Published in final edited form as:

Environ Int. 2016 March ; 88: 86–93. doi:10.1016/j.envint.2015.12.014.

Long-term Exposure to Black Carbon, Cognition and Single Nucleotide Polymorphisms in MicroRNA Processing Genes in Older Men

Elena Colicino¹, Giulia Giuliano¹, Melinda C Power^{1,2}, Johanna Lepeule¹, Elissa H Wilker^{1,3}, Pantel Vokonas⁴, Kasey JM Brennan¹, Serena Fossati^{1,5}, Mirjam Hoxha^{6,7}, Avron Spiro III⁴, Marc G Weisskopf^{1,2}, Joel Schwartz^{1,2}, and Andrea A Baccarelli^{1,2}

Elena Colicino: ecolicin@hsph.harvard.edu; Giulia Giuliano: g.giuliano6@campus.unimib.it; Melinda C Power: melinda.c.power@gmail.com; Johanna Lepeule: jlepeule@hsph.harvard.edu; Elissa H Wilker: ewilker@bidmc.harvard.edu; Pantel Vokonas: pantel.vokonas@va.gov; Kasey JM Brennan: kbrennan@hsph.harvard.edu; Serena Fossati: sfossati@hsph.harvard.edu; Mirjam Hoxha: mirjam.hoxha@unimi.it; Avron Spiro: aspiro3@bu.edu; Marc G Weisskopf: mweissko@hsph.harvard.edu; Joel Schwartz: joel@hsph.harvard.edu; Andrea A Baccarelli: abaccare@hsph.harvard.edu

¹Department of Environmental Health, Harvard T.H. Chan School of Public Health, 677 Huntington Avenue Boston, MA, 02115, USA

²Department of Epidemiology, Harvard T.H. Chan School of Public Health, 677 Huntington Avenue Boston, MA, 02115, USA

³Cardiovascular Epidemiology Research Unit, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA, 02215, USA

⁴VA Boston Healthcare System and Boston University Schools of Public Health and Medicine, 330 Brookline Avenue, Boston, MA 02215 USA

⁵Department of Biomedical and Clinical Sciences “L. Sacco”, University of Milan, Via Festa del Perdono, 7, 20122 Milano, Italy

⁶Department of Clinical Sciences and Community Health, University of Milan, Via Festa del Perdono, 7, 20122 Milano, Italy

⁷Epidemiology Unit, Department of Preventive Medicine, Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico, Via Francesco Sforza, 33, 20122 Milano, Italy

Abstract

Introduction—Air pollution exposure has been linked to impaired cognitive aging, but little is known about biomarkers modifying this association. MicroRNAs (miRNAs) control gene expression and neuronal programming. MiRNA levels vary due to single nucleotide polymorphisms (SNPs) in genes processing miRNAs from precursor molecules.

Correspondence to: Elena Colicino, ecolicin@hsph.harvard.edu.

Competing interests:

The authors declare that they have no competing interests.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Objectives—To investigate whether SNPs in miRNA-processing genes are associated with cognition and modify the relationship between black carbon (BC), marker of traffic-related pollution, and cognitive functions.

Methods—533 Normative Aging Study men (mean±SD 72±7 years) were tested 4 times (mean=1.7 times) using seven cognitive tests between 1995–2007. We tested interactions of 16 miRNA-related SNPs with 1-year average BC from a validated land-use-regression model. We used covariate-adjusted logistic regression for low (< 25) Mini-Mental State Examination (MMSE) and mixed-effect regression for a global cognitive score combining six other tests.

Results—Global cognition was negatively associated with the homozygous minor variant of rs595961 *AGO1* (−0.42SD; 95%CI: (−0.71, −0.13)) relative to the major variant. BC-MMSE association was stronger in heterozygous carriers of rs11077 *XPO5* (OR=1.99; 95%CI: (1.39, 2.85)) and minor variant carriers of *GEMIN4* rs2740348 (OR=1.34; 95%CI: (1.05, 1.7)), compared to their major variant. The BC-global-cognition association was stronger in heterozygous carriers of *GEMIN4* rs4968104 (−0.10SD; 95%CI: (−0.18, −0.02)), and *GEMIN4* rs910924 (−0.09SD; 95%CI: (−0.17, −0.02)) relative to the major variant. Blood miRNA expression analyses showed associations only of *XPO5* rs11077 with miR-9 and miR-96.

Conclusions—Carriers of particular miRNA-processing SNPs had higher susceptibility to BC in BC-cognition associations, possibly due to influences on miRNA expression.

Keywords

Air pollution; black carbon; cognitive function; single nucleotide polymorphisms; microRNA

1. INTRODUCTION

Epidemiology studies have linked air pollution exposure with lower cognitive performance and cognitive decline in older individuals (Ranft et al., 2009; Weuve et al., 2012). Black Carbon (BC) is a marker for primary particles from traffic. These particles are emitted near the ground in populated areas and hence have a higher intake fraction (i.e. a higher probability of getting into a lung) than other particles (Bahner et al., 2007). BC has been associated with lower overall performance on a cognitive battery in a cohort of older men (Power et al., 2011). However, the biological process by which air pollution may impact cognition is yet not fully known.

MicroRNAs (miRNAs) are short non-coding Ribonucleic Acids (RNA)s with key roles in gene expression and neuronal plasticity in the mature nervous system (Kosik, 2006). MiRNAs can inhibit gene expression by targeting complementary messenger RNAs (mRNAs) (Hou et al., 2011; Zhang, 2008). miRNA levels may vary due to single nucleotide polymorphisms (SNPs) in miRNA-processing genes, i.e., genes belonging in the machinery that generates mature miRNAs from longer miRNA precursors (Slezak-Prochazka et al., 2010). miRNAs control pathogenic pathways related to air pollution effects including angiogenesis (Suarez and Sessa, 2009), redox signaling (Brewer and Shah, 2009), and stress response (Leung and Sharp, 2010). Moreover, recent studies have implicated miRNAs as contributing factors in neurodegeneration, often linked with cognitive impairments in older individuals (Bushati and Cohen, 2008; Hebert and De Strooper, 2009).

Previous work in the VA Normative Aging Study (NAS) demonstrated an association between long-term exposure to BC and cognitive functions in the elderly (Power et al., 2011). Herein, we expand on previous analyses by testing the hypothesis that polymorphisms in genes that regulate miRNA-processing have an association with cognition and are able to modify the BC-cognition relationship. We examined an extended panel of SNPs in miRNA-processing genes in NAS participants. To explore the functional relevance of the miRNA-processing SNPs, we also evaluated – in a subset of the study participants – associations of the SNPs with the expression of miRNAs selected due to their roles in oxidative stress and inflammatory pathways. We report here that some of the SNPs in miRNA-processing genes were associated with cognition or modified the association of BC level with cognitive measures. The genes harboring the SNPs have been previously involved in age-related diseases, such as muscular atrophy and tumors, or with other clinical outcomes induced by BC exposure.

2. MATERIALS AND METHODS

2.1 Study sample

NAS is an ongoing longitudinal cohort study of aging men from the greater Boston, Massachusetts, area, established in 1963. Details on the NAS cohort were reported previously (Bell et al., 1966). Briefly, participants 21–81 years of age at entry were enrolled free of known chronic medical conditions and were invited to undergo an in-person examination every 3–5 years. At each visit, participants provided information on medical history, lifestyle, and demographic factors, and underwent a physical examination and laboratory tests. Starting in 1993, participants were invited to complete a battery of cognitive tests (Power et al., 2011). We excluded participants who had experienced a stroke before the first cognitive assessment (3.6% of individuals), leaving a total of 533 individuals with complete BC, SNP and covariate information. At each visit, written informed consent was provided by each participant as approved by the VA Boston Healthcare System Institutional Review Board (IRB). The study was approved by the IRBs of the participating institutions.

2.2 Cognitive testing

At each visit, participants completed a battery of cognitive tests, which included seven tests: the Mini-Mental State Examination (MMSE), the digit span backward test, a verbal fluency task, constructional praxis, immediate recall of a 10-word list, delayed recall of a 10-word list, and a computerized pattern comparison task. The present analysis includes cognitive data from visits between 1995–2007. We defined as baseline visit for each study participant the first cognitive assessment completed on or after July 31st 1995. Multiple assessments (up to 4) of cognitive testing were completed by most study participants (1.7 visits on average).

MMSE is a test of global cognition that assesses orientation, immediate and short-term recall, attention and calculation, word finding, construction reading and writing skills, and ability to follow a 3-step command. The range of scores is 0 to 30. The MMSE has been used in clinical practice and research as a dementia screening tool, and has been extensively validated (Tombaugh and McIntyre, 1992). In this study the maximum possible score on the MMSE was 29, because the question on the county of residence was excluded as counties in

Massachusetts have little political meaning and are generally not known and, thus, not of diagnostic utility (Tombaugh and McIntyre, 1992; Weisskopf et al., 2004).

The other tests assess a variety of domains. In particular digit span backward assesses working memory, though attention and comprehension also contribute to test performances. The range of the score is 0 to 12, based on the numbers read out and repeated correctly in reverse order. The Verbal Fluency task assesses language (vocabulary size, naming), response speed, mental organization, search strategies, short- and long-term memory, letting each participant say as many words as possible from a category in a given time. The constructional praxis test measures the ability to integrate visual-perceptual skills with a motor response. Using a pencil the participant is asked to copy paper drawings of geometric forms. For the immediate recall of a 10-word list, a list of 10 high-frequency, high-imagery words are read at a constant rate of 1 word every 2 seconds, and presented three times in a randomized order to the participant. At the end of each of the three presentations, the participant is asked to recall the list of words. For the delayed recall of a 10-word list the participants must spontaneously recall as many of the 10 words, read in the immediate recall, as she/he can. The pattern comparison task assesses speed of processing by asking participants to discern whether two side by side pictures, presented on a computer screen, are the same or not. The participant is given 90 seconds to respond to as many items as possible (up to a maximum of 130). These tests are part of established batteries, including the Consortium to Establish a Registry for Alzheimer Disease (CERAD) battery (Morris et al., 1989), the Wechsler Adult Intelligence Scales for Adults, Revised (WAIS-R) (Wechsler, 1981), the Neurobehavioral Evaluation System 2 (Letz, 1991), and the Developmental Test of Visual-Motor Integration (Beery, 1997).

2.3 Exposure assessment

We considered estimates of Black Carbon (BC) exposure at the residence of each participant to be surrogates for individual exposure to traffic-related air pollution. The daily estimates were obtained using a validated spatio-temporal land-use-regression model, starting in 1994 and covering the greater Boston, Massachusetts, area. A BC prediction model was constructed from daily average BC measure and predictors based on meteorological conditions (e.g., wind speed), land use (e.g., traffic density), daily BC concentrations at a central monitor, and other descriptors (e.g., day of the week). The model was previously described in detail (Gryparis et al., 2007). We averaged the 365 daily estimates prior to each cognitive assessment completed at the participant's residential address to estimate long-term exposure levels between subsequent visits. BC levels with longer time-window were highly correlated with the 1-year BC exposure levels (Table A.1).

2.4 Single Nucleotide Polymorphisms (SNPs) selection and genotyping

We selected the SNPs based on published work investigating associations between genes involved in miRNA-processing and chronic aging diseases (Horikawa et al., 2008; Yang et al., 2008). The SNPs were chosen because of their influence on pathways linked to clinical outcomes related to degeneration and aging: inflammation, apoptosis, pathological angiogenesis (cancer or macular degeneration) or defective angiogenesis (myocardial ischemia or peripheral vascular disease), and cell signaling (Horikawa et al., 2008; Wilker et

al., 2010). Genotyping was performed on blood DNA using assays designed with Sequenom SpectroDESIGNER software (Sequenom, Inc., San Diego, CA). The extension product was spotted onto a 384-well spectroCHIP followed by analysis in the MALDI-TOF mass spectrometer (Sequenom, Inc.). Duplication was performed on 5% of the samples. The 24 SNPs analyzed for this study were all successfully detected (Wilker et al., 2010). After genotyping, we excluded six SNPs for which the number of participants with homozygous minor variant carriers were less than 10 (rg1106042 in *HIWI*, rs3742330 in *DICER1*, rs417309 in DiGeorge critical region-8 (*DGCR8*), rs636832 in Argonaute 1 (*AGO1*), rs197414 in *DDX20*, rs3744741 in *GEMIN4*), one with a Hardy – Weinberg p-value <0.05 (rs10719 in *DROSHA*), and one with collinearity issue with other covariates (rs197388 in *DDX20*), leaving a total of 16 miRNAs-related SNPs in 10 genes. Linkage disequilibrium (LD) between SNPs in the same gene was previously assessed using LDPlotter tool (<https://www.pharmgat.org/Tools/pbtoldplotfrom>) (Wilker et al., 2010).

2.5 Expression Analysis of candidate MicroRNAs (miRNAs)

We selected a subset of 93 NAS participants due to availability of blood samples specifically collected for RNA isolation. From each sample we measured the expression of 14 candidate miRNAs (Fossati et al., 2014). The selected miRNAs were expressed in blood leukocytes, related to inflammation and oxidative stress, and chosen on the basis of previous literature that highlighted them as differentially expressed in neurodegenerative diseases (Saito and Saito, 2012), and involvement in pathways that impact brain integrity and disease (Abe and Bonini, 2013; Im and Kenny, 2012). MiRNA expression was measured using real-time PCR, as described in the supplemental material.

2.6 Statistical analysis

A sizeable proportion of participants (14.7% of observations) achieved the maximum score in the MMSE and only 8.4% of our observations exhibited scores ≤ 24 , the canonical cutoff value for dementia, thus revealing a ceiling effect for MMSE scores. Therefore, as in our previous work (Power et al., 2011), we considered scores ≤ 25 as low performance (18.3 % of observations). A dichotomous variable for low MMSE performance was created and used in all analyses. The remaining tests were converted into z-scores, using the mean and standard deviation from the baseline. These continuous z-score measures were used in subsequent analyses, with higher scores indicating better performance than the population average at baseline (Power et al., 2011).

We first used logistic regression with generalized estimating equations and empirical variance estimates to account for repeated visits within individuals to estimate the main associations with each SNP, as well as the main association with BC, on the odds of low MMSE. We then evaluated the interaction between BC and each SNP in relation to the odds of low MMSE by adding into the models a multiplicative interaction term between BC and each SNP. Also, we used random-effects linear mixed models to estimate associations with global cognitive function derived from the remaining cognitive tests. As for the MMSE analysis, these models accounted for the multiple visits by fitting a random intercept for the study participant. The models for global cognitive function also fitted a random intercept for each of the multiple cognitive scores for each visit, thus treating each cognitive test score as

a repeated measure of underlying total cognition. The analysis provides one global hypothesis tests for the association between BC and global cognition. Main associations and interactions were fitted in the global cognition models as described above for MMSE.

All models were adjusted for potential confounders or predictors of cognitive function selected *a priori* from previous literature and their significance in each model, including age at cognitive assessment as a continuous variable, and several variables assessed at the baseline visit, i.e., education (<12, 12–16, >16 years), alcohol intake (<2 drinks/day, 2–30 drinks/day), smoking status (never, former, current), physical activity (<12, 12–30, >30 metabolic equivalent hours [MET-hr] per week), obesity (<25, ≥25 body mass index [BMI] Kg/m²), dark fish consumption (<once a week, ≥once a week), computer experience (yes/no), first language (English/not English), percentage of the participant's census tract that is nonwhite, and percentage of the adult residents in the participant's census tract with at least a college degree, an indicator for whether the cognitive data were from the participant's first cognitive assessment (yes/no), and an indicator for whether the participant was a part-time resident of the greater Boston area (yes/no).

Because the relationship between BC and cognitive function in this cohort has been previously shown to be loglinear (Power et al., 2011), we used log-transformed BC in all analyses. To explore the functional relevance of the miRNA-processing SNPs, we evaluated the association of the SNPs with miRNA expression using Student's t-tests because of the relatively high number of SNPs evaluated. All results were considered noteworthy at False Discovery Rates (FDRs) <0.15.

As sensitivity analysis we applied the inverse probability weighting technique to all analysis in order to correct for survival and loss to follow-up bias (Lepeule et al., 2014). We used SAS (version 9.2; SAS Institute Inc., Cary, NC) for all analyses.

3. RESULTS

3.1 Data and Study Participants Characteristics

Among the 533 participants, the number of individuals completing at least one assessment of cognitive testing varied across the cognitive tests as follows: MMSE (n=525), digit span backward test (n=520), verbal fluency task (n=527), constructional praxis (n=484), immediate recall of a 10-word list (n=526), delayed recall of a 10-word list (n=524), and pattern comparison task (n=504).

Table 1 summarizes the baseline characteristics of the sample. The mean age at baseline was 72 years (SD=7; range, 53–97 years). Most men permanently lived in the greater Boston area (96%), had at least some college or graduate-level education (71%), were obese (77%), had hypertension (67%) and were not affected by diabetes mellitus (84%). On the natural scale, 1-year average BC exposure estimates ranged from 0.02 to 1.90 µg/m³ (mean±SD, 0.51±0.26µg/m³) and exhibited a right skewed distribution (for details see (Power et al., 2011)). We log-transformed BC (ln(BC)) and reported associations for a doubling increase in BC concentration on the natural scale, or approximately a 0.69 unit change in ln(BC).

3.2 BC associations with cognition and modification by miRNA-processing SNPs

Each doubling increase in BC concentration on the natural scale was associated with 1.35 times higher odds (95%CI: (1.07, 1.70), Figure 1A; Table A.2; Figure A.1A) of low MMSE score adjusted for clinical and lifestyle factors. BC was not significantly associated with the global cognition scores (a doubling increase in BC level was associated with a change in the global cognition score to -0.01 of its Standard Deviation (SD); 95%CI: $(-0.06, 0.04)$, Figure 1B; Table A.2; Figure A.1B). Global cognition was negatively associated with the homozygous minor variant carriers of rs595961 *AGO1* (-0.42 SD; 95%CI: $(-0.71, -0.13)$) relative to the major variant (Figure 1; Tables A.3–A.4). The BC-cognition relationships were significantly modified by specific miRNA-processing SNPs (Figure 1; Tables A.5–A.6; Figure A.1). In particular, the association of BC with low MMSE was larger in carriers of *XPO5* rs11077 heterozygous variants (OR=1.99; 95%CI: (1.39, 2.85)) than in individuals with the major homozygous carriers (OR=1.20; 95%CI: (0.59, 2.43); p-value for the interaction 0.01, FDR=0.09) (Figure 1A; Table A.5). Also, BC association was stronger in homozygous minor variant carriers *GEMIN4* rs2740348 (OR=1.34; 95%CI: (1.05, 1.70)) than in homozygous minor variant carriers (OR=4.30; 95%CI: (1.86, 9.98); p-value for the interaction 0.01, FDR=0.13) (Figure 1A; Table A.5). There was a significant association of BC with the global cognition scores among heterozygous variant carriers of *GEMIN4* rs4968104 (-0.10 SD for a doubling in BC concentration; 95%CI: $(-0.18, -0.02)$) and among heterozygous variant carriers of *GEMIN4* rs910924 (-0.09 SD a doubling in BC concentration; 95%CI: $(-0.17, -0.02)$) compared with their homozygous major variant genotype (Figure 1B; Table A.6). Additional analyses that adjusted only for age and education were similar to those reported in Tables A.3–A.6 (data not shown). Sensitivity analyses that included weights to correct for survival and loss to follow-up bias did not show major departures from previous results (Tables A.7–A.10).

3.3 Association between miRNA-processing SNPs and the expression of candidate miRNAs

To explore the functional relevance of the miRNA-processing SNPs that were found to modify the BC-cognition relationship, we examined the blood expression levels of 14 selected miRNAs (Table 2). Only *XPO5* rs11077 was positively associated with miR-9 (p-value=0.02, FDR=0.14) and miR-96 (p-value=0.02, FDR=0.14).

4. DISCUSSION

In the present investigation we observed that selected SNPs in some miRNA-processing genes were associated with cognition and modified the association between long-term exposure to BC and cognitive function in a cohort of older men. We also showed that one of the miRNA-processing SNPs that modified BC-cognition relationship also affected miRNA expression levels in blood samples. To our knowledge this is the first study to investigate the role of polymorphisms in miRNA-processing genes as modifiers of the association between traffic-related air pollution and cognition.

Increasing evidence has demonstrated that miRNAs play crucial roles in nervous system developmental phenomena such as neural patterning, establishment and maintenance of cell

identity, as well as adult neurogenesis (Coolen and Bally-Cuif, 2009; Sayed and Abdellatif, 2011). More recently, multiple studies have shown the involvement of miRNA function in neuronal plasticity in the adult and aging nervous system, including the regulation of synaptic protein synthesis, dendritic spine morphogenesis, and plasticity-related diseases. Evidence for miRNA involvement in physiological higher-order brain functions such as learning, memory, and emotions, as well as mental illness, is also emerging (Bredy et al., 2011; Forero et al., 2010; Salta and De Strooper, 2012).

Our analysis focused on potentially functional SNPs located in genes involved in the biogenesis and processing of miRNAs. Bartel has described the transcription and multistep processing of miRNAs in detail elsewhere (Bartel, 2004). In general, miRNAs are generated from primary-miRNAs (pri-miRNAs) of approximately 100 nucleotides (nt). Through several biological steps mediated by specific catalytic proteins, the pri-miRNAs are processed and shortened into pre-miRNAs, which are transferred from the nucleus to the cytoplasm and eventually modified in ~22 nt mature miRNAs. Finally, specific protein complexes guide the mature miRNAs into the RNA-induced silencing complex (RISC), where each miRNA hybridizes to a complementary sequence of a target mRNA, thus leading to degradation of the mRNA and/or suppression of its translation. In our study, five SNPs in three of the genes encoding for proteins (AGO1, XPO5, GEMIN4), operating at various levels between pri-miRNAs generation and mRNA targeting, appeared to be associated with lower global cognition and modify the BC-cognition relationship. In particular, we showed a negative association between SNP in *AGO1* rs595961 and global cognition. We presented interactions of BC with SNPs in *XPO5* rs11077 and *GEMIN4* rs2740348 in relation to low MMSE scores. Additionally SNPs in *GEMIN4* rs4968104 and rs910924 were found to modify the association between BC and the global score of cognitive function.

XPO5 belongs to a large family of karyopherins and mediates the nuclear transport of pre-miRNAs between the nuclear and cytoplasmic compartments. In fact, *XPO5* inactivation, which was first identified in human tumors with microsatellite instability, has been shown to cause the trapping of pre-miRNAs in the nucleus and impair the production of mature miRNAs (Melo et al., 2010). *GEMIN4* contributes to the processing of pre-miRNA by introducing the miRNA precursor into the RISC (Murashov et al., 2007). *GEMIN4* is a core member of the survival of motor neurons (SMN) complex, which is a protein involved in the assembly of small nuclear ribonucleic particles (Cauchi, 2010). A lack of SMN results in widespread splicing defects, especially in spinal motor neurons, and is one cause of spinal muscular atrophy (Charroux et al., 2000). It is worth noting that SNPs in *GEMIN4*, albeit different from the SNPs that modify the BC-cognition association in the present study, have been previously shown to modify the BC-blood pressure relationship (Wilker et al., 2010). Our results indicate that SNPs in *GEMIN4* may have cross-system relevance for clinical outcomes induced by BC exposure. *AGO1* is a core component of the complexes which mediate transcriptional gene silencing in human cells (Kim et al., 2006). *AGO1* was identified as being involved in Wilms' tumors, caused by defects in embryonic kidney development (Carmell et al., 2002) and these kidney defects in children have recently been linked to neurodevelopmental delay and mental illness (Sanna-Cherchi et al., 2012).

For the significant SNPs, we also evaluated their relation to the expression levels in blood of 14 candidate miRNAs. We found that only the SNP in *XPO5* rs11077 was positively associated with the blood expression levels of miR-96 and miR-9. MiR-96 is co-expressed with miR-182 and miR-183 in neurosensory cells and organs, including photoreceptor cells in the eye and hair cells in the ear (Weston et al., 2011; Weston et al., 2006; Wienholds et al., 2005; Xu et al., 2007), which are key components in the peripheral nervous system. Our data suggest that miR-96 expression may represent a pathway through which the SNP could modify the BC-cognition associations. MiR-9 is also highly expressed in the brain and has been indicated to regulate neuronal differentiation (Delaloy et al., 2010). Based on the observation of its downregulation in Huntington's disease, miR-9 has been suggested to be involved in neurodegenerative responses (Packer et al., 2008). Taken together, these previous observations support potential critical roles for the genes and the miRNAs identified in the present work. Our analysis of miRNA expression was conducted in peripheral blood samples and only on a subset of individuals. Further studies are warranted on larger study samples and on more relevant tissues such as for instance brain tissues.

Our study is subject to a number of limitations. Because we performed a high number of statistical tests, caution about false positive findings is warranted. To reduce false positives, we computed the FDRs and we considered noteworthy only the findings at $FDR < 0.15$. We adjusted our results for an extended list of predictors of cognitive function and potential confounders. However, it is possible that other unmeasured variables might have affected our results. To represent personal exposures of traffic-related pollution, we used geospatial models of ambient BC. Prior research indicated that in evaluating air pollution effects most errors are of Berkson type. Classical simulation studies have shown that it is highly unlikely to bias away from the null even in the presence of covariates and indicates that this exposure misclassification may lead to an underestimation of the health effect of air pollution (Zeger et al., 2000). In addition several studies, including one conducted in the Greater Boston area, demonstrated that longitudinal variations of ambient particulate concentrations are representative of longitudinal differences in personal exposure (Rojas-Bracho et al., 2000). BC concentration levels are spatially heterogeneous because of the numerous local (mobile) sources. Therefore, measurement error in our BC exposure metric would likely attenuate the true association. Given that we found significant associations for BC, it is unlikely that this error would affect our conclusions. The data used in the present study differ from our previous work in the NAS (Power et al., 2011) in that we restricted the sample to study participants with SNPs information. Due to this restriction, we did not observe a significant main association of BC with the total cognition scores, possibly due – at least in part – to decreased statistical power. The analyses of miRNA expression were conducted on smaller subset of the study population and have therefore limited statistical power. Finally, since our work was based on a cohort of older, mostly white, men, our findings may apply only to populations with similar characteristics. Additional studies are warranted to confirm our results among females and other ethnicities.

5. CONCLUSIONS

Our results indicate that specific SNPs in miRNAs-processing genes may determine differential susceptibility in the relationship between traffic particles and cognitive

impairments in a population of elderly men. Although more research is needed to clarify the mechanisms underlying the association between BC and neuro-degeneration, these results provide novel hypotheses about the toxic effects of traffic pollution, as measured by BC, and contribute to growing understanding of the roles of miRNAs in relation to environmental stressors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

EC is supported by a grant from the NIEHS (R01ES021733). Other support comes was provided by NIEHS grants R01ES015172, R01ES014663 and R21ES020010, and EPA grant RD832416. Cognitive data collection at the VA NAS was supported by a VA Merit Review and a CSR&D Research Career Scientist award to AS. Additional support was provided by the US Department of Agriculture, Agricultural Research Service (contract 53-K06-510). The VA Normative Aging Study is supported by the Cooperative Studies Program/ERIC, US Department of Veterans Affairs, and is a research component of the Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC). The views expressed in this paper are those of the authors and do not necessarily represent the views of the US Department of Veterans Affairs.

List of Abbreviations

BC	Black Carbon
CERAD	Consortium to Establish a Registry for Alzheimer Disease
CI	Confidence Interval
WAIS-R	Wechsler Adult Intelligence Scales for Adults, Revised
FDR	False Discovery Rate
miRNA	microRNA
mRNA	messenger RNA
MET-hr	metabolic equivalent hours
MMSE	Mini-Mental State Examination
NAS	Normative Aging Study
nt	nucleotide
OR	Odds Ratio
pri-miRNA	Primary-miRNA
RISC	RNA-induces silencing complex
RNA	Ribonucleic Acid
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
SMN US	Survival of Motor Neurons

VA Department of Veterans Affairs

References

- Abe M, Bonini NM. MicroRNAs and neurodegeneration: role and impact. *Trends in cell biology*. 2013; 23:30–36.10.1016/j.tcb.2012.08.013 [PubMed: 23026030]
- Bahner, MA.; Weitz, KA.; Zapata, A.; Deangelo, B. Use of Black Carbon and Organic Carbon Inventories for Projections and Mitigation Analysis. *Emission Inventories: Integration, Analysis, and Communications 16th Annual International Emission Inventory Conference*; Raleigh, NC. May 14–17; 2007.
- Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004; 116(2):281–297. [PubMed: 14744438]
- Beery, K. The Beery-Buktenica Developmental Test of Visual-Motor Integration: VMI, with Supplemental Developmental Tests of Visual Perception and Motor Coordination: Administration, Scoring and Teaching: Administration, scoring and teaching manual. Parsippany, NJ: Modern Curriculum Press; 1997.
- Bell B, Rose CL, Damon A. The Veterans Administration longitudinal study of healthy aging. *The Gerontologist*. 1966; 6:179–184. [PubMed: 5342911]
- Bredy TW, Lin Q, Wei W, Baker–Andresen D, Mattick JS. MicroRNA regulation of neural plasticity and memory. *Neurobiology of Learning and Memory*. 2011; 96:89–94.10.1016/j.nlm.2011.04.004 [PubMed: 21524708]
- Brewer AC, Shah AM. Redox signalling and miRNA function in cardiomyocytes. *Journal of Molecular and Cellular Cardiology*. 2009; 47:2–4.10.1016/j.yjmcc.2009.02.024 [PubMed: 19281814]
- Bushati N, Cohen SM. MicroRNAs in neurodegeneration. *Current Opinion in Neurobiology*. 2008; 18:292–296.10.1016/j.conb.2008.07.001 [PubMed: 18662781]
- Carmell MA, Xuan Z, Zhang MQ, Hannon GJ. The Argonaute family: tentacles that reach into RNAi, developmental control, stem cell maintenance, and tumorigenesis. *Genes & Development*. 2002; 16:2733–2742.10.1101/gad.1026102 [PubMed: 12414724]
- Cauchi RJ. SMN and Gemins: 'we are family'... or are we?: insights into the partnership between Gemins and the spinal muscular atrophy disease protein SMN. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*. 2010; 32:1077–1089.10.1002/bies.201000088
- Charroux B, Pellizzoni L, Perkinson RA, Yong J, Shevchenko A, Mann M, Dreyfuss G. Gemin4. A novel component of the SMN complex that is found in both gems and nucleoli. *The Journal of Cell Biology*. 2000; 148:1177–1186. [PubMed: 10725331]
- Coolen M, Bally-Cuif L. MicroRNAs in brain development and physiology. *Current Opinion in Neurobiology*. 2009; 19:461–470.10.1016/j.conb.2009.09.006 [PubMed: 19846291]
- Delalay C, Liu L, Lee JA, Su H, Shen F, Yang GY, Young WL, Ivey KN, Gao FB. MicroRNA-9 coordinates proliferation and migration of human embryonic stem cell-derived neural progenitors. *Cell Stem Cell*. 2010; 6:323–335.10.1016/j.stem.2010.02.015 [PubMed: 20362537]
- Forero DA, van der Ven K, Callaerts P, Del-Favero J. miRNA genes and the brain: implications for psychiatric disorders. *Human Mutation*. 2010; 31:1195–1204.10.1002/humu.21344 [PubMed: 20725930]
- Fossati S, Baccarelli A, Zanobetti A, Hoxha M, Vokonas PS, Wright RO, Schwartz J. Ambient particulate air pollution and microRNAs in elderly men. *Epidemiology*. 2014; 25:68–78.10.1097/EDE.000000000000026 [PubMed: 24257509]
- Gryparis A, Coull B, Schwartz J, Suh HH. Semiparametric latent variable regression models for spatiotemporal modelling of mobile source particles in the greater Boston area. *Journal of the Royal Statistical Society: Series C*. 2007; 56(2):183–209.
- Hebert SS, De Strooper B. Alterations of the microRNA network cause neurodegenerative disease. *Trends in Neurosciences*. 2009; 32:199–206.10.1016/j.tins.2008.12.003 [PubMed: 19268374]

- Horikawa Y, Wood CG, Yang H, Zhao H, Ye Y, Gu J, Lin J, Habuchi T, Wu X. Single nucleotide polymorphisms of microRNA machinery genes modify the risk of renal cell carcinoma. *Clinical Cancer Research: an Official Journal of the American Association for Cancer Research*. 2008; 14:7956–7962.10.1158/1078-0432.CCR-08-1199 [PubMed: 19047128]
- Hou L, Wang D, Baccarelli A. Environmental chemicals and microRNAs. *Mutation Research*. 2011; 714:105–112.10.1016/j.mrfmmm.2011.05.004 [PubMed: 21609724]
- Im HI, Kenny PJ. MicroRNAs in neuronal function and dysfunction. *Trends in Neurosciences*. 2012; 35:325–334.10.1016/j.tins.2012.01.004 [PubMed: 22436491]
- Kim DH, Villeneuve LM, Morris KV, Rossi JJ. Argonaute-1 directs siRNA-mediated transcriptional gene silencing in human cells. *Nature Structural & Molecular Biology*. 2006; 13:793–797.10.1038/nsmb1142
- Kosik KS. The neuronal microRNA system. *Nature reviews Neuroscience*. 2006; 7:911–920.10.1038/nrn2037 [PubMed: 17115073]
- Lepeule J, Bind MA, Baccarelli AA, Koutrakis P, Tarantini L, Litonjua A, Sparrow D, Vokonas P, Schwartz JD. Epigenetic influences on associations between air pollutants and lung function in elderly men: the normative aging study. *Environmental Health Perspectives*. 2014; 122:566–572.10.1289/ehp.1206458 [PubMed: 24602767]
- Letz R. Use of computerized test batteries for quantifying neurobehavioral outcomes. *Environmental Health Perspectives*. 1991; 90:195–198. [PubMed: 2050061]
- Leung AK, Sharp PA. MicroRNA functions in stress responses. *Molecular Cell*. 2010; 40:205–215.10.1016/j.molcel.2010.09.027 [PubMed: 20965416]
- Melo SA, Moutinho C, Ropero S, Calin GA, Rossi S, Spizzo R, Fernandez AF, Davalos V, Villanueva A, Montoya G, Yamamoto H, Schwartz S Jr, Esteller M. A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells. *Cancer Cell*. 2010; 18:303–315.10.1016/j.ccr.2010.09.007 [PubMed: 20951941]
- Morris JC, Heyman A, Mohs RC, Hughes JP, van Belle G, Fillenbaum G, Mellits ED, Clark C. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology*. 1989; 39:1159–1165. [PubMed: 2771064]
- Murashov AK, Chintalgattu V, Islamov RR, Lever TE, Pak ES, Sierpinski PL, Katwa LC, Van Scott MR. RNAi pathway is functional in peripheral nerve axons. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*. 2007; 21:656–670.10.1096/fj.06-6155com [PubMed: 17209129]
- Packer AN, Xing Y, Harper SQ, Jones L, Davidson BL. The bifunctional microRNA miR-9/miR-9* regulates REST and CoREST and is downregulated in Huntington's disease. *The Journal of Neuroscience: the Official Journal of the Society for Neuroscience*. 2008; 28:14341–14346.10.1523/JNEUROSCI.2390-08.2008 [PubMed: 19118166]
- Power MC, Weisskopf MG, Alexeeff SE, Coull BA, Spiro A 3rd, Schwartz J. Traffic-related air pollution and cognitive function in a cohort of older men. *Environmental Health Perspectives*. 2011; 119:682–687.10.1289/ehp.1002767 [PubMed: 21172758]
- Ranft U, Schikowski T, Sugiri D, Krutmann J, Krämer U. Long-term exposure to traffic-related particulate matter impairs cognitive function in the elderly. *Environmental Research*. 2009; 109(8):1004–11. [PubMed: 19733348]
- Rojas-Bracho L, Suh HH, Koutrakis P. Relationships among personal, indoor, and outdoor fine and coarse particle concentrations for individuals with COPD. *Journal of Exposure Analysis and Environmental Epidemiology*. 2000; 10:294–306. [PubMed: 10910121]
- Saito Y, Saito H. MicroRNAs in cancers and neurodegenerative disorders. *Frontiers in Genetics*. 2012; 3:194.10.3389/fgene.2012.00194 [PubMed: 23056009]
- Salta E, De Strooper B. Non-coding RNAs with essential roles in neurodegenerative disorders. *The Lancet Neurology*. 2012; 11:189–200.10.1016/S1474-4422(11)70286-1 [PubMed: 22265214]
- Sanna-Cherchi S, Kiryluk K, Burgess KE, Bodria M, Sampson MG, Hadley D, Nees SN, Verbitsky M, Perry BJ, Sterken R, Lozanovski VJ, Materna-Kiryluk A, Barlassina C, Kini A, Corbani V, Carrea A, Somenzi D, Murtas C, Ristoska-Bojkovska N, Izzi C, Bianco B, Zaniew M, Flogelova H, Weng PL, Kacak N, Giberti S, Gigante M, Arapovic A, Drnasin K, Caridi G, Curioni S, Allegrì F,

- Ammenti A, Ferretti S, Goj V, Bernardo L, Jobanputra V, Chung WK, Lifton RP, Sanders S, State M, Clark LN, Saraga M, Padmanabhan S, Dominiczak AF, Foroud T, Gesualdo L, Gucev Z, Allegrì L, Latos-Bielenska A, Cusi D, Scolari F, Tasic V, Hakonarson H, Ghiggeri GM, Gharavi AG. Copy-number disorders are a common cause of congenital kidney malformations. *American Journal of Human Genetics*. 2012; 91:987–997.10.1016/j.ajhg.2012.10.007 [PubMed: 23159250]
- Sayed D, Abdellatif M. MicroRNAs in development and disease. *Physiological Reviews*. 2011; 91:827–887.10.1152/physrev.00006.2010 [PubMed: 21742789]
- Slezak-Prochazka I, Durmus S, Kroesen BJ, van den Berg A. MicroRNAs, macrocontrol: regulation of miRNA processing. *RNA*. 2010; 16:1087–1095.10.1261/rna.1804410 [PubMed: 20423980]
- Suarez Y, Sessa WC. MicroRNAs as novel regulators of angiogenesis. *Circ Res*. 2009; 104:442–454.10.1161/CIRCRESAHA.108.191270 [PubMed: 19246688]
- Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. *Journal of the American Geriatrics Society*. 1992; 40:922–935. [PubMed: 1512391]
- Wechsler, D. WAIS-R: Manual: Wechsler Adult Intelligence Scale-Revised. San Antonio, TX: Psychological Corporation; 1981.
- Weisskopf MG, Wright RO, Schwartz J, Spiro A 3rd, Sparrow D, Aro A, Hu H. Cumulative lead exposure and prospective change in cognition among elderly men: the VA Normative Aging Study. *American Journal of Epidemiology*. 2004; 160:1184–1193.10.1093/aje/ [PubMed: 15583371]
- Weston MD, Pierce ML, Jensen-Smith HC, Fritsch B, Rocha-Sanchez S, Beisel KW, Soukup GA. MicroRNA-183 family expression in hair cell development and requirement of microRNAs for hair cell maintenance and survival. *Developmental Dynamics: an Official Publication of the American Association of Anatomists*. 2011; 240:808–819.10.1002/dvdy.22591 [PubMed: 21360794]
- Weston MD, Pierce ML, Rocha-Sanchez S, Beisel KW, Soukup GA. MicroRNA gene expression in the mouse inner ear. *Brain Research*. 2006; 1111:95–104.10.1016/j.brainres.2006.07.006 [PubMed: 16904081]
- Weuve J, Puett RC, Schwartz J, Yanosky JD, Laden F, Grodstein F. Exposure to particulate air pollution and cognitive decline in older women. *Archives of Internal Medicine*. 2012; 172:219–227.10.1001/archinternmed.2011.683 [PubMed: 22332151]
- Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E, de Bruijn E, Horvitz HR, Kauppinen S, Plasterk RH. MicroRNA expression in zebrafish embryonic development. *Science*. 2005; 309:310–311.10.1126/science.1114519 [PubMed: 15919954]
- Wilker EH, Baccarelli A, Suh H, Vokonas P, Wright RO, Schwartz J. Black carbon exposures, blood pressure, and interactions with single nucleotide polymorphisms in MicroRNA processing genes. *Environmental health perspectives*. 2010; 118:943–948.10.1289/ehp.0901440 [PubMed: 20211803]
- Xu S, Witmer PD, Lumayag S, Kovacs B, Valle D. MicroRNA (miRNA) transcriptome of mouse retina and identification of a sensory organ-specific miRNA cluster. *The Journal of Biological Chemistry*. 2007; 282:25053–25066.10.1074/jbc.M700501200 [PubMed: 17597072]
- Yang H, Dinney CP, Ye Y, Zhu Y, Grossman HB, Wu X. Evaluation of genetic variants in microRNA-related genes and risk of bladder cancer. *Cancer research*. 2008; 68:2530–2537.10.1158/0008-5472.CAN-07-5991 [PubMed: 18381463]
- Zeger SL, Thomas D, Dominici F, Samet JM, Schwartz J, Dockery D, Cohen A. Exposure measurement error in time-series studies of air pollution: concepts and consequences. *Environmental Health Perspective*. 2000; 108(5):419–426.
- Zhang C. MicroRNAs: role in cardiovascular biology and disease. *Clinical Science*. 2008; 114:699–706.10.1042/CS20070211 [PubMed: 18474027]

Highlights

- Black Carbon (BC), marker of air pollution, was adversely associated with cognition
- SNPs in miRNAs processing-genes were adversely associated with cognition
- SNPs in miRNAs processing-genes affected susceptibility to BC-cognition association
- Susceptibility possibly due to influences on miRNA expression

FIGURE 1. Association of Black Carbon (BC) with cognitive functions in all participants and by selected Single Nucleotide Polymorphisms in miRNA-processing genes

The figure shows the association of a doubling increase estimated ambient BC concentration with lower Mini-Mental State Examination (MMSE) (Panel A) and on global cognitive function (Panel B), in all subjects or among carriers of selected SNPs in miRNA-processing genes.

*Significant (FDR<0.15) two-sided *p*-value.

Table 1Baseline characteristics of the Normative Aging Study (NAS) cohort ($n=533$).

Characteristics	N (%)	BC concentration (mean $\pm$ SD)
Age (years)		
50–59	35 (6.6)	0.58 $\pm$ 0.31
60–69	223 (41.8)	0.59 $\pm$ 0.27
70–79	216 (40.5)	0.58 $\pm$ 0.25
80–89	56 (10.5)	0.57 $\pm$ 0.30
>90	3 (0.6)	0.47 $\pm$ 0.29
Education (years)		
<12	156 (29.3)	0.63 $\pm$ 0.29
12 to 16	266 (49.9)	0.57 $\pm$ 0.26
>16	111 (20.8)	0.54 $\pm$ 0.25
First Language		
English	460 (86.3)	0.58 $\pm$ 0.27
Not English/bilingual	73 (13.7)	0.59 $\pm$ 0.25
Permanent Resident		
Yes	511 (96.0)	0.48 $\pm$ 0.21
No	22 (4.0)	0.59 $\pm$ 0.27
Computer Experience		
No	312 (58.5)	0.61 $\pm$ 0.28
Yes	221 (41.5)	0.55 $\pm$ 0.25
Physical activity (MET-hr/week)		
<12	300 (56.3)	0.58 $\pm$ 0.27
12 to <30	144 (27.0)	0.60 $\pm$ 0.27
30	89 (16.7)	0.57 $\pm$ 0.25
Alcohol (drinks/day)		
<2	403 (75.6)	0.59 $\pm$ 0.28
2	130 (24.4)	0.57 $\pm$ 0.23
Consumed dark fish (times/week)		
<1	455 (85.4)	0.59 $\pm$ 0.28
1	78 (14.6)	0.55 $\pm$ 0.22
Nonwhite (% of census tract)		
<5%	205 (38.5)	0.55 $\pm$ 0.31
5 to <10%	148 (27.8)	0.53 $\pm$ 0.22
10%	180 (33.8)	0.67 $\pm$ 0.24
25 years of age with at least a college degree (% of census tract)		
<30%	166 (31.1)	0.63 $\pm$ 0.29
30 to <50%	211 (39.6)	0.54 $\pm$ 0.27
50%	156 (29.3)	0.59 $\pm$ 0.23
Smoking Status		
never	153 (28.7)	0.56 $\pm$ 0.23

Characteristics	N (%)	BC concentration (mean±SD)
current	26 (4.9)	0.61±0.19
former	354 (66.4)	0.61±0.19
BMI (Kg/m²)		
<25	123 (23.1)	0.60±0.27
25	410 (76.9)	0.58±0.27
Hypertension		
No	176 (33.0)	0.56±0.28
Yes	357 (67.0)	0.59±0.26
Diabetes		
No	450 (84.4)	0.58±0.27
Yes	83 (15.6)	0.60±0.25

Table 2

Associations between SNPs in microRNA processing-genes and blood expression of candidate miRNAs^a.

miRNA	rs11077 in <i>XPO5</i>					
	AA Major Variant		CC Minor Variant		AC Heterozygous	
	Mean (SE)		Mean (SE)	p-value ^b	Mean (SE)	FDR
<i>miR-21</i>	1.43 (0.33)		1.46 (0.45)	0.96	1.71 (0.30)	0.54 0.88
<i>miR-222</i>	1.58 (0.30)		1.11 (0.40)	0.35	1.71 (0.26)	0.73 0.93
<i>miR-1</i>	1.09 (0.29)		0.89 (0.39)	0.67	0.70 (0.25)	0.31 0.87
<i>miR-125a_5p</i>	2.04 (0.36)		1.21 (0.48)	0.17	1.81 (0.32)	0.63 0.88
<i>miR-125b</i>	1.13 (0.30)		0.87 (0.41)	0.60	1.57 (0.27)	0.28 0.87
<i>miR-126</i>	1.17 (0.21)		1.13 (0.28)	0.90	1.32 (0.19)	0.61 0.88
<i>miR-128</i>	1.87 (0.53)		1.02 (0.72)	0.34	1.90 (0.47)	0.97 0.99
<i>miR-135a</i>	0.73 (0.13)		0.56 (0.18)	0.46	0.73 (0.12)	0.97 0.99
<i>miR-146a</i>	1.25 (0.28)		1.16 (0.37)	0.85	1.54 (0.25)	0.44 0.88
<i>miR-147</i>	4.01 (3.57)		0.95 (4.83)	0.61	7.67 (3.16)	0.44 0.88
<i>miR-218</i>	0.99 (0.89)		0.65 (1.20)	0.82	2.66 (0.79)	0.16 0.75
<i>miR-9</i>	0.68 (0.11)		0.94 (0.15)	0.15	1.03 (0.10)	0.02 0.14
<i>miR-96</i>	1.37 (0.41)		1.46 (0.55)	0.90	2.64 (0.36)	0.02 0.14
<i>miR-155</i>	2.37 (0.40)		2.27 (0.54)	0.88	2.36 (0.35)	0.99 0.99

miRNA	rs595961 in <i>AGO1</i>					
	AA Major Variant		GG Minor Variant		AG Heterozygous	
	Mean (SE)		Mean (SE)	p-value ^b	Mean (SE)	FDR
<i>miR-21</i>	1.77 (0.23)		0.81 (1.34)	0.48	0.97 (0.40)	0.08 0.53
<i>miR-222</i>	1.68 (0.21)		1.05 (1.21)	0.61	1.17 (0.37)	0.22 0.60
<i>miR-1</i>	0.96 (0.20)		0.17 (1.16)	0.51	0.68 (0.35)	0.50 0.60
<i>miR-125a_5p</i>	1.86 (0.25)		1.28 (1.46)	0.69	1.53 (0.44)	0.51 0.60
<i>miR-125b</i>	1.37 (0.21)		0.49 (1.23)	0.48	1.08 (0.37)	0.51 0.60
<i>miR-126</i>	1.35 (0.14)		1.24 (0.84)	0.90	0.87 (0.25)	0.11 0.53
<i>miR-128</i>	1.88 (0.37)		1.07 (2.17)	0.71	1.27 (0.66)	0.42 0.60
<i>miR-135a</i>	0.75 (0.09)		0.35 (0.55)	0.48	0.58 (0.16)	0.36 0.60

rs595961 in AGO1						
miRNA	AA Major Variant		GG Minor Variant		AG Heterozygous	
	Mean (SE)		Mean (SE)	p-value ^b	Mean (SE)	FDR
<i>miR-146a</i>	1.48 (0.19)		0.84 (1.12)	0.57	1.05 (0.34)	0.27
<i>miR-147</i>	5.85 (2.48)		0.42 (14.57)	0.71	3.03 (4.39)	0.58
<i>miR-218</i>	1.83 (0.62)		0.68 (3.65)	0.76	1.29 (1.10)	0.67
<i>miR-9</i>	0.92 (0.08)		0.87 (0.45)	0.93	0.81 (0.14)	0.50
<i>miR-96</i>	2.15 (0.29)		3.41 (1.68)	0.46	1.23 (0.51)	0.12
<i>miR-155</i>	2.54 (0.27)		2.56 (1.61)	0.99	1.74 (0.48)	0.15
rs910924 in GEMIN4						
miRNA	CC Major Variant		TT Minor Variant		CT Heterozygous	
	Mean (SE)		Mean (SE)	p-value ^b	Mean (SE)	FDR
<i>miR-21</i>	1.56 (0.29)		0.47 (0.63)	0.12	1.79 (0.29)	0.58
<i>miR-222</i>	1.38 (0.27)		1.42 (0.57)	0.96	1.74 (0.27)	0.34
<i>miR-1</i>	0.87 (0.25)		0.14 (0.54)	0.23	1.04 (0.25)	0.62
<i>miR-125a_5p</i>	1.46 (0.32)		1.87 (0.68)	0.59	2.06 (0.32)	0.18
<i>miR-125b</i>	1.09 (0.27)		0.97 (0.58)	0.85	1.54 (0.27)	0.24
<i>miR-126</i>	1.03 (0.18)		0.76 (0.39)	0.54	1.53 (0.18)	0.05
<i>miR-128</i>	1.36 (0.47)		1.80 (1.02)	0.70	2.06 (0.47)	0.30
<i>miR-135a</i>	0.67 (0.12)		0.42 (0.26)	0.38	0.79 (0.12)	0.49
<i>miR-146a</i>	1.11 (0.24)		0.84 (0.52)	0.64	1.73 (0.24)	0.07
<i>miR-147</i>	1.99 (3.14)		13.1 (6.79)	0.14	6.43 (3.14)	0.32
<i>miR-218</i>	1.05 (0.78)		4.55 (1.69)	0.06	1.69 (0.78)	0.56
<i>miR-9</i>	0.89 (0.10)		0.75 (0.21)	0.56	0.92 (0.10)	0.81
<i>miR-96</i>	1.50 (0.37)		1.98 (0.79)	0.59	2.41 (0.37)	0.08
<i>miR-155</i>	1.91 (0.35)		2.39 (0.75)	0.56	2.78 (0.35)	0.08
rs4968104 in GEMIN4						
miRNA	TT Major Variant		AA Minor Variant		TA Heterozygous	
	Mean (SE)		Mean (SE)	p-value ^b	Mean (SE)	FDR
<i>miR-21</i>	1.58 (0.29)		0.5 (0.72)	0.17	1.72 (0.29)	0.72
				0.92		0.95

rs4968104 in <i>GEMIN4</i>						
miRNA	TT Major Variant		AA Minor Variant		TA Heterozygous	
	Mean (SE)	FDR	Mean (SE)	p-value ^b	Mean (SE)	FDR
<i>miR-222</i>	1.54 (0.26)		1.02 (0.65)	0.46	1.64 (0.27)	0.79
<i>miR-1</i>	0.92 (0.25)		0.12 (0.62)	0.23	0.95 (0.25)	0.92
<i>miR-125a_5p</i>	1.77 (0.31)		1.87 (0.78)	0.91	1.75 (0.32)	0.96
<i>miR-125b</i>	1.22 (0.26)		0.66 (0.66)	0.43	1.44 (0.27)	0.57
<i>miR-126</i>	1.05 (0.18)		0.86 (0.45)	0.70	1.48 (0.18)	0.10
<i>miR-128</i>	1.86 (0.46)		1.11 (1.16)	0.56	1.68 (0.48)	0.79
<i>miR-135a</i>	0.66 (0.12)		0.48 (0.29)	0.56	0.78 (0.12)	0.49
<i>miR-146a</i>	1.18 (0.23)		0.52 (0.59)	0.30	1.70 (0.24)	0.12
<i>miR-147</i>	4.22 (3.10)		0.32 (7.78)	0.64	6.75 (3.17)	0.57
<i>miR-218</i>	1.11 (0.77)		0.90 (1.94)	0.92	2.40 (0.79)	0.25
<i>miR-9</i>	0.87 (0.10)		0.93 (0.24)	0.81	0.90 (0.10)	0.81
<i>miR-96</i>	1.52 (0.36)		1.40 (0.89)	0.90	2.51 (0.36)	0.06
<i>miR-155</i>	2.14 (0.34)		1.84 (0.86)	0.74	2.65 (0.35)	0.30

rs2740348 in <i>GEMIN4</i>						
miRNA	CC Major Variant		GG Minor Variant		CG Heterozygous	
	Mean (SE)	FDR	Mean (SE)	p-value ^b	Mean (SE)	FDR
<i>miR-21</i>	1.60 (0.23)		1.08 (1.36)	0.71	1.47 (0.44)	0.79
<i>miR-222</i>	1.62 (0.20)		2.10 (1.22)	0.70	1.22 (0.39)	0.38
<i>miR-1</i>	0.87 (0.19)		0.11 (1.16)	0.52	0.98 (0.38)	0.79
<i>miR-125a_5p</i>	1.84 (0.24)		3.91 (1.43)	0.16	1.31 (0.47)	0.31
<i>miR-125b</i>	1.31 (0.20)		2.49 (1.23)	0.35	1.05 (0.40)	0.56
<i>miR-126</i>	1.27 (0.14)		1.03 (0.86)	0.78	1.11 (0.28)	0.61
<i>miR-128</i>	1.81 (0.36)		3.17 (2.17)	0.54	1.22 (0.70)	0.45
<i>miR-135a</i>	0.68 (0.09)		0.37 (0.55)	0.57	0.79 (0.18)	0.59
<i>miR-146a</i>	1.43 (0.19)		0.94 (1.13)	0.67	1.15 (0.37)	0.50
<i>miR-147</i>	6.15 (2.42)		3.97 (14.53)	0.88	1.08 (4.72)	0.34
<i>miR-218</i>	1.83 (0.61)		1.78 (3.65)	0.99	1.09 (1.18)	0.58
<i>miR-9</i>	0.92 (0.07)		0.17 (0.45)	0.10	0.86 (0.15)	0.70

rs2740348 in <i>GEMIN4</i>						
miRNA	CC Major Variant		GG Minor Variant		CG Heterozygous	
	Mean (SE)	FDR	Mean (SE)	p-value ^b	Mean (SE)	p-value ^b FDR
<i>miR-96</i>	2.06 (0.28)		2.12 (1.70)	0.97	1.56 (0.55)	0.42 0.78
<i>miR-155</i>	2.49 (0.27)		1.32 (1.61)	0.48	1.92 (0.52)	0.33 0.78

SE =Standard Error; FDR =False Discovery Rate for SNP main association.

^aSubset of 93 individuals. Table limited to the four SNPs that showed heterogeneity of the relationship between BC and cognitive function.

^bTwo-sided *p*-value for SNP main association coefficient.