# Serum Granulocyte Colony Stimulating Factor and Alzheimer's Disease

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## Abstract

***Background*: Granulocyte colony stimulating factor (GCSF) promotes the survival, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils. Additionally, GCSF acts as a neurotrophic factor, by increasing neuroplasticity and suppressing apoptosis. Alzheimer's disease (AD) is the most common form of age-related dementia and one of the most serious health problems in the world. Due to the role of GCSF as a neurotrophic factor, we sought to determine if peripheral GCSF levels were related to Alzheimer's disease risk or severity. *Methods*: We analyzed levels of GCSF as part of a multiplex assay of serum proteins in 197 patients diagnosed with AD and 200 cognitively normal controls enrolled in a longitudinal study of Alzheimer's disease being conducted by the Texas Alzheimer's Research Consortium (TARCC). Data were analyzed by regression analysis with adjustment for age, years of education, gender and carriage of the ApoE4 allele. *Results*: Serum GCSF was significantly lower among individuals diagnosed with probable Alzheimer's disease, relative to cognitively normal controls (β = -0.124; p = 0.001). However, among AD participants alone, higher serum GCSF was significantly associated with increased disease severity, as indicated by higher scores on the Global Clinical Dementia Rating Scale (β = 0.170; p = 0.018) and lower scores on the Mini-Mental Status Exam (β = -0.176; p = 0.016). *Conclusions*: A significantly lower level of serum GCSF was observed among Alzheimer's patients, relative to cognitively normal individuals. However, among Alzheimer's patients, increased GCSF was associated with greater disease severity. This relationship suggests that GCSF is dysregulated early in the disease process and that the elevation in GCSF observed in more advanced Alzheimer's disease may represent a compensatory response to neuronal damage.**

## Background

Alzheimer's disease (AD) is the most common form of age-related dementia and one of the most serious health problems in the industrialized world.

Granulocyte-colony stimulating factor (G-CSF) is a hematopoietic growth factor that helps regulate the mobilization of bone marrow progenitor cells and promotes neuroprotection and neurogenesis (Schneider et al., 2005, Thomas et al., 2002). In a rodent model of Alzheimer's disease, G-CSF has decreased the amyloid burden in the brain, reversed cognitive impairment (Li et al., 2011, Sanchez-Ramos et al., 2009) and reduces chronic inflammation(Jiang et al., 2010). In other studies G-CSF administered to mice following ischemic injury has been shown to stimulate the proliferation of microglia (Bartolini et al., 2011). In the present study, we sought to determine whether the serum level of G-CSF was correlated with disease status, cognition and dementia in Alzheimer's disease.

## Patient Demographics

| Variable | AD (N=197) | NC (N=203) | P-value |
|---|---|---|---|
| Gender (male) | 34% | 32% | 0.67 |
| Age (yrs) | | | |
| Median | 79 | 70 | |
| Range | 57 - 94 | 52 – 90 | <0.0001 |
| Education (yrs) | | | |
| Median | 14 | 16 | |
| Range | 0-22 | 10-25 | <0.0001 |
| APOE | | | |
| Ex/Ex | 71 | 147 | <0.0001 |
| Ex/E4 | 83 | 48 | |
| E4/E4 | 27 | 5 | |
| Unknown | 16 | 3 | |
| Hispanic Ethnicity | 3.6% | 5.4% | 0.47 |
| Race | | | |
| White | 187 | 190 | |
| Non-White | 10 | 13 | 0.67 |

## Results

Mean serum G-CSF levels were significantly lower in AD cases compared to normal controls. In the AD group, G-CSF levels were significantly negatively associated with scores on the MMSE and significantly positively associated with scores on the CDR Global .

Significant interactions were observed between G-CSF and CRP, BDNF and thrombopoietin. When the sample was split based upon BDNF and thrombopoietin levels, G-CSF was significantly associated with scores on the MMSE and CDR Global only among subjects in the middle tertile. However, when the subjects were stratified on CRP level, G-CSF was significant only among subjects grouped in the highest tertile.

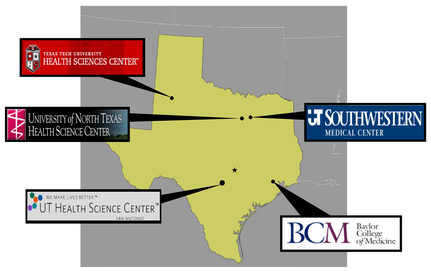
Odds ratio for disease status following adjustment for age, education, gender and ApoE4 status.

| Variable | B | S.E. | EXP(B) | 95% C.I. EXP(B) Lower | Upper | p-value |
|---|---|---|---|---|---|---|
| Age | 0.088 | 0.014 | 1.092 | 1.061 | 1.123 | <0.001 |
| Sex | -0.434 | 0.263 | 0.648 | 0.387 | 1.085 | 0.099 |
| Education | -0.162 | 0.043 | 0.851 | 0.781 | 0.926 | <0.001 |
| APOE4 | 1.623 | 0.255 | 5.066 | 3.074 | 8.349 | <0.001 |
| G-CSF | -0.073 | 0.028 | 0.930 | 0.881 | 0.981 | 0.008 |

Multivariate logistic regression for CDR Global among participants with Alzheimer's disease.

| Variable | B | S.E. | EXP(B) | 95% C.I. EXP(B) Lower | Upper | p-value |
|---|---|---|---|---|---|---|
| Age | 0.011 | 0.007 | 0.126 | 1.701 | 0.091 | 0.011 |
| Sex | 0.081 | 0.116 | 0.052 | 0.704 | 0.482 | 0.081 |
| Education | -0.009 | 0.016 | -0.038 | -0.531 | 0.596 | -0.009 |
| APOE4 | 0.001 | 0.111 | 0.001 | 0.013 | 0.990 | 0.001 |
| G-CSF | 0.030 | 0.012 | 0.170 | 2.379 | 0.018 | 0.030 |

### Texas Alzheimer's Research Consortium Institutions




Multivariate logistic regression for MMSE among participants with Alzheimer's disease.

| Variable | B | S.E. | EXP(B) | 95% C.I. EXP(B) Lower | Upper | p-value |
|---|---|---|---|---|---|---|
| Age | 0.045 | 0.056 | 0.059 | 0.796 | 0.427 | 0.045 |
| Sex | 0.128 | 0.973 | 0.010 | 0.132 | 0.895 | 0.128 |
| Education | 0.098 | 0.140 | 0.051 | 0.701 | 0.484 | 0.098 |
| APOE4 | -0.710 | 0.932 | -0.056 | -0.761 | 0.447 | -0.710 |
| G-CSF | -0.256 | 0.104 | -0.178 | -2.469 | 0.014 | -0.256 |

Multivariate logistic regression analysis of G-CSF and MMSE scores among Alzheimer's disease patients after stratification into tertiles on BDNF, CRP and thrombopoietin.

| Protein | Tertile | Unstandardized Coefficients B | Unstandardized Coefficients Std Error | Standardized Coefficients Beta | t | p-value |
|---|---|---|---|---|---|---|
| Thrombopoietin | Low | 0.024 | 0.182 | 0.018 | 0.132 | 0.896 |
| | Medium | -0.662 | 0.177 | -0.440 | -3.739 | <0.001** |
| | High | -0.252 | 0.207 | -0.159 | -1.218 | 0.228 |
| BDNF | Low | -0.280 | 0.184 | -0.182 | -1.515 | 0.135 |
| | Medium | -0.423 | 0.168 | -0.338 | -2.524 | 0.015* |
| | High | -0.132 | 0.186 | 0.086 | 0.712 | 0.479 |
| CRP | Low | -0.121 | 0.168 | -0.093 | -0.720 | 0.475 |
| | Medium | -0.069 | 0.174 | -0.051 | -0.399 | 0.692 |
| | High | -0.722 | 0.195 | -0.450 | -3.699 | <0.001** |

*Significant at p<0.05. ** Significant at p< 0.001.

## Methods

*Participants.*
Participants included 400 individuals (197 diagnosed with Probable AD and 203 cognitively normal controls; NC) enrolled in the TARC Longitudinal Research Cohort. Each participant completed an annual examination consisting of a medical examination, interview, blood draw, and neuropsychological testing at one of the five TARC sites. These data were reviewed by each site consensus committee and diagnosis is assigned according to NINCDS-ADRDA criteria (McKhann et al., 1984). Controls were judged to be within normal limits on neuropsychological testing by consensus review. Participants with AD were studied at a relatively early stage of the disease. CDR global scores were as follows: 0.5=49, 1=79, 2=53, 3=16. The TARC project received Institutional Review Board approval and all participants and/or caregivers signed written informed consent documents.

*Neuropsychological Testing.*
The TARC neuropsychology core battery includes digit span (WAIS-R, WAIS-III, WMS-R), Trail Making Test, WMS Logical Memory and Visual Reproduction (WMS-R and WMS-III), Boston Naming Test (30- and 60-item versions), verbal fluency (FAS), Clock Drawing Test, the American National Adult Reading Test (AMNART), the Geriatric Depression Scale (GDS-30), Mini-Mental State Examination (MMSE), and ratings on the Clinical Dementia Rating scale (CDR). All raw scores were converted to scale scores based on previously published normative data (Ivnik et al., 1992, Ivnik et al., 1996).

*Assays*
Non-fasting samples were collected in serum-separating tubes during clinical evaluations, allowed to clot at room temperature for 30 minutes, centrifuged, aliquoted, and stored at -80C. Samples were sent frozen to Rules Based Medicine (www.rulesbasedmedicine.com, Austin, TX) where they were thawed for assay without additional freeze-thaw cycles. Rules Based medicine conducted multiplexed immunoassay via their human Multi-Analyte profile (human MAP). For G-CSF, the least detectable dose (LDD) was 5 pg/ml, inter-run coefficient of variation was <10%, dynamic range was 1 – 5000 pg/mL, overall spiked standard recovery for serum was 70%, and cross-reactivity with other human MAP analytes was<1%.

*Analyses*
Statistical analyses were conducted using SPSS version 19.0 (IBM). Assessment of differences in G-CSF levels between diagnostic categories (AD vs. NC) and between G-CSF levels and disease progression (MMSE and CDR Global) was conducted by multivariate regression. In order to determine if serum G-CSF levels were significantly predictive of neuropsychological scores, linear regression models were created using serum G-CSF as the predictor variable and neuropsychological scores as the outcome variable. All regression models included age, gender, years of education, race and APOE4 carrier status as covariates. Statistical significance was declared for p-values <0.05, except for neuropsychological test scores, which were adjusted for multiple testing using a Bonferroni correction. Interactions between G-CSF and several pathways suspected to be involved in Alzheimer's disease pathophysiology were investigated in follow-up analyses. First, proteins were selected to represent the key pathways of inflammation (CRP), neurotrophic factors (BDNF) and vascular function (thrombopoietin). Next, the sample was split into thirds, using tertiles for each of the representative proteins. Finally, associations between serum G-CSF levels were re-assessed in the split sample as before.

## Discussion

G-CSF levels were significantly lower among AD participants, relative to cognitively normal controls. These results are in agreement with Laske and colleagues, who observed lower plasma G-CSF levels in 50 subjects diagnosed with early Alzheimer's disease relative to 50 cognitively intact controls (Laske et al., 2009).

In contrast to Laske et al, we found that increased serum G-CSF was significantly associated with greater disease severity, as indicated by lower MMSE and higher global CDR scores.

Post-hoc analysis of interactions between G-CSF and other serum proteins suggested that the association between G-CSF and disease severity was mediated by inflammation.

G-CSF was associated with MMSE and CDR scores only among AD patients that had neither high nor low levels of BDNF or thrombopoietin, suggesting that the role for G-CSF was not related to dysregulation of neurotrophic factors and vascular function. In contrast, the association between G-CSF and disease severity scores was only apparent among AD patients with elevated serum CRP, an indicator of inflammatory activation.

The alteration of levels in inflammatory proteins and hematopoietic growth factors in the development and progression of AD points to the complex etiology of the disease. The altered levels of G-CSF in AD patients supports a putative pharmacologic target for individuals that exhibit dysregulated levels of G-CSF.

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