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De Novo Gain-Of-Function Variations in *LYN* Associated With an Early-Onset Systemic Autoinflammatory Disorder

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Objective. To identify the molecular basis of a severe systemic autoinflammatory disorder (SAID) and define its main phenotypic features, and to functionally assess the sequence variations identified in *LYN*, a gene encoding a non-receptor tyrosine kinase.

Methods. We used targeted next-generation sequencing and in vitro functional studies of Lyn phosphorylation state and Lyn-dependent NF-κB activity after expression of recombinant Lyn isoforms carrying different sequence variations.

Results. We identified a de novo *LYN* variation (p.Tyr508His) in a patient presenting since birth with recurrent fever, chronic urticaria, atopic dermatitis, arthralgia, increased inflammatory biomarkers, and elevated plasma cytokine levels. We studied the consequences on Lyn phosphorylation state of the p.Tyr508His variation and of the 2 *LYN* variations reported so far (p.Tyr508Phe and p.Tyr508*), and found that all 3 variations prevent phosphorylation of residue 508 and lead to autophosphorylation of Tyr397. Additionally, these 3 *LYN* variations activate the NF-κB pathway. These results show a gain-of-function effect of the variations involving Tyr508 on Lyn activity.

Conclusion. This study demonstrates the pathogenicity of the first 3 *LYN* variations identified in SAID patients and delineates the phenotypic spectrum of a disease entity characterized by severe, early-onset, systemic inflammatory disease affecting neonates with no family history of SAID. All 3 *LYN* variations affect the same tyrosine residue located in the C-terminus of Lyn, thereby demonstrating the critical role of this residue in the proper regulation of Lyn activity in humans.

INTRODUCTION

Lyn, a member of the Src family of protein tyrosine kinases, plays an important role in the regulation of innate and adaptive immune responses (1). Expressed in immune cells (except T lymphocytes) and in nonhematopoietic cells (1), Lyn is involved in

either activation or inhibition of pivotal signaling pathways, such as those dependent on phosphoinositide 3-kinase (PI3K), MAPK, and NF-κB (2,3). Lyn is a nonreceptor kinase conserved throughout evolution. Its activity is tightly regulated by key posttranslational modifications. Lyn phosphorylation at Tyr508 keeps the molecule in an inactive closed conformation, whereas

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dephosphorylation of this residue allows its activation (4) through the subsequent autophosphorylation of Tyr397, which in turn, greatly enhances Lyn enzymatic activity (1). These consequences on Lyn hyperactivation were initially assessed in vivo in a Lyn^{up/up} knockin mouse model of mice carrying a homozygous mutation at Tyr508 (p.Tyr508Phe, also known as Y508F) (5). Lyn^{up/up} mice developed hemolytic anemia, severe autoimmune glomerulonephritis, and circulating autoreactive antibodies (5–7).

In humans, 2 patients carrying a de novo *LYN* variation involving Tyr508 (p.Tyr508Phe and p.Tyr508*) and presenting with an early-onset systemic autoinflammatory disorder (SAID) have been reported (8,9). While the effect of the missense variation p.Tyr508Phe has been studied for the murine Lyn protein, there is so far no study on the functional impact of the nonsense variation p.Tyr508*.

Herein, we report on a patient carrying a novel de novo *LYN* missense variation (p.Tyr508His) and investigate the functional consequences (Lyn phosphorylation state and Lyn-dependent NF- κ B activation) of all 3 variations affecting Tyr508 in humans (p.Tyr508His, p.Tyr508Phe, and p.Tyr508*). Comparison of the phenotypic features of our patient with those of the 2 patients reported previously allowed us to draw a comprehensive description of the *LYN*-associated SAID phenotype.

PATIENT AND METHODS

Patient. Clinical features were collected through a standardized form. Written informed consent was obtained from the patient's parents for genetic tests, data sharing, and publication of the patient's photographs according to French law and the principles of the Declaration of Helsinki.

Molecular studies. Genomic DNA was extracted from peripheral blood leukocytes of the patient and her parents. Next-generation sequencing (NGS) was performed using a custom sequence capture (SeqCap EZ Choice system; Roche NimbleGen) of the exons and flanking intronic sequences of the main SAID-causing genes (i.e., *ADA2*, *AP1S3*, *CARD14*, *IL1RN*, *IL36RN*, *LACC1*, *LPIN2*, *LYN*, *MEFV*, *MVK*, *NCSTN*, *NLRC4*, *NLRP1*, *NLRP12*, *NLRP3*, *NOD2*, *FAM105B*, *PLCG2*, *POMP*, *PSMA3*, *PSMB4*, *PSMB8*, *PSMB9*, *PSTPIP1*, *RBCK1*, *RELA*, *SH3BP2*, *SLC29A3*, *STING1*, *TNFAIP3*, *TNFRSF1A*, *TNFRSF11A*, *WDR1*). Sequencing was performed on a Next-Seq500 (Illumina) platform according to the manufacturer's instructions. The bioinformatics pipeline, previously described (10), was used to detect germline and somatic variations. The results obtained by NGS were confirmed by Sanger sequencing.

LYN exon 13 was amplified by polymerase chain reaction (PCR) and analyzed by Sanger sequencing using a BigDye Terminator sequencing kit (Applied Biosystems) on an ABI 3730XL automated capillary DNA sequencer (Applied Biosystems). Sequences were analyzed against the *LYN* reference sequence (NM_002350).

Microsatellite markers (D3 S1292, D1 S249, D2 S117, D4 S406, D5 S426, and DX S991) were amplified by PCR using fluorescent-labeled primers. The PCR products were analyzed on an ABI 3730XL sequencer (Applied Biosystems).

Plasmid constructs. After reverse transcription using a Transcriptor High Fidelity cDNA Synthesis Kit (Roche), the full-length *LYN* complementary DNA (NM_002350), which was obtained from the control's immortalized B cells, was subjected to PCR amplification using Q5 Hot Start High-Fidelity 2X Master Mix (BioLabs) and was cloned into a pcDNA3.1/V5-His TOPO TA Expression Kit (Invitrogen) following the manufacturer's instructions. Site-directed mutagenesis was performed on the wild-type (WT) *LYN* expression vector (pLYN-WT) to generate the plasmids carrying the Lyn missense and nonsense variations pLYN-Y508H, pLYN-Y508F, and pLYN-Y508*.

Cell culture conditions and transfections. A HEK293T cell line was cultured in DMEM GlutaMAX medium (Invitrogen) supplemented with 10% fetal bovine serum and penicillin/streptomycin (Invitrogen) and maintained at 37°C in a 5% CO₂ atmosphere. HEK293T cells were transfected with different amounts of the above-mentioned plasmids using FuGENE HD transfection reagent (Promega) with a ratio of 1:3 following the manufacturer's instructions. After 24 hours, the transfected cells were collected for immunoblotting or luciferase assay.

Cell lysates preparation. HEK293T cells were lysed in cold lysis buffer (50 mM Tris, 100 mM NaCl, 5 mM EDTA, 1% Triton X-100, phosphatase inhibitor [Roche Diagnostics]) and complete protease inhibitor cocktail (Roche Diagnostics). Lysates were incubated at 4°C under agitation for 1 hour and cleared by 10 minutes of centrifugation at 1,000g and 4°C, then 4× Laemmli buffer (0.2 M dithiothreitol, 0.25 M Tris, 8.4% sodium dodecyl sulfate, 40% glycerol) was added for a final concentration of 1×.

Immunoblotting. Cell lysates were loaded on sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred onto a nitrocellulose membrane, blocked in Tris buffered saline–Tween 0.1% with 5% bovine serum albumin (designated as TBST-BSA 5%) for 1 hour and analyzed by Western blotting with the following antibodies: rabbit anti-human Lyn (#2732; Cell Signaling Technology), phospho-(Y508) Lyn (#2731; Cell Signaling Technology), phospho-(Y397) Lyn (#70926; Cell Signaling Technology) and anti-GAPDH–horseradish peroxidase (MA5-15738-HRP; ThermoFisher). All antibodies were incubated overnight at 4°C in TBST-BSA 5%. The secondary antibody (anti-rabbit IgG–horseradish peroxidase; product no. A0545; Sigma-Aldrich) was incubated for 1 hour at room temperature in TBST-BSA 5%. Proteins were detected using Amersham ECL Select Western Blotting Detection Reagent (GE Healthcare) according to the manufacturer's recommendations.

NF- κ B luciferase reporter assay. HEK293T cells were cotransfected in 12-well plates with the following plasmids: 200 ng of pcDNA3 empty vector or each of the *LYN* expression plasmids, 100 ng of pGL4.32[*luc2P/NF- κ B-RE/Hygro*] vector (Promega), and 10 ng of *Renilla* luciferase control reporter vector (Promega) for normalization. Cells were lysed 24 hours posttransfection and subjected to dual luciferase reporter assays (Promega) using a Tristar LB 941 Multimode Microplate Reader (Berthold Technologies). The firefly luciferase activity was normalized against the *Renilla* signal. Relative firefly/*Renilla* signals are reported.

Statistical analyses. Differences were analyzed using the Student's unpaired 2-tailed *t*-test and were plotted with the Graph-Pad Prism 9 software. A *P* value less than 0.05 was considered statistically significant. Data are presented as the mean \pm SD.

RESULTS

Clinical and biologic phenotype. The patient is a 4-year-old girl born from healthy parents at 26 weeks and 2 days of gestation. Her premature birth was attributable to maternal HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome during pregnancy, and the pregnancy was marked by a second trimester fetal/intrauterine growth restriction (<5th percentile fetal growth size) with cardiomegaly and hepatomegaly associated with persistent Arantius' duct and liver microcalcifications.

The patient's birth weight was 925 gm, birth length was 33.6 cm, head circumference was 25.5 cm, and Apgar scores were 5 at 1 minute and 7 at 5 minutes. At birth, she presented with cardiomegaly, a dilated ascending aorta (without bicuspidy), cholestasis, and hepatosplenomegaly with liver calcifications, as well as several complications related to prematurity (hyaline membrane disease in spite of steroids administered to the mother during pregnancy, sensitive abdominal bloating evocative of enteropathy, and a secondary infection with *Staphylococcus capitis* at 3 weeks of age).

The patient was intubated from birth to day 3, after which non-invasive ventilation (day 3 to day 7) and high-flow oxygen therapy (day 7 to day 21) were required. She received a surfactant (poractant alfa) in the first hours of life due to hyaline membrane disease and received caffeine to prevent apnea. Antibiotic therapy was started due to biologic inflammation (serum C-reactive protein [CRP] level of 40 mg/liter) from birth. Testing for maternal-fetal infection remained negative. The mother was immunized against rubella and cytomegalovirus, and maternal serologic status was negative for toxoplasmosis, syphilis, and HIV. Elevated serum CRP levels (15–63 mg/liter) were reported repeatedly during the patient's first month of life despite the use of several types of antibiotics. She developed recurrent and severe episodes (~1 episode per month) of systemic inflammation lasting ~3–4 days (serum CRP levels up to 366 mg/liter and hyperleukocytosis [white blood cell count 37×10^9 /liter]), with fever, urticarial lesions and atopic dermatitis, arthritis (joint redness and swelling affecting knees and ankles), abdominal pain, and moderate diarrhea. Additionally, acute asthma

Table 1. Genotype and phenotype of 3 patients identified as carrying a de novo *LYN* variation resulting in an early-onset autoinflammatory disorder*

	Patient in the present study	Patient in De Jesus et al 2014 (ref. 8)	Patient in De Jesus et al 2016 (ref. 9)
<i>LYN</i> variation	c.1522T>C, p.(Tyr508His)	c.1524C>G, p.(Tyr508*)	c.1523A>T, p.(Tyr508Phe)
Transmission	de novo	de novo	de novo
Sex	Female	Male	Male
Birth term	26 weeks, 2 days of gestation	31 weeks of gestation	NA
Age at onset	Birth	Birth	Birth
Age at diagnosis	2 years	3 years	15 years
Fever	Yes	Yes	Yes
Facial characteristics	Frontal bossing, hypertelorism, exophthalmia, low set ears	NA	NA
Skin involvement	Urticarial lesions, atopic dermatitis	Purpuric skin rash (neutrophilic vasculitis)	Purpuric skin rash
Hepatosplenomegaly	Yes	Yes	NA
GI involvement	Abdominal pain, diarrhea	NA	Abdominal pain
Ocular involvement	No	Periorbital erythema	Conjunctival erythema
CNS involvement	No	NA	Headaches
Joint involvement	Arthritis	NA	Arthralgia
Other clinical signs	Neonatal cardiomegaly, cholestasis and liver calcifications, severe growth delay, asthma	Hydrops fetalis, testicular pain and swelling, splenectomy (9 months of age), vanishing bile duct disease	Testicular pain, oral ulcers, fatigue
Biologics	Elevated CRP, hyperleukocytosis	Elevated CRP, anemia, severe thrombocytopenia, increased liver enzymes, circulating autoantibodies	Elevated CRP and serum amyloid A
Treatment	Canakinumab 6 mg/kg every 15 days	Oral steroids, dasatinib	Unresponsive to anakinra and tocilizumab; partial response to colchicine, etanercept

* NA = not available; GI = gastrointestinal; CNS = central nervous system; CRP = C-reactive protein.

episodes required several hospitalizations, including 1 hospitalization in intensive care (Table 1, Figure 1A).

At 6 months, she presented with growth retardation (-4 SD on the growth curve) and a psychomotor delay. She started walking at 2 years and 4 months. As shown in Figure 1A, at the age of 14 months, she had distinctive facial features with frontal bossing and hypertelorism. Glucocorticoids (1 mg/kg/day) were started at 19 months after a major inflammatory episode (serum CRP level of 238 mg/liter). Because a relapse occurred after reducing the daily dose, anti-interleukin-1 (anti-IL-1) treatment with anakinra (4 mg/kg/day) was introduced but stopped after cutaneous reaction at the injection point; therefore, canakinumab treatment

(6 mg/kg every 15 days) was started at the age of 2 years, which was effective on clinical symptoms. However, residual biologic inflammation persisted (CRP levels ranging 6–55 mg/liter and hyperleukocytosis [white blood cell count ranging 12.9×10^9 – 17.8×10^9 /liter]) (Figure 1B).

At the age of 2 years, immunophenotyping of peripheral blood lymphocytes revealed B cell and T cell lymphocytosis (B cell count 2,970/ μ l and T cell count 9,035/ μ l), whereas the number of natural killer cells was in normal range. Plasma cytokine measurements performed just before the age of 3 years (blood sampled the day before the next canakinumab injection) revealed a substantial increase in proinflammatory (IL-1 β , IL-6, tumor necrosis factor

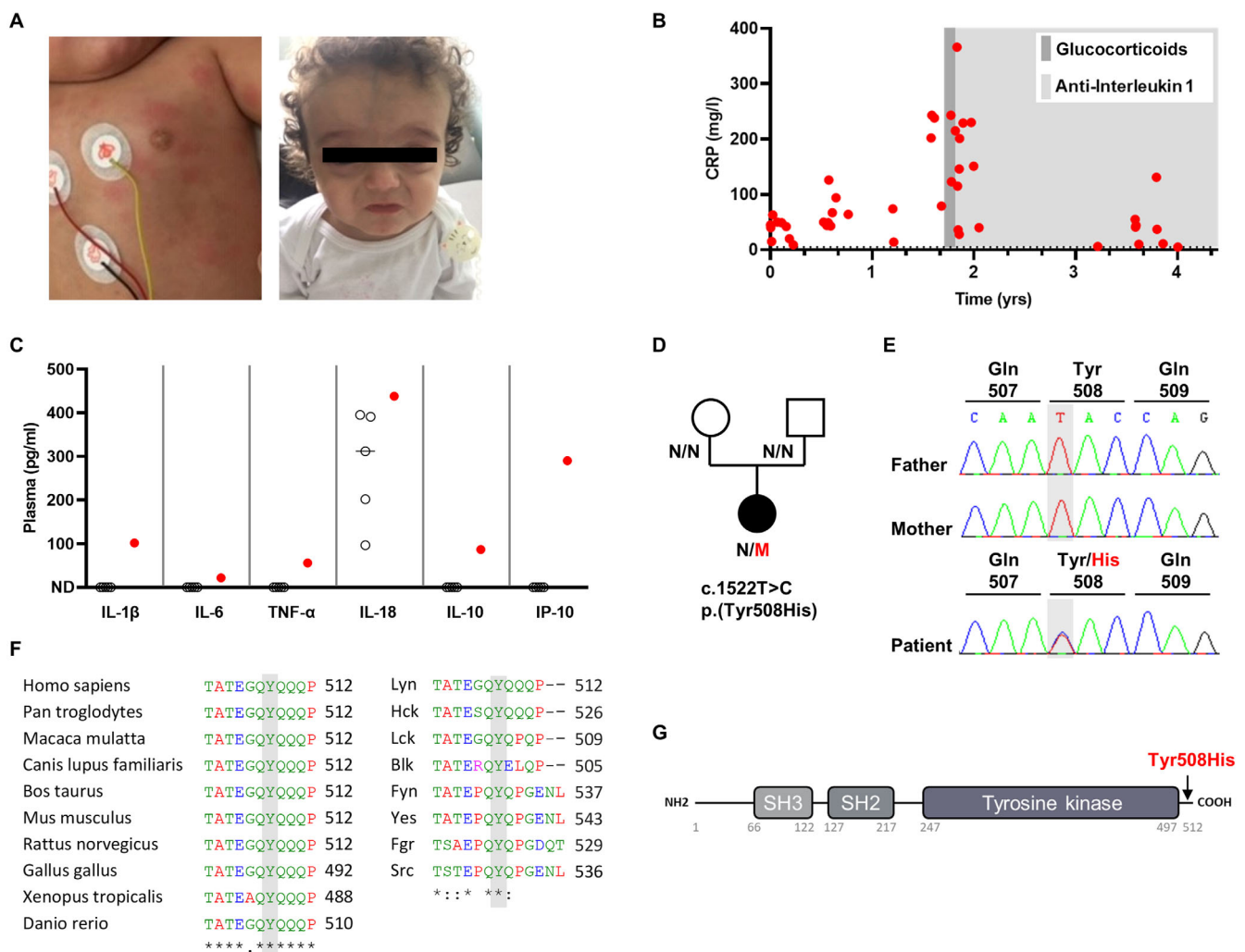


Figure 1. Clinical and genetic description of the patient with a de novo *LYN* variation. **A**, Photograph of the patient at age 7 months (left) and 14 months (right) showing urticarial lesions and distinctive facial features. **B**, Plot showing serum C-reactive protein (CRP) levels measured in the patient at different time points from birth to age 4 years and showing periods of time the patient was receiving glucocorticoids or anti-interleukin-1 treatment. **C**, Enzyme-linked immunosorbent assay–assessed plasma cytokine levels in the patient just before the age of 3 years (red circles) and in 5 healthy donors (open circles). The horizontal line represents the mean of the 5 interleukin-18 (IL-18) values. **D**, Genealogic tree of the patient with her *LYN* genetic status. **E**, Sanger sequencing electropherogram of genomic DNA extracted from peripheral blood leukocytes of the patient and her parents, showing the *LYN* c.1522T>C, p.(Tyr508His) variation present in the heterozygous state in the patient and absent in her parents. **F**, Evolutionary conservation of *Lyn* protein amino acid Tyr508 across species (left) and in other members of the Src family of protein tyrosine kinases (right). **G**, Representation of *Lyn* with domain organization showing the Tyr508His variation.

[TNF], IL-18, and interferon-inducible protein 10 [IP-10]) and anti-inflammatory (IL-10) cytokines relative to 5 healthy donors (Figure 1C). After starting anti-IL-1 therapy, the patient's growth delay was gradually and partly improved (-4 SD until the age of 2 years, -3.5 SD at the age of 3 years, -2 SD at the age of 4 years).

Identification of a de novo *LYN* variation. Study of genomic DNA from the patient's peripheral blood cells through an NGS-based approach (SAID gene panel) allowed identification of the c.1522T>C, p.(Tyr508His) missense variation in the *LYN* gene (herein referred to as Y508H) (Figures 1D and E). Sequencing of parental DNA samples associated with the intrafamilial segregation study of 6 independent microsatellite markers revealed that the Y508H variation occurred de novo. This variation, which is absent from the genome aggregation database (gnomAD), is predicted to be pathogenic according to a Combined Annotation Dependent Depletion (CADD) phred score of 31. Tyr508 is a residue conserved across species as well as among all 7 other members of the Src tyrosine kinase family (Figure 1F), suggesting that amino acid substitutions at this position could be detrimental to Lyn function. In addition, Tyr508 is located in the C-terminal sequence known to be involved in the negative regulation of Lyn activity in mice (5). Notably, the same tyrosine residue is mutated in the mouse model of sustained constitutive activation of Lyn (Lyn^{up/up}) and in the 2 patients previously reported to be carrying de novo *LYN* variation involving Tyr508 (Table 1). Taken together, these data strongly suggest that the Y508H variation is a disease-causing mutation, a conclusion consistent with the classification of this variation according to the guidelines of the American College of Medical Genetics and Genomics (i.e., the PS2, PM1, PM2, PM5, and PP3 criteria can indeed be applied to this de novo mutation, thereby allowing us to classify it as pathogenic) (11).

Functional characterization of *LYN* variations identified in SAID patients. To assess the functional consequences of the *LYN* Y508H, Y508F, and Y508* variations, we studied the phosphorylation state of recombinant forms of human Lyn carrying those variations and compared the results to those obtained with wild-type Lyn (Lyn-WT). Of note, although the Y508* variation introduces a premature stop codon, it should escape the nonsense-mediated messenger RNA (mRNA) decay pathway because of its localization in the last exon of *LYN* (12). After expression of Lyn-WT and each of the mutated Lyn proteins (Lyn-Y508H, Lyn-Y508F, and Lyn-Y508*) in HEK293T cells, we observed an absence of phosphorylation at position 508 and a constitutive phosphorylation of Tyr397 for all mutated proteins (Figure 2A and Supplementary Figure 1, available on the *Arthritis & Rheumatology* website at <https://onlinelibrary.wiley.com/doi/10.1002/art.42354>). Overall, these data clearly show that all 3 variations involving Tyr508 maintain Lyn in its active form, consistent with the gain-of-function effect of the Y508H, Y508F, and Y508* variations.

Furthermore, we sought to evaluate the impact of all 3 *LYN* variations on the NF- κ B signaling pathway by means of a luciferase reporter assay performed under basal conditions. These experiments revealed that, while Lyn-WT did not activate the NF- κ B pathway, all 3 variations led to the activation of this pathway (Figure 2B).

Phenotypic spectrum of *LYN*-associated SAID. With the aim of identifying the main phenotypic features associated with Lyn gain of function, we analyzed the phenotype of the patient reported in our study and compared it to the available phenotypic data of the other 2 individuals previously reported as having a de novo *LYN* variation (Y508F and Y508*) (8,9). The phenotypic data are summarized in Table 1. Two of the 3 patients

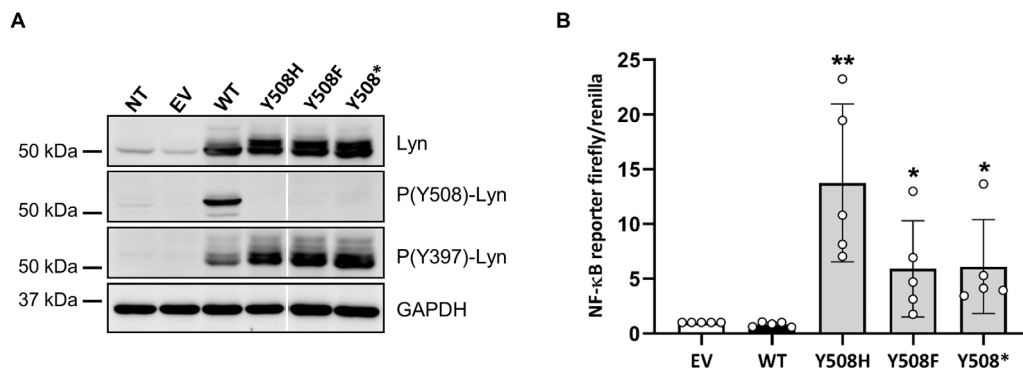


Figure 2. Functional characterization of identified *LYN* variations. **A**, Immunoblot analysis of Lyn, phospho-Y508 Lyn [P(Y508)-Lyn], phospho-Y397 Lyn [P(Y397)-Lyn], and GAPDH (as loading control) in HEK293T cells transiently expressing wild-type (WT) Lyn or each of the mutated Lyn proteins (Lyn-Y508H, Lyn-Y508F, or Lyn-Y508*). Immunoblot analysis represents ≥ 3 independent experiments. **B**, Impact of each Lyn variation on NF- κ B activity. HEK293T cells were transfected with 200 ng of the expression vector encoding wild-type Lyn or each of the mutated Lyn proteins (Lyn-Y508H, Lyn-Y508F, or Lyn-Y508*), or an empty vector (EV) as control, together with an NF- κ B reporter construct (100 ng) and a *Renilla* luciferase control reporter vector for normalization (10 ng). Data represent 5 independent experiments. Values on the vertical axis are the relative firefly/*Renilla* signal. Circles represent individual experiments. Bars are the mean; whiskers show the SD. * = $P \leq 0.05$ and ** = $P \leq 0.01$ by Student's unpaired 2-tailed *t*-test. NT = not transfected.

were born prematurely, and no birth term information is available for the third patient (9). All 3 patients presented with severe inflammatory disease with neonatal onset, characterized by recurrent fever, skin manifestations (such as purpuric skin rash, urticarial lesions, and atopic dermatitis), and arthralgia and/or arthritis, and disease was associated with elevated biologic inflammation markers (high CRP levels and hyperleukocytosis). Other manifestations were observed in 2 of the patients: ocular manifestations (2 of 3 patients), hepatosplenomegaly (2 of 2 patients with available data), abdominal pain (2 of 2 patients with available data), and testicular pain (2 of 2 male patients). Anemia and severe thrombocytopenia requiring splenectomy, as well as circulating autoantibodies and liver damage (increased liver enzymes and, at biopsy a periportal lymphocytic infiltrate, vanishing bile duct disease, and periportal bridging fibrosis) were reported only in the patient carrying the Y508* variation. Of note, our patient presented with facial features reminiscent of CINCA (chronic infantile neurologic, cutaneous, articular) syndrome, which is caused by *NLRP3* variations, that were not reported in the 2 previous patients with a de novo *LYN* variation. Moreover, as mentioned above, the patient reported in the current study also displayed increased plasma levels of proinflammatory (IL-1 β , IL-6, TNF, IL-18, IP-10) and antiinflammatory (IL-10) cytokines (Figure 1C); no cytokine data were available for the other patients.

Therapeutic attempts in *LYN*-associated SAID.

Treatment regimens were different for each patient. The first reported patient with the Y508* variation was treated efficiently with dasatinib (8), a tyrosine kinase inhibitor traditionally used for chronic myeloid leukemia. No response to anti-IL-1 therapy was reported for the patient with the Y508F variation (9), whereas anakinra and canakinumab have shown efficacy on the inflammatory symptoms of our patient who carries the Y508H variation.

DISCUSSION

SAIDs represent a group of diseases that are highly heterogeneous at both the phenotypic and genetic level. The data presented in the current study clearly contribute to the recognition of *LYN* as a SAID-associated gene that should be screened for variations in patients with unexplained, early-onset inflammatory symptoms and no family history of SAID.

The patient reported here carries a de novo *LYN* variation (Y508H) affecting a critical tyrosine residue that modulates Lyn activity. Using in vitro functional assays, we demonstrated the gain-of-function effect on Lyn function of the Y508H variation as well as of the other 2 *LYN* variations (Y508F and Y508*) previously reported in 2 patients (8,9). Indeed, Y508*, which introduces a premature stop codon, does not result in a loss of protein function. Because of its localization in the last exon of *LYN*, it is expected to escape nonsense-mediated mRNA decay, and as

shown by this study, the resulting truncated protein, which lacks 5 residues of the C-terminus of the protein, is activated. It is also noteworthy that all 3 patients carry variations involving the same amino acid. This striking observation underscores the critical role played by Tyr508 in the regulation of Lyn activity.

By studying Lyn phosphorylation under basal conditions, we demonstrated that all 3 mutated proteins involving Y508 (Lyn-Y508H, Lyn-Y508F, Lyn-Y508*) were phosphorylated at Tyr397, consistent with a hyperactive form of the protein. Moreover, we observed an activation of the NF- κ B signaling pathway in basal conditions confirming the constitutive gain of function of the Lyn proteins carrying those variations. It should be noted that these data, which demonstrate the pathogenicity of these 3 Lyn variations, were obtained after expressing the corresponding recombinant Lyn proteins in a human HEK293T cell line. Therefore, it remains possible that, under in vivo conditions, these mutations may have unknown cell type-specific effects.

The 3 patients with *LYN* gain-of-function variations share clinical and biologic features of systemic inflammation, i.e., recurrent fever, arthralgia, skin manifestations, hepatosplenomegaly, and abdominal pain, as well as hyperleukocytosis and elevated CRP levels. Notably, many of these symptoms, including severe neonatal inflammatory syndrome and urticarial lesions, overlap with those observed in CINCA syndrome, a SAID due to de novo gain-of-function variations in *NLRP3* (10). In addition, it should be emphasized that the anti-IL-1 treatment canakinumab, effective in our patient, also shows sustained efficacy in CINCA syndrome (13). Such similarities between CINCA syndrome and *LYN*-associated SAID should prompt physicians and molecular geneticists to look for *LYN* variations in patients presenting with symptoms indicative of CINCA syndrome and having no identifiable variations in *NLRP3*.

It should also be noted that 2 of the 2 patients with a gain-of-function mutation in *LYN* who had available data for birth term were born prematurely, raising the question as to whether such *LYN* defects predispose to miscarriage or premature delivery, or contribute to the rarity of *LYN*-associated SAID. The report of additional patients and families with disease-causing variations in *LYN* should help answer these questions.

In the Lyn^{HP/UP} mouse model with a *Lyn* gain-of-function mutation (Y508F), the adult mice presented with severe autoimmune disease characterized by circulating autoantibodies and severe glomerulonephritis (7). Low titers of circulating autoantibodies have been reported in 1 of the *LYN*-associated SAID patients (8). As the patient of this study is young (4 years old), follow-up is necessary to detect the possible occurrence of an autoimmune disease.

In conclusion, the functional characterization of the 3 *LYN* variations so far identified, together with the description of the associated phenotypes, allowed for development of a comprehensive description of the disease entity resulting from *LYN* gain-of-function variations. To improve the severe and persistent

inflammatory syndrome associated with *LYN* variations, sustained treatment (including anti-IL-1 therapy) is required. Such de novo occurrence of sequence variations in *LYN* also has important implications for genetic counseling.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. Giurgea and Amselem had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Louvrier, El Khouri, Karabina, Amselem, Giurgea.

Acquisition of data. Louvrier, Grall Lerosey, Quartier, Guerrot, Bader Meunier, Chican, Mohammad, Piterboth, Giurgea.

Analysis and interpretation of data. Louvrier, El Khouri, Chican, Assrawi, Daskalopoulou, Arenas Garcia, Copin, Piterboth, Dastot Le Moal, Amselem, Giurgea.

REFERENCES

1. Ingle E. Functions of the Lyn tyrosine kinase in health and disease. *Cell Commun Signal* 2012;10:21.
2. Scapini P, Pereira S, Zhang H, et al. Multiple roles of Lyn kinase in myeloid cell signaling and function [review]. *Immunol Rev* 2009;228:23–40.
3. Tsantikos E, Gottschalk TA, Maxwell MJ, et al. Role of the Lyn tyrosine kinase in the development of autoimmune disease. *Int J Clin Rheumatol* 2014;9:519–35.
4. Hermiston ML, Zikherman J, Zhu JW. CD45, CD148, and Lyp/Pep: critical phosphatases regulating Src family kinase signaling networks in immune cells [review] [published correction appears in *Immunol Rev* 2009;229:387]. *Immunol Rev* 2009;228:288–311.
5. Harder KW, Parsons LM, Armes J, et al. Gain- and loss-of-function Lyn mutant mice define a critical inhibitory role for Lyn in the myeloid lineage. *Immunity* 2001;15:603–15.
6. Slavova-Azmanova NS, Kucera N, Satiaputra J, et al. Gain-of-function Lyn induces anemia: appropriate Lyn activity is essential for normal erythropoiesis and Epo receptor signaling. *Blood* 2013;122:262–71.
7. Hibbs ML, Harder KW, Armes J, et al. Sustained activation of Lyn tyrosine kinase in vivo leads to autoimmunity. *J Exp Med* 2002;196:1593–604.
8. De Jesus AA, Montealegre G, Liu Y, et al. A de novo nonsense mutation in the tyrosine kinase Lyn in a patient with an early onset autoinflammatory phenotype. *Pediatr Rheumatol Online J*; 2014; 12:O25.
9. De Jesus AA, Montealegre GA, Freeman H, et al. Mutations in the tyrosine-protein kinase Lyn cause an early-onset neutrophilic vasculitis syndrome [abstract]. *Arthritis Rheumatol* 2016;68(suppl 10). URL: <https://acrabstracts.org/abstract/mutations-in-the-tyrosine-protein-kinase-lyn-cause-an-early-onset-neutrophilic-vasculitis-syndrome/>.
10. Louvrier C, Assrawi E, El Khouri E, et al. NLRP3-associated autoinflammatory diseases: phenotypic and molecular characteristics of germline versus somatic mutations. *J Allergy Clin Immunol* 2020; 145:1254–61.
11. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405–24.
12. Kurosaki T, Maquat LE. Nonsense-mediated mRNA decay in humans at a glance [review]. *J Cell Sci* 2016;129:461–7.
13. Sibley CH, Chioato A, Felix S, et al. A 24-month open-label study of canakinumab in neonatal-onset multisystem inflammatory disease. *Ann Rheum Dis* 2015;74:1714–9.