SHORT TAKE

Shared signatures of social stress and aging in peripheral blood mononuclear cell gene expression profiles

Noah Snyder-Mackler,1,2 Mehmet Somel3 and Jenny Tung1,2,4
1Department of Evolutionary Anthropology, Duke University, Box 90383, Durham, NC 27708, USA
2Duke University Population Research Institute, Box 90989, Durham, NC 27708, USA
3Department of Biological Sciences, Middle East Technical University, 06800, Ankara, Turkey
4Department of Biology, Duke University, Box 90338, Durham, NC 27708, USA

Summary
Chronic social stress is a predictor of both aging-related disease and mortality risk. Hence, chronic stress has been hypothesized to directly exacerbate the process of physiological aging. Here, we evaluated this hypothesis at the level of gene regulation. We compared two data sets of genome-wide gene expression levels in peripheral blood mononuclear cells (PBMCs): one that captured aging effects and another that focused on chronic social stress. Overall, we found that the direction, although not necessarily the magnitude, of significant gene expression changes tends to be shared between the two data sets. This overlap was observable at three levels: (i) individual genes; (ii) general functional categories of genes; and (iii) molecular pathways implicated in aging. However, we also found evidence that heterogeneity in PBMC composition limits the power to detect more extensive similarities, suggesting that our findings reflect an underestimate of the degree to which age and social stress influence gene regulation in parallel. Cell type-specific data on gene regulation will be important to overcome this limitation in the future studies.

Key words: aging; gene expression; social stress.

The major causes of chronic social stress—low social status, social isolation, and lack of social support—are also linked to higher rates of age-related disease and mortality (House et al., 1988; Shaw et al., 1999; Sapolsky, 2004; Marmot, 2006; Holt-Lunstad et al., 2010). This observation has given rise to the hypothesis that social stress influences the aging process, potentially by affecting the same biological pathways that change during aging. This idea predicts, first, that biomarkers of social stress should also be biomarkers of aging and, second, that the direction of social stress effects on these biomarkers should recapitulate changes with age (Bauer, 2008). Both predictions are supported for a few well-characterized biomarkers, such as IL-6 and telomerase protein levels (Epel et al., 2004; Piazza et al., 2010; Needham et al., 2012; Zalli et al., 2014). However, we do not yet know the extent to which these patterns hold more broadly—information that is key for understanding how social stress impacts the aging process.

Here, we compared two previously published data sets to investigate the relationship between chronic social stress and aging for thousands of genes simultaneously. Both data sets measured genome-wide gene expression levels in peripheral blood mononuclear cells (PBMCs). The first, a study of 1240 humans 15–94 years old, captured the effect of age on gene expression (Görg et al., 2007; Hong et al., 2008). The second involved experimental manipulation of dominance rank (i.e., social status) in 49 rhesus macaques and allowed us to identify genes associated with the response to rank-induced chronic social stress (Tung et al., 2012). Importantly, the physiological consequences of both aging and social stress in nonhuman primates often parallel those observed in humans (Roth et al., 2004; Sapolsky, 2005), including at the level of gene expression (Somel et al., 2010; Tung & Gilad, 2013). Thus, by comparing gene expression levels between the two data sets, we were able to test whether social stress recapitulates the effects of aging.

To do so, we focused on the set of genes (n = 4252) included in both data sets. Overall, we found a significant enrichment of genes that were either consistently upregulated or consistently downregulated in both older and lower status individuals (odds ratio = 1.37, Fisher’s exact test, FET, $P = 3.7 \times 10^{-7}$). This enrichment was even stronger (OR = 2.14, FET $P = 3.0 \times 10^{-7}$) for the 819 genes that were independently and significantly associated with both variables at a 20% false discovery rate (see Data S1 and Table S2 for similar results using alternative FDR thresholds). Interestingly, some of the genes that were identified in this analysis (Table S1) are also known biomarkers of aging [e.g., B2M and NF-IL6: (Ershler & Keller, 2000; Annweiler et al., 2011)]. In contrast, the magnitude of age and rank effects were not significantly correlated, either across all 4252 genes (Spearman’s $\rho = 0.065$, permutation test $P = 0.21$) or among the 819 genes significantly associated with both age and social stress (Spearman’s $\rho = 0.062$, $P = 0.36$). This observation might be interpreted in two, nonmutually exclusive ways. First, while aging and chronic social stress influence similar genes, their exact impact on these genes may differ. A second likely possibility is that parallels at the level of direction rather than magnitude are more readily detectable across data sets, especially those obtained from different species and using different sampling methods.

In addition to directional similarities at the level of individual genes, social stress and aging could affect similar biological pathways. To test this possibility, we used Gene Ontology (GO) terms (specifically, high-level ‘GO Slim’ categories) to identify functionally related sets of genes that were over-represented among significant genes in each of the two data sets (Ashburner et al., 2000). Twenty-nine gene sets were enriched in both cases. Twenty-seven of these ‘co-enriched’ gene categories were similarly affected by social status and age (i.e., either both associated with upregulation or both associated with downregulation with increasing age and lower social status), which was significantly greater than expected by chance (permutation test $P < 0.0001$; Table 1). Only two co-enriched categories, ‘reproduction’ and ‘ATPase activity’, were enriched in both data sets in a manner inconsistent with our motivating hypothesis, no more than expected by chance ($P = 0.79$).
Because the co-enriched categories were quite broad, we also investigated gene sets linked to well-studied aging-related pathways to test whether they, too, were co-enriched across data sets. Specifically, we investigated gene sets connected to known hallmarks of aging, including inflammation, insulin growth factor signaling, mammalian target of rapamycin (mTOR) signaling, RNA processing, telomere maintenance, mitochondrial senescence, and oxidative stress (López-Otín et al., 2013). We found fifteen co-enriched gene sets that were either both upregulated or both downregulated with aging and chronic social stress (Table S3; permutation test \( P = 2.8 \times 10^{-3} \)) and none that exhibited the opposite pattern.

We then asked whether genes within each category show concordance in the direction of effects across data sets (i.e., concordantly increased or concordantly decreased shifts with older age and lower dominance rank). We identified significant concordance within individual co-enriched GO Slim categories for seven gene sets (Table 1). Furthermore, genes in 22 of the 27 co-enriched GO Slim categories were more often concordant than discordant (binomial test: \( P = 1.5 \times 10^{-3} \)). Categories previously linked to aging exhibited a similar pattern (10 of 12, excluding categories with ties; \( P = 3.9 \times 10^{-2} \), Table S3).

Thus, in PBMCs, aging and chronic social stress appear to influence a similar set of both broad categories of genes as well as specific pathways previously implicated in aging. However, we consistently found that directional similarities were more common, and/or easier to detect, than correlations in effect size: only seven of the 27 co-enriched GO Slim categories, and none of the 15 aging-related categories exhibited significant effect size correlations in the predicted direction (Tables 1 and S3). This may be because age and social stress do not alter the same genes within pathways affected by both conditions. Alternatively, discordant changes in PBMC composition between aged and socially stressed individuals might mask parallel changes in gene expression within individual cell types (suggesting that some, but not all, aspects of physiological changes with aging and chronic social stress are shared). Indeed, cell-type composition data from the macaque social stress experiment revealed a significant correlation between cytotoxic T-cell proportions and dominance rank (lower ranking individuals had proportionally fewer of these cells: Tung et al., 2012). While T-cell proportions also change with age, they may not do so in a completely parallel manner to that observed with social stress: Depletion of naïve T cells during aging, for example, has been hypothesized to result from accumulated exposure to pathogens over the life course, a mechanism unlikely to be at work in the social stress data set (Larbi et al., 2008).

To test whether differences in PBMC composition affected our analysis, we therefore quantified how uniformly each gene was...
expressed across PBMC cell types. For each gene, we calculated an ‘evenness’ metric, e. (Haygood et al., 2010) using publicly available gene expression data from each of the five major PBMC cell types in humans (Watkins et al., 2009) (SI). We found that, while the subset of genes that were the most evenly expressed (e > 0.90; n = 555 genes) exhibited strong concordance between the directional effects of aging and social status (OR = 2.45, FET P = 1.1 × 10⁻⁶), this pattern was undetectable among unevenly expressed genes (e < 0.90; n = 257 genes, OR = 1.27, FET P = 0.42). Further, genes in co-enriched categories that were both discordant in direction and significantly correlated between data sets were much more evenly expressed than genes in categories that had discordant, but not significantly correlated effects (Kolmogorov–Smirnov (K-S) test, D = 0.061, P = 1.3 × 10⁻⁹), which were in turn significantly more evenly expressed than genes in discordant, uncorrelated categories (K-S test, D = 0.058, P = 1.2 × 10⁻²). The x-axis is plotted on a negative log scale.

Fig. 1 Genes in concordant and significantly correlated co-enriched categories (solid line) are the most evenly expressed across tissues. The ‘evenness score’ measures the degree to which a gene is expressed at the same level across cell types, ranging from 0 (the gene is expressed in only one cell type) to 1 (the gene is equally expressed across the 5 PBMC cell types we considered). Genes in concordant and significantly correlated categories were significantly more evenly expressed than genes in discordant, uncorrelated categories (Kolmogorov–Smirnov (K-S) test, D = 0.061, P = 1.3 × 10⁻⁹), which were in turn significantly more evenly expressed than genes in discordant, uncorrelated categories (K-S test, D = 0.058, P = 1.2 × 10⁻²).

Acknowledgments
We would like to thank the three anonymous reviewers for their insightful comments on the manuscript. We also thank members of the Tung lab for thoughtful discussion and feedback on an earlier versions of this work.

Funding
This work was supported by National Institutes of Health grants 1R01GM102562 to JT and P30-AG034424 (Center for Aging grant) to James Vaupel. NSM was supported by a postdoctoral fellowship funded through NIH T32AG00139-25 to Ken Land and a National Science Foundation Grant SMA-1306134 to JT and NSM. MS was supported by fellowships from the European Molecular Biology Organization (EMBO ALTF 1475–2010) and The Scientific and Technological Research Council of Turkey (TÜBİTAK 2232, project no. 114C040).

Conflict of interest
None declared.

References
Annweiler C, Bataille R, Ferrière N, Douillet D, Fantino B, Beauchet O (2011) Plasma beta-2 microglobulin as a marker of frailty in older adults: a pilot study. J. Gerontol. A Biol. Sci. Med. Sci. 66, 1077–1079.
Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat. Genet. 25, 25–29.
Bauer ME (2008) Chronic stress and immunosenescence: a review. NeuroimmunoModulation 15, 241–250.
Epel ES, Blackburn EH, Lin J, Dhahbar FS, Adler NE, Morrow JD, Cawthon RM (2004) Accelerated telomere shortening in response to life stress. Proc. Natl Acad. Sci. USA 101, 17312–17315.
Erisler WB, Keller ET (2000) Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Ann. Rev. Med. 51, 245–270.
Göring HHH, Curran JE, Johnson MP, Dyer TD, Charlesworth L, Cole SA, Jowett JBM, Abraham LJ, Rainwater DL, Comuzie AG, Mahaney MC, Almasy L, MacCluer JW, Kissebah AH, Collier GR, Moses EK, Blangero J (2007) Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. Nat. Genet. 39, 1208–1216.
Haygood R, Babitti CC, Fedrigo O, Wray GA (2010) Contrasts between adaptive coding and noncoding changes during human evolution. Proc. Natl Acad. Sci. USA 107, 7853–7857.
Holt-Lunstad J, Smith TB, Layton JB (2010) Social relationships and mortality risk: a meta-analytic review. PLoS Med. 7, e1000316.
Hong M-G, Myers AJ, Magnusson PKE, Prince JA (2008) Transcriptome-wide assessment of human brain and lymphocyte senescence. PLoS ONE 3, e3024.
House J, Landis K, Umberson D (1988) Social relationships and health. Science 241, 540–545.
Larbi A, Franceschi C, Mazzatti D, Solana R, Wikby A, Pawelec G (2008) Aging of the immune system as a prognostic factor for human longevity. Physiology 23, 64–74.
López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153, 1194–1217.
Marmot MG (2006) Status syndrome a challenge to medicine. J. Am. Med. Assoc. 295, 1304–1307.
Needham BL, Fernandez JR, Lin J, Eipel ES, Blackburn EH (2012) Socioeconomic status and cell aging in children. Soc. Sci. Med. 74, 1948–1951.
Piazza JR, Almeida DM, Dmitrieva NO, Klein LC (2010) Frontiers in the use of biomarkers of health in research on stress and aging. J. Gerontol. B Psychol. Sci. Soc. Sci. 65, 513–525.
Roth GS, Mattison JA, Ottinger MA, Chachich ME, Lane MA, Ingram DK (2004) Aging in rhesus monkeys: relevance to human health interventions. Science 305, 1423–1426.

Sapolsky RM (2004) Social status and health in humans and other animals. Annu. Rev. Anthropol. 33, 393–418.

Sapolsky RM (2005) The influence of social hierarchy on primate health. Science 308, 648.

Shaw WS, Patterson TL, Ziegler MG, Dimsdale JE, Semple SJ, Grant I (1999) Accelerated risk of hypertensive blood pressure recordings among Alzheimer caregivers. J. Psychosom. Res. 46, 215–227.

Somel M, Guo S, Fu N, Yan Z, Hu HY, Xu Y, Yuan Y, Ning Z, Hu Y, Menzel C, Hu H, Lachmann M, Zeng R, Chen W, Khaitovich P (2010) MicroRNA, mRNA, and protein expression link development and aging in human and macaque brain. Genome Res. 20, 1207–1218.

Tung J, Gilad Y (2013) Social environmental effects on gene regulation. Cell. Mol. Life Sci. 70, 4323–4339.

Tung J, Barreiro LB, Johnson ZP, Hansen KD, Michopoulous V, Toufexis D, Michelini KM, Wilson ME, Gilad Y (2012) Social environment is associated with gene regulatory variation in the rhesus macaque immune system. Proc. Natl Acad. Sci. USA 109, 6490–6495.

Watkins NA, Gusnanto A, de Bono B, De S, Miranda-Saavedra D, Hardie DL, Angenent WGI, Attwood AP, Ellis PD, Erber W, Foad NS, Garner SF, Isacke CM, Jolley J, Koch K, Macaulay IC, Morley SJ, Rendon A, Rice KM, Taylor N, Thijssen-Timmer DC, Tijssen MR, van der Schoot CE, Wernisch L, Winzer T, Dudbridge F, Buckley CD, Langford CF, Teichmann S, Göttgens B, Ouwehand WH (2009) A HaemAtlas: characterizing gene expression in differentiated human blood cells. Blood 113, e1–e9.

Zalli A, Carvalho LA, Lin J, Hamer M, Erusalisky JD, Blackburn EH, Steptoe A (2014) Shorter telomeres with high telomerase activity are associated with raised allostatic load and impoverished psychosocial resources. Proc. Natl Acad. Sci. USA 111, 4519–4524.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.

Fig. S1 Genes in concordant and significantly correlated co-enriched aging-related categories (solid line) are the most evenly expressed across tissues.

Table S1 The 472 genes that are significantly associated with older age and lower dominance rank in the same direction.

Table S2 Comparison of different false discovery rate thresholds.

Table S3 Co-enriched aging-related gene ontology categories.

Data S1 Supplemental methods.