The Fanconi anemia, complementation group E gene FANCE is differentially expressed in the blood of patients with Crohn's Disease.

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Crohn's Disease is a disorder of the gastrointestinal tract but hematologic disturbances are also found in this patient population (1). We used published datasets (2, 3) to compare the genome-wide transcriptional profiles of blood from patients with Crohn's Disease to that of healthy, non-affected patients. We found that the Fanconi anemia, complementation group E protein FANCE (4) was among the genes whose expression was most different between the whole blood of patients with Crohn's Disease and the blood of healthy, non-affected subjects (2). Analysis of a separate dataset (3) revealed that FANCE was also among the most differentially expressed genes in macrophages derived from the monocytes of patients with Crohn's disease when compared to control monocyte-derived macrophages. This is the first report documenting differential expression of a Fanconi anemia complementation gene in the blood cells of patients with Crohn's Disease.

Keywords: FANCE, Fanconi Anemia, Crohn's Disease

1 Systems level analyses can provide unbiased insights into the most significant changes
2 in genomic structure, gene expression, protein synthesis, metabolic output, and epigenetic
3 modification across the entire complement of genes, mRNAs, proteins, metabolites, and
4 chemical DNA modifications in a cell, tissue, organ or disease state (5). The inflammatory
5 bowel diseases, including Crohn's disease and ulcerative colitis affect 1,810,773 people in the
6 United States (6). The advent of biologic therapies beginning with the introduction of the TNF
7 alpha inhibitor infliximab have improved disease outcomes but there remain populations that
8 are refractory to the most advanced treatment options. The manifestations of patients with
9 Crohn's disease are typically thought of as gastrointestinal but the most common extra
10 intestinal manifestation of Crohn's disease is anemia (7). Enhanced understanding of the
11 hematologic system of patients with Crohn's disease is required. To understand the
12 transcriptional landscape of the hematopoietic tissues of patients with Crohn's disease we
13 compared the transcriptomes of whole blood and monocyte-derived macrophages from CD
14 patients to that of healthy, unaffected controls using two published datasets (2, 3). We
15 identified the Fanconi anemia, complementation group E protein (4) as among the most
16 differentially expressed genes in the blood of patients with Crohn's disease.

18 Fanconi Anemia is an autosomal recessive disease characterized by bone marrow
19 failure in the form of aplastic anemia and susceptibility to cancer (8). In vitro, a key
20 characteristic of the cells of patients with FA is sensitivity to DNA damage-inducing agents
21 such as mitomycin C and ionizing radiation, manifested as chromosomal breakage (8). Genetic
22 complementation studies have identified 22 complementation groups from FANCA to FANCW,
23 8 of which form a nuclear Fanconi anemia complex composed of Fanconi complementation
24 group proteins A, B, C, E, F, G, L and M that mediate signaling of a DNA damage event (8).
25 Specifically, the function of the FA pathway is thought to be in removal of inter-strand DNA
26 crosslinks (ICL) (9). FANCE acts as a bridge between the FA complex and FANCD2, and
27 promotes the nuclear accumulation of the FANCC protein (10, 11). The data here reveal that
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1 FANCE messenger RNA levels are reduced in both the blood of patients with Crohn's
2 Disease, and in macrophages derived from monocytes isolated from CD patients.

3 4 **Methods**

5 We used datasets GSE94648 (2) and GSE60083 (3) for this analysis in conjunction with
6 GEO2R. GSE94648 was generated using Affymetrix Human Genome U133 Plus 2.0 Array
7 technology with n=22 whole blood from controls and n=50 whole blood from patients with
8 Crohn's Disease. GSE60083 was generated using Illumina HumanWG-6 v3.0 expression
9 beadchip technology with n=42 healthy controls and n=58 CD patients. The Benjamin and
10 Hochberg method of p-value adjustment was used for ranking of differential expression but
11 raw p-values were used for assessment of statistical significance of global differential
12 expression. Log-transformation of data was auto-detected, and the NCBI generated category
13 of platform annotation was used.
14

15 A statistical test was performed to evaluate the significance of difference between mRNA
16 expression levels of FANCE in the blood of patients with Crohn's disease as compared to
17 healthy controls, and between monocyte-derived macrophages from CD patients and from
18 control subjects using a two-tailed, unpaired t-test with Welch's correction. Only p-values less
19 than 0.05 were considered statistically significant.
20

21 **Results**

22 We performed global differential gene expression profiling of the blood and monocyte-
23 derived macrophage of patients with Crohn's Disease by comparison to genome-wide
24 transcription data from blood and MDM from healthy controls using published datasets (2, 3).
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26 FANCE is among the genes most differentially expressed in the blood and monocyte-derived
27 macrophages of patients with Crohn's Disease.

28 We identified the Fanconi anemia complementation group E protein, FANCE, as one of
the genes whose expression changed most significantly in the blood of patients to Crohn's
Disease when compared to blood from healthy, non-affected subjects. When ranking all of the
3 OF 13

genes expressed in the blood by the degree of change in expression between patients with Crohn's Disease and non-affected subjects, FANCE ranked 8 out of 10468 total transcripts. Differential expression of FANCE was statistically significant (Table 1; $p=0.0066649$).

In a separate systems analysis of the hematopoietic tissues of patients with Crohn's Disease, now in monocyte-derived macrophage, we again identified FANCE as among the most differentially expressed genes in patients with Crohn's Disease. When ranking all of the genes expressed in monocyte-derived macrophage by the degree of change in expression between patients with Crohn's Disease and non-affected subjects, FANCE ranked 194. This was out of 19674 total transcripts and statistically significant (Table 1; $p=3.9E-07$).

Next, we obtained exact mRNA expression values for the FANCE gene in both whole blood and monocyte-derived macrophage and performed statistical tests to determine if the difference in expression levels were statistically significant.

FANCE is expressed at significantly lower levels in the blood of patients with Crohn's Disease when compared to healthy, non-affected controls.

Gene expression of FANCE was lower in the blood of patients with Crohn's Disease (Table 2; mean mRNA expression of FANCE: 9.602 ± 0.0287) than in the blood of non-affected patients (Table 2; mean mRNA expression of FANCE: 9.874 ± 0.03352) (Figure 1). This difference was statistically significant (Figure 1; $p<0.0001$).

FANCE is expressed at significantly lower levels in the monocyte-derived macrophages of patients with Crohn's Disease when compared to healthy, non-affected controls.

Gene expression of FANCE was also lower in monocyte-derived macrophages of patients with Crohn's Disease (Table 2; mean mRNA expression of FANCE: 6.985 ± 0.02889) than in the blood of non-affected patients (Table 2; mean mRNA expression of FANCE: 7.117 ± 0.04002) (Figure 2). This difference was statistically significant (Figure 2; $p=0.0091$).

Thus, we identified the Fanconi anemia complementation group E protein FANCE as among the genes whose expression was quantitatively most different between the blood and monocyte-derived macrophages of patients with Crohn's Disease and found that FANCE was

expressed at significantly lower levels in both the whole blood and MDM of patients with Crohn's Disease than in non-affected control subjects.

Discussion

We compared the transcriptomes of whole blood from patients with Crohn's Disease with that of whole blood from unaffected, healthy patients. We found that the Fanconi anemia complementation group E protein FANCE was differentially expressed in the blood of patients with Crohn's Disease. The expression of FANCE was also among the genes most differentially expressed in macrophages derived from monocytes isolated from the blood of patients with Crohn's Disease. In both the whole blood and in monocyte-derived macrophages, FANCE was expressed at significantly lower levels in patients with Crohn's Disease.

FANCD2 is one of 10 proteins that form an E3 ubiquitin ligase "core complex" that, together with the E2 UBE2T/FANCT monoubiquitylate FANCD2-I (8). There are three sub-complexes of the FA core complex, including the BL100 complex, the CEF sub-complex, and the AG20 sub-complex. FANCE, together with FANCC and FANCF constitute the CEF sub-complex, which acts as a bridge between the FA core complex and FANCD2-I (8). DNA damage and subsequent repair can be triggered by a number of different events; one of them is the DNA interstrand crosslink (ICL) (9). In the normal, unaffected individual ICLs trigger DNA repair signaling because they can lead to mutations, rearrangements in chromosome structure and cell death (9). The function of the FA pathway is thought to be in ICL removal (9).

There is limited information concerning whether patients with Crohn's Disease exhibit any susceptibility to DNA damage. Single nucleotide polymorphisms in JAK2 and STAT3 have been identified as risk factors for the development of Crohn's Disease (12) and in one study, both alleles were associated with enhanced susceptibility to DNA damage as determined by tail length in a comet assay of peripheral blood leukocytes (13). The A/C and C/C genotypes of the JAK2 SNP rs10758669 conferred a 1.76 and 2.36 fold increased risk for the development of Crohn's Disease in a New Zealand CD patient cohort (13), respectively, and rs10758669 is a

1 tagging SNP for the high-risk haplotype containing the V617F JAK2 variant found in patients
2 with myeloproliferative neoplasms like polycythemia vera (14). Patients with Crohn's Disease
3 have been reported to possess a 2-3 fold increased risk for development of myeloproliferative
4 neoplasms (MPNs) (15). Thus, a genetic risk factor Crohn's Disease is functionally associated
5 with susceptibility to DNA damage, is a tagging SNP for the V617F mutation found in patients
6 with MPNs, and patients with Crohn's Disease have been suggested to possess an increased
7 risk for development of MPNs. We found here that FANCE is differentially expressed in both the
8 whole blood and in the monocyte-derived macrophages of patients with Crohn's Disease, and at
9 significantly higher levels than in the non-affected control subjects. Differential expression of
10 FANCE in patients with Crohn's Disease should be validated in larger and separate cohorts and
11 the susceptibility of patients with Crohn's Disease to DNA damage events should be more
12 rigorously assessed.
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Rank	ID	p-value	t	B	Gene	Gene name
GSE94648						
8 out of 19674	2178_at	3.9E-07	-5.5783687	6.259533	FANCE	Fanconi anemia, complementation group E
GSE60083						
194 out of 10468	ILMN_2043452	0.0066649	-2.770282	-2.401	FANCE	Fanconi anemia complementation group E

Table 1: FANCE is among the genes most differentially expressed in the blood and monocyte-derived macrophages of patients with Crohn's Disease.

The rank of differential expression, probe ID, p-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups, the gene symbol and gene name are represented in this chart.

Figure 1: FANCE is expressed at significantly lower levels in the blood of patients with Crohn's Disease when compared to healthy, non-affected controls.

The expression of FANCE in the blood of non-affected subjects and in the blood of patients with Crohn's Disease is represented with mean value marked and p-value listed. On the right, the same data is presented as a violin plot and the median value, 25th and 75th percentiles marked.

Figure 2: FANCE is expressed at significantly lower levels in the monocyte-derived macrophages of patients with Crohn's Disease when compared to healthy, non-affected controls.

The expression of FANCE in the monocyte-derived macrophages of non-affected subjects and in the monocyte-derived macrophage of patients with Crohn's Disease is represented with mean value marked and p-value listed. On the right, the same data is presented as a violin plot and the median value, 25th and 75th percentiles marked.

	Blood (control)	Blood (CD)
Number of values	22	50
Minimum	9.649	9.123
25% Percentile	9.744	9.482
Median	9.850	9.638
75% Percentile	10.01	9.715
Maximum	10.14	10.03
Mean	9.874	9.602
Std. Deviation	0.1572	0.2029
Std. Error of Mean	0.03352	0.02870
Lower 95% CI	9.805	9.544
Upper 95% CI	9.944	9.659

Table 2: Descriptive statistics for FANCE differential expression in the whole blood of patients with Crohn's Disease.

	Mø-derived macrophages	Mø-derived macrophages (Crohn's)
Number of values	42	58
Minimum	6.597	6.560
25% Percentile	6.954	6.829
Median	7.131	6.956
75% Percentile	7.308	7.112
Maximum	7.744	7.443
Mean	7.117	6.985
Std. Deviation	0.2594	0.2201
Std. Error of Mean	0.04002	0.02889
Lower 95% CI	7.036	6.927
Upper 95% CI	7.198	7.043

Table 3: Descriptive statistics for FANCE differential expression in the monocyte-derived macrophage of patients with Crohn's Disease.