Fbxo6 is a differentially expressed gene in the blood of patients with Crohn's Disease.

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We used published microarray datasets (1-3) to compare global gene expression profiles in whole blood, from patients with Crohn's Disease and from healthy control subjects. We identified the F-box protein 6 Fbxo6 as differentially expressed in the peripheral blood of patients with Crohn's Disease. Patients with Crohn's Disease expressed significantly higher levels of FBXO6 mRNA in the peripheral blood than did control subjects.

Keywords: FBXO6, Crohn's Disease, differential gene expression, gene expression profiling of patient peripheral blood, systems biology of Crohn's Disease.

Crohn's Disease is an inflammatory bowel disease of the gut (4) that can present with anemia as one of its most common extra-intestinal manifestations (5). Gene expression profiling in the blood of patients with Crohn's Disease (6-9) can be useful for diagnostic and prognostic purposes, or for the study of the basic mechanisms which drive formation of the disease. Here we mined published microarray datasets (1-3) to further describe the transcriptional landscape of the peripheral blood and peripheral blood cells of patients with Crohn's Disease by identifying genes whose expression was most quantitatively different in the blood of patients with Crohn's Disease as compared to non-affected control subjects. We found that the gene encoding the F-box protein Fbxo6 (10) was differentially expressed in the blood of patients with Crohn's Disease, in whole blood and in monocyte-derived macrophages. In one patient dataset (1), the quantity of FBXO6 mRNA was present at significantly higher levels in the blood of patients with CD as compared to non-affected controls. FBXO6 has multiple protein partners (11) and multiple cellular functions: in endoplasmic reticulum (ER) stress signaling associated with the unfolded protein responses (12), in the cell cycle (13) and in immunity (14, 15). FBXO6 may be a molecule of interest in studying the multiple factors that contribute to the establishment of the inflammatory phenotype in Crohn's Disease.

Methods

We utilized GSE94648 (1), GSE126124 (2) and GSE60083 (3) and for this study in conjunction with GEO2R. GSE94648 (1) was generated using Affymetrix Human Genome U133 Plus 2.0 Array with n=41 CD patients with active disease, n=9 CD patients with inactive disease, and n=22 healthy controls. GSE126124 (2) was generated using Affymetrix Human Gene 1.0 ST Array technology with n=39 CD patients and n=32 healthy controls. GSE60083 (3) was generated using Illumina HumanWG-6 v3.0 expression beadchip technology with n=58 CD patients and n=42 healthy controls.

1 The Benjamini and Hochberg method of p-value adjustment was used for ranking of
2 differential expression but raw p-values were used for assessment of statistical significance of
3 global differential expression. Log-transformation of data was auto-detected, and the NCBI
4 generated category of platform annotation was used.

5 A statistical test was performed to evaluate the significance of difference between
6 mRNA expression levels of Fbxo6 in the peripheral blood of patients with Crohn's Disease
7 versus in healthy controls using a two-tailed, unpaired t-test with Welch's correction. Only p-
8 values less than 0.05 were considered statistically significant.
9

10 **Results**

11 We mined published microarray datasets generated using peripheral blood (1, 2) or
12 monocyte-derived macrophages (3) from patients with Crohn's Disease (CD) to describe the
13 transcriptional landscape of the blood and immune cells from patients with Crohn's Disease by
14 identifying genes whose expression was most quantitatively different between CD patients and
15 controls.
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17 Fbxo6 is differentially expressed in the blood of patients with Crohn's Disease

18 We identified Fbxo6 as among the genes whose expression was most significantly
19 different when comparing the peripheral blood of patients with Crohn's Disease to that of non-
20 affected controls across two independent microarray datasets (1, 2). When sorting all of the
21 genes expressed in the peripheral blood based on magnitude of change between CD patient
22 blood and control blood, a transcript encoded by the Fbxo6 gene ranked 102 out of 19674 total
23 transcripts. Differential expression of FBXO6 mRNA in the blood of patients with Crohn's
24 Disease was statistically significant (Table 1; $p=2.16E-05$).
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27 Analyzing a separate microarray dataset, we again identified Fbxo6 as differentially
28 expressed in the blood of patients with Crohn's Disease. When sorting all of the genes

expressed in the peripheral blood based on magnitude of change between CD patient blood and control blood, a transcript encoded by the Fbxo6 gene ranked 2540 out of 33297 total transcripts (Table 2). Differential expression of Fbxo6 in the blood of patients with Crohn's Disease in this dataset was also statistically significant (Table 2; $p=2.64E-04$).

Fbxo6 is differentially expressed in monocyte-derived macrophages from patients with Crohn's Disease

We used a third dataset (3) to understand if differential expression of Fbxo6 in the blood cells of patients with Crohn's Disease could be observed in a more uniform population of cells. Comparing the gene expression of monocyte-derived macrophages from patients with Crohn's Disease and MDM from control subjects (3) revealed that Fbxo6 was also differentially expressed in CD patient monocyte-derived macrophages. When sorting all of the genes expressed in monocyte-derived macrophages based on magnitude of change between CD patient macrophages and control macrophages, a transcript encoded by the Fbxo6 gene ranked 470 out of 10468 total transcripts. Differential expression of FBXO6 mRNA in the monocyte-derived macrophages of patients with Crohn's Disease was statistically significant (Table 3; $p=0.0167708$).

Fbxo6 is expressed at significantly higher levels in the peripheral blood of patients with active Crohn's Disease as compared to healthy subjects.

We obtained exact expression levels for a differentially expressed Fbxo6 transcript in the blood of patients with active Crohn's Disease and in the blood of control subjects (1) to directly compare FBXO6 expression levels between groups. We also performed a statistical test to determine if the difference in FBXO6 expression when comparing patient and control blood was statistically significant. FBXO6 was expressed at significantly higher levels in the peripheral blood of patients with active Crohn's Disease when compared to peripheral blood from control

1 subjects (Figure 1; $p < 0.0001$). We calculated, on average, a fold change in FBXO6 expression
2 of 1.1078 ± 0.0921 in CD patient blood relative to control blood (Table 1).

3 4 **Discussion**

5 We compared the transcriptomes of Crohn's Disease patient-derived peripheral blood (1,
6 2) and monocyte-derived macrophages (3) to that of control subjects using three published
7 datasets to identify changes in gene expression in the hematopoietic compartment of patients
8 with Crohn's Disease. We identified the gene encoding the F-box protein 6, Fbxo6, as among
9 the genes expressed most differently in CD patient blood and in CD patient monocyte-derived
10 macrophage as compared to that of controls.

12 FBXO6 is a protein of the F-box family (10), defined by the presence of the F-box motif
13 (16, 17), a protein-protein interaction motif. F-box proteins function as substrate adapters in the
14 site-specific modification of proteins with ubiquitin for protein degradation at the proteasome (4)
15 in the context of SCF (Skp, Cullin, F-box) or CRL (Cullin RING Ligases) complexes (18, 19). By
16 incorporating unique F-box members, each SCF complex possesses unique substrate
17 specificity. FBXO6 is a component of an SCF complex that modifies the chitinbiase moiety in
18 unfolded N-glycoproteins, a modification important in the unfolded protein response (UPR) in
19 the endoplasmic reticulum pathway of protein degradation (ERAD) (20). A study performing
20 mass spectrometry to identify FBXO6 associating proteins after isolation of FBXO6 complexes
21 from HeLa, 293T, and Jurkat cells identified 39 protein partners of FBXO6 (11). In 29 of these
22 binding partners, the interaction was found to be N-glycan dependent. Another study found that
23 FBXO6 was important in apoptotic death associated with the ERAD pathway by ubiquitination
24 and targeting for degradation of Ero1L, an oxidoreductase in the ERAD pathway (12). FBXO6
25 targeting and modification of Ero1L was direct and independent of an SCF complex (12).

26 FBXO6 was found to interact with RIOK1, where it functioned in an antagonistic manner with the

casein kinase II (CKII) to control the stability of R1OK1, as degradation of R1OK1 through FBXO6-mediated ubiquitination was inhibited by CKII phosphorylation of R1OK1 (21). A role for FBXO6 in the cell cycle has also been reported (13). FBXO6 could influence the assembly of the mitotic spindle by specific targeting of spindle-associated proteins, with FBXO6 interacting with BubR1 and Mad2 to target these spindle-associated proteins for degradation (13). FBXO6 phosphorylation was found to be dynamic, with changes in FBXO6 phosphorylation that accompanied cell cycle progression (13).

There are limited studies describing a role for FBXO6 in the immune system (14, 15). In one study, investigators found that in the context of experimental viral infection, FBXO6 targeted the interferon regulatory factor 3 (IRF3) for degradation (14). The authors proposed that FBXO6 degradation of IRF3 functioned to limit the antiviral response by down-regulating interferon signaling and prevent damage by an overactive antiviral response. In a second study using a mouse model of influenza A (IAV) infection (15), the lungs of FBXO6-deficient mice manifested increased Type I interferon (IFN) responses with a concomitant decrease in infection-associated leukocyte recruitment and viral load in the lungs. Alveolar macrophages (AM) from FBXO6 *-/-* mice, when infected with IAV *in vitro*, similarly expressed increased levels of Type I interferons and possessed decreased viral replication levels relative to AMs from wild-type mice (15). While viral infection in wild-type AM was associated with increases in ER stress-associated apoptotic cell death, alveolar macrophage derived from FBXO6-deficient mice displayed a reduction in cell death associated with ER stress. Mechanistically, the authors concluded that FBXO6 supported apoptotic signaling in the context of viral infection-induced ER stress through direct interaction with IRE1 α (15). Thus, FBXO6 has pleiotropic functions: generally, in the cell cycle and in quality control of proteins and stress signaling pathways, as well as at least two specific roles in the immune system: limiting interferon signaling in experimental viral infection

1 by targeting transcription factor IRF3 for degradation, and the regulation of antiviral responses
2 and ER stress-associated cell death during experimental infection with IAV through interactions
3 with IRE1 α .
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5 We found that FBXO6 was a differentially expressed gene in the blood of patients with
6 Crohn's Disease across three separate published microarray datasets. FBXO6 mRNA was
7 present at significantly higher levels in the blood of patients with Crohn's Disease than in
8 healthy, non-affected controls. As FBXO6 has a network of protein interaction partners and
9 functions in multiple cellular processes, drawing conclusions as to a role for FBXO6 in the
10 pathogenesis or biology of Crohn's Disease (e.g., in modulating the antiviral response) will
11 require assessment of FBXO6 function in primary tissues from patients with Crohn's Disease.
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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
102	26270_at	2.16E-05	4.5418403	2.595148	1.1078 ± 0.0921	FBXO6	F-box protein 6

Table 1: The gene encoding FBXO6 is differentially expressed in the blood cells of patients with Crohn's Disease.

The rank of global differential expression in each dataset, the transcript or probe ID, the p-value with respect to differential expression transcriptome-wide, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared (Crohn's Disease and healthy controls), the log fold-change in mRNA expression between the two groups, the gene and gene name are represented in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
2540	7897728	2.64E-04	3.8352861	0.1751136	FBXO6	F-box protein 6

Table 2: The gene encoding FBXO6 is differentially expressed in the blood cells of patients with Crohn's Disease.

The rank of global differential expression in each dataset, the transcript or probe ID, the p-value with respect to differential expression transcriptome-wide, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared (Crohn's Disease and healthy controls), the gene and gene name are represented in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
470	ILMN_1701455	0.0167708	-2.432041	-3.148	FBXO6	F-box protein 6

Table 3: The gene encoding FBXO6 is differentially expressed in monocyte-derived macrophages from patients with Crohn's Disease.

The rank of global differential expression in each dataset, the transcript or probe ID, the p-value with respect to differential expression transcriptome-wide, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared (Crohn's Disease and healthy controls), the gene and gene name are represented in this chart.

Figure 1: FBXO6 is expressed at significantly higher levels in the peripheral blood of patients with active Crohn's Disease as compared to healthy controls.

FBXO6 expression in the whole blood of healthy control subjects (left), and in the whole blood of patients with active Crohn's Disease (right) is displayed with the mean mRNA expression value marked and p-value listed.