



**HAL**  
open science

## **Somatic Mosaic NLRP3 Mutations and Inflammasome Activation in Late-Onset Chronic Urticaria**

Eman Assrawi, Camille Louvrier, Clémence Lepelletier, Sophie Georquin-Lavialle, Jean-David Bouaziz, Fawaz Awad, Florence Moinet, Philippe Moguelet, Marie Dominique Vignon-Pennamen, William Piterboth, et al.

► **To cite this version:**

Eman Assrawi, Camille Louvrier, Clémence Lepelletier, Sophie Georquin-Lavialle, Jean-David Bouaziz, et al.. Somatic Mosaic NLRP3 Mutations and Inflammasome Activation in Late-Onset Chronic Urticaria. *Journal of Investigative Dermatology*, 2020, 140, pp.791 - 798.e2. 10.1016/j.jid.2019.06.153 . hal-03489548

**HAL Id: hal-03489548**

**<https://hal.science/hal-03489548>**

Submitted on 20 May 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1

2 **Somatic mosaic *NLRP3* mutations and inflammasome activation in late-onset chronic**  
3 **urticaria**

4 Eman Assrawi<sup>1</sup>, Camille Louvrier<sup>1,2</sup>, Clémence Lepelletier<sup>3</sup>, Sophie Georgin-Lavialle<sup>1,4</sup>,  
5 Jean-David Bouaziz<sup>3</sup>, Fawaz Awad<sup>1§</sup>, Florence Moinet<sup>5</sup>, Philippe Moguelet<sup>6</sup>, Marie  
6 Dominique Vignon-Pennamen<sup>7</sup>, William Piterboth<sup>2</sup>, Claire Jumeau<sup>1</sup>, Laetitia Cobret<sup>1</sup>,  
7 Elma ElKhouri<sup>1</sup>, Bruno Copin<sup>2</sup>, Philippe Duquesnoy<sup>1</sup>, Marie Legendre<sup>1,2</sup>, Gilles Grateau<sup>1,4</sup>,  
8 Sonia A. Karabina<sup>1</sup>, Serge Amselem<sup>1,2\*</sup>, Irina Giurgea<sup>1,2\*</sup>

9 **ORCID**

10 Eman Assrawi: 0000-0001-8449-2281

11 Camille Louvrier: 0000-0003-2105-1117

12 Clémence Lepelletier: 0000-0003-3204-9194

13 Sophie Georgin-Lavialle: 0000-0001-6668-8854

14 Jean-David Bouaziz: 0000-0002-4993-2461

15 Fawaz Awad: 0000-0002-8214-394X

16 Florence Moinet: 0000-0003-2864-0643

17 Philippe Moguelet: 0000-0003-1996-6406

18 Marie Dominique Vignon-Pennamen: 0000-0002-4632-652X

19 William Piterboth: 0000-0002-0704-2428

20 Claire Jumeau: 0000-0002-6948-1426

21 Laetitia Cobret: 0000-0002-9217-9954

22 Elma ElKhouri: 0000-0002-4851-9632

23 Bruno Copin: 0000-0003-1475-4615

24 Philippe Duquesnoy: 0000-0003-1018-3499

25 Marie Legendre: 0000-0003-2178-0846

26 Gilles Grateau: 0000-0002-8767-7385

27 Sonia A. Karabina: 0000-0002-1865-6185

28 Serge Amelem: 0000-0001-9506-3968

29 Irina Giurgea: 0000-0002-5035-2958

### 30 **Affiliations**

31 1. Sorbonne Université, INSERM, Hôpital Trousseau, Maladies génétiques d'expression  
32 pédiatrique, Paris, 75012, France

33 2. Unité Fonctionnelle de génétique moléculaire, Assistance Publique-Hôpitaux de Paris,  
34 Hôpital Trousseau, Paris, 75012, France

35 3. Service de dermatologie, Assistance Publique-Hôpitaux de Paris, Hôpital Saint-Louis,  
36 Paris, 75010, France

37 4. Service de médecine interne, Assistance Publique-Hôpitaux de Paris, Hôpital Tenon,  
38 Paris, 75012, France

39 5. Service de médecine interne, Centre Hospitalier Universitaire de Martinique,  
40 Martinique, 97200, Fort de France, France

41 6. Unité d'anatomie et cytologie pathologiques, Assistance Publique-Hôpitaux de Paris,  
42 Hôpital Tenon, Paris, 75012, France

43 7. Unité d'anatomie-pathologiques, Assistance Publique-Hôpitaux de Paris, Hôpital Saint-  
44 Louis, Paris, 75010, France

45 <sup>§</sup> Present address: Al-Quds University, Faculty of Medicine, Biochemistry and Molecular  
46 Biology Department, Abu Deis, Jerusalem, Palestine.

47 \* Equally contributing and corresponding authors

48 **Correspondence**

49 Irina Giurgea & Serge Amselem

50 INSERM UMR\_S933,

51 Hôpital Armand Trousseau,

52 26 avenue du Dr. Arnold Netter 75012 Paris,

53 France

54 Email: irina.giurgea@inserm.fr, serge.amselem@inserm.fr

55 Phone: +33 1 44 73 52 3

56 **Short title: Somatic mosaic *NLRP3* mutations and chronic urticaria**

57 **Abbreviations**

58 ASC: Apoptosis-associated Speck-like protein containing a CARD

59 CAPSs: Cryopyrin-Associated Periodic Syndromes

60 CINCA: Chronic Infantile Neurological, Cutaneous and Articular syndrome

61 CRP: C-Reactive Protein

62 EV: empty vector

63 FCAS: Familial Cold Autoinflammatory Syndrome

64 HEK293T: Human Embryonic Kidney cell line

65 IL1 $\beta$ : Interleukin 1 $\beta$

66 IL1R: Interleukin 1 Receptor

67 LRR: Leucine-Rich Repeat

68 MWS: Muckle-Wells Syndrome

69 NACHT domain: nucleotide-binding and oligomerization domain (standing for NAIP,

70 CIITA, HET-E and TP1)

71 NAD: NACHT-Associated Domain

- 72 NGS: Next Generation Sequencing
- 73 NLRP3: Nucleotide-binding domain, Leucine-rich repeat and Pyrin domain-containing
- 74 Protein 3
- 75 *NLRP3*-AID: *NLRP3*-Associated Autoinflammatory Disease
- 76 NUD: Neutrophilic Urticarial Dermatitis
- 77 SNHL: Sensorineural Hearing Loss
- 78 THP1 cells: Human monocytic cell line
- 79 WT: Wild-Type
- 80

81 **Abstract**

82 Chronic urticaria is a common skin disorder with heterogeneous causes. In the absence of  
83 physical triggers, chronic urticarial rash is called idiopathic or spontaneous. The objective  
84 of the current study was to identify the molecular and cellular bases of a disease condition  
85 displayed by two unrelated patients aged over 60 years who presented for two decades with  
86 a chronic urticaria resistant to standard therapy which occurred in the context of systemic  
87 inflammation not triggered by cold. In both patients, a targeted sequencing approach using  
88 a next generation technology identified somatic mosaic mutations in *NLRP3*, a gene  
89 encoding a key inflammasome component. The study of several patients' cell types  
90 showed that despite the late onset of the disease, *NLRP3* mutations were not found to be  
91 restricted to myelomonocytic cells. Rather, the data obtained strongly suggested that the  
92 mutational event occurred very early, during the embryonic development. As shown by  
93 functional studies, the identified mutations –an in-frame deletion and a recurrent *NLRP3*  
94 missense mutation– have a gain-of-function effect on NLRP3-inflammasome activation.  
95 Consistently, a complete remission was obtained in both patients with anti-interleukin 1  
96 receptor antagonists. This study unveils that in late-onset chronic urticaria, the search for  
97 autoinflammatory markers and somatic mosaic *NLRP3* mutations may have important  
98 diagnostic and therapeutic consequences.

99

100

101

102 **KEY WORDS:** chronic urticaria, neutrophilic urticaria, *NLRP3*-associated  
103 autoinflammatory disease, *NLRP3*, somatic mosaic mutations, NLRP3-inflammasome  
104 activation, anti-interleukin 1 receptor antagonists.

105

## 106 INTRODUCTION

107 Urticarial rash is a common dermatological disorder characterized by sudden and recurrent  
108 onset of usually pruritic cutaneous wheals with pale central swelling, surrounded by skin  
109 erythema and edema, affecting about 15-25% of individuals (Kanani et al. 2018). Hives  
110 appears over any part of the body and individual lesions usually last for a few hours and up  
111 to 24 hours (Criado et al. 2015). In its acute form, the urticarial rash lasts for less than 6  
112 weeks, while in the chronic form the rash extends over 6 weeks in a continuous or  
113 intermittent manner (Kaplan and Greaves 2009). Chronic urticaria is more common in  
114 adults, with a peak age of onset between 20 and 40 years, and can be associated with  
115 significant morbidity and decreased quality of life. In the absence of physical triggers,  
116 chronic urticarial rash is called idiopathic or spontaneous (Zuberbier et al. 2014; Beck et al.  
117 2017). Allergy, autoimmune and infectious disorders can underlie chronic urticaria, a  
118 symptom that requires extensive tests for identifying the causative factors (Beck et al.  
119 2017). Urticarial rash can also be one of the symptoms of *NLRP3*-associated  
120 autoinflammatory disease (*NLRP3*-AID), a group of rare monogenic systemic  
121 autoinflammatory disorder previously known as Cryopyrin-Associated Periodic Syndrome  
122 (CAPS) (Kuemmerle-Deschner et al. 2017; Ben-Chetrit et al. 2018). *NLRP3*-AID is  
123 clinically a heterogeneous syndrome and consists of three overlapping entities with  
124 increasing severity: (i) the familial cold autoinflammatory syndrome (FCAS, OMIM  
125 #120100) characterized by neonatal onset of cold-induced inflammatory episodes of fever,  
126 arthralgia and urticarial rash; (ii) the Muckle-Wells syndrome (MWS, OMIM #191900)  
127 characterized by infantile-to-childhood onset of recurrent attacks of fever, urticarial skin  
128 rash and arthritis with subsequent sensorineural hearing loss (SNHL); and (iii) the chronic  
129 infantile neurological, cutaneous and articular syndrome (CINCA, OMIM #607115)  
130 characterized by infantile onset of chronic persistent clinical disease course of ongoing

131 fever, central nervous system involvement, continuous urticarial skin rash and deformative  
132 joint arthritis (Kuemmerle-Deschner 2015).

133 *NLRP3*, the disease-causing gene is expressed mainly in myeloid blood cells, but  
134 also in B and T lymphocytes (Hoffman et al. 2001a; Sutterwala et al. 2006) and in  
135 keratinocytes which could therefore account for the predisposition of patients for urticarial  
136 rash (Kummer et al. 2007; Dai et al. 2017). The encoded NLRP3 protein (also known as  
137 cryopyrin) comprises an N-terminal pyrin domain (PYD), a central nucleotide-binding and  
138 oligomerization domain (NACHT, standing for NAIP, CIITA, HET-E and TP1), a  
139 NACHT-associated domain (NAD) and a C-terminal leucine rich repeats (LRR). NLRP3  
140 initiates the assembly of a multiprotein complex called inflammasome (Martinon et al.  
141 2002; Schroder and Tschopp 2010) through the following steps: NLRP3 interacts with the  
142 adaptor protein ASC (Apoptosis-associated speck-like protein containing a CARD) leading  
143 to the assembly of ASC dimers into multimers designated as ASC specks (Fernandes-  
144 Alnemri et al. 2007). ASC specks, the hallmark of inflammasome activation, form a  
145 scaffold for recruitment and subsequent activation of procaspase-1 into caspase-1. Active  
146 caspase-1 cleaves proinflammatory cytokines, mainly IL1 $\beta$ , into their mature and secreted  
147 forms, which orchestrates the systemic inflammatory response (Martinon et al., 2009).

148 *NLRP3* mutations identified in *NLRP3*-AID patients result in a gain-of-function  
149 effect leading to NLRP3-inflammasome activation. Initially described in affected families  
150 as autosomal dominant disorders due to heterozygous *NLRP3* mutations (Hoffman et al.  
151 2001a), *NLRP3*-AID is now also known as a sporadic disease due to *de novo* germline or  
152 somatic mutations. Primarily identified in leukocytes from pediatric patients (Saito et al.  
153 2005, 2008; Aróstegui et al. 2010; Tanaka et al. 2011; Omoyinmi et al. 2014; Nakagawa et  
154 al. 2015; Lasigliè et al. 2017), somatic mosaic *NLRP3* mutations have been more recently



155 found in some adult patients with a typical *NLRP3*-AID phenotype (Zhou et al. 2015;  
156 Mensa-Vilaro et al. 2016; Rowczenio et al. 2017).

157 We describe here two unrelated elderly patients presenting for two decades with a  
158 chronic urticarial skin rash resistant to standard therapy and associated with systemic  
159 inflammation during the rashes. Two different mosaic *NLRP3* mutations were identified by  
160 means of targeted sequencing using next generation (NGS) technologies. To identify the  
161 origin of these mutational events, we studied the mosaicism level in several cell types from  
162 both patients. In addition, to assess the pathogenicity of the identified variations, we  
163 studied their functional consequences using different *in vitro* assays.

## 164 **RESULTS**

### 165 **Disease phenotype (Table 1)**

166 Proband I, a 65-year-old man, had recurrent episodes of diffuse non-pruritic fleeting  
167 urticarial skin rash involving the trunk and limbs (**Figure 1a**). The rash was intermittent,  
168 lasted for a few hours (4-5 hours) and repeated every 2-3 days since the age of 49 years.  
169 No triggers like cold exposure, physical exercise, stress, nutrition or medications were  
170 noted. The urticarial lesions were accompanied with high-grade fever (39C°), chills, and  
171 flu-like symptoms including arthralgia, fatigue and headache. There was no vasculitis,  
172 adenopathy, organomegaly or ophthalmological, abdominal or neurological manifestations.  
173 During the crises, C-reactive protein (CRP) was elevated (20 mg/L). Immunological and  
174 infectious profile, serum protein electrophoresis and kidney function tests were normal.  
175 Skin biopsy revealed urticarial lesions with a rich inflammatory infiltrate composed mostly  
176 of neutrophils with no signs of vasculitis (**Figure 1c**).

177 Proband II, a 67-year-old woman, developed the first episode of chronic urticarial  
178 skin rash at the age of 46 years. Urticarial lesions manifested as migratory transient  
179 erythematous plaques (**Figure 1b**), with no pruritus. Distributed over the whole body

180 (thighs, abdomen, thorax, upper limbs and face), the rash, which were not triggered by  
181 cold, lasted fewer than 12 hours and occurred two to three times per week. It was  
182 associated with high-grade fever (39-40C°), chills, arthritis, myalgia, headache,  
183 adenopathy and hepatomegaly. No other abdominal or ophthalmological manifestations  
184 were observed. During the attacks, CRP levels ranged from 70 to 120 mg/L. At the age of  
185 50 years, she developed bilateral SNHL. A skin biopsy revealed the presence of dermal  
186 neutrophilic infiltration with no alteration of capillaries and no signs of vasculitis  
187 **(Figure 1d)**. Kidney function tests were normal and extensive analyses of the  
188 immunological and infectious profile were found negative. Serum protein electrophoresis  
189 revealed polyclonal hypergammaglobulinemia.

190         None of the patients had family history of skin, autoimmune or autoinflammatory  
191 disorders. In both probands, second generation H1- antihistamines (the standard therapy),  
192 oral corticosteroids and colchicine showed no efficacy.

### 193 **Molecular analyses**

194 With the aim to identify the etiology of the chronic recurrent urticaria that occurred in a  
195 systemic inflammatory context, we collected the genomic DNA from whole blood samples  
196 of Proband I and II. We looked for systemic autoinflammatory disorders (SAIDs) through  
197 targeted sequencing of the main genes so far involved in these conditions. In both patients,  
198 somewhat unexpectedly, somatic mosaic mutations in *NLRP3* exon 3 were identified.  
199 Proband I carried an in-frame deletion (c.926\_934del p.(Gly309\_Phe311del)), and Proband  
200 II a missense variation (c.1705G>A p.(Glu569Lys)). The mosaicism level in the whole  
201 blood DNA for c.926\_934del was of 17.2% and of 11% for the c.1705G>A transition  
202 **(Figure S1)**.

203         The in-frame deletion of three amino acids (Gly309, Ala310 and Phe311) identified  
204 in Proband I is located within the NACHT domain of *NLRP3* **(Figure 2a)** in close

205 proximity to the Walker B motif of the protein, which is a Mg<sup>2+</sup>-binding loop implicated in  
206 ATP hydrolysis and essential for NLRP3-inflammasome activation (Neven et al. 2004).  
207 The glutamic acid residue at position 569 (Glu569), involved in the mutation identified in  
208 Proband II (p.Glu569Lys), belongs to the NAD domain of the NLRP3 protein, a domain of  
209 so far unknown function (**Figure 2a**). The identified mutations involved evolutionarily  
210 conserved amino acids in mammals (**Figure 2b**). Moreover, both the *NLRP3* transcript,  
211 including exon 3 that contains these *NLRP3* mutations (<https://ecrbrowser.dcode.org>) and  
212 the encoded regions are uniformly conserved across mammalian species (Duéñez-Guzmán  
213 and Haig 2014; Lv et al. 2017).

214 Modelization of the NLRP3 structure, based on a Nod2 crystal (Maekawa et al.  
215 2016), showed that both the three deleted amino acids and Glu569 are located close to the  
216 Walker B motif (**Figure 2c**).

### 217 **Cellular distribution of *NLRP3* mosaicism**

218 To study the cellular distribution of the *NLRP3* mosaicism, different cell types were  
219 subsequently isolated from Probands I and II. The analyzed cells include monocytes,  
220 neutrophils, B and T cells that have a mesodermal embryonic origin, urinary epithelial  
221 cells that are representative of the endodermal embryonic cells and buccal cells that are of  
222 ectodermal origin. Targeted sequencing showed a wide distribution of the mutated alleles  
223 in the studied cells (**Figure 3, lower panel and Figure S1**). We took advantage of these  
224 data to look for the two identified *NLRP3* variants by Sanger sequencing. This study  
225 revealed the presence of a superimposed sequence, mimicking a background noise, but  
226 corresponding to those mutations (**Figure 3, upper Panel**). Mutated alleles were detected  
227 in monocytes, neutrophils and B cells at average levels of 13% in Proband I and 12% in  
228 Proband II. The T cells showed a proportion of the mutated allele of 10% for Proband I and  
229 of 5% for Proband II. In buccal cells, the proportion of the mutated allele was of 3.8% for

230 Proband I and it was estimated around 5% (using Sanger sequencing due to the low  
231 amount of DNA available) for Proband II. In the urinary epithelial cells, the proportion of  
232 the mutated allele was estimated around 5% (using Sanger sequencing) in both probands.

### 233 **Impact of *NLRP3* mutations on ASC speck formation**

234 To assess the pathogenicity of the identified *NLRP3* mutations, we tested their effects on  
235 *NLRP3* function by studying ASC speck formation, a common readout of inflammasome  
236 activation (Man and Kanneganti, 2015). To this end, we transfected HEK293T (ASC-  
237 GFP\_C1-FLAG) cells with plasmids encoding wild-type (WT) *NLRP3* or *NLRP3* carrying  
238 the deletion (p*NLRP3*-Del) or the missense variation (p*NLRP3*-Glu569Lys), or with an  
239 empty vector (EV). In agreement with the known role of *NLRP3* in inflammasome  
240 assembly and ASC speck formation, cells transfected with p*NLRP3*-WT displayed a  
241 significantly higher percentage of ASC specks as compared to cells transfected with the  
242 EV alone. Importantly, cells transfected with the p*NLRP3*-Del or p*NLRP3*-Glu569Lys  
243 construct showed a significantly higher percentage of ASC specks as compared to cells  
244 transfected with p*NLRP3*-WT, thereby revealing a gain-of-function effect of the two  
245 identified mutations on inflammasome activation (**Figure 4 and Figure S2**).

### 246 **Impact of *NLRP3* mutations on IL1 $\beta$ secretion**

247 To test the impact of the p.(Gly309\_Phe311del) and p.(Glu569Lys) mutations on cytokine  
248 secretion, we used THP1 cells, which express endogenously *NLRP3*, ASC, procaspase-1  
249 and IL1 $\beta$ . THP1 cells were first primed with PMA (Phorbol 12-Myristate 13-Acetate) in  
250 order to obtain an adherent macrophage-like phenotype and to induce *IL1B* gene  
251 expression. Cells were then transfected with p*NLRP3*-WT, p*NLRP3*-Del, p*NLRP3*-  
252 Glu569Lys or the EV and treated with lipopolysaccharide (LPS), a well-known activator of  
253 the *NLRP3* inflammasome. IL1 $\beta$  secretion, a hallmark of inflammasome activation, was  
254 assessed in the cell culture supernatants. IL1 $\beta$  levels were found to be significantly higher

255 in the supernatant of cells transfected with pNLRP3-Del or pNLRP3-Glu569Lys as  
256 compared to cells transfected either with pNLRP3-WT or with the EV (**Figure 5**).

## 257 **Treatment**

258 Consistent with the above-described data showing NLRP3-inflammasome activation, both  
259 probands showed complete remission with the anti-IL1R antagonist Anakinra (100mg/day)  
260 administered by subcutaneous injections. One week after Anakinra administration, both  
261 patients reported a significant clinical and biological improvement. A complete resolution  
262 of the urticarial skin rash, the fever and the systemic inflammation (CRP levels <5mg/L)  
263 was observed. Probands I and II are still under treatment today, having received the  
264 treatment for the past two and three years, respectively. During the follow-up period,  
265 kidney function tests were found to be normal in both probands. However, the treatment,  
266 has so far no effect on hearing loss.

## 267 **DISCUSSION**

268 We described two unrelated patients presenting long-term chronic urticarial skin rash of  
269 late-adulthood onset. The clinical presentation of the patients is reminiscent of neutrophilic  
270 urticarial dermatosis (NUD), a condition characterized by an urticarial rash with  
271 predominate neutrophilic infiltration at skin biopsy and systemic inflammation (Kieffer et  
272 al. 2009; Gusdorf and Lipsker 2018). For two decades, our tow patients were refractory to  
273 anti-histamines, steroids and colchicine and, despite broad investigations, the underlying  
274 etiology was not identified. In both patients, the results of serum protein electrophoresis  
275 strongly argued against the diagnosis of Schnitzler syndrome, which is mainly  
276 characterized by the simultaneous occurrence of monoclonal gammopathy and chronic  
277 urticaria (Gusdorf and Lipsker 2017). Taken together, these data prompted us to look for  
278 possible gene mutations through the use of a targeted sequencing approach using NGS  
279 technology focusing on the main genes so far implicated in autoinflammatory disorders.

280 Somewhat unexpectedly, this analysis identified in both patients a somatic mosaic *NLRP3*  
281 variation, raising the question of a *NLRP3*-AID diagnosis in the two patients. The  
282 diagnosis of *NLRP3*-AID is indeed challenging for several clinical and molecular reasons.

283 Firstly, mostly reported in children (either in familial forms or sporadic cases), the  
284 clinical diagnosis of *NLRP3*-AID is puzzling in the absence of a contributive family  
285 history and in adult patients with late-onset chronic urticaria occurring in the context of  
286 systemic inflammation. Although the occurrence of deafness in such context should evoke  
287 the diagnosis of *NLRP3*-AID, hearing loss is common with aging. Of note, SNHL, which is  
288 a hallmark of MWS, was present in both probands. It was identified at the age of 50 years  
289 for Proband II, while, for Proband I, it was identified at the age of 65 years by means of  
290 audiometric tests that were performed once the *NLRP3* mutation was found.

291 Secondly, regarding the molecular investigations, it is important to underline that  
292 mosaic mutations are usually undetectable by Sanger sequencing. Targeted sequencing  
293 using NGS technologies increases the sensitivity of detection of such somatic mutations  
294 and high-depth sequencing is necessary to detect low level mosaicisms. To further  
295 determine the cellular distribution of the mutated alleles, we analyzed the DNA obtained  
296 from patients' cells derived from different embryonic germ layers. A wide cell-type  
297 distribution was observed for both mutations suggesting that the mutational events arose  
298 early during human embryogenesis. Similarly, a wide tissue distribution of the mutated  
299 mosaic *NLRP3* alleles has been also reported in five patients with a late-onset typical  
300 *NLRP3*-AID (Rowczenio et al. 2017). In patients carrying mosaic *NLRP3* mutations,  
301 gonadal cells were not studied for obvious reasons. Consequently, vertical transmission to  
302 the offspring cannot be excluded. The mechanism underlying the late-disease onset in  
303 patients carrying widely distributed somatic mutations is so far not understood. Variable  
304 age of disease onset was also observed in patients with germline *NLRP3* mutations

305 (Hoffman et al. 2001b). As suggested by different authors (Yu and Leslie 2011; Giat and  
306 Lidar 2014; Walker et al. 2016), external factors such as cold, stress or exercise could  
307 trigger the disease development.

308 Finally, to demonstrate the pathogenicity of newly identified *NLRP3* variants and,  
309 therefore, to establish the diagnosis of *NLRP3*-AID, functional studies are mandatory.  
310 Indeed, *NLRP3* variants do not always affect the protein function. The mosaic variations  
311 identified in our patients are located in two regions considered as hot spots for *NLRP3*  
312 mosaicism (Nakagawa et al. 2015). The in-frame deletion (p.Gly309\_Phe311del) is located  
313 in the region of the NACHT domain (305-309 amino acids) that contains 8 out of the 47  
314 reported mosaic mutations. The p.(Glu569Lys) variant is located in the NAD domain (565-  
315 572 amino acids) that includes half of the reported mosaic mutations (n=22/47). The three-  
316 dimensional (3D) model of NLRP3 showed that these two hot spots including the in-frame  
317 deletion and the p.(Glu569Lys) variation are close to the Walker B motif, thereby  
318 suggesting their pathogenicity. Using *in vitro* assays, we found significantly higher ASC  
319 speck formation and IL1 $\beta$  secretion in cells expressing the NLRP3 mutants identified in  
320 our patients, thereby clearly demonstrating the pathogenicity of these mutations.

321 In conclusion, our data support the idea that in chronic urticaria, the presence of  
322 systemic inflammatory markers should evoke a *NLRP3*-related disorder. Beyond the search  
323 for *NLRP3* mutations, functional assays demonstrating a gain-of-function effect of those  
324 variants are essential to confirm their pathogenicity. Consistent with mutations triggering  
325 NLRP3-inflammasome activation, complete remission can be achieved with anti-  
326 interleukin 1 receptor antagonists, thereby, highlighting the importance to identify the  
327 underlying cause of chronic urticaria in those patients.

## 328 **PATIENTS AND METHODS**

### 329 **Patients**

330 Clinical features were recorded on a standardized form. Informed written consent for  
331 genetic studies was given by the participants. Skin punch biopsy was performed on recent  
332 urticarial lesions after local anesthesia. Skin sections were stained with haematoxylin,  
333 eosin and saffron (HES) and examined under a light microscope.

#### 334 **Molecular analyses**

335 Genomic DNA was extracted from peripheral blood leukocytes of Probands I and II using  
336 standard procedures. Peripheral blood mononuclear cells (PBMCs) were isolated from  
337 peripheral blood using Pancoll density gradient. PBMCs were separated by positive  
338 selection using immunomagnetic microbeads (MACS, Miltenyi Biotec), anti-CD14  
339 (monocytes) followed by anti-CD15 (neutrophils), anti-CD19 (B cells) and anti-CD3 (T  
340 cells). DNA was then extracted from monocytes, neutrophils, B cells, T cells, urine and  
341 buccal cells using proteinase K digestion followed by phenol-chloroform extraction  
342 protocol. The DNA samples were sequenced following a targeted sequencing approach  
343 using a custom targeted capture (SeqCap EZ system, Roche) on a NextSeq500 or MiSeq  
344 (Illumina) platform as previously described (Jeanson et al. 2016). The position of the target  
345 oligos is available on request. We used a custom targeted capture which contains the  
346 coding and the untranslated regions (UTR) of the following genes: *ADA2*, *CARD14*,  
347 *IL1RN*, *IL36RN*, *LACC2*, *LPIN2*, *MEFV*, *MVK*, *NLRC4*, *NLRP12*, *NLRP3*, *NOD2*, *PLCG2*,  
348 *PSMB8*, *PSTPIP1*, *RBCK1*, *TMEM173*, *TNFAIP3*, *TNFRSF11A* and *TNFRSF1A*.  
349 Sequence data were analyzed using an in-house double pipeline with sequence reads in  
350 fastq format aligned to the reference human genome (hg19) using BWA and Bowtie2.  
351 Duplicate sequence reads were removed (by Picard and SAMtools, respectively). Variant  
352 calling was performed using GATK and SAMtools respectively. Additional screening of  
353 indels was performed by the Pindel software. Variant calls in VCF format were then  
354 annotated through ANNOVAR. All results obtained by this targeted sequencing were



355 confirmed using Sanger sequencing. *NLRP3* mutation numbering was based on the cDNA  
356 sequence (NM\_004895).

### 357 **Modelization of the NLRP3 structure**

358 The 3D structure of the human NLRP3 protein has been modeled as follows: (i) using the  
359 *HHpred* software, we found that the 3D structure of the NACHT, NAD and LRR domains  
360 of Nod2 (5IRN, *Oryctolagus cuniculus*) (Maekawa et al. 2016) has been identified as the  
361 closest to human NLRP3 protein; (ii) using the *Modeller* software, the predicted structure  
362 of human NLRP3 has been obtained and (iii) using the *Pymol* software, the amino acids  
363 involved in mosaic NLRP3 mutations have been highlighted.

### 364 **Plasmid constructs**

365 Human *NLRP3* cDNA was subcloned into the expression plasmid (pcDNA3.1/V5-His-  
366 TOPO, Invitrogen) to generate the wild-type expression vector (pNLRP3-WT) as  
367 described previously (Jéru et al. 2006). Site-directed mutagenesis was subsequently  
368 performed to generate plasmids carrying either the deletion identified in Proband I  
369 (p.Gly309\_Phe311del) designated as (pNLRP3-Del) or the previously reported  
370 p.(Glu569Lys) mutation that we identified in Proband II (pNLRP3-Glu569Lys), using the  
371 “quick change site-directed mutagenesis kit” (Stratagene, Le Jolla, CA). The resulting  
372 plasmid constructs were confirmed by Sanger sequencing.

### 373 **Cell culture and transfection**

374 HEK293T cells stably expressing FLAG-tagged procaspase-1 (procaspase-1-FLAG) and  
375 green fluorescent protein (GFP)-tagged-ASC (designated ASC-GFP and C1-FLAG) kindly  
376 provided by Professor Emad Alnemri (Thomas Jefferson University, Philadelphia, PA),  
377 were cultured in DMEM-F12 (Gibco). HEK293T cells (ASC-GFP and C1-Flag) were  
378 transfected with 375 ng or 500 ng of the following plasmids: pNLRP3-WT, pNLRP3-Del,

379 pNLRP3-Glu569Lys, or with the EV using FuGENE-HD (Promega) for 24h. THP1 cells  
380 (ATCC®-TIB202) were initially primed with 100 ng/ml PMA (Phorbol 12-Myristate 13-  
381 Acetate) for 3h, then transfected with 500 ng of pNLRP3-WT, pNLRP3-Del, pNLRP3-  
382 Glu569Lys, or with the EV using the FF-100 program in the 4D-Nucleofector™, Amaxa™  
383 (Lonza) for THP1 cells. Following transfection, cells were treated with LPS 100 ng/ml and  
384 incubated at 37C°for 24h. At the indicated time, supernatants of transfected cells were  
385 collected and stored at -80°C for IL1β measurement by ELISA.

### 386 **Speck quantification assay**

387 Following HEK293T cells (ASC-GFP\_C1-FLAG) transfection, the formation of ASC  
388 aggregates (specks) was calculated 24 hours later. Manual counting of cells containing  
389 ASC-GFP specks was performed in 5 representative randomly selected fields at 20×  
390 magnification. The percentage of specks was calculated as the number of specks divided  
391 by the total number of counted cells for three independent experiments. Cells were  
392 observed using a Nikon Eclipse TS100 inverted fluorescent microscope.

### 393 **Statistical analyses**

394 Differences were analyzed statistically using the unpaired student t-test and were plotted  
395 with the GraphPad Prism software. A p value <0.05 was considered as statistically  
396 significant.

### 397 **Data availability statement**

398 All the data sets related to this study are included in the article.

### 399 **Conflict of interest**

400 GG declares fees less than 5000 euros from Novartis, BMS and SOBI. The remaining  
401 authors have no conflict of interest to declare.

402 **Acknowledgments**

403 We are grateful to the affected persons and their families whose cooperation made this  
404 study possible. We would like to thank Pastelle Metura and the M2 Bioinformatics group  
405 of Paris University for their help in developing a 3D NLRP3 model.

406 This study was funded by the Agence Nationale de la Recherche ANR-17-CE17-0021-01.  
407 FA was supported by Alquds University in Palestine and from the “Fondation pour la  
408 Recherche Médicale” (FDT20130928419). EA was supported by An-Najah University in  
409 Palestine. CLo was supported by INSERM (Poste d’accueil, 00552404).

410 **Author contributions**

411 Conceptualization: IG and SA. Data curation: EA and CLo. Formal analysis: EA and CLo.  
412 Funding acquisition: SGL, GG, SAK, SA and IG. Investigation: EA, PM, WP and MDVP.  
413 Methodology: FA, PD, SAK and ML. Project administration: IG and SA. Resources: CLe,  
414 SGL, JDB, FM and GG. Software: BC. Supervision: SAK, IG and SA. Validation: CJ, LC,  
415 EEK and PD. Visualization: EA, CLo and Cle. Writing - original draft preparation: EA,  
416 ML, SAK, IG and SA. Writing - review and editing: EA, CLo, Cle, SGL, JDB, FA, FM,  
417 PM, MDVP, WP, CJ, LC, EEK, BC, PD, ML, GG, SAK, IG and SA.

418 **References**

- 419 Aróstegui JI, Lopez Saldaña MD, Pascal M, Clemente D, Aymerich M, Balaguer F, et al.  
420 A somatic NLRP3 mutation as a cause of a sporadic case of chronic infantile  
421 neurologic, cutaneous, articular syndrome/neonatal-onset multisystem  
422 inflammatory disease: Novel evidence of the role of low-level mosaicism as the  
423 pathophysiologic mechanism underlying mendelian inherited diseases. *Arthritis*  
424 *Rheum.* 2010 Apr;62(4):1158–66.
- 425 Beck LA, Bernstein JA, Maurer M. A Review of International Recommendations for the  
426 Diagnosis and Management of Chronic Urticaria. *Acta Derm Venereol.* 2017 Feb  
427 8;97(2):149–58.
- 428 Ben-Chetrit E, Gattorno M, Gul A, Kastner DL, Lachmann HJ, Touitou I, et al. Consensus  
429 proposal for taxonomy and definition of the autoinflammatory diseases (AIDs): a  
430 Delphi study. *Ann Rheum Dis.* 2018 Nov;77(11):1558–65.
- 431 Criado PR, Criado RFJ, Maruta CW, Reis VMS dos. Chronic urticaria in adults: state-of-  
432 the-art in the new millennium. *An Bras Dermatol.* 2015 Feb;90(1):74–89.
- 433 Dai X, Tohyama M, Murakami M, Sayama K. Epidermal keratinocytes sense dsRNA via  
434 the NLRP3 inflammasome, mediating interleukin (IL)-1 $\beta$  and IL-18 release. *Exp*  
435 *Dermatol.* 2017;26(10):904–11.
- 436 Duéñez-Guzmán EA, Haig D. The evolution of reproduction-related NLRP genes. *J Mol*  
437 *Evol.* 2014 Apr;78(3–4):194–201.
- 438 Fernandes-Alnemri T, Wu J, Yu J-W, Datta P, Miller B, Jankowski W, et al. The  
439 pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory  
440 cell death via caspase-1 activation. *Cell Death Differ.* 2007 Sep;14(9):1590–604.
- 441 Giat E, Lidar M. Cryopyrin-associated periodic syndrome. *Isr Med Assoc J.* 2014  
442 Oct;16(10):659–61.
- 443 Gusdorf L, Lipsker D. Schnitzler Syndrome: a Review. *Curr Rheumatol Rep.* 2017  
444 Aug;19(8):46.
- 445 Gusdorf L, Lipsker D. Neutrophilic urticarial dermatosis: A review. *Ann Dermatol*  
446 *Venereol.* 2018 Dec;145(12):735–40.
- 447 Hoffman HM, Mueller JL, Broide DH, Wanderer AA, Kolodner RD. Mutation of a new  
448 gene encoding a putative pyrin-like protein causes familial cold autoinflammatory  
449 syndrome and Muckle-Wells syndrome. *Nat Genet.* 2001a Nov;29:301–5.
- 450 Hoffman HM, Wanderer AA, Broide DH. Familial cold autoinflammatory syndrome:  
451 phenotype and genotype of an autosomal dominant periodic fever. *J Allergy Clin*  
452 *Immunol.* 2001b Oct;108(4):615–20.
- 453 Jeanson L, Thomas L, Copin B, Coste A, Sermet-Gaudelus I, Dastot-Le Moal F, et al.  
454 Mutations in GAS8, a Gene Encoding a Nexin-Dynein Regulatory Complex  
455 Subunit, Cause Primary Ciliary Dyskinesia with Axonemal Disorganization. *Hum*  
456 *Mutat.* 2016;37(8):776–85.

- 457 Jéru I, Hayrapetyan H, Duquesnoy P, Sarkisian T, Amselem S. PYPAF1 nonsense  
458 mutation in a patient with an unusual autoinflammatory syndrome: role of PYPAF1  
459 in inflammation. *Arthritis Rheum.* 2006 Feb;54(2):508–14.
- 460 Kanani A, Betschel SD, Warrington R. Urticaria and angioedema. *Allergy Asthma Clin*  
461 *Immunol.* 2018;14(Suppl 2):59.
- 462 Kaplan AP, Greaves M. Pathogenesis of chronic urticaria. *Clin Exp Allergy.* 2009  
463 Jun;39(6):777–87.
- 464 Kieffer C, Cribier B, Lipsker D. Neutrophilic urticarial dermatosis: a variant of  
465 neutrophilic urticaria strongly associated with systemic disease. Report of 9 new  
466 cases and review of the literature. *Medicine (Baltimore).* 2009 Jan;88(1):23–31.
- 467 Kuemmerle-Deschner JB. CAPS--pathogenesis, presentation and treatment of an  
468 autoinflammatory disease. *Semin Immunopathol.* 2015 Jul;37(4):377–85.
- 469 Kuemmerle-Deschner JB, Ozen S, Tyrrell PN, Kone-Paut I, Goldbach-Mansky R,  
470 Lachmann H, et al. Diagnostic criteria for cryopyrin-associated periodic syndrome  
471 (CAPS). *Ann Rheum Dis.* 2017 Jun;76(6):942–7.
- 472 Kummer JA, Broekhuizen R, Everett H, Agostini L, Kuijk L, Martinon F, et al.  
473 Inflammasome components NALP 1 and 3 show distinct but separate expression  
474 profiles in human tissues suggesting a site-specific role in the inflammatory  
475 response. *J Histochem Cytochem.* 2007 May;55:443–52.
- 476 Lasigliè D, Mensa-Vilaro A, Ferrera D, Caorsi R, Penco F, Santamaria G, et al. Cryopyrin-  
477 associated Periodic Syndromes in Italian Patients: Evaluation of the Rate of  
478 Somatic NLRP3 Mosaicism and Phenotypic Characterization. *J Rheumatol.* 2017  
479 Nov;44(11):1667–73.
- 480 Lv Z, Wei Z, Zhang Z, Li C, Shao Y, Zhang W, et al. Characterization of NLRP3-like gene  
481 from *Apostichopus japonicus* provides new evidence on inflammation response in  
482 invertebrates. *Fish Shellfish Immunol.* 2017 Sep;68:114–23.
- 483 Maekawa S, Ohto U, Shibata T, Miyake K, Shimizu T. Crystal structure of NOD2 and its  
484 implications in human disease. *Nat Commun.* 2016 10;7:11813.
- 485 Man SM, Kanneganti T-D. Regulation of inflammasome activation. *Immunological*  
486 *Reviews.* 265(1):6–21.
- 487 Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering  
488 activation of inflammatory caspases and processing of proIL-beta. *Mol Cell.* 2002  
489 Aug;10(2):417–26.
- 490 Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev*  
491 *Immunol.* 2009;27:229–65.
- 492 Mensa-Vilaro A, Teresa Bosque M, Magri G, Honda Y, Martínez-Banaclocha H,  
493 Casorran-Berges M, et al. Brief Report: Late-Onset Cryopyrin-Associated Periodic  
494 Syndrome Due to Myeloid-Restricted Somatic NLRP3 Mosaicism. *Arthritis &*  
495 *Rheumatology (Hoboken, NJ).* 2016 Dec;68(12):3035–41.

- 496 Nakagawa K, Gonzalez-Roca E, Souto A, Kawai T, Umebayashi H, Campistol JM, et al.  
497 Somatic NLRP3 mosaicism in Muckle-Wells syndrome. A genetic mechanism  
498 shared by different phenotypes of cryopyrin-associated periodic syndromes. *Annals*  
499 *of the Rheumatic Diseases*. 2015 Mar 1;74(3):603–10.
- 500 Neven B, Callebaut I, Prieur A-M, Feldmann J, Bodemer C, Lepore L, et al. Molecular  
501 basis of the spectral expression of CIAS1 mutations associated with phagocytic  
502 cell-mediated autoinflammatory disorders CINCA/NOMID, MWS, and FCU.  
503 *Blood*. 2004 Apr 1;103(7):2809–15.
- 504 Omoyinmi E, Melo Gomes S, Standing A, Rowczenio DM, Eleftheriou D, Klein N, et al.  
505 Whole-Exome Sequencing Revealing Somatic NLRP3 Mosaicism in a Patient With  
506 Chronic Infantile Neurologic, Cutaneous, Articular Syndrome. *Arthritis Rheumatol*.  
507 2014 Jan;66(1):197–202.
- 508 Rowczenio DM, Gomes SM, Aróstegui JI, Mensa-Vilaro A, Omoyinmi E, Trojer H, et al.  
509 Late-Onset Cryopyrin-Associated Periodic Syndromes Caused by Somatic NLRP3  
510 Mosaicism-UK Single Center Experience. *Front Immunol*. 2017;8:1410.
- 511 Saito M, Fujisawa A, Nishikomori R, Kambe N, Nakata-Hizume M, Yoshimoto M, et al.  
512 Somatic mosaicism of CIAS1 in a patient with chronic infantile neurologic,  
513 cutaneous, articular syndrome. *Arthritis & Rheumatism*. 2005 Nov 1;52(11):3579–  
514 85.
- 515 Saito M, Nishikomori R, Kambe N, Fujisawa A, Tanizaki H, Takeichi K, et al. Disease-  
516 associated CIAS1 mutations induce monocyte death, revealing low-level  
517 mosaicism in mutation-negative cryopyrin-associated periodic syndrome patients.  
518 *Blood*. 2008 Feb 15;111(4):2132–41.
- 519 Schroder K, Tschopp J. The inflammasomes. *Cell*. 2010 Mar 19;140:821–32.
- 520 Sutterwala FS, Ogura Y, Szczepanik M, Lara-Tejero M, Lichtenberger GS, Grant EP, et al.  
521 Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through  
522 its regulation of caspase-1. *Immunity*. 2006 Mar;24:317–27.
- 523 Tanaka N, Izawa K, Saito MK, Sakuma M, Oshima K, Ohara O, et al. High incidence of  
524 NLRP3 somatic mosaicism in patients with chronic infantile neurologic, cutaneous,  
525 articular syndrome: results of an International Multicenter Collaborative Study.  
526 *Arthritis Rheum*. 2011 Nov;63(11):3625–32.
- 527 Walker UA, Hoffman HM, Williams R, Kuemmerle-Deschner J, Hawkins PN. Brief  
528 Report: Severe Inflammation Following Vaccination Against *Streptococcus*  
529 *pneumoniae* in Patients With Cryopyrin-Associated Periodic Syndromes. *Arthritis*  
530 *& Rheumatology (Hoboken, NJ)*. 2016 Feb;68(2):516–20.
- 531 Yu JR, Leslie KS. Cryopyrin-associated periodic syndrome: an update on diagnosis and  
532 treatment response. *Curr Allergy Asthma Rep*. 2011 Feb;11(1):12–20.
- 533 Zhou Q, Aksentijevich I, Wood GM, Walts AD, Hoffmann P, Remmers EF, et al. Brief  
534 Report: Cryopyrin-Associated Periodic Syndrome Caused by a Myeloid-Restricted  
535 Somatic NLRP3 Mutation. *Arthritis & Rheumatology (Hoboken, NJ)*. 2015  
536 Sep;67(9):2482–6.

537 Zuberbier T, Aberer W, Asero R, Bindslev-Jensen C, Brzoza Z, Canonica GW, et al. The  
538 EAACI/GA(2) LEN/EDF/WAO Guideline for the definition, classification,  
539 diagnosis, and management of urticaria: the 2013 revision and update. *Allergy*.  
540 2014 Jul;69(7):868–87.

541

## 542 **Figure legends**

### 543 **Figure 1. Urticarial lesions and skin biopsy**

544 Cutaneous photography of the Probands I **(a)** and II **(b)**.

545 Skin biopsy of the urticarial lesions of Proband I **(c)** and II **(d)**, with scale bar of 200  $\mu\text{m}$   
546 and 100  $\mu\text{m}$ , respectively, showing rich dermal polymorphonuclear cells, mainly  
547 neutophilic infiltration using hematoxylin, eosin and saffron (HES) stain.

### 548 **Figure 2. NLRP3 mutations and their impact at the protein level**

549 **(a)** NLRP3 protein domain-organization diagram. Location of the c.926\_934del  
550 p.(Gly309\_Phe311del) and the c.1705G>A p.(Glu569Lys) mutations identified in this  
551 study in the NACHT and NAD domains, respectively. The Walker B motif is illustrated in  
552 blue. PYD: Pyrin domain, NACHT (NAIP, CIITA, HET-E and TP1) domain, NAD:  
553 NACHT-associated domain, LRR: Leucine-rich repeat domain. **(b)** Partial protein  
554 alignment of NLRP3 in different species showing the evolutionary conservation of amino  
555 acids Gly309\_Phe311, Glu569, and those involved in the previously reported mosaic  
556 NLRP3 mutations (hot spots). (\*) indicates fully conserved residue; (:) indicates strongly  
557 similar properties; (.) indicates similar properties. **(c)** Modelization of the NLRP3 structure  
558 showing the location of the identified mutations (p.(Gly309\_Phe311del) and  
559 p.(Glu569Lys)) and of the previously reported NLRP3 mutations. The NACHT and NAD  
560 domains are represented in light grey and the LRR domain in dark grey. The mutations  
561 identified in this study are in red; the previously reported NLRP3 mutations are in green  
562 and the Walker B motif is in blue.

563 **Figure 3. *NLRP3* DNA sequencing in different cell types**

564 **Upper panel:** Sanger electrophoregrams showing the identified mosaic *NLRP3* mutations  
565 c.926\_934del p.(Gly309\_Phe311del) in Proband I (left) and c.1705G>A p.(Glu569Lys) in  
566 Proband II (right). The mutated amino acids are highlighted in red. The red arrows  
567 indicate the beginning of the deletion (c.926\_934del) sequenced with the reverse primer in  
568 Proband I and the point mutation (c.1705G>A) identified in Proband II. **Lower panel:** The  
569 percentage of the mutated alleles and the ratio of the mutated reads to the total number of  
570 reads identified by targeted sequencing approach. \* indicates the estimated percentage of  
571 the mutated allele based on Sanger sequencing.

572 **Figure 4. Impact of *NLRP3* mutations on ASC speck formation**

573 HEK (ASC-GFP\_C1-FLAG) cells were transfected with different amounts (375 ng or 500  
574 ng) of the expression plasmids encoding the wild-type *NLRP3* (p*NLRP3*-WT) or the  
575 *NLRP3* protein carrying the in-frame deletion p.(Gly309\_Phe311del) called p*NLRP3*-Del  
576 or the missense mutation p.(Glu569Lys) called p*NLRP3*-Glu569Lys, or of the EV. The  
577 percentage of ASC specks was calculated as described in “Patients and Methods”. Results  
578 represent the mean + SD from 3 independent experiments. P values were calculated using  
579 unpaired Student’s t test. \* indicates significant P value (<0.05) as compared to the wild-  
580 type (\*\* P value <0.01, \* P value <0.05).

581 **Figure 5. Impact of *NLRP3* mutations on IL1 $\beta$  secretion**

582 IL1 $\beta$  secretion, as assessed by ELISA in cell culture supernatants of PMA-primed THP1  
583 cells transfected with 500 ng of p*NLRP3*-WT, p*NLRP3*-Del, p*NLRP3*-Glu569Lys or the  
584 empty vector (EV) and treated with LPS, a known stimulus of the *NLRP3* inflammasome.  
585 Results represent the mean + SD of 3 independent experiments performed in triplicates. P  
586 values were calculated using unpaired Student’s t test. \* indicates significant P value  
587 (<0.05) as compared to the wild-type (\*P value <0.05, \*\* P value <0.01).



588 **Supplementary material**

589 **Figure S1. Identification of somatic mosaic *NLRP3* mutations**

590 Visualization using Integrative Genomics Viewer (IGV) of the mosaic *NLRP3* mutations,  
591 c.926\_934del p.(Gly309\_Phe311del) and c.1705G>A p.(Glu569Lys), identified by  
592 Illumina sequencing in different cell types of Proband I and II.

593 **Figure S2. ASC speck formation**

594 Examples of five randomly selected fields at 20× magnification of HEK cells containing  
595 ASC-GFP specks collected through one experiment and plotted in Figure 4. White  
596 arrowheads point examples of ASC specks. Scale bar = 50 μm.

597

598 **Table 1: Phenotype and genotype of patients with chronic urticaria and a mosaic**  
 599 ***NLRP3* mutation**

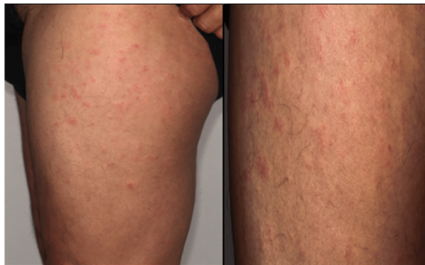
	<b>Proband I</b>	<b>Proband II</b>
<b>Sex</b>	Male	Female
<b>Current age (years)</b>	65	67
<b>Age of urticarial onset (years)</b>	49	45
<b>Fever</b>	+	+
<b>Arthralgia/Arthritis</b>	+	+
<b>Deafness (SNHL)</b>	-*	+
<b><i>NLRP3</i> genotype</b>	c.926_934del p.(Gly309_Phe311del)	c.1705G>A p.(Glu569Lys)**
<b>Percentage of the mutated allele</b>	17%	11%

600 SNHL: SensoriNeural Hearing Loss

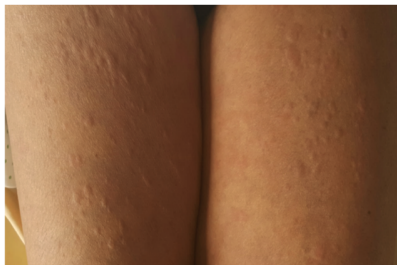
601 \*SNHL, which was not reported at study inclusion, was identified by means of audiometric  
 602 tests that were performed once the *NLRP3* mutation was found.

603 \*\*Also known as c.1699G>A, p.E567K

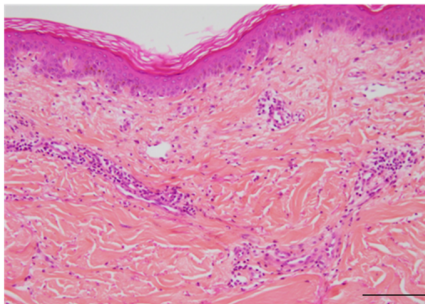
**a**



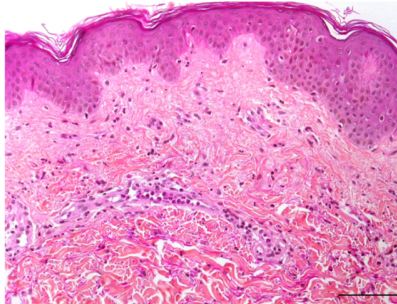
**b**



**c**



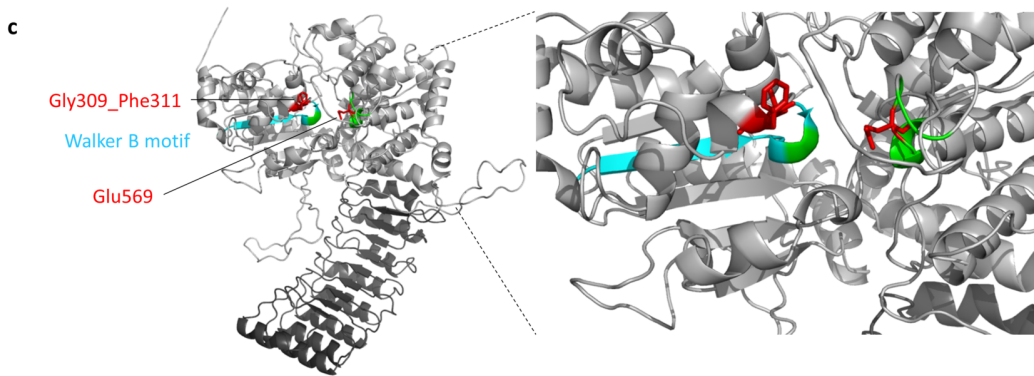
**d**





**b**

Homo_sapiens	KIVRKPS	RILFLMDGFDELQ	GAFDEHIG	(----	DVTVLLEN	YGKFEKGY	LIFVVRFLF
Pan_troglodytes	KIVRKPS	RILFLMDGFDELQ	GAFDEHIG	(----	DVTVLLEN	YGKFEKGY	LIFVVRFLF
Macaca_mulatta	KIVSKPS	RILFLMDGFDELQ	GAFDEHIG	(----	DVTVLLEN	YGKFEKGY	LIFVVRFLF
Canis_lupus	KIVSKPS	RILFLMDGFDELQ	GAFDEHTE	(----	DVKVLLEN	YGKFEKGY	LIFVVRFLF
Bos_taurus	KIVSKPS	RILFLMDGFDELQ	GAFDEHTE	(----	DVKVLLEN	YGKFEKGY	LIFVVRFLF
Mus_musculus	KILRKPS	RILFLMDGFDELQ	GAFDEHIG	(----	DVKVLLEN	YGKFEKGY	LIFVVRFLF
Rattus_norvegicus	KILCKPS	RILFLMDGFDELQ	GAFDEHIE	(----	DVKVLLEN	YGKFEKGY	LIFVVRFLF
	**.	***	*****	**	*****	**	*****

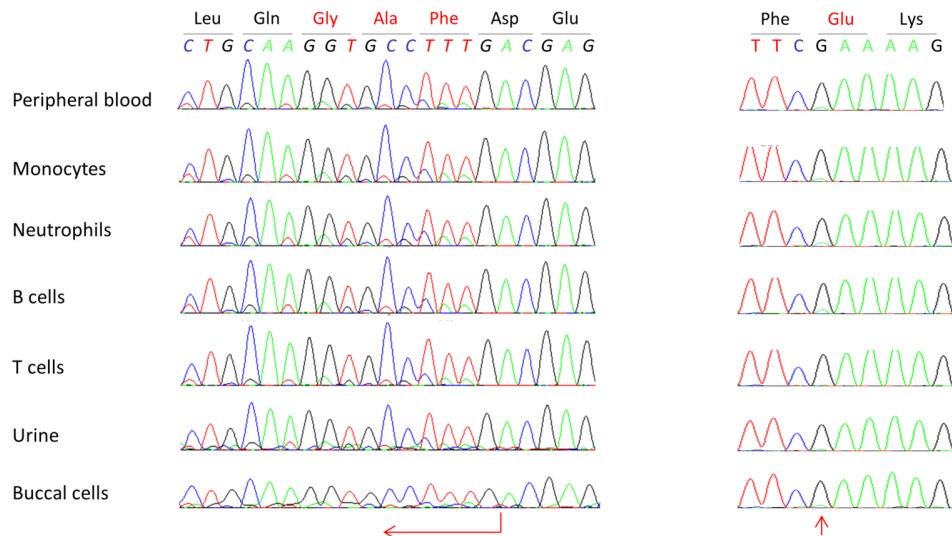


**Proband I**

c.926\_934del, p.(Gly309\_Phe311del)

**Proband II**

c.1705G&gt;A, p.(Glu569Lys)



	<b>Percentage of the mutated allele (mutated reads / total reads)</b>	<b>Percentage of the mutated allele (mutated reads / total reads)</b>
Peripheral blood	17.0% (23/135)	11.0% (26/243)
Monocytes	13.2% (51/385)	12.0% (94/754)
Neutrophils	14.1% (59/368)	12.0% (98/851)
B cells	14.3% (77/540)	11.0% (35/318)
T cells	10.5% (56/531)	5.0% (38/806)
Urine	5.0%*	5.0%*
Buccal cells	3.8% (28/728)	5.0%*

