Multiple inflammatory markers and 15-year incident ADL disability in admixed older adults: The Bambui-Epigen Study

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Abstract

Background: The ability of inflammatory markers to predict disability in later life has received growing attention. However, the current evidence came predominantly from Caucasians and the role of genomic ancestry has not been investigated.
Objective: We investigated the prognostic value of multiple citokynes and chemokines for incident disability in admixed older Brazilians and whether genomic African and Native American ancestry affects the association.
Design: Population-based longitudinal study.
Setting: The Bambui-Epigen (Brazil) Cohort Study of Aging.
Subjects: 1,171 males and females aged ≥60 years over 15-year of follow-up.
Methods: Outcome examined was incident activity of daily living (ADL) disability assessed annually (10,039 measures were performed). Serum levels of citokynes (IL6, IL12, TNF, IL10, and IL1β) and chemokines (CCL2, CCL5, CXCL8, CXCL9 and CXCL10) were measured at baseline. We used 370,539 Single Nucleotide Polymorphisms (SNPs) to estimate each individual genomic ancestry proportions. Potential confounding variables included a wide range of socio-demographic variables and health indicators. Statistical analyses were based on competing risk framework.
Results: The incidence rate of disability was 57.9 per 1,000 person-years. IL6 level at the highest quartile showed an independent association with ADL disability (SRH = 1.32; 95% CI: 1.03, 1.70). Other inflammatory markers showed no statistically significant associations with the outcome. Neither genomic African nor Native American ancestry had an effect modifier on the associations (P for interaction >0.05 for all).
Conclusion: Among multi-inflammatory markers, only IL6 had the potential to identify people at increased risk of ADL disability, independently of ethno-racial background.

Keywords: inflammation; activity of daily living; genomic African ancestry; ageing; disability.

Inflammatory markers have been postulated as a tool to identify people at increased risk of disability.

The role of genome-based ancestry on the ability of inflammatory markers to predict disability is unknown.

We examined whether genome African and Native American ancestries affect the association between inflammatory markers and incident disability in a large cohort of older Brazilian adults.

Elevated levels of Interleukin-6 were associated with increased risk of disability, independently of genome-based ethno-racial background.

Interleukin-6 may be a useful tool for timely prevention of disability in a highly admixed older population.

1. Introduction

Disability in later life is a public health concern worldwide due to increase in demand and cost of long-term care [1]. Identifying predictors of disability can potentially contribute not only to a better understanding of underlying mechanisms, but also to targeting vulnerable groups for timely prevention. Given that there is evidence linking inflammation to the process of ageing and age-related diseases, the ability of inflammatory markers to predict disability in old age has received growing attention [2].

Cytokines and chemokines are important players in the immune responses [2]. There is considerable evidence showing that elevated baseline levels of Interleukin-6 (IL6), a pro-inflammatory cytokine, has a prognostic value for activities of daily living (ADL) disability or impairment in mobility [3–5], muscle strength loss [6–10] and sarcopenia [11] in short and medium terms (up to 5 years). The prognostic value of IL6 for long-term physical functioning is unknown. In the MacArthur Studies of Sucessful Aging, baseline IL6 level did not predict physical functioning performance 7 years later [12]. Studies examining the prognostic value of other cytokines and chemokines for physical functioning are still scarse and have reported inconsistent results [4, 7, 5].

The above mentioned evidence came predominantly from Caucasians [2–12]. To the best of our knowledge, no previous study has specifically examined the influence of genetic ancestry on those associations. Brazil, the largest Latin American country, offers a valuable opportunity to explore this issue. The Brazilian population originates from African, European and Native American ancestral roots [13]. The absence of legal segregation and other factors contributed to an emergence of a highly admixed population [14].

We used 15-year follow-up data from the Bambui-Epigen study [15], the longest running cohort study of aging in Brazil, with two main objectives: (1) to examine the association between multiple inflammatory markers and long term ADL disability; and (2) to investiagate whether genomic African and Native American ancestry levels affects the ability of those

biomarkers to predict the outcome.

2. Methods

2.1 Study Population

The Bambui cohort study of aging is ongoing in Bambuí, a city of approximately 15,000 inhabitants in Southeast Brazil. From an ethno-racial perspective, the cohort population consists of an admixture of African (≅10%), Native American (≅5%), and European (≅85%) genomic ancestries, in similar proportions to that estimated for the Brazilian population, excluding the Amazon region [14]. Detailed information on this cohort can be found elsewhere [15]. Briefly, the population eligible for the cohort consisted of all residents aged 60 years and older on the 1st of January 1997 (92% of the 1,742 inhabitants in this age group participated). Annually, from 1997 to 2011, cohort members underwent subsequent annual follow-up by face-to-face interview. Blood collection and other procedures for the current analysis where performed at the baseline survey. Deaths occurring from study enrollment to December 31, 2011, were considered in this analysis. Deaths were reported by next of kin during the annual follow-up interview and were ascertained through the Brazilian mortality information system. Death certificates were obtained for 95.5% of all deceased participants. Cohort members with an ADL disability at baseline (n = 171) were excluded from the current analysis (see bellow).

2.2 Activity of daily living disability

Annually, from 1997 to 2011, physical functioning was measured as self-reported limitations in the following basic ADL: dressing, walking across a room, bathing or showering, eating, getting in or out of bed, using the toilet. The questions had four possible answers: no difficulty, some difficulty, great difficulty and unable to perform. Onset of disability was considered when

a participant reported, for the first time, great difficulty or unability to perform one or more tasks.

2.3 Inflammatory markers (cytokines and chemokines)

Blood samples for measurement of cytokines and chemokines were collected at the baseline survey in early morning. The Cytometric bread array assay (CBA immunoassay kit; Becton Dickinson, California) was used for the quantitative determination of the serum cytokines (Human Inflammatory kit) and chemokines (Human Chemokines kit) levels according to the instructions of the manufacturer. Data was acquired using a FACSVerse flow cytometer (Becton Dickinson, California). BD FCAP Array 3.0 software (Becton Dickinson, California) was used for sample analysis. The coefficients of variation intra and inter-assays were 5-10% and 7-12%, respectively. Based on their distributions, IL6, CXCL8, CCL2, CXCL9, CCL5 and CXCL10 were log-transformed and considered as continuous variables in our analysis. IL1β, IL10, IL-12 and TNF showed very low detectable levels and were considered as dichotomous variables.

2.4 Genetic and ancestry analyses

Cohort participants were genotyped with the Omni 2.5M array (Illumina, California) [13]. Ancestry inference was performed by using the model-based method [16]. We used 370,539 SNPs to estimate each individual African, European and Native American tri-hybrid ancestry proportions, based on public datasets parental populations. We used the matrix of kinship coefficients and a network-based approach to identify families, and identified them as categorical variables for the association tests described below. Pairs of individuals were considered as related if they had a kinship coefficient >0.1 (first and second-degree relatives). Further details are described elsewhere [13].

2.5 Covariates

Covariates comprised baseline socio-demographic characteristics (age, sex, education and household income), lifestyle (smoking, alcohol consumption and physical activity), body mass index and health conditions. We categorized schooling into <4 years, 4-7 and ≥8 years. Monthly household income per capita was divided into tertiles (<USD 90.00 is the lowest tertile). Current smokers were participants who had smoked at least 100 cigarettes during their lifetime and who still smoke. Leisure-time physical activity was defined as activity of any intensity for 20-30 minutes in the previous 3 months, and categorized into at ≥3 times a week, <3 times a week and never. Alcohol consumption was defined by consumption of 14 doses per week in previous 12 months. Body mass index was categorized into <18.5, 18.6-24.9 and ≥24 kg/m^2. Health conditions considered were: hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg and/or treatment); diabetes (fasting blood glucose ≥126 mg/dL and/or treatment); arthritis (previous medical diagnosis of any joint condition); coronary heart disease (medical diagnosis of myocardial infartion or angina pectoris as assessed by the Rose's questionnaire); intermitent claudication (Rose's questionnaire), stroke (medical diagnosis), fasting high non-HDL cholesterol (≥130 mg/dL); heart failure (plasma B-type natriuretic peptide level >100 pg/mL); anaemia (hemoglobin <13g/dL for men and <12g/dL for women), depressive symptoms (defined by a 12-item version of the General Health Questionnaire score ≥5 [17]); cognitive impairment (defined by a Mini-Mental State Examination score <22 - below the 25th percentile- or by the need for a proxy interview- 87 participants). Based on the above mentioned health conditions (all as dichotomous variables) we used principal component analysis [18] to create a morbidity score that ranged from -∞ to +∞ (higher scores indicated worse health). Scores were divided into quartiles.

2.6 Statistical analysis

We used competing-risks regression [19] to estimate the multivariate subhazard ratios (SHR) to model 15-year survival-time disability data. In order to consider death that could be related to disability, we used the date of death as a competing risk event. All regression models using genomic measures included a clustered (family) robust variance term to avoid potential bias resulting from analysis of individuals who were related (807 participants were first- and second-degree relatives).

First, we examined the association between inflammatory markers with incident ADL disabilit, by estimating sub hazard ratios (SRH) adjusted for socio-demographic, lifestyle, body mass index and health conditions (morbidity score). Then we added genomic ancestry variables to the previous models. Furthermore, we examined separately the statistical significance of multiplicative interactions between the inflammatory marker and genomic African and Native American ancestry on the outcome, controlling for all the above mentioned covariates. A statistically significant association was defined by a P value <0.05 in the log-likelihood test.

An additional analysis was performed to examine the association between baseline quartiles of IL6 serum levels and incident ADL disability. In this analysis we also examined the possibility of reverse causation by excluding those participants whose disability onsets occurred within 12 months. Furhter, we implemented fully adjusted competing-risks regression models to estimate cumulative incidence rates for disability by year, according with baseline IL6 levels in quartiles and plotted the results. Because we did not have enough power to stratify the analysis by gender, men and women were pooled and sex was considered as a covariate in our analyses. Statistical analyses were conducted using STATA 14.2 statistical software (Stata Corporation, Texas).

3. Results

Our analysis was based on 1,171 participants who had no ADL disability at baseline and had complete information for all other study variables. During the study period, 383 cohort participants died, 581 evolved to disability and 104 were lost to follow-up. Along the 15-year follow-up, 10,039 measures of ADL were made. The incidence rate of disability was 57.9 per 1,000 person-years. The mean age was 68.6 years (range 60 to 95) and 61.0% were women. Other baseline characteristics of study participants are shown in Table 1.

Table 1. Baseline socio-demographic and health characteristics of study participants. The Bambui Cohort Study of Aging (1997-2011)

Variables	Overall[a] (n = 1,117)
Socio-demographic	
Age in years, mean (SD)	68.6 (6.7)
Women	61.0
Monthly Family income per capita <USD 90.00	28.5
Lifestyle	
Current smoking	17.1
Alcohol consumptions in previous 12 months (>14 doses per week)	2.9
Physical activities during leisure time in previous 3 months (<3 times a week)	85.2
Body mass index in kg/m^2, mean (SD)	24.9 (4.7)
Health conditions	
Hypertension (SBP ≥140 e/or DBP ≥90 mmHg and/or treatment)	60.4
Diabetes (fasting blood glucose >126 mg/dL and/or treatment)	14.3
Arthritis (medical diagnosis of any joint condition)	24.9
Coronary heart disease (medical diagnosis of myocardial infarction or coronary heart disease as assessed by the Rose's questionnaire)	12.6
Intermittent claudication (Rose's questionnaire)	2.5
Stroke (medical diagnosis)	2.8
Non-HDL cholesterol ≥130 mg/dL	89.4
Heart failure (B-type natriuretic peptide >100 pg/mL)	39.1
Anemia (hemoglobin <13g/dL for men and <12g/dL for women)	3.4
Depressive symptoms (General Health Questionnaire score ≥5, range 0-12)	33.6
Possible cognitive impairment (MMSE score <19, range 0-26, or need for a proxy)	21.7
Genomic ancestry individual proportions	
African, median (p25, p75)	9.6 (4.7, 17.0)
Native American, median (p25, p75)	5.2 (2.7, 8.2)
European, median (p25, p75)	84.3 (74.6, 91.5)
Biomarker plasma levels (pg/mL)	
IL6 , median (p25, p75)	1.0 (0.4, 2.0)
CXCL8, median (p25, p75)	5.1 (2.9, 9.4)
CCL2, median (p25, p75)	38.7 (25.4, 56.2)
CXCL9, median (p25, p75)	2,228 (1,230, 3,913)
CCL5, median (p25, p75)	870 (565, 1,661)
CXCL10, median (p25, p75)	2,970 (1,978, 4,670)
IL10 (≥14.0 pg/mL)[b]	24.9
IL12 (≥ 0.01 pg/mL)[b]	7.7
TNF (≥0.01 pg/mL)[b]	16.9
IL1β (≥0.01 pg/mL)[b]	21.4

[a]Data are presented as percentage, except when specified.
[b]Lowest detectable level.
SD = standard deviation; MMSE = Mini-Mental State Examination; SBP = systolic blood pressure; DBP = diastolic blood; p25, p75 = 25th and 75th percentiles.

As shown in Table 2, IL6 baseline serum level showed a positive statistically significant association with ADL disability in a model adjusted for socio-demographic, lifestyle, body

mass index and morbidity score (SHR = 1.07; 95%CI: 1.01–1.13). Further adjustments for genomic African and Native American ancestry had no impact on the above mentioned estimates. Other biomarkers showed no statistically significant association with the outcome. Additionally, we found no evidence of statistically significant interactions affecting the ability of any biomarker to predict ADL disability neither for African nor for Native American ancestry (P for interaction >0.05 for all).

Table 2. Sub hazard ratios of 15-year incident activity of daily living disability, according with baseline multi-inflammatory serum levels. The Bambui Cohort Study of Aging (1997-2011)

Biomarkers	Model adjusted for sociodemographic, lifestyle, body mass index and morbidity score	Model adjusted for sociodemographic, lifestyle, body mass index, morbidity score, African ancestry proportion and cluster in family	Model adjusted for sociodemographic, lifestyle, body mass index, morbidity score, African ancestry and Native American proportion (continuous) and cluster in family	
	SRH (95%CI)	SRH (95%CI)	SRH (95%CI)	P-value[a]
IL6[b]	1.07 (1.01–1.13)	1.07 (1.01–1.13)	1.07 (1.01–1.13)	0.021
CXCL8[b]	1.05 (0.98–1.11)	1.04 (0.98–1.11)	1.04 (0.98–1.11)	0.224
CCL2[b]	0.99 (0.88–1.12)	0.98 (0.87–1.11)	0.99 (0.88–1.11)	0.859
CXCL9[b]	1.01 (0.99–1.05)	1.02 (0.99–1.05)	1.02 (0.99–1.05)	0.293
CCL5[b]	0.99 (0.92–1.08)	0.99 (0.92–1.08)	0.99 (0.92–1.08)	0.946
CXCL10[b]	0.99 (0.97–1.01)	0.99 (0.97–1.01)	0.99 (0.97–1.01)	0.415
IL10[c]	0.91 (0.74–1.16)	0.92 (0.75–1.13)	0.92 (0.74–1.13)	0.407
IL12[d]	1.27 (0.96–1.68)	1.27 (0.99–1.72)	1.26 (0.93–1.05)	0.141
TNF[d]	1.12 (0.90–1.41)	1.13 (0.90–1.40)	1.12 (0.90–1.39)	0.323
IL1β[d]	0.89 (0.72–1.10)	0.89 (0.73–1.10)	0.90 (0.73–1.11)	0.303

SHR (95%CI) = sub hazard ratios and 95% confidence intervals, estimated by competing-risks regression.

[a]Log-likelihood test.

[b]Log-transformed values (continuous).

[c]≥14.0 pg/mL vs. not detectable.

[d]≥0.01 pg/mL vs. not detectable.

IL6 level at the highest quartile showed statistically significant association with ADL disability in the analysis adjusted for socio-demographic, lifestyle and health variables (SRH = 1.34; 95%CI: 1.04–1.71) (Table 3). Again, the addition of genomic ancestry variables to the previous models had no impact on the predictive value of IL6 levels for ADL disability. The exclusion of onsets prior to 12 months slightly increased the SHR values, and the graded nature of the association became more evident with SRH (95% CI) equal to 1.33 (0.98–1.81), 1.40 (1.04–1.88) and 1.48 (1.09–2.01) for those at the 2nd, 3rd and the highest relative to the lowest quartile. Figure 1 shows the cumulative probability of ADL disability by year, according with quartiles of baseline IL6. The clearly separated lines between the highest and the lowest quartile support the associaton between the biomarker level and the risk of ADL disability showed in Table 3.

Table 3. Incidence rates and sub hazard ratios (95% confidence interval) of 15-year incident activity of daily living disability, according with baseline IL6 serum levels. The Bambui Cohort Study of Aging (1997-2011)

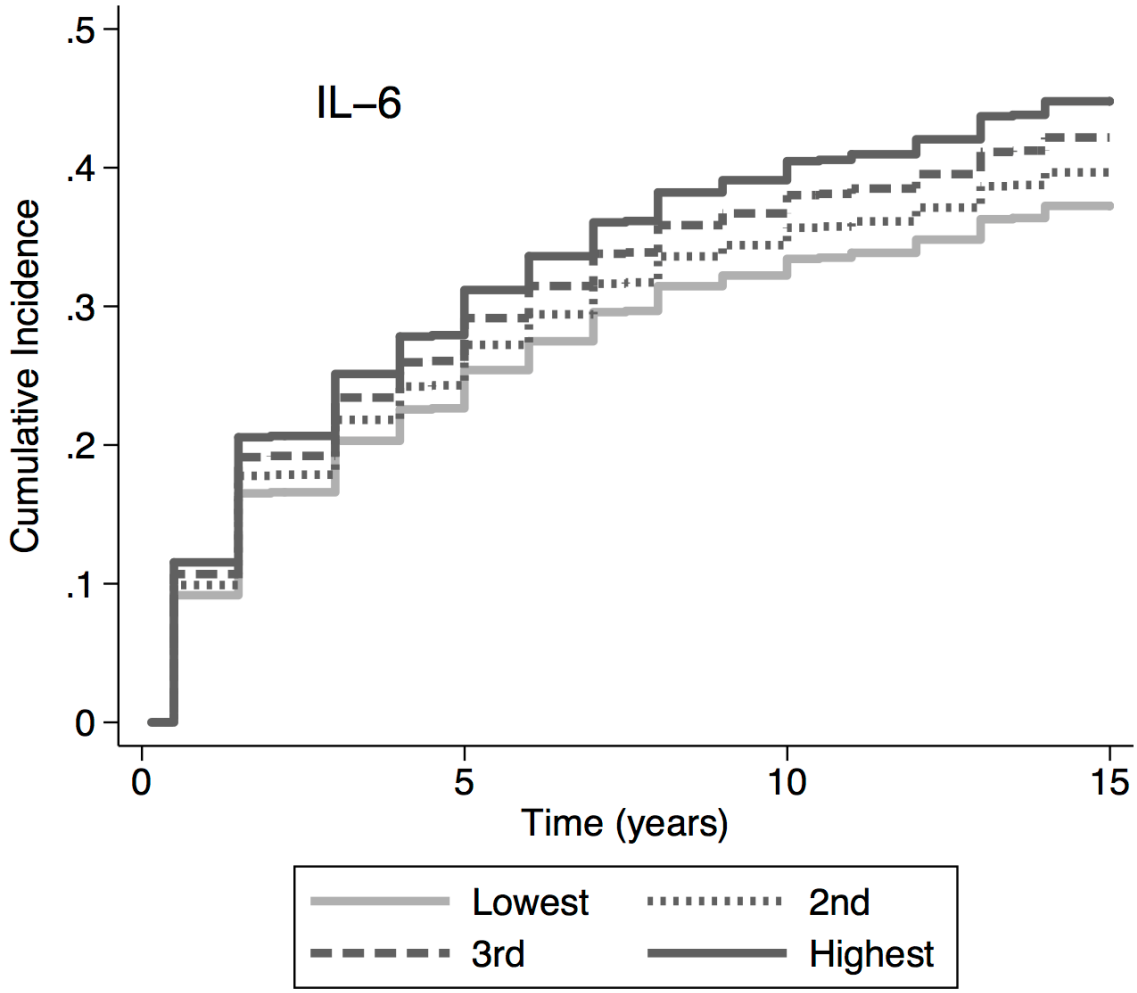
IL6[a]	No. onsets (incidence rate per 1,000 person-years)	SHR (95%CI) adjusted for sociodemographic, lifestyle, body mass index and morbidity score	SHR (95%CI) adjusted for sociodemographic, lifestyle, body mass index, morbidity score, African and Native American ancestry proportions (continuous) and cluster in family	
			All participants	Excluding onsets prior to 12 months
Lowest	128 (39.0)	1.0	1.0	1.0
2nd	143 (55.1)	1.17 (0.91–1.50)	1.18 (0.92–1.51)	1.33 (0.98–1.81)
3rd	156 (69.6)	1.20 (0.94–1.54)	1.19 (0.95–1.53)	1.40 (1.04–1.88)[b]
Highest	154 (79.9)	1.34 (1.04–1.71)[b]	1.39 (1.05–1.72)[b]	1.48 (1.09–2.01)[b]

SHR (95%CI) = sub hazard ratios and 95% confidence intervals, estimated by competing-risks regression.

[a]Lowest: ≤0.5; 2nd: 0.5-1.1; 3rd: 1.2-2.1; highest: ≥2.2 pg/mL.

[b]p<.05 (log-likelihood test).

Figure 1. Fully adjusted cumulative incidence of disability over 15 years by baseline serum levels of IL6 in quartiles. The Bambui Cohort Study of Ageing (1997-2011).



Estimated by competing risk regression, and adjusted for socio-demographic, lifestyle, body mass index, morbidity score and genomic African and Native ancestry (continuous) and cluster in family.

4. Dicussion

Our major finding is that elevated IL6 serum levels predicted very long term incident ADL disability. The observed association persisted after careful controlling for a wide range of covariates, and suggests graded effect. Because this association remained after excluding onsets prior to 12 months, it appears not to be explained by reverse causation, that is, a situation in which the outcome precedes and causes the exposure instead of the other way around [20]. Notably, the individual proportion of genomic African and Native American ancestry did not

affect the predictive value of this biomarker for the outcome.

The association between IL6 levels with ADL disability is in agreement with a number of previous studies conducted in the USA and Europe [2–11]. Two novel findings are that (i) IL6 predicts ADL disability in very long term in an admixed population and that (ii) ancestry background does not play a role on this association. Another issue is the cut off value of the marker. IL6 cut offs of 2.50 pg/mL [3], 2.98 pg/mL [5] and 3.1 or 3.8 pg/mL [10] have been proposed to discriminate persons at risk and those not at risk for disability. Our results showed that persons with IL6 level higher than 2.2 pg/mL were at increased risk.

The exact mechanism linking increased IL6 level to disability is unclear. The catabolic effect of IL6 on muscle has been postulated [6, 7, 11]. However, because IL6 is associated with increased risk of chronic diseases that, in turn, predispose to disability, there is a debate about whether IL6 is a direct cause of physical decline or simply sumarize the burden of illness in older adults [2]. Our results indicate that IL6 predicts ADL disability, independently of several health and health-related measures, but we were not able to capture changes over time. Therefore, the key issue is to disentangle the complex pathway linking IL6 to disability, which was not possible in our analysis.

There are only few studies examining the association between other cytokines and chemokines with physical decline. Two earlier reports, involving around 2,000 participants from the Health, Aging and Body Composition studies showed an association between baseline TNFα with 30-months incident mobility limitation [4] and with a 5-year decline in grip strenght in men (but not in women) [7]. A more recent study, involving 303 participants of the Belfrail Study in Belgium, examined the association between a large battery of cytokines and chemokines with 1.66-year global functioning decline. Except for IL6, no other biomarker had the potential to identify persons at increased risk [5]. Our results, based on a larger number of participants and a longer follow-up period, is in agreement with the latter, that is, among a large battery of

cytokines and chemokines, only IL6 was found to be statistically significantly associated with incident ADL disability.

Strengths of the present study include its well-defined community-based sample followed for a long period. Our study also incorporated a robust set of health and health-related measures that could confound the association between the inflammatory markers and onset of disability. Another important strength was the use of genome measures of ancestry, instead of ethno-racial self-classification, which is prone to misclassification, particularly in admixed populations [14]. A limitation in this analysis is inherent to longitudinal studies of ageing. Older adults are at increased risk of death, which in turn might lead to differential censoring. As an attempt to overcome this limitation, we used competing risk framework in our analysis, considering the data of death as informative censoring. In our initial exploratory analysis of the association between multiple citokynes and chemokines with the outcome, we did not adjust for multiple testing – which could lead to false positive results. However, the association between IL-6 with incident ADL disability was confirmed in subsequent analysis based on the quartile distribution of the biomarker level and, also, in an analysis restrict to onsets after 2 years. Therefore, it is unlekly that those are false positive results. In addition, changes over time in exposure status and ADL trajectories were not investigated. This limitation, however, do not preclude our conclusion that, despite eventual unmeasured changes over time, baseline elevated level of IL6 is an independent predictor for very long-term ADL disability.

In summary, our findings showed that in a cohort of admixed older Brazilian adults, only IL6 has the potential to identify persons at increased risk of future ADL disability, independently of ethnoracial background.

Conflict of interest

The authors declare that they have no competing interests.

Author's contributions

MFL-C: designed the study, conceived the data analysis, and wrote the manuscript. JVMM: performed the data analysis, and participated in the preparation of the manuscript. KCLT, SVP, FBA, CMO, ET-S, AT-C and OAM-F: collaborated in the data analysis and revised the manuscript. All authors reviewed and approved the final version.

Ethics

The Bambui study was approved by the Institutional Review Board (Oswaldo Cruz Foundation, Rio de Janeiro, Brazil). Written informed consent was obtained from all participants at baseline and at all follow-up interviews. Genotyping was approved by Brazil's national research ethics committee, as part of the Epigen-Brazil protocol (CONEP, resolution 15895).

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References

1. National Institute of Aging. National Institute of Health. World Health Organization. Global Health and Aging. NIH Publication no. 11-7737 October 2011 (online).

Available at: http://www.who.int/ageing/publications/global_health.pdf. Accessed March 1, 2016.

2. Singh T, Newman AB. Inflammatory markers in population studies of aging. Ageing Res Rev 2011; 10: 319-329.
3. Ferrucci L, Harris TB, Guralnik JM *et al*. Serum IL-6 level and the development of disability in older persons. J Am Geriatr Soc 1999; 47: 639–646.
4. Penninx BW, Kritchevsky SB, Newman AB *et al*. Inflammatory markers and incident mobility limitation in the elderly. J Am Geriatr Soc 2004; 52: 1105-1113.
5. Adriaensen W, Matheï C, Vaes B, van Pottelbergh G, Wallemacq P, Degryse JM. Interleukin-6 predicts short-term global functional decline in the oldest old: results from the BELFRAIL study. Age (Dordr) 2014; 36: 9723(1-14).
6. Schaap LA, Pluijm SM, Deeg DJ, Visser M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. Am J Med 2006; 119: 526.e9-17.
7. Schaap LA, Pluijm SM, Deeg DJ *et al*. Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength. J Gerontol A Biol Sci Med Sci 2009; 64: 1183–1189.
8. Cesari M, Penninx BW, Pahor M *et al*. Inflammatory markers and physical performance in older persons: the InCHIANTI study. J Gerontol A Biol Sci Med Sci 2004; 59: 242-248.
9. Barbieri M, Ferrucci L, Ragno E *et al*. Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. Am J Physiol Endocrinol Metab 2003; 284: E481-487.
10. Ferrucci L, Penninx BW, Volpato S *et al*. Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. J Am Geriatr Soc 2002; 50: 1947–1954.

11. Payette H, Roubenoff R, Jacques PF *et al*. Insulin-like growth factor-1 and interleukin 6 predict sarcopenia in very old community-living men and women: the Framingham Heart Study. J Am Geriatr Soc 2003; 51: 1237–1243.
12. Taaffe DR, Harris TB, Ferrucci, Rowe J, Seeman TE. Cross-sectional and prospective relationships of interleukin-6 and Creactive protein with physical performance in elderly persons: MacArthur studies of successful aging. J Gerontol A Biol Sci Med Sci 2000; 55: M709–M715.
13. Kehdy FS, Gouveia MH, Machado M *et al*. Brazilian EPIGEN Project Consortium. Origin and dynamics of admixture in Brazilians and its effect on the pattern of deleterious mutations. Proc Natl Acad Sci USA 2015; 112: 8696-8701.
14. Lima-Costa MF, Rodrigues LC, Barreto ML *et al*. Genomic ancestry and ethnoracial self-classification based on 5,871 community-dwelling Brazilians (The Epigen Initiative). Sci Rep 2015; 5: 9812(1-7).
15. Lima-Costa MF, Firmo JO, Uchoa E. Cohort profile: the Bambui (Brazil) Cohort Study of Ageing. Int J Epidemiol 2011; 40: 862–867.
16. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. Genome Res 2009; 19: 1655-1664.
17. Costa E, Barreto SM, Uchoa E, Firmo JOA, Lima-Costa MF, Prince M. Is the GDS-30 better than the GHQ-12 for screening depression in elderly people in the community? The Bambui Health and Aging Study (BHAS). Int Psychogeriatr 2006; 18: 493–503.
18. Ismail K. Unravelling factor analysis. Evid Based Ment Health 2008; 11: 99–102.
19. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. J Amer Statistical Association 1999; 94: 496-509.
20. Szklo M, Javier Nieto E. Epidemiology: Beyond the Basics, 2nd Ed. Massachusets: Jones and Bartlett Publishers, 2007.