Research Paper

Age and poverty status alter the coding and noncoding transcriptome

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ABSTRACT

Emerging evidence indicates that noncoding RNAs play regulatory roles in aging and disease. The functional roles of long noncoding RNAs (lncRNAs) in physiology and disease are not completely understood. Little is known about lncRNAs in the context of human aging and socio-environmental conditions. Microarray profiling of lncRNAs and mRNAs from peripheral blood mononuclear cells from young and old white (n=16) and African American (AA) males (n=16) living above or below poverty from the Healthy Aging in Neighborhoods of Diversity across the Life Span study revealed changes in both lncRNAs and mRNAs with age and poverty status in white males, but not in AA males. We validated lncRNA changes in an expanded cohort (n=40); *CTD-3247F14.2, GAS5, H19, TERC* and *MEG3* changed significantly with age, whereas *AK022914, GAS5, KB-1047C11.2, MEG3* and *XLOC_003262* changed with poverty. Mitochondrial function and response to DNA damage and stress were pathways enriched in younger individuals. Response to stress, viral infection, and immune signals were pathways enriched in individuals living above poverty. These data show that both human age and a marker of social adversity influence lncRNA expression, which may provide insight about molecular pathways underlying aging and social factors that affect disparities in aging and disease.

INTRODUCTION

Recent attention has focused on discovering new biomarkers that may serve as indicators of both health span and life span. DNA methylation, telomere erosion, DNA damage and repair, mitochondrial copy number and function are considered markers of biological aging [1]. However, it has been difficult to unequivocally identify a single universal biomarker to monitor aging differentiated from disease because there is considerable variation in the signs and symptoms of aging. Studies have also identified noncoding RNAs (ncRNAs), such as microRNAs (miRNAs), as relevant biomarkers of aging. We and others have shown that miRNA levels change during human aging [2-6]. Interestingly, these changes in miRNA levels can be modulated by interventions such as calorie restriction or metformin [7, 8]. miRNAs are also key regulators of cellular processes important for aging such as cellular senescence [2]. Data from model systems also support a role for miRNAs in the aging process. For example, the *Caenorhabditis elegans* miRNA *lin-4* regulates lifespan through modulating expression of its target gene, *lin-14* [9]. Since this initial discovery several other miRNAs have been associated with regulating longevity in *C. elegans* [10].

Most recently, increasing interest in another class of ncRNAs, long ncRNAs (lncRNAs), has gained momentum as these molecules can also regulate gene expression at the transcriptional, post-transcriptional and translational levels [11, 12]. Furthermore, lncRNAs have been linked to processes important for aging and age-related disease [13-15]. However, little is known about the global changes in lncRNA expression that occur with aging. Aging is a multi-factorial process marked by a fundamental decline in physiological

responses and maintenance of tissue homeostasis and integrity. This decline of cell, tissue and organ function leads to an increase in a myriad of age-related diseases including cancer, type 2 diabetes mellitus, auto-immunity, infections, cardiovascular disease and ultimately mortality. Recent findings from model systems suggest that longevity and health span can be modulated by specific changes in gene expression patterns [10, 16]. These patterns could be useful markers for identifying individuals in at-risk populations for the premature development of disease or monitoring adverse outcomes that may lead to early mortality.

Social determinants of health significantly influence the trajectory of aging, health status and outcomes among populations at risk for health disparities. It is well known that poverty and low socioeconomic status remain major risk factors for cardiovascular disease, chronic kidney disease and early mortality [17-19]. The weathering hypothesis suggests that socioeconomic disadvantage results in accelerated aging or premature declines in health status among African Americans (AAs) [20]. In the United States, AA men are particularly vulnerable to early mortality [21-24]. Therefore, it is important to identify the biological mechanisms and biologic risk factors through which social determinants of health accelerate aging phenotypes and trigger premature mortality. Adverse social conditions, including poverty, poor neighborhood conditions, discrimination, and crime are robust risk factors for negative health outcomes. Social adversity has been linked to gene expression changes in both children and adults [25-27]. Specifically, challenging social-environmental conditions initiate changes in gene expression among specific gene sets. The observed increase in mRNAs encoding inflammatory proteins and the decrease in mRNAs encoding immune-response proteins, termed the Conserved Transcriptional Response to Adversity (CTRA) [25-27], has been documented in several human and non-human models (for review [28]). These data hint that differences in gene expression may underlie racial and socioeconomic disparities in health.

Here we profiled both lncRNAs and mRNAs in the context of human aging, race and poverty. These lncRNAs may serve as potential biomarkers of susceptibility to age-related diseases. In addition, lncRNAs may be important promoters of the change in gene expression that results from exposure to various social-environmental conditions.

RESULTS

lncRNA changes with age and poverty

Although lncRNAs have been studied in the context of senescence and various other hallmarks of aging, little is known about whether lncRNA expression is altered with human age. Furthermore, since poverty is well known to influence accelerated development of age-associated chronic disease and premature mortality [29, 30], we chose a sub-cohort of young and old AAs and

Table 1. Demographic information for human cohorts.

Microarray cohorts		
White males	**Young**	**Old**
n	8	8
Age (mean(SD))	30.6 (0.5)	63.6 (0.5)
PovStat = Below (%)	3 (37.5)	4 (50)
AA males	**Young**	**Old**
n	8	8
Age (mean(SD))	30.6 (0.5)	63.5 (0.5)
PovStat = Below (%)	4 (50)	4 (50)
Validation cohort		
White males	**Young**	**Old**
n	20	20
Age (mean(SD))	32.1 (1.7)	62.6 (1.2)
PovStat = Below (%)	9 (45)	9 (45)

whites from the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study that were both below and above poverty (Table 1). Poverty was designated as below poverty if the self-reported household income was below 125% of the 2004 Health and Human Services Poverty Guidelines at baseline recruitment. For our initial assessment of lncRNAs, we used the Arraystar human lncRNA microarray V3.0, which profiles about 30,586 lncRNAs and 26,109 coding transcripts. We selected young (30.6 yrs) and old males (63.3 years) that were either white or AA both below or above poverty (n=8/group Table 1).

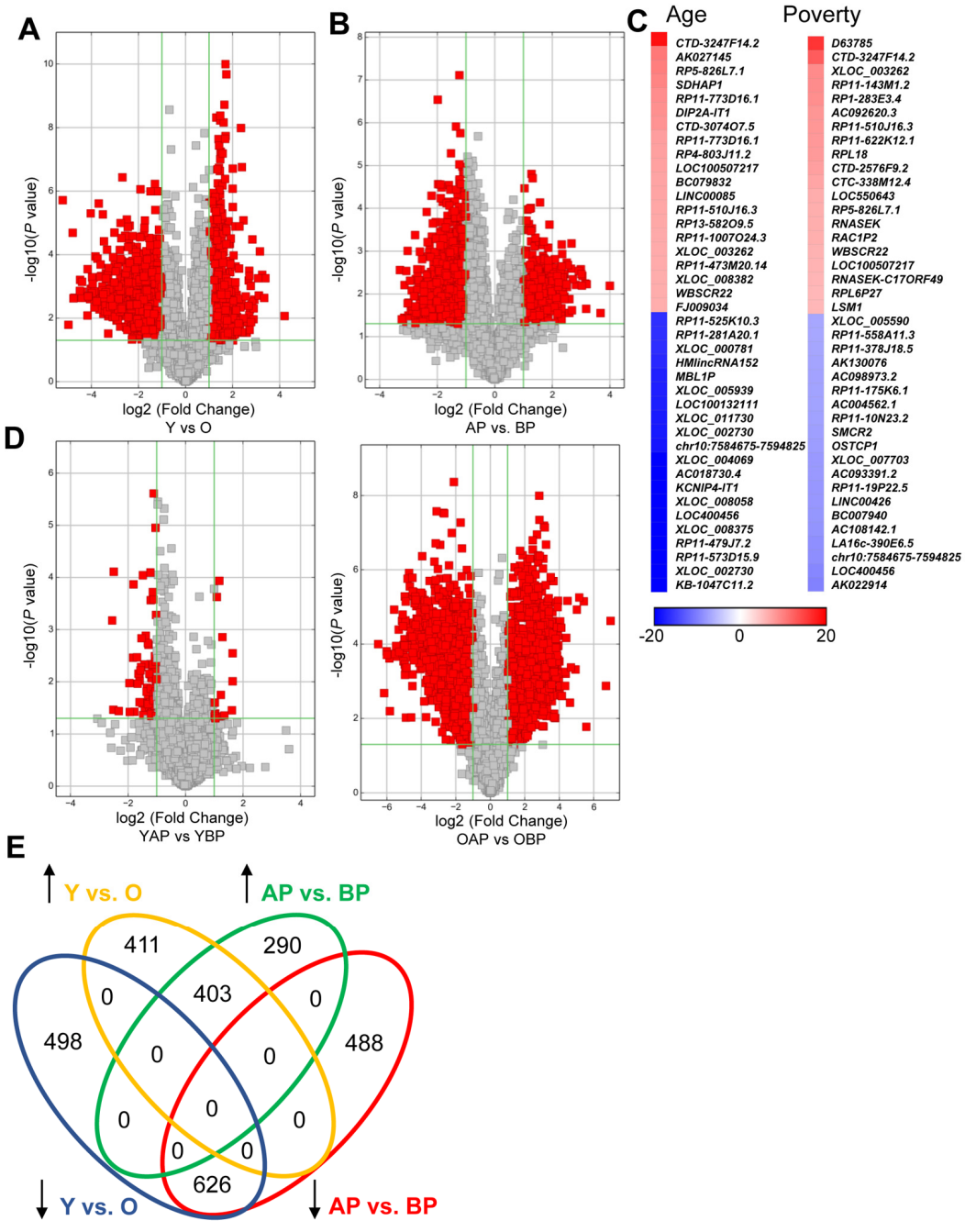

Figure 1. Changes in lncRNA abundance with age and poverty. The lncRNA expression levels in white young and old males (**A**) living above or below poverty (**B**) were assessed using microarrays (Table 1 for demographic details). Volcano plots show log2 fold change and *P* value for each lncRNA. Red indicates lncRNAs that were ≥2-fold change and $P \leq 0.05$. (**A**,**B**,**D**) lncRNA comparisons between groups are indicated. (**C**) Heat maps indicate the top fold changed lncRNAs with age and poverty. These lncRNAs are also listed in Tables 2 and 3. (**E**) Venn diagram of the total number of significantly increased ↑ or decreased ↓ lncRNAs for each comparison. Y, young; O, old, AP, above poverty; BP, below poverty.

We found that a substantial number of lncRNAs were significantly altered with human age in white males (1938 lncRNAs; Figure 1A; Supplementary Tables 1-2). In addition, poverty status also significantly changed the levels of lncRNAs in white males (1807 lncRNAs; Figure 1B; Supplementary Tables 3-4). The top changes in lncRNA abundance with age and poverty are listed in Tables 2 and 3, respectively and shown in the heat maps in Figure 1C. A list of all significantly altered lncRNAs with age and poverty are found in Supplementary Tables 1-4. Intriguingly, lncRNA changes with poverty were not observed in young individuals, but were robust among old individuals, consistent with the idea that poverty over the life span may greatly influence gene expression (Figure 1D). However, we did not assess childhood poverty or socioeconomic status. Comparison of the lncRNAs changed in abundance between poverty and age indicate that 1,029 lncRNAs that were significantly altered overlap with poverty and age (Figure 1E). We observed a different pattern for lncRNA differences with poverty and age in AA males (Supplementary Figure 1), where far fewer lncRNAs were significantly changed in abundance with age (only 26) and with poverty (245).

To determine the effects of race on lncRNA expression, we compared lncRNAs that were altered by race. We found that 291 lncRNAs were altered between white and AA males. Furthermore, if we categorize based on race, poverty and age, we identified changes in lncRNA levels that occurred with both races comparing above and below poverty status. There were 12 lncRNAs that were altered with poverty in both races and these are indicated in gray boxes in Figure 2. Only one lncRNA, *RNF157-AS1*, changed significantly with age in both whites and AAs, and one lncRNA, *C6orf3,* changed significantly with both poverty and age in AAs (Figure 2A).

Comparison to age-associated lncRNAs

Aging and poverty associated changes in lncRNA levels in whites were also compared to lncRNAs that have previously been described in the literature to be age-associated. Since senescent cells accumulate with age, and have been linked to a number of age-related pathologies, we first compared our dataset to lncRNAs that previously were found to be significantly changed in abundance during cellular senescence, termed senescence-associated lncRNAs (SAL-RNAs) [31]. Several poverty-associated lncRNAs (14) and aging-related lncRNAs (13) overlapped with the various SAL-RNAs (Figure 2B). There were 10 SAL-RNAs that overlapped with both comparisons (Figure 2B).

We also further compared the lncRNAs we found to be significantly different to previously reported age-associated lncRNAs. These lncRNAs were compiled

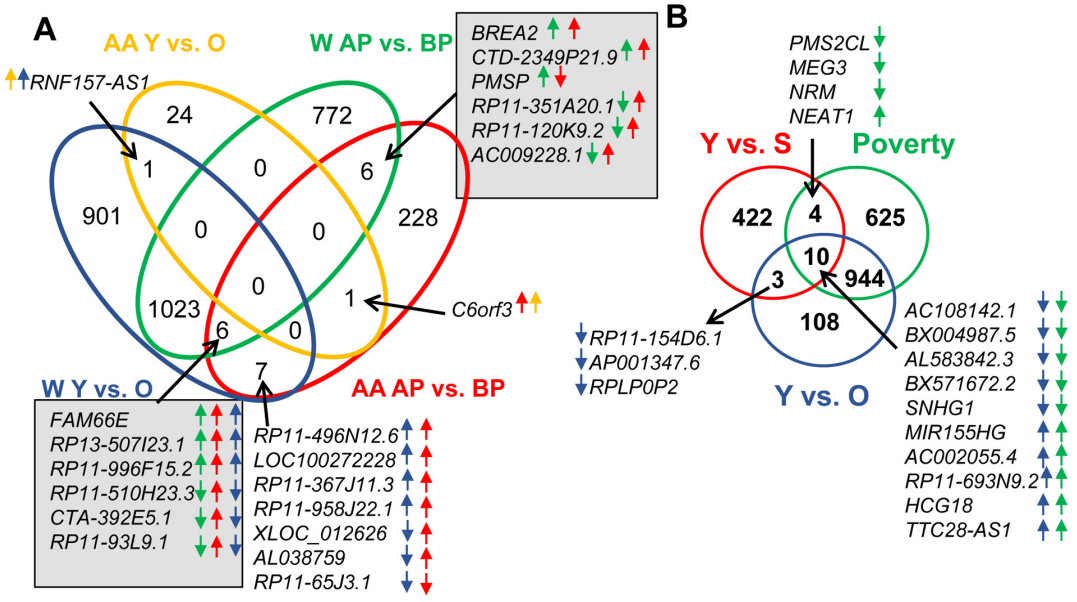

Figure 2. Comparison of lncRNA level changes with age, poverty and senescence. (**A**) Significantly changed lncRNA abundance was compared between African American (AA) males living below poverty (BP) and above poverty (AB) to white males (W) living below and above poverty. (**B**) Changes in lncRNAs levels in whites with poverty and age were also compared to significantly changed senescence-associated lncRNAs (SAL-RNAs) identified previously [31]. The comparisons in (**A**) were done using the Seqname and (**B**) using Gene symbol, hence the slight differences in numbers. Arrows indicate the direction of change (higher or lower) in the comparisons. The color of the arrow indicates the comparison examined.

based on the regulation of various hallmarks of aging [13] or age-associated functions, including differences with aging in the achilles tendon [32], specific lncRNAs associated with regulating senescence [33, 34], mouse premature aging and muscle wasting [35]. This list consisted of 118 "Age-associated lncRNAs" (Supplementary Table 5). Three lncRNAs were overlapping between these Age-associated lncRNAs and our poverty and aging lncRNAs and three were overlapping just with poverty and four just with age (Figure 3A).

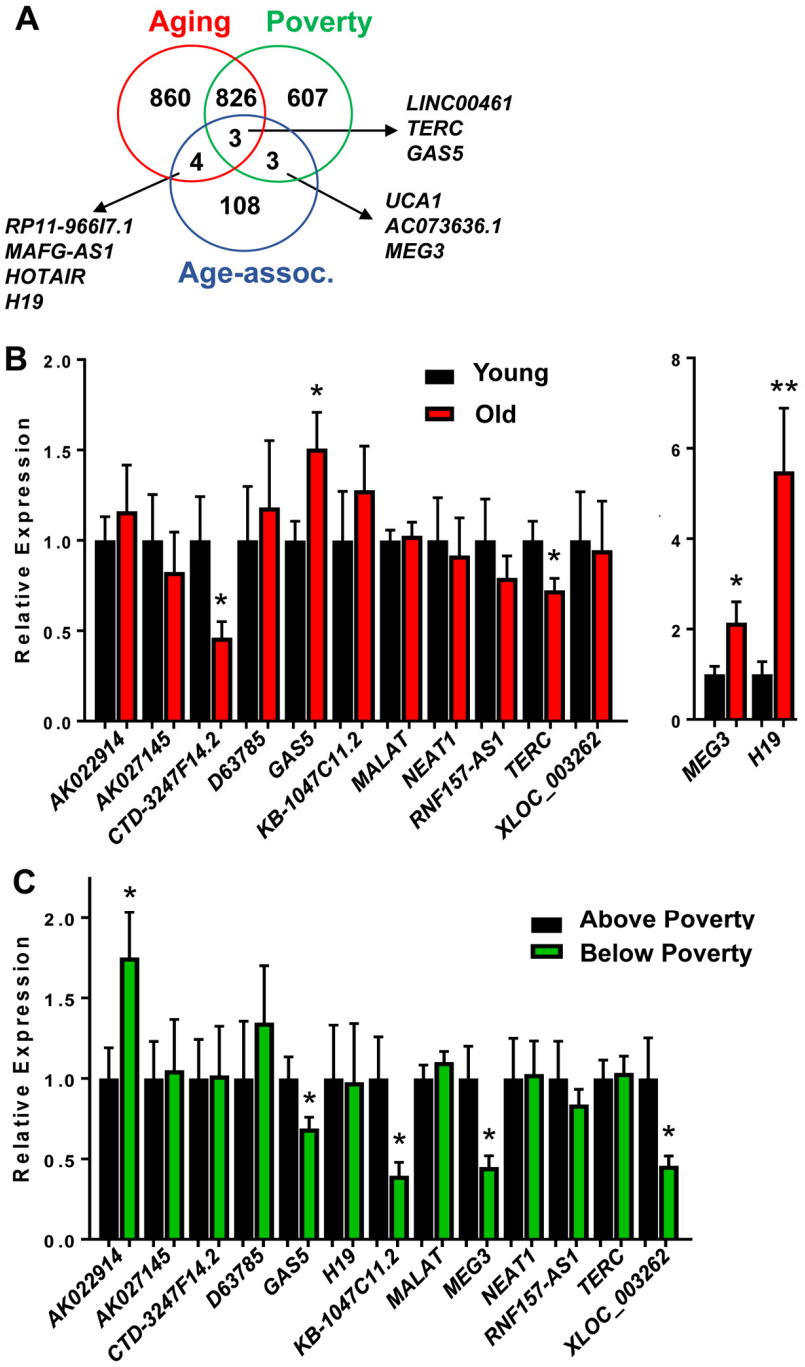

Figure 3. Validation of age and poverty associated changes in lncRNAs abundance. (**A**) lncRNAs significantly changed in abundance with age and poverty in white males were compared to lncRNAs identified in the literature to be age-associated. Overlapping lncRNAs are shown. (**B**-**C**) RNA was isolated from PBMCs from young (mean=32.1 yrs) and old (mean=62.6 yrs) white males above and below poverty (n=40; see Table 1 for demographics). RNA was reverse transcribed and lncRNA levels were quantified by RT-qPCR.

Validation of lncRNA changes with age and poverty

Several of these overlapping lncRNAs were chosen to further validate in an expanded cohort of individuals (n=40; Table 1). In addition, we also validated the top lncRNAs that were significantly changed in abundance with either age or poverty (Tables 2, 3). All significantly changed lncRNAs are listed in Supplementary Tables 1-4. We designed primers for the top lncRNAs showing the most robust changes in levels and for various "Age-associated lncRNAs" including *H19, MEG3, TERC* and *GAS5*. We designed multiple primer sets for *XLOC-002730* and *LOC400456*, but we were unable to amplify these lncRNAs. We chose to further validate in the expanded cohort a total of 13 lncRNAs. *RNF157-AS1* was included since levels of this lncRNA were changed differentially with age in AAs and whites, but it was not significantly altered in this expanded cohort. Importantly, *CTD-3247F14.2 (*LNCipedia: *lnc-EGR3-1), GAS5, H19, TERC* and *MEG3* levels were all significantly changed with age (Figure 3B). *AK022914 (DUXAP9), GAS5, KB-1047C11.2 (*LNCipedia: *C8orf37-AS1:1), MEG3* and *XLOC_003262* (LNCipedia: *lnc-KY-1*) levels were significantly different with poverty (Figure 3C). Interestingly, *GAS5* binds and acts as a cytoplasmic decoy for the glucocorticoid receptor (GR) [36]. This observation is intriguing given that transcriptional changes in GR signaling pathways are part of the Conserved Transcriptional Response to Adversity (CTRA) in mice and humans [28].

mRNA changes with age and poverty

In addition to examining lncRNA levels, we also wanted to further understand differences in the levels of expressed mRNAs with poverty and age. To do this, we examined mRNA expression levels by microarray and found that, similar to the lncRNAs, there were many significantly different mRNAs with both age and poverty in white males (Figure 4A,B; Supplementary Tables 6-9). The top changes in mRNA abundance with age and poverty are listed in Tables 4 and 5, respectively and shown in the heat maps in Figure 4C. A list of all significantly altered mRNAs with age and poverty are found in Supplementary Tables 6-9. Changes in expressed mRNAs with poverty were also more robust in the older individuals compared to the younger individuals, similar to our results with lncRNAs (Figure 4D). Many of the differentially abundant mRNAs were overlapping between poverty

Table 2. Top changes in lncRNA abundance with age.

Increased in Young					Decreased in Young				
Gene Symbol	Fold	*P* value	Seqname	Source	Gene Symbol	Fold	*P* value	Seqname	Source
CTD-3247F14.2	18.50	0.008	ENST00000566457	GENCODE	***KB-1047C11.2***	37.34	0.000	ENST00000517655	GENCODE
AK027145	10.52	0.001	uc002ywy.3	UCSC_knowngene	***XLOC_002730***	31.53	0.016	TCONS_00006917	LincRNAs; Cabili et al
RP5-826L7.1	10.14	0.002	ENST00000446476	GENCODE	***RP11-573D15.9***	27.85	0.000	ENST00000577781	GENCODE
SDHAP1	9.57	0.000	ENST00000440850	pseudogene	***RP11-479J7.2***	27.24	0.002	ENST00000425271	GENCODE
RP11-773D16.1	8.94	0.000	ENST00000496359	GENCODE	***XLOC_008375***	26.51	0.002	TCONS_00018115	LincRNAs; Cabili et al
DIP2A-IT1	8.67	0.002	NR_046400	RefSeq	***LOC400456***	22.55	0.001	NR_034095	RefSeq
CTD-3074O7.5	8.47	0.001	ENST00000533502	GENCODE	***XLOC_008058***	22.39	0.001	TCONS_00017457	LincRNAs; Cabili et al
RP11-773D16.1	7.60	0.003	ENST00000488805	GENCODE	***KCNIP4-IT1***	21.32	0.002	NR_002813	RefSeq
RP4-803J11.2	7.46	0.005	ENST00000418348	GENCODE	***AC018730.4***	21.06	0.001	ENST00000454183	GENCODE
LOC100507217	7.39	0.011	NR_037600	RefSeq	***XLOC_004069***	20.96	0.001	TCONS_00008575	LincRNAs; Cabili et al
BC079832	7.16	0.000	uc003nhj.3	UCSC_knowngene	***chr10:7584675-7594825***	18.28	0.002	chr10:7584675-7594825-	LincRNAs; Cabili et al
LINC00085	7.10	0.003	ENST00000573896	GENCODE	***XLOC_002730***	18.03	0.008	TCONS_00006916	LincRNAs; Cabili et al
RP11-510J16.3	6.89	0.013	ENST00000564138	GENCODE	***XLOC_011730***	17.92	0.000	TCONS_00024847	LincRNAs; Cabili et al
RP13-582O9.5	6.83	0.001	ENST00000521207	GENCODE	***LOC100132111***	17.58	0.002	NR_024237	RefSeq
RP11-1007O24.3	6.64	0.005	ENST00000565181	GENCODE	***XLOC_005939***	17.49	0.002	TCONS_00011248	LincRNAs; Cabili et al
XLOC_003262	6.59	0.023	TCONS_00006666	LincRNAs; Cabili et al	***MBL1P***	17.35	0.002	ENST00000480805	GENCODE
RP11-473M20.14	6.55	0.000	ENST00000575139	GENCODE	***HMlincRNA152***	16.72	0.002	HMlincRNA152-	LincRNAs; Cabili et al
XLOC_008382	6.53	0.000	TCONS_00018121	LincRNAs;Cabili et al	***XLOC_000781***	16.36	0.000	TCONS_00001451	LincRNAs; Cabili et al
WBSCR22	6.52	0.008	NR_037776	RefSeq	***RP11-281A20.1***	16.34	0.002	ENST00000449730	GENCODE
FJ009034	6.41	0.001	uc010wyg.2	UCSC_knowngene	***RP11-525K10.3***	16.31	0.001	ENST00000568776	GENCODE

Top lncRNAs differentially expressed with age and *P*<0.05, Fold change >2 and FDR <0.1.

and age (Figure 4E). Interestingly, comparison of the top mRNAs that were more abundant in both young individuals and those living above poverty showed that out of the top 20 hits 6 overlapped including *IL1B, EIF1AY, CCL3L3, FAM96A, HLA-DRB1,* and *ATRAID* (Figure 4C; Tables 4,5). Out of the top 20 mRNAs that were less abundant with age and poverty status *ETS1, C20orf152, NANOG,* and *SLC18A3* were all overlapping (Figure 4C; Tables 4, 5).

Differences in mRNA abundance were also examined in AA males. There were very few mRNAs showing significant changes in abundance with age in AA males (Supplementary Figure 1). More mRNAs were significantly different comparing individuals above and below poverty (Supplementary Figure 1).

Age and poverty pathway analysis

To gain additional information about the biological pathways that may be regulated by the mRNAs changing in abundance with poverty and age, we performed gene ontology (GO) analysis. In younger individuals, we found enrichment of pathways associated with mitochondrial function and response to DNA damage and stress (Figure 5A). In older individuals, pathways related to chemokine production and development were enriched. In the context of poverty, pathways related to development and differentiation were enriched in individuals living below poverty (Figure 5B). We found that the top pathways enriched among those living above poverty were those implicated in the response to stress, immune stimuli, and viral

Table 3. Top changes in lncRNA abundance with poverty.

Increased in Above Poverty					Decreased in Above Poverty				
Gene Symbol	Fold	*P* value	Seqname	Source	Gene Symbol	Fold	*P* value	Seqname	Source
D63785	15.93	0.006	uc002hlt.1	UCSC_knowngene	***AK022914***	9.82	0.003	uc001vvr.1	UCSC_knowngene
CTD-3247F14.2	12.74	0.028	ENST00000566457	GENCODE	***LOC400456***	9.41	0.039	NR_034095	RefSeq
XLOC_003262	9.13	0.006	TCONS_00006666	LincRNAs; Cabili et al	***chr10:7584675-7594825***	8.88	0.036	chr10:7584675-7594825-	LincRNAs; Cabili et al
RP11-143M1.2	9.02	0.022	ENST00000442069	GENCODE	***LA16c-390E6.5***	8.81	0.006	ENST00000566287	GENCODE
RP1-283E3.4	8.86	0.006	ENST00000577672	GENCODE	***AC108142.1***	8.39	0.048	ENST00000507869	GENCODE
AC092620.3	8.23	0.004	ENST00000453636	GENCODE	***BC007940***	8.22	0.038	uc002xzq.1	UCSC_knowngene
RP11-510J16.3	8.15	0.006	ENST00000564138	GENCODE	***LINC00426***	8.17	0.001	ENST00000447147	GENCODE
RP11-622K12.1	8.05	0.003	ENST00000423617	GENCODE	***RP11-19P22.5***	8.14	0.017	ENST00000583179	GENCODE
RPL18	7.91	0.003	NR_073022	RefSeq	***AC093391.2***	8.01	0.016	ENST00000446492	GENCODE
CTD-2576F9.2	7.55	0.003	ENST00000568835	GENCODE	***XLOC_007703***	8.01	0.032	TCONS_00016773	LincRNAs; Cabili et al
CTC-338M12.4	7.04	0.006	ENST00000506340	GENCODE	***OSTCP1***	7.70	0.005	ENST00000522287	GENCODE
LOC550643	6.54	0.005	NR_015367	RefSeq	***SMCR2***	7.56	0.025	ENST00000456090	GENCODE
RP5-826L7.1	6.39	0.024	ENST00000446476	GENCODE	***RP11-10N23.2***	7.38	0.008	ENST00000520562	GENCODE
RNASEK	6.35	0.001	NR_037715	RefSeq	***AC004562.1***	7.27	0.024	ENST00000420427	GENCODE
RAC1P2	6.26	0.003	ENST00000511164	pseudogene	***RP11-175K6.1***	7.26	0.027	ENST00000523301	GENCODE
WBSCR22	6.01	0.013	NR_037776	RefSeq	***AC098973.2***	7.21	0.019	ENST00000414382	GENCODE
LOC100507217	6.00	0.028	NR_037600	RefSeq	***AK130076***	7.17	0.039	uc001kfc.1	UCSC_knowngene
RNASEK-C17ORF49	5.81	0.002	NR_037717	RefSeq	***RP11-378J18.5***	6.96	0.011	ENST00000433391	GENCODE
RPL6P27	5.66	0.007	ENST00000583065	GENCODE	***RP11-558A11.3***	6.91	0.040	ENST00000568458	GENCODE
LSM1	5.65	0.002	NR_045493	RefSeq	**XLOC_005590**	6.898	0.035	TCONS_00012060	LincRNAs; Cabili et al

Top lncRNAs differentially expressed with poverty and *P*<0.05, Fold change >2 and FDR <0.1.

infection (Figure 5B). This finding is not unexpected because mRNAs increased in abundance in response to social adversity, part of the inflammatory response cascade, and mRNAs decreased in abundance with social adversity are noted to be related to the innate interferon antiviral response as well as synthesis of antibodies [27].

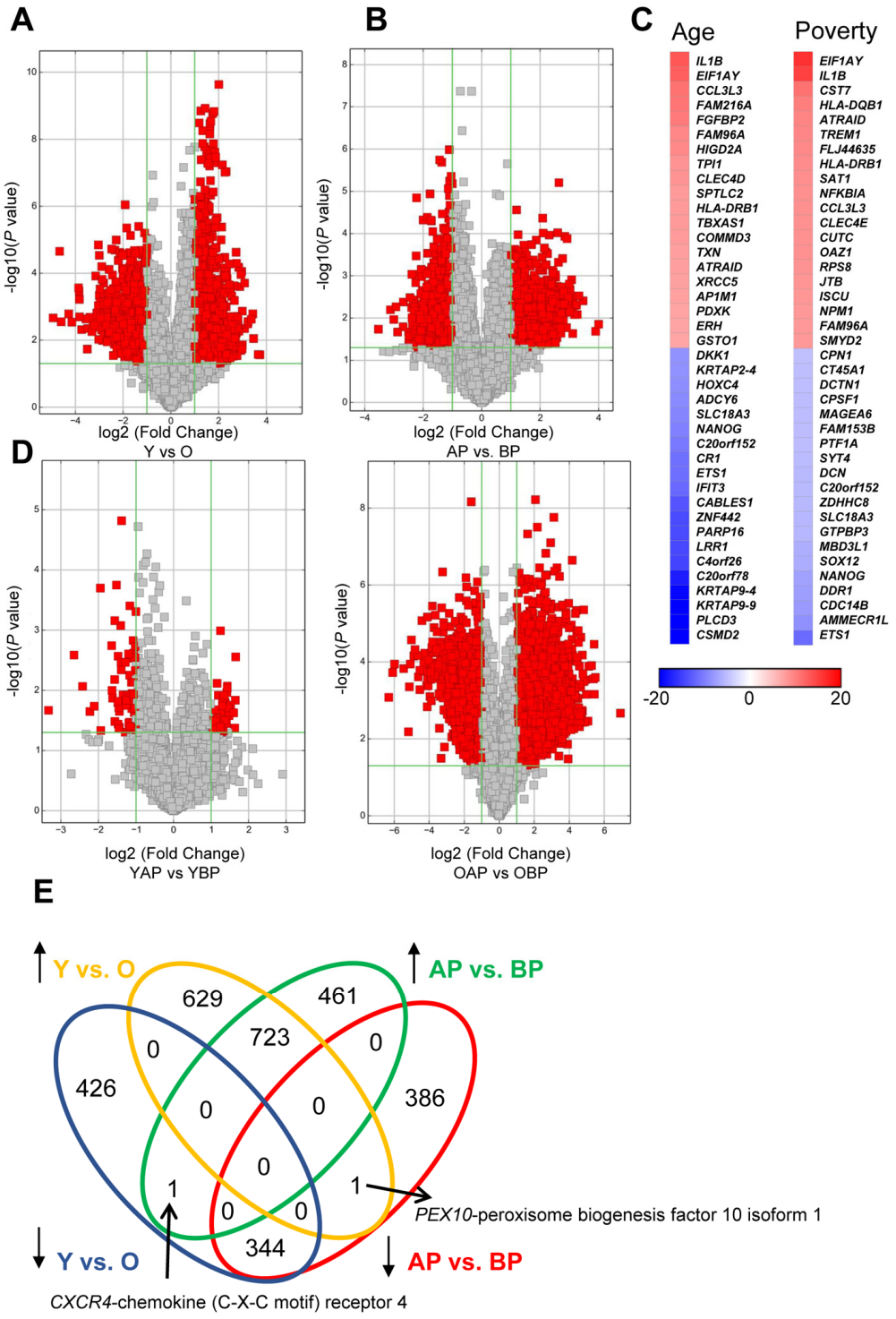

Figure 4. Changes in mRNA expression levels with age and poverty. The levels of expressed mRNAs in white young and old males (**A**) living above or below poverty (**B**) were assessed using microarrays. Volcano plots show log2 fold change and *P* value for each mRNA. Red indicates mRNAs that changed were ≥2-fold, with $P \leq 0.05$. (**A**,**B**,**D**) Comparisons of significantly different mRNAs between groups are indicated. (**C**) Heat maps indicate the top fold changed mRNAs with age and poverty. These mRNAs are also listed in Tables 4 and 5. (**E**) Venn diagram of the total number of significantly increased or decreased mRNAs for each comparison. Y, young; O, old, AP, above poverty; BP, below poverty.

Table 4. Top changes in mRNA abundance with age.

Increased in Young				Decreased in Young			
Gene Symbol	Fold	*P* value	Seqname	Gene Symbol	Fold	*P* value	Seqname
IL1B	13.23	0.027	NM_000576	***CSMD2***	30.48	0.002	ENST00000241312
EIF1AY	12.58	0.027	ENST00000361365	***PLCD3***	25.30	0.000	NM_133373
CCL3L3	11.02	0.009	NM_001001437	***KRTAP9-9***	24.11	0.003	ENST00000394008
FAM216A	10.80	0.002	NM_013300	***KRTAP9-4***	19.41	0.002	NM_033191
FGFBP2	10.32	0.002	NM_031950	***C20orf78***	17.73	0.003	NM_001242671
FAM96A	9.42	0.003	NM_001014812	***C4orf26***	14.59	0.000	NM_178497
HIGD2A	9.33	0.003	NM_138820	***LRR1***	14.36	0.000	NM_203467
TPI1	8.50	0.000	NM_000365	***PARP16***	14.22	0.001	NM_017851
CLEC4D	8.46	0.004	NM_080387	***ZNF442***	14.17	0.002	NM_030824
SPTLC2	7.95	0.001	NM_004863	***CABLES1***	13.31	0.005	NM_138375
HLA-DRB1	7.93	0.014	NM_002124	***IFIT3***	11.77	0.002	NM_001549
TBXAS1	7.89	0.018	NM_001166254	***ETS1***	11.35	0.018	NM_005238
COMMD3	7.84	0.000	NM_012071	***CR1***	11.21	0.004	NM_000651
TXN	7.63	0.000	NM_003329	***C20orf152***	10.51	0.002	ENST00000349339
ATRAID	7.63	0.020	NM_016085	***NANOG***	9.89	0.005	NM_024865
XRCC5	7.45	0.016	NM_021141	***SLC18A3***	9.55	0.003	NM_003055
AP1M1	7.41	0.000	ENST00000291439	***ADCY6***	9.17	0.004	NM_015270
PDXK	7.21	0.005	NM_003681	***HOXC4***	8.89	0.000	ENST00000430889
ERH	7.15	0.000	NM_004450	***KRTAP2-4***	8.60	0.002	NM_033184
GSTO1	7.03	0.004	NM_001191003	***DKK1***	8.56	0.002	NM_012242

Top mRNAs differentially expressed with age and *P*<0.05, Fold change >2 and FDR <0.1.

DISCUSSION

Here, we have examined differences in expressed lncRNAs and mRNAs with age and poverty status in both white and AA males. In our initial genome-wide profiling, we found that white males had larger significant differences in expression levels of lncRNAs and mRNAs with poverty and age, as compared to AA males, where these changes were far more modest. This indicates that the influence of age and poverty on gene expression patterns differs between racial groups. It is not clear what drives these differences, but they may correlate with the wide gap in health status and health outcomes between AAs and whites particularly between AA men and white men. AA males living below poverty in the HANDLS cohort, and in other studies, are particularly vulnerable to early mortality compared to their white counterparts and even when compared to AA men who live above the poverty line despite adjustments for various lifestyle cofactors [21, 22]. Given this disparity, it is important to examine whether and how gene expression or other genomic factors may act as a biologic transduction pathway for the social determinants of health.

We found altered expression levels in a number of lncRNAs with age and poverty in white males. Although lncRNAs have been studied in the context of aging, in general most studies have focused on a specific lncRNA or on those that relate to various hallmarks of aging. Here, we have performed a comprehensive study of lncRNAs that change in abundance with human age in men whose life expectancy at birth and life span is shorter than women. We also compared these lncRNAs to a growing list of age-associated lncRNAs from the literature. Several of these overlapping lncRNAs were further validated in an expanded cohort of individuals; among these, *GAS5, H19, TERC* and *MEG3* levels were all significantly changed with age (Figure 3B). *GAS5 and MEG3* levels were significantly different with poverty. These data further confirm that these are true age-associated lncRNAs, particularly since these lncRNAs were identified as being aging-associated in other tissues or

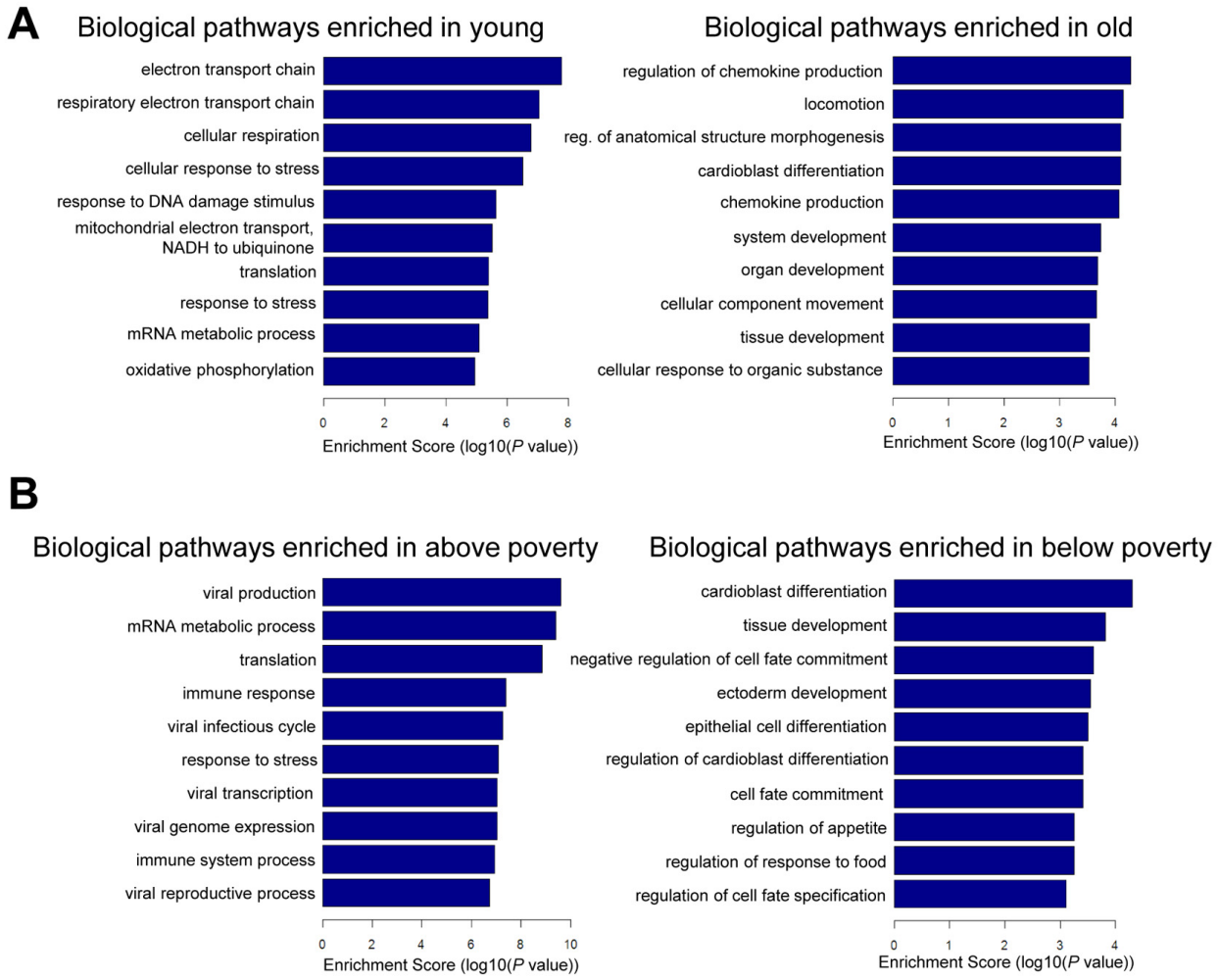

Figure 5. Pathway analysis for age and poverty. Differentially expressed mRNAs with age and poverty were used for gene ontology (GO) analysis using categories derived from Gene Ontology. Top biological pathways are shown for age (**A**) and poverty (**B**).

model systems. Several of these lncRNAs regulate aging processes including telomere function (*TERC*), autophagy (*MEG3*), protein trafficking (*GAS5*), and epigenetic change and proliferation (*H19*) [13]. Investigators have found that many of these lncRNAs participate in regulating age-related disease [15].

In addition to confirming these age-associated lncRNAs, we also identified many novel age- and poverty-associated lncRNAs (Tables 2, 3). The lncRNAs displaying the largest increases and decreases in abundance were further validated in our expanded cohort; *CTD-3247F14.2 (*LNCipedia: *lnc-EGR3-1)* levels were significantly changed with age, while *AK022914 (DUXAP9), KB-1047C11.2 (*LNCipedia: *C8orf37-AS1:1),* and *XLOC_003262* (LNCipedia: *lnc-KY-1*) levels were significantly changed with poverty. These data shed new light on various lncRNAs that may be important biological transducers of stressors related to poverty and age, as well as to the loss of homeostasis that may result from both. Future work lies in determining whether the expression of these lncRNAs is a consequence or contributor to the aging process. Interestingly, we found that the levels of one lncRNA, *RNF157-AS1*, were altered differentially with age in AAs and in whites. It will be interesting in the future to further investigate how this lncRNA expression is altered with age and race.

In summary, we have identified lncRNAs that are altered in abundance with both poverty and age in whites and AAs. As our knowledge of the functional roles of lncRNAs expands, it will be important to determine how these ncRNAs contribute to the aging process and to the biological transduction of social adversity and social determinants that result in health disparities.

Table 5. Top changes in mRNA abundance with poverty.

Increased in Above Poverty				Decreased in Above Poverty			
Gene Symbol	Fold	*P* value	Seqname	Gene Symbol	Fold	*P* value	Seqname
EIF1AY	16.03	0.014	ENST00000361365	***ETS1***	11.59	0.018	NM_005238
IL1B	14.94	0.020	NM_000576	***AMMECR1L***	8.72	0.006	NM_031445
CST7	11.06	0.004	ENST00000480798	***CDC14B***	8.02	0.011	NM_003671
HLA-DQB1	10.06	0.003	NM_002123	***DDR1***	7.64	0.005	NM_001954
ATRAID	9.66	0.008	NM_016085	***NANOG***	7.34	0.021	NM_024865
TREM1	9.46	0.005	NM_018643	***SOX12***	6.43	0.016	NM_006943
FLJ44635	9.26	0.005	NM_207422	***MBD3L1***	6.39	0.004	ENST00000305625
HLA-DRB1	9.05	0.008	NM_002124	***GTPBP3***	5.66	0.022	NM_133644
SAT1	8.75	0.004	NM_002970	***SLC18A3***	5.63	0.035	NM_003055
NFKBIA	8.73	0.005	NM_020529	***ZDHHC8***	5.53	0.026	NM_013373
CCL3L3	8.67	0.023	NM_001001437	***C20orf152***	5.50	0.043	ENST00000349339
CLEC4E	8.66	0.003	NM_014358	***DCN***	5.47	0.003	NM_133504
CUTC	8.64	0.004	NM_015960	***SYT4***	5.31	0.029	NM_020783
OAZ1	8.62	0.004	NM_004152	***PTF1A***	5.28	0.041	NM_178161
RPS8	8.44	0.001	NM_001012	***FAM153B***	5.27	0.006	ENST00000253490
JTB	8.31	0.004	NM_006694	***MAGEA6***	5.24	0.001	NM_175868
ISCU	8.16	0.002	NM_213595	***CPSF1***	5.23	0.010	NM_013291
NPM1	8.07	0.007	NM_199185	***DCTN1***	5.14	0.015	NM_001135041
FAM96A	8.01	0.003	NM_032231	***CT45A1***	5.07	0.005	NM_001017417
SMYD2	7.97	0.002	NM_020197	***CPN1***	4.98	0.005	NM_001308

Top mRNAs differentially expressed with poverty and $P<0.05$, Fold change >2 and FDR <0.1.

MATERIALS AND METHODS

Clinical participants

Participants were chosen from the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study of the National Institute on Aging Intramural Research Program, National Institutes of Health (NIH). The study has been approved by the Institutional Review Board of the National Institute of Environmental Health Sciences, NIH. All participants provided written informed consent. HANDLS is a Baltimore, Maryland based longitudinal epidemiologic study focused on examining the interaction of race and socioeconomic status on aging and age-related health disparities [37]. A sub-cohort of young (~30 yrs) and old (~64 yrs) white or African American (AA) males above or below poverty were chosen for the microarray (n=8/group) (Table 1). An expanded cohort of white males above and below poverty were chosen for RT-qPCR validation (n=20/group; Table 1). Below poverty was designated if the self-reported household income was below 125% of the 2004 Health and Human Services Poverty Guidelines at baseline recruitment.

PBMCs

Fasting blood samples are obtained from participants in the morning and collected in 8-ml Vacutainer® heparinized vials (BD, Franklin Lakes, NJ). Peripheral blood mononuclear cells were isolated within 3 hours of phlebotomy as detailed previously [4]. After isolation, PBMCs are aliquoted and stored at -80^{o}C.

RNA isolation and microarray

Total RNA from PBMCs was isolated using TRIzol™ according to manufacturer's instructions. RNA quality and quantity were analyzed using a NanoDrop ND-1000 spectrophotometer and standard denaturing agarose gel electrophoresis.

lncRNA and mRNA microarrays were performed by Arraystar Inc. The Arraystar Human LncRNA Micro-

array V3.0 contains lncRNAs identified through public transcriptome databases (Refseq, UCSC knowngenes, Gencode, etc), as well as from the literature. In brief, sample preparation and microarray hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology) with minor modifications. mRNA was purified from total RNA after removal of rRNA (mRNA-ONLY™ Eukaryotic mRNA Isolation Kit, Epicentre). Subsequently, each sample was amplified and transcribed into fluorescent cRNA along the entire length of the transcripts without 3' bias utilizing a random priming method (Arraystar Flash RNA Labeling Kit, Arraystar). The labeled cRNAs were hybridized onto the Human LncRNA Array v3.0 (8 x 60K, Arraystar). After washing and fixing of the slides, the arrays were scanned by the Agilent Scanner G2505C.

Acquired array images were analyzed using the Agilent Feature Extraction software (version 11.0.1.1). Quantile normalization and subsequent data processing were performed using the GeneSpring GX v12.0 software package (Agilent Technologies). After quantile normalization of the raw data, ComBat was used to adjust batch effects. After normalization of the raw data, lncRNAs and mRNAs that at least 8 out of 16 samples have flags in present or marginal were chosen for further data analysis. Differentially expressed lncRNAs and mRNAs with statistical significance with Fold Change >= 2.0, *P* value <= 0.05 were identified through Volcano Plot filtering between two groups. All significantly changed lncRNAs are listed in Supplementary Tables 1-4 and mRNAs in Supplementary Tables 6-9. Differentially expressed mRNAs were used for gene ontology (GO) analysis using categories derived from Gene Ontology (http://www.geneontology.org). Biological pathways are shown. Fisher's exact test was used to calculate significance and *P* value denotes the significance of GO term enrichment in the differentially expressed genes. Microarray data can be accessed at GEO (Accession Number: GSE123500).

RT-qPCR

Cryopreserved PBMCs were thawed quickly and washed with PBS. Total RNA was isolated using TRIzol™ according to manufacturer's instructions with an inclusion of a DNase treatment step. RNA quality and quantity were analyzed using a NanoDrop 2000c. RNA was reverse transcribed using random hexamers and reverse transcriptase (Invitrogen). RT-qPCR was performed using SYBR green master mix and lncRNA specific primers. LncRNA primer sequences were designed using NCBI reference sequences or sequences from LNCipedia (www.lncipedia.org). Primer sequences and detailed information are listed in Supplementary Table 10. In some cases, the LNCipedia nomenclature differs from the nomenclature used here and these alternative names are also listed in the text and in Supplementary Table 10. *NEAT1* and *MEG3* primers were described previously [38, 39]. *GAS5* has 29 different transcripts and primers were designed using sequences from microarray. Similar data was observed for transcript variants *GAS5_019, GAS5_24* and *GAS5_026* and data only from *GAS5_019* is shown. For other lncRNAs with multiple variants, primers were designed against exons that overlapping between the variants. Reactions were run on an Applied Biosystems 7500 Real-Time PCR System using default settings. lncRNA expression was normalized to an average of *HPRT* and *UBC*. Previously, we have found that these mRNAs are least variable for aging studies in PBMCs [40]. lncRNA levels were examined for Gaussian distribution by measuring kurtosis and skewness and outliers for each lncRNA were excluded from the analysis using Grubb's test with an alpha of 0.05.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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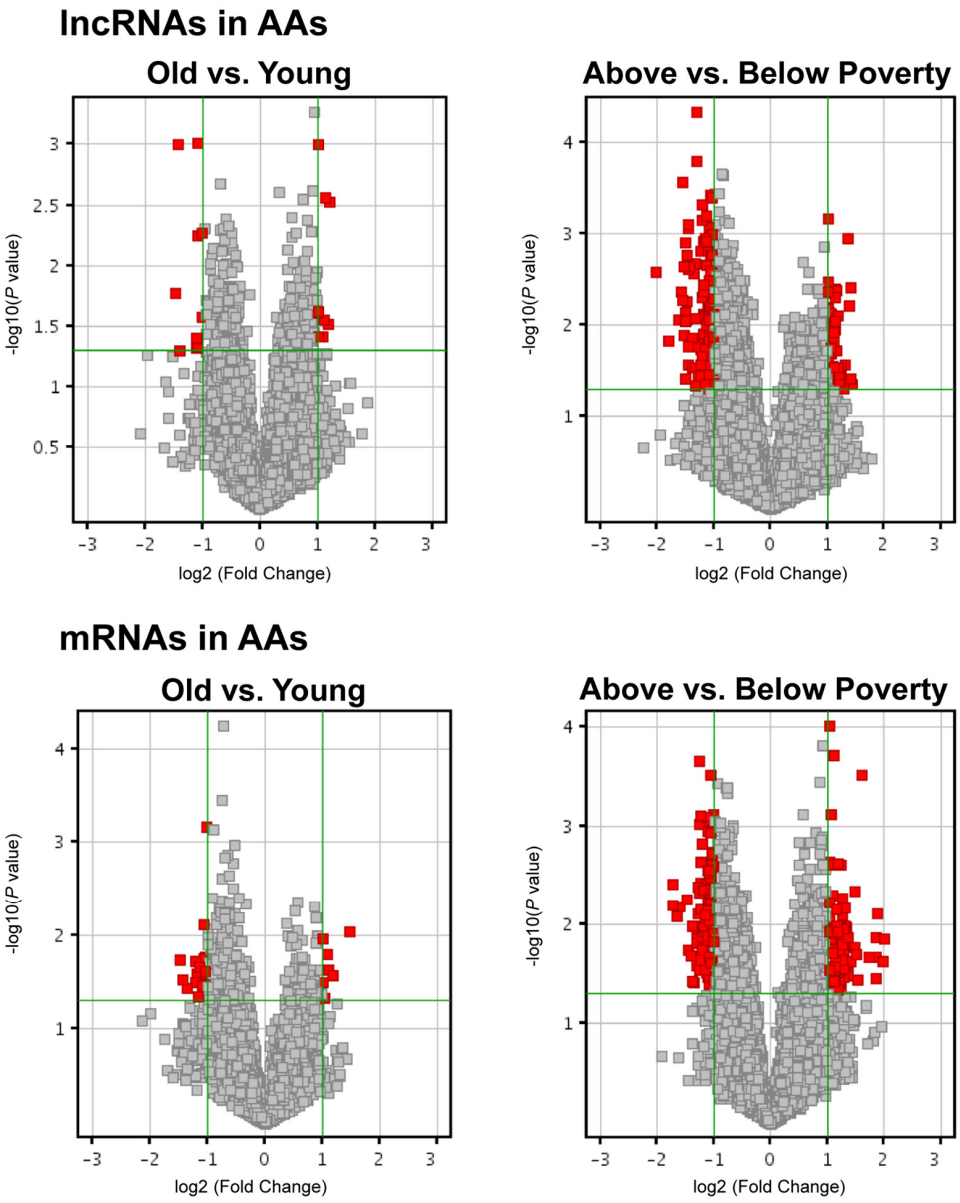
SUPPLEMENTARY MATERIAL

Please follow the links in Full Text version to see the Supplementary Tables of this manuscript:

Supplementary Table 1. lncRNAs significantly higher in abundance in young white males.
Supplementary Table 2. lncRNAs significantly lower in abundance in young white males.
Supplementary Table 3. lncRNAs significantly higher in abundance in white males living above poverty.
Supplementary Table 4. lncRNAs significantly lower in abundance in white males living above poverty.
Supplementary Table 5. Age-associated lncRNAs.
Supplementary Table 6. mRNAs significantly higher in abundance in young white males.
Supplementary Table 7. mRNAs significantly lower in abundance in young white males.
Supplementary Table 8. mRNAs significantly higher in abundance in white males living above poverty.
Supplementary Table 9. mRNAs significantly lower in abundance in white males living above poverty.
Supplementary Table 10. Primer sequences for RT-qPCR validation.



Supplementary Figure 1. lncRNA and mRNA level changes with age and poverty in African American males. lncRNA and mRNA expression from African American (AA) young and old males living above or below poverty was assessed using microarray (Table 1A for demographic details). Volcano plots show log2 fold change and P value for each lncRNA and mRNA. Red indicates lncRNAs and mRNAs that were >2 fold change and *P* value for each lncRNA and mRNA. Red indicateslncRNAs and mRNAs that were >2 fold change and *P*<0.05. Comparisons between groups are indicated.