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Article

ARTICLE

Molecular Correlates of Socioeconomic Status and Clinical Outcomes Following Hematopoietic Cell Transplantation for Leukemia

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Abstract

Background: Clinical outcomes among allogeneic hematopoietic cell transplant (HCT) recipients are negatively affected by low socioeconomic status (SES), yet the biological mechanisms accounting for this health disparity remain to be elucidated. Among unrelated donor HCT recipients with acute myelogenous leukemia, one recent pilot study linked low SES to increased expression of a stress-related gene expression profile known as the conserved transcriptional response to adversity (CTRA) in peripheral blood mononuclear cells, which involves up-regulation of pro-inflammatory genes and down-regulation of genes involved in type I interferon response and antibody synthesis.
Methods: This study examined these relationships using additional measures in a larger archival sample of 261 adults who received an unrelated donor HCT for acute myelogenous leukemia to 1) identify cellular and molecular mechanisms involved in SES-related differences in pre-transplant leukocyte transcriptome profiles, and 2) evaluate pre-transplant CTRA biology associations with clinical outcomes through multivariable analysis controlling for demographic-, disease-, and transplant-related covariates.
Results: Low SES individuals showed increases in classic monocyte activation and pro-inflammatory transcription control pathways as well as decreases in activation of nonclassic monocytes, all consistent with the CTRA biological pattern. Transplant recipients in the highest or lowest quartiles of the CTRA pro-inflammatory gene component had a more than 2-fold elevated hazard of relapse (hazard ratio [HR] = 2.47, 95% confidence interval [CI] = 1.44 to 4.24), $P = .001$; HR = 2.52, 95% CI = 1.46 to 4.34, $P = .001$) and more than 20% reduction in leukemia-free survival (HR = 1.57, 95% CI = 1.08 to 2.28, $P = .012$; HR = 1.49, 95% CI = 1.04 to 2.15, $P = .03$) compared with the middle quartiles.
Conclusions: These findings identify SES- and CTRA-associated myeloid- and inflammation-related transcriptome signatures in recipient pre-transplant blood samples as a potential novel predictive biomarker of HCT-related clinical outcomes.

Hematopoietic cell transplant (HCT) patients with low socioeconomic status (SES) have reduced survival and increased transplant-related mortality (TRM) (1,2). These differences are partially attributable to differential health behaviors and medical care access (3), but SES-related disparities in cancer outcomes remain even after accounting for such differences (4,5). Psychobiological processes involving stress physiology and immune activation may also contribute to SES-related disparities in treatment outcomes (6,7). Social environmental conditions influence a variety of physiological processes (8–11)

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including gene regulation in both healthy and diseased tissues (12–14). One genomic response of particular significance for HCT involves sympathetic nervous system-mediated activation of a leukocyte transcriptional program known as the conserved transcriptional response to adversity (CTRA). The CTRA biological pattern can be assessed in transcriptome profiling data in several ways, including 1) an a priori-specified composite score involving 53 indicator genes for inflammation and Type I interferon response, 2) transcript origin analyses (TOA) assessing increased activity of monocytes in general and classic monocytes in particular, and 3) activity of pro-inflammatory and antiviral transcription factors (15–18). This transcriptional shift is observed during extended periods of stress, threat, or uncertainty (15,16,19–21) and is mediated by both per-cell changes in gene expression and by myelopoietic up-regulation of the pro-inflammatory $CD16^{-}$ classic monocyte subpopulation (19).

CTRA profiles are elevated in circulating leukocytes from healthy low-SES individuals (12,19). In a small pilot study (N = 78), we recently observed that low SES is also associated with elevated CTRA profiles in pre-transplant peripheral blood mononuclear cell (PBMC) samples from HCT recipients with acute myelogenous leukemia (AML) (14). Univariate analyses demonstrated increased relapse and decreased leukemia-free survival (LFS) in individuals showing high CTRA patterning. SES-related differences in CTRA gene expression may represent one biological pathway underlying socioeconomic disparities in HCT outcomes. Independent of SES, CTRA profiles may constitute a useful molecular biomarker for predicting treatment failure risks. This study includes a larger sample size, multivariable statistical analyses, and additional measures of CTRA biology to further explore the relationships among SES, leukocyte gene regulation, and clinical outcomes in unrelated donor HCTs for AML. These analyses also allowed mapping of the molecular and cellular pathways previously implicated in CTRA expression as well as population dynamics of $CD16^{-}$ classic monocytes.

Methods

Data Sources

The Center for International Blood and Marrow Transplant Research (CIBMTR) is a working group of more than 450 HCT centers that report all transplants consecutively with compliance audits and longitudinal patient follow-up. CIBMTR studies comply with federal regulations protecting human research participants.

Participants

This study included patients older than 18 years with AML in first or second complete remission who underwent a T-replete, unrelated myeloablative fully matched (8 of 8) adult donor peripheral blood or bone marrow transplant from 1995 to 2005 with available residential US zip code information and blood samples at transplant time (N = 264). Mean household income was estimated from patient residential zip code 2006–2010 Census Bureau data (22). Three patients had zip codes without a correlative match and were excluded. Race was reported by transplant centers and categorized according to the US Office of Management and Budget classification. The institutional review board of the National Marrow Donor Program approved all study procedures. All recipients consented to participate in the CIBMTR Research Repository and database.

Analysis of CTRA Biology

Total RNA was extracted from cryopreserved PBMCs (RNeasy; Qiagen), tested for integrity (Bioanalyzer; Agilent), and converted to fluorescent cRNA for hybridization to Illumina Human HT-12 v4 BeadArrays (23). All 264 samples yielded valid results as assessed by endpoint quality assurance metrics (median hybridization intensity >100 fluorescence units). We analyzed the CTRA physiological pattern using three different convergent analyses. The first examined a prespecified 52-gene composite score comprised of 19 pro-inflammatory genes (*IL1A*, *IL1B*, *IL6*, *IL8*, *TNF*, *PTGS1*, *PTGS2*, *FOS*, *FOSB*, *FOSL1*, *FOSL2*, *JUN*, *JUNB*, *JUND*, *NFKB1*, *NFKB2*, *REL*, *RELA*, and *RELB*) weighted positively to indicate their direct association with the CTRA pattern and 30 type I interferon genes (*GBP1*, *IFI27*, *IFI27L1-2*, *IFI30*, *IFI35*, *IFI44*, *IFI44L*, *IFI6*, *IFIH1*, *IFIT1-3*, *IFIT5*, *IFIT1L*, *IFITM1-3*, *IFITM4P*, *IFITM5*, *IFNB1*, *IRF2*, *IRF7-8*, *MX1-2*, *OAS1-3*, and *OASL*) and three antibody synthesis genes (*IGJ*, *IGLL1*, and *IGLL3*) weighted negatively to indicate their inverse association (16–18,24). These indicators represent the entire 53-gene CTRA indicator set used in previous research with the exception of *IFI16*, which was unavailable due to random variations in microarray synthesis (25).

The second analytic approach used the transcriptome data to derive estimates of transcription control pathway activity for prespecified pro-inflammatory and antiviral transcription factors. These analyses employed the TELiS promoter-based bioinformatic analysis to assess activity of NF-κB, activator protein 1, and interferon response factors (26). Ancillary analyses also examined activity of neuroendocrine signaling pathways upstream of the CTRA (cAMP response element-binding protein and the glucocorticoid receptor).

The third analytic approach assessed the specific leukocyte subsets mediating the observed differences in gene expression using TOA as previously described (14,23). Briefly, TOA forms cell-specific diagnostic scores for each gene using reference transcriptome profiles of isolated cell samples, and the specific gene sets derived from this study's analyses are tested for overrepresentation of diagnostic scores for each cell type to identify the predominant cellular origin of SES effects (eg, due to differential abundance of that cell and/or selective transcriptional activation of that cell type). Analyses used reference data on isolated monocytes, B cells, $CD4^{+}$ and $CD8^{+}$ T cells, natural killer cells, and plasmacytoid dendritic cells (Gene Expression Omnibus series GSE1133) as well as classic ($CD16^{-}$) and nonclassic ($CD16^{+}$) monocyte subsets (GSE25913). The original TOA method uses group-level identification of differentially expressed genes. To provide an individual-specific measure of cell subset-related gene expression suitable for clinical outcome prediction, we applied TOA to the set of genes that were relatively up-regulated in each patient's sample (≥2 SD above the sample average for that gene) or relatively down-regulated (≥2 SD below the sample average) after screening out genes that showed minimal expression above background (average < 7.04 $\log_2$ RNA abundance) or minimal variation in expression across samples (SD < 0.074 $\log_2$ RNA abundance, corresponding to <1.10-fold variation across the 4-SD range spanning 95% of individuals under a normal distribution). Interested investigators can submit a CIBMTR proposal for data access approval.

Clinical Outcomes

Primary clinical outcomes were relapse (with TRM as the competing risk) and LFS (survival in complete remission after HCT, with events being either disease relapse or treatment-related death). Additional outcomes included neutrophil engraftment at day +28 (absolute neutrophil count $>0.5 \times 10^9$/L sustained for three consecutive days), acute and chronic graft-vs-host disease (GVHD; death without GVHD is the competing risk, with patients censored at subsequent transplant or last follow-up), TRM (death from any cause by day +28 days regardless of relapse status; death beyond day +28 considered transplant related if disease was in remission), and overall survival (death from any cause). Time-to-event outcomes all start at time of HCT. The median follow-up time is about 10 years (Table 1).

Statistical Analyses

SES and CTRA Biology

SES was represented using annual income value cut-points previously reported (14): lowest quartile (<$34 700), middle two quartiles ($34 700–$56 300), and highest quartile (>$56 300). Analyses controlled for age, race, sex, body mass index category, AML type, prior autologous transplant, Karnofsky performance score, and interval from diagnosis to transplant. When a given outcome was tested for association with multiple hypothesized predictors, analyses compared for multiple comparisons following standard statistical protocols in biomedical research (27). Where indicated, ancillary analyses also controlled for specific gene mRNA transcripts marking the prevalence of T lymphocytes and their subsets (CD3D, CD3E, CD4, CD8A), B lymphocytes (CD19), natural killer cells (CD16/FCGR3A, CD56/NCAM1), and monocytes (CD14) (20) to ensure results were not confounded by variations in leukocyte subset prevalence (28). Throughout all bioinformatics analyses, standard errors for summary statistics were derived from 200 bootstrap resamples of residual vectors to account for potential correlation among residuals across genes; *P* values were derived from *t* statistics based on these bootstrap-estimated standard errors (29). To evaluate SES association with CTRA, analyses tested 1) an a priori-defined contrast score representing up-regulated expression of 19 pro-inflammatory genes and down-regulated expression of 30 genes involved in type I interferon responses and three in antibody synthesis, as described above; 2) a transcription factor-based analysis in which the promoter DNA sequences of all genes showing greater than 1.2-fold differential expression in low- vs high-SES transcriptome profiles were scanned for transcription factor-binding motifs (TFBMs) for pro-inflammatory and Type I interferon-related transcription factors using TRANSFAC position-specific weight matrices V$CREL_01, V$AP1_Q6, and V$ISRE_01 (as well as V$CREB_02 and V$GR_Q6 to assess ancillary hypotheses about related neuroendocrine signaling pathways) (26), with differential activity inferred from the ratio of TFBM prevalence in up- vs down-regulated gene sets and $\log_2$-transformed ratios averaged over nine parametric variations of TRANSFAC MatInspector scan stringency and promoter length (26,30); and 3) a cell-based analysis in which all genes showing more than 1.2-fold differential expression in low- vs high-SES transcriptome profiles were mapped to cell diagnostic scores using TOA as previously described (14,23,30) (reference data derived from GSE1133 and GSE25913 as described above). Point estimates of TFBM effect size served as inputs into bioinformatics analyses because

Table 1. Patient, disease, and treatment characteristics*

Characteristic	No. (%)
Patients, n	261
Recipient age, median (range), y	44 (18–68)
Recipient age at transplant, y	
18–19	3 (1)
20–29	55 (21)
30–39	40 (15)
40–49	105 (40)
50–59	53 (20)
60–69	5 (2)
Recipient sex	
Male	115 (44)
Female	146 (56)
Recipient race	
White	248 (95)
African-American	4 (2)
Asian	1 (<1)
Other	3 (1)
Unknown	5 (2)
Recipient BMI	
Underweight (<18.5 kg/m^2)	6 (2)
Normal (18.5–25 kg/m^2)	85 (33)
Overweight (25–30 kg/m^2)	83 (32)
Obese (≥30 kg/m^2)	85 (33)
Missing	2 (<1)
Karnofsky score before transplant	
<90	50 (19)
≥90	185 (71)
Missing	26 (10)
Type of AML	
De-novo AML	205 (79)
Therapy-related AML	15 (6)
Secondary AML with previous diagnosis of MDS/MPS	39 (15)
Missing	2 (<1)
Prior autologous HCT	
No	251 (96)
Yes	10 (4)
Donor age, median(range), y	34 (20–59)
Graft type	
Bone marrow	128 (49)
Peripheral blood	133 (51)
Donor age at transplant, y	
18–32	122 (47)
33–49	128 (49)
≥50	11 (4)
Donor-recipient sex match	
M-M	84 (32)
M-F	82 (31)
F-M	31 (12)
F-F	64 (25)
Donor race	
White	225 (86)
African-American	4 (2)
Asian	3 (1)
Native American	5 (2)
More than one race	8 (3)
Other	7 (3)
Unknown	9 (3)
Donor BMI	
Underweight (<18.5 kg/m^2)	3 (1)
Normal (18.5–25 kg/m^2)	45 (17)
Overweight (25–30 kg/m^2)	66 (25)

(continued)

Table 1. (continued)

Characteristic	No. (%)
Obese (≥30 kg/m^2)	45 (17)
Missing	102 (39)
Donor-recipient CMV match	
−/−	79 (30)
−/+	96 (37)
+/−	34 (13)
+/+	51 (20)
Missing	1 (<1)
ABO incompatibility	
Matched	118 (45)
Minor mismatch	53 (20)
Major mismatch	70 (27)
Bi-directional	19 (7)
Missing	1 (<1)
Cytogenetics scoring	
Favorable	34 (13)
Intermediate	108 (41)
Poor	66 (25)
Not tested	20 (8)
Missing	33 (13)
Conditioning regimen (MA only)	
TBI+Cy	113 (43)
TBI+other	37 (14)
Bu+Cy	91 (35)
Mel+Thio	2 (<1)
Bu+Flud	14 (5)
Mel+Flud	3 (1)
Bu+Clad	1 (<1)
Median income, US$	
<34 700	21 (8)
34 700–43 600	48 (18)
43 601–56 300	77 (30)
>56 300	115 (44)
GVHD prophylaxis	
Tac-based	140 (54)
CSA-based	116 (44)
Missing/other	5 (2)
Year of transplant	
1995–2000	68 (26)
2001–2003	80 (31)
2004–2005	113 (43)
CD34 cell dose, 10^6/kg	6 (<1–34)
Time from diagnosis to transplant	8 (<1–133)
Median follow-up of survivors (range), mo	120 (27–218)

*AML = acute myelogenous leukemia; BMI = body mass index; Bu = busulfan; Clad = cladribine; CMV = cytomegalovirus; CSA = cyclosporine; Cy = cyclophosphamide; Flud = fludarabine; GVHD = graft-vs-host disease; MA = myeloablative; MDS = myelodysplastic syndrome; Mel = melphalan; MPS = myeloproliferative syndrome; Tac = tacrolimus; TBI = total body irradiation; Thio = thiotepa.

previous research finds that it yields more reliable gene lists and bioinformatic results than does *P*/*q*-value screening (24,30–35).

CTRA Biology and Clinical Outcomes

Patients were grouped based on quartiles of CTRA indicator gene composite scores (and subcomponents in follow-up analyses), and these associations were also tested on a continuous scale. Continuous TOA scores (cellular origin scores for genes up- and down-regulated in association with SES) were evaluated in association with HCT outcomes. Due to the impact of CTRA inflammatory patterns on monocyte biology, total monocyte TOA scores were also assessed as predictors of clinical outcomes. (No attempt was made to relate transcription factor activity to clinical outcomes because that analysis utilizes group-based estimates of differential gene expression and thus does not yield a patient-specific score that could be used to predict clinical outcomes.) The association of patient-, disease-, and transplant-related characteristics with CTRA contrast scores (and their subcomponents) in quartiles was tested by standard χ^2 test (or Fisher exact test for counts ≤5) for categorical variables or Kruskal-Wallis test for continuous variables. We stabilized variance across genes by z-score transformation to eliminate heteroscedasticity before computing the CTRA or TOA composite score for each individual. The association of SES or CTRA composite score quartiles with overall survival and LFS was tested using the log-rank test. Probabilities of overall survival and LFS were estimated using the Kaplan-Meier estimator, and the probabilities of TRM, relapse, neutrophil engraftment, and a/cGVHD were assessed by the cumulative-incidence function method. Cox proportional-hazards regression models were used to control for patient baseline characteristics in quantifying relationships between CTRA or TOA scores and clinical outcomes. All the patient-, disease-, and demographic-related variables considered in the SES analysis were examined. The proportional hazards assumption was assessed for each candidate variable and outcome using a time-dependent covariate approach. Prognostic variables were selected for each outcome separately using stepwise model selection ($P < .05$ for entry and retention). All *P* values are two-sided. Data analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Results

Patient Characteristics

Each variable (Table 1) was tested for association with SES, with most not statistically significant. However, a minority showed association and were therefore controlled for in subsequent analyses of CTRA biology, including more male recipients in the highest SES quartile (N = 39 in Q4 vs N = 27, 25, 24 in Q1–Q3, respectively; $P = .03$). There was also a statistically significant difference in donor-recipient sex match (more female-male and fewer male-female matches in the highest SES quartile; N = 15 in Q4 vs N = 5, 7, 4 in Q1–Q3, respectively; $P = .02$) and GVHD prophylaxis (tacrolimus-based regimens were more prevalent in the highest SES quartile; N = 39 in Q4 vs N = 29, 21, 21 in Q1–Q3, respectively; $P = .03$).

Molecular Correlates of SES

Low SES was not associated with the 52-gene CTRA composite score (or its subcomponents) utilized in our previous study (14) despite the fact that gene-specific SES association measures derived from this sample correlated $r = 0.50$ with those observed previously (Figure 1A) (14). However, low SES was associated with two other bioinformatic indicators of CTRA-related transcription factor activation: increased NF-κB and activator protein 1 (Figure 1, B and C). As in the previous study, genes up-regulated in low SES samples derived predominantly from monocytes ($P = .03$; Figure 1D) and from classic (CD16$^-$) monocytes more specifically ($P < .001$; Figure 1E). Reciprocally, genes up-regulated in high-SES recipients derived predominantly from nonclassic (CD16$^+$) monocytes ($P = .04$; Figure 1E).

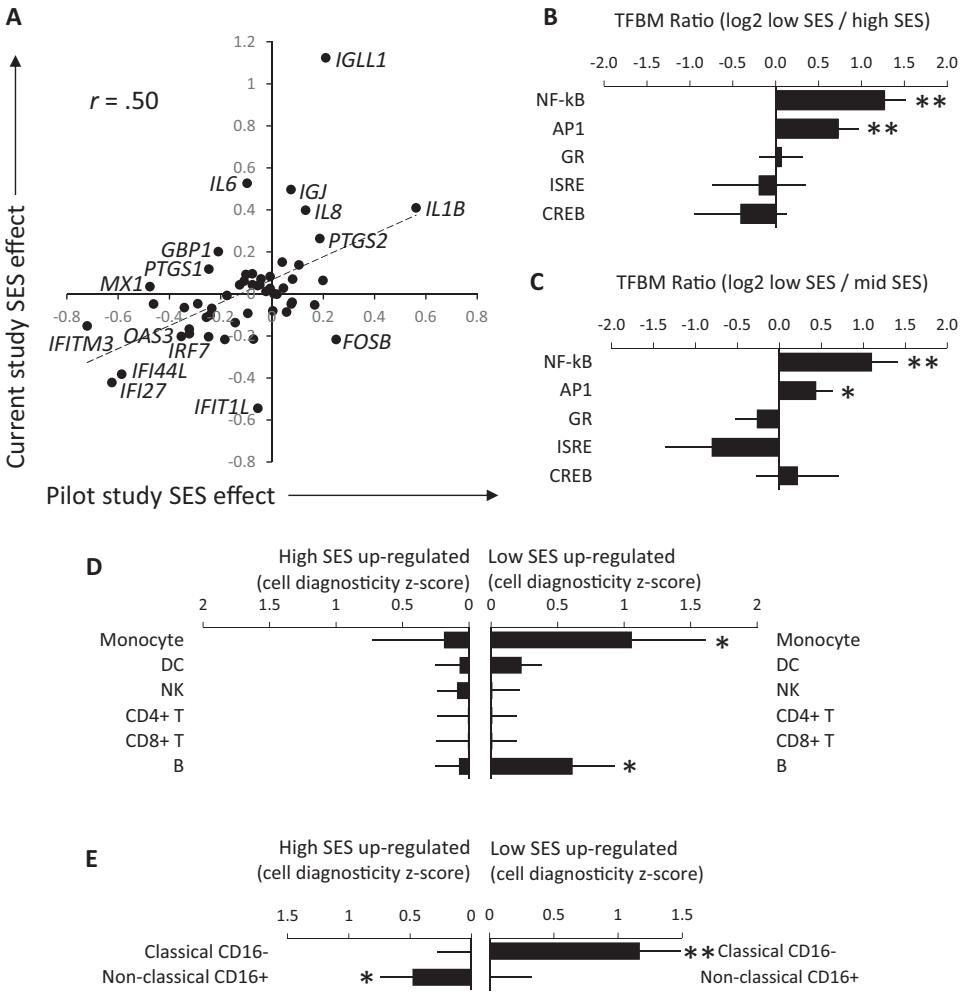

Figure 1. A–E) Expression of the conserved transcriptional response to adversity gene set, transcription control pathways, and cellular origin. **A**) Gene-specific socioeconomic status (SES) associations derived from current sample vs prior pilot sample (14). Genes showing ≥20% difference in expression between hematopoietic cell transplant recipients of low- vs high-SES (**B**) and low- vs middle-SES (**C**) groups were tested for differential activity of specific transcription factors as indicated by Transcription Element Listening System analysis of transcription factor-binding motifs in proximal promoter sequences of up- vs down-regulated genes (26). Genes up-regulated in low-SES samples generally derive from monocytes (**D**), and more specifically from classic ($CD16^-$) monocytes (**E**). Genes down-regulated in low SES derive predominantly from nonclassic ($CD16^+$) monocytes (**E**). *$P < .05$, **$P < .01$. In D and E, ** values would remain statistically significant after correction for multiple testing, whereas * would not. Displayed data (**B–E**) are single model-derived parameter estimates with associated standard errors.

CTRA Associations with Transplant Outcomes

The association of SES with clinical outcomes did not reach statistical significance likely due to limited power to detect the previously observed effect size (1). The CTRA composite score was not statistically significant when its association was tested with clinical outcomes. However, when the 19-gene pro-inflammatory subcomponent and 30-gene antiviral/antibody-related subcomponent were examined separately, the pro-inflammatory subcomponent was associated with relapse (Figure 2A; Table 2, $P = .002$) and LFS (Figure 3; Table 2, $P = .03$) when examined categorically. Univariate modeling revealed similar patterns. As shown in Figures 2–3 and Table 2, outcome risks were relatively elevated in the lowest and highest quartiles of inflammatory gene expression compared with those in the middle two quartiles (ie, a U-shaped or bipolar risk profile). Outcome risks did not differ between the lowest and highest quartiles. As such, transplant recipients in the highest or lowest quartiles of the CTRA pro-inflammatory gene component had a more than 2-fold elevated hazard of relapse (hazard ratio [HR] = 2.47, 95% confidence interval [CI] = 1.44 to 4.24), $P = .001$; HR = 2.52, 95% CI = 1.46 to 4.34, $P = .001$) and greater than 20% reduction in LFS (HR = 1.57, 95% CI = 1.08 to 2.28, $P = .012$; HR = 1.49, 95% CI = 1.04 to 2.15, $P = .03$) compared with the middle quartiles. Quadratic trend analysis of the continuous pro-inflammatory CTRA score also yielded a statistically significant association with relapse (Table 2, $P = .01$) and LFS (Table 2, $P = .02$).

Total monocyte activation (monocyte TOA scores) was also significantly associated with clinical outcome risk. Relapse risk was related to categorical TOA scores representing down-regulation of total monocyte-derived transcripts (Figure 2B; Table 3; $P = .01$), with high TOA scores indicating more monocyte down-regulation. Continuous monocyte TOA scores showed similar risk patterning with up-regulated scores associated with decreased relapse (Table 3; $P = .001$) and down-regulated scores associated with increased relapse (Table 3; $P = .001$). When evaluated continuously, monocyte TOA scores were additionally associated with LFS as well, with

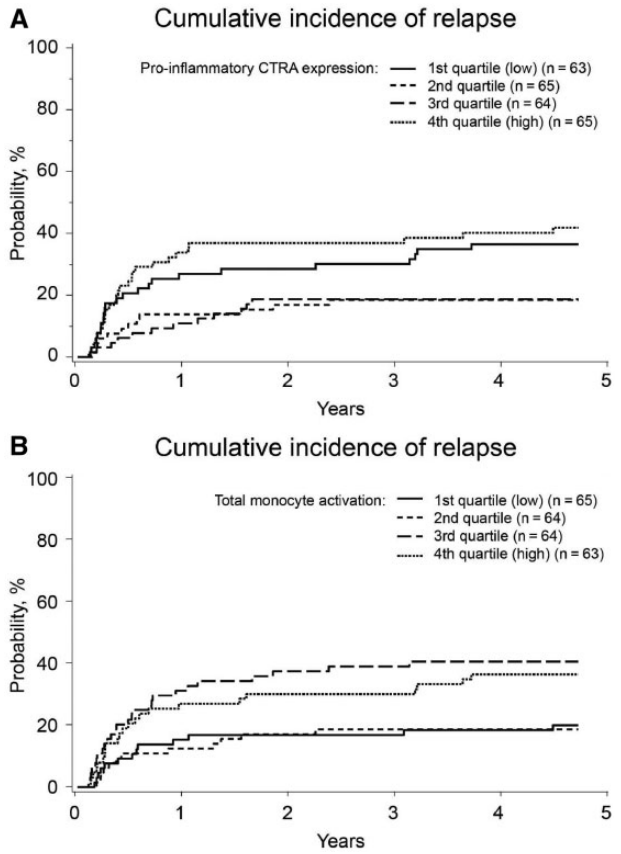

Figure 2. Cumulative incidence of relapse by molecular indicator. **A)** Pro-inflammatory conserved transcriptional response to adversity subcomponent is associated with relapse. **B)** Down-regulation of total monocyte-derived transcripts is associated with relapse (again, high scores indicate more down-regulation). CTRA = conserved transcriptional response to adversity.

up-regulated scores associated with decreased LFS (Table 3; $P = .01$) and down-regulated scores associated with increased LFS (Table 3; $P = .01$). Overall survival, TRM, neutrophil engraftment, and a/cGVHD did not reveal a statistically significant association with monocyte biology in general.

Discussion

These data link low SES to two distinct aspects of leukocyte transcriptome alteration in the blood of AML patients before HCT: increased activity of pro-inflammatory transcription factors (NF-κB and AP-1) and increased activity of monocytes, in general, and classic monocytes in particular. Moreover, some aspects of this CTRA molecular profile appear to portend increased risk of adverse clinical outcomes during HCT for low-SES patients, because the complementary profiles of reduced inflammatory gene expression and monocyte activation more broadly are both associated with decreased post-transplant relapse and enhanced LFS. Some aspects of this profile are consistent with our previous pilot data (14) linking low SES to enhanced expression of the stress-related CTRA biological pattern, which is mediated by pro-inflammatory transcription factor activation and increases in classic monocytes at the expense of nonclassic monocytes. However, other aspects of the present results are not consistent with those earlier findings (14), including the absence of a statistically significant SES relationship with a prespecified 52-gene CTRA indicator score. Regardless of the specific mechanisms involved, these data identify

Table 2. Clinical outcome models for pro-inflammatory CTRA molecular predictors of clinical outcomes

	No. of patients	HR (95% CI)	P
Relapse* (categorical; overall $P = .002$)			
Pro-inflammatory CTRA score			
≤−5.47	63	1.00 (reference)	
(>−5.47, 0.14)	65	0.38 (0.20 to 0.75)	.005
(>0.14, 5.83)	64	0.41 (0.21 to 0.80)	.009
>5.83	65	0.98 (0.57 to 1.68)	.95
Relapse* (continuous)			
Parameter			
Pro-inflammatory CTRA linear		0.95 (0.78 to 1.15)	.60
Pro-inflammatory CTRA quadratic		1.10 (1.02 to 1.18)	.01
Leukemia-free survival† (categorical; overall $P = .025$)			
Pro-inflammatory CTRA score			
≤−5.47	63	1.00 (reference)	
(>−5.47, 0.14)	64	0.56 (0.36 to 0.87)	.01
(>0.14, 5.83)	62	0.79 (0.52 to 1.20)	.26
>5.83	61	1.06 (0.70 to 1.61)	.80
Leukemia-free survival† (continuous)			
Parameter			
Pro-inflammatory CTRA linear		1.00 (0.87 to 1.16)	.97
Pro-inflammatory CTRA quadratic		1.08 (1.01 to 1.15)	.02

*No adjusted covariates entered into the model. CI = confidence interval; CTRA = conserved transcriptional response to adversity; HR = hazard ratio.
†Disease, GVHD prophylaxis, and graft types were adjusted in the model.

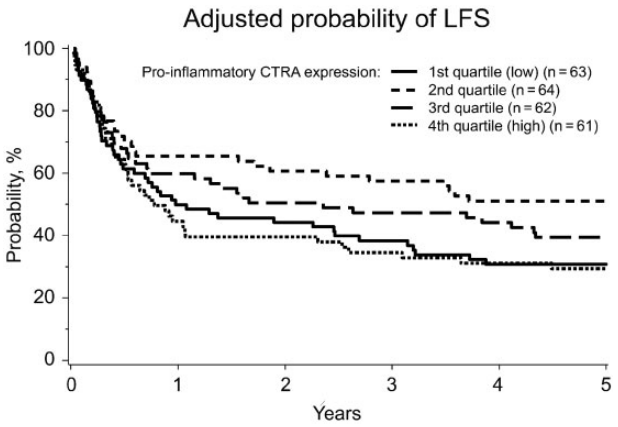

Figure 3. Probability of leukemia-free survival by pro-inflammatory conserved transcriptional response (CTRA) to adversity expression. LFS = leukemia-free survival.

inflammation and myeloid lineage-related transcriptome profiles that may serve as predictive biomarkers to prospectively identify patients at risk of HCT treatment failure. Alterations in myeloid lineage-related immunoregulation could also represent an intervention target to reduce the risk of adverse HCT outcomes generally, and particularly for low-SES individuals.

Although the present results are broadly consistent with our previous findings in relating low-SES and HCT outcomes to myeloid lineage gene regulation (14), the results from this more robust analysis differ in several respects. They provide greater

Table 3. Clinical outcome models for total monocyte population molecular predictors of clinical outcomes

	No. of patients	HR (95% CI)	P
Relapse* (categorical; overall P = .012)			
Down-regulated gene total monocyte TOA score			
≤−0.5029	65	1.00 (reference)	
(>−0.5029, −0.1039)	64	1.08 (0.52 to 2.30)	.84
(>−0.1039, 0.3700)	64	2.39 (1.24 to 4.61)	.01
>0.3700	63	2.15 (1.09 to 4.22)	.03
Relapse* (continuous)			
Up-regulated gene total monocyte TOA score (linear)		0.52 (0.35 to 0.78)	.001
Down-regulated gene total monocyte TOA score (linear)		1.71 (1.23 to 2.36)	.001
Leukemia-free survival† (continuous)			
Up-regulated gene total monocyte TOA score (linear)		0.71 (0.54 to 0.92)	.01
Down-regulated gene total monocyte TOA score (linear)		1.32 (1.06 to 1.63)	.01

*No adjusted covariates entered into the model. CI = confidence interval; HR = hazard ratio; TOA = transcript origin analyses.
†Disease, GVHD prophylaxis, and graft types were adjusted in the model.

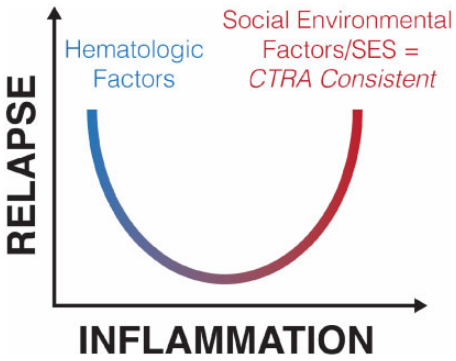

Figure 4. The conserved transcriptional response to adversity (CTRA) inflammation index demonstrates a U-shaped bipolar risk profile for relapse. Social environmental- or socioeconomic-related factors (CTRA consistent) may be responsible for one pole of influence, and hematologic or hematopoietic factors reflective of overall dyspoiesis may be associated with the other risk pole. SES = socioeconomic status.

evidence of pro-inflammatory transcription factor activation, possibly due to increased statistical power in this larger sample. They also yield less pronounced association of the previous 53-gene CTRA indicator gene profile with clinical outcomes, possibly due to a subset of rogue transcripts in this data set (see off-diagonal gene values in Figure 1A; this finding likely relates to effects of more heterogenous disease status in this sample, although specific mechanisms remain a topic for future research). Another difference is that these data identified a U-shaped bipolar risk for the CTRA inflammation index (Figure 4). This curvilinear relationship may be a reflection of SES-related gene regulation at one pole and general post-transplant dyspoiesis related to adverse HCT outcomes at the other (36). The U-shaped relationship may suggest that, in addition to SES- and CTRA-related biological influences, inflammatory molecular patterns may also detect occult residual AML influences on hematopoiesis and/or leukocyte population dynamics. The present findings do not identify a statistically significant effect of classic vs nonclassic monocyte expression on HCT outcomes despite their association with SES; however, this may be due to limited statistical power as was observed in the relationship between SES and clinical outcomes in this sample. Importantly, the genomic indicators statistically significantly related to both SES and clinical outcomes—relapse and LFS—are consistent in both this analysis and our previous study (14).

This study's findings are limited in several respects. They focus on a one-time, cross-sectional, pretransplant assessment of PBMC gene expression during a specified period; future studies are needed to determine whether the recipient SES-related gene expression profiles vary throughout the transplant process or differentially affect outcomes in a time-dependent manner and in a more contemporary cohort. The observed associations may also be attributable in part to other related chronic stress factors associated with SES, including but not limited to depressed mood (37) and low social support (38); genomic alterations as presented here may be indicative of a more comprehensive overall stress biomarker. Future work should use larger samples to conduct formal statistical mediation analyses evaluating a direct mechanistic model between SES and clinical outcomes. Other potential factors influencing differences in HCT immune function and biology, such as adolescent and young adult status, should also be considered. Although zip code has been robustly associated with both HCT (1,14) and general health outcomes (39), future research should consider more sensitive and individual-specific SES measures, including address, self-reported income, insurance status, and employment (40,41). Because this study tests several distinct substantive hypotheses, the multiple findings reported here will be important to replicate.

In summary, this study identifies SES-related gene expression among unrelated donor HCT recipients with AML to include increased activity of pro-inflammatory transcription control pathways, increased activation of classic monocytes, and a complementary decrease in activity of nonclassical monocytes, all of which are consistent with the CTRA physiologic pattern relating stress and sympathetic nervous system activation to altered regulation of gene expression in myeloid lineage immune cells. Multivariable modeling indicates a statistically significant effect for this general molecular pattern as predictive of worse clinical outcomes, including relapse and LFS. These findings provide a molecular framework within which to understand social environmental influences on immunobiology and clinical outcomes in the setting of cancer and HCT.

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JMK designed and performed research and analyzed data. JDR designed research. TW and BRL designed research, contributed vital analytical tools, and analyzed data. TW performed research. NH analyzed data. JDR, SRS, SJL, and MRV designed research and analyzed data. JMGA performed research. SWC designed and performed research, contributed vital analytical tools, and analyzed data. All authors contributed to manuscript preparation.

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References

1. Baker KS , Davies SM, Majhail NS, et al. Race and socioeconomic status influence outcomes of unrelated donor hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2009;15(12):1543–1554.
2. Silla L, Fischer GB, Paz A, et al. Patient socioeconomic status as a prognostic factor for allo-SCT. *Bone Marrow Transplant*. 2009;43(7):571–577.
3. Brookfield KF, Cheung MC, Lucci J, et al. Disparities in survival among women with invasive cervical cancer: a problem of access to care. *Cancer*. 2009;115(1):166–178.
4. Matthews KA, Gallo LC. Psychological perspectives on pathways linking socioeconomic status and physical health. *Annu Rev Psychol*. 2011;62(1):501–530.
5. Paulsen MS, Andersen M, Munck AP, et al. Socio-economic status influences blood pressure control despite equal access to care. *Fam Pract*. 2012;29(5):503–510.
6. Owen N, Poulton T, Hay FC, et al. Socioeconomic status, C-reactive protein, immune factors, and responses to acute mental stress. *Brain Behav Immun*. 2003;17(4):286–295.
7. Steptoe A, Marmot M. The role of psychobiological pathways in socio-economic inequalities in cardiovascular disease risk. *Eur Heart J*. 2002;23(1):13.
8. Black PH. The inflammatory consequences of psychologic stress: relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II. *Med Hypotheses*. 2006;67(4):879–891.
9. Chen E, Miller GE, Kobor MS, et al. Maternal warmth buffers the effects of low early-life socioeconomic status on pro-inflammatory signaling in adulthood. *Mol Psychiatry*. 2011;16(7):729–737.
10. Friedman EM, Herd P. Income, education, and inflammation: differential associations in a national probability sample (The MIDUS Study). *Psychosom Med*. 2010;72(3):290–300.
11. Gruenewald TL, Cohen S, Matthews KA, et al. Association of socioeconomic status with inflammation markers in black and white men and women in the Coronary Artery Risk Development in Young Adults (CARDIA) study. *Soc Sci Med*. 2009;69(3):451–459.
12. Miller GE, Chen E, Fok AK, et al. Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *Proc Natl Acad Sci*. 2009;106(34):14716–14721.
13. Miller GE, Chen E, Sze J, et al. A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-[kappa] B signaling. *Biol Psychiatry*. 2008;64(4):266–272.
14. Knight JM, Rizzo JD, Logan BR, et al. Low socioeconomic status, adverse gene expression profiles, and clinical outcomes in hematopoietic stem cell transplant recipients. *Clin Cancer Res*. 2016;22(1):69–78.
15. Cole S. Social regulation of gene expression in the immune system. In: *The Oxford Handbook of Psychoneuroimmunology*, Segerstrom S, ed. New York: Oxford University Press; 2012:254–273.
16. Irwin MR, Cole SW. Reciprocal regulation of the neural and innate immune systems. *Nat Rev Immunol*. 2011;11(9):625–632.
17. Antoni MH, Lutgendorf SK, Blomberg B, et al. Transcriptional modulation of human leukocytes by cognitive-behavioral stress management in women undergoing treatment for breast cancer. *Biol Psychiatry*. 2012;71(4):366–372.
18. Cole SW, Conti G, Arevalo JMG, et al. Transcriptional modulation of the developing immune system by early life social adversity. *Proc Natl Acad Sci*. 2012;109(50):20578–20583.
19. Powell ND, Sloan EK, Bailey MT, et al. Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via beta-adrenergic induction of myelopoiesis. *Proc Natl Acad Sci USA*. 2013;110(41):16574–16579.
20. Cole SW, Hawkley LC, Arevalo JM, et al. Social regulation of gene expression in human leukocytes. *Genome Biol*. 2007;8(9):R189.
21. Cacioppo JT, Hawkley LC. Social isolation and health, with an emphasis on underlying mechanisms. *Perspect Biol Med*. 2003;46(3 suppl):S39–S52.
22. University of Michigan Population Studies Center IfSR. *Zip Code Characteristics: Mean and Median Household Income*. 2017. https://www.psc.isr.umich.edu/dis/census/Features/tract2zip/.
23. Cole SW, Hawkley LC, Arevalo JMG, et al. Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes. *Proc Natl Acad Sci*. 2011;108(7):3080.
24. Fredrickson BL, Grewen KM, Coffey KA, et al. A functional genomic perspective on human well-being. *Proc Natl Acad Sci USA*. 2013;110(33):13684–13689.
25. Illumina. *Imputing HumanHT-12 Expression BeadChip Data*. 2011. https://support.illumina.com/content/dam/illumina-marketing/documents/products/technotes/technote_humanht_12_imputation.pdf.
26. Cole SW, Yan W, Galic Z, et al. Expression-based monitoring of transcription factor activity: the TELiS database. *Bioinformatics*. 2005;21(6):803–810.
27. Cao J, Zhang S. Multiple comparison procedures. *JAMA*. 2014;312(5):543–544.
28. Cole SW. Elevating the perspective on human stress genomics. *Psychoneuroendocrinology*. 2010;35(7):955–962.
29. Efron B, Tibshirani RJ. *An Introduction to the Bootstrap*. Dordrecht: Spring Science+Business Media; 1993.
30. Cole SW, Galic Z, Zack JA. Controlling false-negative errors in microarray differential expression analysis: A PRIM approach. *Bioinformatics*. 2003;19(14):1808.
31. Witten D, Tibshirani R. A comparison of fold-change and the t-statistic for microarray data analysis. *Analysis*. 2007;1776:58–85.
32. Shi L, Jones WD, Jensen RV, et al. The balance of reproducibility, sensitivity, and specificity of lists of differentially expressed genes in microarray studies. *BMC Bioinformatics*. 2008; Abstract 9(S9):S10. BioMed Central.
33. Shi L, Campbell G, Jones WD, et al. The MicroArray Quality Control (MAQC)-II study of common practices for the development and validation of microarray-based predictive models. *Nat Biotechnol*. 2010;28(8):827.

34. Shi L, Reid LH, Jones WD, et al. The MicroArray Quality Control (MAQC) project shows inter-and intraplatform reproducibility of gene expression measurements. *Nat Biotechnol*. 2006;24(9):1151.
35. Guo L, Lobenhofer EK, Wang C, et al. Rat toxicogenomic study reveals analytical consistency across microarray platforms. *Nat Biotechnol*. 2006;24(9):1162.
36. Wells DA, Benesch M, Loken MR, et al. Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. *Blood*. 2003;102(1):394–403.
37. Williams DR. Socioeconomic differentials in health: a review and redirection. *Soc Psychol Q*. 1990;53(2):81–99.
38. Pinquart M, Sorensen S. Influences on loneliness in older adults: a meta-analysis. *Basic Appl Soc Psychol*. 2001;23(4):245–266.
39. Everson SA, Maty SC, Lynch JW, et al. Epidemiologic evidence for the relation between socioeconomic status and depression, obesity, and diabetes. *J Psychosom Res*. 2002;53(4):891–895.
40. Winkleby MA, Jatulis DE, Frank E, et al. Socioeconomic status and health: how education, income, and occupation contribute to risk factors for cardiovascular disease. *Am J Public Health*. 1992;82(6):816–820.
41. Adler NE, Boyce T, Chesney MA, et al. Socioeconomic status and health: the challenge of the gradient. *Am Psychol*. 1994;49(1):15.