Diagnosis of autoimmune lymphoproliferative syndrome caused by FAS deficiency in adults

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ABSTRACT

A diagnosis of autoimmune lymphoproliferative syndrome caused by FAS deficiency during adulthood is unusual. We analyzed 17 cases of autoimmune lymphoproliferative syndrome caused by FAS deficiency diagnosed during adulthood in French reference centers for hereditary immunodeficiencies and for immune cytopenias. Twelve of the 17 patients had developed their first symptoms during childhood. The diagnosis of autoimmune lymphoproliferative syndrome had been delayed for a variety of reasons, including unusual clinical manifestations, late referral to a reference center, and the occurrence of somatic *FAS* mutations. The 5 other patients presented their first symptoms after the age of 16 years. In these patients, three germline heterozygous *FAS* mutations were predicted to be associated with haploinsufficiency and a somatic event on the second *FAS* allele was observed in 2 cases. Autoimmune lymphoproliferative syndrome may well be diagnosed in adulthood. The occurrence of additional genetic events may account for the delayed disease onset.

Introduction

Autoimmune lymphoproliferative syndrome caused by FAS/TNFRSF6 deficiency (ALPS-FAS) is characterized by: i) early-onset, benign splenomegaly and lymphadenopathy; ii) the accumulation of a mature, polyclonal population of TCR$\alpha\beta^+$ $CD4^-CD8^-$ T cells (referred to as double-negative (DN) T cells); iii) multilineage cytopenia caused by peripheral autoimmune destruction or splenic sequestration; and iv) an increased risk of B-cell lymphoma.[1-4]

Most cases of ALPS-FAS result from heterozygous dominant germline *FAS* mutations. However, homozygous germline mutations have occasionally been reported.[2] Somatic heterozygous *FAS* mutations are also common and account for 10-15% of ALPS-FAS cases. Lastly, when germline *FAS* heterozygous mutations lead to haploinsufficiency,[5] combinations of germline and somatic modifications of the *FAS* gene have been observed.[6] A FAS-mediated apoptosis defect may be assessed *in vitro* in patients carrying germline mutations but not in those affected by somatic mutations. Plasma biomarkers are now routinely assayed in the pre-diagnosis of ALPS-FAS and include soluble FAS ligand (sFASL), interleukin-10 (IL-10), IL-18 and vitamin B12.[7,8]

The onset of ALPS-FAS is typically observed in the first few years of life (on average at 2.5 years of age).[1,3] Late ALPS-FAS onset (i.e. during adulthood) has only been described in a few cases.[9,10]

Here, we present data on the clinical, biological and molecular aspects of 17 ALPS-FAS patients diagnosed during adulthood. The data were collated by the French national reference centers for hereditary immunodeficiencies and immune cytopenias.

Design and Methods

We checked patient records at the French National Reference Center for Hereditary Immunodeficiencies (CEREDIH) and the French National Reference Center for Immune Cytopenias for cases of ALPS diagnosed after the age of 16 years. Only confirmed ALPS-FAS patients were selected, i.e. patients with a molecular diagnosis of germinal and/or somatic *FAS* mutations.[1] All participants or their parents/guardians gave their signed, informed consent to the study in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee Comité de Protection des Personnes Ile de

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France (Number DC2011-1338). The relative DN T-cell counts were obtained from the local immunology laboratories and confirmed in the Centre d'Etude des Déficits Immunitaires (CEDI) reference laboratory. The CEDI laboratory also identified FAS mutations, analyzed *in vitro* T-cell FAS-mediated apoptosis, and assayed plasma levels of sFASL and IL-10, as previously described.[6,7]

Results

We identified 17 cases of ALPS-FAS in which a molecular diagnosis (i.e. identification of a *FAS* mutation) had been made after the age of 16 years (Table 1). We then divided this small cohort of adult ALPS-FAS patients into two groups according to the time at which the first manifestations of the condition occurred.

In a first group of 12 patients (group 1), clinical symptoms of ALPS had been present during childhood but the molecular diagnosis had only been made in adulthood (at a mean age of 33 years). In childhood, all but one of the patients had presented with splenomegaly, enlarged lymph nodes and cytopenia. However, the diagnosis had been delayed by a variety of factors. In 6 cases, the molecular diagnosis was made following the identification of *FAS* mutations in an ALPS-FAS proband with pediatric onset (P1.1; 1.4; 1.6-1.9). In 3 cases (P1.2; 1.3 and 1.5), diagnosis of ALPS-FAS was delayed by interference from a previous diagnosis of Evans syndrome (P1.2 and 1.3) or by an unusual clinical presentation such as hyperviscous syndrome and nephrotic syndrome (P1.5). The diagnosis of ALPS-FAS was made after consultation in a tertiary hospital and analysis of ALPS-FAS-related biomarkers (such as the DN T-cell count and plasma sFASL and IL-10 levels).

For 3 patients (P1.10-1.12) a molecular diagnosis was made following DN T-cell sorting and the identification of somatic FAS mutations.

Interestingly, lymph node and liver biopsies in 3 of these 12 patients had revealed follicular hyperplasia and had prompted a diagnosis of 'atypical' Castleman's disease. When a splenectomy was performed, the histology results were not conclusive and showed only lymphoid hyperplasia.

A variety of *FAS* mutations were identified (Table 2). In one patient (P1.3), the mutation affected the extracellular domain of FAS and was probably associated with haploinsufficiency. In 11 patients, the *FAS* mutations affected the intracellular domain. One mutation was predicted to lead to a truncated death domain, whereas 10 were missense mutations.

The second group (group 2) was made up of 5 patients

Table 1. Patients' characteristics and clinical features.

	Pt	Gender	Age at first symptoms (years)	Age at ALPS diagnosis (years)	Condition leading to ALPS diagnosis	ALPS symptoms in childhood	ALPS symptoms in adulthood	Age (years)/ status at last follow up
ALPS-FAS with symptoms in childhood	1.1	M	0.3	27	ALPS diagnosis in a family member	SM ++, LN ++, H, A, skin vasculitis, growth failure	Intermittent LN +	34/asymptomatic
	1.2	M	2	29	NHL in a patient with Evans syndrome and CLP	SM++, LN ++, H, CITP, Evans, skin vasculitis	B-NHL (29y) intermittent LN+	39/died (stroke)
	1.3	M	12	29	Evans syndrome and CLP	SM +++, LN ++, A, CITP, Evans	CITP relapse	29 / CITP
	1.4	M	13	37	ALPS diagnosis in a family member	SM+++, LN +	Asymptomatic	50/asymptomatic
	1.5	F	14	26	CLP	SM ++, LN ++, A, hyperviscous sd, nephrotic sd	Intermittent SM+ and LN+	31/asymptomatic
	1.6	M	8	36	ALPS diagnosis in a family member	SM, LN, A, CITP	CITP relapse AIHA	39/mild thrombopenia AIHA, steroids + splenectomy
	1.7	M	childhood	59	ALPS diagnosis in a family member	SM, LN, AIHA, CITP	CITP relapse	62/asymptomatic
	1.8	M	NA	55	ALPS diagnosis in a family member	NA	B-NHL (51y)	57/asymptomatic
	1.9	M	childhood	30	ALPS diagnosis in a family member	SM, LN, A	None	33/asymptomatic
	1.10	M	2	25	CLP	SM+++, LN++, AIHA	SM+, persistent mild AIHA	28/mild A, low-dose steroids
	1.11	M	0.8	19	CLP	SM+++, LN++, A, hepatitis, skin vasculitis	Asymptomatic	20/asymptomatic
	1.12	F	1.5	27	HL with previous history of CLP and AIHA	SM+++, AIHA	HL (23 y)	27/asymptomatic
ALPS-FAS with first symptom in adulthood	2.1	M	18	19	Exploration for Evans and LPS	none	LN, S +, purpura, Evans	30/asymptomatic
	2.2	F	20	26	Son diagnosed with ALPS	none	S ++	32/asymptomatic
	2.3	M	24	27	Cold agglutinin disease	none	LN, S +, AIHA	32/persistent mild AIHA, MMF
	2.4	M	29	31	Son diagnosed with ALPS	none	LN, S+, AIHA	32/asymptomatic
	2.5	M	25	43	Son diagnosed with ALPS	none	S+, AIHA	47/asymptomatic

M: male; F: female; CLP: chronic lymphoproliferative syndrome; NHL: non-Hodgkin's lymphoma; HL: Hodgkin's lymphoma; SM: splenomegaly (+++ splenomegaly below umbilicus; ++ below half distance between the costal margin and umbilicus; + above half distance between costal margin and umbilicus) LN: lymph nodes (++ lymph node diameter between 3 and 5 centimeters; + between 1 and 2 centimeters); H: hepatomegaly; A: anemia; AIHA: autoimmune hemolytic anemia; CITP: chronic immune thrombocytopenia; NA: not available; sd: syndrome; MMF: mycophenolate mofetil.

Table 2. Biomarkers at diagnosis and FAS mutations.

Patient		FAS-mutation	γGlobulins (g/L)	% DNTC	FasL (ng/mL)	IL-10 (pg/mL)
1.1	-FAS	P217fsX220	18	5	0.39	76
1.2	-FAS	K193fsX211	34.6	1	ND	ND
1.3	-FAS	F134fsX186	ND	6	0.8	61
1.4	-FAS	G253D	ND	7	ND	ND
1.5	-FAS	R250X	32.1	6	3.1	31
1.6	-FAS	I262T	ND	ND	ND	ND
1.7	-FAS	I262T	15.4	ND	ND	ND
1.8	-FAS	I262T	9.57	7	ND	ND
1.9	-FAS	I262T	20.8	11.1	0.24	33
1.10	-sFAS	Q273K	19.3	5	1.27	97
1.11	-sFAS	D260V	40.2	ND	0.8	215
1.12	-sFAS	D225Y	24	11	0.91	27
2.1	-FAS	R250Q	High	14	ND	ND
2.2	-FAS	G66D + PUD	ND	6	2	79
2.3	-FAS	A290E	11	8	0.3	5
2.4	-FAS	D108G	9.5	4.8	0.35	19
2.5	-FAS	C157X + PUD	15.7	11	7.3	244

FAS: ALPS with a germline TNFRSF6 mutation; sFAS: ALPS with a somatic TNFRSF6 mutation; PUD: parental unidisomy; DNTC: double-negative T cells (normal proportion < 2%); FasL: Fas Ligand (normal level < 0.2 ng/mL); IL10: interleukin 10 (normal level < 20 pg/mL); ND: not determined.

who had only displayed their first clinical symptoms of ALPS after the age of 16 years. Four of the 5 patients presented with autoimmune cytopenias (mainly hemolytic anemia). Lymphoproliferation was reported in all 5 patients but was milder than in typical pediatric ALPS-FAS patients. Gamma globulin level was normal in 2 patients, contrasting with frequent hypergammaglobulinemia in infants. In 3 cases (P2.2; 2.4; 2.5), a diagnosis of ALPS was only confirmed after diagnosis of the same condition in a related child. In 2 cases (P2.1 and P2.3), a diagnosis of ALPS-FAS was made after screening for autoimmune manifestations (Evans syndrome with lymphoproliferation and cold agglutinin disease).

In all tested patients, levels of ALPS-specific markers (DN T cells, sFASL and IL-10) were moderately (P2.3 and 2.4) or greatly (P2.2 and P2.5) elevated. The administration of immunosuppressant agents attenuates the elevation of these markers.

Interestingly, three mutations (P2.3; 2.4 and 2.5) were predicted to be associated with haploinsufficiency. This type of mutation is often associated with a secondary somatic event affecting the second *FAS* allele.[6] In 2 patients (P2.2 and 2.5), molecular screening of DNA extracted from sorted DN T cells revealed the loss of the wild-type allele and duplication of the mutated allele.

Discussion

We analyzed 17 cases of ALPS-FAS diagnosed during adulthood in French reference centers for hereditary immunodeficiencies and for immune cytopenias.

Twelve of the 17 patients had developed their first symptoms during childhood. Consideration of this group of patients shows that early-onset ALPS-FAS patients can be misdiagnosed in childhood. The 5 other patients had their first symptoms after the age of 16 years, although we can not exclude the possibility that minor symptoms in childhood went unreported. Unusual clinical manifestations, and the occurrence of somatic *FAS* mutations which were first described only a few years ago, have led to delayed diagnosis in both groups. In 9 patients, the molecular diagnosis was made following the identification of *FAS* mutations in a related child with ALPS-FAS. Six of these 9 patients had been symptomatic in childhood more than twenty to thirty years previously, a time at which knowledge of ALPS was not widespread. Although this diagnosis is now an expected consequence of thorough family screening, these cases showed that ALPS symptoms can be less prominent in adulthood than in childhood (Table 1). In such cases, an indolent autoimmune lymphoproliferative syndrome remains in adulthood and constitutes a tricky diagnosis for non-pediatricians who are unfamiliar with ALPS-FAS. Indeed, there are several differential diagnoses for ALPS in adults such as multicentric Castelman's disease, angioimmunoblastic T-cell lymphoma, and Rosai-Dorfman disease. Chronic lymphoproliferative syndrome is usually present in these diseases and auto-immune cytopenia is not rare. Physicians should consider ALPS in these situations and also in Evans's syndrome, which has now been recognized as a heterogeneous syndrome that includes patients with ALPS-FAS.[10]

The occurrence of lymphoma in 3 of the adult patients reported highlights the need to consider a diagnosis of ALPS-FAS in lymphoma patients with immune cytopenias or chronic benign lymphoproliferation during infancy. The relapse of immune cytopenias in adulthood should also prompt the physician to consider a diagnosis of ALPS.

Assay for plasma biomarkers (such as sFASL, IL-10 and vitamin B12) should facilitate the correct diagnosis of ALPS-FAS in adult patients or in those with unusual presentations.

Interestingly, in the 5 patients with ALPS-FAS late onset, three germline heterozygous *FAS* mutations were predicted to be associated with haploinsufficiency and a somatic event on the second *FAS* allele was observed in 2 cases. The occurrence of this type of somatic event could account for the delayed onset of ALPS symptoms in these 2 individuals. Indeed a delayed onset of ALPS symptoms was observed in pediatric patients with combined germline and somatic FAS mutations.[3] This may be related to a later occurrence of the somatic events during embryonic development or even after birth. Hence, a limited number of progenitor lymphocytes might carry the causal genetic element thereby delaying the accumulation of pathogenic lymphocytes and onset of first symptoms. The lack of somatic events within the second FAS allele in the other 3 patients might be explained by intronic FAS mutations or mutations in other genes involved in the FAS/FASL signaling pathways, such as the *caspase-10* gene.[11]

Taken as a whole, our data show that ALPS-FAS symptoms can occur in adulthood and not just in misdiagnosed pediatric ALPS-FAS patients. Some patients really do appear to present their first symptoms in adulthood. Biomarker assays are critical in guiding the physician towards a diagnosis of ALPS-FAS. In patients with elevated biomarker levels, molecular screening for somatic events contributed to the diagnosis of ALPS-FAS in 25% of the cases in group 1 and revealed a second genetic event in

2 of the 5 patients in group 2. This indicated that somatic events might account for delayed onset of clinical symptoms in ALPS patients and could pave the way for the characterization of somatic mutations in known genes involved in the FAS/FASL pathway or in newly identified genes involved in key checkpoints of self-tolerance.

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