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Phenotype and cytokine profile description in a TRAPS Syndrome family with *TNFRSF1A* p.(Thr79Met): association with sacro-iliitis.

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ABSTRACT

Objective: A comparative retrospective and analytic study was performed in 13 members of a family, affected or not by tumor necrosis factor TNF- α receptor-associated periodic syndrome (TRAPS), including one patient with sacro-iliitis.

Methods: Clinical features and *TNFRSF1A* gene analysis were reported for each family member, symptomatic or asymptomatic. Biological features including CRP/SAA, IgD and ex vivo T lymphocytes and myeloid-derived cytokine profile (IL1- β , IL-1 α , IL-1ra, IL-4, IL-10, IL-17, IFN- γ , TNF- α , IL-6) were characterized for all family members. CRP and IgD only were compared with those of 13 axial SA patients with TNF-blockade indication.

Results: Symptomatic TRAPS patients presented p.(Thr79Met) variant and generally complained of abdominal pain. They displayed higher SAA/IgD levels and IL-6/IL-1RA/IL-10 production by PBMC compared to asymptomatic family members. IgD serum level was higher in symptomatic members compared to SA patients. The patient with sacro-iliitis displayed the highest cytokine production and IgD serum levels.

Conclusion: This study describes clinical heterogeneity in a family examined for TRAPS syndrome and reports the first sacro-iliitis in a patient with pathogenic *TNFRSF1A* variant. Dysregulated PBMC-derived cytokine and IL-10/IgD dysregulation in the patient with sacro-iliitis raises the issue of sacro-iliitis pathophysiology.

KEYWORDS

TRAPS; Cytokine profile; Sacro-iliitis; Spondyloarthritis.

INTRODUCTION

Tumor necrosis factor (TNF)- α receptor-associated periodic syndrome (TRAPS) is an autosomal dominant auto-inflammatory disease due to variants in the *TNFRSF1A* gene (1).

As illustrated in the EUROTRAPS cohort, clinical features of TRAPS are fever lasting from 1 to 3 weeks combined with abdominal pain, periorbital edema, conjunctivitis, migratory erythematous plaque simulating erysipelas with underlying myalgia, and arthralgia (1).

Thirteen family members, outside the Eurofever cohort, from the Poitou-Charentes region in France were seen in an outpatient clinic for TRAPS syndrome. One of them was simultaneously diagnosed with axial spondyloarthritis (SA). Inflammatory back pain (IBP) has been reported only marginally in TRAPS syndrome (2–4). We formulated the hypothesis that clinical heterogeneity was driven by cytokine profile heterogeneity. The aim of this work was to collect and compare data from 13 members of the same family, including clinical and biologic features, genotype and cytokine profile. A biological comparison was then conducted between symptomatic TRAPS patients in a group of SA patients.

METHODS

The study was a comparative retrospective and analytic study at Poitiers University Hospital.

1. PATIENTS

1.1. TRAPS Family members

Thirteen members of the family including 7 symptomatic members (presenting TRAPS related/pathogenic variant and the clinical features described in TRAPS) and 6 asymptomatic members were enrolled in the study (5).

Clinical characteristics were collected in an outpatient clinic using a questionnaire mentioning clinical items suggestive of TRAPS.

1.2. SA patients

The sera of patients, seen in outpatient clinic, for SA matching ASAS criteria [with typical axial disease and inflammatory syndrome (at diagnosis and/or during follow-up)] (6), prior to biotherapy initiation were retrospectively retrieved. The sex ratio W:M was 5:1 and mean age was 47.1 years. NSAIDs had failed in all patients and serum was frozen at anti-TNF pre-therapeutic evaluation.

1.3. Ethics

This study complied with the tenets of the Declaration of Helsinki and the protocol was approved by the Poitiers CHU Research Department. Patients were enrolled after comprehensive information checking that they (or their legal guardians) were not opposed to the study and the storage of their personal data. Genetical analyses were performed after written consent was obtained for each family member or legal authority for children under 18 years of age .

2. Biological study: Genotype, Cytokine profile, Biological phenotype.

2.1. GENOTYPE :

TNFRSF1A analysis was performed by Sanger sequencing.

2.2. Cytokine profile in TRAPS family members:

Human peripheral blood mononuclear cells (PBMCs) were isolated from peripheral heparinized blood by density gradient centrifugation using Ficoll-Paque (GE Healthcare) and counted for each family member. Collection occurred during remission time in symptomatic members with diagnosed TRAPS syndrome. Isolated PBMCs were cultured in RPMI 1640 containing 2mM glutamine, 100 U/mL penicillin-streptomycin (Invitrogen Life Technologies), and 10% heat-inactivated fetal calf serum (Gibco Life Technologies). Cells were seeded at 1×10^6 cells/mL in 24-well plates and incubated 24 h at 37°C in a humidified atmosphere

containing 5% CO₂. After incubation, plates were centrifuged, and supernatants were stored at -80°C until analysis.

The levels of IL-1 family cytokines (IL1- β , IL-1 α , IL-1ra), pro-inflammatory cytokines (IL-4, IL-17, IFN- γ , TNF- α and IL-6) and anti-inflammatory cytokine (IL-10) were detected in serum and supernatants using the MILLIPLEX MAP Human Cytokine/Chemokine magnetic bead panel kit (Millipore) coupled to Luminex 200™ xPONENT™ software. All samples were assayed in duplicate. Concentrations below the detection limit were considered undetectable.

2.3. Inflammatory markers in TRAPS Syndrome family members and SA patients:

Blood samples were drawn in dry Vacutainer® tubes to determine blood count, C Reactive Protein (CRP), Serum Amyloid A protein (SAA) and IgD levels. IgD level was raised over 100mg/l. Sera were obtained by centrifugation and stored at -80°C until analysis. Ten mL of blood were collected in Vacutainer® tubes containing heparin for the isolation of PBMC and genetic analysis.

CRP and SAA were determined routinely by laser immunonepehelemetry (Siemens). IgD level was determined in sera by Enzyme Linked Immunosorbent Assay (ELISA) as previously described (7).

For SA patients, CRP was retrospectively retrieved, while IgD measurement was performed from frozen sera.

3. STATISTICAL ANALYSIS

All the statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc). Descriptive statistics were presented as mean \pm standard error (SEM). The unpaired

nonparametric Mann-Whitney U test was used to evaluate the differences between TRAPS patients, control groups and asymptomatic patients. Spearman correlation was used to study correlation. p values inferior to 0.05 were considered statistically significant.

RESULTS

1. TRAPS FAMILIAL STUDY

Genetical and clinical description

Thirteen family members of Caucasian ancestry were included in the study. Among them, 7 were symptomatic (patients 1-7) and with diagnosed TRAPS syndrome. The patients were aged from 5 to 69 years and displayed the same pathogenic p.(Thr79Met) variant (formerly T50M)(8). Six patients displayed typical clinical phenotypes (table). Of note, patients 1 and 2 experienced flares only in their 3rd decades of life.

The other six family members (patients 8-13), aged from 6 months to 39 years, were asymptomatic. Only one patient was adult. The p.(Thr79Met) variant of *TNFRSF1A* was found in 2 asymptomatic patients with ages 6 months and 8 years. No clinical complaint or abnormal biological feature was found (table).

Focus on Patient 4

Patient 4 displayed an atypical clinical phenotype that started at 33 years of age, characterized by musculo-articular phenotype without fever, abdominal pain and skin manifestation. Clinical examination showed pain on right sacroiliac and gluteal medius enthesitis. Her symptomatology resolved under Non-Steroid Anti-inflammatory drug (NSAID). HLA B27 antigen screening was negative. Magnetic resonance imaging showed

sacro-iliitis, in agreement with the SpondyloArthritis International Society (ASAS) criteria for SA.

Ex vivo cytokine production

Mean IL-6 levels in PBMC supernatants were significantly enhanced in symptomatic members compared to asymptomatic members (176 pg/mL vs 4 pg/mL; p inferior to 0.01) (figure 1C). Mean IL-1ra and IL-10 levels were increased in symptomatic members compared to asymptomatic members (figure 1E and 1F). In contrast, IL-1 α , IL-1 β and TNF- α levels in PBMC supernatants were not statistically different between symptomatic members and asymptomatic members, despite a discrete IL-1 β increase in TRAPS patients compared to asymptomatic members (figure 1A, 1B and 1D). Th1, Th2, Th17 specific cytokines, IFN- γ , IL-4 and IL-17 PBMC excretion did not differ between TRAPS patients and asymptomatic family members (data not shown).

Patient 4 with sacro-iliitis displayed the highest cytokine PBMC production: 132 pg/mL for IL-1 β , 1210 pg/mL for IL-6, 127 pg/mL for IL-10 and 481 pg/mL for TNF- α respectively.

2. COMPARISON OF TRAPS FAMILY MEMBERS AND SA PATIENTS

CRP, SAA and IgD were assessed in symptomatic members during remission period, while CRP and IgD were assessed in actively diseased SA patients (table). CRP was normal in asymptomatic members. CRP was higher in symptomatic TRAPS patients, with a mean level of 19 mg/l.

IgD was significantly higher in symptomatic TRAPS patients with a mean level of 143mg/L compared to asymptomatic members, with a mean level 24mg/L (<0.05) (table).

Patient 4 with sacro-iliitis: Biology revealed higher CRP and the highest IgD level (table).

While CRP was higher in SA patients, with a mean level of 11.7mg/l, IgD was normal.

Subsequently, correlation analysis was performed. Whatever the group within the TRAPS family, there was no correlation between IL-6 level in PBMC supernatant and SAA or CRP.

Despite the absence of correlation, all patients with increased CRP displayed higher IL-6 in PBMC supernatant (figure 2B). No correlation was found between IgD level and CRP levels (data not shown).

In a pooled analysis including symptomatic and asymptomatic members, there was a correlation between IL-10 PBMC excretion in supernatants and IgD serum level, with a 0.62 ($p < 0.05$) correlation index (figure 2A).

DISCUSSION

This study shows clinical phenotype heterogeneity among a family seen for TRAPS syndrome with the p.(Thr79Met) variant (formerly T50M). It is also the first description of sacro-iliitis in a patient with a TRAPS syndrome carrying a pathogenic mutation.

Patient 4 aside, the clinical features observed in our study did not differ from literature data (1). Interestingly, abdominal pain was the most frequent feature, not fever. Even if fever remains a key symptom in TRAPS syndrome, phenotypes without fever have been reported

(9). Of note, two of our patients did not display any symptoms at the time of the study, but subsequently developed fever(9,10).

One of the original findings of this study was increased excretion of the anti-inflammatory cytokine IL-10 by TRAPS patients PBMCs, associated with IL-6 and IL-1RA overproduction without IL-1Beta enhancement. IL-10 excretion in symptomatic patient PBMCs had never previously been reported as increased in TRAPS patients. Increased IL-10 excretion was observed in all patients in remission. The pathophysiology behind IL-10 excretion remains unclear. IL-10 is an anti-inflammatory cytokine produced mainly by monocytes and, to a lesser extent, by T cells, more particularly Tregs. IL-10 production by monocytes is delayed when compared to the pro-inflammatory cytokines (11). IL-6 excretion from PBMCs in TRAPS patients had previously been reported and appears in most patients regardless of their age and might play an important role in the pro-inflammatory state of TRAPS patients(12). Although IL-1RA has not been measured in TRAPS patients, IL-1 blockade by anakinra and increased IL-1-beta excretion could predict this finding(13).

In our study, mean IgD levels were significantly higher in the symptomatic group of patients when compared to asymptomatic patients and SA patients. While increased IgD serum level is characteristic of MKD (mevalonate kinase deficiency) disease, it has also been reported in TRAPS and Familial Mediterranean Fever (FMF)(14). The precise role of IgD and its pathophysiological role in auto-inflammatory conditions is still unknown (14). In our study, IgD raise was correlated to IL-10 PBMC excretion in supernatants. This finding had previously been described in PBMC of healthy people (7). IL-10 and IgD were significantly higher in symptomatic patients, even in patients who no longer experienced flares. This

finding raises the issue of clinical remission with continuous biological activity. Lachman et al reported clinical remissions among TRAPS patients in the EUROFEVER cohort, especially in those with variant of uncertain significance (VUS). The definition of remission did not include biological information(1,15). The p.(Thr79Met) gene variant is a pathogenic mutation, associated with longer flares and higher amyloidosis risk, and to our knowledge it is the first description of clinical remission with p.(Thr79Met) *TNFRSF1A* variant (9,13). As these 2 patients maintained increased IL-10 excretion and IgD sera levels, we can hypothesize that these features reflect the continuous inflammatory process and that the cessation of flares is only the tip of the iceberg.

Patient 4 raises question regarding sacro-iliitis. Studies focusing on sacro-iliitis in SAID have been performed, but mostly in FMF and scarcely in TRAPS. While three sacro-iliitis cases have been reported in TRAPS patients, all carried VUS for *TNFRSF1A*. The first one was reported in a 9-year-old African-American boy, carrying the p.(Phe46Leu) variant, a VUS *TNFRSF1A* variant without HLA B27 antigen carriage(2). Sacro-iliitis was resolute after methylprednisolone flash. The second reported case involved a 27-year-old Caucasian woman, carrying the p.(Arg121Gln) (formerly R92Q) variant considered as a VUS *TNFRSF1A* variant, without HLA B27 antigen carriage or *MEFV* gene variant. Sacro-iliitis was resolute after TNF Alpha blockade (3). The third reported a 28-year-old Caucasian male, once again carrying the p.(Arg121Gln) variant, a VUS *TNFRSF1A* variant with HLA B27 antigen carriage (4). This study is the first description of sacro-iliitis in TRAPS with a pathogenic mutation, namely p.(Thr79Met). IBP did not relapse in this patient. Moreover, as the patient exhibited the highest IL-10/IgD ratio, the question as to whether sacro-iliitis could be attributed to

p.(Thr79Met) pathogenic variant for *TNFRSF1A* rather than co-existing spondyloarthritis remains unresolved.

As a conclusion, this description illustrated the heterogeneous clinical phenotype of TRAPS in a single family, including sacro-iliitis. We also found increased PBMC production for IL-10 and IgD serum level in TRAPS patients compared to SA patients.

Competing interests: NA

Funding: NA

Authors' contributions: GD and ESG interviewed, examined, performed immunological tests and analyzed patients or data. AD performed immunological tests. LC performed genetic tests and provided genetic expertise. JCL was a major contributor in data immunological analysis and document writing. All authors read and approved the final manuscript.

Ethics approval and consent to participate: Analyses were carried out after written consent was obtained for each patient or legal authority. Those analyses were considered as part of a familial study and were approved by Poitiers CHU Research Direction Department.

Consent for publication: Analyses and data collection were carried out after written consent was obtained for each patient or legal authority in outpatient clinic. Each family member gave his consent for publication.

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Table 1 : Clinical features, biological features and genotype of the family members

SYMPTOMATIC PATIENTS										
Patient			Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	All
			F	M	M	F	F	M	M	
Age (years old)			57	69	42	33	30	29	5	
Age at onset (decade)			2nd	2nd	1st	1st	2nd	2nd	1st	
Clinical and biological phenotype at onset	<i>Clinical</i>	Fever	Yes	No	Yes	No	Yes	No	Yes	57%
		Abdominal Pain	Yes	Yes	Yes	Yes	Yes	No	Yes	85%
		Myalgia	Yes	No	Yes	No	Yes	Yes	Yes	71%
		Skin manifestations	Yes	No	No	No	No	Yes	Yes	42%
		Arthralgia	Yes	No	Yes	Yes	Yes	No	Yes	57%
	<i>Biological</i>	CRP (mg/l)	21	<1	<1	42	67	<1	<1	
SAA (mg/l)		24	22	<4.0	<4.0	<4.0	<4.0	<4.0		
IgD (mg/l)		141	73	194	371	35	35	160		
Genotype			T50M	T50M	T50M	T50M	T50M	T50M	T50M	
Evolution	Continuous		No	No	Yes	No	No	No	No	
	Flares		Yes	Yes	Yes	Yes	Yes	Yes	Yes	
	Trigger		Pregnancy	No	No	Asthenia/ Stress/ Infections	Asthenia/ Stress/ Infections	Asthenia/ Stress/ Infections	None	

Spontaneous Clinical Remission	Yes/No		Yes	Yes	No	No	No	No	No	
	Age (decade)		3rd	3rd-4th	--	--	--	--	--	
Follow-up beginning	Age (decade)		2 nd	2 nd	2nd-3rd	2nd	2nd	2nd	1st	
Corticosteroids		No	No	Yes	No	Yes	Yes	Yes	Yes	
Biologics		No	No	IL-1 blockade	No	No	TNF- α blockade	No	No	
ASYMPTOMATIC PATIENTS										
Patient			Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	All	
Sex			F	F	M	M	M	F	12.41	
Age (years old)			39	14	8	8	5	0.5		
Age at onset (decade)										
	Clinical	Fever	No	No	No	No	No	No	0%	
		Abdominal Pain	No	No	No	No	No	No	0%	
		Myalgia	No	No	No	No	No	No	0%	
		Skin manifestations	No	No	No	No	No	No	0%	
		Arthralgia	No	No	No	No	No	No	0%	
	Biological	CRP (mg/l)	<1	<1	<1	1.2	4.4	<1		
		SAA (mg/l)	<4.0	9	24	<4.0	<4.0	<4.0		
		IgD (mg/l)	4	6	42	41	51	4.5		

Genotype			No	No	No	T50M	No	T50M	
Treatment			No	No	No	No	No	No	

Figure 1: Cytokine production (pg/mL) after 24 hours PBMC culture, without stimulation in symptomatic members and asymptomatic family members Mann Whitney Test *: $p < 0.05$ **: $p < 0.01$

1A IL-1 α level

1B IL-1 β level

1C IL-6 level

1D TNF- α level

1E IL-RA level

1F IL-10 level

Figure 2: Correlation studies. Spearman Correlation: $p < 0.01$

2A IL-10 PBMC production (pg/mL) and IgD serum levels (mg/L)
levels(mg/L) and IL-10 PBMC production (pg/mL)

2B SAA serum



