Phenotypic and genotypic characteristics of cryopyrin-associated periodic syndrome: a series of 136 patients from the Eurofever Registry

Phenotype and genotype of Cryopyrin~~e~~ Associated Periodic syndrome (CAPS) : a series of 136 patients from the Eurofever international registry

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ABSTRACT

Objective: ~~to~~ To evaluate genetic, demographic and clinical features in patients with cryopyrin-associated periodic syndrome (CAPS) from the Eurofever registry. To evaluate genotype-phenotype correlation and define predictive markers of disease-severity.

Methods: A web-based registry collected retrospective data on patients with CAPS. Experts in the disease independently validated cases. Patients carrying *NLRP3* variants and germline mutation-negative patients (with complete gene screening) were included.

Results: 136 patients were analysed. Median age at disease onset was 9 months; median duration of follow-up was 15 years. Skin rash, articular involvement and fever were the most prevalent features; neurological involvement including severe complications were noticed in 40% and 12% respectively; ophthalmological involvement was observed in 71% and neurosensorial hearing loss in 42%. 133 patients carried heterozygous germline mutation while 3 patients were mutation negative. Thirty-one different NLRP3 mutations were recorded, ~~including~~ 7 ~~recurrent~~ mutations ~~accounting~~ accounted for 78% of the patients while 24 rare variants were reported in 27 cases. The later variants were significantly associated with early disease onset, neurological complications (including severe ones) and severe articular involvement. T348M variant was associated with disease onset before 6 months, a chronic course and hearing loss. Neurological involvement was significantly less associated with V198M, E311K and A439V alleles. Early onset was ~~predictable~~ predictive of severe neurological complications and hearing loss.

Conclusion: Patients carrying rare NLRP3 variants are at risk of severe disease; ~~early onset <~~onset before 6 months ~~exposes to~~is associated with an increase in neurological involvement and neurosensorial hearing loss. These findings may impact on treatment decisions.

INTRODUCTION :

The cryopyrin-associated periodic syndromes (CAPS) belong to the growing family of inherited autoinflammatory syndromes characterised by recurrent bouts of systemic and organ specific inflammation related to inappropriate activity or regulation of the innate immune system. CAPS are usually dominantly inherited but *de novo* cases are also reported. They encompass ~~3~~ three distinct diseases with a continuum of severity from the mildest, familial cold autoinflammatory syndrome (FCAS, OMIM 120100), to the most severe, neonatal-onset multisystem inflammatory disease (NOMID) [or chronic infantile neurologic cutaneous articular (CINCA) syndrome, OMIM 607115], while patients with Muckle-Wells syndrome (MWS, OMIM 191900) have an intermediate phenotype. Patients with FCAS are characterised by recurrent cold-induced episodes of fever, urticaria-like rash, arthalgia and ~~conjonctivitis~~conjunctivitis; in MWS, the disease-pattern is more chronic than recurrent, patients often complain of fever, rash and arthritis or arthralgia without specific triggers, sensorineural hearing loss and AA amyloidosis can developed in adulthood[1]; CINCA/NOMID patients present with unconstant fever, urticaria-like rash and neurosensorial involvement characterised by chronic aseptic meningitis, ~~papillary oedema~~papilloedema, papillitis and deafness. Hypertrophic arthropathy with contractures and bone deformity can occur in severely affected patients. Early onset is frequently but not exclusively reported in CINCA~~, but not exclusively~~[2, 3].

CAPS are ~~genetically related to~~caused by dominantly ~~inhereted~~ inherited or *de novo* gain of function mutations of *NLRP3* (nucleotide-binding domain, leucine-rich repeat family, pyrin domain containing 3)[4-6]. Somatic mutations have been recently described in CINCA/NOMID germline mutation-negative patients[7,8, 9]. ~~*This*~~ *NLRP3* ~~gene~~ encodes ~~for a protein, called~~ cryopyrin, a key coponent of the inflammasome. This multimeric cytosolic protein complex ~~triggers~~ controls activation of caspase-1, which in turns catalyses the cleavage of pro-interleukin-1β (Il-1) into IL-1β, a potent pro-inflammatory cytokine[10]. Most of the recognised NLRP3 variants ~~described today~~ are missense mutations found in exon 3 that encode for the nucleotide-binding domain of cryopyrin[11]. ~~Very~~ A few mutations ~~were~~ have been found in exons 4, 6 and 8 encoding ~~for~~ the leucin-rich-repeat domain[12]. *NLRP3* mutations are associated with constitutive activation of the inflammasome ~~with~~ and overexpression of IL-1β. The better understanding of both the genetic basis and physiopathology of CAPS, underlines~~ing~~ the major role of~~r~~ IL-1β, ~~offer new~~and the potential of targeted therapeutics. ~~perspectives. In fact i~~Inhibition of IL-1β ~~in CAPS~~has dramatically improved all clinical

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Commentato [HJL1]: This doesn't really work in English - do you mean intermittant or episodic?

Commentato [HJL2]: This isn't clear - Variable, intermittent, persistent

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manifestations related to inflammation[13] but treatments are very costly and decisions around timing of treatment initiation in young children can be complex particualrly as prediction of the course of the disease and the risk of long-term organ damage remains extremely challenging. ~~-~~

~~Thanks to the EUROFEVER registry, an international survey of autoinflammatory diseases~~W~~,~~ ~~w~~e undertook a detailed phenotype and genotype description of a large international cohort of CAPS patient enrolled in the EUROFEVER registry, an international survey of autoinflammatory diseases and ~~s. We~~ searched for genetic and phenotypic predictive markers of a more severe outcome.

PATIENTS AND METHODS :

Patients and study design

All patient data were extracted from The EUROFEVER registry. This international survey of autoinflammatory diseases[17] has been enrolling patients suffering from various autoinflammatory syndromes including Cryopyrin-associated periodic syndromes (CAPS) since November 2009. This Project was sponsored by the Autoinflammatory Diseases' Working Group of the Paediatric Rheumatology European Society (PRES) and supported by the Executive Agency For Health and Consumers (EAHC, Project No 2007332, http://ec.europa.eu/eahc/projects/database.html)

Ethical committee approval for entering patients in the registry was obtained in the participating countries according to local regulatory requirements.

Patients with diagnosis of CAPS (as validated by experts BN, JKD and MG) and carrying germline *NLRP3* mutation or without germline mutation despite complete screening of the gene were included in this study. Demographic data, clinical manifestations, laboratory findings, data from other diagnostic procedures and information about molecular genetic variants were collected anonymously. The database cut-off for analysis was June 2012.

Phenotype and genotype characterization:

Detailed epidemiological and demographic data were collected. The clinical characteristics were also recorded including: disease pattern defined by either recurrent acute episodes, chronic disease or chronic with acute exacerbations; constitutional symptoms including fever, fatigue, malaise, mood disorders or failure to thrive; cutaneous involvement and detailed characteristics of skin rash. Organ specific involvement ~~was arbitrarily classified a~~were

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divided into~~s~~ mild or severe groups. M~~ild m~~usculo-skeletal manifestations were ~~considered in~~ included: ~~of~~ myalgia ~~and~~ or arthralgia; severe musculo-skeletal manifestations encompassed: overgrowth, joint contractures, bone deformity, bone erosion or osteolytic lesions. Mild neurological involvement was defined by the presence of morning headache, papilloedema or aseptic meningitis; while the presence of epilepsy, hydrocephalus or mental retardation ~~items~~ classified as severe. Mild ocular manifestations were defined by conjunctivitis or uveitis while severe ocular involvement ~~was defined by~~included: optic nerve atrophy, cataract, impaired vision. Neurosensorial hearing loss was based on the result of at least one pathologic audiogram.

The most relevant and well documented clinical characteristics were grouped into ~~9~~ nine main items: age of onset before 6 months, chronic disease course, positive familial history, cold trigger, mild neurological involvement, severe neurological involvement, mild articular involvement, severe articular involvement, hearing loss.

~~Germline sequencing of *NLRP3* gene was performed and documented in all patients. Informations about sequencing were also collected.~~

Statistical analysis:

Descriptive statistics were reported as medians with interquartile range (IQR) values, or numbers with percentage (%). To test the association between the main clinical phenotypes and genotype pattern, nine genotype subgroups were defined. Seven subgroups were defined with patients bearing one of the most frequent mutations (~~occurring for~~found in ≥ 5 unrelated patients): R260W, E311K, V198M, T348M, D303N, Q703K, A439V. Patients with rare mutations (≤ 2 unrelated patients) were pooled in a separate group, and patients with no mutation accounted for the last group. Clinical characteristics of the patients were compared on the basis of the presence or not of each mutation.~~.~~ Univariate association between each of the main clinical characteristics was conducted in a secondary analysis. Comparison between groups was performed using of the Fisher's exact test and the chi-squared test, when appropriate. All tests were two-sided. The Benjamini-Hochberg procedure was used to correct for multiple comparisons, and $p < 0.01$ was considered significant. All analyses were conducted using Stata, version 11.0 (StatCorp Inc., College Station, TX, USA)

Commentato [HJL5]: I have reservations about this. 5 of these are clear cut mutations which have been previously described, V198M is difficult - again with a literature discussing that it is a common variant and can be disease causing in a minority and Q703K is really contentious and is very common in healthy Caucasians. I think this needs to be clearly discussed in the introduction and a justification for lumping them together put in here. Without that it reads as if equal weight is given to all these sequence variants and I think that just isn't true. Given the diffilulties here I also think the phenotype of the excluded patients and the role of the validating clinicians has to be expanded upon - there needs to be a clear description of why the Q703K patients in particular were included in the cohort.

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RESULTS:

Demographic data:

In June 2012, 157 patients were available in the registry, 9 patients were excluded during the validation process and 12 patients were excluded because of absence of genetic screening (n= 10) or because of incomplete screening of the gene in mutation negative patients (n= 2). A total of 136 patients (67 female, 69 male) were analyzed and were enrolled by 27 centers from 16 countries: United Kingdom (49), France (29), Italy (28), Germany (10), Netherlands (4), Spain (4), India (2), Switzerland (1), Turkey (1), Russia (2), Pakistan (1), Australia (1), South Africa (1), Saudi Arabia (1), Chile (1), and Denmark (1). One hundred and twenty nine patients were Caucasian, 7 were Asian. A p~~P~~ositive familial history was reported in 66 patients while 54 cases were sporadic (status unknown for 6).

Demographic data are summarized in table 1. Median age at disease onset and at disease diagnosis was respectively 9 months (IQR, 44 days to 5 years) and 15 years (IQR, 5 to 36 years). The median age at last visit was 15 years (IQR, 8 to 35 years).

Clinical characteristics:

Clinical characteristics of the patients are detailed in ~~table~~ Table 2. Seventy-eight patients (57%) had a chronic course while 58 (43%) experienced only acute episodes. Skin rash, articular involvement and fever were the most prevalent features, observed respectively in 97%, 86% and 84%. Six patients (4%) suffered from severe articular involvement as previously defined: flexion contractures (n = 3), patella overgrowth (n = 1), bone deformity (n = 4), bone erosions (n = 1), or osteolytic lesions (n = 2). Neurological involvement was noticed in 55 patients (40%) including 16 individuals with severe neurological involvement (seizures (n = 2), hydrocephalus (n = 10), mental retardation (n= 9)). Ophthalmological involvement was observed in 71%. Severe ophthalmological involvement was documented in 16 patients (12%) (Optic nerve atrophy (n = 6), cataract (n = 4), glaucoma (n = 2), or impaired vision (n = 8)). Neurosensorial hearing loss was reported in 42% documented by an audiogram in all cases. AA amyloidosis was identified in 5 patients carrying R260W (3 patients) V198M (1 patient) and A439V (1 patient) mutations at a median age of 29 years old (range 21 to 52 years). Ninety-five patients (70%) suffered from various constitutional symptoms. A total of 83 patients suffered from recurrent episodes. Characteristics of these bouts are given in ~~table~~ Table 3. Overall, 77% of patients received anti-IL-1 drugs during the course of the disease, anakinra or canakinumab (figure 2 b).

Commentato [HJL6]: Probably needs some clinical details here

Commentato [HJL7]: This could really do with defining - perhaps something along the lines of 'symptoms alomst every day'.

Commentato [HJL8]: Not sure what this means - in relation to chronic disease course or acute attacks

Genotypic characterization:

Heterozygous germline mutation was reported in 133 patients. Of the ~~while 3~~ three patients without ~~did not carried~~ germline mutations t~~. In the later, 2~~wo ~~patients turned out~~were subsequently found to carry ~~a~~ somatic *NLRP3* mutations. Sequencing of the whole gene was performed in 13 patients (10%) while most relevant exons were sequenced in 93 cases (68%) and most relevant point mutations searched in 9 (7%) cases (information on *NLRP3* sequencing exhaustivity was not available in 21 cases (15%)). Thirty-one germline ~~different~~ NLRP3 mutations were ~~recorded and showed~~detected (~~ in f~~Figure 1b). Seven mutations ~~were recurrent and occurred~~ were found in 106 (78%) patients, including 5 patients ~~with both~~carrying both the~~ variants~~ R260W and V198M variants. Twenty-seven patients (20%) carried other rare mutations (F~~f~~igure1a.) including ~~2~~ two previously unreported *NRLP3* variants (I572S, L1016F). ~~A non recurrent~~Twenty-one mutations ~~was~~ were found in a single individual~~reported in 21 cases~~, ~~3~~ three mutations (M662T, P350M and I572F) were ~~documented~~ each found in ~~2~~ two unrelated patients~~ each~~. All mutations were nonsense. All except~~ed one~~ ~~(~~L1061F~~)~~ were located in *NLRP3* exon 3.

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Association between genotype and clinical characteristics:

Distribution of the clinical characteristics in each of the genotype~~s~~ subgroups is detailed in ~~table~~ Table 4, illustrating positive or negative significant associations between each clinical item and a specific genotype. The R260W variant was associated with ~~the presence of a~~ cold triggered symptoms~~ing~~ ($p < 10^{-3}$), a positive familial history ($p < 10^{-3}$) and a trend to ~~a delayed age at~~symptom onset after 6 months (p= 0.04). The T348M variant was associated with ~~an early~~ disease onset before 6 months (p= 0.01), a chronic course (p= 0.007) and ~~a frequent~~ hearing loss (p= 0.01). V198M, E311K and A439V alleles were rarely associated with neurological involvement. Finally, young age at onset (p= 0.003); neurological manifestations (p= 0.008) including severe ones ($p < 10^{-3}$); severe articular involvement (p= 0.01) and sporadic pattern ($p < 10^{-3}$) were significantly commoner in ~~associated with~~ patients bearing a rare mutation. In this later group, we found a trend for a higher risk ~~for~~ of neurosensorial hearing loss (p= 0.02). Distribution of the main clinical features in patients carrying rare variants is reported in supplementary Table 1.

Percentage of patients treated by anti-IL1 drugs in each genotype group is given in F~~f~~igure 2b. Low~~er~~est rates of treatment ~~was~~ were observed in patients carrying Q703K (11%), V198M (60%) and R260W (70%) mutations.

Association among clinical manifestations:

Univariate associations between each of the main clinical phenotypes were tested in the entire cohort. A chronic disease course was more frequently observed in patients with disease onset before the age of 6 months compared to patients with later onset ($p= 2.10^{-3}$). Early onset was also ~~predictable for~~predictive of severe neurological complications ($p < 10^{-3)}$ and hearing loss ($p= 0.01$). Patients with a positive familial history developed less severe disease compared to patients without, ~~by means of a~~specifically with a lower risk for severe articular involvement ($p= 0.01$), and both mild and severe neurological involvement ($p= 10^{-3}$ and $p < 10^{-3}$, respectively). A cold trigger was more frequently identified in patients with familial history of CAPS ($p < 10^{-3}$) and less frequently in patients with a chronic course of disease ($p < 10^{-3}$). Finally, neurological involvement was frequently associated with hearing loss ($p < 10^{-3}$). The other clinical phenotypes ~~were not associated~~showed no associations with ~~to~~ each other.

DISCUSSION:

The present work provides a detailed description of clinical and genotype features of the largest series yet of patients suffering from cryopyrin-associated periodic syndromes (CAPS). There were collected via the EUROFEVER registry, designed to set up an international web based registry of autoinflammatory diseases. This large collection of data allowed statistically and clinically meaningful analysis of phenotype/genotype correlations and identified risk factors of severe disease. It also highlights the very wide spectrum of these diseases. Importantly, patients with rare mutations were found to have a more severe phenotype. An early disease onset before the age of 6 months was predictive of a more severe outcome.

~~Because of~~As the phenotypes overlap~~ping phenotypes~~, the older subdivisions of ~~strict definitions of~~ CAPS syndromes into ~~so called~~ FCAS, MWS or CINCA were felt to be inappropriate and not applied ~~to~~ in this study. Instead, ~~relevant~~ clinical symptoms were used each type of involvement. In many respects, our data confirm clinical descriptions from earlier publications[18,19,20]. Disease course was pre~~valently~~dominantly chronic. R~~.~~~~Skin r~~ash frequent clinical manifestation, being present in 97% of patients. ~~Skin r~~Rash was ~~not~~ patients and classical urticarial rash was reported in only 89% of patients. ~~Disease course was~~ involvement as defined by presence of at least one of: morning headache, ~~and/or~~ chronic or ~~and/or~~ papillary oedema was identified in 40% of patients. Because documentation of CSF examination and fundoscopy were not exhaustive (in only 64% and 79% of patients respectively) we ~~can not~~cannot exclude ~~miss estimation~~underestimation of neurological

involvement. However, morning headaches ~~were~~ have been reported to be highly predictive chronic meningitis in earlier studies in CAPS ~~as previously reported~~[21] ~~as meningitis was~~ arthropathy) ~~noticed~~ in six~~6~~ patients was less frequently reported than in previous series[6,16,20]. amyloidosis was ~~rare and~~ reported in ~~5~~ five patients ~~only~~. ~~This~~ AA amyloidosis is known to onset complication (median age of onset of 31 years[22]) ~~is rare~~and its rarity compared to reports (where it developed in up to 10%)[22]~~. This~~ might be related to the ~~relative~~ young cohort (18 years) or to early introduction of therapeutic interventions that prevent chronic inflammation~~impact on this severe complication~~.

We detected a total of 31 missense mutations including ~~2~~ two previously unreported mutations. Distribution of the *NLRP3* variants documented in this study was very similar to previous studies; the frequent *NLRP3* mutations: R260W~~,~~; T348M~~,~~; V198M~~,~~; A439V~~,~~; D303N~~,~~; Q703K and E311K accounted for 71% of patients in our cohort and rare variants accounted for 20%. This distribution is very similar to Cuisset and al[11]. Surprisingly, only ~~3~~ three patients with typical severe CAPS phenotype were screened without any germline mutation which is much ~~. It's~~ less common than previously reported [6,9].

Since the discovery of the gene responsible for FCAS, MWS and CINCA/NOMID~~–~~[4], and the understanding of their common molecular basis, some degree of genotype/phenotype correlation has been raised by several studies, even though phenotypic variability was also not~~ic~~ed[14,6,15,16]~~–~~, suggesting ~~the possible~~a role for~~of~~ additional genetic and environmental factors. Assessment of genotype/phenotype correlations if difficult and can be limited by several factors. In CAPS

~~The~~ We found the T348M mutation was significantly associated with early onset, a chronic course and a frequent hearing loss. These findings are in accordance with previous reports [12, 14, 15, 29-31]. R260W was the most frequently identified variant in 36 patients of our cohort, with age at last visit of 32.2 years. It is the most frequently reported mutation in the literature and was found associated with FCAS, MWS or atypical autoinflammatory syndrome[32,14,11]. In our cohort, this variant was significantly associated with presence of a cold trigger and an autosomal dominant pattern of inheritance. There was a trend for a later onset and a recurrent pattern of evolution. A439V has been previously reported in patients with mild phenotype, FCAS or MWS~~–~~[4]. In our study, the ~~among~~ 14 patients with a median age of 51 years old at last visit, hearing loss and neurological involvement ~~developed significantly less frequently~~was significantly less frequent (identified in one patient each), while autosomal dominant pattern of inheritance was the rule. Interestingly, patients with E311K displayed a high rate of hearing loss, as previously reported[33].

Even though D303N variant and germline mutation negative CAPS ~~effectives~~ were too ~~low to~~ statisticall~~y~~ly~~~~ significance analysis, they were associated with severe phenotype as previously reported [5, 14, 16, 34, 35,29,12].

The *NLRP3* V198M and Q703K alleles deserve special comments. They are both found in healthy Caucasian controls (with alleles frequencies of 0.7 and 5% respectively ref 16) and are considered as low penetrant variants associated with mild disease [16, 32, 36] also Rowczenio DM, Arthritis Res Ther. 2013 Feb 19;15(1):R30. They were identified in 12 and 9 patients of this cohort respectively and were associated with a mild phenotype. Neurological involvement and hearing loss were rarely described despite long follow-up. In addition, 5 patients were reported with both V198M and R260W, these patients did not differ in phenotype and severity.

~~Because of the~~The retrospective design of this work produces a number of limitations ~~should also be discussed~~. Some data were missing and patient~~s were heterogeneously~~ documentation was heterogeneous~~ed~~. Details about treatment were incomplete ~~for~~ in some patients. In addition, the fact that a very large number of centers and clinicians included small numbers of patients each, may introduce bias of data interpretation.

In conclusion, we provide a detailed description of both clinical and genetic features of patient with CAPS in a large cohort of patients and have shown associations between genotype and phenotypic~~e~~ severity although the significance of common population variants such as Q703K still remains unclear. ~~–~~This study highlights new insights ~~on~~ in CAPS that may help in every day clinical practice. While it remains challenging ~~to appreciate the outcome and~~to anticipate potential complications, we suggest that sporadic cases, starting at an early age are at risk of severe neurological complications, and neurosensorial hearing loss especially in patients carrying rare germline *NLRP3* variants, T348M, D303N or somatic *NLRP3* mutation. These patients should be ~~investigated and~~ monitored closely and treatment should be discussed early to prevent long-term complications. In contrast, an autosomal dominant pattern of inheritance and a cold triggering seem to predict a better outcome with a ~~decrease~~ lower risk of severe organ damage.

Commentato [HJL10]: the largest and most recent series

Commentato [HJL11]: to patients with R260W or V198M?

Commentato [HJL12]: This needs expanding. There is a literature describing V198M and clearly in some indivuduals it is suffiecnt to cause classical CAPS and in other to cause other autoinflammatory symptoms. The role of Q703K is very much less clear - it is very frequent in Caucasian alleles - it is not at all clear that it is pathogenic - and it may be that it's finding here is incidental reflecting extensive genetic sequencing in patients with symptoms of unknown aetiology. I really think you need to expand on the Q703K clnical phenoptype here - it is very soft indeed with no FH, later onset and no deafness.

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Legends of figures

Figure 1: distribution of genotype among CAPS patients

(a.) Distribution of NLRP3 mutations in the cohort of 136 patients and (b.) representation along crypyrin protein. Mutation in bold are the frequent one's. Mutations in gray belong to the group of rare mutations, mutations underlined were identified in two unrelated patients of the cohort.

Figure 2: distribution of age at last visit and treatment according to the genotype

(a.) Age at onset of the disease for the whole cohort and for each genotype subgroups. Median values are represented with horizontal bars.

(b.) Percentage of patients treated with anti-IL-1 drugs for the whole cohort and for each genoptype groups.

(c.) Age at last visit for the whole cohort and for each genotype subgroups. Median values are represented with horizontal bars.

Table 1: characteristics of CAPS patients

	n (%)
Sex	
Male	69 (50)
Female	67 (50)
Sexe ratio	1
Origin	
Caucasian	128 (94)
Asian	7 (5)
Other	1 (1)
Mode of inheritance	
AD	76 (58.5)
De novo	54 (41.5)
	Median (IQ25-75)
Age onset (years)	0,8 (0,1-5)
Age diagnosis (years)	15 (5-36)
Age at last visit (years)	15 (8-35)

IQ denotes inter-quartile

Commentato [HJL13]: Unless family screening has been performed you don't know that. This is particuarly true for the more difficult variants V198M, Q703K etc. It might be better to say No Family History

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Table 2: clinical characteristics

	n	%
Fever	108	84
Disease Pattern		
Recurrent	58	43
Chronic with ~~access~~exacerbations	25	18
Chronic	53	39
Constitutional Symptoms	95	70
Skin Involvement	132	97
Urticarial rash	121	89
Maculo-papular rash	36	26
Articular Involvement	117	86
Myalgia	60	44
Arthralgia	107	79
Arthritis	47	36
Severe articular involvement**	6	4
Neurological Involvement	55	40
Papilloedema	29	27
Meningitis	23	26
Severe neurological involvement***	16	12
Headache morning	39	29
Hearing loss	56	42
Ophthalmologic involvement	97	71
Conjunctivitis	87	66
Uveitis	9	7
Severe ophtalmological involvement****	16	12

Table 3: characteristics of access

	n	%
Periodic Disease		
Recurrent Disease	58	42
Chronic with ~~recurrent access~~exacerbations	25	18
Duration of access		
< 24h	27	48
1- 3 d	9	16
>3 d	20	36
Number of access		
<12/y	32	52
12-24/y	5	8
>24/y	24	39
Fever during access	83	100
Fever type		
>38°	48	58
<38°	35	42
Trigger	40	56
Type of Trigger		
Cold	34	85
Other*	6	15
Seasonal change		
Yes	20	33
No	41	67

h denotes hours, d : days ; y : years
* : stress (n=1) ; infection (n=1) ; trauma (n=2) ; food (n=1) ; fatigue (n=2)

Commentato [HJL14]: I don't know what this means????
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Table 4: Genotype-phenotype association analysis

Genotype/phenotype	Age onset < 6 mo	Chronic course	Relatives affected	Neurological	Severe Neurological	Articular	Severe articular	Hearing loss	Cold trigger
R260W (n= 36)									
yes	11 (30,6)	15 (41,7)	32 (91,4)	15 (41,7)	2 (5,6)	33 (91,7)	0 (0)	12 (33,3)	17 (47,2)
no	50 (50)	63 (63)	44 (46,3)	40 (40)	14 (14)	83 (83,8)	6 (6)	44 (44,9)	17 (17)
p	0,04	0,03	< 10-3	0,9	0,24	0,25	0,34	0,23	< 10-3
T348M (n=20)									
yes	14 (70)	17 (85)	11 (57,9)	11 (55)	0 (0)	15 (75)	0 (0	13 (68,4)	2 (10)
no	47 (40,5)	61 (52,6)	65 (58,6)	44 (38)	16 (13,8)	101 (87,8)	6 (5,2)	43 (37,4)	32 (27,6)
p	0,01	0,007	0,96	0,15	0,13	0,16	0,59	0,01	0,09
V198M (n=13)									
yes	5 (38,5)	8 (61,5)	8 (66,7)	1 (7,7)	0 (0)	11 (84,6)	1 (7,7)	4 (30,8)	3 (23)
no	56 (45,5)	70 (56,9)	68 (57,6)	54 (44)	16 (13)	105 (86)	5 (4,1)	52 (43)	31 (25,2)
p	0,63	0,75	0,76	0,01	0,36	1	0,46	0,4	1
A439V (n= 14)									
yes	5 (35,7)	6 (42,9)	13 (92,9)	1 (7,1)	0 (0)	12 (85,7)	0 (0)	1 (7,1)	7 (50)
no	56 (46)	72 (59)	63 (54,3)	54 (44,3)	16 (13,1)	104 (86)	6 (4,9)	55 (45,8)	27 (22,1)
p	0,47	0,25	0,006	0,007	0,37	1	1	0,005	0,04
E311K (n=9)									
yes	2 (22,2)	5 (55,6)	8 (88,9)	0 (0)	0 (0)	9 (100)	0 (0)	6 (66,7)	0 (0)
no	59 (46,5)	73 (57,5)	68 (56,2)	55 (43,3)	16 (12,60)	107 (84,9)	6 (4,7)	50 (40)	34 (26,8)
p	0,19	1	0,08	0,01	0,6	0,36	1	0,16	0,11
D303N (n= 5)									
yes	2 (40)	2 (40)	0 (0)	4 (80)	1 (20)	5 (100)	0 (0)	3 (60)	1 (20)
no	59 (45)	76 (58)	76 (60,8)	51 (39)	15 (11,5)	111 (85,4)	6 (4,6)	53 (41,1)	33 (25,2)
p	1	0,65	0,01	0,16	0,47	1	1	0,65	1
Q703K (n= 9)									
yes	0 (0)	3 (33,3)	0 (0)	3 (33,3)	0 (0)	7 (77,8)	0 (0)	0 (0)	2 (22,2)
no	61 (100)	75 (59,1)	76 (62,3)	52 (41)	16 (12,6)	109 (86,5)	6 (4,7)	56 (44,8)	32 (25,2)
p	0,004	0,17	0,001	0,74	0,6	0,61	1	0,01	1
No mutation (n= 3)									
yes	3 (100)	3 (100)	0 (0)	3 (100)	3 (100)	3 (100)	1 (33,3)	1 (33,3)	0 (0)
no	58 (43,6)	75 (56,4)	76 (59,8)	52 (39,1)	13 (9,8)	113 (85,6)	5 (3,8)	55 (42)	34 (25,6)
p	0,09	0,26	0,07	0,06	0,001	1	0,13	1	0,57
Rare mutations (n= 27)									
yes	19 (70,4)	19 (70,4)	4 (16)	17 (63)	10 (37)	21 (80,8)	4 (14,8)	16 (61,5)	2 (7,4)
no	42 (38,5)	59 (54,1)	72 (68,6)	38 (34,9)	6 (5,5)	95 (87,2)	2 (1,9)	40 (37)	32 (29,4)
p	0,003	0,13	< 10-3	0,008	< 10-3	0,4	0,01	0,02	0,02
Total = 136 (n/%)	61 (44,85)	78 (57,35)	76 (58,46)	55 (40,44)	16 (11,76)	116 (85,93)	6 (4,41)	56 (41,79)	34 (25)

Commentato [HJL15]: I think this would be much easier to understand with just the positive number and percentage (the no and percentage is extremely confusing and doesn't contriute much to understanding the phenotype associated with each mutation)

Patients bearing the mutation of interest were compared to the other patients of the cohort in regards to their status for 9 clinical variables, using a chi-squarred or Fisher test, according to the effectives. A p value of 0,01 or less was considered significant.

Figure 1

a.

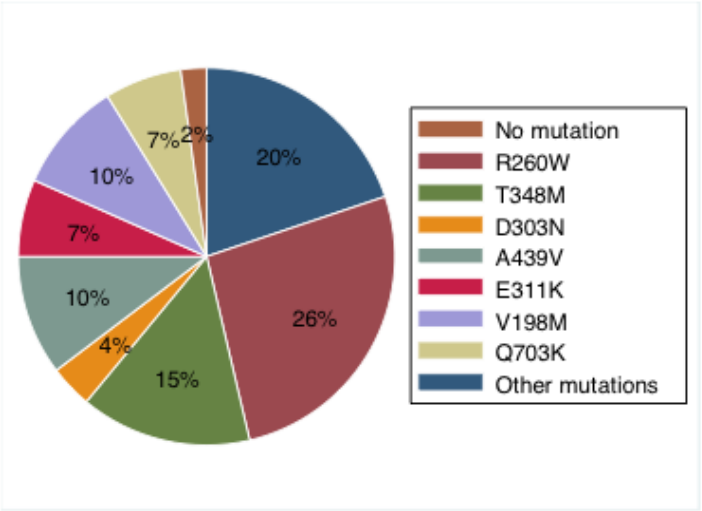


Figure 1

b.

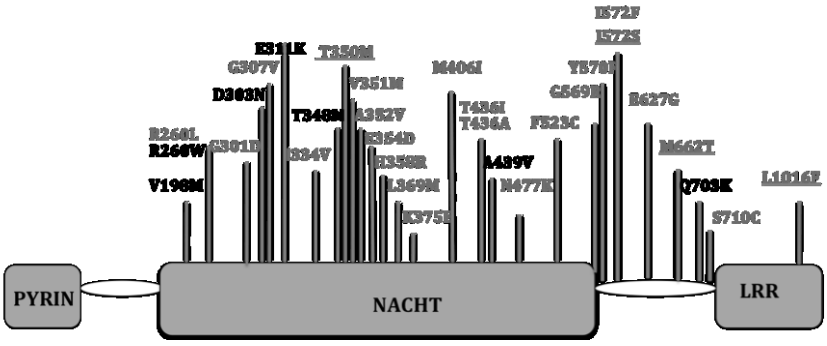
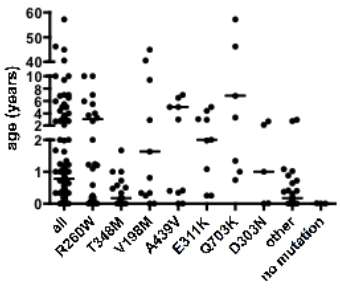
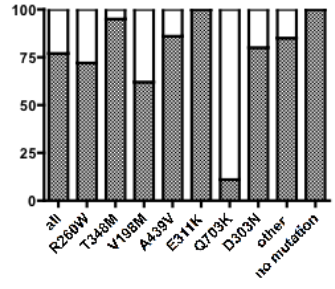


Figure 2

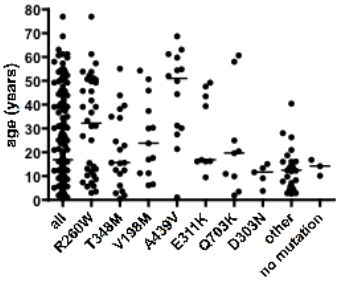
a.


age (years)
all
R260W
T348M
V198M
A439V
E311K
Q703K
D303N
other
no mutation

b.


all
R260W
T348M
V198M
A439V
E311K
Q703K
D303N
other
no mutation

c.


age (years)
all
R260W
T348M
V198M
A439V
E311K
Q703K
D303N
other
no mutation

Supplem. Table 1: Characteristics of patients with rare variant of *NLRP3*

Age onset: median in years (IQ25-75)	0,9	(0-0,7)
Duration of follow up: median in years (IQ25-75)	13	(5-16)
	n	**%**
Mode of inheritance		
AD	4	15
De novo	21	78
NK	2	7
Disease Pattern		
Recurrent	8	30
Chronic with access	11	40
Chronic	8	30
Age at onset < 6 month	19	70
Fever	19	70
~~Constitutionnal~~Constitutional Symptoms	22	81
Skin Involvement	27	100
Urticarial rash	24	89
Maculo-papular rash	9	33
Articular Involvement	21	78
Arthritis	9	33
Severe articular involvement	4	15
Neurological Involvement	17	63
Severe Neurological Involvement	10	37
Hearing loss	16	59
~~Ophtalmological~~Ophthalmological involvement	20	74
~~Conjonctivitis~~Conjunctivitis	10	37
Uveitis	5	19
Severe ~~ophtalmological~~ophthalmological Involvement	10	37
Cold trigger	2	7

Formattato: Tipo di carattere: Corsivo