment permettent désormais d'identifier les points de cassure plus rapidement(278).

La question de l'implication de ce remaniement dans la pathogénèse reste non résolue. En effet, la FMF, qui est le prototype mais également la plus fréquente des MAI monogéniques, est une pathologie complexe. Le modèle classique monogénique est parfois mis en échec chez certains patients(236,237,279). L'hypothèse d'un deuxième gène responsable de la FMF est ancienne mais n'a jamais été confirmée(280). Des variants controversés selon le modèle monogénique pourraient agir sur un mode multifactoriel, faisant de cette pathologie un modèle non purement monogénique.

L'identification de cette duplication met en lumière cette complexité. La patiente porteuse de la duplication, ainsi que de la mutation sévère M694V héritée de son père, est atteinte, sans que l'on puisse confirmer si la duplication joue un rôle dans la pathogénèse. Des études d'expression allèle spécifique pourraient préciser l'impact de cette duplication chez la patiente. Il n'est pas exclu qu'elle ne joue pas de rôle, et que seulement 1/3 du pool de protéine MEFV mutée en 694 soit responsable de la pathologie.

Etude du mosaïcisme dans les MAI

Cette étude m'a permis de mieux caractériser le phénomène de mosaïcisme dans les MAI, et dans les CAPS notamment. Les mosaïques étaient très largement sous-estimées à l'ère du séquençage Sanger, pour des raisons de seuil de détection peu performant (environ 20%).

Nous avons montré qu'il n'y a pas de lien entre la proportion de cellules portant la mutation, et les manifestations cliniques du CAPS, notamment neurologiques. L'âge d'apparition de la maladie ne semble pas non plus lié à la profondeur du mosaïcisme.

Néanmoins, les limites de cette étude sont le faible nombre de cas étudiés – le mosaïcisme reste rareté dans les maladies rares - et la non exhaustivité des tissus étudiés (cellules musculaires, neuronales...).

Nous avons également montré qu'il existe des points chauds dans *NLRP3*, qui sont plus volontiers le siège de mutations somatiques. On peut aussi émettre l'hypothèse que des mutations somatiques dans d'autres domaines de la protéine sont cliniquement silencieux.

Le phénomène de mosaïcisme est probablement plus répandu qu'on le pensait, et peut même concerner des maladies récessives (281).

Série de cas DADA2 et expansion du spectre des mutations

J'ai rapporté dans ce travail une série de cas de DADA2 à travers le centre de référence des maladies autoinflammatoires et de l'amylose inflammatoire (CEREMAIA) en France.

Cette publication a permis d'augmenter le spectre des mutations connues dans le gène *ADA2*. En accord avec les données de la littérature, les mutations sont variées dans leur mécanisme (substitutions, décalage du cadre de lecture) aussi bien que dans leur localisation (pas de hotspot).

Grâce à l'analyse statistique des signes cliniques plus fréquemment retrouvés chez les patients confirmés, j'ai proposé un arbre décisionnel diagnostique, permettant une prise en charge géénétique plus rapide et moins onéreuse. En effet, la combinaison d'un signe d'inflammation systémique, d'un signe de vascularite et d'une répétition des épisodes, est plus souvent associée à la découverte de mutation bi alléliques dans *ADA2*. Dans ce cas, un séquençage ciblé, plus rapide qu'un panel, permettra souvent de poser un diagnostic positif. Cet arbre décisionnel souligne aussi l'importance de chercher les remaniements par PCR quantitative, et propose d'évaluer l'activité enzymatique pour les cas non résolus génétiquement.

Cet arbre présente des limites, issues d'un biais de collecte d'informations de l'étude. En effet, nous n'avons pas systématiquement collecté les signes de dysimmunité ou hématologiques. Il est bien connu à présent qu'il existe des formes purement dysimmunitaire ou hématologique (282,283). On peut aussi considérer que ces présentations cliniques étaient moins bien connues et moins bien repérées par les cliniciens. Cette critique de l'arbre décisionnel a fait l'objet d'une correspondance dans *European Journal of Human Genetics*(284).

J'ai commencé un travail d'évaluation systématique de l'activité enzymatique chez les patients suspectés de DADA2 qui sont adressé dans notre laboratoire pour étude génétique. Il y a plusieurs bénéfices à réaliser cette mesure dans le sérum des patients : pourvoir comparer la concordance entre la biochimie et la génétique, évaluer l'impact sur la protéine de variants de signification incertaine, et enfin identifier les patients susceptibles de porter une mutation non détectée par le séquençage ou la recherche de remaniements (comme des mutations introniques profondes, des mutations affectant l'épissage, ou des mutations affectant la régulation de l'expression)

Identification de *PSMB10* : un nouveau gène de MAI

Un des buts principaux de ce travail de thèse était d'identifier de nouveaux gènes des MAI. J'ai réalisé des études de WES, dans des familles soigneusement sélectionnées, sur des critères cliniques (richesse du phénotype, début néonatal...) ou familiaux (antécédents familiaux, consanguinité).

Jusqu'ici, des mutations des gènes *PSMB8* et *PSMB9* (2 sous-unités de l'immunoprotéasome) ont déjà été décrites dans les PRAAS. La sous-unité *PSMB10* est la 3^{ème} sous-unité de l'immunoprotéasome, et la seule à ne pas y avoir été associée.

Ainsi, j'ai rapporté dans cette thèse le cas d'une jeune patiente consanguine, avec une maladie inflammatoire évocatrice de PRAAS, à début néonatal et à retentissement phénotypique important. Elle portait une mutation homozygote du gène *PSMB10*, localisée dans la portion pro-peptidique, censée être clivée lors de la maturation de la protéine, avant l'assemblage du protéasome. J'ai pu montrer l'impact de cette mutation *in vitro* sur la maturation de la protéine *PSMB10* et sur les fonctions enzymatiques de l'immunoprotéasome. Les études *ex vivo* ont permis de confirmer l'impact sur les activités enzymatiques, ainsi que sur le défaut de dégradation des protéines ubiquitinylées. Les résultats de ces études fonctionnelles étaient ainsi en faveur de l'implication de cette mutation dans la maturation et les fonctions du protéasome. La limite la plus importante de l'étude est l'absence d'un deuxième cas, malgré une recherche extensive aux plans local (banque d'ADN), « régional » en interrogeant le registre européen Eurofevers(285), et mondial par l'utilisation de l'outil GeneMatcher (<https://www.genematcher.org>).

On peut néanmoins tempérer cette limite par deux éléments de réponse : i) il s'agit d'une sous-unité d'un complexe protéique déjà connu pour être associé au PRAAS ii) il existe un modèle murin muté dans *psmb10*, qui présente un tableau clinique proche de la patiente. Les souris mutantes dans *psmb10* présentent néanmoins un déficit immunitaire qui n'est pas retrouvé chez la patiente. Ce type de discordance entre l'humain et la souris a déjà été rapporté dans le modèle murin du SAVI(286).

L'identification du prochain patient portant une mutation de *PSMB10* pourra définitivement confirmer son implication dans les PRAAS ; néanmoins il n'est pas exclu qu'il s'agisse d'une mutation privée, voire qu'il n'existe pas à ce jour d'autre patient vivant, porteur de mutation dans *PSMB10*.

Conclusions et perspectives

Si l'approche de diagnostic génétique par étude de panel phénotypique a permis de montrer une amélioration du rendement diagnostique, l'amenant à 10%, il faut aller plus loin dans cette démarche. En effet, les MAI ont des formes cliniques qui peuvent être chevauchantes avec des déficits immunitaires ou des maladies auto-immunes. L'ajout de ces gènes pourrait permettre d'augmenter sensiblement le taux de diagnostic, notamment pour les diagnostics différentiels. L'approche par whole-exome devenant de moins en moins chère, on peut aussi se poser la question d'un switch au « tout-exome ». Néanmoins, les capacités actuelles des séquenceurs ne permettraient pas d'assurer un débit aussi important qu'avec les panels, qui gardent encore leur place en génétique moléculaire diagnostique.

Les études de WES ont en revanche encore de l'intérêt pour découvrir de nouveaux gènes impliqués dans les MAI. La nécessité de pouvoir valider par des études fonctionnelles les nouvelles mutations représente un nouveau challenge pour les généticiens. Il y a beaucoup plus de mutations à caractériser qu'il n'y a de laboratoires qui peuvent prendre en charge ces analyses longues, spécifiques, et « à façon ».

J'ai pu mettre en place au cours de ce travail de thèse des outils pour apporter des arguments biologiques pouvant étayer le niveau de pathogénicité des mutations.

Ainsi, nous avons à présent la possibilité de mesurer l'activité enzymatique d'ADA2, qui permet de compléter le diagnostic génétique en routine, et de caractériser les nouveaux (ou anciens) VUS identifiés.

Concernant les PRAAS, nous pouvons désormais réaliser des études enzymatiques *ex vivo*, ou *in vitro* pour PSMB10. Des mutations touchant les autres sous-unités pourront être également testées en construisant les vecteurs adéquats. L'évaluation globale du taux d'ubiquitylation des protéines est un autre outil permettant d'évaluer la fonction protéolytique du protéasome.

Je souhaite continuer à développer cette plateforme d'études fonctionnelles, en implémentant par exemple le western-blot natif pour l'étude de l'assemblage du protéasome, ou en étudiant les réponses des inflammasomes en fonction des mutations de gènes d'inflammasomopathies.

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Résumé :

Les maladies autoinflammatoires monogéniques sont des pathologies mendéliennes causées par des mutations de gènes impliqués dans l'immunité innée. Elles se traduisent chez les patients par des poussées inflammatoires systémiques, des atteintes tissulaires, sans production d'autoanticorps. La première partie des résultats rapporte une étude comparative entre le séquençage Sanger et le séquençage nouvelle génération (NGS). Les 297 patients étaient inclus prospectivement et randomisés en 2 bras : Sanger et NGS. Nous avons conclu que l'approche NGS doublait le rendement diagnostique (10.1%) par rapport au Sanger (4.1%). Nous avons également montré que les 4 fièvres récurrentes héréditaires historiques n'étaient pas les plus fréquentes dans notre série. La deuxième étude rapporte la première duplication entière du gène *MEFV* chez une patiente atteinte de FMF. Nous avons montré qu'il s'agit d'une duplication en tandem et avons pu séquencer le point de cassure. L'implication de la duplication dans l'apparition de la maladie n'est pas claire et des études d'expression seraient nécessaires pour pouvoir conclure. Le troisième volet traite du phénomène de mosaïcisme dans les MAI, notamment les CAPS, par une revue de la littérature, enrichie par de nouveaux cas identifiés à Montpellier. Nous n'avons pas mis en évidence d'impact du taux de mosaïcisme sur les manifestations cliniques ou sur l'âge de début des symptômes. La quatrième étude rapporte une série de cas de patients atteints de déficit en adénosine déaminase de type 2, nous permettant d'enrichir le spectre des mutations connues. Nous avons également proposé un arbre décisionnel afin de proposer un diagnostic génétique plus rapide. Enfin, la cinquième étude est l'identification d'un nouveau gène responsable de syndrome autoinflammatoire associé au protéasome (PRAAS) chez une patiente consanguine. Nous avons montré l'implication du gène *PSMB10* dans la physiopathologie de la patiente, en conduisant des études *in vitro* et *ex vivo*. Les résultats *in vitro* montraient, dans une lignée cellulaire transfectée, une altération des fonctions enzymatiques du protéasome et une anomalie du clivage normal de la protéine, empêchant probablement l'assemblage correct de l'immunoprotéasome. Les études *ex vivo* sur cellules nucléées de la patiente montraient également des anomalies enzymatiques, ainsi qu'une accumulation de protéine ubiquitinylées, témoignant d'un dysfonctionnement de l'immunoprotéasome.

Abstract

Monogenic systemic autoinflammatory diseases (SAIDs) are Mendelian pathologies caused by gene mutations involved in innate immunity. Autoinflammatory patients exhibit inflammatory flares, tissue damage, without production of autoantibodies. The first part of the thesis is a comparative study between Sanger sequencing and Next Generation Sequencing (NGS). The 297 patients were included prospectively and randomized into 2 arms: Sanger and NGS. We concluded that the NGS approach doubled the diagnostic yield (10.1%) compared to the Sanger (4.1%). We also showed that the 4 historical hereditary recurrent fevers were not the most frequent in our series. The second study reports the first duplication of the *MEFV* gene in a FMF patient. We have shown that this is a tandem duplication and have been able to sequence the breakpoint. The implication of this duplication in the onset of the disease is unclear and studies of expression would be necessary. The third part deals with the phenomenon of mosaicism in SAIDs (cryopyrin associated periodic syndrome (CAPS) in particular), by a literature review, enriched by new cases identified in Montpellier. We did not show any impact of the mosaicism rate on clinical manifestations or age of onset of symptoms. The fourth study reports a series of cases of patients with type 2 adenosine deaminase deficiency, allowing us to expand the mutation spectrum. We also proposed a decision tree to fasten the genetic diagnosis. Finally, the fifth study is the identification of a new gene responsible for proteasome-associated autoinflammatory syndrome (PRAAS) in a consanguineous patient. We have shown the involvement of the *PSMB10* gene in the pathophysiology of the patient, conducting *in vitro* and *ex vivo* studies. The *in vitro* results showed, in a transfected cell line, an alteration of the enzyme functions of the immunoproteasome, and defects of the normal cleavage of the protein, probably preventing the correct assembly of the immunoproteasome. The *ex vivo* cell studies of the patient also showed enzymatic abnormalities, as well as ubiquitinated protein accumulation, indicating immunoproteasome dysfunction.