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Gene expression markers of age-related inflammation in two human cohorts

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ABSTRACT

Introduction: Chronically elevated circulating inflammatory markers are common in older persons but mechanisms are unclear. Many blood transcripts (>800 genes) are associated with interleukin-6 protein levels (IL6) independent of age. We aimed to identify gene transcripts statistically mediating, as drivers or responders, the increasing levels of IL6 protein in blood at older ages.
Methods: Blood derived *in-vivo* RNA from the Framingham Heart Study (FHS, n = 2422, ages 40–92 yrs) and InCHIANTI study (n = 694, ages 30–104 yrs), with Affymetrix and Illumina expression arrays respectively (>17,000 genes tested), were tested for statistical mediation of the age-IL6 association using resampling techniques, adjusted for confounders and multiple testing.
Results: In FHS, *IL6* expression was not associated with IL6 protein levels in blood. 102 genes (0.6% of 17,324 expressed) statistically mediated the age-IL6 association of which 25 replicated in InCHIANTI (including 5 of the 10 largest effect genes). The largest effect gene (*SLC4A10*, coding for NCBE, a sodium bicarbonate transporter) mediated 19% (adjusted CI 8.9 to 34.1%) and replicated by PCR in InCHIANTI (n = 194, 35.6% mediated, p = 0.01). Other replicated mediators included *PRF1* (perforin, a cytolytic protein in cytotoxic T lymphocytes and NK cells) and *IL1B* (Interleukin 1 beta): few other cytokines were significant mediators.
Conclusions: This transcriptome-wide study on human blood identified a small distinct set of genes that statistically mediate the age-IL6 association. Findings are robust across two cohorts and different expression technologies. Raised IL6 levels may not derive from circulating white cells in age related inflammation.
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1. Introduction

Chronically elevated levels of pro-inflammatory biomarkers are a core feature of aging, and a risk factor for many diseases and adverse phenotypes that are frequent in older persons (De Martinis et al., 2006). Increased levels of pro-inflammatory markers in blood and other tissues with aging are paralleled by a progressive decline in overall immune responsiveness (Franceschi and Campisi, 2014).

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Elevated interleukin-6 (IL6) and C-reactive protein (CRP) have emerged as robust age-related risk factors for multiple adverse outcomes including several diseases, disability, cognitive impairment and death (Singh and Newman, 2011). IL6 is a pleiotropic cytokine produced by several cell types, including immune cells, hepatocytes, vascular endothelial cells, adipocytes and skeletal muscle (Singh and Newman, 2011). IL6 levels are often undetectable in young individuals but increase with advancing age, even in the absence of detectable causes, including cardiovascular risk factors and disease (Ferrucci et al., 2005). High levels of IL6 predict all major age-associated diseases, physical and cognitive disability and mortality (Economos et al., 2013; Ferrucci et al., 1999; Heikkila et al., 2008; Ridker et al., 2000). The mechanisms that cause and sustain high levels of inflammatory markers in aging are largely unknown.

The overproduction of pro-inflammatory markers may occur in many sites beyond circulating leukocytes; candidates include tissue resident macrophages, adipocytes, endothelial cells, cells within atherosclerotic plaques and muscle cells (Howcroft et al., 2013). The accumulation of cells expressing a senescence-associated secretory phenotype (Campisi, 2013; Young and Narita, 2009) in different tissues, possibly induced by age-related NF-kB dysregulation (Bektas et al., 2013), may also be a possible cause. Data collected in animal models have provided inconsistent results and there is a scarcity of data in humans.

In a recent study, the authors determined the whole blood gene expression transcripts associated with IL6 levels in 2 human populations, independent of age (Lin et al., 2014). That analysis identified 4139 genes that were significantly associated with interleukin-6 levels (FDR < 0.05), independent of age, sex and blood cell components, of which 807 genes replicated in the smaller InCHIANTI cohort. Many of the top genes generally associated with blood IL6 protein levels (independent of age) are in inflammation-related pathways or erythrocyte function, including the JAK/Stat signaling pathway and interleukin-10 signaling pathway.

In the study presented here, we aimed to identify the gene transcripts in whole blood that might be specific to the chronic inflammation of aging. In particular, we hypothesized that genes with expression profiles that statistically mediate "age-related inflammation" – that account for a proportion of the statistical association between age and IL6, a proxy for age-related inflammation - may reflect the most relevant molecular pathways in aging and may help to identify the specific cell subtypes most closely involved. We therefore estimated the degree to which each whole blood gene transcript statistically explained the age-IL6 association, using mediation models in 2 independent community-based cohorts in a discovery (Framingham Heart Study, FHS) and replication (InCHIANTI) analysis. The cohorts utilize two different microarray platforms, allowing replication of findings robust to cohort and microarray differences.

2. Methods

Our study was performed in two independent and well-characterized human cohorts. The discovery cohort was the Framingham Heart Study (FHS) Offspring cohort, USA (Kannel et al., 1979), with replication of the significant mediators in the InCHIANTI (Invecchiare in Chianti, aging in the Chianti area) study, a community-based cohort study of aging in Florence, Italy (Ferrucci et al., 2000).

FHS participants targeted in this analysis were from the Offspring Cohort enrolled in 1971 as the offspring (and offspring spouses) of the Original FHS cohort. Offspring participants who attended examination 8 (2005–2008) and had blood-derived RNA prepared were included in this analysis (n = 2422, see Table 1 for further cohort details). This study was approved by the Institutional Review Boards at Boston University Medical Center, and all participants gave written informed consent (Feinleib et al., 1975).

InCHIANTI participants were originally enrolled in 1998–2000, and were interviewed and examined every 3 years. Ethical approval was granted by the Instituto Nazionale Riposo e Cura Anziani Institutional Review Board in Italy. Participants gave informed consent to participate. RNA was available at wave 4 (year 9) of the study, with IL6 also measured at year 9. All the data required for the full analyses were available for 694 individuals (see Table 1 for further cohort details).

Table 1
Summary statistics of the Framingham and InCHIANTI cohort characteristics.

Framingham heart study offspring			InCHIANTI		
	N	%		*N*	%
Gender			Gender		
Males	1093	45.1	Males	313	45.1
Females	1329	54.9	Females	381	54.9
Age			Age		
30–49	50	2.1	30–49	88	12.7
50–69	1508	62.3	50–69	100	14.4
70–89	859	35.5	70–89	477	68.7
90–104	5	0.2	90–104	29	4.2
Mean (SD)	2422	66.4 (9.0)	Mean (SD)	694	72.2 (15.3)
Tobacco exposure			Tobacco exposure		
None	775	32	None	380	54.8
Former smoker	1444	59.6	Former smoker	240	34.6
Current smoker	203	8.38	Current smoker	74	10.7
	N	Mean (SD)		*N*	Mean (SD)
BMI (kg/m^2)	2422	28.5 (5.4)	BMI (kg/m2)	694	27.1 (4.3)
Interleukin 6 (pg/mL)	2422	2.7 (3.0)	Interleukin 6 (pg/mL)	694	3.8 (2.9)
Leukocyte composition			Leukocyte composition		
Neutrophils	2422	59.8 (7.9)	Neutrophils	694	57.5 (9.1)
Lymphocytes	2422	27.0 (7.5)	Lymphocytes	694	30.8 (8.7)
Monocytes	2422	9.2 (1.9)	Monocytes	694	8.0 (2.1)
Eosinophils	2422	3.3 (1.6)	Eosinophils	694	3.2 (2.1)
Basophils	2422	0.8 (0.2)	Basophils	694	0.6 (0.2)

2.1. RNA collection and extraction

2.1.1. FHS

The methods for gene expression profiling were previously published (Joehanes et al., 2013). Briefly, peripheral blood samples were extracted using the PAXgene Blood mRNA kit (PreAnalytiX, Hombrechtikon, Switzerland), and amplified by the WT-Ovation Pico RNA Amplification System (NuGEN, San Carlos, CA), according to manufacturer's instructions. cDNA was then hybridized to the Human Exon 1.0 ST Array (Affymetrix, Inc., Santa Clara, CA) for quantification. The raw data were quantile-normalized and natural-log transformed, followed by summarization using Robust Multi-array Average (Irizarry et al., 2003). The gene annotations were obtained from Affymetrix NetAffx Analysis Center (version 31). We excluded transcript clusters that were not mapped to RefSeq transcripts, resulting in 17,873 distinct transcripts (17,324 unique gene identifiers) for downstream analysis.

2.1.2. InCHIANTI

Peripheral blood samples were also extracted using the PAXgene Blood mRNA kit according to the manufacturer's instructions (Debey-Pascher et al., 2009), which preserves transcript expression levels as at the time of blood sampling. Samples were collected in 2008/9 (wave 4) from 733 participants. Whole genome expression profiling of the samples was conducted using the Illumina Human HT-12 microarray (Illumina, San Diego, USA) as previously described (Zeller et al., 2010). Data processing was done using the Illumina and BeadStudio software (Illumina, San Diego, USA) as previously described (Zeller et al., 2010). All microarray experiments and analyses complied with MIAME guidelines (Brazma et al., 2001). Participants were excluded if mean signal intensities across all probes with $p \leq 0.01$ were >3 standard deviations from the cohort mean; probes with <5% of participants

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