# Distinct blood protein profiles associated with the risk of short-term and mid/long-term clinical relapse in Crohn's disease patients stopping infliximab: when the remission state hides different types of residual disease activity

Nicolas Pierre[1], Vân Anh Huynh-Thu[2], Thomas Marichal[3], Matthieu Allez[4], Yoram Bouhnik[5], David Laharie[6], Arnaud Bourreille[7], Jean-Frédéric Colombel[8], Marie-Alice Meuwis[1,9,#], Edouard Louis[1,9,#], GETAID (Groupe d'Etude Thérapeutique des Affections Inflammatoires du tube Digestif)

[1]Laboratory of Translational Gastroenterology, GIGA-institute, University of Liege, Liege, Belgium; [2]Department of Electrical Engineering and Computer Science, University of Liege, Liege, Belgium; [3]Laboratory of Immunophysiology, GIGA-institute, University of Liege, Liege, Belgium; [4]Service d'Hépato-Gastroentérologie, Hôpital Saint Louis, APHP, Université de Paris, Paris, France; [5]Service de Gastroentérologie et Assistance Nutritive, Hôpital Beaujon, Clichy, France; [6]Service d'Hépato-Gastroentérologie, Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France; [7]Institut des maladies de l'appareil digestif, Centre Hospitalier Universitaire de Nantes, Hôtel-Dieu, Nantes, France; [8]Division of Gastroenterology, Icahn School of Medicine at Mount Sina, New York, USA; [9]Hepato-Gastroenterology and Digestive Oncology Department, Liege University Hospital, Liege, Belgium. [#]*Equally contributed to this work.*

**Corresponding author:**

Nicolas Pierre

Address: Translational Gastroenterology, GIGA institute Bât. B34 Quartier Hôpital, avenue de l'Hôpital 11, 4000 Liege, Belgique

Tel:+32 4 3662538; Fax:+32 4 3667889

Email: nicolas.pierre@uliege.be

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## ABSTRACT

### Objective

Despite being in sustained and stable remission, Crohn's disease (CD) patients stopping anti-TNFα show a high rate of relapse (~50% within 2 years). Characterising non-invasively the biological profiles of those patients is needed to better guide the decision of anti-TNFα withdrawal.

### Design

Ninety-two immune-related proteins were measured by proximity extension assay in serum of CD patients (n=102) in sustained steroid-free remission and stopping anti-TNFα (infliximab). As previously shown, a stratification based on time to clinical relapse was used to characterise the distinct biological profiles of relapsers (short-term relapsers: <6 months vs mid/long-term relapsers: >6 months). Associations between protein levels and time to clinical relapse were determined by univariable Cox model.

### Results

The risk (HR: hazard ratio) of mid/long-term clinical relapse was specifically associated with a high serum level of proteins mainly expressed in lymphocytes (LAG3, SH2B3, SIT1; HR: 2.2-4.5; $p<0.05$), a low serum level of anti-inflammatory effectors (IL-10, HSD11B1; HR: 0.2-0.3; $p<0.05$) and cellular junction proteins (CDSN, CNTNAP2, CXADR, ITGA11; HR: 0.4; $p<0.05$). The risk of short-term clinical relapse was specifically associated with a high serum level of pro-inflammatory effectors (IL-6, IL12RB1; HR: 3.5-3.6; $p<0.05$) and a low or high serum level of proteins mainly expressed in antigen presenting cells (CLEC4A, CLEC4C, CLEC7A, LAMP3; HR: 0.4-4.1; $p<0.05$).

### Conclusion

We identified distinct blood protein profiles associated with the risk of short-term and mid/long-term clinical relapse in CD patients stopping infliximab. These findings constitute an advance for the development of non-invasive biomarkers guiding the decision of anti-TNFα withdrawal.

**What is already known on this topic?**

- Crohn's disease (CD) patients achieving sustained remission and stopping anti-TNFα show a high rate of relapse (~50% within 2 years).
- Characterising non-invasively the biological profiles of relapsers is needed to better guide the decision of anti-TNFα withdrawal.

**What this study adds**

- Blood proteins involved in inflammatory processes, anti-inflammatory defences, cellular junctions and immunoregulation of immune cells showed distinct associations with the risk of short-term (<6 months) and mid/long-term (>6 months) clinical relapse following anti-TNFα withdrawal in CD patients.
- The biological profiles of short-term and mid/long-term relapsers can be considered as different degrees of unstable biological remission characterised by different types of residual disease activity.

**How this study might affect research, practice or policy**

- These findings represent a biological basis to develop non-invasive biomarkers guiding the decision of anti-TNFα withdrawal in CD patients.

## INTRODUCTION

In Crohn's disease (CD), anti-tumour necrosis factor α (TNFα) therapy is well-known to induce stable remission in a subset of patients[1]. When this objective is achieved, anti-TNFα withdrawal can be considered and this choice could offer substantial benefits in terms of safety and cost. Indeed, chronic exposure to anti-TNFα has been linked to serious complications (eg, lymphoma, infections, melanoma and non-melanoma skin cancers)[2–5] and this treatment is a burden for the healthcare system[6]. These arguments need to be balanced by the fact that CD patients stopping anti-TNFα present a high rate of relapse (~50% within 2 years according to a meta-analysis)[7]. This risk shows a remarkable homogeneity across studies and arises despite that, at time of anti-TNFα withdrawal, most patients (>80%) are in sustained remission according to clinical, endoscopic and biomarker (CRP and feacal calprotectin) criteria classically used[7]. This situation underlines that the current definition of remission is not well adapted to guide the decision of anti-TNFα withdrawal. Such option could be better considered by knowing the biological profiles of relapsers.

We previously reported that, in CD patients stopping anti-TNFα (infliximab), the risk of short-term (<6 months) and mid/long-term clinical relapse (>6 months) were associated with specific serum proteins reflecting distinct pathological process[8]. This result was a first advance to identify the biological profiles of relapsers. The aim of the present study was to improve this knowledge by further characterising the blood protein profiles of short-term and mid/long-term relapsers following infliximab withdrawal.

In our above mentioned study, the relatively low analytical sensitivity (down to ~1 $\mu g.mL^{-1}$) of the measurement method (crude serum analysed by selected reaction monitoring, SRM, a mass spectrometry-based technology)[8,9] led us to measure mainly the abundant and liver-produced proteins of the serum (54 out of the 72 measured proteins). In the present study, we hypothesised that targeting low abundance serum proteins which do not mainly reflect a liver

response could reveal novel information complementary to our previous work. To test this assumption, we took advantage of the proximity extension assay (PEA) technology (Olink) which, by using the amplification system of polymerase chain reaction (PCR), reaches a sensitivity allowing to measure low-abundant proteins of human blood (down to ~1 pg.mL$^{-1}$)[10,11]. We selected the immune response panel (n=92 proteins) which targets some processes related to the development and perpetuation of CD: inflammation, cytokine-mediated signalling pathways, adaptive immune response, defence response to virus, lymphocyte activation (Olink). This panel contains a low number of liver-produced proteins (3/92). As in our previous work[8], proteins were measured in the baseline serum of patients included in the study of infliximab diSconTinuation in CrOhn's disease patients in stable Remission on combined therapy with Immunosuppressors (STORI), a trial set up to identify risk factors for clinical relapse in CD patients stopping infliximab[12].

## METHODS

### Patients and samples

STORI is a study designed by the Groupe d'Etude Thérapeutique des Affections Inflammatoires du tube Digestif (GETAID) in which CD patients were prospectively recruited across 20 centres in Belgium and France between March 2006 and December 2009[12]. The inclusion and exclusion criteria have been detailed previously[12]. Briefly, included patients were under a combined therapy (infliximab and antimetabolites for at least 1 year) and in corticosteroid-free clinical remission (CD activity index: CDAI<150 for at least 6 months) when they stopped infliximab (baseline). At baseline, sera were obtained after centrifugation of coagulated blood and stored at −80 °C until analysis. A clinical relapse was declared when patients presented a CDAI higher than 250 or a CDAI between 150 and 250 with an increase of 70 point from baseline over two consecutive weeks. The data presented in this study were obtained from 102

out of the initial 115 patients of the STORI cohort (Table 1). The study was approved by the French Ethics Committee-Hôpital Saint-Louis (CPP 2005/14) and the AFSSAPS (0809/ALV/EG05). The investigational review board at each of the participating centre approved the protocol and, before screening, all patients gave their written informed consent. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

**Proximity extension assay**

The serum abundance of 92 proteins were measured by PEA (immune response panel, Olink, Sweden). For each protein, the analytic performances of the assay (limit of detection, lower limit of quantification, upper limit of quantification, hook effect, dynamic range, intra-assay and inter-assay run precision) are available on the manufacturer's website (https://www.olink.com/). The principle of PEA technology has been previously explained[11]. Briefly, this method relies on dual recognition of protein by a pair of antibodies labelled with complementary single-stranded DNA oligonucleotides. These PEA probes become close when antibody pair binds to its target, this allows their hybridization and subsequent elongation by a DNA polymerase thus resulting in the synthesis of a double-stranded DNA with a unique sequence for each targeted protein (barcode). This DNA template is then amplified and quantified by quantitative PCR. Data are normalised with internal and external controls to reduce technical variability, they are finally reported on a $Log_2$ scale with an arbitrary unit called normalized protein expression (NPX)[11].

**Statistical analysis**

Among the 102 studied patients, 44 were declared relapsers (R) and 58 were declared non-relapsers (NR). To study separately the short-term relapsers (<6 months) from the mid/long-

term relapsers (>6 months), the cohort was stratified according to a time to clinical relapse of 6 months as previously described[8]. In the short-term clinical relapse dataset, the follow-up of non-relapsers was censored at 6 months and mid/long-term relapsers (n=29) were considered as non-relapsers. In the mid/long-term clinical relapse dataset, non-relapsers presenting a follow-up inferior to 6 months (n=13) and short-term relapsers (n=15) were excluded. Thus, the short-term clinical relapse dataset and the mid/long-term clinical relapse datasets were composed of 102 (15 R, 87 NR) and 74 (29 R, 45 NR) patients, respectively.

For each targeted protein, an optimal cut-off value was selected as the one maximizing the sum of sensitivity and specificity (Youden's index) in classifying relapsers versus non-relapsers. By using these cut-offs, the univariable Cox model was applied to determine the association of each protein with the time to clinical relapse, this relation was depicted by the hazard ratio (HR=hazard in patients above the cut-off/hazard in patients under the cut-off). The proportional hazard assumption was verified by a statistical test (Schoenfeld's residuals) and by a graphical criterion (crossing of survival curves). The non-respect of this assumption (HR not constant over time) led to exclusion of some variables (mid/long-term clinical relapse dataset: ITM2A, LAMP3; non-stratified dataset: TRIM21, IL-10, IRAK1, ITGA6, TRIM5, PRDX5, IRF9, CDSN, DDX58).

The lifelines Python library and the ggplot2 R package were used to perform the Cox model and to generate volcano plots, respectively[13,14]. The p-values inferior to 0.05 were considered significant.

**Strategy of analysis to search biological patterns differentiating the short-term relapsers from the mid/long-term relapsers**

Proteins only associated with the risk of short-term clinical relapse (n=25) were compared to those only associated with the risk of mid/long-term clinical relapse (n=17) (online

supplemental table 1). This research was conducted by investigating heterogeneous information related to the studied proteins: 1) functions, cellular origin and involvement in signalling pathways which were determined with the help of literature and databases (Uniprot, Human Protein Atlas, Kyoto Encyclopedia of Genes and Genomes, Reactome) (online supplemental table 1); 2) association between the risk of clinical relapse and the serum level (high: HR>1; low: HR<1; online supplemental table 1); 3) links with CD pathophysiology; 4) biological convergences with our previous findings[8]. By integrating this prior knowledge, we found relevant to focus our analysis on: pro-inflammatory and anti-inflammatory effectors, proteins mainly expressed in antigen presenting cells (APCs) or lymphocytes, cellular junction proteins, downstream signalling of cytokine receptors and pattern recognition receptors. The information used to classify proteins in the abovementioned categories are provided in online supplemental tables 2, 3, 4, 5.

The proteins not highlighted in the core manuscript and showing a significant association with the risk of short-term and/or mid/long-term clinical relapse (n=19) were included in the online supplemental table 1 and the online supplemental figure 1.

## RESULTS

### Study population, experimental design and outcomes of patients after infliximab withdrawal

The clinical characteristics of the study population are presented in table 1. The Figure 1 presents the experimental design and outcomes of patients after infliximab withdrawal. After stopping infliximab, 44 patients relapsed and 58 did not relapse with a median (IQR) time of clinical relapse of 6.7 (4.2-11.2) months and a median (IQR) time of follow-up of 25.4 (7.9-30.1) months, respectively. Among relapsers, 15 were considered short-term relapsers (< 6

months) and 29 mid/long-term relapsers (> 6 months) with a median (IQR) time of clinical relapse of 3.6 (2.8-4.1) and 9.8 (6.7-12.5), respectively (figure 1).

**Risk of clinical relapse associated with pro-inflammatory and anti-inflammatory effectors**

Among the 6 pro-inflammatory or anti-inflammatory effectors of the PEA panel (online supplemental table 2), 4 showed an association with the risk of clinical relapse: interleukin-6 (IL-6); interleukin-12 receptor subunit beta-1 (IL12RB1); corticosteroid 11-beta-dehydrogenase isozyme 1 (HSD11B1), interleukin-10 (IL-10) (online supplemental table 1). Remarkably, the risk of mid/long-term clinical relapse was associated with a low serum level of anti-inflammatory effectors (IL-10 and HSD11B1) while the risk of short-term clinical relapse was associated with high serum level of pro-inflammatory effectors (IL-6 and IL12RB1) (figure 2A,B; online supplemental table 1).

**Risk of clinical relapse associated with proteins mainly expressed in antigen presenting cells or lymphocytes**

Some proteins of the PEA panel are mainly expressed in lymphocytes or APCs where they exert immunoregulatory functions since, most of them (17/18), are known to promote immunity and/or tolerance (online supplemental table 3). Among the proteins mainly expressed in APCs, 2 were associated with the risk of mid/long-term clinical relapse (C-type lectin domain family 4 member G, CLEC4G; Allergin-1, MILR1; figure 3A,B) while 6 were associated with the risk of short-term clinical relapse (C-type lectin domain family 4 member A, CLEC4A; C-type lectin domain family 4 member C, CLEC4C; CLEC4G; C-type lectin domain family 7 member A, CLEC7A; MILR1; lysosome-associated membrane glycoprotein 3, LAMP3; figure 3A,B). Three of these proteins presented the strongest statistical associations with the risk of short-term clinical relapse (CLEC4C; CLEC4G; LAMP3; figure 3A). Among the proteins mainly

expressed in lymphocytes, 1 was associated with the risk of short-term clinical relapse (Natural killer cells antigen CD94, KLRD1) while 3 were associated with the risk of mid/long-term clinical relapse (lymphocyte activation gene 3 protein, LAG3; SH2B adapter protein 3, SH2B3; signaling threshold-regulating transmembrane adapter 1, SIT1) (figure 3A,B). Of note, SIT1 showed the strongest statistical association with the risk of mid/long-term clinical relapse (figure 3B).

**Risk of clinical relapse associated with cellular junction proteins**

The PEA panel contains 6 cellular junction proteins which are either implicated in cell-cell junctions (corneodesmosin: CDSN; contactin-associated protein-like 2: CNTNAP2; coxsackievirus and adenovirus receptor: CXADR) or in cell-matrix junctions (integrin α-11: ITGA11; integrin alpha-6: ITGA6; integrin beta-6: ITGB6) (online supplemental table 4). The risk of mid-long/term clinical relapse was characterised by a low serum level of 4 cellular junction proteins (CDSN; CNTNAP2; CXADR; ITGA11) (figure 4A,B). Finally, a low or a high serum level of ITGA6 was associated with respectively the risk of short-term clinical relapse or the risk of mid/long-term clinical relapse (figure 4A,B).

**Risk of clinical relapse associated with downstream signalling of cytokine receptors and pattern recognition receptors**

In the PEA panel, some proteins are part of immune pathways such as nuclear factor-κB (NF-κB) (n=10), interferon (n=3) and mitogen-activated protein kinases (n=1) (online supplemental table 5). All those proteins belong to the downstream signalling of cytokine receptors and pattern recognition receptors, and remarkably, most of them (10/12) exhibited an opposite pattern (HR<1 vs HR>1) between the short-term clinical relapse dataset and mid/long-term clinical relapse dataset (figure 5).

## DISCUSSION

The present work supports that, in CD patients stopping infliximab, the risk of short-term clinical relapse (<6 months) and mid/long-term clinical relapse (>6 months) are associated with distinct pathophysiological processes. Previously, we came to the same conclusion by using another technology (SRM) and by evaluating other biological phenomena[8]. These converging results extend and consolidate the knowledge on the distinct biological profiles of short-term relapsers, mid/long-term relapsers and, by contrast, non-relapsers. As a corollary, we now propose to consider that non-relapsers experience a stable biological remission, while short-term relapsers and mid/long-term relapsers present different degrees of unstable biological remission characterised by different types of residual disease activity. This identification and characterisation of CD patient subsets could constitute a basis to develop non-invasive biomarkers guiding the decision of anti-TNFα withdrawal.

Our previous study revealed that monitoring inflammation as currently performed in clinics can only have a limited utility when contemplating anti-TNFα withdrawal. Indeed, inflammatory markers were essentially associated with the risk of short-term clinical relapse[8], while the majority of relapsers present a time to relapse greater than 6 months after anti-TNFα withdrawal (66% in the present work and >80% in another study[15]). Thus, characterising the biological profile of mid/long-term relapsers is a research priority that will require to study other targets than inflammatory markers. Our study constitutes a progress in this direction since we highlighted that the risk of mid/long-term clinical relapse is associated with markers involved in anti-inflammatory defences, cellular junctions, immunoregulation of APCs and lymphocytes.

Our study also adds new elements in the understanding of clinical relapse after infliximab withdrawal. According to our results, a reduced anti-inflammatory capacity could be implicated

in the pathophysiological mechanisms leading to clinical relapse. Indeed, we showed an association between the risk of mid/long-term clinical relapse and a low circulating levels of potent anti-inflammatory effectors (IL-10, the master anti-inflammatory cytokine; HSD11B1, an enzyme converting inactive cortisone to active cortisol thus promoting the anti-inflammatory effect of glucocorticoids[16,17]). This goes along the same line as our previous results showing a similar association with the apolipoproteins A-I and A-II which are known to mediate the anti-inflammatory properties of high density lipoprotein[8,18]. Although our study cannot establish causal relationships, our results are concordant with the hypothesis that a reduction of anti-inflammatory effectors could be a prelude to the inflammatory flare characterising the relapse. This hypothesis needs to be tested in a dedicated work.

Despite its capital role in regulating the action of endogenous and synthetic glucocorticoids[17], HSD11B1 has received little attention in the IBD literature so far. An increase of HSD11B1 mRNA level has been reported in inflamed versus non-inflamed intestinal biopsies of IBD and non-IBD patients[19]. In mice, similar results were reported in dextran sulfate sodium (DSS)-induced colitis model[20]. Actually, up-regulation of HSD11B1 is a well-known homeostatic response to inflammatory stimuli which is observed in non-immune and immune cells (monocytes, macrophages, leukocytes, neutrophils)[17]. In contrast, it is intriguing and new to report in the present study that a future risk for patients (clinical relapse) is associated with a low serum level of HSD11B1. Taken together, these observations suggest to more deeply study the role of HSD11B1 in CD.

When compared to mucosa of healthy individual, the macroscopically normal mucosa of CD patients showed abnormalities of the cellular junctions which were revealed through microscopy techniques (confocal endomicroscopy, confocal and electron microscopy)[21–25] and also supported by molecular-based evidence showing a decreased mRNA levels of α-cathenin (adherens junction) and tight junction protein ZO-1[26]. Remarkably, our results

showed that a perturbation of cellular junctions could be an early defect affecting the relapsing patients. Indeed, we found that low serum levels of 4 cellular junction proteins were specifically associated with the risk of mid/long-term clinical relapse. It now remains to elucidate whether these results obtained in the blood reflect an alteration of the gut epithelium.

In a coherent manner, our previous and present studies well converge to support that acute inflammation characterises the short-term relapsers. Indeed, we previously reported that a high serum level of acute-phase reactants is specifically associated with the risk of short-term clinical relapse[8] and, this outcome was herein associated with a high serum level of IL-6 which is a canonical inducer of the acute-phase response[27].

Our study showed an association between the risk of short-term clinical relapse and a high serum level of CLEC4C, a highly specific marker of plasmacytoid dendritic cells (pDCs) which exerts a tolerogenic effect[28]. This result deserves to be discussed in the light of what is already known on pDCs in CD. In mice, ablation of pDCs reduced DSS-induced colitis[29]. In CD patients with acute flare ups, evidence supported that peripheral pDCs migrate to colonic mucosa and mesenteric lymph nodes and this is accompanied by a modification of their phenotype toward a pro-inflammatory profile[30,31]. According to our results on CLEC4C, we can reasonably speculate that changes in peripheral pDCs could be detected, not only during, but also before acute flare ups.

Prediction and causality are aims commonly confused in clinical studies, this situation leads to inappropriate methodologies and erroneous conclusions[32]. In this context, it has been recommended that researchers clearly explain and state their objective: causality vs prediction[32]. Etiological research generally aims to determine the causal effect of an independent variable X on a dependent variable Y (outcome). In this situation, adjustment for confounders is typically performed to reduce the omitted-variables bias which can distort the relation between X and Y. On the other hand, prediction research aims to forecast Y by knowing

a set of variables called predictors and this whatever their relations (causal or not) with Y and with omitted variables. The present study does not pursue a causal aim, i.e., we do not pretend that modulating a PEA marker (X) will influence the clinical relapse (Y). Our objective clearly concerns the prediction of a clinical outcome. More precisely, we aimed to characterise the biological states of patients (depicted by PEA markers) predicting their clinical relapse and this whatever the effect of other variables (eg, age, sex, treatments, infliximab trough level, disease extension, smoking). In other words, considering other variables than the PEA markers is not relevant to achieve our objective. Besides, adjusting for confounders would have been a methodological error since this approach is appropriate to deal with causality but not prediction[32].

The limitations of our work include the fact that the definition of relapse was only clinical (CDAI), i.e., not confirmed by objective markers (CRP or calprotectin). Thus, the classification of patients as relapsers can be affected by the subjectivity of symptoms used to calculate the CDAI.

We also acknowledge a limitation regarding the interpretation of results obtained on non-secreted proteins (79/92 in the PEA dataset according to UniProt and Human Protein Atlas, including some proteins highlighted in the discussion: HSD11B1, CLEC4C). Indeed, the role of non-secreted proteins in the serum is largely unknown and their presence outside the cells probably depends on complex combination of mechanisms regulating cells death, protein cleavage, cellular population and gene expression. For those proteins, the main interest of our study was to show their distinct patterns in the short-term relapsers and mid/long-term relapsers rather than to provide clear pathophysiological insights. In contrast, our results obtained on IL-6 and IL-10 (secreted proteins) can be better interpreted given that function of these cytokines in the blood is well known and their roles in CD are largely documented in the literature.

Another limitation of our work is the absence of independent cohort to generalise the results, this prevents to directly determine the clinical interest of individual markers or their combinations in statistical models. However, we think that part of our results can be generalisable in a much broader context than our study. This proposal is supported by the idea that, whatever the cause of a relapse, this is a failure of both treatments and human homeostasis which is associated with some invariable pathophysiological processes. For instance, this is the case of acute inflammation which is generally associated with a relapse whatever the clinical context. In the present study, a high level of IL-6 was specifically associated with the risk of short-term clinical relapse after infliximab withdrawal. Similar finding was reported in inactive CD patients who were not treated with biologics and, for some of them, receiving no treatments[33]. Hence, a high circulating level of IL-6 can annunciate a short-term clinical relapse and this result is generalisable far beyond the STORI cohort and the specific context of infliximab withdrawal. A similar reasoning can be applied with the association between a high serum level of acute-phase reactants and the risk of short-term clinical relapse[8]. Even not independently validated, the generalisability of this finding cannot be really contested given its coherence with the CD pathophysiology. To our point of view, the main message of our study (distinct biological profiles between short-term relapsers and mid/long-term relapsers) could be also generalisable given that any disease flare is preceded by a succession of biological states. In our study, these biological changes have been objectified and allow to better understand why, after stopping the infliximab, the clinical relapse occurred in a wide period of time (2-26 months). Reasonably, we think that similar observations could be found in heterogeneous contexts and that, time to relapse or more generally time to event, is a useful parameter to reveal biological profiles.

Our method of analysis was built to anchor the results in a biological reality and, to our opinion, this is an essential condition for external validity. Indeed, our findings rely on a

validated biological hypothesis (time to clinical relapse is related to the pathophysiological sate of patients) and a simple univariable statistics approach where variables were grouped based on their biological rather than mathematical relations. On the other hand, data-driven approaches performing complex mathematical relations between variables are prone to overfitting and the biological meaning of their outputs is generally unknown or difficult to understand (black box effect). These criticisms notably concern machine learning tools[34,35].

In summary, we identified distinct biological profiles between short-term relapsers and mid/long-term relapsers following anti-TNFα withdrawal in CD patients. This knowledge is essential to develop biomarkers guiding the therapeutic strategy.

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**Contributors**

M-AM, YB, DL, J-FC, MA and EL designed the study. The GETAID provided the samples and the clinical information. VAH-T performed the statistical analysis. NP analysed and interpreted the data with the help of M-AM, VAH-T, TM and EL. NP wrote the initial draft of the manuscript, with the assistance of M-AM, TM, VAH-T and EL.

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**Competing interests**

None declared.

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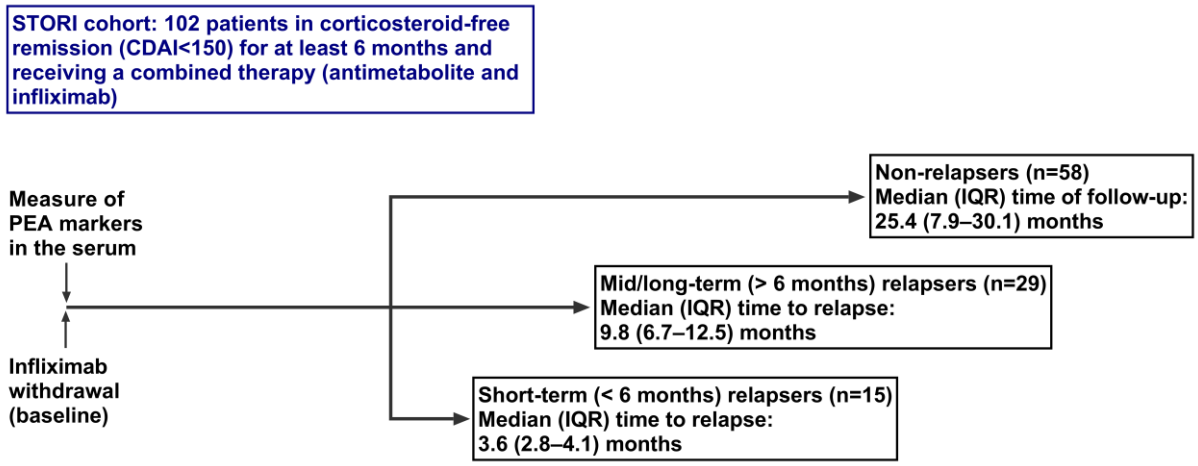



**Figure 1. Experimental design and outcomes of patients after infliximab withdrawal**

IQR, interquartile range; PEA, proximity extension assay; STORI, diSconTinuation in CrOhn's disease patients in stable Remission on combined therapy with Immunosuppressors.

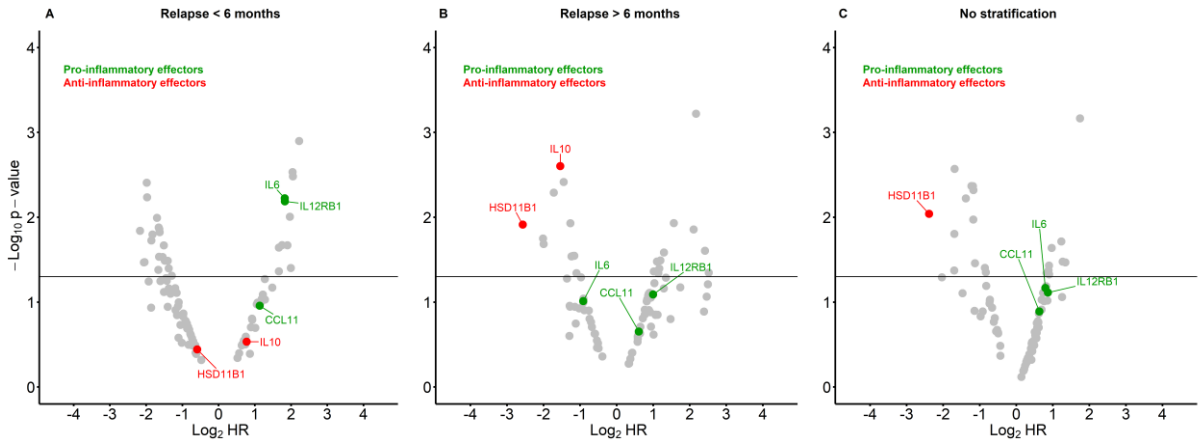



**Figure 2. Risk of clinical relapse associated with pro-inflammatory and anti-inflammatory effectors.** The risk of clinical relapse (HR: hazard ratio) associated with each protein was determined by univariable Cox model and represented by plotting its degree of significance (-$Log_{10}$ p-value) against its effect size ($Log_2$ HR) in the short-term clinical relapse (<6 months) dataset (A) mid/long-term clinical relapse (>6 months) dataset (B) and non-stratified dataset (C). The significance threshold (p-value=0.05) is represented by the horizontal lines. CCL11, eotaxin; HSD11B1, corticosteroid 11-beta-dehydrogenase isozyme 1; IL6, interleukin-6; IL10, interleukin-10; IL12RB1, interleukin-12 receptor subunit beta-1.

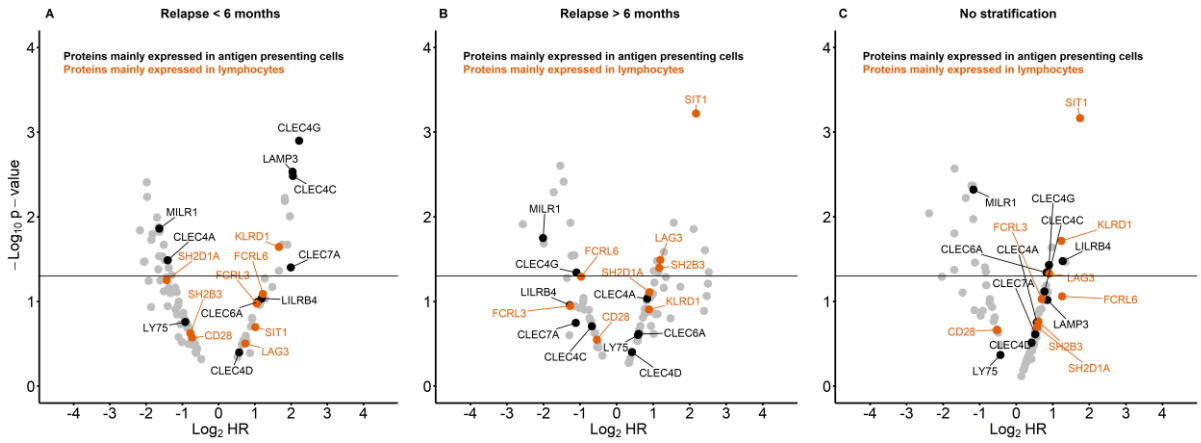



**Figure 3. Risk of clinical relapse associated with proteins mainly expressed in antigen presenting cells or lymphocytes** The risk of clinical relapse (HR: hazard ratio) associated with each protein was determined by univariable Cox model and represented by plotting its degree

of significance (-$Log_{10}$ p-value) against its effect size ($Log_2$ HR) in the short-term clinical relapse (<6 months) dataset (A) mid/long-term clinical relapse (>6 months) dataset (B) and non-stratified dataset (C). The significance threshold (p-value=0.05) is represented by the horizontal lines. CD28, T-cell-specific surface glycoprotein CD28; CLEC4A, C-type lectin domain family 4 member A; CLEC4C, C-type lectin domain family 4 member C; CLEC4D, C-type lectin domain family 4 member D; CLEC4G, C-type lectin domain family 4 member G; CLEC6A, C-type lectin domain family 6 member A; CLEC7A, C-type lectin domain family 7 member A; FCRL3, Fc receptor-like protein 3; FCRL6, Fc receptor-like protein 6; KLRD1, natural killer cells antigen CD94; LAG3, lymphocyte activation gene 3 protein; LAMP3, Lysosome-associated membrane glycoprotein 3; LILRB4, leukocyte immunoglobulin-like receptor subfamily B member 4; LY75, lymphocyte antigen 75; MILR1, allergin-1; SIT1, signaling threshold-regulating transmembrane adapter 1; SH2B3, SH2B adapter protein 3; SH2D1A, SH2 domain-containing protein 1A.

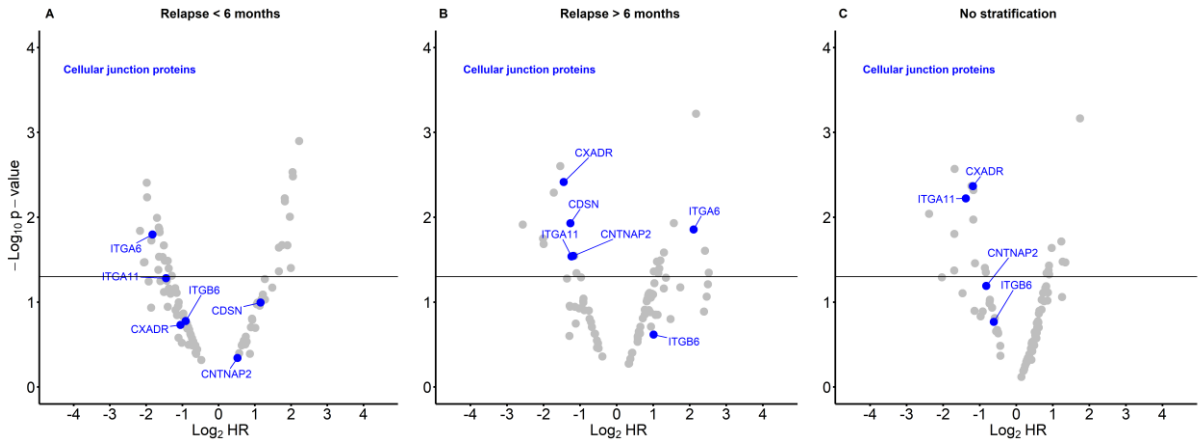



**Figure 4. Risk of clinical relapse associated with cellular junction proteins.** The risk of clinical relapse (HR: hazard ratio) associated with each protein was determined by univariable Cox model and represented by plotting its degree of significance (-$Log_{10}$ p-value) against its effect size ($Log_2$ HR) in the short-term clinical relapse (<6 months) dataset (A) mid/long-term clinical relapse (>6 months) dataset (B) and non-stratified dataset (C). The significance

threshold (p-value=0.05) is represented by the horizontal lines. CDSN, corneodesmosin; CNTNAP2, contactin-associated protein-like 2; CXADR, coxsackievirus and adenovirus receptor; ITGA6, integrin alpha-6; ITGA11, integrin alpha-11; ITGB6, integrin beta-6.

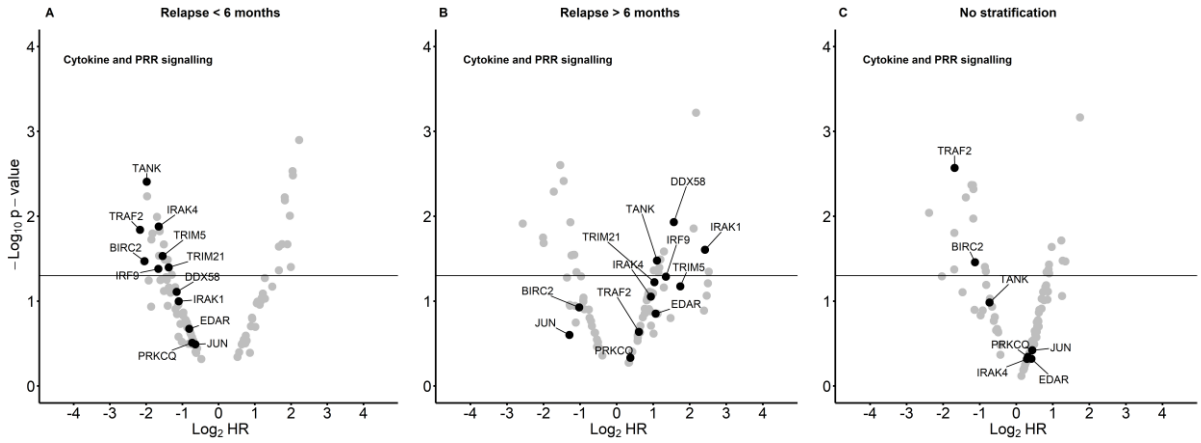



**Figure 5. Risk of clinical relapse associated with downstream signalling of cytokine receptors and pattern recognition receptors.** The risk of clinical relapse (HR: hazard ratio) associated with each protein was determined by univariable Cox model and represented by plotting its degree of significance (-$Log_{10}$ p-value) against its effect size ($Log_2$ HR) in the short-term clinical relapse (<6 months) dataset (A) mid/long-term clinical relapse (>6 months) dataset (B) and non-stratified dataset (C). The significance threshold (p-value=0.05) is represented by the horizontal lines. BIRC2, baculoviral IAP repeat-containing protein 2; DDX58, antiviral innate immune response receptor RIG-I; EDAR, tumor necrosis factor receptor superfamily member EDAR; IRAK1, interleukin-1 receptor-associated kinase 1; IRAK4, interleukin-1 receptor-associated kinase 4; IRF9, interferon regulatory factor 9; JUN, transcription factor AP-1; PRKCQ, protein kinase C theta type; TANK, TRAF family member-associated NF-kappa-B activator; TRAF2, TNF receptor-associated factor 2; TRIM5, tripartite motif-containing protein 5; TRIM21, E3 ubiquitin-protein ligase TRIM21.

**Table 1. Patients' characteristics (n=102)**

| | |
|---|---|
| Male, n (%) | 46 (45) |
| Age, median years (IQR) | 31 (25-39) |
| Disease duration, median years (IQR) | 8 (4-12) |
| Active smoker, n (%) | 38 (37) |
| CDAI (IQR) | 36.2 (16.1-59.5) |
| Disease site | |
| Ileal, n (%) | 13 (13) |
| Colonic, n (%) | 32 (31) |
| Ileocolonic, n (%) | 56 (55) |
| Upper gastrointestinal tract, n (%) | 8 (8) |
| Perianal lesions, n (%) | 36 (35) |
| ***Treatment history*** | |
| Methotrexate, n (%) | 17 (17) |
| Azathioprine/mercaptopurine, n (%) | 85 (83) |
| Duration of antimetabolite treatment, median years (IQR) | 2.6 (1.7-4.5) |
| Duration of infliximab treatment, median years (IQR) | 2.2 (1.6-3.1) |
| Previous surgical resection, n (%) | 22 (22) |
| ***Endoscopy*** | |
| CDEIS (IQR) | 0.6 (0.0-2.8) |
| CDEIS=0, n (%) | 38 (37) |
| Remaining ulcers, n (%) | 32 (31) |
| ***Biologic variables*** | |
| Haemoglobin level, g/L, median (IQR) | 136 (127-144) |
| Haematocrit, %, median (IQR) | 40 (37-43) |
| Leukocyte count, $10^9$/L, median (IQR) | 6.0 (4.9-7.3) |
| Platelet count, $10^9$/L, median (IQR) | 265 (225-313) |
| hsCRP level, mg/L, median (IQR) | 1.9 (0.8-4.2) |
| Infliximab trough level, mg/L, median (IQR) | 3.7 (1.9-7.9) |
| Faecal calprotectin, µg/g, n=77, median (IQR) | 49.4 (29.6-200.8) |

CDAI: Crohn's disease activity index; CDEIS: Crohn's disease endoscopic index of severity; hsCRP: high-sensitivity CRP; IQR: interquartile range

| Protein names | Gene names | Uniprot accession number | Main cellular expression | Functions | Presentation in volcano plots | Association with the risk of short-term and/or mid/long-term relapse | HR (95% CI) in short-term relapse dataset | HR p-value in short-term relapse dataset | HR (95% CI) in mid/long-term relapse dataset | HR p-value in mid/long-term relapse dataset | HR (95% CI) in non-stratified dataset | HR p-value in non-stratified dataset |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amphiregulin | AREG | P15514 | Widely expressed | Growth factor | Not shown | No association | 0.6(0.2-1.7) | 0.318 | 0.7(0.3-1.5) | 0.344 | 1.2(0.7-2.1) | 0.506 |
| Aryl hydrocarbon receptor nuclear translocator | ARNT | P27540 | Ubiquitous | Response to hypoxia (component of HIF-1) | Sup Figure 1 | Short-term relapse | 3.4(1.2-9.5) | 0.021 | 1.9(0.9-3.9) | 0.088 | 1.8(1-3.2) | 0.065 |
| Transcription regulator protein BACH1 | BACH1 | O14867 | Ubiquitous | Antioxidant defence (repressor) | Sup Figure 1 | Mid/long-term relapse | 0.3(0.1-1) | 0.057 | 2.2(1-4.8) | 0.046 | 1.4(0.8-2.6) | 0.285 |
| Baculoviral IAP repeat-containing protein 2 | BIRC2 | Q13490 | Ubiquitous | NF-κB pathway | Figure 5 | Short-term relapse | 0.2(0.1-0.9) | 0.034 | 0.5(0.2-1.2) | 0.119 | 0.5(0.2-0.9) | 0.035 |
| Butyrophilin subfamily 3 member A2 | BTN3A2 | P78410 | Ubiquitous | Adaptive immunity | Not shown | No association | 0.6(0.2-1.6) | 0.323 | 0.6(0.3-1.2) | 0.126 | 0.7(0.4-1.2) | 0.233 |
| Eotaxin | CCL11 | P51671 | Widely expressed | Pro-inflammatory, chemotaxis | Figure 2 | No association | 2.2(0.8-5.8) | 0.110 | 1.5(0.8-3) | 0.222 | 1.5(0.9-2.7) | 0.129 |
| T-cell-specific surface glycoprotein CD28 | CD28 | P10747 | T cells | Immunity, receptor for CD80 and CD86 | Figure 3 | No association | 0.6(0.2-1.5) | 0.267 | 0.7(0.3-1.4) | 0.283 | 0.7(0.4-1.2) | 0.217 |
| CD83 antigen | CD83 | Q01151 | Widely expressed | Antigen presentation | Not shown | No association | 2.4(0.9-6.9) | 0.094 | 0.5(0.2-1.2) | 0.124 | 0.5(0.2-1.2) | 0.129 |
| Corneodesmosin | CDSN | Q15517 | Keratinocytes | Cellular junction | Figure 4 | Mid/long-term relapse | 2.2(0.9-5.8) | 0.101 | 0.4(0.2-0.8) | 0.012 | 0.7(0.4-1.2) | 0.211 |
| Cytoskeleton-associated protein 4 | CKAP4 | Q07065 | Widely expressed | Cytoskeleton | Not shown | No association | 0.6(0.2-1.8) | 0.394 | 1.8(0.9-3.6) | 0.088 | 1.3(0.7-2.4) | 0.352 |
| C-type lectin domain family 4 member A | CLEC4A | Q9UMR7 | APCs | Tolerance, PRRs, ligands not well known | Figure 3 | Short-term relapse | 0.4(0.2-0.9) | 0.033 | 1.8(0.9-3.5) | 0.093 | 1.5(0.8-2.6) | 0.177 |
| C-type lectin domain family 4 member C | CLEC4C | Q8WTT0 | pDCs | Tolerance, PRRs, ligands not well known | Figure 3 | Short-term relapse | 4.1(1.6-10.7) | 0.003 | 0.6(0.3-1.3) | 0.196 | 1.7(0.9-3.1) | 0.076 |
| C-type lectin domain family 4 member D | CLEC4D | Q8WXI8 | APCs | Immunity, PRRs, ligands not well known | Figure 3 | No association | 1.5(0.6-3.7) | 0.400 | 1.3(0.7-2.6) | 0.397 | 1.3(0.8-2.3) | 0.307 |
| C-type lectin domain family 4 member G | CLEC4G | Q6UXB4 | LSECs, APCs | Tolerance, PRRs, virus recognition | Figure 3 | Short and mid/long-term relapse | 4.7(1.8-11.9) | 0.001 | 0.5(0.2-1) | 0.046 | 1.9(1-3.3) | 0.037 |
| C-type lectin domain family 6 member A | CLEC6A | Q6EIG7 | APCs | Immunity, PRRs, fungi recognition | Figure 3 | No association | 2.1(0.9-5.1) | 0.102 | 1.5(0.8-3) | 0.242 | 1.8(1-3.1) | 0.046 |
| C-type lectin domain family 7 member A | CLEC7A | Q9BXN2 | APCs | Immunity and tolerance, PRRs, fungi and DAMPs recognition | Figure 3 | Short-term relapse | 4(1.1-14.9) | 0.040 | 0.5(0.1-1.4) | 0.179 | 1.4(0.8-2.6) | 0.244 |
| Contactin-associated protein-like 2 | CNTNAP2 | Q9UHC6 | Nervous system cells | Cellular junction | Figure 4 | Mid/long-term relapse | 1.4(0.6-3.7) | 0.454 | 0.4(0.2-0.9) | 0.029 | 0.6(0.3-1) | 0.064 |
| Coxsackievirus and adenovirus receptor | CXADR | P78310 | Widely expressed | Cellular junction, regulate entry of virus | Figure 4 | Mid/long-term relapse | 0.5(0.2-1.4) | 0.186 | 0.4(0.2-0.7) | 0.004 | 0.4(0.2-0.8) | 0.004 |
| Stromal cell-derived factor 1 | CXCL12 | P48061 | Widely expressed | Chemotaxis | Not shown | No association | 1.6(0.7-4) | 0.298 | 0.7(0.3-1.3) | 0.236 | 2.5(1.1-6) | 0.034 |
| Dual adapter for phosphotyrosine and 3-phosphotyro | DAPP1 | Q9UN19 | Widely expressed | Not well defined | Sup Figure 1 | Mid/long-term relapse | 0.3(0.1-1) | 0.057 | 5.7(1-31.7) | 0.045 | 0.4(0.1-1.1) | 0.079 |
| Discoidin, CUB and LCCL domain-containing prote | DCBLD2 | Q96PD2 | Widely expressed | Not well defined | Sup Figure 1 | Mid/long-term relapse | 1.6(0.6-3.8) | 0.317 | 0.3(0.1-0.7) | 0.005 | 0.4(0.2-0.8) | 0.004 |
| Dynactin subunit 1 | DCTN1 | Q14203 | Ubiquitous | Pleiotropic effects | Not shown | No association | 0.4(0.1-1.1) | 0.069 | 1.8(0.9-3.5) | 0.098 | 1.3(0.7-2.3) | 0.410 |
| Antiviral innate immune response receptor RIG-I | DDX58 | O95786 | Ubiquitous | NF-κB pathway | Figure 5 | Mid/long-term relapse | 0.4(0.2-1.1) | 0.078 | 3(1.3-6.9) | 0.012 | 1.6(0.9-3) | 0.128 |
| DNA fragmentation factor subunit alpha | DFFA | O00273 | Ubiquitous | Apoptosis | Sup Figure 1 | Short-term relapse | 0.3(0.1-0.8) | 0.019 | 2.8(0.7-11.5) | 0.158 | 1.2(0.6-2.1) | 0.640 |
| Diacylglycerol kinase zeta | DGKZ | Q13574 | Widely expressed | Pleiotropic effects | Sup Figure 1 | Short-term relapse | 0.3(0.1-0.9) | 0.029 | 1.9(0.8-4.3) | 0.131 | 1.4(0.7-2.6) | 0.316 |
| Inactive dipeptidyl peptidase 10 | DPP10 | Q8N608 | Widely expressed | Potassium homeostasis | Sup Figure 1 | Mid/long-term relapse | 0.5(0.1-1.9) | 0.302 | 0.4(0.2-0.9) | 0.028 | 0.3(0.1-0.8) | 0.016 |
| Tumor necrosis factor receptor superfamily member | EDAR | Q9UNE0 | Widely expressed | NF-κB pathway | Figure 5 | No association | 0.6(0.2-1.4) | 0.212 | 2.1(0.8-5.6) | 0.140 | 1.3(0.6-3) | 0.478 |
| Egl nine homolog 1 | EGLN1 | Q9GZT9 | Widely expressed | Response to hypoxia (inactivate HIF-1α) | Sup Figure 1 | Mid/long-term relapse | 0.4(0.1-1.1) | 0.076 | 2(1-4) | 0.044 | 1.5(0.9-2.7) | 0.136 |
| Eukaryotic translation initiation factor 4 gamma 1 | EIF4G1 | Q04637 | Ubiquitous | Translation | Sup Figure 1 | Short-term relapse | 0.3(0.1-0.8) | 0.010 | 5.6(0.9-34.7) | 0.062 | 0.2(0.1-1) | 0.051 |
| Eukaryotic translation initiation factor 5A-1 | EIF5A | P63241 | Ubiquitous | Translation | Not shown | No association | 1.8(0.4-7.5) | 0.407 | 1.3(0.6-2.6) | 0.532 | 1.2(0.7-2.3) | 0.467 |
| Protein FAM3B | FAM3B | P58499 | Widely expressed | Apoptosis | Not shown | No association | 0.7(0.2-1.8) | 0.407 | 5.2(0.6-44.6) | 0.129 | 1.2(0.7-2.2) | 0.519 |
| Fc receptor-like protein 3 | FCRL3 | Q96P31 | Lymphocytes | Immunity and tolerance, receptor for IgA | Figure 3 | No association | 2.1(0.9-5) | 0.106 | 0.4(0.1-1.2) | 0.113 | 1.6(0.9-2.8) | 0.094 |
| Fc receptor-like protein 6 | FCRL6 | Q6DN72 | Lymphocytes | Tolerance, receptor for MHC II antigens | Figure 3 | No association | 2.3(0.9-6.1) | 0.082 | 0.5(0.3-1) | 0.051 | 2.4(0.9-6.4) | 0.087 |
| Fibroblast growth factor 2 | FGF2 | P09038 | Widely expressed | Pleiotropic effects | Sup Figure 1 | Mid/long-term relapse | 2.8(0.9-8.4) | 0.067 | 0.3(0.1-0.8) | 0.021 | 0.6(0.3-1.1) | 0.096 |
| FXYD domain-containing ion transport regulator 5 | FXYD5 | Q96DB9 | Ubiquitous | Ion transport, cell adhesion | Not shown | No association | 0.4(0.2-1.3) | 0.124 | 0.7(0.3-1.3) | 0.236 | 0.7(0.4-1.2) | 0.154 |
| Polypeptide N-acetylgalactosaminyltransferase 3 | GALNT3 | Q14435 | Widely expressed | Protein glycosylation | Not shown | No association | 1.6(0.7-3.9) | 0.284 | 0.5(0.3-1.1) | 0.101 | 0.7(0.4-1.4) | 0.327 |
| Beta-galactosidase | GLB1 | P16278 | Ubiquitous | Metabolisme | Not shown | No association | 0.5(0.2-1.3) | 0.142 | 0.5(0.2-1.2) | 0.114 | 0.3(0.1-1) | 0.042 |
| Hematopoietic lineage cell-specific protein | HCLS1 | P14317 | Immune cells | Immune response | Not shown | No association | 0.6(0.2-1.4) | 0.196 | 1.5(0.7-3) | 0.269 | 1.1(0.6-2) | 0.646 |
| Protein HEXIM1 | HEXIM1 | O94992 | Ubiquitous | Pleiotropic effects | Not shown | No association | 0.4(0.1-1.1) | 0.080 | 1.5(0.8-3) | 0.255 | 0.5(0.2-1.3) | 0.127 |
| Histamine N-methyltransferase | HNMT | P50135 | Widely expressed | Histamine metabolism | Not shown | No association | 1.6(0.6-3.8) | 0.323 | 0.5(0.3-1.1) | 0.091 | 0.7(0.4-1.3) | 0.220 |
| Corticosteroid 11-beta-dehydrogenase isozyme 1 | HSD11B1 | P28845 | Widely expressed | Anti-inflammatory | Figure 2 | Mid/long-term relapse | 0.7(0.3-1.6) | 0.361 | 0.2(0-0.7) | 0.012 | 0.2(0.1-0.7) | 0.009 |
| Islet cell autoantigen 1 | ICA1 | Q05084 | Widely expressed | Not well known | Not shown | No association | 0.4(0.1-1.3) | 0.114 | 1.3(0.7-2.7) | 0.394 | 0.5(0.2-1.3) | 0.147 |
| Interferon lambda receptor 1 | IFNLR1 | Q8IU57 | Widely expressed | Antiviral defense | Not shown | No association | 0.6(0.3-1.5) | 0.291 | 1.8(0.9-3.6) | 0.080 | 1.7(0.9-2.9) | 0.078 |
| Interleukin-10 | IL10 | P22301 | Widely expressed | Anti-inflammatory | Figure 2 | Mid/long-term relapse | 1.7(0.6-4.6) | 0.293 | 0.3(0.2-0.7) | 0.002 | 0.4(0.2-0.8) | 0.005 |
| Interleukin-12 receptor subunit beta-1 | IL12RB1 | P42701 | Widely expressed | Pro-inflammatory | Figure 2 | Short-term relapse | 3.6(1.4-8.9) | 0.006 | 2(0.9-4.3) | 0.081 | 1.8(0.9-3.5) | 0.077 |
| Interleukin-5 | IL5 | P05113 | Widely expressed | Adaptive immunity | Not shown | No association | 0.5(0.2-1.3) | 0.148 | 2.2(0.8-6.1) | 0.141 | 0.6(0.4-1.1) | 0.116 |
| Interleukin-6 | IL6 | P05231 | Widely expressed | Pro-inflammatory | Figure 2 | Short-term relapse | 3.5(1.4-8.7) | 0.006 | 0.5(0.3-1.1) | 0.097 | 1.7(1-3.1) | 0.068 |
| Interleukin-1 receptor-associated kinase 1 | IRAK1 | P51617 | Ubiquitous | NF-κB pathway | Figure 5 | Mid/long-term relapse | 0.5(0.2-1.2) | 0.100 | 5.3(1.2-23.1) | 0.025 | 1.9(0.8-4.5) | 0.173 |
| Interleukin-1 receptor-associated kinase 4 | IRAK4 | Q9NWZ3 | Ubiquitous | NF-κB pathway | Figure 5 | Short-term relapse | 0.3(0.1-0.8) | 0.013 | 1.9(0.9-4) | 0.088 | 1.2(0.7-2.2) | 0.479 |
| Interferon regulatory factor 9 | IRF9 | Q00978 | Ubiquitous | Interferon pathway | Figure 5 | Short-term relapse | 0.3(0.1-1) | 0.042 | 2.5(1-6.5) | 0.051 | 1.4(0.7-2.8) | 0.285 |
| Integrin alpha-11 | ITGA11 | Q9UKX5 | Widely expressed | Cellular junction | Figure 4 | Mid/long-term relapse | 0.4(0.1-1) | 0.052 | 0.4(0.2-0.9) | 0.029 | 0.4(0.2-0.8) | 0.006 |
| Integrin alpha-6 | ITGA6 | P23229 | Widely expressed | Cellular junction | Figure 4 | Short and mid/long-term relapse | 0.3(0.1-0.8) | 0.016 | 4.3(1.3-13.8) | 0.014 | 1.8(0.9-4) | 0.118 |
| Integrin beta-6 | ITGB6 | P18564 | Widely expressed | Cellular junction | Figure 4 | No association | 0.5(0.2-1.3) | 0.167 | 2(0.6-6.5) | 0.242 | 0.7(0.4-1.2) | 0.171 |
| Integral membrane protein 2A | ITM2A | O43736 | Widely expressed | Not well defined | Sup Figure 1 | Short-term relapse | 3.9(1.4-11) | 0.010 | 1.7(0.8-3.4) | 0.174 | 1.8(1-3.3) | 0.042 |
| Transcription factor AP-1 | JUN | P05412 | Ubiquitous | MAPKs | Figure 5 | No association | 0.6(0.3-1.5) | 0.323 | 0.4(0.1-1.9) | 0.250 | 1.4(0.7-2.7) | 0.379 |
| Natural killer cells antigen CD94 | KLRD1 | Q13241 | NK cells | Immunity and tolerance, receptor for MHC I antigens | Figure 3 | Short-term relapse | 3.2(1.2-8.5) | 0.023 | 1.8(0.8-4) | 0.124 | 2.3(1.1-4.8) | 0.019 |
| Importin subunit alpha-5 | KPNA1 | P52294 | Ubiquitous | Cellular transport | Not shown | No association | 0.5(0.1-1.8) | 0.263 | 1.5(0.7-3.1) | 0.293 | 1.3(0.7-2.4) | 0.454 |
| Keratin, type I cytoskeletal 19 | KRT19 | P08727 | Widely expressed | Cytoskeleton | Not shown | No association | 1.9(0.8-4.6) | 0.156 | 1.6(0.8-3.2) | 0.234 | 1.7(0.9-3.2) | 0.097 |
| Lymphocyte activation gene 3 protein | LAG3 | P18627 | Lymphocytes | Tolerance, receptor for MHC II antigens | Figure 3 | Mid/long-term relapse | 1.7(0.6-4.5) | 0.314 | 2.3(1.1-4.9) | 0.032 | 1.9(1-3.5) | 0.047 |
| Lysosome-associated membrane glycoprotein 3 | LAMP3 | Q9UQV4 | DCs | Unknown | Figure 3 | Short-term relapse | 4.1(1.6-10.4) | 0.003 | 0.8(0.4-1.5) | 0.421 | 1.8(0.9-3.6) | 0.096 |
| Leukocyte immunoglobulin-like receptor subfamily I | LILRB4 | Q8NHJ6 | pDCs | Tolerance, MHC II response | Figure 3 | No association | 2.3(0.9-5.9) | 0.092 | 0.4(0.1-1.2) | 0.111 | 2.4(1.1-5.4) | 0.033 |
| Lymphocyte antigen 75 | LY75 | O60449 | APCs | Immunity and tolerance, PRRs, bacteria and DAMPs recognitior | Figure 3 | No association | 0.5(0.2-1.3) | 0.175 | 1.5(0.8-3) | 0.249 | 0.7(0.4-1.6) | 0.429 |
| Mannan-binding lectin serine protease 1 | MASP1 | P48740 | Liver | Complement pathway (lectin) | Sup Figure 1 | Short-term relapse | 0.3(0.1-0.7) | 0.006 | 0.6(0.3-1.2) | 0.172 | 0.4(0.2-0.8) | 0.011 |
| Methylated-DNA--protein-cysteine methyltransferas | MGMT | P16455 | Ubiquitous | DNA repair | Not shown | No association | 1.7(0.7-4.1) | 0.255 | 1.6(0.8-3.1) | 0.195 | 1.4(0.8-2.5) | 0.225 |
| Allergin-1 | MILR1 | Q7Z6M3 | APCs | Tolerance, Ig-like receptor with unknown ligand | Figure 3 | Short and mid/long-term relapse | 0.3(0.1-0.8) | 0.014 | 0.2(0.1-0.8) | 0.018 | 0.4(0.3-0.8) | 0.005 |
| Natural cytotoxicity triggering receptor 1 | NCR1 | O76036 | NK cells | Activation of NK cells, virus recognition | Not shown | No association | 2.4(1-5.9) | 0.053 | 1.5(0.8-3.1) | 0.224 | 2(1.1-3.5) | 0.023 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Merlin | NF2 | P35240 | Ubiquitous | Cytoskeleton, hippo pathway | Not shown | No association | 0.3(0.1-1.4) | 0.116 | 1.3(0.6-2.6) | 0.528 | 1.1(0.6-2.1) | 0.762 |
| Nuclear factor of activated T-cells, cytoplasmic 3 | NFATC3 | Q12968 | Ubiquitous | Immune response | Not shown | No association | 0.5(0.2-1.2) | 0.136 | 0.6(0.3-1.2) | 0.157 | 0.6(0.3-1.1) | 0.094 |
| Neurotrophin-4 | NTF4 | P34130 | Widely expressed | Growth factor | Not shown | No association | 0.6(0.2-1.4) | 0.244 | 0.7(0.4-1.4) | 0.305 | 0.7(0.4-1.2) | 0.228 |
| Protein-arginine deiminase type-2 | PADI2 | Q9Y2J8 | Widely expressed | Pleiotropic effects | Not shown | No association | 0.5(0.2-1.2) | 0.113 | 1.8(0.9-3.6) | 0.084 | 1.5(0.8-2.9) | 0.199 |
| Phosphoinositide 3-kinase adapter protein 1 | PIK3AP1 | Q6ZUJ8 | Widely expressed | Adaptive immunity | Not shown | No association | 0.4(0.2-1.1) | 0.069 | 1.7(0.9-3.4) | 0.123 | 1.2(0.7-2.1) | 0.562 |
| Plexin-A4 | PLXNA4 | Q9HCM2 | Widely expressed | Pleiotropic effects | Not shown | No association | 0.5(0.2-1.4) | 0.204 | 0.4(0.2-1) | 0.053 | 1.5(0.8-2.7) | 0.169 |
| Neurabin-2 | PPP1R9B | Q96SB3 | Widely expressed | Pleiotropic effects | Sup Figure 1 | Short-term relapse | 0.3(0.1-0.8) | 0.015 | 5.5(0.8-38.9) | 0.086 | 0.6(0.3-1) | 0.045 |
| Peroxiredoxin-1 | PRDX1 | Q06830 | Ubiquitous | Antioxidant defence | Sup Figure 1 | Short and mid/long-term relapse | 0.4(0.1-0.9) | 0.021 | 2.5(1.1-5.4) | 0.026 | 0.6(0.3-1) | 0.039 |
| Thioredoxin-dependent peroxide reductase, mitocho | PRDX3 | P30048 | Ubiquitous | Antioxidant defence | Not shown | No association | 1.9(0.8-4.7) | 0.161 | 1.9(0.7-5.1) | 0.194 | 1.4(0.7-2.8) | 0.322 |
| Peroxiredoxin-5, mitochondrial | PRDX5 | P30044 | Ubiquitous | Antioxidant defence | Sup Figure 1 | Short-term relapse | 0.4(0.2-1) | 0.049 | 2(0.9-4.1) | 0.080 | 1.2(0.7-2.1) | 0.617 |
| Protein kinase C theta type | PRKCQ | Q04759 | Widely expressed | NF-κB pathway | Figure 5 | No association | 0.6(0.2-1.6) | 0.309 | 1.3(0.6-2.6) | 0.465 | 1.2(0.7-2.2) | 0.453 |
| PC4 and SFRS1-interacting protein | PSIP1 | O75475 | Ubiquitous | Not well defined | Sup Figure 1 | Short-term relapse | 0.4(0.1-0.9) | 0.032 | 1.8(0.9-3.6) | 0.094 | 1.2(0.6-2.1) | 0.615 |
| Parathyroid hormone/parathyroid hormone-related p | PTH1R | Q03431 | Widely expressed | Pleiotropic effects | Not shown | No association | 0.7(0.3-1.8) | 0.481 | 1.7(0.8-3.5) | 0.140 | 1.6(0.9-2.7) | 0.127 |
| SH2B adapter protein 3 | SH2B3 | Q9UQQ2 | Lymphocytes | Tolerance, adaptor protein | Figure 3 | Mid/long-term relapse | 0.6(0.2-1.4) | 0.236 | 2.2(1-4.9) | 0.040 | 1.5(0.8-2.8) | 0.173 |
| SH2 domain-containing protein 1A | SH2D1A | O60880 | Lymphocytes | Immunity, adaptor protein | Figure 3 | No association | 0.4(0.1-1) | 0.056 | 1.9(0.9-3.7) | 0.078 | 1.5(0.8-2.7) | 0.199 |
| Signaling threshold-regulating transmembrane adapt | SIT1 | Q9Y3P8 | Lymphocytes | Tolerance, transmembrane adaptor | Figure 3 | Mid/long-term relapse | 2(0.7-5.9) | 0.201 | 4.5(1.9-10.7) | 0.001 | 3.4(1.7-6.7) | 0.001 |
| Protein sprouty homolog 2 | SPRY2 | O43597 | Widely expressed | Inhibitor of the fibroblast growth factor signaling | Sup Figure 1 | Short-term relapse | 3.2(1-9.7) | 0.043 | 0.7(0.3-1.5) | 0.350 | 1.6(0.9-3) | 0.123 |
| SRSF protein kinase 2 | SRPK2 | P78362 | Ubiquitous | Pleiotropic effects | Not shown | No association | 0.5(0.2-1.3) | 0.179 | 1.9(0.9-3.8) | 0.085 | 1.3(0.8-2.4) | 0.297 |
| Stanniocalcin-1 | STC1 | P52823 | Widely expressed | Pleiotropic effects | Sup Figure 1 | Short-term relapse | 3.7(1.2-11.4) | 0.021 | 2.4(0.9-6.4) | 0.069 | 1.5(0.8-2.8) | 0.229 |
| TRAF family member-associated NF-kappa-B activa | TANK | Q92844 | Ubiquitous | NF-κB pathway | Figure 5 | Short and mid/long-term relapse | 0.3(0.1-0.6) | 0.004 | 2.2(1.1-4.4) | 0.033 | 0.6(0.3-1.1) | 0.104 |
| Tryptase alpha/beta-1 | TPSAB1 | Q15661 | Mast cells | Protease | Not shown | No association | 0.5(0.2-1.2) | 0.110 | 0.8(0.4-1.5) | 0.438 | 0.7(0.4-1.2) | 0.213 |
| TNF receptor-associated factor 2 | TRAF2 | Q12933 | Ubiquitous | NF-κB pathway | Figure 5 | Short-term relapse | 0.2(0.1-0.7) | 0.014 | 1.5(0.8-3) | 0.230 | 0.3(0.1-0.7) | 0.003 |
| Triggering receptor expressed on myeloid cells 1 | TREM1 | Q9NP99 | Widely expressed | Pro-inflammatory | Not shown | No association | 1.9(0.7-4.7) | 0.197 | 1.6(0.8-3.3) | 0.155 | 1.5(0.8-2.7) | 0.168 |
| E3 ubiquitin-protein ligase TRIM21 | TRIM21 | P19474 | Ubiquitous | NF-κB and interferon pathways | Figure 5 | Short-term relapse | 0.4(0.2-1) | 0.040 | 2(1-4.3) | 0.060 | 1.1(0.6-2) | 0.665 |
| Tripartite motif-containing protein 5 | TRIM5 | Q9C035 | Ubiquitous | NF-κB and interferon pathways | Figure 5 | Short-term relapse | 0.3(0.1-0.9) | 0.029 | 3.3(0.9-12.2) | 0.067 | 1.1(0.5-2.4) | 0.716 |
| Zinc finger and BTB domain-containing protein 16 | ZBTB16 | Q05516 | Widely expressed | Pleiotropic effects | Sup Figure 1 | Short-term relapse | 0.2(0.1-0.9) | 0.034 | 2.1(0.8-5.6) | 0.141 | 1.5(0.7-2.9) | 0.266 |

**Supplementary table 2. Pro-inflammatory and anti-inflammatory effectors**

| Protein names | Gene names | Uniprot accession number | Main cellular expression | Inflammatory balance |
|---|---|---|---|---|
| Interleukin-6 | IL6 | P05231 | Ubiquitous | Pro-inflammatory[1] |
| Eotaxin | CCL11 | P51671 | Ubiquitous | Pro-inflammatory[2] |
| Interleukin-12 receptor subunit beta-1 | IL12RB1 | P42701 | Ubiquitous | Pro-inflammatory[3] |
| Interleukin-10 | IL10 | P22301 | Ubiquitous | Anti-inflammatory[4] |
| Corticosteroid 11-beta-dehydrogenase isozyme 1 | HSD11B1 | P28845 | Ubiquitous | Anti-inflammatory[5] |

**Supplementary table 3. Proteins mainly expressed in antigen presenting cells or lymphocytes**

| Protein names | Gene names | Uniprot accession number | Main cellular expression | Immune function | Protein function and ligands |
|---|---|---|---|---|---|
| T-cell-specific surface glycoprotein CD28 | CD28 | P10747 | T cells[6] | Immunity[7] | Receptor for CD80 and CD86[8] |
| Natural killer cells antigen CD94 | KLRD1 | Q13241 | NK cells[9] | Immunity and tolerance[9] | Receptor for MHC I antigens[9] |
| Lymphocyte activation gene 3 protein | LAG3 | P18627 | Lymphocytes[10] | Tolerance[7] | Receptor for MHC II antigens[8] |
| Signaling threshold-regulating transmembrane adapter 1 | SIT1 | Q9Y3P8 | Lymphocytes[11] | Tolerance[12] | Transmembrane adaptor[12] |
| SH2B adapter protein 3 | SH2B3 | Q9UQQ2 | Lymphocytes[13] | Tolerance[13] | Adaptor protein[13] |
| SH2 domain-containing protein 1A | SH2D1A | O60880 | Lymphocytes[14] | Immunity[14] | Adaptor protein[14] |
| Fc receptor-like protein 6 | FCRL6 | Q6DN72 | Lymphocytes[15] | Tolerance[16] | Receptor for MHC II antigen[15] |
| Fc receptor-like protein 3 | FCRL3 | Q96P31 | Lymphocytes[17] | Immunity and tolerance[17] | Receptor for IgA[17] |
| C-type lectin domain family 4 member C | CLEC4C | Q8WTT0 | pDCs[18] | Tolerance[19] | PRRs, ligands not well known[20] |
| Leukocyte immunoglobulin-like receptor subfamily B member 4 | LILRB4 | Q8NHJ6 | pDCs[21] | Tolerance[22] | Receptor for BST2[21] |
| Lysosome-associated membrane glycoprotein 3 | LAMP3 | Q9UQV4 | DCs[23] | Unknown | MHC II response[23] |
| C-type lectin domain family 4 member G | CLEC4G | Q6UXB4 | LSECs, APCs[20] | Tolerance[7] | PRRs, virus recognition[20] |
| C-type lectin domain family 4 member A | CLEC4A | Q9UMR7 | APCs[24] | Tolerance[20] | PRRs, ligands not well known[20] |
| C-type lectin domain family 7 member A | CLEC7A | Q9BXN2 | APCs[24] | Immunity and tolerance[18,20] | PRRs, fungi and DAMPs recognition[20] |
| C-type lectin domain family 6 member A | CLEC6A | Q6EIG7 | APCs[20] | Immunity[20] | PRRs, fungi recognition[25] |
| C-type lectin domain family 4 member D | CLEC4D | Q8WXI8 | APCs[26] | Immunity[18] | PRRs, ligands not well known[20] |
| Allergin-1 | MILR1 | Q7Z6M3 | APCs[27] | Tolerance[27] | Ig-like receptor with unknown ligand[27] |
| Lymphocyte antigen 75 | LY75 | O60449 | APCs[28] | Immunity and tolerance[29] | PRRs, bacteria and DAMPs recognition[30] |

APCs: antigen presenting cells; BST2: bone marrow stromal cell antigen 2; CD80: cluster of differentiation 80; DAMP: damage associated molecular pattern; DCs: dendritic cells; LSECs: liver sinusoidal endothelial cells; MHC I; major histocompatibility complex class I; MHC II: major histocompatibility complex class II; pDCs: plasmacytoid dendritic cells; PRRs: pattern recognition receptors.

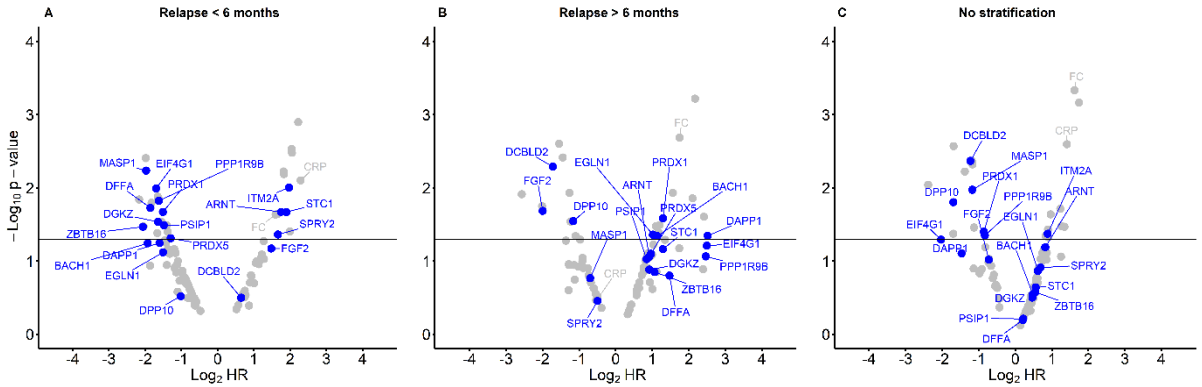
**Supplementary table 4. Cellular junction proteins**

| Protein names | Gene names | Uniprot accession number | Main cellular expression | Functions |
|---|---|---|---|---|
| Corneodesmosin | CDSN | Q15517 | Keratinocytes[31] | Cell-cell junction (corneodesmosomes)[31] |
| Contactin-associated protein-like 2 | CNTNAP2 | Q9UHC6 | Nervous system cells[32] | Cell-cell junction (synapse)[32] |
| Coxsackievirus and adenovirus receptor | CXADR | P78310 | Ubiquitous | Cell-cell junction (tight junction)[33] |
| Integrin alpha-6 | ITGA6 | P23229 | Ubiquitous | Cell-matrix junction[34] |
| Integrin alpha-11 | ITGA11 | Q9UKX5 | Ubiquitous | Cell-matrix junction[34] |
| Integrin beta-6 | ITGB6 | P18564 | Ubiquitous | Cell-matrix junction[34] |

**Supplementary table 5. Downstream signalling of cytokine receptors and pattern recognition receptors**

| Protein names | Gene names | Uniprot accession number | Main cellular expression | Pathways |
|---|---|---|---|---|
| Antiviral innate immune response receptor RIG-I | DDX58 | O95786 | Ubiquitous | NF-κB[a] |
| Interleukin-1 receptor-associated kinase 1 | IRAK1 | P51617 | Ubiquitous | NF-κB[a] |
| Protein kinase C theta type | PRKCQ | Q04759 | Ubiquitous | NF-κB[a] |
| TNF receptor-associated factor 2 | TRAF2 | Q12933 | Ubiquitous | NF-κB[a] |
| Baculoviral IAP repeat-containing protein 2 | BIRC2 | Q13490 | Ubiquitous | NF-κB[a] |
| TRAF family member-associated NF-kappa-B activator | TANK | Q92844 | Ubiquitous | NF-κB |
| Interleukin-1 receptor-associated kinase 4 | IRAK4 | Q9NWZ3 | Ubiquitous | NF-κB[a] |
| Tumor necrosis factor receptor superfamily member EDAR | EDAR | Q9UNE0 | Ubiquitous | NF-κB[a] |
| E3 ubiquitin-protein ligase TRIM21 | TRIM21 | P19474 | Ubiquitous | Interferon[b]; NF-κB[35] |
| Tripartite motif-containing protein 5 | TRIM5 | Q9C035 | Ubiquitous | Interferon[b]; NF-κB[36] |
| Interferon regulatory factor 9 | IRF9 | Q00978 | Ubiquitous | Interferon[b] |
| Transcription factor AP-1 | JUN | P05412 | Ubiquitous | MAPKs[c] |

MAPKs: mitogen-activated protein kinases; NF-κB: nuclear factor-κB. [a]KEGG pathway hsa04064 (NF-kappa B signaling pathway); [b]Reactome pathway R-HSA-877300 (interferon gamma signalling); [b]KEGG pathway hsa04010 (MAPK signaling pathway).




**Supplementary Figure 1. Proteins not highlighted in the core manuscript and showing a significant association with the risk of short-term and/or mid/long-term relapse.**

The risk of relapse (HR: hazard ratio) associated with each protein was determined by univariable Cox model and represented by plotting its degree of significance (-$Log_{10}$ p-value) against its effect size ($Log_2$ HR) in the short-term relapse (<6 months) dataset (A) mid/long-term relapse (>6 months) dataset (B) and non-stratified dataset (C). The significance threshold (p-value=0.05) is represented by the horizontal lines. The protein names, their main cellular expression and function are presented in online supplemental table 1. ARNT, Aryl hydrocarbon receptor nuclear translocator; BACH1, Transcription regulator protein BACH1; DAPP1, Dual adapter for phosphotyrosine and 3-phosphotyrosine and 3-phosphoinositide; DCBLD2, Discoidin, CUB and LCCL domain-containing protein 2; DFFA, DNA fragmentation factor subunit alpha; DGKZ, Diacylglycerol kinase zeta; DPP10, Inactive dipeptidyl peptidase 10; EGLN1, Egl nine homolog 1; EIF4G1, Eukaryotic translation initiation factor 4 gamma 1; FGF2, Fibroblast growth factor 2; ITM2A, Integral membrane protein 2A; MASP1, Mannan-binding lectin serine protease 1;

PPP1R9B, Neurabin-2; PRDX1, Peroxiredoxin-1; PRDX5, Peroxiredoxin-5, mitochondrial; PSIP1, PC4 and SFRS1-interacting protein; SPRY2, Protein sprouty homolog 2; STC1, Stanniocalcin-1; ZBTB16, Zinc finger and BTB domain-containing protein 16.

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