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# Case Report: Clinical, molecular, and functional characterization of autoimmune lymphoproliferative syndrome—a family study with a multimodal diagnosis

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Autoimmune Lymphoproliferative Syndrome (ALPS) is a rare immunological disorder caused by defective apoptosis, commonly due to pathogenic variants in the *FAS* gene. We report a comprehensive clinical, immunophenotypic, molecular, and functional evaluation of nine members of a consanguineous Brazilian family harboring the pathogenic *FAS* (NM\_000043.6:c.748C>T) variant. The index case, an 11-year-old boy, presented with recurrent cytopenias, splenomegaly, and increased double-negative T cells. Genetic analysis identified additional variants in *CASP10* (NM\_032977.4: c.1202\_1208del), and *LRBA* (NM\_001364905.1:c.2450-7C>T), evidencing a complex genotype. Functional assays confirmed different levels of impaired *FAS*-mediated apoptosis in some affected individuals. Among nine family members studied, four out of them met clinical and molecular criteria for ALPS, demonstrating incomplete penetrance and variable phenotype. All affected individuals share the same variants in *FAS* and *CASP10*, yet their clinical presentations differ significantly. Clinical manifestations and elevated double-negative T cells were observed exclusively in male individuals. Notably, a female family member harboring both *FAS* and *CASP10* variants remained asymptomatic, supporting previous findings of incomplete penetrance and suggesting that sex-related factors—possibly including hormonal influences—may modulate clinical expression in ALPS. Introduction of sirolimus therapy led to sustained remission in the index case. This study reports a successful integration of multimodal diagnostic strategy for accurate identification and management of ALPS, and it highlights the potential role of targeted therapies in improving outcomes.

## KEYWORDS

autoimmune lymphoproliferative syndrome (ALPS), diagnosis, immunophenotyping, *FAS* variant, *CASP10* variant, apoptosis defects, functional assay

## 1 Introduction

Autoimmune Lymphoproliferative Syndrome (ALPS) is a rare immunological disorder characterized by defective apoptotic mechanisms that disrupt lymphocyte homeostasis. Clinical manifestations include multilineage cytopenias, lymphadenopathy, hepatomegaly, splenomegaly as well as autoimmune diseases and increased risk of lymphoma (1, 2). Its prevalence remains uncertain, although it is estimated that there are several hundred cases globally (3).

Pathogenic variants in the *FAS* gene are the main cause of ALPS. In addition to *FAS*, other variants in apoptosis-related genes such as *FASLG*, *CASP10*, *CASP8*, *FADD* and *NRAS* are described in a small group of patients (4, 5). In this context, the *FAS*:c.748C>T (p.Arg250Ter) variant emerges as a genetic alteration of interest, since it is associated with the clinical severity of ALPS (6–8). This variant results in the replacement of the arginine codon by a premature termination codon, p.Arg250Ter, affecting FAS receptor functioning and leading to cellular resistance to programmed death. This resistance is particularly relevant in the pathogenesis of ALPS, since the accumulation of autoreactive lymphocytes translates into an intensification of both autoimmune activity and clinical severity (9). In parallel, although the pathogenicity of some *CASP10* variants remains controversial (10, 11), some studies suggest that they may act as disease modifiers by subtly impairing apoptotic signaling in synergy with other genetic alterations (12).

Patients with ALPS have an expansion of TCRαβ+CD4-CD8- cells (DNT cells), which correlates with dysfunction in the regulation of the immune response. Increased levels of interleukin-10, vitamin B12, and the presence of defective FAS-mediated apoptosis are also frequent and suggest impairment in the programmed cell death mechanism, essential for immune homeostasis (13).

Although *FAS* variants are a well-established cause of defective apoptotic signaling in ALPS, their impact on apoptosis is not always the same. In this study, we observed different levels of apoptotic activity among patients carrying the same *FAS* variants. The phenotypic variability observed among patients can be attributed to genotypic events that affect the expression of other genes involved in apoptosis and in the immune response, which can result in cases ranging from asymptomatic to severe clinical manifestations (14, 15).

Among the emerging therapeutic options, Sirolimus has stood out as a promising intervention, modulating the immune response and reducing the clinical manifestations of ALPS (16, 17). Previous studies, including systematic reviews have described its effectiveness in improving patients' quality of life, as well as its ability to induce remission in refractory conditions to conventional therapy (18).

The diagnosis of ALPS remains challenging due to its heterogeneous clinical presentation, variable penetrance, and the overlap of symptoms with other lymphoproliferative and autoimmune disorders (2, 19–21). This study aimed to add a piece in a comprehension of a complex disease, providing a clinical, immunophenotypic, and genetic assessment of a family

with multiple members affected by ALPS. Here, we present a unique and comprehensive investigation of a large Brazilian family with ALPS. Among the nine individuals studied, four of them met both the clinical and molecular criteria for the disease, evidencing the importance of familial screening in identifying affected individuals who might otherwise remain undiagnosed. By analyzing these relatives, we aimed to characterize the phenotypic variability of ALPS, explore its incomplete penetrance, and identify potential genotype-phenotype correlations, thereby contributing to a deeper understanding of the disease and its diagnostic challenges. This family-based study highlights the value of integrating multimodal diagnostic approaches to enhance the detection and management of ALPS cases.

## 2 Case description

This study was conducted at Brasília Children's Hospital (Brazil) and was approved by the local Research Ethical Committee (FEPECS-2.332.383). Informed consent was obtained from the patient's caregivers and from adult relatives.

An 11-year-old boy, who was born from consanguineous Brazilian parents, at 38 weeks gestational age, by cesarean section, in good conditions with an Apgar score of 9 and birth weight of 2,675 g. His medical history was negative for recurrent infections, and he had normal psychomotor development. His mother had hypothyroidism. His father was diagnosed with splenomegaly, although no other clinical features, as autoimmune cytopenia, was identified until the end of data collecting. The index patient has two siblings, one asymptomatic boy from his father's side and one girl from her mother's side. His sister has attention deficit disorder and intellectual disability. The patient also had a positive family history of autoimmune diseases, including psoriasis, autoimmune thyroiditis and benign nodules in mammary glands. However, no family history of pediatric cancer was described.

At the age of 3 years and 7 months, the patient was referred to Brasília Children's Hospital, where he began follow-up with our team, after presenting with a three-day fever and a cutaneous rash on the face and scalp, initially treated with oral cephalixin. Despite antibiotic treatment, after a couple of days his condition worsened, and he presented with asthenia and pallor. The patient presented with a clinical feature of cardiac decompensation secondary to severe anemia, as well as abdominal pain. He was then hospitalized for diagnostic elucidation. He received blood transfusion, but with persistence of fever, in association with neutropenia, intravenous antibiotic was prescribed. During that time, tests for infectious diseases were performed, including HIV, viral Hepatitis, Toxoplasmosis, Leishmaniasis, Parvovirus, and others, which were negative.

The patient condition progressed to multilineage cytopenia (immune neutropenia and autoimmune hemolytic anemia) and he was sent to pediatric oncology service. Bone marrow aspirate ruled out hematological malignancies. Oral corticosteroid was prescribed due to a presumed diagnosis of autoimmune hemolytic anemia, and he was discharged after 12 days of hospitalization. The patient had partial response to corticosteroids, and he was sent to an

immunologic evaluation, due to high levels of vitamin B12 (>2,000 pg/mL) in association with cytopenia and benign lymphoproliferative disease with suspicion of ALPS diagnosis.

### 3 Diagnostic assessment headings

#### 3.1 Clinical and laboratory evaluation

Clinical and laboratory data were collected from medical records. At the first immunological evaluation at 3 years and 8 months of age, the proband exhibited normal growth and development, with no dysmorphic features. However, physical examination revealed splenomegaly and cervical lymphadenopathy. Baseline immunological assessments showed increased immunoglobulin serum levels, a normal vaccine response to rubella and measles as well as normal levels of complement protein. Autoimmune screening showed negative antinuclear and antiphospholipid antibodies, but a positive direct Coombs test was detected. A previous abdominal ultrasound performed at the age of 3 years and 7 months had already evidenced lymphadenopathy and splenomegaly (9 cm).

#### 3.2 Immunophenotyping

To further characterize the immunological profile of the patient and some family members, immunophenotyping was performed. Peripheral blood samples were processed using standard flow cytometry protocols. A comprehensive panel of monoclonal antibodies targeting B and T cell markers (CD19, IgD, IgM, CD38, CD27, CD3, CD4, CD8, CD45RA and CCR7) was used to characterize lymphocyte subsets, with data acquisition performed on a BD FACSCanto™ II cytometer and analyzed using FlowJo™ FACS Diva™ software. Notably, the patient presented with reduced CD4+ T cell counts and an increased percentage of circulating CD3+ TCRαβ+CD4–CD8– double-negative T (DNT) cells (10.8%).

A segregation study was performed and his father had elevated double-negative T (DNT) cells (6.9%), low IgM levels, and anemia, with no clinical manifestations of lymphoproliferation. He was sent to a clinical immunologist and an abdominal ultrasound evidenced splenomegaly. The patient's parents are first cousins, and given the consanguinity, a comprehensive familial investigation was initiated. The study included eight additional paternal relatives: the mother, father, half-brother (paternal), grandmother (paternal), two great-aunts, one paternal uncle, and one paternal aunt.

#### 3.3 Genetic sequencing

The patient fulfilled the clinical criteria for ALPS, and the diagnosis was confirmed by Sanger sequencing, which identified the pathogenic variant *FAS*:c.748C>T (p.Arg250Ter) in heterozygosity. Segregation analysis demonstrated that this variant was inherited from the patient's father.

To gain deeper insights into the genetic basis of the disease and to validate the immunophenotypic findings, next-generation sequencing (NGS) was performed using a targeted panel covering part of the genes associated with inborn errors of immunity, representing a subset of those listed by the 2024 IUIS classification (Supplementary Table 1). NGS was conducted on the proband and his parents, identifying variants related to ALPS in the *FAS*, *CASP10*, and *LRBA* genes. These variants were subsequently confirmed by polymerase chain reaction (PCR) with gene-specific primers, followed by Sanger sequencing. Additionally, Sanger sequencing of these variants was performed for all family members included in the study. Electropherogram analyses were conducted using CLC Workbench Software v6.4 (QIAGEN).

#### 3.4 Functional apoptosis assay

A functional apoptosis assay was performed to evaluate FAS-mediated apoptosis in the proband, his father (both with variants in *FAS* and *CASP10*), and his half-brother (*FAS* variant only). PBMCs were isolated via Ficoll gradient, cultured in RPMI-1640 with supplements, and incubated under standard conditions following the protocol provided in the datasheet of the CD95 antibody (BD Biosciences, Cat# 555670). Cells were exposed to control, rProtG, CD95 + rProtG, and monastrol (positive control). After incubation, samples were stained with antibodies (CD3, CD4, CD8, CD27, CD45, CD45RA, TCRγδ, CD5) and analyzed by flow cytometry to assess apoptosis in T effector memory and double-negative T cell subsets.

### 4 Therapeutic intervention

The therapeutic intervention is summarized in Figure 1. The proband received standard treatment with corticosteroids and initially demonstrated a favorable clinical response. However, due to relapses, regular intravenous immunoglobulin (IVIG) therapy was prescribed, at an immunomodulatory starting dose of 25 g, that was adjusted according to the patient's weight. No adverse events were reported. The patient is currently receiving a reduced replacement dose of immunoglobulin (0.6 g/kg/month), following an initial immunomodulatory regimen, with the aim of managing associated immunodeficiency and reducing infection risk, in association with Sirolimus. Sirolimus was introduced in May 2023, with clinical improvement, and no relapses was noted since then. The starting dose was 2 mg/m<sup>2</sup>/day from November 2023 to March 2024, but unfortunately it was stopped due to difficulties to get the medication from the Brazilian health department. The medication was prescribed again in April 2024 at the same starting dose.

### 5 Follow-up and outcomes

The patient has been followed for seven years and he was hospitalized twice during this period, with a total length of stay

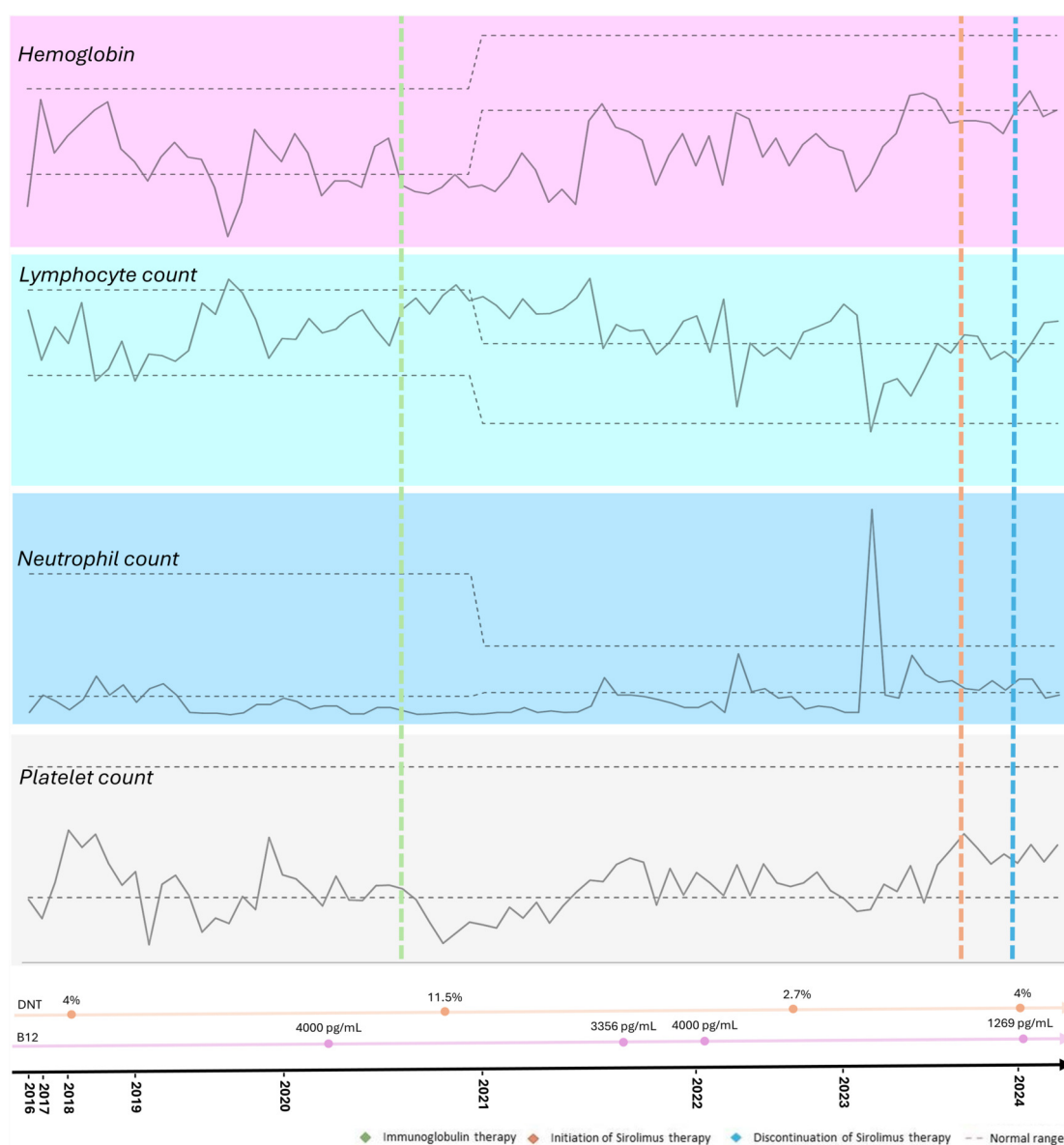


FIGURE 1

Timeline of the proband's hematological parameters from diagnosis of autoimmune lymphoproliferative syndrome (ALPS) to the present, highlighting key clinical events, treatments, and their impact on blood cell counts.

of 20 days. The cause of hospital admissions was febrile neutropenia associated with oral thrush. Intravenous antibiotics were used with good outcomes. Apart from that, he was also treated with oral antibiotics 12 times because of upper respiratory tract infections. The most common antibiotics used were Azithromycin and Amoxicillin-clavulanate.

Relapses of cytopenia were noted but with response to oral corticosteroids, and he also had fluctuating splenomegaly. Since the introduction of sirolimus at the age of 9 years, no abnormal images suggestive of active lymphoproliferative disease or cytopenia in the complete blood count (CBC) has been observed. He is still under IVIG therapy in association with sirolimus with good adherence. No adverse events to the therapy have been noted. The proband is currently undergoing

a reduction in the dose of immunoglobulin due to the use of sirolimus, showing improvements in blood cell counts, decreased lymphoproliferation, without requiring high doses of corticosteroids (Figure 1).

The patient, his father and his half-brother exhibited elevated double-negative T (DNT) cells, whereas the mother had DNT levels within the reference range (Figure 2A). Next-generation sequencing (NGS) analysis of the proband identified a pathogenic variant in *FAS*:c.748C>T (p.Arg250Ter), a variant of uncertain significance in *CASP10*:c.1202\_1208del:p.(Cys401Leufs\*15), both in heterozygosity, with an autosomal dominant inheritance pattern, inherited from the father. Additionally, a variant in *LRBA*:c.2450-7C>T:p.? was detected in heterozygosity, with an autosomal recessive inheritance pattern, inherited from the mother (Figure 2B).

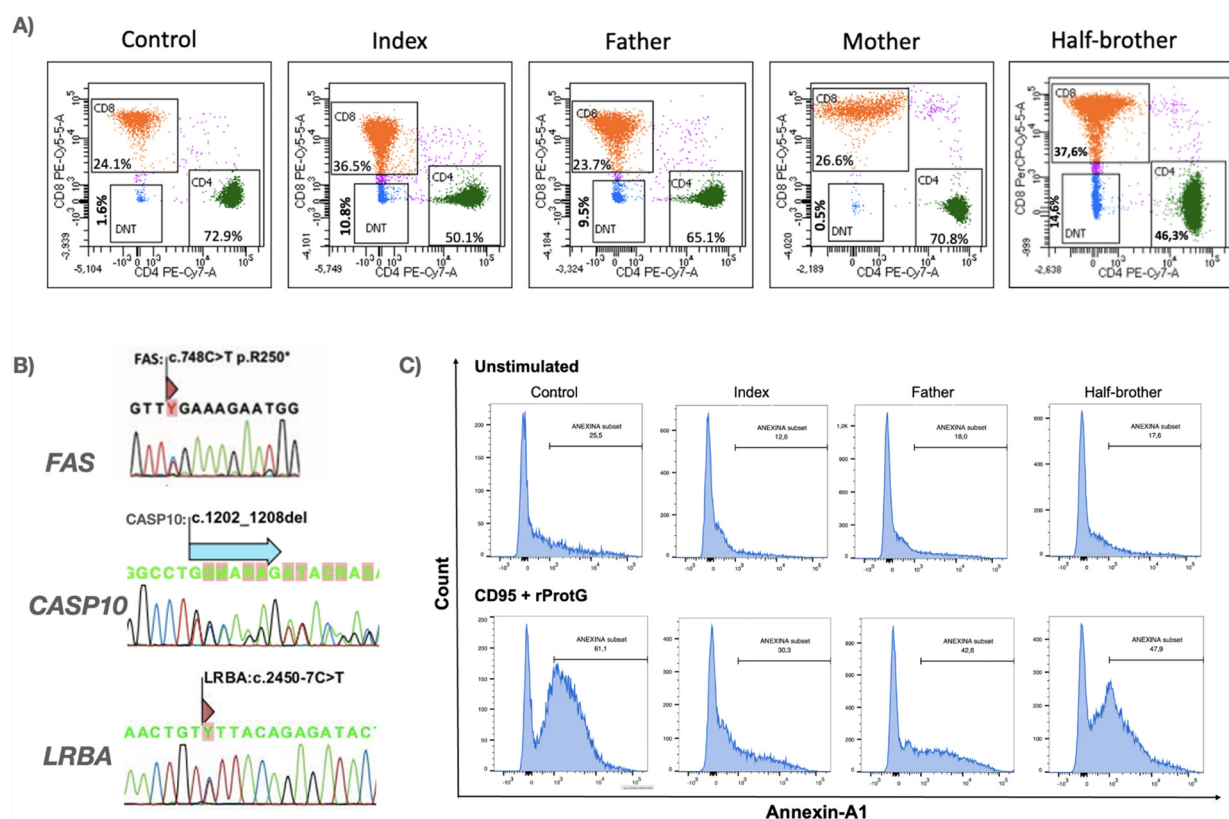


FIGURE 2

Immunophenotypic, molecular, and functional characterization of the proband and his family. (A) Immunophenotyping of circulating lymphocytes demonstrating elevated percentages of CD3+TCR $\alpha/\beta$ +CD4-CD8- double-negative T (DNT) cells in the index case and affected family members. Total lymphocyte counts at the time of immunophenotyping were as follows: index—994/ $\mu$ L, father—1,100/ $\mu$ L, mother—3,072/ $\mu$ L, and half-brother—1,942/ $\mu$ L. (B) Genetic analysis of *FAS*, *CASP10*, and *LRBA* by RT-PCR followed by Sanger sequencing. *FAS* variant (*FAS*:c.748C>T) was identified in all paternal relatives, while a deletion in *CASP10*:c.1202\_1208del) was detected in three paternal family members. The index case and his mother carried a *LRBA* variant (*LRBA*:c.2450-7C>T). (C) Functional apoptosis assay evaluating Fas-mediated apoptotic responses in peripheral blood mononuclear cells (PBMCs). Cells from the index case, relatives, and a healthy donor were stimulated with agonistic anti-Fas antibody CD95 (5  $\mu$ g/100  $\mu$ L) plus rProtG (1  $\mu$ g/100  $\mu$ L) for 21 hours. Apoptosis was assessed via Annexin V staining and flow cytometric analysis of CD45, CD45RA, CD3, CD4, CD8, CD27, and TCR $\gamma/\delta$  markers, revealing defects in apoptotic signaling.

Sanger sequencing of *FAS*, *CASP10* and *LRBA* was performed for all family members included in the study, revealing the presence of the *FAS* variant in all paternal family members ( $n=8$ ; 4 males and 4 females). Additionally, a concomitant *CASP10* variant was identified in half of these individuals ( $n=4$ ; 3 males and 1 female) (Table 1). Correlating molecular findings with clinical presentation and DNT elevation, only male individuals exhibited characteristic symptoms of the disease. Notably, the paternal grandmother, who carried variants in both *FAS* and *CASP10*, remained asymptomatic. Furthermore, disease-associated phenotypic expression varied significantly among male carriers, despite sharing the same variants (Table 1). The proband displayed the most severe clinical manifestations, requiring retherapeutic interventions.

Additionally, memory B cell analysis (CD19<sup>+</sup>CD27<sup>+</sup>), revealed reduced absolute counts in four of the nine family members evaluated—namely, the index case (most markedly), his father, paternal uncle, and grandmother. Three of these individuals had a confirmed ALPS diagnosis, while the grandmother remained asymptomatic. In contrast, the half-brother, despite meeting

diagnostic criteria for ALPS, showed normal memory B cell levels. This finding is consistent with recent evidence indicating that memory B cell reduction is a common immunophenotypic feature in ALPS patients (22).

The functional apoptosis assay further highlighted this pattern of incomplete penetrance. Both the proband and his father carried variants in *FAS* and *CASP10*, while the half-brother harbored only a *FAS* variant. All three exhibited chronic lymphadenopathy. Notably, the apoptotic activity levels varied among them, with the proband exhibiting the most impaired apoptosis, followed by his father and then his half-brother (Figure 2C).

## 6 Discussion

Autoimmune Lymphoproliferative Syndrome (ALPS) is a rare and often underdiagnosed disorder with less than several hundred cases reported globally (8). In Brazil, the number of documented cases remains scarce, with few studies focusing on this condition (23). Since 2020, scientific literature has advanced



TABLE 1 Clinical and laboratory findings of nine Brazilian patients of the same family according to revised 2009 HIH diagnostic criteria for autoimmune lymphoproliferative syndrome.

Patient information			Required criteria		Accessory criteria				Diagnosis status
					Primary		Secondary		
Sample	Family member	Sex	Chronic (>6 mo), nonmalignant, noninfectious lymphadenopathy or splenomegaly or both	Elevated DNT (>2,5% of CD3 +/TCRαβ+)	Defective lymphocyte apoptosis	Somatic or germline pathogenic variants in <i>FAS</i> , <i>FASL</i> or <i>CASP10</i>	Elevated plasma-soluble FAS ligand levels (>200 pg/mL) OR IL-10 (>20 pg/mL) OR vitamin B12 (>1,500 ng/L) OR IL-18 (>500 pg/mL)	Autoimmune cytopenias and elevated IgG level	
ALPS 15	Index	M	Present	Present	Present	<i>FAS/CASP10</i>	Present	Present	Confirmed ALPS
ALPS 37	Father	M	Present	Present	Present	<i>FAS/CASP10</i>	Present	Present	Confirmed ALPS
ALPS 38	Mother	F	Absent	Absent	Absent	Absent	Absent	Absent	Unaffected
ALPS 73	Half-brother	M	Present	Present	Absent	<i>FAS</i>	Absent	Absent	Confirmed ALPS
ALPS 74	Grandmother	F	Absent	Absent	Not Evaluated	<i>FAS/CASP10</i>	Absent	Absent	Unaffected
ALPS 77	Aunt	F	Absent	Absent	Not Evaluated	<i>FAS</i>	Absent	Absent	Unaffected
ALPS 78	Uncle	M	Present	Present	Not Evaluated	<i>FAS/CASP10</i>	Absent	Absent	Confirmed ALPS
ALPS 80	Great aunt	F	Absent	Absent	Not Evaluated	<i>FAS</i>	Absent	Absent	Unaffected
ALPS 85	Great aunt	F	Absent	Absent	Not Evaluated	<i>FAS</i>	Absent	Absent	Unaffected

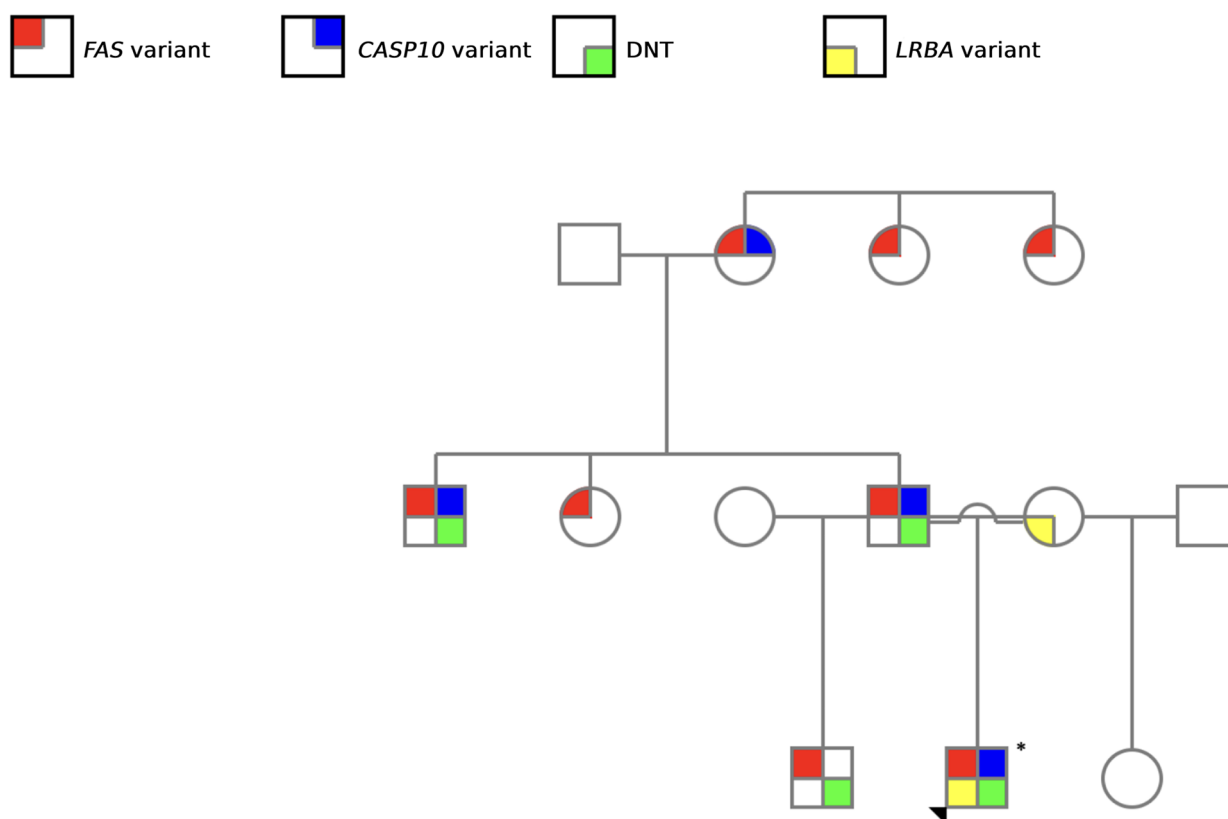


FIGURE 3

Family pedigree illustrating the screened genetic alterations. Red: *FAS* variant; blue: *CASP10* variant; yellow: *LRBA* variant, and green: elevated DNT cells. The arrowhead indicated the proband.

in understanding the mechanisms underlying this condition, revealing intricate interactions between genetic, environmental, and immunological factors that favor its manifestation, but the multifaceted clinical presentation of ALPS requires further understanding (24, 25).

Our study is particularly relevant as it encompasses nine members of the same family, allowing the diagnosis of four ALPS patients based on clinical and molecular criteria (Figure 3). Notably, aside from the proband, all other affected family members were only diagnosed as a result of the comprehensive evaluation undertaken in this study. This highlights the challenges associated with ALPS diagnosis, which arise from the heterogeneity of its clinical manifestations—both in terms of symptom presentation and severity—as well as the complexity of establishing a definitive diagnosis (2, 26).

The variable phenotypic expression observed within the family highlight the importance of molecular and functional assays in addition to clinical criteria. It allows to identify asymptomatic or oligosymptomatic individuals who might otherwise remain undiagnosed. This emphasizes the importance of greater awareness and routine family screening in suspected cases, which can support earlier diagnosis, better patient management, and timely interventions.

This family-based analysis reinforces key characteristics of ALPS previously described in the literature, such as incomplete penetrance and a male predominance in clinical manifestations

(27–29). ALPS has a higher prevalence in males and epidemiological studies suggest a possible interaction between genetic and hormonal factors that favor the manifestation of the disease in this group (16, 30). In our study, only male individuals exhibited clinical signs of the disease, and a striking example was a female family member who, despite carrying both *FAS* and *CASP10* variants, remained asymptomatic.

Although the *FAS* variant identified in this family is located within the death domain, typically associated with autosomal dominant ALPS, our findings illustrate incomplete clinical penetrance, as not all carriers developed symptoms. This variability suggests that, beyond the location and type of variant (31), additional genetic, epigenetic, or environmental factors likely modulate disease expression. The differing clinical presentations among individuals with identical *FAS* and *CASP10* variants underscore the complexity of genotype-phenotype correlations in ALPS and reinforce the need for further studies to elucidate the mechanisms influencing penetrance and disease severity.

Furthermore, the functional apoptosis assay provided additional support for the diagnosis, confirming the pathogenic role of the identified variants. Our findings suggest that additional variants, such as those in *CASP10* and in *LRBA*, may play a key role in modulating apoptosis in ALPS. The *CASP10* variant identified in our study *CASP10*:c.1202\_1208del:p.(Cys401Leufs\*15) has been previously reported and functionally

evaluated, showing reduced protein expression without impairing FAS-mediated apoptosis (10). These findings support the notion that this alteration alone is unlikely to cause ALPS. However, considering the co-occurrence of this variant with a pathogenic FAS variant and the known influence of additional genetic factors on disease penetrance, its potential modifier role cannot be excluded (12, 19).

Although not directly involved in apoptosis like FAS or CASP10, LRBA dysfunction may contribute to immune dysregulation and phenotypic variability. The LRBA variant found in this family, despite being of uncertain significance, may act as a modifier. As LRBA regulates CTLA4 trafficking, its deficiency has been linked to autoimmunity and lymphoproliferation (32, 33), which could help explain the clinical heterogeneity among carriers of the same FAS variant.

The identification and diagnosis of ALPS are critical not only for the treatment of autoimmune manifestations and lymphoproliferation but also for long-term surveillance due to the significantly increased risk of malignancies, particularly lymphomas. It is already known that individuals with heterozygous germline FAS variants have high risk for both non-Hodgkin and Hodgkin lymphomas, with incidences 14 and 51 times greater than the general population, respectively (34, 35). Thus, early diagnosis including clinical evaluation, molecular tests, and functional assays, is crucial for patients survival. Moreover, the persistence of defective FAS-mediated apoptosis in both lymphoid cells and tumor tissues evidences the role of impaired apoptotic pathways as a key factor in lymphomagenesis, justifying the clinical follow-up of asymptomatic carriers of FAS variants (35, 36).

Standard therapeutic management of ALPS includes corticosteroids and immunosuppressive drugs. However, in refractory cases, new approaches have focused on the use of mTOR inhibitors, such as sirolimus. The therapeutic course of the proband underscores the challenges of managing relapsing autoimmune cytopenias and lymphoproliferative disease in ALPS. Initial treatment with corticosteroids provided temporary benefit, but frequent relapses require use of regular IVIG and, later, sirolimus, which led to a sustained remission. This case supports sirolimus as an effective treatment option for ALPS, consistent with previous reports (18).

Clinical studies have shown the efficacy of sirolimus in ALPS, particularly in patients with ALPS-FAS variants, as observed in the proband. A systematic review in India reported that more than half of the 94 ALPS patients evaluated exhibited a fast, complete, and sustained response to sirolimus, with resolution of lymphoproliferation and cytopenias (18). Additionally, sirolimus has a more favorable safety profile compared to corticosteroids and IVIG, which were previously used in the proband's treatment. Given the risks associated with prolonged corticosteroid use—such as metabolic complications, hypertension, and immunosuppression—and the temporary nature of symptom relief provided by immunoglobulin, sirolimus represents a targeted and less toxic alternative (18).

This study highlights the value of integrating genetic testing and immunophenotyping into the diagnostic approach for ALPS, particularly given its clinical and genetic heterogeneity. Identifying

asymptomatic carriers supports the need for long-term monitoring, as these individuals may develop symptoms or transmit the disease. Early diagnosis allows timely interventions, potentially reducing complications. Advances in targeted therapies and a better understanding of genotype-phenotype correlations are key to refining diagnostic criteria and improving patient care.

The patient and his family have been actively engaged in the therapeutic approach, and since sirolimus treatment was initiated, no severe relapses of autoimmune cytopenia have been observed. Consequently, this has led to an overall improvement in their quality of life.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

## Ethics statement

The studies involving humans were approved by Research Ethical Committee (FEPECS-2.332.383). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor (s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

## Author contributions

BG: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. RC: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing. CS: Data curation, Methodology, Writing – original draft, Writing – review & editing. AD: Data curation, Methodology, Writing – review & editing. RP: Data curation, Methodology, Writing – review & editing. AS: Data curation, Methodology, Writing – review & editing. RT: Investigation, Writing – review & editing. FT: Investigation, Writing – review & editing. AA: Investigation, Writing – review & editing. KM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fped.2025.1639749/full#supplementary-material>

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