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Association between plasma CCL11 (eotaxin-1) and cognitive status in older adults: Differences between rural and urban dwellers

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ABSTRACT

The chemokine CCL11 has been implicated in age-related cognitive deterioration in mice, yet evidence on the relationship between CCL11 and cognitive function in humans is limited. This study explored associations between CCL11 and cognition in rural and urban community-dwelling older adults. Participants were 515 urban dwellers from the 3C-Bordeaux cohort and 318 rural dwellers from the AMI cohort. Plasma CCL11 was measured using an enzyme-linked immunoassay. Mini Mental State Examination (MMSE) test scores were used as the main measure of cognitive performance. Multivariate regression analysis was used to evaluate the cross-sectional association between CCL11 and cognitive performance. CCL11 was significantly higher in rural dwellers compared to city dwellers (median [IQR]: 145 [115-201] pg/mL vs. 103 [85-129] pg/mL; $p < 0.001$). After adjustment for confounders, CCL11 was found to be negatively associated with cognitive performance in rural dwellers but not in city dwellers. These results suggest that CCL11 may be an independent determinant of cognitive function in older rural dwellers and that the residential environment modifies this association.

Keywords: Cognitive impairment; Aging; CCL11; Dwelling environment; Cohort

1. Introduction

Chronic systemic inflammation is increasingly recognised as a contributing factor in the etiology of age-related cognitive decline and neurodegenerative diseases (Bourassa and Sbarra, 2017; Sankowski et al., 2015; Weaver et al., 2002). However, epidemiological studies exploring relationships between markers of inflammation and cognitive function have produced mixed and often inconsistent results (Bettcher and Kramer, 2014; Sharma et al., 2016). In this context, among a number of putative circulating inflammatory mediators, the chemokine C-C motif ligand 11 (CCL11), also known as eotaxin-1, has recently emerged as a potential pathophysiologically relevant age-related negative regulator of neurogenesis and cognitive function in mice (Villeda et al., 2011). Widely recognized as an eosinophil chemoattractant, CCL11 plays an important role in the pathogenesis of allergic conditions such as asthma and in inflammatory diseases of the gastrointestinal tract (Williams, 2015). More recently, circulating levels of CCL11 have been shown to rise with normal human ageing (Bradburn et al.; 2018; Shurin et al., 2007; Villeda et al., 2011) and in neurodegenerative diseases (Huber et al., 2018). In particular, small studies have linked higher blood levels of CCL11 with Alzheimer's disease (AD) (Choi et al., 2008) and with memory deficits in older adults diagnosed with mild cognitive impairment (MCI) and AD dementia phenotypes (Bettcher et al., 2016). In contrast, high CCL11 levels were found to correlate with a delayed age at onset in AD (Lalli et al., 2015) suggesting a complex role of this chemokine in neurodegeneration.

Little is known about the relationship between CCL11 and cognitive function in healthy older humans, particularly in the context of varying socio-demographic factors. Population-based studies indicate that cognitive performance in older adults is influenced by socio-demographic characteristics, with level of education, gender, age and income showing consistent associations (Wight et al., 2006). Few studies have also examined the influence of the residential environment (rural or urban) on cognition but the findings have been inconsistent (Crowe et al., 2010; Nunes, et al.; 2010; Saenz et al., 2018; Xu et al., 2017).

Elucidating how the residential context impacts on the biological processes associated with cognitive decline may help to better understand the pathophysiological mechanisms underlying this condition. Therefore, in this study we examined if circulating levels of plasma CCL11 are associated with cognitive performance in older adults living in the community and if the relationship would differ between rural and urban dwellers.

2. Methods

2.1. Study population

Participants in this cross-sectional study were men and women aged 65 and older from two well characterised population-based French cohorts, namely, Three-City (3C) (The 3C Study Group, 2003) and Approche Multidisciplinaire Intégrée (AMI) (Peres et al., 2012), who were enrolled in 2013 to the exploratory phase of the FRAILOMIC initiative, a European project investigating biomarkers of frailty (Erusalimsky et al., 2016). The 3C is a population-based prospective cohort formed during 1999-2000 to study the vascular risk factors of dementia in non-institutionalized city dwellers randomly selected from the electoral roles of three urban areas, namely Dijon, Montpellier and Bordeaux (The 3C Study Group, 2003). Only participants from the Bordeaux 10-year-follow-up wave (n=1214), for whom a comprehensive set of parameters were recorded, were considered for enrolment to FRAILOMIC. The AMI is a population-based prospective cohort (n=1002) started in 2007 to study health and ageing in elderly farmers living in rural South West France, which were selected randomly from the database of the French Farmer Health Insurance System (Peres et al., 2012). All participants gave written informed consent and the study was approved by the Ethics committees of the participating institutions: the 3C study protocol was approved by the Consultative Committee for the Protection of Persons participating in Biomedical Research at Kremlin-Bicêtre University Hospital (Paris) and the AMI study was approved by the Ethics Committee of the CHU (University Hospital) of Bordeaux. The research followed the principles embodied in the Declaration of Helsinki.

Participants from the two cohorts were recruited and investigated following similar procedures, therefore allowing for comparative analyses. In both cohorts, a wide range of data was collected by a neuropsychologist during a home visit, including anthropometric measurements, frailty assessment, neuropsychological testing and recording of sociodemographic characteristics, lifestyle habits, medication and medical history. Blood sampling was also conducted in both cohorts. Individuals were eligible for inclusion in FRAILOMIC if a blood specimen was available for biomarker evaluation and if the frailty status could be determined using the frailty criteria proposed by Fried et al. (Fried et al., 2001). A total of 525 subjects from 3C-Bordeaux fulfilled the aforementioned selection criteria. In AMI, among 695 participants with available blood specimens, all subjects diagnosed as frail (n=80) were included in the study, whereas selection of the non-frail participants was constrained to achieve a ratio of 1 case to 3 controls and a similar cardiovascular risk profile in both groups. This led to a total AMI subset of 320 subjects. Using 25 variables including socio-demographic and clinical data, disability and death, an inverse probability weighting was applied to the AMI subset in order to limit the selection bias which had led to an over-representation of the frail subjects in this group.

2.2. Cognitive Tests

At the time of the FRAILOMIC sampling, three cognitive tests had been administered in both cohorts, namely the Mini-mental State Examination (MMSE), the Free and Cued Selective Reminding Test RL/RI-16 items (FCSRT) and the Isaacs Set Test (IST). The MMSE test was considered as the main outcome since it is a questionnaire assessing global cognitive performance. The MMSE score ranges from 0 to 30, with a higher score indicating better performance (Folstein et al., 1975). The FCRST is used to assess episodic memory with a score ranging from 0 to 48, also increasing with the level of cognitive performance (Grober et al., 1988). Finally, the IST is a verbal fluency assessment test that requires subjects to recall items in four different semantic categories (fruits, animals, colours and cities). This test was limited to 15 seconds in the present analysis, with a maximum score of

10 words per category and consequently a maximum total score of 40 (Isaacs and Akhtar, 1972).

2.3. Measurement of CCL11

Plasma levels of CCL11 were determined from fasting blood samples stored at -80°C using a commercially available sandwich ELISA (Quantikine Human CCL11/Eotaxin Immunoassay, R&D Systems, Abingdon, UK). The intra- and inter-assay coefficients of variation were 1.2% and 3.1%, respectively.

2.4. Other variables

Sociodemographic information recorded at baseline included age, gender, monthly income and level of education, which in the present analysis was subdivided into three categories: low (Primary School or less, ages 3-11), intermediate (Secondary School, cycles 1 and 2, ages 12-18) and high (University, age >18). Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. Frailty was evaluated using Fried's criteria (Fried et al., 2001). The degree of disability was assessed using a hierarchical index which distinguishes four levels: no disability, mild, moderate and severe disability (Barberger-Gateau et al., 2000). Depressive symptoms were assessed using the Center for Epidemiologic Studies-Depression scale (CES-D, 20-item version). A score ≥17 for men and ≥23 for women was considered as an indicator of a clinically relevant depression (Fuhrer and Rouillon, 1989). Medication use was categorised as taking <5 drugs, 5-9 drugs or >9 drugs.

2.5. Statistical analysis

2.5.1. Comparison of sociodemographic characteristics and health indicators.

Continuous variables are reported as means ± SD for normally distributed data or as medians with IQR for skewed data. Categorical variables are presented as percentages. Differences in characteristics between groups were compared by χ^2 test or by Fisher's exact test for categorical variables, and by Student's t test or Mann-Whitney test for continuous

variables, as appropriate. Missing data (around 17% of the total database) were imputed by multiple regression imputation (Seaman et al., 2012).

2.5.2. Regression analyses.

The association between CCL11 and cognition was evaluated by linear regression analysis when the results of the cognitive tests were considered in their continuous form. In addition, for the two cognitive tests for which a clinically relevant threshold was available (i.e. MMSE and FCSRT), and hence scores could be dichotomised, logistic regression models were applied. For the MMSE test we used the square root of the error number to respect the Gaussian distribution (Jacqmin-Gadda et al., 1997) and a score of 24/30 as the clinically relevant threshold (Tombaugh and McIntyre, 1992). For the FCSRT a threshold of 17/48 was used (Auriacombe et al., 2010). Analyses were initially performed on the pooled data subsets. Models were adjusted for age, gender, level of education and living environment (cohort). Due to the cohort effect (see Results section), further analyses were carried out separately on each cohort subset. Given the skewed distribution of CCL11, for its evaluation as a continuous variable it was natural log-transformed (ln CCL11) before further analysis. Analyses were performed using Statistical Analysis System (SAS) version 9.4® (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Characteristics of the participants

Figure 1 shows a flow chart depicting the selection of participants from the 3C and AMI cohorts enrolled in FRAILOMIC and their progression through the current study. A total of 517 participants from 3C-Bordeaux and 320 participants from AMI had sufficient blood available to have their plasma CCL11 determined. Of these, two participants in each subset was excluded from the analysis for giving an out of range CCL11 concentration (i.e. >700 pg/mL). The characteristics of the participants from the two cohort subsets are presented in Table 1. In addition to the living environment, the two subsets showed significant differences in the majority of the studied characteristics. Thus, compared with participants from 3C, AMI

participants were significantly younger, with the majority being male, having a lower income and lower levels of education. Furthermore, significant differences in the clinical characteristics were also observed, with AMI participants showing a higher average BMI and a lower prevalence of disabilities or depression. In addition, the AMI participants showed poorer cognitive performances than those of 3C on all tests used. For example, the mean MMSE score of the AMI subset was 24.9 compared to 27.6 in the 3C subset, with 27% of AMI participants scoring below the threshold of 24 compared to only 6.7% in the 3C subset. Finally, median CCL11 concentrations were significantly higher in AMI compared to 3C (145.1 pg/mL vs 103.3 pg/mL, respectively).

3.2. Relationship between CCL11 levels and cognitive performance

In order to explore the relationship between CCL11 levels and cognitive performance, multivariate regression analyses were performed on the pooled cohort subsets (Table 2). After adjusting for age, gender and level of education (model 2) the odds of exhibiting global cognitive impairment (i.e. MMSE score <24) increased more than two-fold per unit increment of ln CCL11 (OR = 2.40, 95% CI 1.43;4.02, $p < 0.001$). Results remained significant when the MMSE score was considered as a continuous variable (β = 0.29, 95% CI 0.15;0.44, $p < 0.001$). However, after further adjustment for the living environment (rural/urban) these associations were no longer significant (Table 2, model 3). Significant associations were also found between ln CCL11 and the FCSRT scores (OR = 1.67, 95% CI 1.01;2.75, $p = 0.046$ and β = -1.72, 95% CI -2.99;-0.44, $p = 0.009$) or the IST scores (β = -1.51, 95% CI -2.56;-0.45, $p = 0.005$). Like in the case of the MMSE test results, after further adjustment for the living environment, the association between ln CCL11 and either the FCSRT or the IST results was no longer significant (Table 2, model 3).

Given the considerable difference in CCL11 concentrations between the two cohort subsets (Table 1), and the effect of the living environment (Table 2, model 3), further analyses were conducted on the two cohort subsets separately (Table 3). In the AMI cohort significant associations persisted between ln CCL11 and MMSE scores (OR = 2.23, 95% CI

1.92;2.58, $p < 0.001$ and β = 0.22, 95% CI 0.14;0.29, $p < 0.001$). Similarly, in this cohort an increase in ln CCL11 was associated with greater odds of scoring poorly for episodic memory, i.e. FCSRT score <17/48 (OR = 1.33, 95% CI 1.13;1.56, $p = 0.001$), except that in this case the relationship was lost when the FCSRT score was considered as a continuous variable. In contrast, no relationship was observed for verbal fluency in the AMI cohort. None of the cognitive tests scores investigated as continuous or dichotomic variables were significantly associated with CCL11 levels in the 3C cohort (Table 3).

3.3. Secondary analysis

To our knowledge there is no clinically relevant threshold for the categorization of CCL11 concentrations. In the absence of such a threshold, the distribution of CCL11 concentrations measured in the AMI cohort subset was divided by tertiles in order to assess the extent to which increasing cutoff values could distinguish participants who displayed greater odds of performing poorly in the cognitive tests. As shown in Table 4, for the MMSE test the odds of exhibiting lower cognitive performance (i.e. MMSE score <24) increased almost 1.6 fold for participants in the intermediate tertile of CCL11 (124.72 – 177.44 pg/mL) and almost 2.8 fold for those falling in the highest tertile ($p < 0.001$ vs. the lowest tertile). Results also remained significant when the MMSE score was considered as a continuous variable. Similarly, participants falling in the higher ranges of CCL11 exhibited higher odds of performing poorly in the FCSRT. In contrast this analysis did not show significant associations with IST scores (Table 4).

4. Discussion

The present study provides evidence relating plasma levels of the chemokine CCL11 with cognitive performance in community-dwelling older adults from South Western France. Specifically, it shows that in a cohort of rural dwellers increased CCL11 is associated with poorer cognitive performance independently of relevant covariates. Importantly, this study did not find such a relationship in a cohort of city dwellers from the same region of France,

despite the fact that both population samples were tested following the same procedures. Thus, our study suggests that the living environment influences the association between CCL11 and cognitive performance. To our knowledge, no significant association between CCL11 and cognition had been previously reported in elderly population samples of the types involved in this study.

The association between CCL11 and cognitive performance was clearly demonstrated when assessing global cognitive function (MMSE test) and to a lesser extent when evaluating episodic memory (FCSRT); the latter was manifested only when the results of the FCSRT were dichotomised. Noticeably, no relationship was observed between CCL11 and verbal fluency as measured by the IST. Both the FCSRT and the IST measure more narrow aspects of cognitive function, namely, memory and recall, respectively. Thus, it is possible that the association with CCL11 levels is more robustly manifested when assessing global cognition.

The relationship between measures of cognition and CCL11 has not been widely explored. Small studies in patients diagnosed with MCI or AD have generated mixed findings (Bettcher et al., 2016; Leung et al., 2013; Westin et al., 2012). Bettcher et al. found that higher levels of CCL11 in combination with MCP-1 predict worse memory functions in MCI and AD phenotypes (Bettcher et al., 2016), whereas Leung and co-workers reported significantly higher levels of CCL11 in patients with AD compared to those with MCI (Leung et al., 2013). However, in sharp contrast, another study found that levels of CCL11 were reduced in patients with MCI who developed AD within 4-7 years compared to those who remained stable during the same period (Westin et al., 2012). In addition, a study by Bradburn et al. showed that CCL11 was negatively associated with episodic memory and global cognition scores in relatively healthy elderly adults. However, this association was lost after adjusting for confounders (Bradburn et al., 2018). Thus, so far the evidence relating blood CCL11 with cognitive function in humans is limited and inconclusive.

In our study, it was striking that CCL11 levels were associated with cognitive performance in rural dwellers but not in city dwellers. One possibility to explain this difference is that CCL11 would be detrimental to cognitive function only if it was elevated above a pathophysiologically relevant threshold. In support of this possibility we found that (i) the median CCL11 was ~50% higher in the AMI subset compared to the 3C subset, and (ii) AMI participants in the middle and upper tertiles of CCL11 had greater odds of exhibiting lower cognitive performance scores (as measured by the MMSE test and the FCSRT) than those in the lowest tertile.

The reason as to why rural dwellers exhibited higher levels of CCL11 compared to city dwellers is not known. Importantly, CCL11 determinations for both cohorts were undertaken in the same laboratory, at the same time and with the origin of the samples blinded to the operator. Although the AMI subset had a much larger proportion of males than the 3C subset, CCL11 levels in plasma are not influenced by gender (Hoefer et al., 2017; Larsson et al., 2015). In contrast, CCL11 has been reported to be higher in older individuals (Bradburn et al., 2018; Shurin et al., 2007; Villeda et al., 2011). However, participants included in the AMI subset were on average 6.2 years younger than those in the 3C subset (Table 1). Therefore, gender or chronological age cannot account for the difference in CCL11 seen between the two cohort subsets. Levels of this chemokine are known to be raised by exposure to chemical, food and air-borne allergens (Matsuura et al., 2009; Meller et al., 2007; Minshall et al., 1997). Whether rural dwellers were subjected to higher and/or more persistent levels of exposure to such substances than their city counterparts remains to be established. If that were to be the case and considering that long term exposures to inflammatory environments negatively affect cognitive status (Baldi et al., 2011; Marshall et al., 2000), we hypothesize that this association might be in part mediated by CCL11. Thus, the current findings could expand our understanding of how the environment influences the pathophysiological processes underlying cognitive decline.

The precise biological mechanisms through which plasma CCL11 might be related to cognitive performance are yet to be delineated. CCL11 is able to cross the blood brain barrier and hence it is possible that systemic elevation of this chemokine will affect brain functions (Erickson et al., 2014). Consistent with this possibility there is evidence to suggest that adverse changes in brain morphology may mediate the association of peripheral inflammation with cognitive performance (Marsland et al., 2015). Indeed, studies in mice show that CCL11 impairs hippocampal neurogenesis and learning and memory (Villeda et al., 2011). In addition, *in vitro* studies have demonstrated that CCL11 induces microglial cells to produce reactive oxygen species, promoting excitotoxic neuronal death (Parajuli et al., 2015). Thus, CCL11 might cause neuronal damage and impair cognitive functions independent of eosinophil recruitment.

The present study has important strengths, including that it was carried out in well-characterised cohorts and with relatively large sample sizes. Nonetheless, it also has several limitations. First, although AMI and 3C are longitudinal cohorts, the present analysis is cross-sectional so a causal conclusion cannot be drawn as to whether increased plasma CCL11 contributes towards the deterioration of cognitive function or if it is simply a marker of another biological process that affects cognition. Second, although we controlled for age, gender and level of education, other non-recorded variables could be affecting the association, for example, vitamin D levels (Kubeczko et al., 2016). Third, when considering the applicability of the results from this study to other populations it should be born in mind that ethnicity and socioeconomic position are generally known to impact on cytokine/chemokine profiles. In the particular case of CCL11, there is evidence of ethnic variation in its circulating concentrations, with individuals of African ancestry having lower levels than those of Eurasian Ancestry (Castillo et al., 2010; Coussens et al., 2013). In the current study rural dwellers were mostly Caucasians from the same region of France and of a mixed socioeconomic background, albeit with a higher proportion being on lower incomes. Hence, the present findings might not generalize to other rural communities with different

ethnic and/or socioeconomic composition. A final limitation of our study is its inability to establish if the observed relationship between cognitive status and CCL11 is part of a more generalised heightened inflammatory state, since we had no further biological data on the inflammatory status of the participants.

5. Conclusions

In summary, despite the above-mentioned limitations, our study adds to the growing literature relating drivers of inflammation to cognitive decline, and might have implications for understanding the biological pathways through which social and environmental factors influence cognition. We report for the first time that in older individuals living in a rural environment, and otherwise free from clinically diagnosed neurodegenerative diseases, increased levels of CCL11 are associated with a lower cognitive performance. Longitudinal studies are needed to establish the causal relationships between this chemokine and cognitive function and if it can be used as a biomarker to predict cognitive decline.

Disclosure statement

The authors have no conflicts of interest to disclose.

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References

Auriacombe, S., Helmer, C., Amieva, H., Berr, C., Dubois, B., Dartigues, J.F., 2010. Validity of the free and cued selective reminding test in predicting dementia: the 3C study. Neurology 74, 1760-1767.

Baldi, I., Gruber, A., Rondeau, V., Lebailly, P., Brochard, P., Fabrigoule, C., 2011. Neurobehavioral effects of long-term exposure to pesticides: results from the 4-year follow-up of the PHYTONER study. Occup. Environ. Med. 68, 108-115.

Barberger-Gateau, P., Rainville, C., Letenneur, L., Dartigues, J.F., 2000. A hierarchical model of domains of disablement in the elderly: a longitudinal approach. Disabil. Rehabil. 22, 308-317.

Bettcher, B.M., Fitch, R., Wynn, M.J., Lalli, M.A., Elofson, J., Jastrzab, L., Mitic, L., Miller, Z.A., Rabinovici, G.D., Miller, B.L., Kao, A.W., Kosik, K.S., Kramer, J.H., 2016. MCP-1 and eotaxin-1 selectively and negatively associate with memory in MCI and Alzheimer's disease dementia phenotypes. Alzheimers Dement. (Amst) 3, 91-97.

Bettcher, B.M., Kramer, J.H., 2014. Longitudinal inflammation, cognitive decline, and Alzheimer's disease: a mini-review. Clin. Pharmacol. Ther. 96, 464-469.

Bourassa, K., Sbarra, D.A., 2017. Body mass and cognitive decline are indirectly associated via inflammation among aging adults. Brain. Behav. Immun. 60, 63-70.

Bradburn, S., McPhee, J., Bagley, L., Carroll, M., Slevin, M., Al-Shanti, N., Barnouin, Y., Hogrel, J.Y., Paasuke, M., Gapeyeva, H., Maier, A., Sipila, S., Narici, M., Robinson, A., Mann, D., Payton, A., Pendleton, N., Butler-Browne, G., Murgatroyd, C., 2018. Dysregulation of C-X-C motif ligand 10 during aging and association with cognitive performance. Neurobiol. Aging 63, 54-64.

Castillo, L., Rohatgi, A., Ayers, C.R., Owens, A.W., Das, S.R., Khera, A., McGuire, D.K., de Lemos, J.A., 2010. Associations of four circulating chemokines with multiple atherosclerosis phenotypes in a large population-based sample: results from the dallas heart study. J. Interferon Cytokine Res. 30, 339-347.

Coussens, A.K., Wilkinson, R.J., Nikolayevskyy, V., Elkington, P.T., Hanifa, Y., Islam, K., Timms, P.M., Bothamley, G.H., Claxton, A.P., Packe, G.E., Darmalingam, M., Davidson, R.N., Milburn, H.J., Baker, L.V., Barker, R.D., Drobniewski, F.A., Mein, C.A., Bhaw-Rosun, L., Nuamah, R.A., Griffiths, C.J., Martineau, A.R., 2013. Ethnic variation in inflammatory profile in tuberculosis. PLoS Pathog. 9, e1003468.

Choi, C., Jeong, J.H., Jang, J.S., Choi, K., Lee, J., Kwon, J., Choi, K.G., Lee, J.S., Kang, S.W., 2008. Multiplex analysis of cytokines in the serum and cerebrospinal fluid of patients with Alzheimer's disease by color-coded bead technology. J. Clin. Neurol. 4, 84-88.

Crowe, M., Sartori, A., Clay, O.J., Wadley, V.G., Andel, R., Wang, H.X., Sawyer, P., Allman, R.M., 2010. Diabetes and cognitive decline: investigating the potential influence of factors related to health disparities. J. Aging Health 22, 292-306.

Erickson, M.A., Morofuji, Y., Owen, J.B., Banks, W.A., 2014. Rapid transport of CCL11 across the blood-brain barrier: regional variation and importance of blood cells. J. Pharmacol. Exp. Ther. 349, 497-507.

Erusalimsky, J.D., Grillari, J., Grune, T., Jansen-Duerr, P., Lippi, G., Sinclair, A.J., Tegner, J., Vina, J., Durrance-Bagale, A., Minambres, R., Viegas, M., Rodriguez-Manas, L., 2016. In Search of 'Omics'-Based Biomarkers to Predict Risk of Frailty and Its Consequences in Older Individuals: The FRAILOMIC Initiative. Gerontology 62, 182-190.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12, 189-198.

Fried, L.P., Tangen, C.M., Walston, J., Newman, A.B., Hirsch, C., Gottdiener, J., Seeman, T., Tracy, R., Kop, W.J., Burke, G., McBurnie, M.A., Cardiovascular Health Study Collaborative Research, G., 2001. Frailty in older adults: evidence for a phenotype. J. Gerontol. A Biol. Sci. Med. Sci. 56, M146-M156.

Fuhrer, R., Rouillon, F., 1989. La version française de l'échelle CES-D (Center for epidemiologic studies-depression scale). Description et traduction de l'échelle d'autoévaluation. Psychiatr. Psychobiol. 4, 163-166.

Grober, E., Buschke, H., Crystal, H., Bang, S., Dresner, R., 1988. Screening for dementia by memory testing. Neurology 38, 900-903.

The 3C Study Group, 2003. Vascular factors and risk of dementia: design of the Three-City Study and baseline characteristics of the study population. Neuroepidemiology 22, 316-325.

Hoefer, J., Luger, M., Dal-Pont, C., Culig, Z., Schennach, H., Jochberger, S., 2017. The "Aging Factor" Eotaxin-1 (CCL11) Is Detectable in Transfusion Blood Products and Increases with the Donor's Age. Front. Aging Neurosci. 9, 402.

Huber, A.K., Giles, D.A., Segal, B.M., Irani, D.N., 2018. An emerging role for eotaxins in neurodegenerative disease. Clin. Immunol. 189, 29-33.

Isaacs, B., Akhtar, A.J., 1972. The set test: a rapid test of mental function in old people. Age Ageing 1, 222-226.

Jacqmin-Gadda, H., Fabrigoule, C., Commenges, D., Dartigues, J.F., 1997. A 5-year longitudinal study of the Mini-Mental State Examination in normal aging. Am. J. Epidemiol. 145, 498-506.

Kubeczko, M., Nowara, E., Karwasiecka, D., Siewior, G., Czajka-Francuz, P., Chudek, J., Wojnar, J., 2016. C-C motif ligand 11 reduction in CLL patients serum after vitamin D supplementation. Hematology 21, 343-50.

Lalli, M.A., Bettcher, B.M., Arcila, M.L., Garcia, G., Guzman, C., Madrigal, L., Ramirez, L., Acosta-Uribe, J., Baena, A., Wojta, K.J., Coppola, G., Fitch, R., de Both, M.D., Huentelman, M.J., Reiman, E.M., Brunkow, M.E., Glusman, G., Roach, J.C., Kao, A.W., Lopera, F., Kosik, K.S., 2015. Whole-genome sequencing suggests a chemokine gene cluster that modifies age at onset in familial Alzheimer's disease. Mol. Psychiatry 20, 1294-1300.

Larsson, A., Carlsson, L., Gordh, T., Lind, A.L., Thulin, M., Kamali-Moghaddam, M., 2015. The effects of age and gender on plasma levels of 63 cytokines. J. Immunol. Methods 425, 58-61.

Leung, R., Proitsi, P., Simmons, A., Lunnon, K., Guntert, A., Kronenberg, D., Pritchard, M., Tsolaki, M., Mecocci, P., Kloszewska, I., Vellas, B., Soininen, H., Wahlund, L.O., Lovestone, S., 2013. Inflammatory proteins in plasma are associated with severity of Alzheimer's disease. PLoS One 8, e64971.

Marshall, P.S., O'Hara, C., Steinberg, P., 2000. Effects of seasonal allergic rhinitis on selected cognitive abilities. Ann. Allergy Asthma Immunol. 84, 403-410.

Marsland, A.L., Gianaros, P.J., Kuan, D.C., Sheu, L.K., Krajina, K., Manuck, S.B., 2015. Brain morphology links systemic inflammation to cognitive function in midlife adults. Brain Behav. Immun. 48, 195-204.

Matsuura, H., Ishiguro, A., Abe, H., Mamada, Y., Suzuki, T., Kohda, K., Shimbo, T., 2009. Elevation of plasma eotaxin levels in children with food allergy. Nihon Rinsho Meneki Gakkai Kaishi 32, 180-185.

Meller, S., Lauerma, A.I., Kopp, F.M., Winterberg, F., Anthoni, M., Muller, A., Gombert, M., Haahtela, A., Alenius, H., Rieker, J., Dieu-Nosjean, M.C., Kubitza, R.C., Gleichmann, E., Ruzicka, T., Zlotnik, A., Homey, B., 2007. Chemokine responses distinguish chemical-induced allergic from irritant skin inflammation: memory T cells make the difference. J. Allergy Clin. Immunol. 119, 1470-1480.

Minshall, E.M., Cameron, L., Lavigne, F., Leung, D.Y., Hamilos, D., Garcia-Zepada, E.A., Rothenberg, M., Luster, A.D., Hamid, Q., 1997. Eotaxin mRNA and protein expression in chronic sinusitis and allergen-induced nasal responses in seasonal allergic rhinitis. Am. J. Respir. Cell Mol. Biol. 17(6), 683-90. doi:10.1165/ajrcmb.17.6.2865.

Nunes, B., Silva, R.D., Cruz, V.T., Roriz, J.M., Pais, J., Silva, M.C., 2010. Prevalence and pattern of cognitive impairment in rural and urban populations from Northern Portugal. BMC Neurol. 10, 42.

Parajuli, B., Horiuchi, H., Mizuno, T., Takeuchi, H., Suzumura, A., 2015. CCL11 enhances excitotoxic neuronal death by producing reactive oxygen species in microglia. Glia 63, 2274-2284.

Peres, K., Matharan, F., Allard, M., Amieva, H., Baldi, I., Barberger-Gateau, P., Bergua, V., Bourdel-Marchasson, I., Delcourt, C., Foubert-Samier, A., Fourrier-Reglat, A., Gaimard, M., Laberon, S., Maubaret, C., Postal, V., Chantal, C., Rainfray, M., Rascle, N., Dartigues, J.F., 2012. Health and aging in elderly farmers: the AMI cohort. BMC Public Health 12, 558.

Saenz, J.L., Downer, B., Garcia, M.A., Wong, R., 2018. Cognition and Context: Rural-Urban Differences in Cognitive Aging Among Older Mexican Adults. J. Aging Health 30, 965-986.

Sankowski, R., Mader, S., Valdes-Ferrer, S.I., 2015. Systemic inflammation and the brain: novel roles of genetic, molecular, and environmental cues as drivers of neurodegeneration. Front. Cell Neurosci. 9, 28.

Seaman, S.R., White, I.R., Copas, A.J., Li, L., 2012. Combining multiple imputation and inverse-probability weighting. Biometrics 68, 129-137.

Sharma, M., Fitzpatrick, A.L., Arnold, A.M., Chi, G., Lopez, O.L., Jenny, N.S., DeKosky, S.T., 2016. Inflammatory Biomarkers and Cognitive Decline: The Ginkgo Evaluation of Memory Study. J. Am. Geriatr. Soc. 64, 1171-1177.

Shurin, G.V., Yurkovetsky, Z.R., Chatta, G.S., Tourkova, I.L., Shurin, M.R., Lokshin, A.E. 2007. Dynamic alteration of soluble serum biomarkers in healthy aging. Cytokine 39, 123-129.

Tombaugh, T.N., McIntyre, N.J. 1992. The mini-mental state examination: a comprehensive review. J. Am. Geriatr. Soc. 40, 922-935.

Villeda, S.A., Luo, J., Mosher, K.I., Zou, B., Britschgi, M., Bieri, G., Stan, T.M., Fainberg, N., Ding, Z., Eggel, A., Lucin, K.M., Czirr, E., Park, J.S., Couillard-Despres, S., Aigner, L., Li, G., Peskind, E.R., Kaye, J.A., Quinn, J.F., Galasko, D.R., Xie, X.S., Rando, T.A., Wyss-Coray, T., 2011. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 477, 90-94.

Weaver, J.D., Huang, M.H., Albert, M., Harris, T., Rowe, J.W., Seeman, T.E., 2002. Interleukin-6 and risk of cognitive decline: MacArthur studies of successful aging. Neurology 59, 371-378.

Westin, K., Buchhave, P., Nielsen, H., Minthon, L., Janciauskiene, S., Hansson, O., 2012. CCL2 is associated with a faster rate of cognitive decline during early stages of Alzheimer's disease. PLoS One 7, e30525.

Wight, R.G., Aneshensel, C.S., Miller-Martinez, D., Botticello, A.L., Cummings, J.R., Karlamangla, A.S., Seeman, T.E., 2006. Urban neighborhood context, educational attainment, and cognitive function among older adults. Am. J. Epidemiol. 163, 1071-1078.

Williams, T.J., 2015. Eotaxin-1 (CCL11). Front. Immunol. 6, 84.

Xu, H., Dupre, M.E., Gu, D., Wu, B., 2017. The impact of residential status on cognitive decline among older adults in China: Results from a longitudinal study. BMC Geriatr. 17, 107.

Table 1. Baseline sociodemographic, health and cognitive characteristics of the study participants

		3C		AMI		*P*
		n		*n*		
Sociodemographic parameters						
Living environment, %		515		318		<0.001
Urban			100.0		0.0	
Rural			0.0		100.0	
Age, mean ± SD, years		515	82.2 ± 4.3	318	76.0 ± 6.7	<0.001
Gender, % male		515	36.5	318	62.0	<0.001
Level of education, %		515		318		<0.001
Primary or less			26.6		80.8	
Intermediate			50.3		17.3	
High			23.1		1.9	
Monthly income (€), %		482		254		<0.001
≤1500			38.6		71.9	
>1500			61.4		29.1	
Laboratory and biomedical parameters						
CCL11, median [IQR], pg/mL		515	103.3 [84.8 – 128.6]	318	145.1 [114.5 – 201.2]	<0.001
BMI, mean ± SD, kg/m^2		505	25.7 ± 4.3	316	27.6 ± 4.6	<0.001
Hierarchical disability index, %		502		308		0.002
No			28.7		32.8	
Mild			45.0		38.6	
Moderate			22.3		18.5	
Severe			4.0		10.1	
Frailty, %		510		318		<0.001
Robust			21.2		49.1	
Pre-frail			53.9		26.1	
Frail			24.9		24.8	
Medication (number per day), %		515		318		0.075
<5			38.1		44.7	
5-9			49.9		41.8	
>9			12.0		13.5	
Depression, %		507	10.7	295	5.4	0.011
Cognitive performance						
MMSE	Mean ± SD	509	27.6 ± 2.4	311	24.9 ± 4.3	<0.001
	% scoring <24/30		6.7		27.0	<0.001
FCSRT	Mean ± SD	467	25.2 ± 7.2	249	21.1 ± 6.9	<0.001
	% scoring <17/48		13.1		23.7	<0.001
IST	Mean ± SD	507	28.3 ± 6.0	293	24.4 ± 6.5	<0.001

Abbreviations: CCL11, C-C Motif Chemokine Ligand 11; BMI, body mass index; MMSE, Mini-Mental State Examination; FCSRT, Free and Cued Selective Reminding Test; IST, Isaacs Set Test

Table 2. Multivariate regression models of the relationship between natural log-transformed CCL11 and cognitive performance scores in the pooled cohort subsets

MMSE	**<24/30**			**Square root of error number**		
	OR	**95% CI**	***P***	**β**	**95% CI**	***P***
Unadjusted	3.19	2.01;5.05	<0.001	0.50	0.35;0.65	<0.001
Model 1	3.72	2.30;5.60	<0.001	0.54	0.38;0.69	<0.001
Model 2	2.40	1.43;4.02	0.001	0.29	0.15;0.44	<0.001
Model 3	1.60	0.92;2.78	0.098	0.11	-0.04;0.25	0.149
FCSRT	**<17/48**					
	OR	**95% CI**	***P***	**β**	**95% CI**	***P***
Unadjusted	1.96	1.23;3.14	0.005	-2.83	-4.16;-1.51	<0.001
Model 1	2.15	1.32;3.49	0.002	-3.08	-4.40;-1.75	<0.001
Model 2	1.67	1.01;2.75	0.046	-1.72	-2.99;-0.44	0.009
Model 3	1.35	0.80;2.31	0.273	-0.68	-1.99;0.64	0.315
IST						
				β	**95% CI**	***P***
Unadjusted				-2.65	-3.75;-1.55	<0.001
Model 1				-2.86	-3.97;-1.75	<0.001
Model 2				-1.51	-2.56;-0.45	0.005
Model 3				-0.63	-1.72;0.46	0.258

Model 1: Multivariate model adjusted for age and gender

Model 2: Model 1 additionally adjusted for level of education

Model 3: Model 2 additionally adjusted for cohort

Abbreviations: MMSE, Mini-Mental State Examination; FCSRT, Free and Cued Selective Reminding Test; IST, Isaacs Set Test.

Table 3. Multivariate regression models of the relationship between natural log-transformed CCL11 and cognitive performance scores in the AMI and 3C cohort subsets

	AMI cohort			3C cohort		
Cognitive tests						
MMSE (<24/30)	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Unadjusted	2.10	1.82;2.41	<0.001	1.03	0.38;2.82	0.953
Model 1	1.98	1.72;2.28	<0.001	1.00	0.34;2.96	0.995
Model 2	2.23	1.92;2.58	<0.001	0.93	0.31;2.77	0.892
MMSE (square root of error number)	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
Unadjusted	0.25	0.17;0.33	<0.001	0.03	-0.18;0.23	0.794
Model 1	0.21	0.14;0.29	<0.001	0.02	-0.18;0.22	0.868
Model 2	0.22	0.14;0.29	<0.001	0.01	-0.19;0.19	0.963
FCSRT (<17/48)	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Unadjusted	1.42	1.21;1.66	<0.001	1.41	0.67;2.97	0.367
Model 1	1.30	1.11;1.53	0.001	1.41	0.64;3.08	0.391
Model 2	1.33	1.13;1.56	0.001	1.36	0.62;2.98	0.449
FCSRT	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
Unadjusted	-0.52	-1.20;0.16	0.134	-1.05	-2.91;0.82	0.270
Model 1	-0.06	-0.70;0.58	0.855	-0.97	-2.77;0.84	0.292
Model 2	0.08	-0.54;0.70	0.796	-0.87	-2.63;0.88	0.328
IST	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
Unadjusted	-0.42	-0.98;0.15	0.150	-0.86	-2.37;0.64	0.260
Model 1	-0.16	-0.70;0.38	0.554	-0.82	-2.30;0.65	0.274
Model 2	-0.12	-0.65;0.40	0.643	-0.72	-2.14;0.71	0.322

Model 1: Multivariate model adjusted for age and gender

Model 2: Model 1 additionally adjusted for level of education

Abbreviations: MMSE, Mini-Mental State Examination; FCSRT, Free and Cued Selective Reminding Test; IST, Isaacs Set Test.

Table 4. Multivariate regression analysis of the relationship between cognitive performance scores and CCL11 by ascending tertiles in the AMI cohort subset

Cognitive tests				
MMSE (<24/30)		**OR**	**95% CI**	***P***
CCL11 (ρg/mL)	*< 124.72*	-	-	-
	124.72 – 177.44	1.56	1.33;1.82	<0.001
	> 177.44	2.77	2.38;3.23	<0.001
MMSE (square root of error number)		**β**	**95% CI**	***P***
CCL11 (ρg/mL)	*< 124.72*	-	-	-
	124.72 – 177.44	0.13	0.06;0.20	0.001
	> 177.44	0.23	0.16;0.30	<0.001
FCSRT (<17/48)		**OR**	**95% CI**	***P***
CCL11 (ρg/mL)	*< 124.72*	-	-	-
	124.72 – 177.44	1.33	1.13;1.57	0.001
	> 177.44	1.77	1.50;2.08	<0.001
FCSRT		**β**	**95% CI**	***P***
CCL11 (ρg/mL)	*< 124.72*	-	-	-
	124.72 – 177.44	0.04	-0.56;0.63	0.897
	> 177.44	-0.81	-1.41;-0.22	0.007
IST		**β**	**95% CI**	***P***
CCL11 (ρg/mL)	*< 124.72*	-	-	-
	124.72 – 177.44	0.80	0.29;1.31	0.002
	> 177.44	-0.06	-0.57;0.45	0.823

The multivariate model was adjusted for age, gender and level of education

Abbreviations: BMI, body mass index; MMSE, Mini-Mental State Examination; FCSRT, Free and Cued Selective Reminding Test; IST, Isaacs Set Test; CCL11, C-C Motif Chemokine Ligand 11

Figure legends

Fig. 1. Flow chart of the study sample selection. The criteria used for the selection of participants is described in the main text

Highlights

- Plasma CCL11 is significantly higher in older rural dwellers than in urban dwellers
- In older rural dwellers CCL11 is negatively associated with cognitive performance
- CCL11 is not associated with cognitive performance in older urban dwellers
- The living environment modifies the relationship between CCL11 and cognitive status

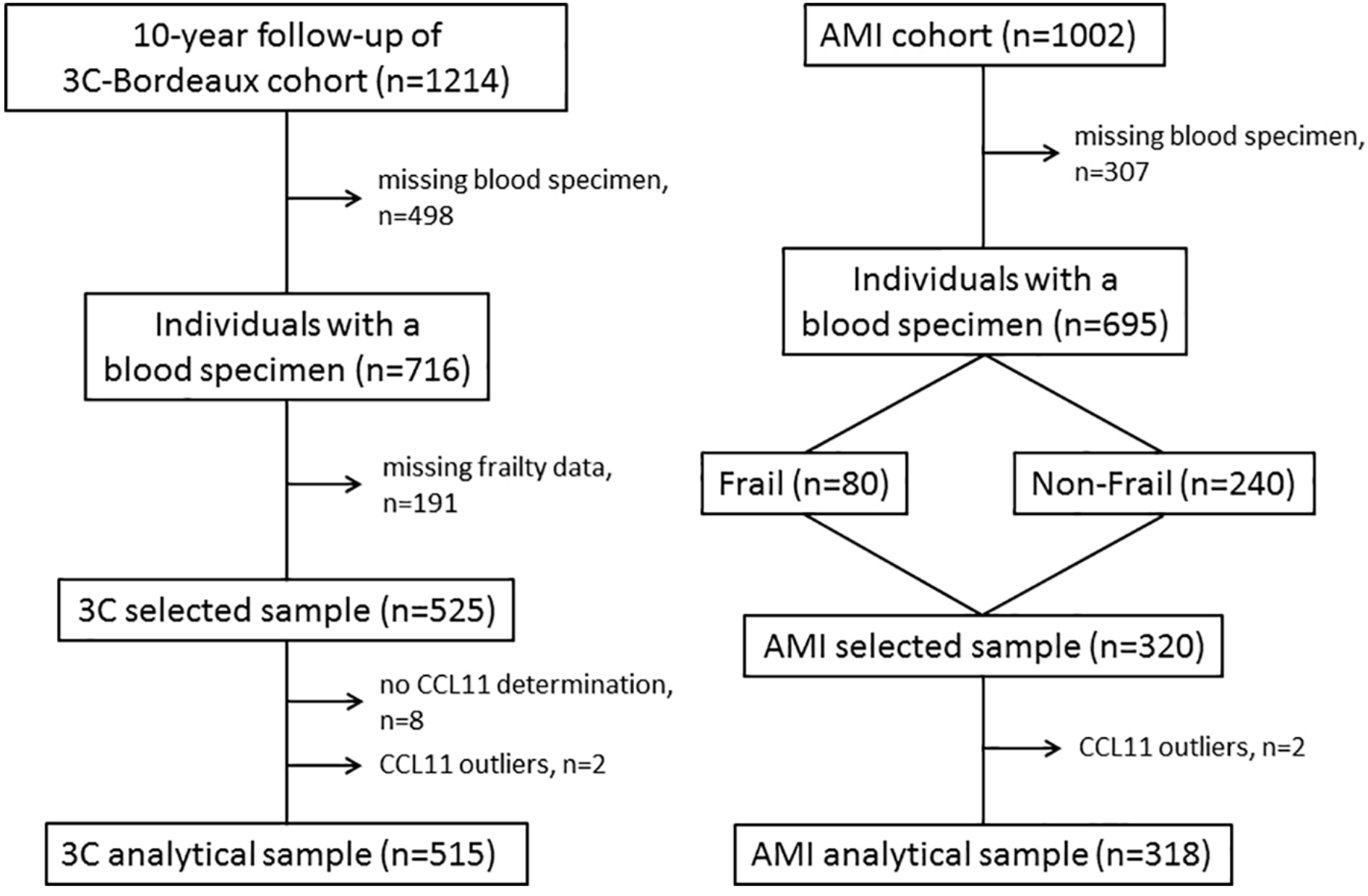
10-year follow-up of 3C-Bordeaux cohort (n=1214)
missing blood specimen, n=498
Individuals with a blood specimen (n=716)
missing frailty data, n=191
3C selected sample (n=525)
no CCL11 determination, n=8
CCL11 outliers, n=2
3C analytical sample (n=515)
AMI cohort (n=1002)
missing blood specimen, n=307
Individuals with a blood specimen (n=695)
Frail (n=80)
Non-Frail (n=240)
AMI selected sample (n=320)
CCL11 outliers, n=2
AMI analytical sample (n=318)

Figure 1