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The genetic landscape of the FAS pathway deficiencies

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Abstract

Dysfunction of the FAS-FASLG pathway causes a lymphoproliferative disorder with autoimmunity called Autoimmune lymphoproliferative syndrome (ALPS) mainly caused by FAS mutations. The goal of this review is to describe the genetic bases of the autoimmune lymphoproliferative syndrome and to underline their genetic complexity with the contribution of both germline and somatic events accounting for the variable clinical penetrance of the FAS mutations. Starting from the cohort of patients studied in the French cohort (>165 cases), we also reviewed the literature cases in order to depict a full description of the mutations affecting the FAS-FASLG pathway involved in the outcome of this rare non-malignant and non-infectious pediatric lymphoproliferative disease. We also discussed the variable clinical penetrance associated with mutations affecting the extracellular domain of the protein. Such non-penetrant germline mutations are frequently associated with an additional somatic event impacting the second allele of FAS. Moreover, the uncomplete clinical penetrance associated with mutations affecting the intracellular domain of FAS, in patient lacking additional FAS somatic event, suggested a potential digenic inheritance with a FAS mutation accompanied by a genetic modifier possibly impacting another player of the lymphocytes homeostasis (affecting the survival, activation or apoptosis of the peripheral leukocytes).

The Autoimmune lymphoproliferative syndrome (ALPS), also called Canal and Smith syndrome [1], is a rare pediatric disorder. It is defined by a chronic (>6month) non-malignant and non-infectious lymphoproliferation with blood elevated numbers of TCRβ+ CD4- CD8+ lymphocytes called double negative (DN) T cells [2]. They also accumulate in the secondary lymphoid organs (lymph node, spleen) leading to splenomegaly, diffuse adenopathy, and sometimes...
hepatomegaly. DN T cells have a unique and characteristic differentiation profile with features of highly active terminally differentiated cells. This profile could be also observed in a fraction of single positive CD4+ or CD8+ T cells, which, together with identical CDR3 sequences, confirms that these pathognomonic DN T cells arose from mature single positive peripheral T cells [3]. In healthy individuals, they represent a small subset of mature peripheral T cells (less than 2 percent of TCRαβ T cells). The ALPS usually develops before five years of age and is accompanied by autoimmune manifestations (mainly autoimmune cytopenia) in about 2/3 of the cases [4].

In addition of elevated DN T cells, biological markers have been identified, such as elevated plasma levels of soluble FAS-Ligand (FASLG), Interleukin-10 (IL-10) and Vitamin B12 [5]. Elevated levels of IL-10 in plasma is mainly originating from the DN T cells [6]. The Vitamin B12 results from the increased synthesis and secretion of haptocorrin by circulating leukocytes in ALPS, particularly the DN T cells [7].

In addition to the autoimmune manifestations, an increased risk of hematological malignancies, both Hodgkin and non-Hodgkin lymphoma, is observed in ALPS patients. This risk is estimated to be up to 61 to 149 times that in the general population [8].

The main genetic defects involved in ALPS are mutations affecting the death receptor FAS, its ligand, or the apoptosis pathway [2]. Over the past 15 years, ALPS-like disorders have been described, including RAS-associated leucoproliferative disorder (RALD) [9]; CASPASE-8 deficiency state (CEDS) [10]; gain-of-function (GOF) signal transducer and activator of transcription 3 (STAT3) mutations [11]; CTLA-4 haploinsufficiency with autoimmune infiltration (CHAI) [12,13]; and lipopolysaccharide-responsive vesicle trafficking, beach and anchor containing (LRBA) deficiency with autoantibodies, regulatory T-cell defects, autoimmune infiltration, and enteropathy (LATAIE) [14]. Although patients with ALPS-like disorders may initially present with symptoms similar to the original ALPS, they differ by a later onset, an absence of apoptosis defect, frequent lymphocytic tissue infiltration, and a broader spectrum of autoimmune diseases (including organ-specific ones) [15,16].

The present work is focused on the mutations affecting the FAS pathway (FAS, FASLG and FADD) associated with ALPS identified in the French reference center and/or described in the literature.

The FAS pathway

FAS (TNFRSF6) is the sixth member of the Tumor Necrosis Factor Receptor (TNFR) superfamily (NCBI gene ID:355). It is a membrane-bound trimeric protein playing a key role in apoptosis. The FAS gene, located on the long-arm of the chromosome 10 (10q23.3–4) in human and on the chromosome 19 in mice, is composed of 9 exons encoding a type-I protein. Exons 1 to 5, 6 and 7 to 9 encode the extracellular domain (ECD), the transmembrane domain (TD) and the intracellular domain (ICD) respectively [Fig. 1]. A functional domain called the Death Domain (DD), entirely encoded by the exon 9, defines the subfamily of Death Receptors such as the TNF-Receptor 1 (TNFRSF1A), DR3 (TNFRSF25), FAS (TNFRSF6), and the TRAIL receptors TRAILR1 and TRAILR2 (TNFRSF10-A and -B respectively) [17]. The mature FAS protein has 319 amino acids and a predicted molecular weight of 48 kD. FAS is expressed as a trimer mainly on activated lymphocytes and virtually in all tissues. Upon interaction with its cognate

![Fig. 1 From the FAS gene organization to the membrane-bound form of FAS in human. FAS (TNFRSF6, located in 10q24 in human) is composed of 9 exons. It encodes a type I protein. The ECD including the three Cysteine-Rich-Domain (defining its belonging to the TNFR super family) is encoded by the 5 first exons (depicted in blue). The transmembrane domain (represented in green) is encoded by the exon 6 and the ICD is encoded by the exons 7 to 9. The exons 7 and 8 encoded for the calcium-inducing domain (CID) highlighted in yellow. The functional death domain (DD), which is crucial for the apoptotic signal transmission, is entirely encoded by the exon 9 and is represented in orange. The FAS death receptor is a transmembrane protein ubiquitously expressed at the surface of the cells as a trimer.](image-url)
ligand FASLG, FAS triggers a biochemical cascade leading to the organized cell destruction.

FASLG belongs to the tumor necrosis factor (TNF) family and is encoded by a gene located on chromosome 1q23 in human (NCBI Gene ID:356); it is normally stored in the cytoplasm in secretory vesicles and can be expressed as two different isoforms: a 37 kDa membrane-bound protein (mFASLG) and a 26–29 kDa soluble variant (sFASLG), produced by a metalloproteinase-mediated cleavage of the mFASLG [18]. The FASLG gene is composed of four exons and is located on chromosome 1 in human and mice. It encodes a type II protein, meaning that the N-terminal domain is intracellular [Fig. 2].

The FAS-FASL interaction induces a signaling cascade resulting in programmed cell death (for review [19]). The initial event following FAS/FASLG interaction is the recruitment of an adapter molecule called FAS-Associated Death Domain (FADD). This recruitment is mediated by the homotypic interaction of the Death Domains (DD) of FAS and FADD. FADD also contains a domain called death-effector domain (DED), mediating the recruitment of DED-containing cysteine proteases such as PRO-CASPASE-8 and PRO-CASPASE-10 (also called Flice and Flice-2, respectively) in humans. The caspases are cysteine proteases including initial caspases (CASPASE-8,-10 and -2) and effector caspases (CASPASE-3,-6,-7,-9). CASPASE-8 and -10 and -10 are processed in their active form into a death-inducing signaling complex (DISC) which, in turn, triggers the activation of other pro-caspases in a biochemical cascade culminating in apoptosis. Initiation of this process can be inhibited by the recruitment of an inactive caspase analog, the Flice inhibitory protein (FLIP). Finely tuned stoichiometry of FLIP, CASPASE-8 and CASPASE-10 regulates the stability and pro-apoptotic activity of the death-inducing signaling complex, leading to a high sensitivity to apoptosis of activated T and B cells as compared to their resting counterpart [Fig. 3].

If the FAS mutations are the most frequent causes of ALPS in humans, few examples of FALG, FADD, CASP8 and CASP10 mutations have also been reported. However, the causal roles of the CASP8/10 mutations are still a matter of debate and will be discussed below.

The first FAS mutations have been identified by analogy with natural or engineered animal models. We will briefly summarize these models before developing the human cases.

**Mice models**

Natural animal models of the autoimmune lymphoproliferative syndrome have been reported with the description of the lpr and gld mice [20]. The lpr and gld mutations affect the Fas (on chromosome 19) and Faslg (on chromosome 1) genes respectively. The lpr and gld mutations behave as loss-of-function (LOF) recessive mutations. The homozygous animals develop lymphoproliferation (and autoimmunity in a strain dependent manner) whereas the heterozygous animals remain free of symptoms.

Lpr mice are representative of Fas dysfunction [21]. A retrotransposon insertion into the intron 2 of the Fas gene was identified in the MRL (Murphy Roths Large) background. This insertion disrupts the normal splicing of the exon 3 leading to an unstable transcript destroyed through an mRNA decay process. The lpr mutation is leaky, as a normal intron 2 splicing may occur at low level. Therefore, the lpr mice are able to express a very low level of Fas protein.

Fig. 2 From the FASLG gene organization to the membrane-bound form of FASLG in human. FASLG (located in 1q24.3 in human) is composed of 4 exons. The first exon is encoding both the ICD (highlighted in light orange) and the transmembrane part of the protein (in green). The amino acids 17 to 24 contained the Casein kinase I (CKI) substrate motif (highlighted in yellow). The domain including amino acids 37 to 70 is called PRD since it’s a Prolin Rich Domain and is highlighted in dark orange. The exons 2 to 4 are encoding the extracellular part of the protein (highlighted in brown) including: -1) a metalloprotease cleavage site (between Ser126 and Leu127, highlighted with a hatched red line), -2) a self-assembly domain (highlighted in hatched brown), and 3)- the TNF Homology Domain (THD) involved in FASLG-FAS interaction (highlighted in brown). FASLG is a type II transmembrane protein expressed as a trimmer at the surface of activated T cells, or by some cells in privilege sites.
The lpr<sup>9</sup> mutation is a missense mutation in exon 9, leading to a defect of the apoptosis signal transduction by disrupting the Fas–FADD interaction [22]. Again, only homozygous lpr<sup>9</sup> mice develop the lymphoproliferative syndrome.

The gld mutation is a missense mutation in the ECD of the Fasl<sup>9</sup> gene impairing the Fasl<sup>9</sup>–Fas interaction [20]. Interestingly, gld-lpr<sup>9</sup>-double heterozygous (gld/wt; lpr<sup>9</sup>/wt) animals develop a mild lymphoproliferation [23], suggesting that accumulation of genetic defects affecting the same pathway could be a potential digenic form of the disease.

The autoimmune features in the lpr mice strongly depend on the genetic background of the animal. Indeed, Fas wt MRL mice develop a lupus like disease by 20 weeks of age, whereas homozygous lpr MRL animals develop symptoms as early as 8 weeks of age. This indicates that the Fas mutation is accelerating the autoimmune disease related to the MRL background. Accordingly, a Dnase1L3 mutation was later described in the MRL background. DNase deficiency leads to an accumulation of DNA thereby triggering cellular DNA sensors and promoting the overt production of type I interferon, one of the key cytokine involved in the pathophysiology of lupus. In other genetic backgrounds, such as the C57BL/6 and the BALB/c strains, a variable expression of autoimmunity is observed, with a most severe phenotype observed in lpr/lpr BALB/c animals. Complete Fas or Fasl KO mice have also been engineered and exhibit more severe and earlier symptoms than the natural models (lpr, lpr<sup>9</sup> and gld) [24].

There is no murine model of Casp10 deficiency as the mouse counterpart is a pseudogene. The FADD and Caspase-8 genes ablation are embryonic lethal [25,26], whereas heterozygous animals do not exhibit any particular phenotype. Conditional knock-out in lymphocytes or transgenic animal with a dominant negative form of FADD exhibited a defective lymphocyte proliferation [27–29], indicating a non-apoptotic role of FADD in the antigen receptor signaling pathway.

Conditional Casp8 KO in T lymphocytes led to T cell lymphopenia in young mice, and as mice age, B cell and T cell compartments expand, producing lymphoproliferation and a lethal T cell infiltrating disorder [30] indicating that Casp8 is also involved in non-apoptotic functions.

**To humans**

The genetic etiology of the ALPS was described in 1995 with the discovery of the FAS gene mutations [31]. The related apoptosis defect accounts for the accumulation of autoreactive lymphocytes as well as for specific clinical and biological features that distinguish the ALPS-FAS from other monogenic defects of this apoptosis pathway, such as FADD and Caspase-8 deficiencies. The recognition of these genetic diseases demonstrated the key role of this apoptotic pathway in controlling the adaptive immune response in humans. The functional integrity of the apoptosis pathway can be assessed.
in vitro. This assays allow to evaluate the capacity of the activated T lymphocytes to enter apoptosis: 1) after induction by an agonist of the death receptor FAS or by an agonist of the death receptor TRAIL, allowing the assessment of the extrinsic apoptosis pathway; 2) after re-activation of the cells by stimulating the TCR, which induces the expression of membrane FAS-L, leading to AICD (activation induced cell death); or 3) after deprivation of interleukin-2, leading to ACAD (activated cell autonomous death) involving the intrinsic pathway of apoptosis, also called the mitochondrial pathway. This test has been described in detail in a methods journal [32].

**Germline heterozygous mutations**

According to the revised criteria for the diagnosis and classification of ALPS from the 2009 International Workshop at the National Institutes of Health (NIH) [33], approximately 500 patients with ALPS originating from more than 300 families have been investigated all over the world. In the French cohort, the ALPS-FAS diagnosis came up for more than 130 patients by the identification of nearly one hundred heterozygous FAS mutations [Fig. 4].

The heterozygous mutations affecting the ICD of FAS are highly more frequent as they affect 77% of the ALPS-FAS patients [Fig. 4]. They are usually associated with a preserve protein expression and follow an autosomal dominant inheritance meaning that one mutant allele lead to the disease development. These mutations are associated with a profound apoptosis defect in vitro as a consequence of an impaired FAS-FADD interaction [Fig. 5]. Of note, mutations affecting the Alanine at the position 237 and the Arginine at the position 250 were identified in 8 and 16 patients respectively (corresponding to 6.3% and 12.6% of the patients). This observation suggests that they are key residues in the FAS-FADD interaction or that they are hot-spot of mutations associated with a particular DNA structure. The crystal structure of FAS confirmed that these residues were indeed central in the DD structure and therefore in the interaction with FADD [34]. This does not rule-out the hot-spot hypothesis but so far there was no study published on this potential phenomenon.

Heterozygous mutation of FAS affecting the ECD account for 23% of the genetic cause of ALPS-FAS in the French cohort [Fig. 4]. Few missense mutations affected either the structure of the ECD (mutations affecting cysteine residues thereby disrupting the 3D structure of FAS) or domains involved in the FAS/FASLG binding. However, most of them lead to a premature stop codon, predicted to produce a truncated protein lacking the TM domain. Since these mutant proteins cannot anchor to the membrane, the dominant nature of such mutations remained elusive for a long time. The hypothesis of a soluble form of the mutated FAS able to compete with the normal FAS with regards to the FASLG binding was initially proposed. However, the resolution of the 3D structure of FAS
and additional experimental works ruled-out this hypothesis. Indeed, the activated lymphocytes of individuals carrying a FAS mutation affecting the ECD exhibited a reduced FAS expression at the membrane, supporting the notion that these mutations lead to the absence of protein expression. Since the mutant allele is not expressed these mutations act by haploinsufficiency [Fig. 5]. In total, 77% (n = 101) of heterozygous germline mutations affected the ICD of FAS. Among them, 74 are localized in the DD and the others led to loss of DD by premature stop or frame-shift. Only 23% (n = 22) affected the ECD and 5% (n = 7) had TM defect. These genetic characteristics are very similar to those described by Price et al. in the description of the cohort followed at the NIH [8].

Interestingly, all the germline mutations are functionally penetrant as activated T cells from all carriers exhibit an apoptosis resistance after induction by an agonistic anti-FAS antibody. However, some of these mutations exhibited a clinical non-penetrance, as some FAS mutation carriers remain free of symptoms or elevated biological markers all lifelong, even if their cells exhibit the same magnitude of the apoptosis defect as those of the affected individual [5]. The clinical penetrance is calculated as the percentage of ill patients among the total of carriers for a given mutation (i.e the penetrance is 66% in a family with 2 affected peoples among 3 carriers of the same FAS mutation). Importantly the penetrance is low (<30%) for mutations associated with haploinsufficiency, whereas it is high (>80% and up to 100%) for mutations affecting the ICD. The low clinical penetrance of some mutations suggests that additional factors are required to account for the onset of the disease in limited number of carriers in a given family. It could be environmental factors, including infectious history. However, in families presenting with multiple affecting individuals -even at different generation-we favored the hypothesis of genetic modifiers, that turned to be the good hypothesis at least in some families (see below).

**Somatic dominant mutations**

Somatic FAS mutations are the second most common genetic etiology of this disease (ALPS-sFAS) [35–38]. The somatic mutations likely appear during the embryologic development, in hematopoietic progenitors or earlier, and confers a selective advantage to the mutated cells. The proportion of mutated cells is similar in all circulating lineages of hematopoietic origin (granulocytes, T-, B- lymphocytes, NK cells or monocytes), but these mutations remained undetectable in the hair, skin or mucosal epithelial cells. The mutation affected about 4% of the hematopoietic CD34+ cells, 10–30% of the peripheral leucocytes, and 80–100% of the DN T cells. Because, most if not all DN T cells are mutated, it allows the detection of the mutation by sequencing the genomic DNA isolated from sorted DN T cells. To date, 27 patients have been identified in the

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**Fig. 5 Consequences of the presence of mutations affecting FAS on the expression and the function of the protein depending of their localization.** In the wild-type (WT) situation (left panel) FAS is expressed as a trimer. When FAS interacts with is ligand FASLG, FAS multimerizes and initiates the apoptosis cascade leading to FAS- FADD interaction (highlighted in pink). It is followed by CASPASE-8/10 (in violet and blue) recruitment and the DISC formation, leading to apoptosis in more than 80% of the activated T cells. As the consequence of the FAS mutations affecting the ICD of the receptor, the amount of receptors is normal at the cell surface (middle panel). However, the mutated form of FAS inhibits the recruitment of FADD and the DISC formation, exhibiting a dominant negative effect on the apoptosis signal transmission. The apoptosis is profoundly decreased. The mutations affecting the ECD of the protein (right panel) lead to a FAS expression defect. The activated T cells expressed only the FAS receptor encoded by the wild type allele, leading to half of the normal amount of the receptor, resulting to a partially decreased apoptosis (between 50 and 70% of death). These mutations are associated with haploinsufficiency.
French cohort [Fig. 6]. Of note, the apoptosis function of the activated T cells of these patients was preserved as a consequence of in vitro artefact. Since T cells are expanded for 9 days in order to reach the maximal sensitivity to the FAS-induced cell death [see Fig. 3], the mutated cells -poorly proliferating in vitro, possibly as a consequence of an in vivo-acquired exhausted profile-disappeared.

Importantly, the clinical features of the patients harboring a somatic FAS mutation is undistinguishable from those of the ALPS-FAS patients carrying a germline mutation [4,36]. Interestingly, all but one patient in the French cohort (among 27) exhibited a somatic FAS mutation affecting the ECD of the protein, leading to the expression of a mutant protein predicted to exert a dominant negative effect [Fig. 6]. One can hypothesize that, in the unique case of somatic mutation affecting the ECD of FAS, the mutation appeared in the context of an unidentified germline mutation (i.e. a deep intronic mutation) or in a genetic background favoring the disease outcome.

Accumulation of germline and somatic mutations of FAS

We more recently described that germline haploinsufficient FAS mutations can be associated with somatic events on the second FAS allele in the patients, but not in the healthy relatives carrying the germline mutation alone [39]. This new mechanism provided a molecular explanation for the variable penetrance of the FAS mutations affecting the ECD of FAS. In patient, we identified a somatic event on the second FAS allele, in addition to the previously identified familial germinal heterozygous FAS mutation. The combined germline and somatic mutations led to a complete absence of FAS expression in double mutant cells, and a full-blown disease. In contrast, healthy relatives carry only the germline FAS mutation, their lymphocytes exhibit a reduced but detectable FAS expression, and they remain free of any ALPS symptoms.

The somatic second event can be a missense or nonsense mutation of the second allele of FAS, or a loss of the wild type allele compensated by a duplication of the mutated allele, a genetic mechanism called uniparental disomy (UPD), finally leading to a loss of heterozygosity (LOH), a phenomenon well-known in malignant tumors development. Regions of up to >100 kbp of the chromosome 10 (encompassing FAS) have been shown to be lost and replaced by a copy of the mutated sister chromosome. The UPD is the most frequent event occurring in the ALPS-FAS/sFAS condition and lead to the presence of the FAS mutation at the homozygous state. In the French cohort, only 3 ALPS-FAS/sFAS patients harbor a point mutation in the second allele of FAS, whereas the other 15 exhibited somatic UPD [Fig. 7].

Germain homozygous mutations or compound heterozygous mutations

Autosomal recessive (AR) form of ALPS can be caused by homozygous germline FAS mutations in rare examples of consanguineous pedigrees.

As above described, FAS mutations leading to haploinsufficiency behave as recessive mutations, that is why a second somatic event is required for the disease onset. Of note, in rare consanguineous pedigrees, homozygous germline haploinsufficient mutations have been reported [31,40–44] [Table 1]. Such mutations can impact either the extracellular or ICD of the receptor, and are all associated with complete expression defect at the cell surface level, and thus to a complete apoptosis defect in vitro. Such homozygous patients are the human counterparts of the Fas null mice. They develop a very severe early-onset (sometimes in utero) lymphoproliferation requiring hematopoietic stem cell transplantation in most cases as the disease could not be controlled by immunosuppressive treatments [45,46].

The heterozygous parents usually remain healthy but may sometimes develop minor ALPS symptoms suggesting the
potential occurrence of additional somatic events in those heterozygous carriers. Since the heterozygous mutant cells of the parents express the wild type FAS allele their lymphocytes FAS protein expression at the cell surface is half that of healthy controls, an expression level allowing a sufficient apoptosis function to maintain lymphocyte homeostasis in vivo [Fig. 5].

A unique example of compound FAS heterozygous mutations has been described in the literature [47]. Each healthy parent was heterozygous for one of the 2 mutations found in 3 siblings who developed a severe form of ALPS. One of these mutations affected the ECD and was thus haplo-insufficient. This description of compound heterozygous mutations is further supporting the notion that additional genetic events are required to account for the disease onset in patients carrying haploinsufficient mutations.

**Non-FAS mutations in the apoptosis pathway**

**FASLG**

Recessive mutations in the FASLG gene have been identified in few patients then called ALPS-FASLG [Table 2] (OMIM 134638). To date, only four different homozygous mutations have been found in four unrelated patients, all exhibiting an autosomal recessive inheritance mode [48–51]. Two heterozygous FASLG mutations have been also reported yet [52,53]. Only one (p.R156G) affecting the ECD of FASLG was associated with ALPS features -mainly adenopathy-the second (p.M158-E185del) lead to lupus-like feature which did not fulfill the classical criteria for ALPS. These data and the infrequency of FASLG defect in ALPS patients suggest a

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**Table 1 Reported Homozygous FAS mutation associated with ALPS-FAS in the literature.**

| Affected exon | Consequence at cDNA level | Consequence at protein level | FAS expression | number of patients | reference |
|--------------|---------------------------|------------------------------|----------------|-------------------|-----------|
| 3            | c.320G > A                | p.Cys107Tyr                  | no             | 2                 | [37]      |
| 4            | c.361C > T                | p.Arg121Trp                 | no             | 1                 | [38]      |
| 4            | c.427T > G                | p.Cys143Gly                 | no             | 1                 | [39]      |
| 6            | c.506-16A > G             | p.Gly169_Trp189del          | no             | 1                 | [40]      |
| 6            | c.538C > T                | p.Gly169_Trp189del          | no             | 1                 | [40]      |
| 9            | c.797A > G                | p.Asn266Ser                 | residual       | 1                 | [38]      |
| 9            | c.968_987dup              | p.Glu330LysfsTer38          | no             | 1                 | [41]      |
| 9            | c.916_1550delinsTTGT      | p.Ala307Tyrfs*6             | no             | 1                 | [29]      |

**Table 2 Reported FASLG mutation associated with ALPS-FASLG in the literature.**

| Affected exon | Consequence at protein level | FASLG expression | FAS-induced apoptosis | DN T cells count | inheritance | clinical manifestations | reference |
|--------------|------------------------------|------------------|-----------------------|-------------------|-------------|-------------------------|-----------|
| 1            | p.F87fsX95                   | Abolished        | Normal                | Increased         | Autosomal recessive | ALPS       | [47]      |
| 1            | p.69AsfsX75                  | Abolished        | Normal                | Increased         | Autosomal recessive | ALPS       | [48]      |
| 4            | p.A247E                      | Normal           | Normal                | Normal            | Autosomal recessive | ALPS       | [45]      |
| 4            | p.C202S                      | Normal           | ND                    | Increased         | Autosomal recessive | ALPS       | [46]      |
| 4            | p.R156G                      | Normal           | Normal                | Normal            | Dominant negative  | ALPS       | [49]      |
| 4            | p.M158-E185del               | Not done         | Not done              | Normal            | Dominant negative  | SLE + Lymphadenopathy | [50]      |
recessive trait of the FASLG mutations. In mice, the gld model also develop the disease only at the homozygous state. Moreover, the double heterozygous model (gld/+; lprª/tº), animals developed autoimmune lympho-proliferation suggesting that additional genetic event in the FAS pathway are required for the development of the disease in the context of a heterozygous FASLG mutation.

**FADD**

FADD deficiency (OMIM: 602457) is an autosomal recessive disorder. To date, only six patients exhibiting FADD deficiency has been described. They demonstrated a clinical phenotype mainly overlapping with ALPS-FAS, including decreased FAS-induced apoptosis, increased circulating DT cells, elevated plasma FAS ligand, IL-10, and vitamin B12, variable degrees of lymphadenopathy or splenomegaly, but also recurrent febrile episodes with encephalopathy and seizures, cerebral atrophy, and structural cardiac abnormalities. Five patients carried a homozygous mutation affecting the same amino acid (p.C105W) [54,55] and one exhibited two compound heterozygous mutations (p.C105R and c.52-58delCACGAGC), one inherited from each parents [56].

**CASPASE-10**

Four mutations of the CASPASE-10 (CASP10) gene have been reported in ALPS-like patients [57,58]. However, one of these mutations (p.V410I) is a polymorphic variant carried by 2% of the general population (Global minor allele frequency in clinVar = 0.01917). Moreover, activated T cells from healthy individuals carrying this mutation at the heterozygous or even homozygous state, display normal FAS-induced apoptosis (authors personal unpublished results). The p.Y446C and the p.V410I variants did not exhibited any dominant negative effect by inhibiting the apoptotic signal transduction from severe disease by CASPASE-10 in 63 families with ALPS-FAS due to dominant FAS mutations [56]. Thus, different genetic variations in CASPASE-10 can produce contrasting phenotypic effects.

Combined germline heterozygous FAS and germline heterozygous CASP10 mutations (p.Y446C or p.P50IL) have been reported in one ALPS patient [60]. FAS expression was reduced and CASPASE-10 activity was decreased. More recently, the first ALPS patient with a combination of a somatic FAS mutation and a germline CASP10 substitution was also reported [61]. All these data do not definitively prove the pathological role of CASP10 variants in the ALPS outcome, but highlighted once again that the accumulation of genetic defects in the FAS pathway could lead to the onset of the disease. Of note, a functional Caspase-10 does not exist in mice (as Casp10 is a pseudogene), thereby questioning its physiological role.

**CASPASE-8**

CASPASE-8 deficiency (OMIM 601763) has been described in 4 patients from the same family harboring an homozygous autosomal recessive mutation in the CASPASE-8 gene [10,62]. They suffered from immunodeficiency with recurrent pulmonary infections. They also had lymphocytic infiltrations in multiple organs such as liver, spleen, lung, and brain. The lymphocytic infiltration seen in the older patients is reminiscent to that seen in parenchymal organs of older mice lacking Caspase-8 within their T cells [30]. The DT cell counts were in normal range and the clinical phenotype associated a mild immunodeficiency. More recently, homozygous mutations leading to CASPASE-8 deficiency were described in three patients presenting with very early onset IBD (VEO-IBD) [63]. Consequently, this CASPASE-8 deficiency is no longer included in the ALPS classification.

**Conclusion**

The ALPS-FAS was the first description of a monogenic cause of autoimmunity, but its non-Mendelian expression remained elusive until the description of germline and somatic mutations in ALPS patients.

The complete ALPS condition can be linked to mutations in different genes involved in the FAS-FASLG signaling pathway. However more than 90 percent of the patients exhibited a mutation in the TNFRSF6 gene encoding for the death receptor FAS. This observation indicated that the elimination of the rogue autoimmune lymphocytes - mainly B-lymphocytes, as the autoimmune features of ALPS are antibody mediated (autoimmune hemolytic anemia (AHAI), Immune thrombocytopenic purpura (ITP) and neutropenia) is tightly regulated by the FAS-FASLG pathway.

The FAS mutations identified in the ALPS patients can be germline (ALPS-FAS), somatic (ALPS-sFAS) or both (ALPS-FAS/sFAS). The patients carrying a homozygous mutation display a very severe and early phenotype and had to be treated by hematopoietic stem cells transplantation. The clinical penetrance of the heterozygous mutations is quite variable, as some individuals carry mutation of FAS without exhibiting any clinical feature. The less penetrant mutations affect the ECD of the protein. In such cases, the mutated allele is not expressed, but the protein produced by the wild type allele remained able to maintain the apoptotic function necessary to the homeostasis of the cells in vivo, despite a mild apoptosis defect assessed in vitro. In most of the ALPS-FAS families exhibiting a mutation with partial clinical penetrance, a second event in the second allele of FAS was identified. These data indicated that the ALPS-FAS could be the consequence of the accumulation of genetic defects in human, as previously observed in the mice model which remain healthy at the heterozygous state. The mutations affecting the ICD of the protein present a high clinical penetrance, reaching overall 80% of the carriers. In these cases, the mutant act as a dominant negative effect by inhibiting the apoptotic signal transmission.
However, in some ALPS-FAS families carrying a mutation with partial clinical penetrance and no second event in the second allele of FAS (excluded by sequencing of the cDNA of the DN T cells) another genetic modifier is suspected. In such patients, one can assumes that the disease could occur as the consequence of a digenic inheritance. The FAS mutation is inherited from one parent whereas the other parent would transmit a mutation in a gene yet to be identified, and possibly affecting the FAS pathway.

The challenge is now to identify which mutated gene that, combined with a FAS mutation, could be involved in the ALPS onset. Since the FAS mutations are rare events with frequencies compatible with a rare disease, the second event could eventually be a more frequent mutation, rendering its identification more complex. It can affect a gene involved in cell-death, survival, proliferation or regulation of important function for the immune system homeostasis.

In conclusion, most cases of ALPS are associated with a defective FAS-induced apoptosis, thus defining this biochemical pathway as a master check-point of self-tolerance. The ALPS-FAS is clinically homogeneous but genetically heterogeneous most likely because the genetic modifiers could affect numerous molecules involved in cell death or survival. The identification of such factors should help in deciphering the pathophysiological basis of ALPS and may lead to uncover new genetic defects associated with the ALPS in patients with a functional FAS pathway.

**Conflicts of interest**

Authors declare no conflict of interest.

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