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BRIEF COMMUNICATION

Somatic loss of heterozygosity, but not haploinsufficiency alone, leads to full-blown autoimmune lymphoproliferative syndrome in 1 of 12 family members with *FAS* start codon mutation☆

Fabian Hauck [a, b, c, *, 1, 2], Aude Magerus-Chatinet [a, b, 1], Stephanie Vicca [d], Anne Rensing-Ehl [e], Angela Roesen-Wolff [c], Joachim Roesler [c, 3], Frédéric Rieux-Laucat [a, b, 3]

[a] *INSERM U768, Laboratoire du Développement Normal et Pathologique du Système Immunitaire, Hôpital Necker-Enfants Malades, Paris, France*
[b] *Université Paris Descartes, Sorbonne Paris Cité, Paris, France*
[c] *Section of Immunology, University Children's Hospital Carl-Gustav Carus, Dresden, Germany*
[d] *Laboratoire de Biochimie Générale, Hôpital Necker-Enfants Malades, Paris, France*
[e] *Centre of Chronic Immunodeficiency, University Medical Centre, Freiburg, Germany*

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Abstract We describe a family with 12 members carrying a heterozygous germline *FAS* c.3G>T start codon mutation leading to FAS haploinsufficiency. One patient had autoimmune

Abbreviations: ALPS, autoimmune lymphoproliferative syndrome; DN T cell, double-negative T cell; ICD, intracellular domain; ECD, extracellular domain; LOH, loss of heterozygosity; MNR, mutation-negative relative; MPR, mutation-positive relative; sFASL, soluble FAS ligand; SP T cell, single-positive T cell

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* Corresponding author at: University Children's Hospital, Ludwig Maximilian University, Lindwurmstrasse 4, 80336 München, Germany. Fax: +49 8951607942.

E-mail addresses: fabian.hauck@med.uni-muenchen.de (F. Hauck), aude.magerus@inserm.fr (A. Magerus-Chatinet), stephanie.vicca@nck.aphp.fr (S. Vicca), anne.rensing-ehl@uniklinik-freiburg.de (A. Rensing-Ehl), angela.roesen-wolff@uniklinikum-dresden.de (A. Roesen-Wolff), joachim.roesler@uniklinikum-dresden.de (J. Roesler), frederic.rieux-laucat@inserm.fr (F. Rieux-Laucat).

[1] Fabian Hauck and Aude Magerus-Chatinet contributed equally to this work.

[2] Permanent address: Department of Pediatric Hematology, Oncology and Immunology, University Children's Hospital, Ludwig Maximilian University Munich, Germany.

[3] Joachim Roesler and Frédéric Rieux-Laucat contributed equally to this work.

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lymphoproliferative syndrome (ALPS), one had recovered from ALPS, and ten mutation-positive relatives (MPRs) were healthy. FAS-mediated apoptosis and surface expression of FAS in single-positive T cells were lower for MPRs but did not discriminate between them and the ALPS patient. However, double-negative (DN) T cells of the ALPS patient had no FAS expression due to somatic loss of heterozygosity. Our results in this kindred suggest that FAS haploinsufficiency does not cause ALPS-FAS, but that modifying genetic events are crucial for its pathogenesis. FAS surface expression on DN T cells should be assessed routinely and FAS haploinsufficient patients should be followed as its potential for lymphomagenesis is not well defined and a second hit might occur later on.

1. Introduction

FAS is a single-pass type I membrane protein belonging to the tumor necrosis factor receptor superfamily [1]. Homohexameric soluble FAS ligand (sFASL) or membrane-bound FASL binds to preassembled FAS homopenta- to homoheptamers [2,3] and induces extrinsic programmed cell death [4]. Apoptosis is crucial for the homeostasis of the immune system [5] as it confines an ongoing immune response after the elimination of antigen. It is also involved in thymic selection by removing futile and autoreactive cells and in tumor surveillance by extinguishing malignant lymphatic cells [6–8].

Fifteen years ago, defects in genes regulating apoptosis have been found to cause the autoimmune lymphoproliferative syndrome (ALPS, OMIM #601859) [9–12] and ALPS-like disorders [13–18]. ALPS, initially termed Canale–Smith syndrome [19], is characterized by childhood onset chronic and benign lymphadenopathy, splenomegaly with hypersplenism, and autoimmune phenomena with the latter two resulting in multilineage cytopenia [20–23]. Less frequently, ALPS is associated with malignant lymphoma [24,25]. Numbers of $CD3^+TCR\alpha\beta^+CD4^-CD8^-$ double-negative (DN) T cells, Igs, and ALPS biomarkers such as IL-10, sFASL and vitamin B_{12} are increased in clinically active ALPS [26,27] (for diagnostic criteria see [15]).

In rare cases, homozygous *FAS* germline mutations [28] cause the most severe form of ALPS-FAS (for classification see [15]). More frequently, heterozygous germline [9,10] and, to a lesser extent, somatic mutations [29] are responsible for ALPS-FAS and ALPS-sFAS, respectively. These mutations, predominantly affecting the intracellular domain (ICD) of FAS, exert graduated dominant negative effects that lead to variable penetrance and expression [3,30,31]. Additionally, a functional loss of one *FAS* allele has been described in ALPS-FAS patients that were carrying a mutation affecting the extracellular domain (ECD) of FAS. This lead to a monoallelic FAS expression defect and resulted in ALPS with low clinical penetrance [32,33]. Therefore, haploinsufficiency has been assumed as another possible pathogenic mechanism. However, under these particular conditions, the observed low penetrance suggested that a second event was necessary for the clinical manifestation of ALPS. Indeed, we recently identified such second events in ALPS patients with *FAS* ECD mutations as either an independent somatic mutation on the second allele, i.e. compound heterozygosity, or a recombination event with the defective allele, i.e. loss of heterozygosity (LOH) [34].

Here, we describe a large family with 12 of its members carrying a heterozygous germline *FAS* c.3G>T start codon mutation. As no alternative in frame start codon is present in the *FAS* gene, no mutated FAS message is transcribed or translated. This implicated the absence of any negative effect of the mutated *FAS* allele and provided us with the opportunity to study pure FAS haploinsufficiency.

2. Material and methods

2.1. Approval

We obtained written informed consent for participation in the study from the parents and/or the patients. The study was approved by the institutional review board of the University Hospital Carl-Gustav Carus Dresden (*Ethikkommission an der Technischen Universität Dresden, EK 320122008*) and all experiments were carried out in accordance with the Declaration of Helsinki.

2.2. Biomarker quantification

Plasma sFASL, plasma IL-10 and plasma vitamin B_{12} were quantified as recently described [26].

2.3. Apoptosis assay

PBMCs were isolated by Ficoll-hypaque density gradient centrifugation, activated for 3 days with PHA 12 μg/ml (Sigma Aldrich) and cultivated for 7 days with IL-2 100 IU/ml (Proleukin, Chiron Corp). At day 10, T cell blasts were cultured with activating FAS antibody Apo-1.3 10 ng/ml or 100 ng/ml (Alexis Biochemicals) and cross-linking rabbit-anti-mouse IgG 5 μg/ml (Jackson Laboratories) for 18–24 h. Cells were suspended in a hypotonic solution (0.1% sodium citrate, 0.1% Triton X-100) (Sigma-Aldrich) with propidium iodide 50 μg/ml (Sigma-Aldrich). Apoptotic cells were detected by flow cytometry (BD FACS Canto II) and counted as hypodiploid nuclei. Data was analyzed with FlowJo 8.8.6 software (TreeStar Inc.) and apoptosis was calculated as follows: (% apoptosis observed in the sample − % spontaneous apoptosis) / (100 − % spontaneous apoptosis) × 100. p-Values for inter-group differences were calculated with the Mann–Whitney test-tool of PRISM statistical software (Graphpad Software, Inc., San Diego, USA).