Correspondance on 'Clinical characteristics and genetic analyses of 187 patients with undefined autoinflammatory diseases'

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We read with great interest the article by Ter Haar *et al*[1] who describe the clinical characteristics, treatment response and genetic findings in a cohort of patients (n=187) with undefined systemic autoinflammatory diseases (sAIDs). The authors observed that patients with pericarditis were older at disease onset and had milder disease whereas patients with intellectual impairment were younger at disease onset and often had affected relatives. Of note, among patients with recurrent disease course, 30% (n=53/180) had aphthous stomatitis. Moreover, after complete gene screening, screening of most relevant exons or screening of most relevant point mutations, 9.4% (n=15/159) of patients carried likely pathogenic variants or variants of uncertain significance. Our recent French multicentre study on targeted next-generation sequencing (NGS) gives further insight into the clinical characteristics and genetic findings in patients with sAIDs associated with recurrent aphthous stomatitis (RAS).

RAS (ie, adult onset, association with other features such as fever and malaise) can represent a diagnostic challenge. RAS especially when associated with fever, pharyngitis and lymphadenopathy may be the first presentation of sAIDs.[2] sAIDs arise from impaired inflammatory responses due to variants in gene coding proteins involved in innate immunity. commonly of monogenic inheritance.[3] The most common sAIDs associated with RAS are periodic fever, aphthous stomatitis, pharyngitis and adenopathy syndrome, NLRP3-associated autoinflammatory disease (NLRP3-AID), tumour necrosis factor receptor-associated periodic syndrome (TRAPS), A20 haploinsufficiency (HA20), mevalonate kinase deficiency (MKD) and deficiency of adenosine deaminase 2 (DADA2).[4] NGS allows for investigating multiple genes simultaneously and has become the preferred method for molecular testing of sAIDs.[5]

In our study, we aimed to determine the diagnostic performance of a targeted NGS panel of 55 genes in patients with suspected sAID with RAS.

A total of 631 adult and paediatric patients were referred for genetic diagnosis of sAID to the reference laboratory for autoinflammatory diseases of Montpellier, France, between 2014 and 2019. Patients of whom clinical information was available ((n=338), 53.3% female, median age 15 years, 107 (31.7%) with RAS) were included in the final analysis (table 1).

We detected pathogenic or likely pathogenic variants compatible with a final diagnosis in 41/338 (12.1%) of patients. These variants were found in the following genes: *MEFV* (n=7), *MVK* (n=6), *NLRP3* (n=4), *TNFRSF1A* (n=4), *ADA2* (n=3), *NOD2* (n=3), *NLRC4* (n=2), *SLC29A3* (n=2), *TMEM173* (n=2), others (n=8) (table 1). Based on the results of the genetic screening, probable/confirmed diagnosis were: familial Mediterranean fever, (n=5), MKD (n=6), *NLRP3*-AID (n=4), TRAPS (n=4), DADA2 (n=3), Blau syndrome (n=3), *NLRC4*-associated autoinflammatory disease (*NLRC4*-AID) (n=2), H syndrome (n=2), STING-associated vasculopathy with onset in infancy (n=2), pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) (n=2), HA20 (n=1), others (n=7).

Pathogenic or likely pathogenic variants were detected in 9/107 (8.4%) of patients with RAS: *MVK* (n=3), *TNFRSF1A* (n=2), *MEFV* (n=1), *ADA2* (n=1), *NLRP3* (n=1), *TRNT1* (n=1). Probable/confirmed diagnosis of these patients were: MKD (n=3), TRAPS (n=2), PAAND (n=1), DADA2 (n=1), *NLRP3*-AID

Table 1 Demographic and clinical characteristics of patients with suspected systemic autoinflammatory disease (sAIDs) and genotypes of the 41 patients with a probable or confirmed diagnosis of monogenic sAIDs using a targeted next-generation sequencing panel

Variable	Total (n=338)	No RAS (n=231)	RAS (n=107)	P value
Age (years), median (IQR)	15 (8–32)	15 (8–32)	16 (11–30)	0.54
Females, n (%)	182 (53.3)	124 (53.7)	58 (54.2)	1
Consanguinity, n (%)	8 (2.4)	6 (2.6)	2 (1.9)	1
Frequency of attacks>1/month, n (%)	189 (55.9)	100 (50)	49 (53)	0.35
Fever, n (%)	265 (78.4)	185 (76)	92 (82)	0.40
Arthritis, n (%)	99 (29.3)	75 (31)	31 (27)	0.44
Genital ulcers, n (%)	18 (5.3)	0 (0.0)	18 (16.8)	**<0.0001**
Pleuritis/pericarditis, n (%)	41 (12.1)	34 (14.7)	7 (6.5)	**0.032**
Diarrhoea, n (%)	95 (28.1)	52 (22.5)	43 (40.2)	**0.0001**
Erythema nodosum, n (%)	19 (5.6)	11 (4.8)	8 (7.5)	0.32
Uveitis, n (%)	24 (7.1)	12 (5.2)	12 (11.2)	0.066
Meningitis, n (%)	21 (6.2)	10 (4.3)	11 (10.3)	0.05
Vasculitis, n (%)	30 (8.9)	18 (7.8)	12 (11.2)	0.31
Cytopenia, n (%)	19 (5.6)	16 (6.9)	3 (2.8)	0.20
Immune deficiency, n (%)	36 (10.7)	21 (9.1)	15 (14.0)	0.19
Genetic diagnoses, n (%):	41 (12.1)	32 (13.9)	9 (8.4)	0.21
Blau syndrome	*NOD2*: p.(Lys225Met);(Lys225=)			
Blau syndrome	***NOD2*: p.(Arg334Gln);(Arg334=)**			
Blau syndrome	*NOD2*: p.(Cys483Arg);(Cys483=)			
DADA2	***ADA2*: p.(Cys159Tyr);(Tyr453Cys)**			
DADA2	***ADA2*: p.(Gly47Arg);(Gly47Arg)**			
DADA2*	***ADA2*: p.(Ile143Serfs*41);(?)**			
DITRA	***IL36RN*: p.(His32Arg);(His32Arg)**			
FMF	*MEFV*: p.(Met694Ile);(Met694=)			
FMF	*MEFV*: p.(Met694Val);(Met694=)			
FMF	*MEFV*: p.(Met694Val);(Met694=)			
FMF	*MEFV*: p.(Met694Val);(Met694=)			
FMF	*MEFV*: p.(Met694Val);(Met694=)			
H syndrome	***SLC29A3*: p.(?);(?)**			
H syndrome	*SLC29A3*: p.Gly427Ser(;)Gly427Ser			
HA20	***TNFAIP3*: p.(Pro274Hisfs*13);(Pro274=)**			
JIA	***LACC1*: p.(Asp125Metfs*12);(Asp125Metfs*12)**			
Majeed syndrome	*LPIN2*: p.Gln44Hisfs*13(;) Gln44Hisfs*13			
MKD	***MVK*: p.(Ile268Thr);(Val377Ile)**			
MKD	***MVK*: p.(Lys13Gln);(Val377Ile)**			
MKD*	***MVK*: p.(Thr237Ser);(Val377Ile)**			
MKD*	***MVK*: p.(Thr237Ser);(Val377Ile)**			
MKD	***MVK*: p.(Ile268Thr);(Val377Ile)**			
MKD*	***MVK*: p.(Thr342Ile);(Val377Ile)**			
NLRC4-AID	***NLRC4*: p.(Thr177Ser);(Thr177=)**			
NLRC4-AID	***NLRC4*: p.(Thr337Asn);(Thr337=)**			
NLRP12-AID	*NLRP12*: p.(?);(=)			
NLRP3-AID*	***NLRP3*: p.(Tyr570=/Asn)**			

Continued

Table 1 Continued

Variable	Total (n=338)	No RAS (n=231)	RAS (n=107)	P value
NLRP3-AID	***NLRP3*: p.(Arg260Pro);(Arg260=)**			
NLRP3-AID	*NLRP3*: p.(Ser726Gly);(Ser726=)			
NLRP3-AID	*NLRP3*: p.(Glu688Lys);(Glu688=)			
PAAND*	***MEFV*: p.(Ser242Arg);(Ser242=)**			
PAAND*	***MEFV*: p.(Ser242Arg);(Ser242=)**			
PAPA	***PSTPIP1*: p.(Glu250Gln);(Glu250=)**			
PRAAS	***PSMB8*: p.(Thr75Met);(Thr75Met)**			
SAVI	***TMEM173*: p.(Val155Met);(Val155=)**			
SAVI	***TMEM173*: p.(Val155Met);(Val155=)**			
SIFD*	***TRNT1*: p.(His391Arg);(His391Arg)**			
TRAPS*	***TNFRSF1A*: p.(Cys72Tyr);(Cys72=)**			
TRAPS	*TNFRSF1A*: p.(Gly204Cys);(Gly204=)			
TRAPS	***TNFRSF1A*: p.(Cys72Tyr);(Cys72=)**			
TRAPS*	***TNFRSF1A*: p.(Thr79Met);(Thr79=)**			

P values are from $\chi 2$ test or Student's t test.
In bold: patients with a confirmed sAID (typical clinical presentation associated with a confirmatory genetic test).
*Patients with recurrent aphthous stomatitis (RAS).
AID, associated autoinflammatory disease; DADA2, deficiency of adenosine deaminase 2 ; DITRA, deficiency of interleukin thirty-six receptor antagonist; FMF, familial Mediterranean fever; HA20, A20 haploinsufficiency; JIA, juvenile idiopathic arthritis; MKD, mevalonate kinase deficiency; PAAND, pyrin-associated autoinflammation with neutrophilic dermatosis; PAPA, pyogenic arthritis-pyoderma gangrenosum-acne syndrome; PRAAS, proteasome-associated autoinflammatory syndrome; SAVI, STING-associated vasculopathy with onset in infancy; SIFD, syndrome of congenital sideroblastic anaemia, B-cell immunodeficiency, periodic fevers, and developmental delay; TRAPS, tumour necrosis factor receptor-associated periodic syndrome .

(n=1), syndrome of congenital sideroblastic anaemia, B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD) (n=1). None of the patients had a diagnosis of HA20. Details about single patients are reported in table 1.

When comparing the characteristics of patients with suspected sAID with and without RAS, the latter was associated with more severe illness with genital ulcers, diarrhoea and uveitis being more frequent in these patients. There were no statistically significant differences between the two groups with respect to age, gender, heredity, consanguinity or other clinical symptoms. Furthermore, the presence of RAS was not associated with better diagnostic performances of NGS panel with 9/107 (8.4%) and 32/231 (13.9%) probable/confirmed diagnosis in patients with and without RAS, respectively (p=0.21).

Failure to diagnose and delay in diagnosis of sAIDs can result in life-threatening consequences. Having an accurate genetic diagnosis is essential to initiate early treatment. Currently, NGS coupled to clinical information has become the preferred method for diagnosing sAIDs. However, the diagnostic utility of targeted genetic panels is usually low (4%–22% diagnostic yield).[6 7] Since RAS is a frequent manifestation of sAIDs, we hypothesised that its presence could increase the genetic diagnosis yield of NGS. When focusing on patients with RAS, the diagnostic yield did not increase compared with patients without RAS, thus suggesting that this is not a good clinical feature to focus on. Therefore, identifying better clinical factors should be a priority to guide DNA screening.

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