Successful treatment of *JAK1* associated inflammatory disease

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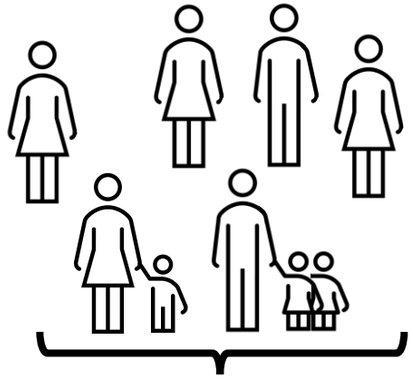
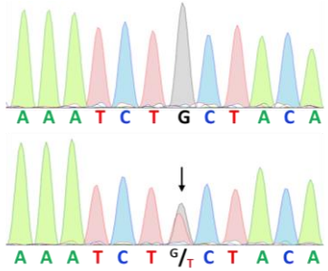
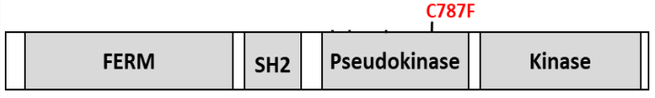
C787F *JAK1* mutation

A large French family



Dominant autosomal transmission





Main features

Atopic dermatitis

Allergies & asthma

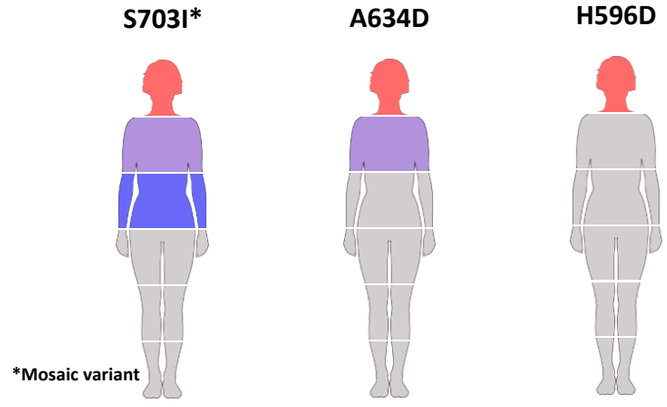
Chronic diarrhea

Calcifying fibrous tumors

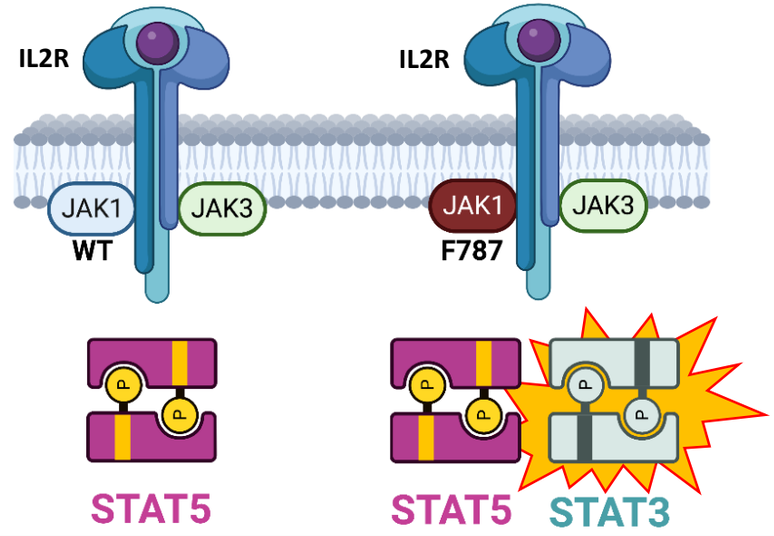
Arthralgia

Basophilia

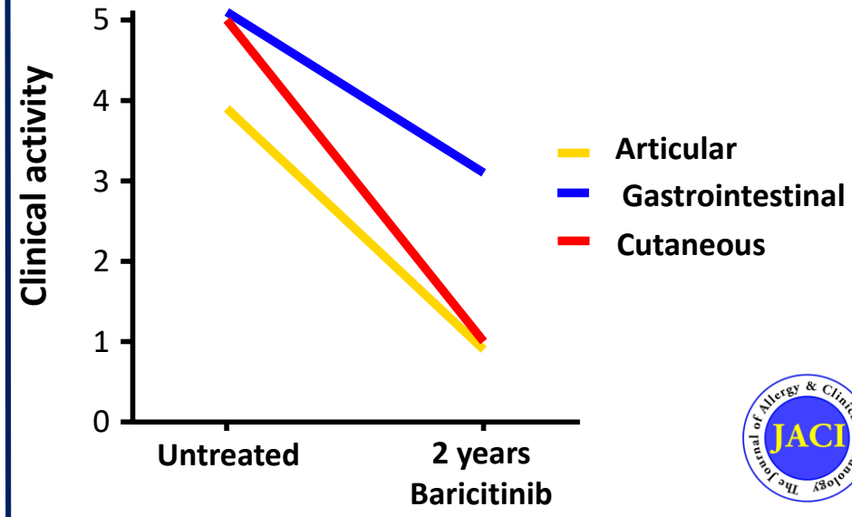
Other *JAK1* mutations



Atypical phosphorylation of STAT3 in 5 out of 6 patients



Improved by baricitinib



Successful treatment of *JAK1* associated inflammatory disease

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Background: Gain of function (GOF) variants of JAK1 drive a rare immune dysregulation syndrome associated with atopic dermatitis, allergy and eosinophilia.

Objectives: To describe the clinical and immunological characteristics associated with a new GOF variant of *JAK1* and report the therapeutic efficacy of JAK inhibition.

Methods: We identified a family affected by *JAK1* associated autoinflammatory disease and performed clinical assessment and immunological monitoring on 9 patients. JAK1 signalling was studied by flow and mass cytometry in patients' cells at basal state, or after immune stimulation. A molecular disease signature in the blood was studied at the transcriptomic level. Patients were treated with one of two JAK inhibitors; either baricitinib or upadacitinib. Clinical, cellular, and molecular response were evaluated over a 2-year period.

Results: Affected individuals displayed a syndromic disease with prominent allergy including atopic dermatitis, ichthyosis, arthralgia, chronic diarrhoea, disseminated calcifying fibrous tumours and elevated whole blood histamine levels. A variant of *JAK1* localized in the pseudokinase domain was identified in all 9 affected tested patients. Hyper-phosphorylation of STAT3 was found in 5 out of 6 patients tested. Treatment of patients' cells with baricitinib controlled most of the atypical hyper-phosphorylation of STAT3. Administration of baricitinib to patients led to rapid improvement of the disease in all adults and was associated with reduction of systemic inflammation.

Conclusions: Patients with this new *JAK1* GOF pathogenic variant displayed very high levels of blood histamine and showed a variable combination of atopy with articular and gastrointestinal manifestations as well as calcifying fibrous tumours. The disease, that appears to be linked to STAT3 hyper-activation, was well controlled under treatment by JAK inhibitors in adult patients.

Clinical implication – This study significantly expands the clinical spectrum of *JAK1* associated autoinflammatory disease and report the clinical benefit of two distinct JAK inhibitors over a 2-year period in a large family.

Capsule summary– This study opens new avenues in the diagnosis and treatment of JAK1 associated autoinflammatory disease. It should help to reduce diagnostic delay of *JAK1* mutated patients.

Keywords: JAK 1, atopic dermatitis, JAK inhibitors, inborn errors of immunity, allergy

Abbreviations

CyTOF: cytometry by time of flight = mass cytometry
CT-scan: computer tomography scan
ENT: ear-nose-throat
FACS: fluorescence activated cell sorting
GOF: gain of function
IBD: inflammatory bowel disease
IFN: interferon
IL: interleukin
JAK: Janus kinase
JAID: JAK1 associated inflammatory disease
NGS: next generation sequencing
PBMCs: peripheral blood mononuclear cells
STAT: signal transducer and activator of transcription

Introduction:

Cytokines are soluble effector proteins produced by a variety of cells of hematopoietic or stromal origin that act as key regulators of both the innate and the adaptive immune response. As such, a significant number of pathogenic variants of cytokines, receptors to cytokines or proteins involved in downstream signalling, such as proteins of the Janus kinase (JAK)- Signal transducer and activator of transcription (STAT) (JAK-STAT) pathway, have been associated with rare inborn errors of immunity (1). Inborn errors of immunity are heterogeneous rare diseases secondary to monogenic germline pathogenic variants resulting in autoimmunity, autoinflammation, allergy, and increased susceptibility to infectious diseases and/or malignancy (2–4).

Beyond simply increasing our understanding of the immune system, identifying such disorders has an obvious interest in selecting the most appropriate management strategy for patients that often have a long personal history of multiple treatment failures (5,6). Indeed, cytokines, their receptors and members of the JAK/STAT pathway are the targets of an increasing number of innovative treatments developed in recent years that are now used in daily clinical practice (7–9). These drugs, originally developed to treat more common immunological or haematological diseases, are therefore broadly available for prompt and targeted treatment of these rare patients, achieving an efficient translational medicine approach.

Here, we investigated 9 patients of a large French family affected by a dominantly inherited early-onset immune dysregulation syndrome with prominent allergy and autoinflammation related to a previously unreported heterozygous p.Cys787Phe gain of function (GOF) variant of *JAK1* gene**.** JAK1 is involved in the signalling of multiple cytokine receptors including those of the interferon (IFN), gp130, γ_c type, IL-3/β_c and single chain families (10). JAK1 associated inflammatory disease (JAID)(11) is an extremely rare and poorly understood condition previously reported in only 5 individuals from 3 kindreds (12–14). We describe the unique clinical and immunological characteristics associated with the same Cys787Phe pathogenic variant and report the therapeutic efficacy of two JAK inhibitors with a follow-up of 2 years.

Methods

Patients and study approval

All patients, or parents for children, and healthy relatives provided written informed consent for participation, genetic testing, and blood samples. As part of their routine care, patients underwent a series of complete physical examinations and several biological, radiological and pathological studies.

Genetic investigation

We performed a Next Generation Sequencing (NGS) panel targeting 300 genes causal of PID (15). This strategy led to the identification of a heterozygous missense variant in the exon 19 of *JAK1* gene. This *JAK1* variant was confirmed by Sanger sequencing for the index case and several members of his kindred.

Functional Studies

We obtained blood and tissue samples from the study participants to assess the inflammatory profile. Circulating cytokine and allergic related mediator dosages were performed on plasma according to standard procedures. PBMCs or whole blood cells were incubated at 37°C with IFN-α2, IL-2, IL-4 or IL-6 for 30 min. Cellular response was then assessed using fluorescence activated cell sorting (FACS) or mass cytometry (CyTOF). For treated conditions, cells were incubated at 37°C with baricitinib (200 nM) for 15 min prior to cytokine stimulation.

To study the transcriptomic signature of the disease, and the response to baricitinib treatments a total of 100 ng RNA per sample was used to assess the expression of 750 genes involved in immunity with the nCounter Human Autoimmune Profiling Panel (Nanostring).

Additional details are provided in the Supplementary Appendix.

Results

Disease manifestations

From birth, the index patient (V-1) had presented severe dermatitis and multiple food allergies, leading to the identification of a complex immune dysregulation syndrome of apparently dominant inheritance within his family. All affected individuals displayed diffuse ichthyosiform skin lesions and atopic dermatitis-like presentation (Table 1 and S1, Fig. 1 and S1). The ichthyosisform skin lesions were characterized by scaling and hyperkeratosis, with occasional cracks in the palms and erythematous areas. None of the individuals had bullous or erythrodermal lesions as part of the ichthyosis. The skin was also lichenified with areas of intense xerosis and infiltrated erythematous lesions especially on the face in most patients. CT scan in all affected adults identified disseminated nodules of soft tissue density with occasional calcification involving both thoracic and abdominal cavities as well as testis (Fig. 1E). Though most patients did not report symptoms related to the nodules, patient IV-5 underwent orchidectomy due to concerns about possible testicular cancer and developed dysphagia due to a large lower esophageal lesion. Pathological examination of these tumors revealed hypocellular hyalinized collagen with uniform proliferation of fibroblastic spindle cells, inflammatory infiltrate, and large ranges of calcifications, consistent with benign calcifying fibrous tumors (Fig. S2). Besides these common features, patients displayed various other manifestations: oligoarthritis, asthma, susceptibility to warts and ENT infections and chronic diarrhea. Fecal calprotectin, digestive endoscopies with systematic gastrointestinal biopsies were normal except in patient IV-7 in whom exulcerated nodular lesions of the lower esophagus and aphthoid lesions of the jejunum and ileum were found. Biopsies of the ileal lesions showed aspecific inflammation. The diagnosis of unclassified IBD was made in this patient, while the others were diagnosed with motility-related diarrhea. Of note, most patients also had food allergies that started in childhood and improved spontaneously over time. Failure to thrive was noted in two children with severe food allergies and two adults had a short stature. Additionally, two patients displayed unique features: III-6 had a voluminous nodular splenomegaly related to sclerosing angiomatoid nodular transformation (Fig. S2) and IV-5 had Addison's disease.

All patients displayed biological features of allergy: all had extremely high whole blood histamine levels, and most of them displayed mild basophilia, high total IgE levels, and mild eosinophil elevation between 500 and 2000/mm^3. Mild hypogammaglobulinemia was noted in 4 patients. Blood lymphocyte phenotyping revealed inconstant CD3 and CD19 lymphocytosis (Fig. S3). Broad autoimmunity screening was inconclusive (Table S2).

For dermatitis, all patients were treated with emollients and antihistamines, and some also received topical corticosteroids and topical tacrolimus with only moderate relief of symptoms. Patient IV-7 had previously been treated with several lines of immunosuppressants and biologic therapies for IBD/inflammatory rheumatism association (Table S1). Only infliximab led to a partial response of the inflammatory rheumatism, while both digestive and cutaneous involvements remained unchanged. Her father, patient III-6, had previously received adalimumab for oligoarthritis, which had no effect.

Genetic investigations

Next generation sequencing in the proband, his brother and father using a panel for primary immunodeficiencies and revealed the presence of a heterozygous variant: c.2360 G>T; p.(Cys787Phe) in *JAK1* (NM_002227.4). The variant was then confirmed using Sanger sequencing (Fig 2). This variant has not yet been implicated in a pathological condition and is absent from the human gene mutation database. Moreover, this variant was considered private to this family as it was not found in the public gnomAD 2.1.1 database. Subsequent targeted Sanger sequencing of *JAK1* gene in relatives revealed that all the tested affected subjects carried the same variant c.2360G>T in a heterozygous state, while the variant was absent in all the tested healthy siblings (Fig. 2A). The co-segregation of this variant with the clinical phenotype was fully consistent with the autosomal dominant inheritance model of the disease in this family. The variant leads to replacement of an evolutionary highly conserved cysteine and was predicted to be deleterious by all tested models. (Fig 2C). This amino acid is localized in the pseudokinase domain of JAK1, *i.e.* the domain affected by the three previously described GOF *JAK1* pathogenic variants (Fig 2D and S4A). Modelling of the impact of the variant on human JAK1 protein predicted that the phenylalanine residue in position 787 formed new stabilizing interactions with Cys817 and Tyr788 (Fig. 2E).

Patients with JAK1 pseudo kinase domain pathogenic variant Cys787Phe demonstrate altered JAK/STAT signaling.

All tested patients demonstrated an upregulation of the Th1 (IL-12p70, IFNγ) and Th17 (IL-6, IL-12p19, IL-1β) cytokines as well as increased IL-3, IL-33 and TSLP. Th2 cytokines IL-4, IL-13 and IL-10 were significantly lower in the patients compared to healthy donors. (Fig 3A). Nine out of the 10 tested patients returned negative Type I interferon signature (16). III-13 was positive on a first occasion and negative on a second (Fig. S5 A, B, C and D). Targeted transcriptomic analysis of peripheral blood of 4 patients prior to treatment and 4 healthy donors, was performed using a panel of 750 immune genes. Again, no enrichment of a Type I interferon signature was observed. Most enriched pathways concerned over expression of genes related to JAKs/STATs (*JAK1, JAK2, JAK3, STAT3, STAT5b)* signaling (Figs. 3B and 3C). Consistently, immunochemistry analysis on ichtyosiform skin lesions from the patients revealed overexpression of STAT3 and JAK1 (Fig. 3D). Both JAK1 and STAT3 were also highly expressed in calcifying fibrous tumors of JAK1 patients but not in digestive biopsies (Fig. S2A and S2B).

Previoulsy reported *JAK1* pseudokinase domain pathogenic variants led to aberrant STAT activation (12–14). While we noted high STATs activation in whole blood cells from V4, the phosphorylation status of STAT1, STAT3, STAT5 and STAT6 was comparable in the other tested patients to the levels observed in healthy donors for most cell types (Fig 4A, and Fig S6A) and no shared differences were observed across the 5 tested patients. Consistent results were found when studying basal STATs phosphorylation status in frozen PBMC by flow cytometry (Fig S6 B). To study JAK1 hyper and biased activation we stimulated PBMCs from patients with 5 cytokines of which the receptors are known to signal via JAK1: IFNα, IFNγ, IL-2, IL-4 and IL-6 (Fig 4B and Fig S7). Heterogeneous results were observed when comparing the phosphorylation of the canonical STATs for each of these cytokines between patients and controls (IFNα and IFNγ / STAT1, IL-2 / STAT5b, IL-4 / STAT6 and IL-6 / STAT3). Following IFNα stimulation, STAT1 phosphorylation was higher in T cells from III-6, IV-5, IV-9 and II-16 than in T cells from 4 tested healthy donors. The same was true for B cells from patients IV-9 and II-16 and NK cells from patients IV-5, IV-9 and II-16. Similarly, STAT3 phosphorylation was higher in B cells, NK cells and monocytes from patients III-6 following IL-6 stimulation compared to healthy donors. Interestingly, PBMCs of 5 out of the 6 tested patients demonstrated hyper-

phosphorylation of STAT3 in response to stimulation with IFN-α2 (Patients IV-7 and IV-9) or IL-2 (Patients III-6, IV-5 and V-1) when compared to PBMCs from healthy donors. PBMCs from Patients IV-7 and IV-9 also demonstrated hyper-phosphorylation of STAT5b under IFN-α2 stimulation (Fig. 4B). This atypical activation of STAT3 under IL-2 stimulation was confirmed in whole blood (Fig 4C) and appeared to predominantly affect NK cells (both $CD56^{dim}$ and $CD56^{hi}$ populations) (Fig 4C, S8A and S8B). Taken together, our *in vitro* and *ex vivo* experiments reveal a heterogeneous inflammatory profile between patients that converge towards an overexpression and an atypical activation of STAT3 in individuals bearing the $JAK1^{Cys787Phe}$ variant in this kindred.

Patients with *JAK*1 pathogenic variant have an altered basophilic phenotype

All patients displayed extremely high levels of blood histamine level and 6 out of 9 of them had mild basophilia. Comparison with 12 consecutive patients with familial Mediterranean fever, 10 consecutive patients with moderate-to-severe atopic dermatitis and 10 consecutive all-coming patients seen for routine outpatient clinical exams confirmed a significant elevation of basophils in $JAK1^{Cys787Phe}$ patients' blood (Fig. 5A). This basophilic expansion was also observed at the transcriptomic level. Indeed, differential gene expression analysis from peripheral blood of 4 untreated patients compared to healthy donors revealed a basophilic signature defined by the over expression of *IL4, MS4A2*, *HDC* and *CPA3.* (Fig. 5B). Histamine levels was quantified in sorted leukocyte populations from affected individuals and HD showing that basophils contained the largest amount (Fig. 5C). No significant variation in the cellular histamine content between affected individuals and HD including basophils was detected (Fig. 5D). Altogether, this suggests that the high blood histamine levels observed in $JAK1^{Cys787Phe}$ patients mostly results from a mild chronic basophil expansion. In order to test the activation status of circulating basophils we looked at the expression of the following activation markers: CD63, CD69; CD107a, CD193 and CD203c. None of these markers were over expressed on basophils from tested patients compared to healthy donors (Fig 5E).

Treatment with baricitinib

Considering their previously reported efficacy in patients with *JAK1*-pseudokinase domain-GOF variants (12,13), and their indication in atopic dermatitis (17,18), JAK inhibitors were considered for the treatment of our patients. Baricitinib was first tested because of the availability of pharmacokinetic data in children (19). Six adults (II-16, III-4, III-6, III-13, IV-5 and IV-7) and 1 child (V-1) received baricitinib at a dose of 4 and 2 mg once daily respectively. All adults displayed a dramatic skin improvement within a few days following the treatment introduction. Beyond the complete resolution of almost all symptoms related to atopic dermatitis, ichthyosiform lesions gradually improved leading to a drastic reduction in the daily usage of emollient cream and ultimately to a better quality of life (Figs. 6A and 6B and S9). Three out of four patients with chronic diarrhea and two out of three with arthromyalgia experienced substantial improvement of their symptoms. Calcifying fibrous tumors, however, did not regress under baricitinib. V-1, the only treated child, displayed a less positive response with slightly improved ichthyosis and persistent atopic features (Fig. 6B). Moreover, baricitinib did not restore normal ponderal and statural growth.

Inflammatory phenotype modifications with baricitinib treatment were assessed *ex vivo* and *in vitro*. Under baricitinib, all the elevated cytokines decreased except for the V-1 patient. Similarly, IL-4, IL-10 and IL-13 plasmatic levels increased (Fig. 6C , Fig S10 and Table S3). The blood transcriptional analysis under baricitinib treatment revealed a loss of all the previously enriched gene sets related to JAKs/STATs signaling (Fig. S11). *In vitro,* baricitinib achieved a good control of the STAT3 and STAT5b atypical activation following IFN α2 stimulation (Fig. S12). However, STAT3 hyper-phosphorylation following IL-2 stimulation was poorly controlled *in vitro* by the treatment.

Interestingly, despite clinical improvement of atopic features in adults, neither blood histamine levels nor basophilia decreased under baricitinib treatment (Fig. S13A). Accordingly, differential gene expression analysis in whole blood showed a persistent basophilic transcriptional signature (Fig. S13B).

Thus, treatment with baricitinib substantially attenuated the clinical manifestations of the disease and reverted most of the cellular and molecular inflammatory phenotypes driven by JAK1^{Cys787Phe}.

Treatment with upadacitinib

Although clinically effective, treatment with baricitinib was not sufficient to fully control all symptoms of the disease. Upadacitinib, a specific JAK1 inhibitor, was tested in 4 adult patients (III-4, III-6, IV-5 and IV-7) and was able to control canonical STAT1 and STAT5 activation following IFNα and IL-2 stimulation of patients' whole blood (Fig S14). Cutaneous and articular involvements worsened in 3 (III-6, IV-7 and III-4) and 2 (IV-7 and III-4) patients respectively. Two patients who displayed persistent gastrointestinal manifestations under baricitinib (III-6 and IV-7) experienced a substantial reduction of stool frequency and abundance with upadacitinib. (Fig. S15) Overall, due to the dissociated response under upadacitinib, patients III-6, IV-7 and III-4 were switched back to baricitinib; while only patient IV-5 remained under upadacitinib. He displayed an overall diminution of most inflammatory cytokine levels under upadacitinib compared to baricitinib (Fig S16). After resuming treatment with baricitinib, cutaneous (III-6, IV-7 and III-4) and articular (IV-7 and III-4) symptoms improved again.

Discussion

We report a large French family displaying a complex immune dysregulation syndrome with predominant cutaneous involvements perfectly segregating with the heterozygous *JAK1*: p.Cys787Phe variant. The main features, mostly related to atopy and/or autoinflammation, are close to what has been reported in the previously described *JAK1* pathogenic variants (12–14). This large family with this novel *JAK1* pathogenic variant harbors some specific features such as diffuse ichthyosiform skin rash, very high blood histamine levels associated with mild basophilia and multiple profound benign calcified tumours. These clinical and biological specificities are simple to identify on clinical examination or through blood sampling and could constitute markers indicative of JAK1-associated inflammatory disease (JAID) for the clinician. As a result, we expect them to be valuable in guiding the clinical diagnosis of a JAID among patients with familial atopic dermatitis/ichthyosis.

Our observation highlights that the clinical heterogeneity reported in patients with *JAK1* GOF variants is mirrored by a variable dysregulation of the JAK/STAT signalling pathway. Indeed, despite careful assessment using three different assays, and in contrast to the patient with the S703I mosaic, we did not observe any consistent type I IFN signature in our family. This is reminiscent of what is observed in STAT1 GOF variants, in which type I interferon signature is

variably present across patients or variants (20). Increase of the constitutive basal activation of the JAK/STAT signalling pathway was variable across patients. Cytokine-induced hyperphosphorylation of canonical JAK/STAT pathways was variably observed across patients, in opposition to previous report on patients with A634D and 703I *JAK1* GOF variants (12,13). This discrepancy might be partly explained by the larger group of patients and controls (heathy donors) used in our study compared to the previous reports, better encompassing the inter-individual variability of both control and patient groups. Together, our observation of JAK/STAT signalling in our patients imply that lack of type I interferon signature and more generally lack of over activation of canonical JAK/STAT pathway should not be considered as excluding criteria for *JAK 1* GOF diagnosis.

Besides this clinical and molecular heterogeneity, there are compelling associations pointing toward a common pathophysiology. The first are the above-mentioned shared clinical features, which mainly consist in atopic manifestations such as atopic dermatitis, food allergy, asthma, and eosinophilia (Fig. S17). The second is the existence of common dysregulations of the STATs phosphorylation by JAKs in patients with a *JAK1* GOF variant. Indeed, as for *JAK1* S703I mosaic variant, we observed a consistent atypical phosphorylation of STAT3 and STAT5b in cells from patients with the Cys787Phe pathogenic variant. This finding is highly consistent with the phenotypic overlap of JAK1 associated disease with manifestations observed in patients with *STAT3* and *STAT5b* GOF pathogenic variants (21,22) (Table 2).

It might be important however to highlight that we observed a trend toward hyperphosphorylation of STAT6 following IL-4 treatment. Given the clinical overlap of STAT6 GOF (23–25) patients with JAK1 GOF patients (Table2), it is reasonable to speculate that hyperphosphorylation of STAT6 might contribute to the allergic manifestation in JAK1 GOF patients.

While we do not provide molecular evidence of the pathogenicity of the Cys787Phe variant we provide compelling genetic, clinical and cellular data strongly supporting the association between this variant and the disease. Indeed, this variant not only affects a highly conserved residue and is absent from all public databases, but it also segregates perfectly with the disease over 4 generations in this family. Our cellular observation confirms biased STAT3 and STAT5b signaling as previously reported for the JAK1 S703I patients (13). Finally, as discussed above, there is a strong clinical overlap with the other patients with JAK1 mutant already reported in the literature (Fig S17).

Consistently, treatment with the JAK1/2 inhibitor baricitinib treatment resulted in a major improvement of most of atopic and inflammatory manifestations among adults, especially ichthyosis-related and atopic dermatitis-related symptom, achieving a substantial improvement of quality of life. This good clinical response was consistent with the partial correction or normalization of all dysregulated cytokines and the extinction of the JAKs/STATs signaling transcriptomic signatures. However, in some patients, treatment with baricitinib was not sufficient to fully control digestive and articular features leading us to switch to upadacitinib, a selective JAK1 inhibitor, in 4 patients. Digestive manifestations improved (n=2), however skin and/or articular involvements worsened (n=3). As suggested *in vitro* (13), our results support that selective JAK1 inhibition might not be an optimal therapeutic approach in patients with *JAK1* associated disease. Indeed, it remains unclear how a variant in the JAK1 pseudokinase domain could lead to atypical STAT3 and STAT5b activation. To date, the function of JAK1 pseudokinase domain in the overall protein function, including regulation of the catalytic activity or binding/recruitment of signalling partners, remains largely unknown (26). Disease-causing variants of the *JAK1* pseudokinase domain could favour JAK2 trans-activation. This hypothesis is partly supported by our observations that in most patients the disease was better controlled by the JAK1/JAK2 inhibitor baricitinib than by the selective JAK1 inhibitor upadacitinib. Futures directions to complete our study might include in-depth molecular characterisation in order to demonstrate how the JAK1 Cys787Phe variant alter cytokine receptors' signaling.

Interestingly, AD was a common feature of our patients, suggesting that JAK1 dysregulation might account for some common forms of AD. The spectacular efficacy of baricitinib on our patients' dermatitis suggest that a proportion of AD patients with altered JAK1 signalling would have particularly high benefit from this therapy.

Despite the above-mentioned efficacy of JAKinibs to treat skin atopic manifestations, both baricitinib and upadacitinib failed to control the basophilic signature of the disease, suggesting a pathway independent of JAK1/2 kinase activity might drive this basophilia. Thus, the precise basophil contribution to the disease pathogenesis is not yet elucidated. One child received baricitinib resulting in decreased pruritus and better sleep without improvement of his food allergy. The treatment improved the skin involvement. However, considering the potential risk on growth due to blocking of growth hormone signalling, baricitinib was discontinued.

The description of this large family with inherited JAID expends the clinical spectrum of the disease and can guide the future diagnosis of such patients. Two years follow-up of our patients under baricitinib treatment reveals a clear benefit of JAK1 and JAK2 inhibition for the control of most disease manifestation. Finally, the clinical response to various JAK inhibitor treatment regimens of JAID patients provides useful information on their benefits among patients suffering from more common diseases such as atopic dermatitis, chronic inflammatory rheumatism, or inflammatory bowel diseases.

Authors contributions

MPR and SGL conceived and designed the study. AF, MPR and SGL wrote the paper. VH and JML contributed to writing the paper. AF, CPo, AC, CLL, JLC, SD, MF, BH, PM, MP, JML, JC, NS, YYJZ, SM and MPR performed experiments, AF, CPo, AC, CLL, MC, MM, SD, MF, LLC, TRJM, PM, CPi, SV, DD, JML, JC, JPH, MPR, NS, YYJZ, SM, AH, RRB, TTM and SGL performed data analysis. AF, VH, CL, JDK, VL, TM, MHS, SGL were involved in the clinical study and sample collection. All authors reviewed the manuscript and gave final approval for the version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Competing interests : none

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Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figure 1: Clinical findings.

A: Bilateral cheek involvement of severe atopic dermatitis-like inflammatory skin lesions in patient V-1. **B:** Inflammatory Linear Verrucous Papules of the forearm in patient IV-5. **C:** Extensive ichthyosiform skin lesions of the breast and forearm in patient II-16. **D:** Digital recurrent warts in patient IV-7. **E:** Sagittal reconstruction of patient IV-5 CT-scan showing multiple calcified nodules of both thoracic and abdominal cavities, including a voluminous lesion of the lower esophagus responsible for dysphagia

Figure 2: Variant in JAK1 pseudokinase domain.

A: Pedigree of the family. Solid symbols indicate affected individuals, open symbols: unaffected individuals, asterisk: genotyped individuals, arrow: proband, slashes: deceased individuals. Non-genotyped individuals were considered to be affected if they displayed early onset dermatitis. JAK1-Cys787Phe variant was found in all tested symptomatic individuals and in none of the clinically asymptomatic relatives. B: Electropherogram of patient V-1 and healthy relative IV-11. C: Alignment of JAK1 homologs illustrates the strict evolutionary conservation of the Cysteine in position 787. D: Representation of the JAK1 domains with localizations of known inflammatory disease-causing variants of the pseudokinase domain. E: Prediction of the impact of the variant on the interactions between the residue in position 787 and other residues.

Figure 3: Patients demonstrate systemic and local inflammation.

A) Representation of the plasmatic concentration of various cytokines in the plasma of healthy donors and JAK1 patients. Results are presented as log2 fold change normalized on the mean of the healthy donors. B) Significantly enriched pathways in patients compared to healthy donors identified by gene set enrichment analysis from transcriptomic study on whole blood. C) Detail of the genes from the identified pathways. D) JAK1 and STAT3 immunostaining of a normal skin biopsy from a healthy donor and an ichthyous skin biopsy from patient III-13

(magnification x400). JAK1 cytoplasmic overexpression of the entire epidermis and STAT3 nuclear overexpression of the upper part of the epidermis were observed in the patient's skin.

Figure 4: Dysregulated phosphorylation of STAT3 in patient's cells.

A)**.** Bar graph showing the phosphorylation of STAT3 in unstimulated fresh whole blood from 4 donors and 5 untreated patients. For IV-7 : Baricitinib discontinuation >72h. B) Phosphorylation status of STAT1, STAT3, STAT5b and STAT6 was measured by flow cytometry on frozen PBMCs from 4 healthy donors and 6 patients stimulated with IFN-α2 (100 UI/mL) and IL-2 (50 ng/mL) C) STAT3 phosphorylation in response to IL-2 assessed by mass cytometry in blood leukocyte subsets from 3 healthy donors (left) and patient IV-7 (right). Each solid circle indicates a cluster. Circle diameter indicates cluster's relative frequency in the sample(s). The continuous color scale depicts the fold change of pSTAT3 mean signal intensity after IL-2 stimulation, compared to baseline pSTAT3 signal intensity assessed in non-stimulated conditions.

Figure 5: Moderate basophilia in JAK1^{C787F} patients.

A) Quantification of circulating basophils in 7 JAK1^{C787F} patients, 10 patients with atopic dermatitis (AD), 12 patients with familial Mediterranean fever (FMF) and 10 consecutive all-coming patients. B) Volcano plot representation of the gene expression analysis comparing patients to healthy donors. *IL4*, a cytokine gene known to be largely expressed in basophils, and 3 of the 4 basophilic genes included in the transcriptomic panel (*MS4A2*, *HDC* and *CPA3*) were among the 7 top upregulated genes in JAK1-Cys787Phe patients. C) Intracellular histamine quantification in circulating leukocyte subsets from patients and healthy donors. D) Intracellular histamine quantification in circulating basophils from patients and healthy donors. E) Evaluation by flow cytometry of the expression of activation markers by basophils from patients and healthy donors.

Figure 6: Response to baricitinib.

A) Three key cutaneous manifestations from two patients are presented before (top) and during treatment with baricitinib (bottom). From left to right: improvement of atopic dermatitis-like lesions of the face in patient III-4 (I); improvement of the inflammatory linear verrucous papules of patient IV-5's forearm (II); and near disappearance of xerosis and lichenification of icthyosiform atopic dermatitis lesions on patient III-4's hand (III). **B)** Effect of baricitinib on the articular, gastrointestinal, and skin involvements using a subjective clinical scale ranging from 0 (no activity) to 5 (severe involvement). **C)** Plasmatic concentration of cytokines in patients before and under treatment with baricitinib. All patients had a partial or total improvement of cytokine levels except V-1.

Table 1:

Summary of the manifestations of the JAK1-associated autoinflammatory disease in this family.

ENT: ear-nose-throat

* all adult patients, children did not undergo systematic CT scans

	Main features	Frequency no./total no
Allergy	Ichthyosis	9/9
	Atopic dermatitis	9/9
	Elevated blood histamine level	9/9
	Moderate basophilia	6/9
	Moderate eosinophilia	5/9
	High total IgE	5/9
Inflammation	Arthralgia	6/9
	Mild inflammatory syndrome	7/9
Immune deficiency	Recurrent ENT infections	5/9
	Susceptibility to warts	4/9
	Mild hypogammaglobulinemia	~~5~~ 4/9
Other	Calcifying fibrous tumors	7/7*
	Diarrhea	5/9
	Short stature / failure to thrive	5/9

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613 **Table 2**
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		JAK1-Cys787Phe	STAT3 AD GOF[$]	STAT5b AD GOF [£*]	STAT6 AD GOF[€]	JAK3 AD GOF[&]
Onset		Neonatal	Childhood	Neonatal	Early chilhood	?
Failure to thrive / short stature		+	+	+	+	?
Atopy	**Atopic dermatitis**	+	+	+	+	-
	Food allergies	+	-	+	+	-
	Asthma	+	-	+	+	-
	Eosinophil count	Moderately elevated	Normal	High	High	-
	Eosinophilic GI disease	-	-	+	+	-
	Basophilia	+	-	-	-	-
	Hyper-IgE	+	-	+	+	-
Auto-immunity / inflammation	**Enteropathy / IBD**	+/-	+	-	-	-
	Arthritis	+	+	-	-	-
	Autoimmune cytopenia	-	+	-	-	+
	Interstitial lung disease	-	+	-	-	-
	Endocrinopathies	Addison's disease	T1D, thyroiditis	-	-	-
Imm. def.	**Hypogammaglobulinemia**	+/-	+/-	-	+/-	+
	Infection susceptibility	Mild	+	-	+	+
	Warts	+	-	-	-	-
Onco-hemato.	**Lymphadenopathy**	+/-	+	-	-	+
	Hepatosplenomegaly	-	+	-	-	+
	Multiple CFT	+	-	-	-	-
	Risk of malignancy	?	+	?	?	?
Other feature(s)		Ichthyosis, CFT, Diarrhea of uncertain cause	-	-	-	CLPD-NK

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616 T1D: Type-1 diabetes melitus; Imm. def.: immune deficiency; CFT: calcifying fibrous tumors; onco-hemato.: onco-hematological features.
617 GI:gastro intestinal; IBD : intestinal bowel disease
618 $: adapted from Fabre *et al. J Allergy Clin Immunol Pract* 2019
619 £: adapted from Ma *et al. Blood* 2017 ; *: somatic mutation
620 €: adapted from Sharma *et al. J Exp Med* 2023
621 &: adapted from Lesmana *et al.* Blood 2020
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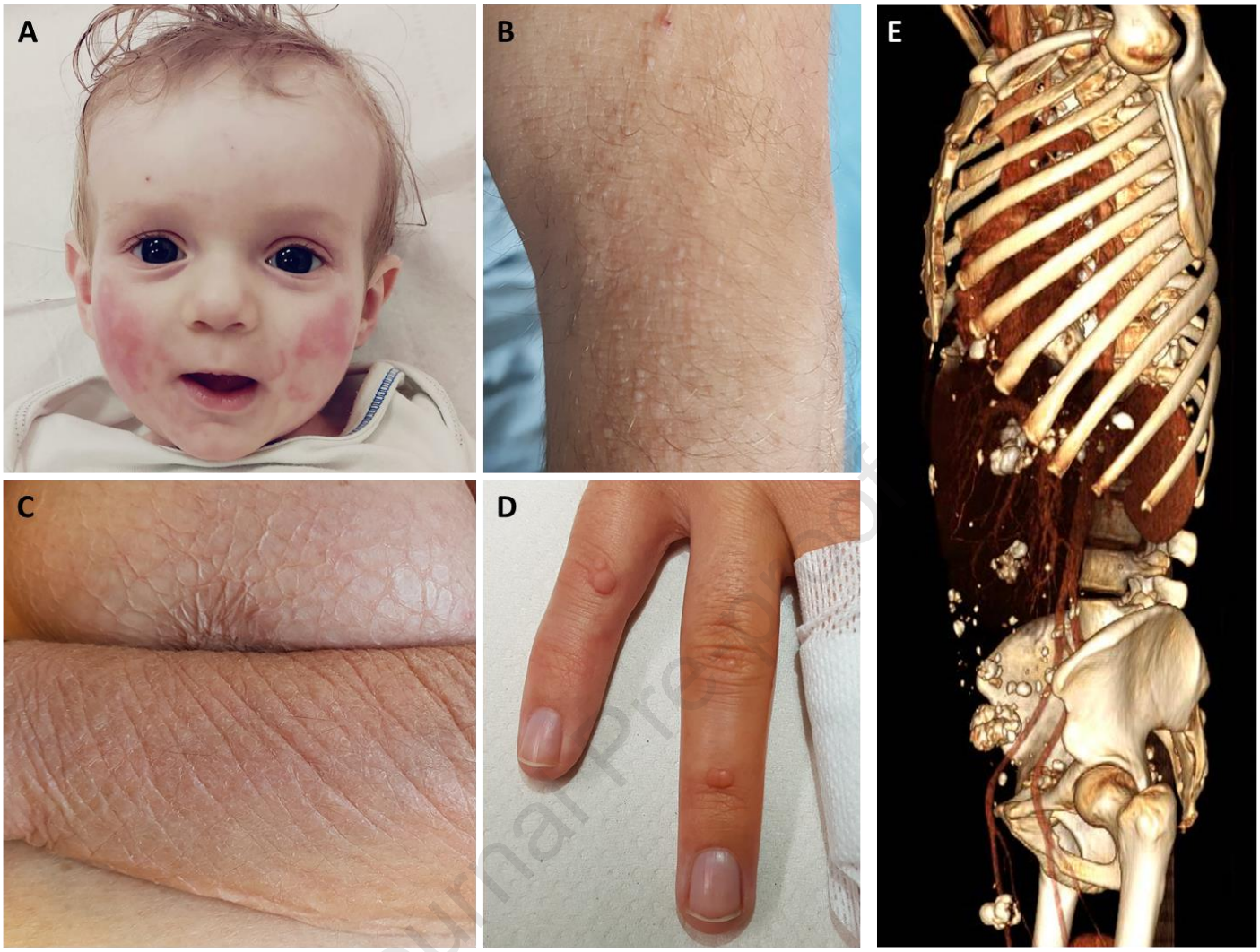

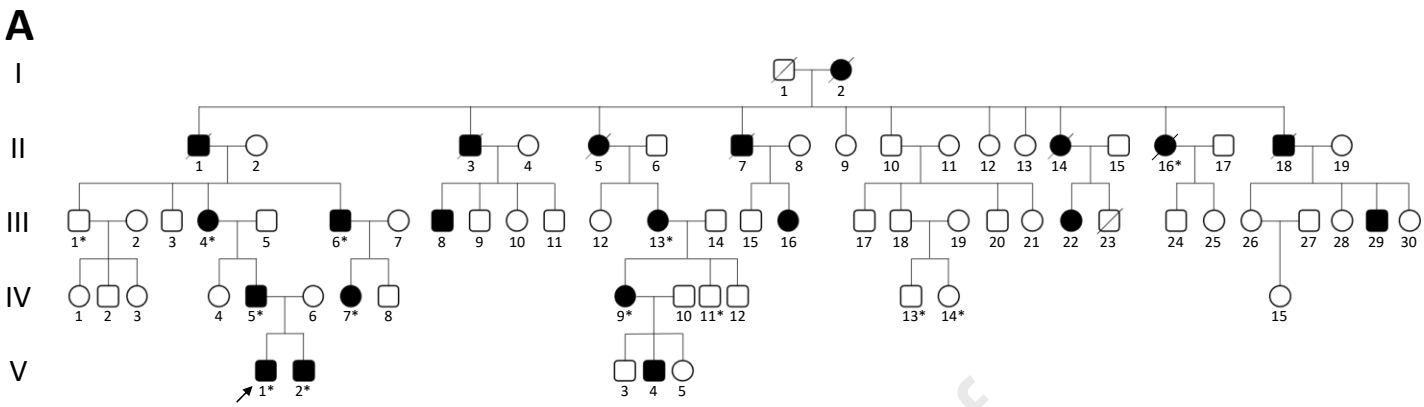

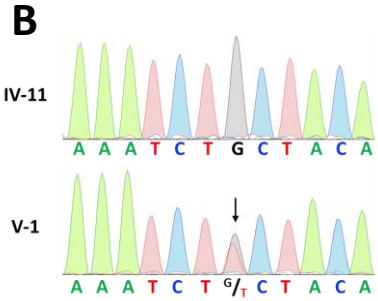

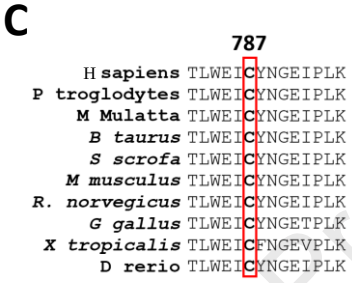

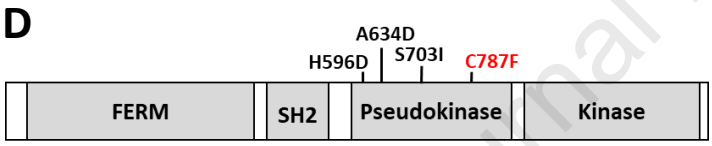

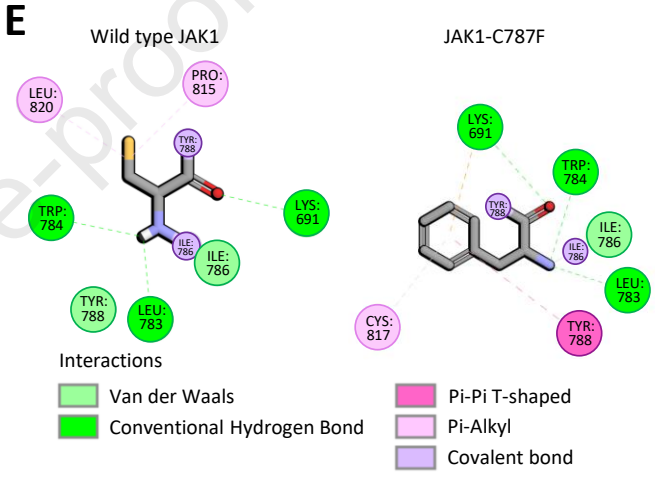

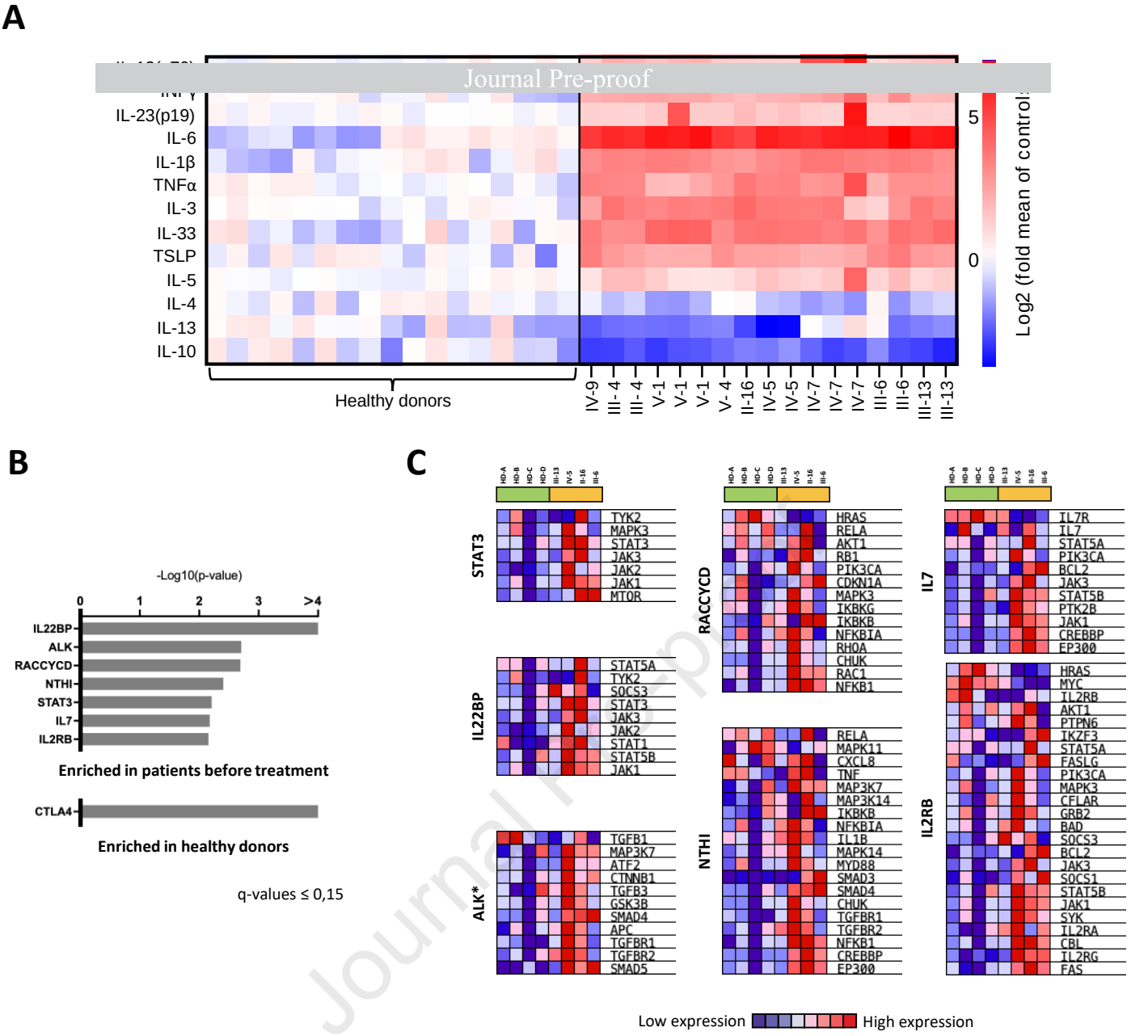

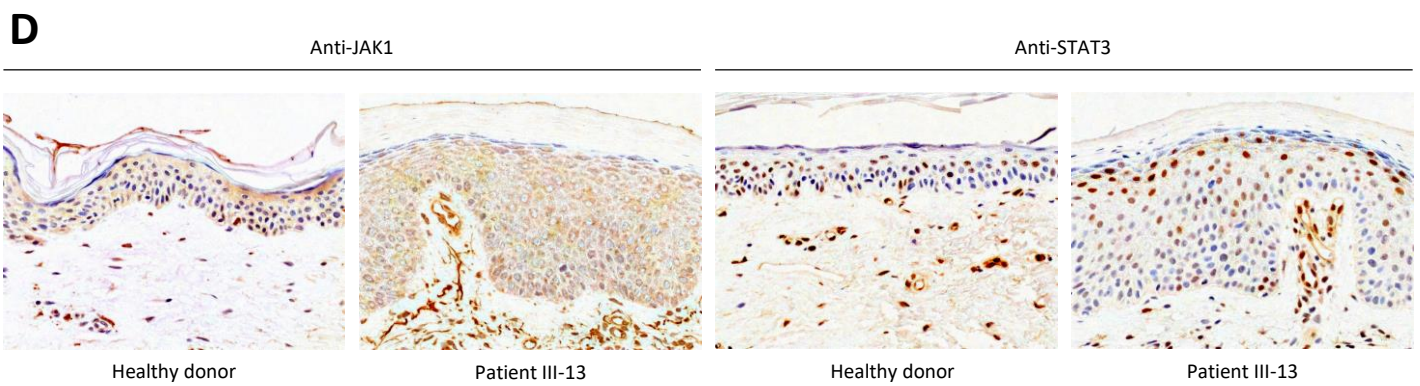

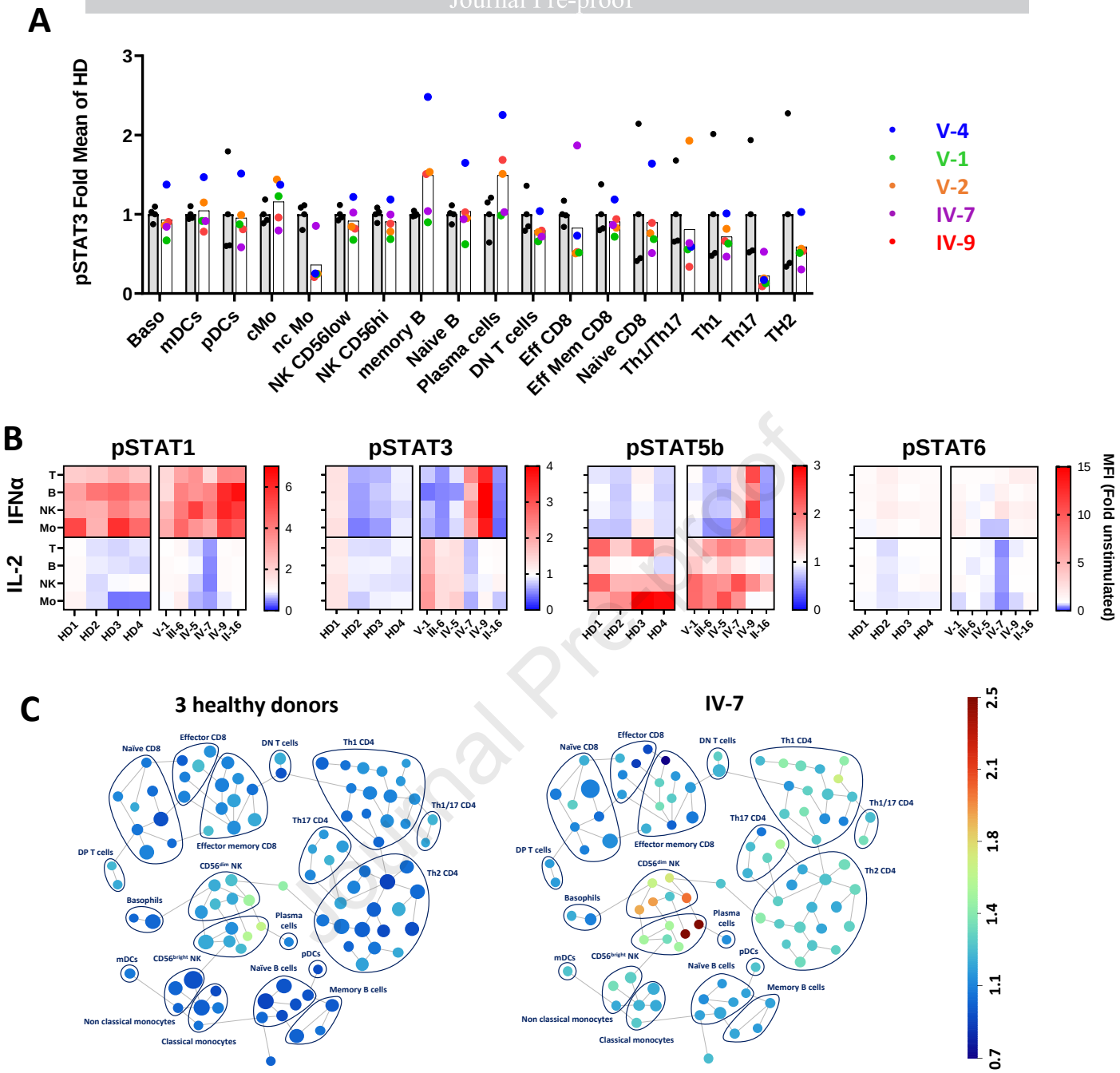

Figure 5