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Association of Long-term Exposure to Air Pollution and Dementia Risk: The Role of Homocysteine, Methionine, and Cardiovascular Burden

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ABSTRACT

Background and objectives: Growing evidence links air pollution with dementia risk, but the biological mechanisms are largely unknown. We investigated the role played by homocysteine (tHcy)

and methionine in this association and explored whether this could be explained by cardiovascular diseases (CVDs).

Methods: Data were extracted from the ongoing Swedish National study on Aging and Care in Kungsholmen (SNAC-K), a longitudinal population-based study. At baseline, 2512 dementia-free participants were examined up to 2013 (mean follow-up: 5.18±2.96 years). Two air pollutants (particulate matter ≤2.5μm [$PM_{2.5}$] and nitrogen oxides [NO_X]) were assessed yearly from 1990 until 2013 using dispersion models at residential addresses. The hazard ratio of dementia over air pollution levels was estimated using Cox models adjusted for age, sex, education, smoking, socioeconomic status, physical activity, retirement age, creatinine, year of assessment and use of supplements. The total effect of air pollutants on dementia was decomposed into four pathways involving tHcy/methionine: 1. Direct effect; 2. Indirect effect (mediation); 3. Effect due to interaction; and 4. Effect due to both mediation and interaction. To test whether the association was independent from CVDs (ischemic heart disease, atrial fibrillation, heart failure and stroke), we repeated the analyses excluding those individuals who developed CVDs.

Results: Mean age of the study participants was 73.4 (SD:10.4) and 62.1% were females. During an average period of five years (mean:5.18; SD:2.96 years), 376 incident dementia cases were identified. There was a 70% increased hazard of dementia per unit increase of $PM_{2.5}$ during the 5-years before baseline (HR:1.71;95%CI:1.33-2.09). Overall, 50% (51.6%;95%CI:9.0;94.1) of the total effect of $PM_{2.5}$ on dementia was due to mediation of tHcy (6.6%;95%CI:1.6;11.6) and/or interaction (47.8%;95%CI:4.9;91.7) with tHcy and 48.4% (p=0.03) to the direct effect of $PM_{2.5}$ on dementia. High levels of methionine reduced the dementia hazard linked to $PM_{2.5}$ by 31% (HR:0.69;95%CI:0.56;0.85) with 24.8% attributable to the interaction with methionine and 25.9% (p=0.001) to the direct effect of $PM_{2.5}$. No mediation effect was found through methionine. Attenuated results were obtained for NOx. Findings for tHcy were attenuated after excluding those who developed CVDs, while remained similar for methionine.

Discussion: High levels of homocysteine enhanced the dementia risk attributed to air pollution, while high methionine concentrations reduced this risk. The impact of homocysteine on cardiovascular

conditions partly explains this association. Alternative pathways other than cardiovascular mechanisms may be at play between methionine and dementia.

INTRODUCTION

A growing body of evidence has linked air pollution to negative cognitive outcomes, including dementia[1-4]. Since air pollution is universal, this observation is of paramount importance as, even with small-to-moderate effect sizes, actions that aim to reduce air pollution would have an enormous public health impact in terms of dementia prevention.

Although most of the studies indicate an increased dementia risk linked to ambient air pollution, the mechanisms through which air pollution impacts the brain are poorly understood. Findings from animal[5] and human[6] studies support the hypothesis that being exposed to polluted air can result in higher brain amyloid-β (Aβ) deposition and neurodegeneration. In addition, stroke is deemed as a relevant intermediate condition between air pollution and dementia, suggesting that vascular pathologies may also play a role in this relationship[7, 8].

Oxidative stress, endothelial dysfunction and systemic inflammation have all been implicated in the pathogenesis of dementia and have been involved in both Aβ deposition and vascular damage[9]. Homocysteine (tHcy), an amino acid generated via demethylation of methionine, may be an important contributor to these pathological processes[10] and it has been linked to the development of cardiovascular diseases[10, 11] as well as to dementia[12, 13]. Conversely, high levels of methionine, an essential amino acid, and a precursor of tHcy, are associated with decreased risk of cardiovascular and neurological conditions including dementia[14-16]. Few studies have explored the impact of air pollution on these amino acids and a recent study found a positive association between high levels of particulate matter (PM) and tHcy and contrasting findings for methionine[17].

In this study, we aimed to explore the impact of tHcy and methionine on the pathway linking air pollution to dementia. Furthermore, we examined whether cardiovascular diseases (CVDs) could have a role in explaining such association. We base our analyses on a clinically characterized population-based cohort with spatially highly resolved information on air pollution and longitudinal clinical evaluations of dementia.

METHODS

Study population

For the present study we employed data from the ongoing *Swedish National study on Aging and Care in Kungsholmen* (SNAC-K), a population-based longitudinal study[18] including individuals aged 60+ and residents of the Kungsholmen district in central Stockholm. At baseline (2001-2004), 3,363 (response rate 73.3%) participants were evaluated. Participants have then been followed every six (young-old cohorts; 60–78 years) or three years (older cohorts; ≥78 years).

Standard Protocol Approvals, Registrations, and Patient Consents

All participants or a proxy provided a written informed consent. The Regional Ethical Review Board in Stockholm, Sweden, approved the protocols of the SNAC-K study.

This study was reported in keeping with the STROBE Recommendations (**eTable 1**).

Data collection

At each study visit, data were collected at a dedicated research center following standard procedures that included face-to-face interviews and clinical and laboratory examinations performed by trained physicians, nurses and psychologists. Participants were assessed at home or in institution if they agreed to participate but were unable to reach the research center.

Information on age, sex, education, and retirement age were collected during the nurse interview. The highest level of formal education was categorized as elementary school, high school, and college/university or above. Socioeconomic position was operationalized considering the longest

occupation held and was categorized into three groups[19]: blue and white collar workers and entrepreneurs. Retirement that occurred before the age of 65 was considered as early retirement. Smoking was considered as current, former, or never smoker. Use of any type of B vitamin supplement was also collected during the physician interview, coupled with the drug register. Creatinine was obtained from laboratory tests at baseline. The level of engagement in physical activities was derived from a questionnaire assessing both the frequency and intensity of different activities and physical inactivity was defined whether the participant was active for less than once/week in light/intensive activity. DNA was extracted from venous blood and Apolipoprotein E (APOE) alleles genotyped. Participants have been categorized as ε4 and ε4-non carriers.

For a sub-sample of SNAC-K participants (N=1976), a self-administered semi-quantitative questionnaire assessed food frequency and was employed to retrieve dietary habits over the year prior to the assessment. The national food composition database was used to compute nutrients intake by multiplying each dish portion by the expected nutrient standard content[20].

Dementia diagnosis

The clinical diagnosis of dementia was performed in keeping with the criteria of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), based on the clinical examination conducted by physicians. Briefly, it includes medical- with questions regarding comorbidities- and drug history as well as general and neurological physical examination. Physicians assess also cognitive functioning by administering tests related to subjective cognitive complaints, problem solving, abstract thinking, self- and time-space orientation, and general knowledge. In addition, the MMSE, Clock Drawing test, counting forward and backward and a short story assessing frontal lobe are administered. Finally, independence of daily living both basic and instrumental are assessed. The diagnosis follows a procedure consisting in three steps[21]. A first and preliminary diagnosis was made by the examining physician who met the participant; secondly, a second preliminary diagnosis was made by a reviewing physician from the data collection team. In case of disagreement between the first and the second diagnoses, the final diagnosis was made by senior neurologists not involved in the data collection. To further ascertain possible diagnoses of dementia among individuals who died

between the SNAC-K follow-up examinations, clinical charts of those who died were collected with their death certificates and examined by the same physicians.

Cardiovascular disease burden

As cardiovascular diseases (CVDs) we considered the following conditions: ischemic heart disease, heart failure, atrial fibrillation, and stroke.

A comprehensive clinical procedure was followed to detect all diseases, as detailed elsewhere [22]. Briefly, diagnoses were based on medical history collected by physicians during the interviews, clinical examinations, diagnostic tests (instrumental and blood tests), inpatient and outpatient records, medical journals and registers from the Swedish National Patient Register [22]. Diagnoses were coded in accordance with the International Classification of Diseases, 10th revision (ICD-10) upon a clinical review performed by trained physicians.

Air pollution assessment

We calculated annual mean levels of $PM_{2.5}$ and NO_x at the home addresses of the participants with dispersion modelling according to local emission inventories[23]. The inventories consist of local emissions of traffic as well as non-traffic sources, for the following years: 1990, 1995, 2000, 2005, and 2011. The local emission of NOx mainly consisted of exhaust emissions from road traffic, while residential wood burning, road traffic exhaust and particles from road wear were the dominant sources of $PM_{2.5}$. A Gaussian dispersion model was applied to the local emission databases. Annual mean levels of $PM_{2.5}$ and NO_x were obtained from linear interpolation over the four years between each model simulation. In order to obtain total levels of $PM_{2.5}$ and NO_x, annual long-range contributions, homogeneous over the model domain, were added to the simulated locally generated levels. The long-range contributions were based on measurements at the rural monitoring site Norr Malma, located outside the calculation domain, 60 km northeast of Stockholm. We compared the model that calculated yearly levels with the one that measured the values at three curbside (traffic) monitoring sites and one urban background site in Stockholm City, and we obtained r2-values of 0.97 for NOx and 0.86 for $PM_{2.5}$.

Out of the 2512 participants included at baseline, 248 moved outside the district of Kungsholmen during the entire follow-up time. The exposure level of the pollutants for these individuals was calculated at the new home address. No major differences were detected between those who were residents of the Kungsholmen district for the entire period and those who moved outside the study area (5-year average before baseline of $PM_{2.5}$ and NOx were 8.4±0.7 and 8.3±0.7 and 33.9±11.7 and 32.7±11.7 for Kungsholmen residents and those who moved, respectively). Those who moved outside of Stockholm County (n=9) were instead excluded from the study.

Serum methionine and homocysteine

At baseline, non-fasting venous blood samples were collected. Routine analyses were carried out within two hours, through a chemiluminescence microparticle folate-binding protein assay at the Sabbatsberg Hospital, Stockholm, Sweden. Plates in dry ice were then shipped to the University of Oxford, United Kingdom. The levels of tHcy and methionine were measured using tandem mass spectrometry after treatment of serum with a reducing agent, as detailed previously[24]. Interassay coefficients of variation ranged between 5%-10%. tHCy and methionine values above 15 µmol/L[25] and 20.7 µmol/L (upper two tertiles) were considered as high, respectively. We also considered the ratio between methionine and tHcy (Met:tHcy) as a possible indicator of methylation activity with high ratios considered proxy for greater methylation activity[15]. The two upper tertiles of Met:tHcy levels were defined as a high level (cut-off: 1.47 µmol/L).

Statistical analyses

Cox models were employed to derive hazard ratios (HRs) and 95% confidence intervals (CIs) of dementia in relation to 5-years average $PM_{2.5}$ and NOx before baseline assessment. Individuals were considered at risk until dementia diagnosis, death, or end of follow-up, whichever came first. We assumed a linear relationship between $PM_{2.5}$, NOx and the log(HR). The proportional hazard assumption was assessed by regressing the Scaled Schoenfeld's residuals against survival time. No deviation from proportionality was detected (p: 0.3313 for $PM_{2.5}$ and p: 0.1649 for NOx).

The potential mediating and interactive effects of serum markers of methylation status were analysed through the counterfactual approach[26, 27] by decomposing the total effect of $PM_{2.5}$ (and NOx) on dementia into four potential causal pathways: 1. A direct effect (pathways associating air pollution and dementia independently of serum markers of methylation status); 2. The effect due to mediation alone (pathways associating air pollution and dementia only through serum markers induced by air pollution); 3. The effect of interaction between air pollution and serum markers (pathways that only operate when both increased serum biomarkers and air pollution are present without mediation effect); and 4. The effect due to both mediation and interaction (pathways that only operate when both increased serum biomarkers and air pollution are present with mediation effect). The decomposition allows estimating and testing the proportion of the total effect due to each of these four components. The four-way decomposition of the total effect requires jointly testing the associations between the exposures and the mediators/modifiers, which were assessed using linear or logistic regression, and the associations between the exposures and outcome, which were assessed using survival model. **eFigure 1** shows the four different pathways model.

To test whether CVDs played a role in the associations, we repeated the analyses after excluding those who developed CVDs during the follow-up (incident CVDs cases: 364).

Potential confounding factors were *a priori* identified based literature review[2] and available data from the study population: age, sex, educational level, year of assessment, smoking, early retirement, socioeconomic position, physical activity, creatinine and use of supplements.

Secondary analyses: To evaluate the potential modifier effects of sex and APOE, we tested for interaction between air pollutants and both sex and *APOE* genotype and reported the analyses by sex and *APOEε4* carriers/non carriers. As sensitivity analysis, we additionally adjusted the models for food intake of folate and vitamin B12.

All statistical analyses were performed with Stata, version 17 (StataCorp, TX, USA).

Data Availability

Access to the data for the current study (snac-k.se) will be possible upon request and approval by the SNAC-K data management and maintenance committee at the Aging Research Center, Karolinska Institutet, Stockholm, Sweden.

RESULTS

The analytical sample resulted in 2512 dementia-free individuals as we excluded 240 persons affected by dementia at baseline, one person with intellectual disability, and four participants with tHcy levels >69.97 μmol/L or creatinine > 400 μmol/L. In addition, 410 individuals had missing information on biomarkers or air pollution and 196 had missing dementia information at follow-up. Those with missing information on exposure or covariates were more likely to be older ($p<0.001$), while no differences concerning sex and education arose ($p=0.865$ and $p=0.920$, respectively)

During an average period of five years (mean:5.18; SD:2.96 years; range 2.1–10.3 years), 376 incident dementia (incidence rate per 1000 person/year:28.9;95%CI:26.1-31.9) cases were identified. Baseline characteristics of the analytic sample overall and by incident dementia are reported in **Table 1**. Participants who developed dementia were less educated, less likely to be male, more likely to be/have been blue collar, and retired before age 65. Higher tHcy and lower methionine concentrations were observed among participants who developed dementia compared to those who did not.

Table 1 here

Figure 1 shows the average concentration (μg/m^3) of $PM_{2.5}$ and NOx during the five years preceding baseline assessment. During the five years before baseline there was a slight decrease in the concentration of both air pollutants, specifically -2.04 μg/m3 (SD:0.02) for $PM_{2.5}$ and -4.68 μg/m3 (SD: 2.03) for NOx.

Figure 1 here

A higher 5-year mean residential outdoor $PM_{2.5}$ concentration was associated with higher tHcy and methionine at baseline, by 0.36 μmol/L for tHcy (95%CI:0.07-0.66) and 0.36 μmol/L (95%CI:0.02-0.70) for methionine, per 1 μg/m^3 $PM_{2.5}$, after adjusting for potential confounders (age, sex, education,

smoking, retirement status, socioeconomic position, physical activity, creatinine and use of supplements). Similarly, higher levels of NOx were associated with a higher tHcy concentrations, by 0.31 (95%CI:0.07-0.5) per 10 μg/m^3 increase in 5-year mean NOx. NOx levels were not associated with a difference in methionine (mean difference μmol/L:0.15;95%CI:-0.13;0.42 per 10 μg/m^3 increase in NOx). Neither $PM_{2.5}$ nor NOx concentrations showed a significant association with Met:tHcy.

In adjusted models, a tHcy concentration above 15 μmol/L was associated with 55% higher hazard of dementia (HR=1.55;95%CI:1.23-1.95), and methionine above 20.7 μmol/L was associated with approximately 30% lower hazard to develop dementia (HR=0.69,95%CI:0.56-0.85). Met:tHcy ratio above 1.47 μmol/L was associated with 37% lower hazard of developing dementia (HR=0.63,95%CI:0.51-0.79).

After considering potential confounders, 70% higher incidence of dementia was found per 1μg/m^3 increase of $PM_{2.5}$ (HR=1.71;95%CI:1.33-2.09), whereas a 30% increased risk of dementia was found per 10 μg/m^3 increase of NOx (HR:1.33;95%CI:1.19-1.49).

Table 2 presents the association between $PM_{2.5}$ and incident dementia decomposed by tHcy and methionine. We found that 51.6% of the total effect of $PM_{2.5}$ on dementia was due to mediation and/or interaction with tHcy. Overall, 47.8% of the association was explained only by interaction, while 6.6% only by mediation. Furthermore, 48.4% of the association between $PM_{2.5}$ and dementia could be attributed to a direct effect.

High concentrations of methionine reduced dementia risk linked to $PM_{2.5}$ exposure by 26%.
No statistically significant mediation effect was found through methionine in the association between $PM_{2.5}$ and dementia. A direct effect of $PM_{2.5}$ on dementia was also found here.

Table 2 here

Similar, but attenuated, results were obtained for NOx (**eTable 2**).

eTable3 shows the association between $PM_{2.5}$ and NOx and incident dementia decomposed by Met:tHcy. High Met:tHcy ratio was associated with a reduced dementia risk (-44.5%;95%CI:-83.0%;-

5.9%) per 1μg/m^3 increase of $PM_{2.5}$. This was mainly due to a large proportion attributable to interaction between $PM_{2.5}$ and Met:tHcy, while no mediation effect was detected. Similar results were obtained for NOx.

The results for tHcy were attenuated and no longer statistically significant, whereas the results remained similar for methionine when we repeated the analyses after excluding individuals with incident CVDs (**Table 3**). For $PM_{2.5}$, neither the mediating nor the interaction roles were significant, while a direct effect was present (65.6%; 95%CI:6.4;100%). High concentrations of methionine reduced the dementia risk linked to $PM_{2.5}$ exposure by 30% but mediation remained non-significant. Similar results were obtained for NOx (**eTables 4**).

Secondary analyses: We tested the modifying effect of sex and *APOE* genotype and report the results in **eTable 5**. Overall, 1μg/m3 increase of $PM_{2.5}$ was associated with an 87% (1.61-2.16) higher hazard for dementia in women and a 66% (1.32-2.09) increased hazard in men, while a 10 μg/m3 increase of NOx was linked with a 3% increased dementia risk both in women (1.02-1.04) and men (1.01-1.05). Concerning *APOE* genotype, 1μg/m3 increase of $PM_{2.5}$ was associated with a 78% (1.53-2.0) higher hazard for dementia in APOEε4 non carriers and an 83% (1.48-2.26) increased hazard in ε4 carriers, while a 10 μg/m3 increase of NOx was linked with a 3% increased dementia risk both in non-carriers (1.02-1.04) and carriers (1.01-1.05).

When we further adjusted the analyses for food intake of folate and vitamin B12 the results were consistent for homocysteine while slightly attenuated for methionine (**eTable 6**).

DISCUSSION

The present study led to the following main findings: First, similar to previous studies, air pollution emerged as a risk factor for dementia development; Second, homocysteine and methionine, as well as the Met:tHcy ratio (an indirect sign of methylation activity), played a relevant role in this association; Third, their role was mainly as effect-modifiers, with homocysteine exacerbating, while methionine mitigating the effect; Finally, the role played by homocysteine was only observed in participants who

had developed CVDs, whereas the protective role of methionine was likely independent of CVDs development.

Air pollution and dementia risk

Air pollution has recently been included in the list of modifiable risk factors for dementia[1]. Despite methodological heterogeneity in study designs, differences in exposure assessments and different settings, most recent studies provide evidence in support of an association, especially when considering $PM_{2.5}$ exposure[2]. In our study, we observed a 70% and 30% increased dementia risk per 1μg/m^3 and 10 μg/m^3 increase in $PM_{2.5}$ and NO_x, respectively. These results come from an urban area in central Stockholm where substantial improvements in air quality have occurred in the last decade, and where mean concentrations of air pollutants are quite low in comparison to the average in the rest of Europe, US or China. As a comparison to our mean concentration of 8.3 μg/m3, a recent study[28] in Europe reported an average annual concentration of $PM_{2.5}$ in 2019 of 13.8 μg/m3 but it is noteworthy that mean concentrations in our study were higher compared to those of 5.7 μg/m3 reported in the Nordic countries. Our results are in line with other Swedish studies investigating air pollution and dementia onset[29, 30] and suggest once again that even low concentrations of air pollution are associated with poor health outcomes[31, 32]. However, unlike previous studies that have reported stronger associations between $PM_{2.5}$ and dementia in women as compared to men [33], we were unable to detect such a difference. Identifying vulnerable subgroups who might particularly benefit of air quality improvements continues to be important to safeguard public health and in setting efficient and appropriate air quality standards[34].

The modifier role of homocysteine and methionine in the air pollution-dementia risk association

In the present study, we found that higher concentrations of tHcy increased the detrimental effect of air pollution by 50%, whereas raised methionine reduced the air-pollutant related dementia risk by 30%. We also found that a higher Met:tHcy ratio was associated with a reduced dementia risk related to air pollution exposure. Met:tHcy ratio can be considered a possible indicator of methylation activity

and reduced values of this ratio reflect impaired methylation activity. Hcy reflects the functional status of three B vitamins (folate, vitamin B12 and B6)[13] and a number of factors can, directly or indirectly, raise blood concentration of tHcy, including age, renal impairment, and B vitamin insufficiency[10]. Notably, it has been suggested that PM exposure may raise Hcy levels by inducing systemic inflammation and oxidative stress, reducing the activity of enzymes implicated in Hcy metabolism and/or competing with methyl groups with the Hcy re-methylation process[17].

According to our findings, for both homocysteine and methionine, interaction with air pollution seemed to be stronger than mediation. Our findings on interaction demonstrate an interplay between hyper-homocysteinemia and low methionine levels with air pollution determining the individual´s dementia risk. This is consistent with the fact that hyper-homocysteinemia and low levels of methionine can be generated through pathways other than high air pollution exposure, as mentioned above[10].

Possible mechanisms underlying the impact of air pollution on cognitive aging

The mechanisms by which air pollution impacts brain health are still mostly unknown. A study including transgenic mice exposed to urban nanosized PM (nPM) showed increased cerebral Aβ deposition[5]. This evidence was corroborated by a study from the US including 18,000 cognitively impaired individuals where [6] the authors found that exposure to $PM_{2.5}$ was associated with deposition of brain Aβ plaques. According to our findings, a direct effect of air pollution on dementia was responsible for up to 46.7% of the total effect, suggesting that alternative pathways may be at place, among which it can be hypothesized a deposition of amyloid plaques. Air pollution can also affect the brain through indirect pathways; e.g., a close heart-brain connection is at play in dementia development[35] and it is well established that different components of air pollution increase cardiovascular morbidity and mortality [36, 37]. In line with these observations, we and others previously found that the presence of CVDs enhanced the detrimental effect of air pollution[8] and, in particular, stroke was an important intermediate condition between air pollution and dementia[7]. Current knowledge supports two main pathways, namely a direct damage of air pollution to the brain and

indirect pathways, including the development of CVDs. It is plausible to hypothesize that these pathways act in synergy being more complementary than alternative in affecting brain health.

In our study, we did not find any relevant mediation/interaction through tHcy when we excluded individuals who developed CVDs and this sheds light on the possible mechanisms through which tHcy acts in this association, adding weight to the crucial role played here by CVDs. In an autopsy study investigating the impact of tHcy on different neuropathological outcomes, the relationship between tHcy and neurofibrillary tangles was limited to individuals who also had cerebral infarcts, suggesting that cerebral perfusion may modulate the impact of homocysteine on tau pathology[38]. In our study, after the exclusion of incident CVDs cases, the proportion attributable to the direct effect of pollution rose to 65% of the total effect, thus suggesting that air pollution acts through multiple pathways including direct effects without the involvement of tHcy in addition to indirect effects through CVDs partly influenced by tHcy.

An attenuation of the effect after excluding CVDs cases was not detectable for methionine, suggesting that its protective role could act through pathways other than reduced cardiovascular burden. Methionine may in fact have a more pleiotropic role than tHcy, being potentially involved in processes not primarily linked with CVDs, but eventually affecting brain aging. Indeed, methionine is involved in processes such as protein synthesis and polyamine metabolism and serves as the precursor to produce amino acids involved in normal brain function[39, 40]. Methionine deficiency has been linked with glutathione deficiency, a major antioxidant, associated with the development of several diseases[41]. Interventions that lower tHcy and increase methionine, such as improving B vitamin status [13], might thus modify the association between air pollution and dementia.

Strengths and limitations

Our findings are based on a large, clinically characterized, population-based study with spatially detailed information on long-term exposure to air pollution, biomarkers and clinical evaluations including dementia diagnosis. Some limitations need to be acknowledged. In SNAC-K, we lack a

biological characterization of dementia subtypes and further studies are needed to better understand whether air pollution raises specific subtypes risk (e.g., AD, vascular dementia, Lewy Body dementia). We, however, would like to point out that in SNAC-K most of the dementia cases are late onset, and previous studies have shown that dementia occurring after the age of 75 is rarely purely AD but more commonly demonstrating a mixed pathology [43]. In addition, the geographical area included, the Kungsholmen district of Stockholm, is small and limits spatial contrasts in air pollutants. Further, SNAC-K includes older adults who are generally wealthy, fit and healthy and live in an area with relatively low air pollution levels, which might limit the generalizability of our findings to other populations. However, this would most likely lead to a possible underestimation rather than inflation of the association between air pollution and dementia and coupled with the observations of harmful effects at low exposure levels, this strengthens the clinical and public health message. In the present study, air pollutant exposure was assessed since 1990 and is assumed to temporally overlap with the long preclinical and prodromal stages of dementia during which lesion deposition and accumulation can occur. Future studies challenging that assumption with even longer observational period should be undertaken, but irrespectively our findings likely support the role of air pollution as an exacerbation of dementia development. We were limited to using serum biomarkers from a single time point, and to confirm our findings further evaluations on persistent levels of high homocysteine and low methionine during follow-up time are needed. In addition, even if we were able to adjust the analyses for several covariates, including use of supplements as well as for food intake of folate and vitamin B12, we did not have available data on frequency and dosage of supplements. Finally, alternative or complementary biological pathways might be at play in the air pollution/dementia link, for example inflammation, and these hypotheses should be tested in future studies.

Conclusion

Collectively, our findings support the evidence of air pollution as a risk factor for dementia in older adults. This was evident in an area with comparatively high standard of air quality and argues for additional actions in further reducing air pollution in our cities. We here found, once again, that an

indirect link between air pollution and dementia may exist, and that the homocysteine/methionine cycle, as well as cardiovascular burden may be implicated. In addition to these pathways, our results indicated a substantial direct effect of air pollution on dementia suggesting that air pollution affects the development of dementia through multiple pathways. This highlights the need to further elucidate the exact biological mechanisms behind the brain damage of air pollution.

Tables and Figures

Table 1. Baseline characteristics of the study population overall and by dementia status at follow-up

	No Dementia (N=2136)	Dementia (N=376)	Total (N=2512)
Age (years, mean±SD)	71.9 (10.1)	82.2 (7.3)	73.4 (10.4)
Female, n (%)	1295 (60.6)	266 (70.7)	1561 (62.1)
Education, n (%)			
Elementary	308 (14.4)	81 (21.6)	389 (15.5)
High School	1032 (48.3)	224 (59.7)	2256 (50.0)
University or above	796 (37.3)	70 (18.7)	866 (34.5)
Smoking, n (%)			
Never smoker	954 (44.8)	205 (54.6)	1159 (46.3)
Former smoker	847 (39.8)	125 (33.3)	972 (38.8)
Current smoker	326 (15.4)	45 (12.1)	371 (14.9)
Age retirement, n (%)			
After 65 years	482 (22.6)	4 (1.1)	486 (19.3)
SES status, n (%)			
Blue collar workers	439 (20.6)	121 (32.3)	560 (22.4)
White collar workers	1466 (68.7)	226 (60.1)	1692 (67.4)
Entrepreneurs	227 (10.7)	28 (7.6)	255 (10.2)
Physical activity, n (%)			
No or mild activity	1447 (67.7)	253 (67.3)	1700 (67.7)
High activity	528 (24.7)	36 (9.6)	564 (22.5)
tHcy, median (IQR), µmol/L	12.7 (5.4)	14.7 (6.8)	12.9 (5.5)
tHcy, n (%)			
<15 µmol/L	1497 (70.1)	205 (54.5)	1702 (67.8)
≥15 µmol/L	639 (29.9)	171 (45.5)	810 (32.3)
Methionine µmol/L, median (IQR)	23.1 (7.3)	21.7 (10.0)	22.9 (7.3)
Methionine- tHcy ratio µmol/L, median (IQR)	1.82 (1.0)	1.51(0.8)	1.78 (1.0)
Creatinine, median (IQR), µmol/L	86.0 (19.0)	86.0 (22.0)	86.0 (19.0)
$PM_{2.5}$*, mean (SD) µg/m^3	8.3 (0.7)	8.4 (0.6)	8.3 (0.7)
NOx*, mean (SD) µg/m^3	25.6 (9.1)	26.1 (7.1)	25.7 (8.8)

Abbreviations: SES, socio economic status; SD, standard deviation; IQR, interquartile range; PM, particulate matter; NO: nitrogen oxides; tHcy: total serum homocysteine

Missing values: 0.04% (n=1) for education; 0.4% (n=10) for smoking; 0.7% (17) for early retirement; 0.2% (n=5) for SES; 9.9% (n=248) for physical activity.

*Average exposure during the 5-years period preceding the baseline assessment

Figure 1 Concentration levels of PM2.5 and NOx during the five years preceding baseline assessment

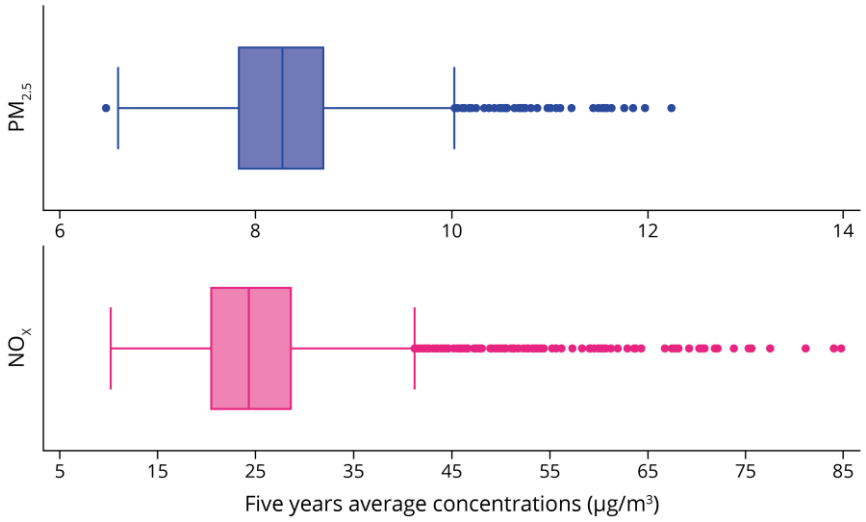

Table 2 Decomposition of the association between $PM_{2.5}$ and incident dementia into pathways involving homocysteine (Panel A) and methionine (Panel B)

Homocysteine	**Proportions**	**P-value**	**95% CI**
Proportion attributable to direct effect	48.4%	0.026	5.9%;91.0%
Proportion attributable to interaction	47.8%	0.029	4.9%;91.7%
Proportion attributable to mediation	6.6%	0.009	1.6%;11.6%
Overall proportion eliminated*	51.6%	0.017	9.0%;94.1%
Methionine			
Proportion attributable to direct effect	25.9%	0.000	3.2%;48.7%
Proportion attributable to interaction	-24.8%	0.030	-47.3%; -2.4%
Proportion attributable to mediation	-2.1%	0.346	-6.6%; 2.3%
Overall proportion eliminated*	-25.9%	0.026	-48.7%; -3.2%

Results are derived from Cox regression model with four-way decomposition by levels of homocysteine (cut-off: 15 µmol/L) and methionine (cut-off: 20.7 µmol/L).

Models adjusted for age, sex, education, socioeconomic position, retirement age, smoking, physical activity, creatinine, year of assessment and use of supplements.

*This proportion includes the effect attributed to both interaction and mediation.

Table 3 Decomposition of the association between $PM_{2.5}$ and incident dementia into pathways involving homocysteine (Panel A) and methionine (Panel B) after excluding incident cardiovascular diseases.

Homocysteine	**Proportions**	**P-value**	**95% CI**
Proportion attributable to direct effect	65.6%	0.030	6.4%;100%
Proportion attributable to interaction	30.8%	0.311	-28.8%;90.5%
Proportion attributable to mediation	5.1%	0.059	-0.2%; 10.3%
Overall proportion eliminated*	34.4%	0.255	-24.8%; 93.6%
Methionine			
Proportion attributable to direct effect	30.0%	0.000	4.9%; 55.0%
Proportion attributable to interaction	-29.2%	0.020	-53.8%; -4.6%
Proportion attributable to mediation	-1.6%	0.532	-6.6%; 3.4%
Overall proportion eliminated*	-30.0%	0.019	-55.0%; -4.9%

Results are derived from Cox regression models with four-way decomposition by levels of homocysteine (cut-off: 15 µmol/L) and methionine (cut-off: 20.7 µmol/L).

Model adjusted for age, sex, education, socioeconomic position, retirement age, smoking, physical activity, creatinine, year of assessment, use of supplements cardiovascular diseases at baseline.

*This proportion includes the effect attributed to both interaction and mediation.

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Association of Long-term Exposure to Air Pollution and Dementia Risk: The Role of Homocysteine, Methionine, and Cardiovascular Burden

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