Pathogenic variants in the NLRP3 LRR domain at position 861 are responsible for a boost-dependent atypical CAPS phenotype.

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Conflict of interest

All the authors declare that they have no relevant conflicts of interest.

Abstract

- **Background:** Cryopyrin-associated periodic syndrome (CAPS) is associated with *NLRP3* pathogenic variants, mostly located in the NACHT domain. Cold induced urticarial rash is one of the main clinical features. We however identified a series of 14 patients with pathogenic variants of the Y861 residue (p.Tyr861) of the LRR domain of NLRP3 and minimal prevalence of cold-induced urticarial rash.
- **Objective:** We aimed to address a possible genotype / phenotype correlation for CAPS patients and to investigate at the cellular levels the impact of the Y861C substitution (p.Tyr861Cys) on NLRP3 activation.
- **Methods:** Clinical features of 14 CAPS patients with heterozygous substitution at position 861 in the LRR domain of NLRP3 were compared to clinical features of 48 CAPS patients with pathogenic variants outside the LRR domain of NLRP3. IL-1β secretion by PBMCs and purified monocytes from patients and healthy donors was evaluated following LPS and MSU stimulation.
- **Results:** Patients with substitution at position 861 of NLRP3 demonstrated a higher prevalence of sensorineural hearing loss while being less prone to skin urticarial. In contrast to classical CAPS patients, cells from patients with a pathogenic variant at position 861 required an activation signal to secrete IL-1β but produced more IL-1β during the early and late phase of secretion than cells from healthy donors.
- **Conclusion:** Pathogenic variants of Y861 of NLRP3 drive a boost-dependent over secretion of IL-1β associated with an atypical CAPS phenotype.

Clinical Implications

NLRP3 pathogenic variants should be considered as a possible cause of early onset hearing loss in patients with elevated inflammatory biomarkers, even in the absence of skin urticarial rashes.

Capsule Summary

Pathogenic variants of the residue 861of NLRP3 drive an atypical CAPS phenotype associated with increased prevalence of hearing loss but less frequent skin urticaria compared to typical CAPS.

Key Words

NLRP3, CAPS, inflammasome, LRR domain, Interleukin-1, hear loss, deafness, urticaria

Abbreviations

ASC: Apoptosis-associated speck-like protein containing a CARD domain

ATP: adenosine triphosphate

CAPS: cryopyrin-associated periodic syndrome

CD14: Cluster of differentiation 14

CRP: C-reactive protein

cryoEM: cryogenic electron microscopy

ELISA: enzyme-linked immunosorbent assay

FMF: Familial Mediterranean fever

HC: healthy control

LPS: lipopolysaccharide

MD: Molecular Dynamics

NACHT: NAIP (neuronal apoptosis inhibitor protein), C2TA (MHC class 2 transcription activator), HET-E (incompatibility locus protein from Podospora anserina), TP1 (telomerase-associated protein)

NEK7: NIMA Related Kinase 7

NLRP3: NOD-like receptor family, pyrin domain containing 3

PBMC: Peripheral blood mononuclear cell

PRR: Pattern recognition receptors

RPMI: Roswell Park Memorial Institute medium

SEM: standard error of the mean

SD: standard deviation

TSM1: target of methylation-induced silencing

LRR: Leucine-Rich Repeat

IL-1β: interleukine 1 beta

IL-18: interleukine 18

MSU: monosodium urate crystals

WT: Wild-Type

Introduction:

NOD-like receptor family, pyrin domain containing 3 (NLRP3) protein is probably the most studied pattern recognition receptors (PRRs), that assemble inflammasome complex upon activation (1). The full canonical activation of the NLRP3 inflammasome is dependent on a two steps process (2). The first step, named "priming", controls the transcriptional upregulation of NLRP3 and the inflammasome substrates pro-IL-1β, pro-IL-18 , as well as license NLRP3 for later activation through post-translational modifications including phosphorylations (3,4) and deubiquitinations (5) linked to structural changes and subcellular relocalisation. The second step, named "activation" initiates the final conformation change of NLRP3 protomers. During the priming/activation process NLRP3 transient from an inactive ADP-bound 10-12mers NLRP3 "cage" structure to an active ATP-bound pentamers inflammasome (6–9) leading to caspase-1 activation, and therefore the maturation and release of IL-1β and IL-18 as well as pyroptotic inflammatory cell death. Heterozygous missense gain of function variants in *NLRP3* have been associated with an autoinflammatory syndrome called cryopyrin-associated periodic syndrome (CAPS) (10). Besides systemic inflammation, intermittent fever and arthralgia, CAPS patients display very specific features including pseudo-urticarial rash often triggered by exposure to cold, neuroinflammatory features such as aseptic meningitidis, sensorineural hearing loss, various ocular manifestations like conjunctivitis and uveitis, and less frequently oral aphtous and digestive features(11). Interestingly, *NLRP3* pathogenic variants found among CAPS patients are almost exclusively located in the NACHT domain of NLRP3. These mutations are thought to impact ATPase activity or key subdomain interaction surface that intervene in the final opening of the inflammasome structure (8,9) As a result, priming step alone is sufficient to drive significant IL-1β production and this *in vitro* dysregulation profile is therefore considered as a signature of CAPS (12). The most effective treatment for CAPS patients are IL-1β inhibitors (13)(14).

Methods

Human sample collection.

The blood from healthy donors was obtained from *"Etablissement Français du Sang"* (convention # 07/CABANEL/106), Paris, France. For CAPS patients' blood, the study was approved by the Comité de Protection des Personnes (N° EudraCT: 2018-A01358-47) in France. Experimental procedures with human samples were done according to the European Union guidelines and the Declaration of Helsinki. Clinical data were extracted from the JIR (Juvenile Inflammatory Rheumatism)- cohort; an international multicenter data repository established by the National Commission on Informatics and Liberty (CNIL; authorization number N◦: 914677). Patients consented to be included in the JIR-cohort and were informed that data collected in medical records might be used for research studies in accordance with privacy rules.

Culture conditions

In vitro experiments were performed using frozen human mononuclear cells from peripheral blood isolated by centrifugation in density gradient medium (STEMCELL Technologies). Human monocytes were purified by positive selection with Human CD14 microbeads (Miltenyi). Peripheral blood mononuclear cells (PBMC) and monocytes were cultured in RPMI 1640 (Invitrogen, Gaithersburg, MD) (R10) containing 10% heat-inactivated fetal bovine serum.

Measurement of ASC protein aggregates in serum

Sera ASC aggregates were determined using an adaptated previously described method adapted for our purposes (24). One hundred microliters of patients or HC serum were incubated with 2.5 µL of phycoerythrin anti-ASC (TMS-1) antibody (Biolegend) for 1 hour at room temperature. Analyses were performed on the BD LSRFortessa™ X-20 flow cytometer instrument (BD Biosciences). Nonfluorescent 1-µm microspheres (Thermo Fisher Scientific) were used as a guide to gate around ASC specks. Total events in the gated area were divided by 100 for the evaluation of ASC speck per microliter.

IL-1β and IL-18 detection by ELISA

PBMCs were seeded at 1.10^6 cells /mL and monocytes isolated from PBMCs at 1.10^5 cells /mL. Standard protocol consists in a priming with LPS 10ng/ml. After 3h PBMC were activated with MSU (monosodium urate crystals) 200µg/ml. After 3 hours the media were collected and analyzed by ELISA. For kinetic experiments, the media were collected 30 minutes, 1 hour, 2 hours, 3 hours and 6 hours after MSU. For basal production on unstimulated cells, PBMC or purified monocytes were cultured overnight. IL-1β and IL18 were measured in cell supernatant and/or patient's plasma using a kit from R&D (DY201-05 and DY318-05 respectively) according to supplier recommendations.

Legendplex:

Cytokines in patients' plasma were quantified using bead-based multiplex assays (LEGENDplex 13-plex HU Essential Immune Response Panel). The experiment was conducted according to the manufacturer protocol. Briefly, diluted plasma were incubated with Capture Beads for 2h. Following centrifugation, supernatants were discarded and biotinylated detection antibodies were added for 1h. After incubation with SA-PE, supernatants are discarded and samples are resuspended in the provided wash buffer to read on the flow cytometer.

In silico Modeling:

All *in silico* studies were performed within the Biovia Discovery Studio (DS 2022) suite. Mutants models were generated using the cryo-EM structure of the wt NLRP3 in complex with NEK7 (PDB: 6NPY) and the Modeller program (Version: 9.24) implemented in the Building Mutant protocol of DS. Short Molecular Dynamics MD simulations (5 ns) were run using the NAMD program (25) implemented in DS and the CHARMM36m force field. The average interfacial interaction energy between NLRP3 and NEK7, based on the sum of the van der Waals and electrostatic interactions was calculated on the last ns of the 5ns MD simulations using the Calculate Interaction Energy tool of DS. Analysis of the NLRP3/NEK7 interactions was conducted with the Analysis Protein Interface tool.

Statistics

All statistical analyses were performed using GraphPad Prism (version 9.1.1). Data on the figures are presented as mean (± SD for the kinetic experiments). For experimental data, statistical analysis were performed either using non-parametric One-way ANOVA followed by Kruskal-Wallis multiple comparison test or non-parametric 2-tailed Mann-Whitney test (IL-18 plasma assay). For clinical information, non-categorical data were analyzed using Mann Whitney test and categorical data using Fisher test. P values less than 0.05 were considered significant.

Results

Pathogenic variants of the residue 861 in NLRP3 drive an atypical CAPS disease.

Nine patients carrying a heterozygous Y861C missense variant (NM_004895.5 : c.2582A>G p.(Tyr861Cys)) of *NLRP3*, and 5 carrying a heterozygous Y861H variant (NM_004895.5 : c.2581T>C p.(Tyr861His)) were included in France, Holland, and Portugal (15,16). Depending on the first methionine used for numbering, these variants are also known as c.2576A>G p.(Tyr859Cys) and c.2575T>C p.(Tyr859His) using NM_001243133.2. These variants are respectively reported pathogenic and likely pathogenic in the ClinVar database.

Unlike most CAPS causing pathogenic variants that are located in the NACHT domain, these substitutions involve a tyrosine residue in the LRR domain of NLRP3 (**Figure1A**). All patients belonged to large families with dominant transmission of the mutation (**Figure 2**).

While these patients display typical features of CAPS as ophthalmological manifestations such as recurrent conjunctivitis, uveitis or papillary edema (93%), arthromyalgia (79%) and aseptic meningitidis (43%) (**Figure 1 B, C, D and E**), some other main features are significantly differentially observed compared to our cohort of 48 CAPS patients with pathogenic variants outside of the LRR domain. Indeed, they have more sensorineural hearing loss (100% Vs 63%, p=0.006), more headaches (86% Vs 45%, p=0.013), less urticarial rash (29% Vs 94%, p<0.001) and less aphtosis (7% Vs 53%, p=0.004). Of note, 3 patients from a same family had hypereosinophilia, a feature not found in our typical CAPS cohort (21% Vs 0%, p=0.037) (**table I and sup table I**).

In order to confirm that the patients with substitution of the tyrosine residue at position 861 of NLRP3 were affected by an inflammasomopathy we quantified ASC speck and IL-18 in their plasma. ASC speck concentration in plasma of NLRP3^{Y861C} patients was higher than in healthy donors and comparable to the levels observed in typical CAPS patients or patients with FMF

(**Figure 1 F**). Similarly, IL-18 concentration was significantly higher in NLRP3^{Y861C} patients compared to controls (mean: 216,4 pg/ml ± 9,1 pg/ml and 84,1 pg/ml ± 4,1 pg/ml respectively; p=0,0023 – Two-tail Mann-Whitney test) (**Figure 1 G**). No other cytokine were found to be significantly over express in typical CAPS or NLRP3^{Y861C} patients (Sup Fig1).

Finally, in line with these results and what is known in classical CAPS, IL-1β inhibitors were very efficient in all patients. Most clinical symptoms quickly resolved, with marked improvement in general condition and almost complete resolution of headache, ophthalmic manifestations and arthralgia. Additionally, hearing ceased to deteriorate. Accordingly, biological response was immediate, with normalization of CRP, SAA and blood count within 8 days.

Activation signal is required to fully activate inflammasome in cells from NLRP3^{Y861C} patients.

To date, the only characterisation of primary immune cells from NLRP3^{Y861C} mutated patients suggests that patients' PBMCs spontaneously produced IL-1β, in the absence of any stimulus, either prime and/or boost signal (15). However, in our hands, neither PBMC nor isolated monocytes from NLRP3^{Y861C} produced IL-1β in the absence of a priming or activating signal (**Figure 3A and B**). Thus, we tested the response of NLRP3^{Y861C} mutated cells to priming signal only. As for cells from healthy donors, priming with LPS alone induced a very slight production of IL1-β in NLRP3^{Y861C} mutated PBMCs and monocytes while NLRP3 NACHT domain mutated cells were producing higher quantities (2,4 ± 0,48 fold HD, p >0.999 and 21,7 ± 0,99 fold HD, p = 0,0050 respectively for PBMC, One-way ANOVA, Kruskal-Wallis test) (**Figure 3C and D**). These results strongly suggest that NLRP3^{Y861C} patients have a distinct pathophysiological mechanism compared to the more common CAPS driving NACHT pathogenic variants of NLRP3. Finally, the comparison of IL1β production after prime plus boost stimulation in cells from healthy donors, NLRP3^{Y861C} mutated patients and typical CAPS patients returned comparable levels (**Figure 3C and D**). Overall, despite compelling clinical and genetic evidence that NLRP3^{Y861C} drives an atypical CAPS phenotype in most patients, our in vitro results could not differentiate NLRP3 activation between healthy donors and NLRP3^{Y861C} mutated patients.

Cells from NLRP3^{Y861C} patients demonstrate sustained IL-1β production compared to cells from healthy donors.

Recently, *Caseley et al* reported a patient with a NLRP3^{R920D} substitution in the LRR domain of NLRP3 resulting in an atypical inflammatory syndrome (17). Based on modelling studies, the authors suggested that the mutation enhanced the electrostatic complementarity between the LRR portion of NLRP3 and NEK7 leading to an increased binding affinity between the two partners. Since Y861 is located at the interface of NLRP3 and NEK7 **(Figure 4A)**, we hypothesized that Y861 pathogenic variants might potentially enhance the NLRP3/NEK7 binding as well.

To evaluate the impact of the Y861C and Y861H substitution on the stability of the interaction between NLRP3 and NEK7, we performed short Molecular Dynamics (MD) simulations starting from the cryoEM structure of the NLRP3/NEK7 complex (PDB:6NPY, (18)). We examined the binding interface of C861 and H861 mutant models and calculated the interaction energy for each NLRP3/NEK7 complex. A model of the R920Q mutation was also generated for the sake of comparison. Our results with R920Q model are in agreement with those previously reported by *Caseley et al* (17). R920Q mutation resulted in an enhanced binding between NLPR3 and NEK7 (**Figure 4A**). Compared to WT, Y861C model had a positive effect on stability between NLRP3 and NEK however the Y861H mutation proved to be detrimental to the stability of the NLRP3/NEK interface. Thus, given the phenotypic similarities between Y861C and Y861H patients, increased binding affinity between NLRP3 and NEK7 probably does not explain the atypical phenotypes observed in most of these patients. This might also be supported by the strong phenotypic difference between the NLRP3 R920Q mutated patient and NLRP3 Y861C, and Y861H patients.

Previous work in mice and human derived monocytic cell lines (19) suggest that the Y861 residue (Y859 in mice) is central for the control of NLRP3 activation. It is proposed that phosphorylation of Y861 promotes the addressing of activated NLRP3 to phagolysosome leading to a reduction of the NLRP3 pool available for inflammasome assembly (20). We anticipate that such mechanism should results in increased early production of IL-1β by patient's cells. In order to test this hypothesis, we performed a kinetic of IL-1β production following LPS and MSU stimulation on isolated monocytes from healthy donors and 3 NLRP3^{T861C} mutated patients. Our results highlight a strongest production of IL1β during the first two hours post boost in monocytes from the 3 NLRP3^{T861C} mutated patients compared to healthy donors supporting our hypothesis **(Figure 4B)**. We also observed a sustained

production of IL1β in monocytes from patients. Indeed, while the secretion stopped after 3 hours in cells from most healthy donors, we could still observe a production of IL-1β between 3 and 6 hours post boost in the monocytes from the 3 tested NLRP3^{T861C} mutated individuals **(Figure 4C)**. These latest results also support the hypothesis of a defective deactivation of the inflammasome complex.

Discussion

Altogether, we describe 14 patients with pathogenic variants located in the LRR domain of NLRP3 at position 861. This series highlights a clear genotypes /phenotypes correlation with all patients displaying hearing loss, and 10 out of 14 devoid of pseudo-urticarial rash (**Figure 5**). This remarkable difference in phenotype observed in patients with pathogenic variants outside the LRR domain (21) might be explained by the distinct activation profile of NLRP3 depending on the localization of the disease-causing variants. Indeed, in contrast to cells from typical CAPS, PBMCs and monocytes from NLRP3^{T861C} mutated patients required a boost signal for full inflammasome activation. However, patients' cells demonstrated exacerbated early IL1β secretion and sustained IL1β secretion following "prime and boost" activation when compared to healthy donors. It remains unclear what causes this exacerbated response to stimuli. Previous studies in mice suggest that phosphorylation of the Y859 residue promote NLRP3 degradation (19). In their models, defective phosphorylation of Y859 is associated with defective addressing of activated NLRP3 to phagolysosomes (20). Nevertheless, another study performed using human NLRP3 expressed in mice cells suggests that it is not the phosphorylation of Y861, but the phosphorylation of Y918 that control NLRP3 activity (22). Further experiments will be needed on human cells to confirm or exclude the contribution of Y861 to NLRP3 degradation. Another nonexclusive hypothesis is that the modification of the electrostatic charge of NLRP3 LRR domain caused by the Y861C substitution either stabilizes the active inflammasome complex or destabilizes the inactive cage conformation. Indeed, charges in the LRR concave side plays a major role in NEK7 recruitment to NLRP3 to form the inflammasome and Y861C may drives an increase affinity of NLRP3 for NEK7, thus resulting in an overactivated inflammasome. A similar mechanism was already reported for a R920Q substitution causing an atypical auto inflammatory disease (17). Further experimental approaches are needed to fully understand how these mutation impact NLRP3 function and regulation and contribute to the CAPS phenotypes. Of note, we observed a non-statistically significant trend towards an overall increase IL-1β secretion in cells from Y861C patients

compare to cells from healthy donors upon LPS + MSU treatment. Future experiment on a larger group of patients and control should help to determine if this observation is due to inter-individual variability or is a hallmark of this mutation.

The low prevalence of skin manifestation and aphtosis associated to the high prevalence of deafness and headaches in NLRP3^{Y861C} and NLRP3^{Y861H} mutated patients might be explained by the distinct pathophysiological mechanism compared to typical CAPS. Skin pseudo-urticarial rash seen in most CAPS could result from a local production of IL-1β triggered by an uncontrolled priming signal in patients with mutation leading to activation signal independent NLRP3 inflammasome formation (*ie* mutation in the NACHT domain). Conversely, the preserved dependence on the boost of Y861 pathogenic variants could protect these patients from the cutaneous phenotype of CAPS. On the other hand, NLRP3^{Y861C} and NLRP3^{Y861H}

mutated patients show high prevalence of sensorineural hearing loss which is reminiscent of what was observed in two families with a NLRP3 ^{R920D} mutation which is in the close vicinity of Y861 (23). Better understanding of the impact of these variants on local and systemic inflammation in humans might help to explain these phenotypic specificities.

In conclusion, it appears that the differential diagnosis of a dominantly inherited sensorial hearing loss should include cryopyrinopathy in individuals with elevated CRP, even in the absence of urticaria. This is particularly true in patients with ophthalmological (conjunctivitis) and neurological (headache) features and hypereosinophilia. A genetic sequencing looking for *NLRP3* pathogenic variants by next generation sequencing could reduce the diagnostic delay and prevent the risks of hearing or visual sequelae and AA amyloidosis.

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Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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461 **Tables**

462 ***Table I*** *: Clinical features of CAPS patients associated with non LRR pathogenic variants and bearing Y861H or*
463 *Y861C variants*

General features	**Non LRR mutated CAPS (n = 48)**	**LRR mutated CAPS (n = 14)**	**P-value**
Mean age at symptoms onset and range (years old)	4,5 (0-46)	10,1 (3-32)	**<0,0001**
Mean age at diagnosis and range (years old)	33,7 (0-73)	32,6 (7-54)	0,9015
Mean diagnostic delay and range (years)	29,2 (0-73)	22,4 (4-39)	0,3842
Familial form	35/48 (73%)	14/14 (100%)	**0,0290**
Mosaicism	4/48 (8%)	0/14 (0%)	0,5659
Clinical features			
Sensorineural hearing loss	29/46 (63%)	14/14 (100%)	**0,0060**
Urticarial rash	45/48 (94%)	4/14 (29%)	**<0,0001**
Aphtosis	23/43 (53%)	1/14 (7%)	**0,0039**
Hypereosinophilia before treatment	0/26 (0%)	3/14 (21%)	**0,0368**
Headache	21/46 (45%)	12/14 (86%)	**0,0128**
Meningitis and/or intracerebral hypertension	12/46 (26%)	6/14 (43%)	0,3188
Intellectual impairment	4/47 (9%)	0/14 (0%)	0,5650
Arthromyalgia	42/46 (91%)	11/14 (79%)	0,3375
Conjunctivitis	29/47 (62%)	11/14 (79%)	0,3421
Uveitis and/or papillary edema	18/46 (39%)	7/14 (50%)	0,5434
IBD-like symptoms	4/45 (9%)	1/12 (8%)	>0,9999
Other digestive involvement (abdominal pain, nausea, hepatosplenomegaly)	4/21 (19%)	4/10 (40%)	0,3809
Presence of amyloidosis AA	3/47 (6%)	0/14 (0%)	>0,9999
Mean age at amyloidosis diagnosis and range (years old)	26 (14-38)	/	
Treatment			
Mean age at start of treatment and range (years old)	35,4 (4-74)	34,5 (14-54)	0,9739
Use of anakinra	20/25 (80%)	7/11 (63%)	0,4088
Use of canakinumab	33/33 (100%)	10/10 (100%)	>0,9999
Good response to treatment	41/45 (91%)	14/14 (100%)	0,5635

464 *CAPS: cryopyrin-associated periodic syndrome, LRR: leucine-rich-repeat, IBD: inflammatory*
465 *bowel disease*

466

467

Figure 1: Mutations of residue 861 of NLRP3 drive an inflamasommopathy.

A) Representation of NLRP3 with known mutated amino acid in CAPS patients highlighted in red and the Y861 in light blue. **B)** Picture of P6 left eye showing conjunctivitis **C)** Fundus color photograph of patient P1 right eye showing an optic disc atrophy. **D)** Finger of P1 showing digital clubbing fingers. **E)** Audiograms of P1. **F)** Quantification of ASC speck in the plasma of healthy donors, typical CAPS patients, NLRP3 Y861C patients and FMF patients. **G)** Quantification of IL-18 in the plasma of healthy donors and NLRP3 Y861C patients

Figure 2: Pedigrees of 4 families with NLRP3 Y861C and Y861H pathogenic variants.

Figure 3: Cells from patients with Y861C substitution required a boost signal to be activated.

A) Quantification of basal production of IL1β during overnight culture from PBMC or **B)** monocytes of healthy donors, typical CAPS patients and NLRP3 Y861C patients. **C)** quantification of IL1β production of PBMC or **D)** monocytes of healthy donors, typical CAPS patients and NLRP3 Y861C patients following LPS and LPS + MSU stimulation (3 hours priming + 3 hours activating signal)

Figure 4: Cells from patients with Y861C substitution demonstrate sustained IL-1β production.

A) Up: Structure of the NLRP3 (yellow)/NEK7 (red) interface (PDB: 6NPY) near the residue Y861 (cyan). Basic residues of NEK7 (ie K128, K130, R131) are involved in electrostatic interactions (dashed bond) with W776, E802 and E864 of NLRP3. Down: Predicted impact of selected mutation on NEK7/NLRP3 interactions through MD simulations. The table display the key interface residues involved in electrostatic interactions and energies calculations. The interfacial interaction energy is considered in van der Waals and electrostatic terms. **B)** As for the previously describe R920Q mutation, Y861C is predicted to stabilize NLRP3/NEK7 interaction compared to WT. However, Y861H is predicted to be detrimental **B)** Quantification of IL1β production over time of monocytes from healthy donors and 3 NLRP3 Y861C patients. **C)** Quantification of the production of IL1β between 3 hours and 6 hours post boost in monocytes healthy donors and NLRP3 Y861C patients.

Figure 5: Graphical abstract. CAPS patients with pathogenic variant at position 861of NLRP3 are more susceptible to headache and hearing loss than patients with variants in the NACHT domain. Inversely, NACHT mutated patients are more susceptible to aphtosis and urticarial rash.

Figure 1

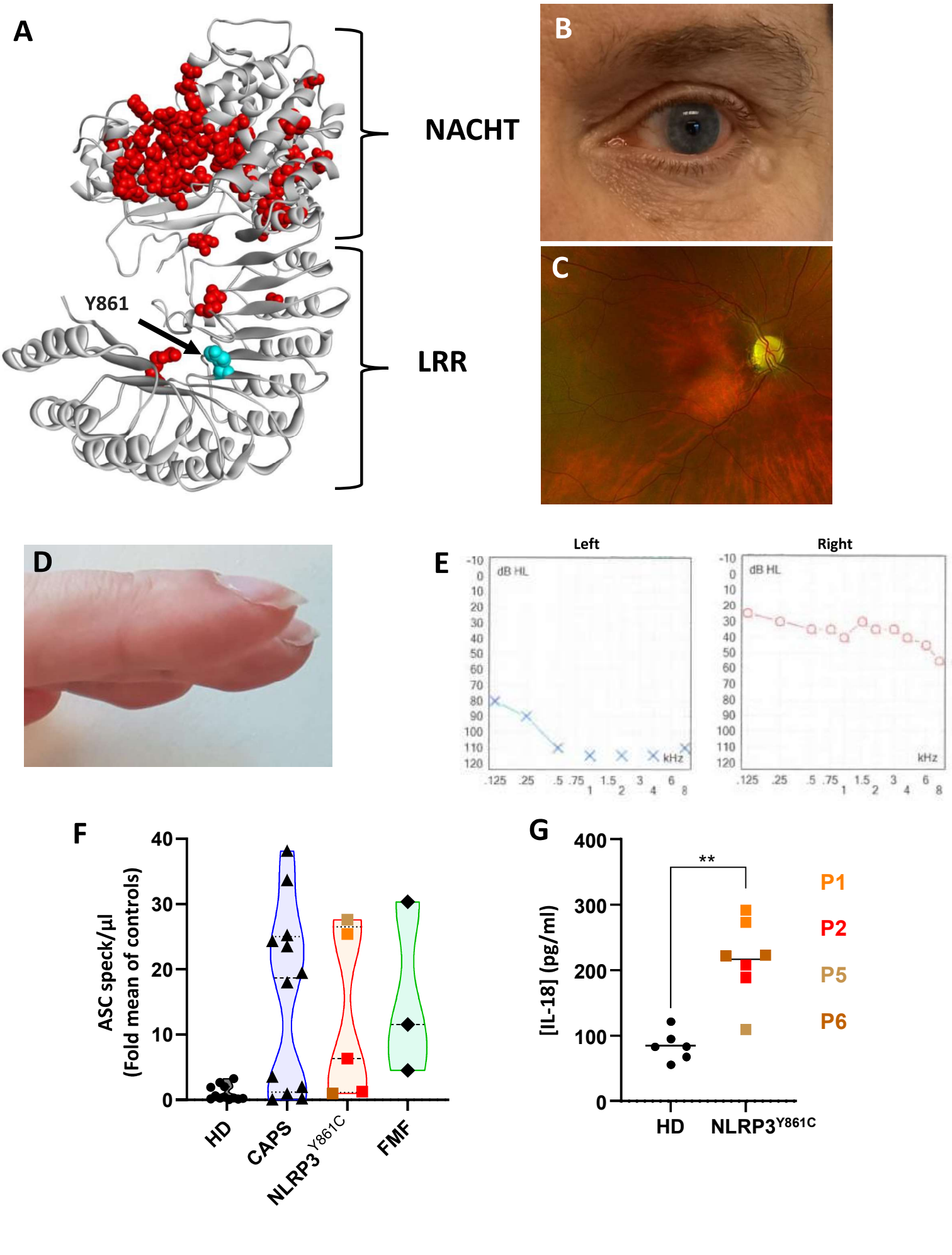

A
NACHT
LRR
Y861
B
C
D
E
Left
Right
dB HL
kHz
F
ASC speck/µl
(Fold mean of controls)
HD
CAPS
NLRP3 Y861C
FMF
G
[IL-18] (pg/ml)
**
P1
P2
P5
P6
HD
NLRP3Y861C

Figure 2

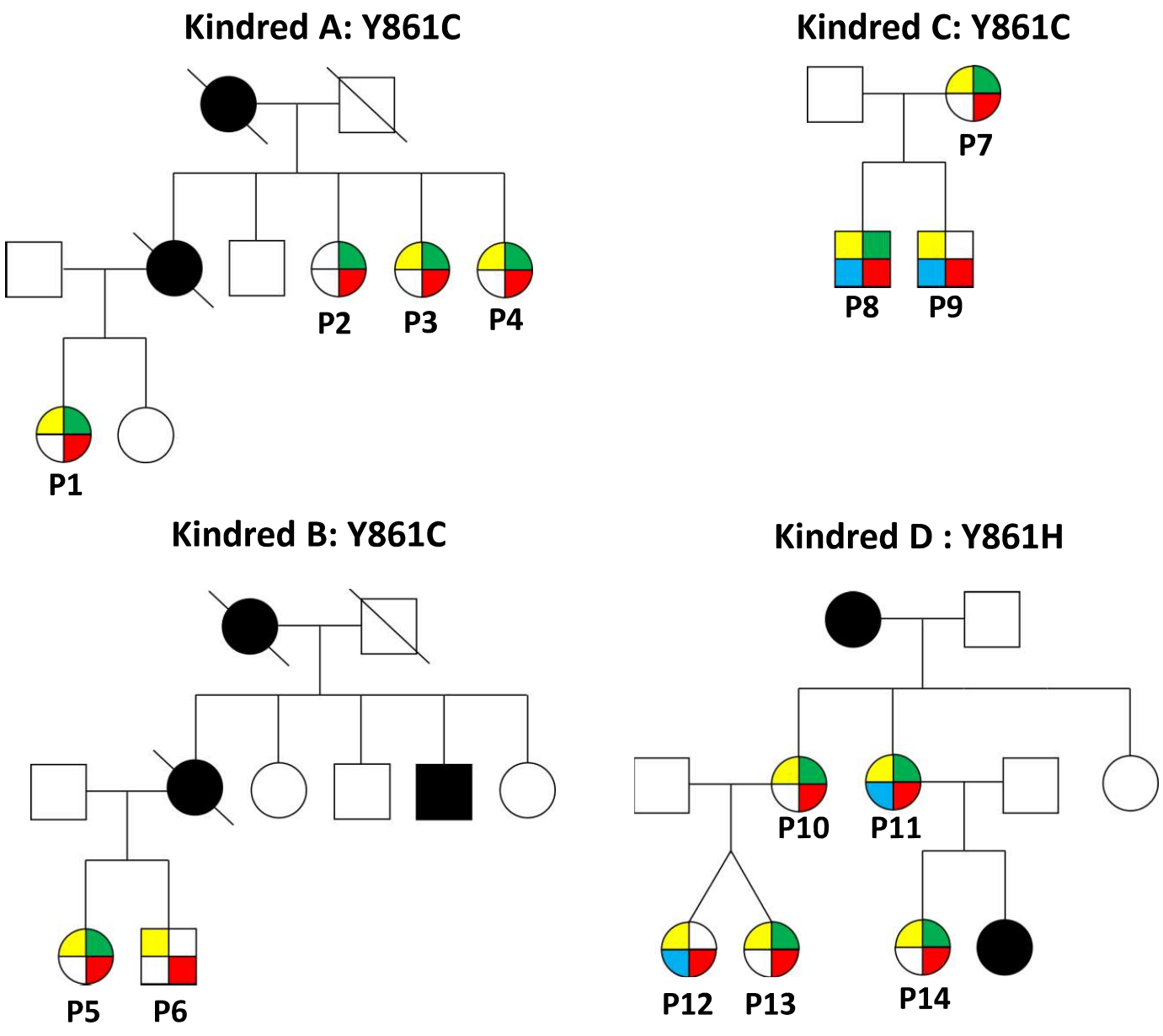

Kindred A: Y861C
P1
P2
P3
P4
Kindred C: Y861C
P7
P8
P9
Kindred B: Y861C
P5
P6
Kindred D : Y861H
P10
P11
P12
P13
P14
Sensorineural hearing loss
Urticarial rash
Arthromyalgia
Ocular involvement
Individual without CAPS related symptoms
Individual with CAPS related symptoms not assessed

Figure 3

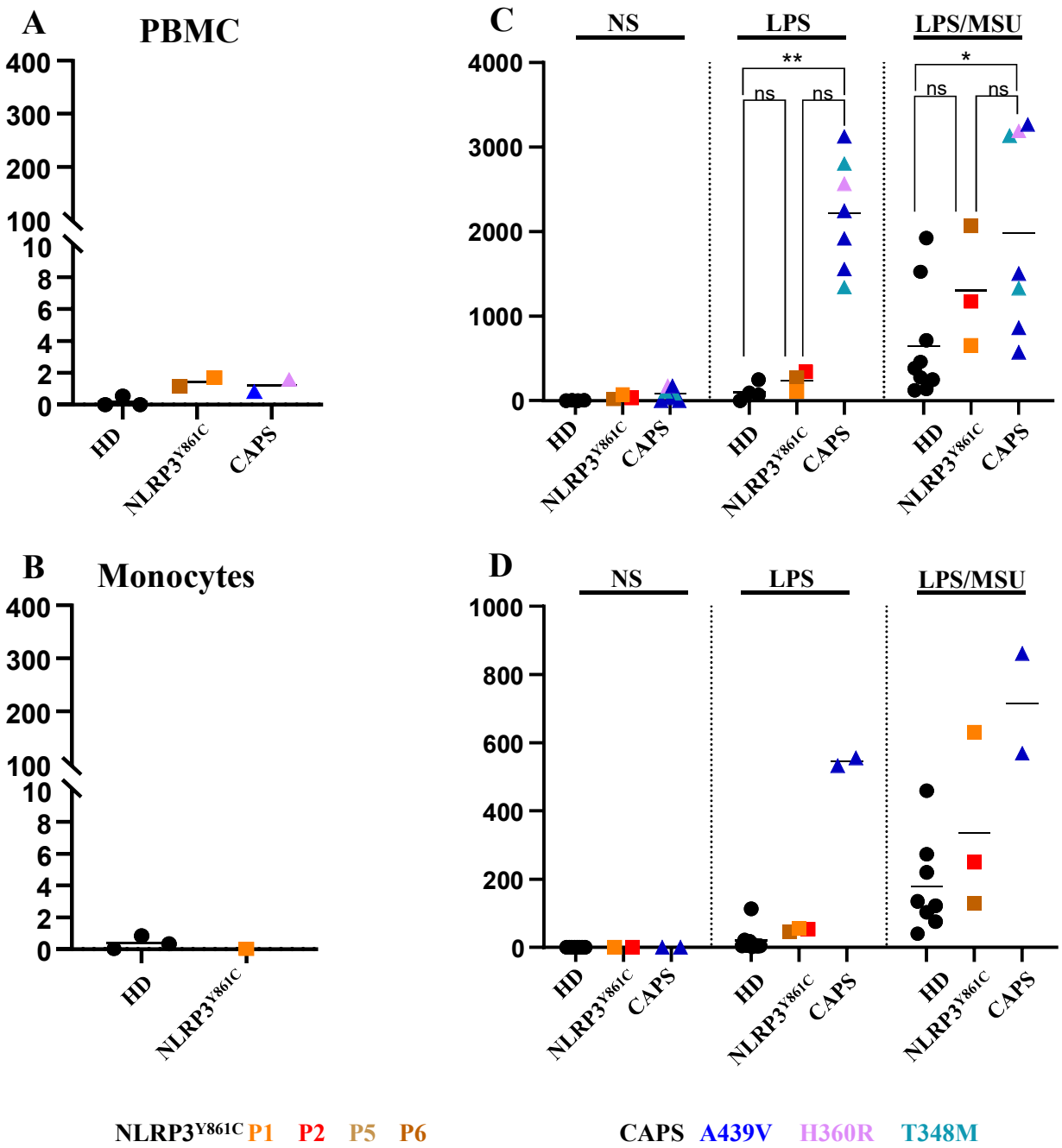
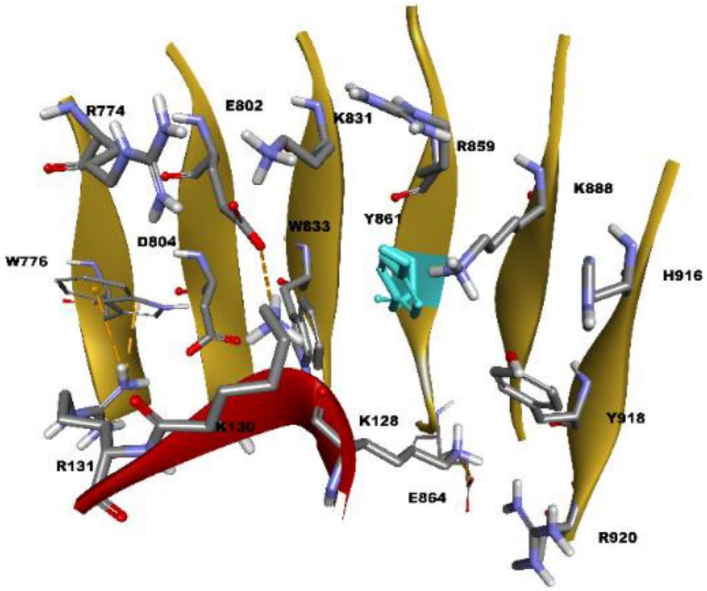

A
PBMC
400
300
200
100
10
8
6
4
2
0
HD
NLRP3Y861C
CAPS
B
Monocytes
400
300
200
100
10
8
6
4
2
0
HD
NLRP3Y861C
C
NS
LPS
LPS/MSU
4000
3000
2000
1000
0
**
*
ns
ns
ns
ns
HD
NLRP3Y861C
CAPS
D
NS
LPS
LPS/MSU
1000
800
600
400
200
0
HD
NLRP3Y861C
CAPS
NLRP3Y861C P1 P2 P5 P6
CAPS A439V H360R T348M

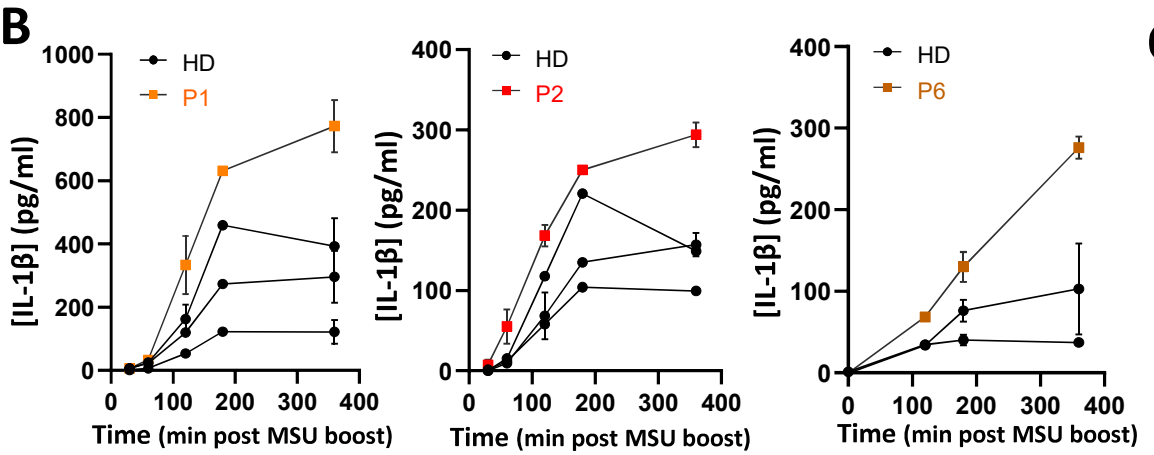
Figure 4

A



NLRP3 Genotypes	Interacting AA from NLRP3	Interacting AA from NEK7	Interaction energy (kcal/mol)	Electrostatic energy (kcal/mol)	Van der Waals energy (kcal/mol)
Y861 (WT)	D750, D804, D807, E864	R121, K128, R131, K140	-383.0	-326.9	-56.1
C861	E356, K696, D747, D750	R131, K140, D261, R294	–501.9	-424.2	-77.7
H861	K696, D750, E864	K128, K140, D261	–351.2	–310.1	-41.1
Q920	K696, D750, D804, E864, E1007, E1033,	K124, K127, K128, R131, K140, D261	-451.8	-413.0	-38.8

B



C

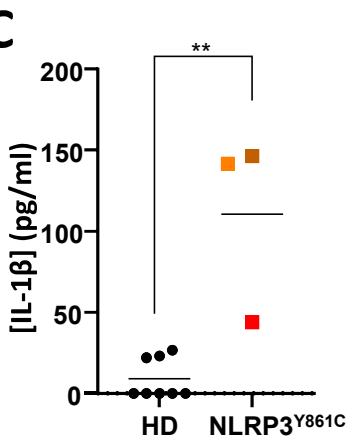
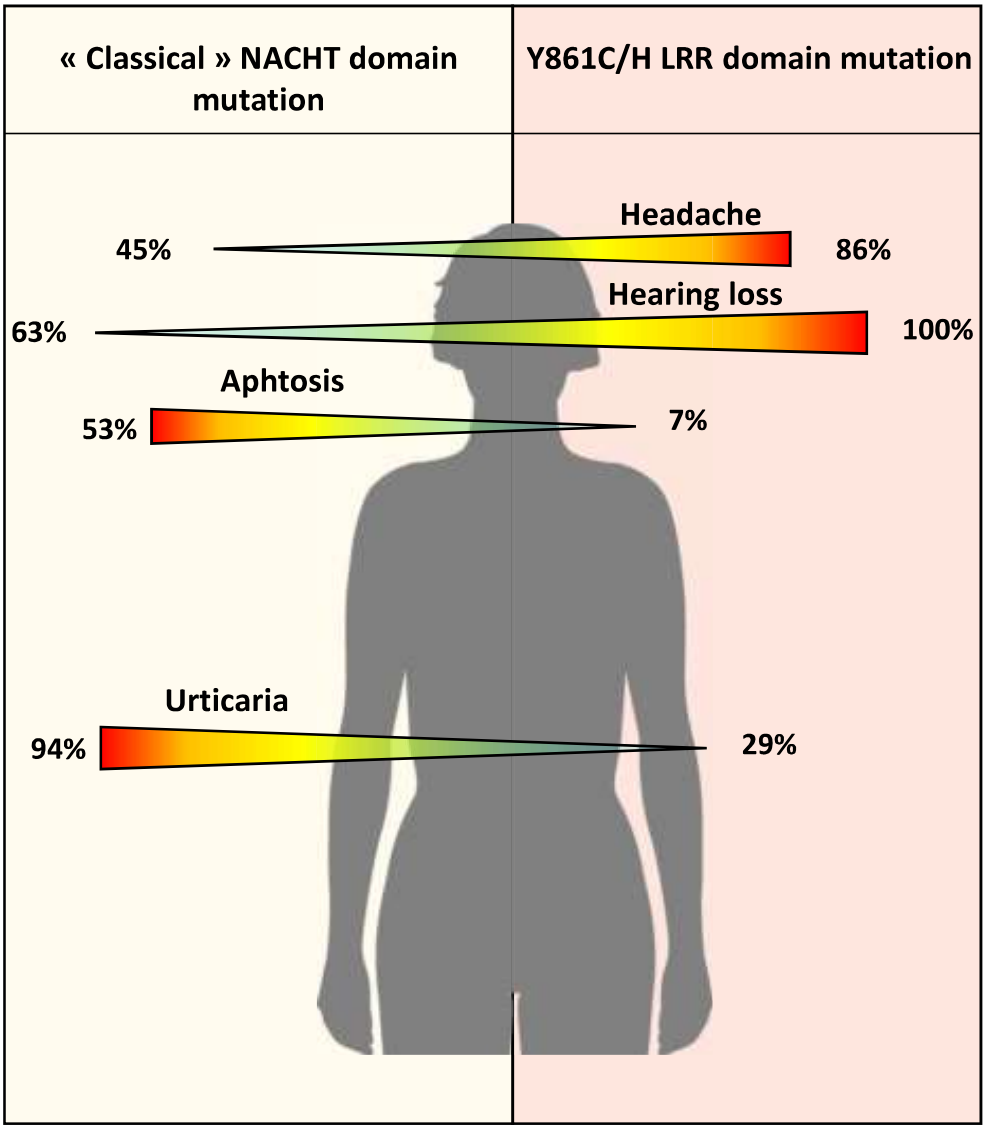
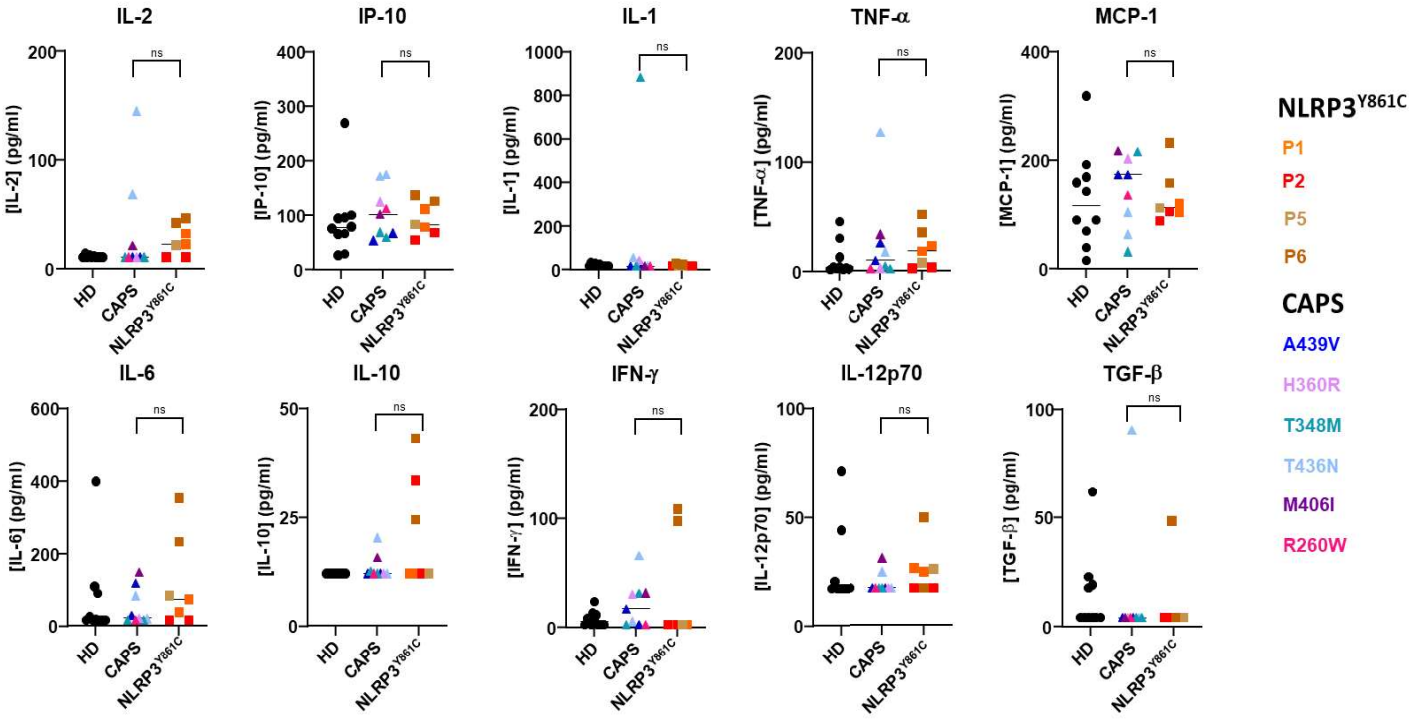


Figure 5


« Classical » NACHT domain mutation
Y861C/H LRR domain mutation
Headache
45%
86%
Hearing loss
63%
100%
Aphtosis
53%
7%
Urticaria
94%
29%

Sup Fig 1



Sup fig 1: Screening of the plasmatique levels of 10 inflammatory cytokines did not reveals any significant difference between typical CAPS and NLRP3 Y861C patients.